A Contribution to the Morphology of Bacteria.

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With Plate 1.

Since the classical papers on Bacteria published by Professor F. Cohn in the 'Zeitschrift der Biologie d. Pflanzen,' vols. i to iii, all bacteriologists have accepted the subdivision of the Schizomycetes into cocci, bacilli, and vibrios or spirilla, as representing the main morphological fundamental types. But by Lankester[1] and Zopfs' researches on Cladothrix dichotoma, and by Hauser's well-known and exhaustive work on 'Putrefactive Bacteria' ('Ueber Fäulnissbacterien,' Leipzig, 1885), it has become recognised that the shape under which a particular bacterial species presents itself depends both on the medium in which it grows, as also on certain inherent characters of the organism itself. Thus it has become recognised that while the elements of one species appear as often in the form of oval as of cylindrical cells, those of another retain, under almost all conditions, pre-eminently that of cylindrical cells. To name a few instances: (*a*) the Proteus vulgaris of Hauser. This organism—the organism of putrefaction par excellence—is known to occur in the most varied shapes, as cocci, oval forms, cylindrical and vibrionic forms; but when growing in gelatine plates at 20° C. it will be found that in the

[1] 'Quart. Journ. Micr. Sci.,' vol. xiii, 1873; vol. xvi, 1876.

first twenty-four to forty-eight hours the colonies are made up entirely of cylindrical bacilli, some of extreme length and forming very characteristic threads. The "swarming" out and the development of strands from such a colony are uniformly due to, and consist of, thread-like bacilli (see my article in Stevenson and Murphy's 'Treatise on Hygiene,' II, pl. ii); but later on, say after three or four days, when liquefaction of the gelatine has become extensive, the forms one meets are those various kinds described by Hauser as coccus forms, ovals, cylindrical cells, and vibrionic forms.

(*b*) The Bacillus filamentosus (which I found in sewage, and which I described in my article in Stevenson and Murphy's 'Treatise on Hygiene,' II, fig. 14) is, under all conditions of culture (gelatine, agar, broth, serum, &c.), always made up of cylindrical cells, either singly or in pairs, or forming longer and shorter chains.

The same applies to the Bacillus subtilis, the bacillus of swine erysipelas, and the bacillus of human typhoid fever.

(*c*) The Bacillus prodigiosus, on the other hand, remains under artificial cultivation in the various media pre-eminently of the spherical or slightly oval shape, while there are always present a few cylindrical forms, and it is owing to the greatly prevalent number of coccus forms that in former years this organism was described (Cohn, Schrötter) as the Micrococcus prodigiosus.

Another interesting point connected with the unstable shape under which some species appear is that while some, growing in one medium, appear under one particular shape, this changes when growing in a different medium. A group of bacteria are known, the essential biological character of which is that most of them produce acute septicæmic infection in one or the other rodent. To this group belong the bacillus of fowl cholera, of fowl enteritis, of Frettchenseuche, of Wildseuche, of swine fever, of the Middlesborough pneumonia, of grouse disease, the Bacillus coli, and others.

Now all these in their cultural characters in the different media have many points in common, as also the close resem-

blance of the acute disease they are capable of producing in rodents. But it is noticeable amongst them that while some preserve the same definite shape when grown in one species of animals or one kind of medium, this becomes changed under other conditions. Take, for instance, the bacillus of grouse disease: in the grouse itself, taken from the liver or cultivated in gelatine, it appears in a short oval form; but in the guinea-pig or in the mouse the cells are more commonly of a cylindrical form, and so also in the gelatine culture from the blood of these animals.

Amongst the best known examples of permanency of shape is that of the Bacillus anthracis; so much so that its cylindrical elements, single and in short and long chains in the blood of an animal dead of anthrax, have become as much the classical illustrations of typical bacilli as those of the Bacillus subtilis.

A. In the year 1883 (see this Journal, vol. xxiii) I have described a peculiar change of the anthrax bacilli in culture, in the course of which the typical cylindrical cells constituting the well-known threads become transformed into oval and spherical corpuscles, some containing vacuoles. This change was named a torula form, because some of the threads resemble in a remarkable manner the chains of cylindrical, oval, and spherical cells which are observed on Saccharomyces mycoderma of thrush. The direct connection between the typical cylindrical cells and the spherical and oval corpuscles (three and more times the diameter), and the division of these into similar corpuscles, was traced in many filaments through all intermediate stages (fig. 1).

At that time I insisted on these changes not being due to involution and degeneration, but belonging to an active phase of growth in the artificial media. In the first place it was then shown that this morphological change is observed already in an early stage of growth, when of degeneration there can be no question; besides, in later phases, after two, three, and more days' growth, the progress of the growth and the resulting filaments are again of the characteristic appearances. I have

within recent years almost constantly observed a similar change in early phases of the growth of anthrax bacilli in gelatine plates (beef bouillon; gelatine 10 per cent.; peptone 1 per cent.; salt 1 per cent.). Already in very early phases, when the colonies are only just visible as angular greyish spots, and when by their numerous outgrowing filamentous prolongations they become more and more connected with one another—that is, between twenty-four to forty-eight or seventy-two hours' incubation at 20° C.—numbers of these filamentous sproutings, examined in impression cover-glass specimens, are seen to be made up entirely or partially, not of the typical filaments composed of the cylindrical typical bacilli, but of large spindle-shaped spherical or oval elements, the protoplasm of which showing abundant vacuolation. In figs. 2 and 3 such growing outrunners of young colonies are accurately represented by photograms. There can be no question, then, of these forms being indicative of active growth; as a matter of fact, later on—that is after four days and more, as growth proceeds and liquefaction becomes pronounced—such forms do not obtain any more; the threads are all uniformly made up of the typical cylindrical bacilli.

When comparing the colonies of the thrush fungus, Saccharomyces mycoderma, or Oidium albicans, growing on gelatine plates, it will be found that the impression preparations obtained therefrom show in many filaments the very identical appearances; and that while some threads or parts of threads are composed of cylindrical cells, others are made up of oval, spindle-shaped, and spherical cells; the same local accumulation of the growing protoplasm as huge spindles or spheres, and the same vacuolation of the protoplasm are observed in both. I conclude from this that, although the Bacillus anthracis is a typical bacillus in the blood of animals infected with, or dead from, anthrax, and also in most conditions of artificial cultivation, it nevertheless under certain conditions (early stages of growing colonies on gelatine) assumes a character by which it closely resembles a Saccharomyces mycoderma, or perhaps Oidium, and thereby

probably it returns to an atavistic stage in its evolutional history.

B. The second microbe, by which a similar marked change is exhibited, is the Bacillus diphtheriæ. Löffler ('Mitth. aus d. k. Ges.,' vol. ii) first drew attention to the fact that the diphtheria bacillus, discovered by Klebs, shows on cultivation a curious segregation of its protoplasm, and a knob-like or club-shaped enlargement of one or both ends. Löffler, and after him others (Flügge, 'Mikroorganismen;' Baumgarten, 'Pathologische Mycologie,' and others), considered these changes as due to involution. I have already, in the 'Report of the Medical Officer of the Local Government Board, 1889–90,' and 'Centralblatt f. Bakt. und Parasitenkunde,' vol. vii, 1890, shown that this view cannot be correct, for the following reasons:

(*a*) In the diphtheritic membrane, in which the progress of the disease is still active, an abundance of diphtheria bacilli occur, which show this change in a marked degree, viz. segregation of the protoplasm into spherical, cubical, or cylindrical particles, and terminal knobs or clubs, sometimes of great size and containing vacuoles.

(*b*) On agar cultures, already after twenty-four to thirty-six hours, when the growth is in its initial and most active phase, an abundance of bacilli are seen, which are shorter or longer threads, in which the segregation of the protoplasm and the terminal knobs and clubs are already very marked (fig. 4).

(*c*) In the subcutaneous necrotic tumour of the cow, produced by subcutaneous injection of virulent culture of the Bacillus diphtheriæ, there occur connected masses and clumps in which at the growing margin the diphtheria bacilli appear all in the form of threads, in which the spherical or oval swellings and terminal knobs are most conspicuous, and strikingly resemble the ends of growing hyphæ; the subjacent muscular fibres become invaded and gradually destroyed by the growth of the threads into their substance. This process of the gradual growth and penetration of the diphtheria threads with swellings and club-shaped ends into the muscular

fibres is very marked, and occurs in a large number of places. It was described and illustrated in the 'Report of the Medical Officer of the Local Government Board, 1889–90,' pp. 173 and 174, plates xiv, xv, and xvi; and I have to add here that sections of the tumour stained in a mixture of eosin and methyl blue show this in beautiful contrast, the growing threads blue, the muscular substance red; and it seems to me that one has only to examine such a specimen to at once see that the threads are actively growing; of an involution there can be no question.

Dr. Abbott, in the 'Journal of Pathology and Bacteriology,' vol. ii, while agreeing that the thread-like bacilli with terminal swellings are not involution forms, and are present in the growing artificial cultures (on serum), does not agree to their being comparable to the growing ends of hyphæ. This difference seems, however, merely a question of words; all that I maintained was that the threads with knobbed ends strongly resemble growing ends of hyphæ, and that such a change in bacilli as I described in artificial cultures, and particularly in the growing threads in the cow's tumour, if it is not due to involution, as it certainly is not, is only explained by its representing a relationship to a mycelial fungus, perhaps the Saccharomyces mycoderma or an Oidium form.

(*d*) From the milk of cows successfully inoculated with cultures of the diphtheria bacilli (Report, 1889–90 and 1890–91) I have isolated by culture the diphtheria bacillus, and in the gelatine cultures they showed this change in a conspicuous manner; the colonies in their young state are almost entirely made up of thread-like forms with terminal knob-like and club-shaped swellings (figs. 5 and 6) quite unlike the typical bacilli. Here the bacilli are actively growing, and therefore it is quite out of place to regard these forms as due to involution; and if they are not involutions, their similarity in growth and shape strongly suggests the view that I have put forward, viz. that they are comparable to the hyphæ of a mycelial fungus, e.g. Saccharomyces mycoderma. In these threads with local accumulations of their substance, and with terminal knob-like or club-shaped enlargement of their proto-

plasm, we have a condition of things which does not harmonise with the fundamental characters of a typical bacillus, but rather suggests that this microbe, though under many conditions conforming with what corresponds to a typical bacillus, may after all not be one, or at any rate the boundary between it and a mycelial fungus is not a severe one.

C. The most instructive organism, showing a similar and perhaps more pronounced morphological change, is the tubercle bacillus of Koch. As is well known, this microbe presents itself in the tubercular deposits and in serum and agar glycerine cultures in a form which has vindicated to itself the term of a typical cylindrical bacillus. But already in preparations made of the human pulmonary (tubercular) sputum forms occur which appear more of the character of threads composed of unequal—i. e. not uniform—elements; some of these threads show not unfrequently a terminal element of the same knob-shaped or club-shaped character as those mentioned of the diphtheria bacilli. It was on account of such forms that Metschnikoff expressed the opinion that perhaps the tubercular bacillus is not a bacillus at all, but belongs to the group of mycelial fungi. I have already in 1889–90 shown that in glycerine agar cultures of the tubercle bacilli, after some weeks' growth at 37° C., there occur large numbers of such thread-like forms with club-shaped ends; some short, others long, some smooth, others made up of unequal elements; further, long and short threads occur which show undoubtedly and markedly branchings, these latter either of great length or only as just commencing sprouts (see figs. 7 and 8). That all these forms are undoubtedly the tubercle parasite is shown by the transitional forms between the typical cylindrical tubercle bacilli and the long-branched threads (homogeneous or segmented), with smaller or longer lateral buddings, and by the fact that all these forms behave in staining (fuchsin, washing with nitric acid, 1 : 3) like the true tubercle bacilli. I have seen the same forms already after three to five weeks' growth on solidified hydrocele fluid; here most of the organisms were the typical cylindrical bacilli, but there were some undoubted

threads with knobbed ends, some branched, others as yet unbranched.

There can be no question about involution, because, as I have pointed out (l. c., and 'Centralblatt f. Bakteriology,' vol. vii, No. 25), the branched nature of the threads and the presence of the small lateral buddings conclusively prove the active growth. Later, Mafucci ('Archiv f. Hygiene und Infect.,' xi, p. 445) described the same forms in the culture of the tubercle bacilli of the fowl, and Fischel ('Fortschr. d. Med.,' Bnd. x, No. 22, p. 908) also of the human tubercle cultures; and this latter observer arrived at the same conclusion as myself, viz. that we are dealing with forms which are comparable to a mycelial fungus.

From all these facts I think we are justified in concluding that the above three species are not so well-marked typical bacilli as has always been assumed; that is to say, well-defined species of desmo-bacteria in the sense of F. Cohn. True, under many conditions they show morphological characters of the same kind as the typical bacilli; but under other conditions they easily revert to or assume forms by which their relation to the Saccharomyces or Oidium (anthrax, diphtheria), or a still higher mycelial fungus (tubercle) becomes evident.

EXPLANATION OF PLATE 1,

Illustrating Dr. E. Klein's paper, "A Contribution to the Morphology of Bacteria."

All figures are reproductions from photograms magnified 1000.

FIG. 1.—Cover-glass specimen of Bacillus anthracis growing in gelatine plate, two days old. Many of the bacilli are changed into spherical or oval masses, containing vacuoles.

FIG. 2.—Cover-glass specimen from an impression of a gelatine-plate cultivation of Bacillus anthracis, two days' growth. Instead of the typical threads of cylindrical bacilli, there are threads made up of thick spindles, the protoplasm in many of these vacuolated.

FIG. 3.—From the same plate cultivation as the previous figure. Copious vacuolation.

FIG. 4.—From an agar culture of the Bacillus diphtheriæ, grown at 37° C. for two days, showing typical club-shaped filamentous bacilli.

FIGS. 5 and 6.—Diphtheria bacilli, derived from the milk of a cow infected with diphtheria. The bacilli had been growing on gelatine. Typical club-shaped filamentous forms.

FIGS. 7 and 8.—From glycerine-agar cultivations of the tubercle bacilli. Filamentous bacilli with terminal knob-like enlargements, some showing distinct branching.