VI.—*The Life-history of the Meningococcus.*

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(Read April 18, 1917.)

PLATES XXIV AND XXV.

IN November of last year I enjoyed the privilege of demonstrating to this Society, with the aid of Mr. Martin Duncan's microphotographic skill, some morphological phases in the life-history of certain bacteria, such as the *Bacillus exanthematicus* of typhus fever, and the organisms of the enteric group.

You will perhaps remember that the object of my demonstration was to present evidence, admittedly incomplete, that the lower bacteria are not necessarily what they seem to be, and as we are taught, namely, unicellular organisms with a simple life-history. So far, indeed, from their life-cycle being a simple affair, beginning and ending with transverse binary fission into two equal parts, the evidence, such as it was, suggested that the life-history of bacteria is one of great complexity, and includes, in some cases at least, a minute filterable, and invisible, or almost invisible, stage.

To advance such a doctrine as this in the face of up-to-date bacteriological teaching is to proclaim oneself a heretic, and to invite the doom that awaits all heretics.

As, however, I have not yet been led to the stake, I venture to ask you once again to examine very briefly, as you did last November, the articles of faith of the orthodox bacteriologist, who

EXPLANATION OF PLATES XXIV. AND XXV.

FIGS.

- 1.—Meningococcus. Case 4. Cerebro-spinal fluid incubated two nights, then on serum-agar one night. Benian's Congo-red film. $\times 1500$.
- 2.—Case 3. Plymouth. Cerebro-spinal fluid incubated two nights, then placed in acid broth two nights; then in serum-broth one night. $\times 1500$.
- 3, 4.—Meningococcus. One colony, on serum-agar from incubated cerebro-spinal fluid containing budding cysts and meningococci. Fermenting glucose only. Spread on serum-agar. Confluent growth A.S.B. $\times 60$ to Phen; then incubated five days. Centrifuged, and examined in Congo-red. $\times 1500$.
- 5.—Case 3. Plymouth. Cerebro-spinal fluid incubated two nights, then placed in acid broth two nights. $\times 1500$.
- 6.—Case 3. Plymouth. Cerebro-spinal fluid incubated two nights, then placed in acid broth two nights; then in serum broth one night. $\times 1500$.
- 7, 8.—Meningococcus. Serum broth. Benian's Congo-red film. $\times 1650$.

very properly supports the teaching of the schools. If, says he, an aberrant morphological type of organism appears in my cultures, it is one of three things—it is either a contaminant, or it is an involution form, or, if he is a brave man, it is a mutant. But whichever of the three he thinks it is, he throws away his cultures and starts afresh. And he does this because he is firmly convinced that the lower bacteria can only reproduce themselves—apart from endosporulation of a special type in certain cases—by equal binary fission, this belief being itself founded on the unquestioned fact that bacteria do breed true to type in standardized laboratory media.

But, as you may remember, I ventured in answer to these arguments to formulate four propositions:—

1. That it is just as dangerous to assume that a given organism is a contaminant, without the most searching enquiry, as it is to assume that it is not.

2. That to call an aberrant morphological type an involution form, especially when it occurs in young and suitable media, is to take refuge behind a label which under such circumstances means nothing at all.

3. That the fact that bacteria breed true to type (to laboratory type) in test-tubes under the highly artificial condition of laboratory culture cannot reasonably be taken as evidence that the test-tube method of reproduction is the sole method of reproduction under natural conditions.

4. That if the orthodox view as to the simplicity of the life-cycle of bacteria be true, the phenomenon has practically no parallel in comparative biology.

On theoretical grounds alone, therefore, I urged that the present view is not likely to be the correct view, and that in order to examine the evidence for the opposite view with an impartial mind, it is absolutely essential that we should not invoke too easily the contaminant, the involution form, the mutant, *et hoc genus omne*, in order to escape our difficulties.

This, of course, is not to say that the most rigid precautions against error should not be taken, as the description of the technique to be presently described will, I hope, show.

It is, of course, fully recognized that innumerable observations have been recorded in the past of aberrant morphological types occurring in apparently pure cultures of bacteria, especially when their cultural environment was altered. Excellent illustrations of this may be seen, for example, in Besson's pictorial description of the *Bacillus pyocyaneus*, and of the *B. anthracis*. The first point, however, I wish to make is, that if patience be employed it is often possible to show the presence of aberrant morphological types without change of cultural environment. It is therefore unnecessary, provided that optimum conditions of cultivation are

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employed, to invoke the theories of involution forms, or of mutation, in order to explain these genuine types of pleomorphic activity. A more reasonable, and much simpler, explanation of bacterial pleomorphism lies, in short, in the view that these aberrant types merely represent different stages in the life-history of bacteria. And this view is strongly supported, if not absolutely proved, by actual observation, on the warm-stage, of the development of single individuals. And in this connexion it is well to remember that excellent precedent is afforded for this explanation by demonstration of the fact that the *Discomyces* and *Sporotricha*, to mention only two groups, are not bacterial at all, as they were for long considered to be.

So much by way of introduction.

To-day Mr. Martin Duncan and I propose to put before you fresh evidence in support of my thesis that the lower bacteria are not necessarily what they seem to be. And the organism I have selected for this purpose is the meningococcus of Weichselbaum.

It is generally taught, and it is widely believed, that the meningococcus of Weichselbaum is the direct casual agent of that form of epidemic cerebrospinal fever with which the meningococcus is usually associated.

It is also taught, and it is generally accepted, that the meningococcus—as one of the “lower” bacteria—is an organism with a simple life-history, reproduction taking place exclusively by the process of binary fission into two equal parts, a meningococcus always arising from a meningococcus, and in no other way.

As regards the first of these propositions, that the meningococcus is itself the cause of epidemic cerebrospinal fever, I showed by experiment in 1915—and this, in conjunction with Captain Caulfeild, I amply confirmed in the following year—that the theory cannot be accepted for this simple reason, viz. that cultures of the meningococcus in cerebrospinal fluid, or in laboratory media, frequently contain a filterable virus which is just as pathogenic to monkeys (though not necessarily productive of the disease) as are unfiltered cultures.

It follows from this, notwithstanding many statements to the contrary, that the disease has not yet been produced experimentally in animals by injection of pure cultures of Weichselbaum's organism. There is, in fact, no evidence that the meningococcus as such is responsible for the disease.

It is true that an attempt was made to discredit my results by the suggestion that the filterable virus referred to was a toxin. But there is no toxin known to us which is directly capable of producing continued fever lasting for many weeks, after the lapse of a well-marked latent period. And completely negative bacteriological examinations of the animals in question excluded the

indirect action of a theoretical toxin in producing the results observed.

The demonstration of the presence of this filterable virus in meningococcal cultures had, in addition, this important result, that it showed quite clearly that the second proposition of the text-books to which I have referred—namely, that the meningococcus is a bacterium with a simple life-history, beginning and ending with simple binary fission—is probably not true. It unmistakably suggests, in fact, that the meningococcus represents one phase only in the life-cycle of an organism which has not yet been described.

The object of the present communication is, as I have said, to show yet another phase in the life-history of the unknown causal organism of epidemic cerebrospinal fever, though I wish to make it perfectly clear that even now the whole of the mystery is not cleared up, and that there are still other phases which require further study.

I shall, therefore, here confine myself to what may be learnt by study of the so-called giant meningococcus, which is generally believed to be a large involution form of Weichselbaum's organism, but which is in reality, as you will see, not a bacterium at all—even a diseased bacterium, as your Secretary humorously would put it. Before, however, Mr. Martin Duncan shows you his slides, it is necessary very briefly to summarize what is at present known as regards the morphology of the meningococcus and its alleged involution forms.

The meningococcus, which is usually only studied in cultures on solid media, is a Gram-negative coccus, occurring singly or in pairs, often with flattened opposed surfaces, sometimes in triads or in tetrads, but never—the text-books say—in chain formation, except as a result of accidental apposition. Heiman and Feldstein also describe giant cocci, four to five times the size of a normal coccus, which in diplococcoid form measures roughly 1 m. in diam. These giant cocci stain intensely with whatever counterstain is employed in Gram preparations, and are believed by these authors to be involution forms, and are said to be only seen in cultures up to forty-eight hours old. In some cultures, as I recorded in 1914, extremely minute cocci are also to be seen mixed with the larger normal forms, and with the so-called giant cocci. And between these very minute forms and the normal forms, intermediate forms of every grade in size are sometimes to be made out, sometimes in diplococcoid form, and at others in single coccus form. In some of the normal-sized cocci, and occasionally in the giant forms, a central bright spot has been described (staining deeply with alkaline-methylene blue)—not to be confused with the clear space, sometimes remarkably wide, which may be seen between the two members of a dividing coccus.

The presence of the giant forms appears to receive no notice in

the English text-books, faithful to the general tradition that bacterial morphology does not merit serious study. And in no description of the meningococcus that I have seen in any language has there appeared any account of observations of growth of this organism, or of the giant forms, on the warm-stage, an omission, as will be seen, which explains a good deal.

From this brief résumé of the present state of knowledge of the morphology of the meningococcus, there is clearly a good deal to suggest that further enquiry was necessary as to the supposed botanical position of that organism. We have here, for example, an organism which varies very greatly in size, which sometimes occurs in chains, whether accidentally produced or not, and which sometimes has a central bright spot, giving in unstained preparations a clear space, strongly suggestive of a false spore. And, finally, we have giant forms appearing repeatedly in young suitable cultures of a few hours old, which, on that account alone, are most unlikely to be involution forms; as well as very great variation in size of "normal" meningococci, not only in cultures from one case, but also in cultures from one case as compared with cultures from another case. Anyone, for example, who has studied the morphology of Gordon's so-called strain No. 2 in liquid cultures cannot but have been struck with the large size of the normal individuals present, as compared with those often seen in cultures from strain No. 1. So aberrant, in fact, are they often found to be, that it is difficult to believe that they are true meningococci, either in size or shape.

So much for current descriptions of meningococcal morphology, as studied mainly on solid media.

We come now to my own studies of the morphology of the meningococcus in liquid media, subcultured from solid media. And here let me say, that in attempting to determine the true botanical position of the meningococcus, or of any other reputed bacterium, it is often fatal to restrict one's observations to growth on solid media only, however convenient these may be for purposes of identification.

In the case of the meningococcus, I have so far only once succeeded in obtaining free growth on solid media of the organism we will now show you, although I had no difficulty in obtaining free growth in thirty-six consecutive acute cases of cerebrospinal meningitis from primary colonies on serum-agar.

In every case the meningococcus has been isolated and fully identified, by its staining reactions, its morphology, its sugar reactions, and its serological reactions, and by certain well-defined cultural reactions, many of which are not in the text-books. I have not found the largest forms in any case apart from the meningococcus, and in no case has the meningococcus been found without these organisms being also found. I have never been able to cultivate

them in pure culture apart from the meningococcus, and in every apparently pure culture of the meningococcus these organisms have always been found if diligently searched for.

They can be demonstrated in incubated cerebrospinal fluid, collected with every precaution against contamination; and they can be demonstrated, if searched for, in single colonies on serum-agar plates, provided that these have been inoculated with incubated cerebrospinal fluid. The best and most certain method, in fact, of demonstrating their presence has been, in my hands, by subculture from single colonies on serum-agar, itself inoculated with incubated cerebrospinal fluid. This subculture, however, must be into liquid media, an excellent medium being equal parts of tested horse-serum and broth, the reaction of which to phenolphthalein may be + 10, + 30, or even + 50.

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

1. Cerebrospinal 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 each case only samples of fluid withdrawn at first lumbar puncture 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 $37\cdot0^{\circ}$ C. for a period varying from forty-eight to seventy-six hours.

4. A further volume of the fluid is now withdrawn from the flask, and is centrifuged and examined.

5. Of six cases in which prolonged examination was carried out of films prepared from the deposit of the incubated fluid, the organism has been found in three.

6. Two or more serum-agar slopes, and sometimes, in addition, three serum-agar plates, are now incubated; the plates in series with considerable volumes of the incubated cerebrospinal fluid.

7. 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.

8. On the following day the entire growth of each slope is inoculated into serum-broth tubes, which are incubated for forty-eight hours.

9. 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.

10. Samples of the serum-broth tubes are at the same time, and subsequently every day, centrifuged and the deposits examined.

11. The 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 is possible to obtain by observation of cultures from single colonies is assured.

However useful for purposes of identification, and for ensuring sterilization as far as may be, cultivation from single colonies cannot, nevertheless, give an absolute guarantee of sterility. 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 each individual into a separate tube containing a suitable liquid medium, in the hope of successful cultivation therefrom.

This 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, and even to be seen as very minute organisms, 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 one of these smaller forms. Now, the smallest of these forms are, as I have repeatedly tested, quite invisible under a dry lens, and the highest compensating ocular that will allow of good illumination. Even with a magnification of 3000, as 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 this point, and this 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.

After innumerable trials I have however partially succeeded, as you will see.

Owing, however, to the fact that these giant forms are apparently loth to grow on solid media, it became necessary to devise a method of observing growth in liquid cultures, the hanging-drop method of course being useless for the purpose owing to the shape and depth of the drop.

There are two ways, both of which you will see illustrated on the screen, of studying growth in liquid cultures on the warm stage.

The first—not very satisfactory, owing to rapid drying—is to make a thin film of serum-broth culture under a cover-slip on an ordinary sterile slide covered with a thin layer of fresh serum-agar. In successful cases growth of the giant forms, showing exogenous gemmation and endosporulation, can be observed in the thin layer

of liquid before this has dried, and before the meningococci present have had time to "bite" into the solid medium. Flexible collodion makes a good seal, but drying at incubator temperature still takes place in three to five hours, and owing to the use of an oil-immersion lens the employment of a moist chamber does not appear practicable.

The alternative method of watching growth in liquid culture was to make shallow cells, as suggested by Mr. Martin Duncan, who made several for me of shellac varnish on an ordinary slide, and to cover with a sterile slip, inserting sufficient medium to ensure flooding of the top of the walls of the cell, and, in consequence, automatic sealing thereof by capillary attraction. I now employ cells made with black enamel, which stands sterilization better than the shellac, each cell being about one-tenth of a millimetre in depth.

By this method relative immobilization of the organism under observation is secured, and on the whole the method gives satisfactory results.

These results, however, require the greatest care in interpretation, owing to the presence—firstly—of the almost invisible organism referred to, early gemination and endosporulation 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 a fresh bud or endospore.

In all cases, therefore, most tedious and prolonged observation is necessary, which ought to be repeated again and again with fresh cultures.

By the use of these cells cultures can be kept liquid for forty-eight hours without difficulty. Disappointment is, however, frequent, owing to death, or arrest of growth, of an individual organism under observation.

ILLUSTRATIVE FERMENTATION AND SEROLOGICAL TESTS OF SERUM-BROTH CULTURES OF THE MENINGOCOCCI 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 was 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
„ 2 ..	C	C	C	C	C	P	nil	nil
„ 3 ..	C	C	C	C	P	nil	nil	nil
„ 4 ..	C	C	C	C	C	nil	nil	nil

In the case of these four cultures the dilutions were made from undiluted meningococcal serum supplied by the Lister Institute.

Titre, 1/250 ; No. Cl. ; date of tubing, 9/2/16 ; dates of agglutination, 8/3/16, 4/3/16, 29/4/16, 29/4/16.

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

C = complete agglutination.

P = partial

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.

	Normal	I.			II.			III.			IV.		
		<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>
Cult. 5	—	++	++	++	—	—	—	+	—	—	—	—	—
„ 6	—	—	—	—	++	++	++	—	—	—	—	—	—
„ 7	—	—	—	—	—	—	—	—	—	—	—	—	—
„ 8	—	++	++	++	—	—	—	—	—	—	—	—	—
„ 9	—	++	++	+	—	—	—	—	—	—	—	—	—
„ 10	—	—	—	—	++	+	—	—	—	—	++	++	+
„ 11	—	—	—	—	++	++	+	—	—	—	—	—	—
„ 12	—	—	—	—	++	++	++	++	(+)	—	—	—	—
„ 13	—	—	—	—	++	++	++	—	—	—	—	—	—
„ 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 1/100, 1/200, 1/400.

In summing up this demonstration of the morphology of the meningococcus and of the so-called giant forms, I suggest the following order of events, by no means dogmatically or finally, as the subject is one of the greatest difficulty. Starting from one of the giant forms, whatever its true botanical nature, whether

related to the yeasts or not, what appears to happen is this:—A giant form will start the process of single, or multiple, gemination. I have not seen one of these buds actually separate from the mother-cell. But I have seen them grow from extremely minute buds to a considerable size, and in so doing give off secondary and even tertiary buds. Each, or all, of these buds, as well as the original mother-cell, may at the same time, or independently in point of time, show one or more small rounded bright bodies, by a clear process of endogenous segmentation. They may be centrally placed or peripherally. Some of them will rapidly enlarge, but they never appear to attain the size of a freely growing, exogenously produced bud. Sometimes they will suddenly disappear from the mother-cell, and as suddenly reappear either at the periphery of the mother-cell or away from it. Sometimes they may be seen in what appears to be a process of extrusion, when they may take on a diplococcal form of about the size and appearance of a normal meningococcus. In some of the mother-cells, whilst this is going on, minute black spots will appear in the mother-cell, sometimes centrally and sometimes peripherally. Eventually they tend to become aggregated, in symmetrical fashion, round the periphery of the cell, where they will often assume a definitely minute bacillary form. In some cases, whilst situated centrally, these minute black spots will move about with incredible rapidity (? Brownian movements) inside the mother-cell, and several times I have seen what appears to be a sudden extrusion of one or more of these (? metachromatic) bodies, after which they are no more seen.

Whether these bodies may possibly be of the nature of swarm-spores I cannot say. There is still a highly significant point to mention, which is this. In every meningococcal culture I have examined on the warm-stage in the liquid culture cell described, I have noticed the development of rounded single cocci from the normal size of a normal meningococcus into what appear to be giant cocci, no evidence of binary fission being present. On the contrary, if they are focused in the small stage, the appearance of diplococcoid formation is often simulated, whilst on still further observation it is seen that the segments are unequal in size, the smaller segment being in reality a minute bud in the process of formation. These organisms appear, therefore, to be detached buds, developing again into giant forms.

And there, incomplete as the story is, I must for the present leave it.