FURTHER STUDIES ON THE MORPHOLOGY OF BACTERIA

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Official bacteriology, if I may venture to use the expression, still regards the morphology of bacteria as very simple. The cells of a certain species are supposed to be fairly uniform and to multiply, only by fission. As a matter of fact the very system of classification of bacteria is based on this theory. Many investigations have shown, however, that the theory needs revision. A thorough review of the literature concerning this problem is to be found in the excellent work by F. Löhnis[1] recently issued.

Besides reviewing the literature Löhnis discusses a theory of his own, according to which bacteria develop cyclic changes called life-cycles, including an organized and an amorphous stage. During the latter stage the cell-walls disintegrate and the protoplasm of different cells mixes. From this unstainable "symplasm" new individual cells develop. If these claims could be proved to be true, it would, I think, be a most interesting discovery, throwing a new light on the problem of variation of bacteria. As is well known, studies of the Twort-d'Herelle phenomenon have shown the great importance of the variation of bacteria. It has been found that not only the lytic agent but the ageing of the cultures induces variation. How does this happen? What rôle do the metabolic products play and how do the hereditary factors work? All these problems are very difficult to approach before we know whether there is any exchange of protoplasm between the cells of the bacteria.

In studying the morphology of *Coryneb. diphtheriae* I have been very anxious to find the structures figured by Löhnis

[1] Memoirs of the National Academy of Sciences, vol. xvi, 1921.

especially since Almquist, an author of my own country, describes the same things under the name of "plasmodia." He claims that very small and numerous organized individual cells develop from the "plasmodia." I am able to confirm the statement that the "plasmodia" or "symplasm" are easily seen for example with *Coryneb. diphtheriae*, in cultures of some age; but I cannot abandon the old view that they are merely slimy agglomerations of cells or, in other words, a "zoogloea." My reasons are that the masses always contain some cells, perhaps empty-looking which, however, are possibly able to grow, and that the production of slime, sometimes in gorgeous masses, in ageing cultures is very common with bacteria. It may be true that the cells growing from the "plasmodia" are sometimes very numerous; but this can be explained in another way, as we shall see later.

Löhnis also describes one mode of interaction between the plasmatic substances of the bacterial cells called "conjunction." This process consists of a "direct union of two or more individual cells" exactly comparable with the copulation of yeasts. The well-known V-forms of *B. subtilis* and *Coryneb. diphtheriae* are the result of such a conjunction, the cells being connected "by bridges or by beaks." With regard to the *Coryneb. diphtheriae* I have followed the development of these bridges and have found that they are not secondary conjunctions, but residues left when the new cell forms from the old one just like the threads connecting the cells of a yeast mycelium.

Nevertheless, though I cannot adduce any evidence in support of Löhnis' discoveries, I am very much inclined to believe in them, since it is improbable that bacteria form an exception to the rule that a species dies at length without sexual rejuvenescence.

Beside the above-mentioned processes Löhnis describes several kinds of asexual multiplication common to all bacteria. One of these is the formation of gonidia. In analogy with the large trichobacteria, as for example Cladothrix and Leptothrix, bacteria form within the cells small bodies which are able to grow, either while still in the mother cell, or when liberated by the bursting of the cell wall. If the cells containing gonidia have

changed their shape into giant forms they are called gonidangia. The club form of the Actinomyces is regarded as such a gonidangium. "Very many of the so-called involution forms of spherical, pearshape, and clubshape are, in fact, gonidangia." I am convinced, however, that many of the pictures explained by Löhnis as growing gonidia are only budding and branching vegetative cells. He says that the great multitude of forms seen with *Bact. coli* and *Bact. typhi* and especially in the pictures of these bacteria published by Hort are due to the development of gonidia before they are liberated from the mother cell-wall. As I have pointed out in numerous earlier papers, these "fantastic" forms are just the same as occur in yeast and must be regarded as normal vegetative cells. A proof that Löhnis exaggerates the importance of the gonidia is to be found on page 144 of his monograph, where he says that "it still remains to be studied whether the eosinophilic granules, which are present in blood-cells, are actually cell-products or bacterial gonidia."

On the other hand, it cannot be denied that fishing from old bacterial cultures, containing no intact cells, or very few, but including masses of easily stainable small granules, often gives an abundance of colonies on the agar plates. It is then very tempting to explain this growth, as many have done, as originating from the granules. But to supply a proof is exceedingly difficult, and I doubt whether it is possible to provide convincing pictures. In diphtheroids and other bacteria I have often seen the organisms in the form of a pure culture of filaments, which in growing old have become more and more granulated, and at last have broken up into granulated masses. Plating from such a culture has, as has been mentioned above, given numerous short bacillary forms. I have supposed that the granules are contracted masses of protoplasm separated by empty spaces in the ageing filament and not definite organized gonidia, since they differ widely in size and shape. The name I have found most adequate is "fragmentation spores" a term used by Bostroem in Actinomyces. These formations are, I think, the cause of the striped appearance of *Coryneb. diphtheriae*. They are not to be confused with the polar granules, being real granulas and lying in vacuoles, where they exhibit a dancing movement.

Furthermore Löhnis tells us about some other bodies serving the purpose of multiplication which he calls regenerative bodies. They are of different nature and are produced either by the gonidia or by the symplasm and they are "generally characterized by their being easily and deeply stained by aqueous dyes, by their different appearance as compared with the vegetative cells (globular, oval, or irregular), by their ability to reproduce normal cells immediately or after having propagated as such by fission or by budding, by their distinctly increased resistance against drying, heat, or other detrimental influences." Beside the "regenerative bodies" he describes "arthro-spores" and "microcysts," the latter being formed by "transformation of a whole vegetative cell into one, usually relatively large, resting body" the former "by the segmentation of the vegetative cell and the transformation of these segments into fairly resistant reproductive organs." The thickening of the wall is characteristic of both, but usually the arthro-spores are more cubical and the microcysts more rounded in shape. The microcysts can grow to vegetative cells or change into gonidangia or divide into several equal parts, each able to give rise to a new cell. The author says that "what we now call regenerative bodies is a collection of different reproductive organs, whose proper separation and classification must be left for the time when more data shall be available."

In studying bacteria I have always followed as a guide the theory that bacteria are closely connected with the fungi proper. This theory at once throws light on the pleomorphism of the bacteria and also on many problems of variation. According to this theory, it is natural that the bacteria cells should differ widely in shape and that new variants of more or less constancy, should rise in a culture. In the same way I have tried to explain the structure of the bacterial cell and finally also some peculiar bodies which I have seen in the case of *Coryneb. diphtheriae*, *Bact. coli* and *Bact. typhi*. These bodies evidently belong to one of the above mentioned groups of Löhnis. For my part, however, I should prefer another name to those used by Löhnis since these bodies are most similar I think to the so-called chlamydospores of the fungi. What then is a chlamydospore? This name is

given to a peculiar kind of resting form having thick walls and being a little more resistant than common vegetative cells. Many of the Eumycetes, for example Favus, show mycelial filaments with circumscript swellings or bulges. Such swellings are often transformed into chlamydospores by the flowing of the protoplasm from other parts of the filament to the swelling, which afterwards surrounds itself with a thick wall and becomes granulated. Owing to the abundance of protoplasm and the presence of the thick membrane, the body takes stain in a higher degree than the other cells. The remaining parts of the filament soon die and the spore is free. The "resting cells" of yeast show a very great resemblance to these chlamydospores. The only difference is that here the whole cell is transformed into a resting form. Chlamydospores according to Plaut are demonstrated on the text-figure below. Now it may be mentioned, first of all, that many bacteria show just the same swellings, as is well known to every one familiar with ageing bacterial cultures. Sometimes the swellings are situated in the end of the filament, which then assumes a club-like shape, common to many bacteria, for example, *Mycob. tuberculosis* and *Coryneb. diphtheriae*. In other cases the swelling has a peculiar triangular form. The formations just described are mostly to be seen on pictures of "involution forms;" but many writers have accepted them as organs of multiplication. It may be remembered that Plaut utters a warning against the confusion of these swellings and spores and other higher formations found in fungi. How much more easily does such a confusion take place when we are dealing with bacteria which are of so small a size.[2]

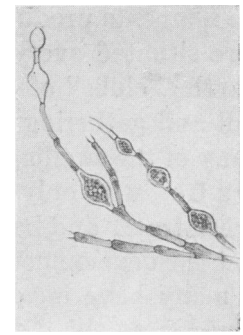


FIG. 1. CHLAMYDOSPORES ACCORDING TO PLAUT

Sometimes, however, you will see the whole development of chlamydospores from these swellings even in the case of bacteria. *Bact. typhi* shows these forms, especially when grown at low temperature and on drying agar as Almquist has pointed out, and

[2] In Kolle-Wasserman, Handl. der path. Mikroorganismen, Bd. v, Jena, 1913.

Coryneb. diphtheriae shows the same forms when left for a week or more on Löffler serum. Figures 8, 12 and 16 demonstrate such bodies from a culture of *Bact. typhi.* As to *Coryneb. diphtheriae* I refer to an earlier article. Neither of these bacteria, however, is very suitable for studies of these formations, since they appear rather irregularly and only in ageing cultures. I have found excellent material in a Corynebacterium originally isolated by Rosenow from a case of Hodgkins disease and kindly provided me by R. R. Mellon. The corpuscles appear in the cultures of this bacterium in great abundance even after twenty-four hours' growth on plain agar as is to be seen on figures 1, 7 and 13. A scrutiny of the living microörganism under high magnification showed that the filaments produced swellings which, just like those of Favus, were situated everywhere, even in the ends, which then looked like the "clubs" of *Coryneb. diphtheriae.* Thickening of the cell-wall and gathering of the protoplasm inside was clearly seen in many of the swellings. On the other hand, such bodies were also seen free, with only faint traces of the filament attached to them. These traces and bodies on the ends of branched filaments prove that the development is that hinted at above and not the reverse, as many have imagined. As a matter of fact the pictures obtained from the development of the chlamydospores in a hypha is sometimes strikingly similar to those of the outgrowth of a spore to a filament, especially when dealing, not with long filaments, but with rods or bacillary forms and when the body is placed at the end. But the observation of bodies in both the ends of the rod immediately solves the question. As far as I can see, Meirowsky[3] has made such a mistake in interpreting his pictures of *Mycob. tuberculosis.*

But those bodies, are they not gonidangia, asci, or whatever you like to call them? With the very same microörganism Mellon[4] has found bodies containing small motile corpuscles getting free by the bursting of the wall and giving rise to new individuals. I have not been able to cultivate these bodies; and

[3] Studien über Fortplanzung von Bacterien, Spirillen und Spirochäten, Berlin, 1914.

[4] Journ. Med. Research, 1920, **62**, 61.

they are not the same as those described above. The chlamydospores, as I like to call them, never contain any formations indicating a multiplication, and they grow by budding just as yeast does. The evidence of budding was easy to obtain, since the bodies put in new broth and removed from this in a high dilution of thionin showed beautiful "budding discs" just like those of yeast and other microörganisms described by me in an earlier paper.

Finally I want to emphasize the fact that I do not deny the existence of other more complicated forms in bacteria, but merely report what I have seen and have not seen. In vain I have tried to find gonidangia or asci. Even with *V. cholerae*, where large round, thick-walled granulated cells very suspiciously similar to the organs of multiplication, are common in ageing cultures, I have never found anything of that kind. On figures 2, 4 and 5 some of these bodies of *V. cholerae* are demonstrated. It is, as will be seen, big enough to be easily studied.

SUMMARY

The author discusses several theories concerning peculiar forms in bacteria supposed to be organs of sexual and asexual multiplication. He also describes some spherical, ovoid or club-shaped bodies found by himself with *Bact. typhi*, *V. cholerae*, *Coryneb. diphtheriae* and other species, which he regards as chlamydospores.

EXPLANATION OF THE PLATE

Figures 3, 6, 9, 14 and 15 are taken from wet preparations obtained by transferring the microörganisms from the culture to a drop of the staining fluid, which was then covered with a glass. In such a thin layer of fluid the microörganisms lie motionless at least for a time long enough to allow photographing. The other pictures are taken from preparations fixed by drying the slides in the usual way. The staining fluid was in all preparations a highly diluted solution of thionin in water.

Figures 1, 7 and 13 (*Corynebacterium lymphogranulomatis* Rosenow). Slanted agar culture twenty-four hours old. Many chlamydospores in different stages of development.

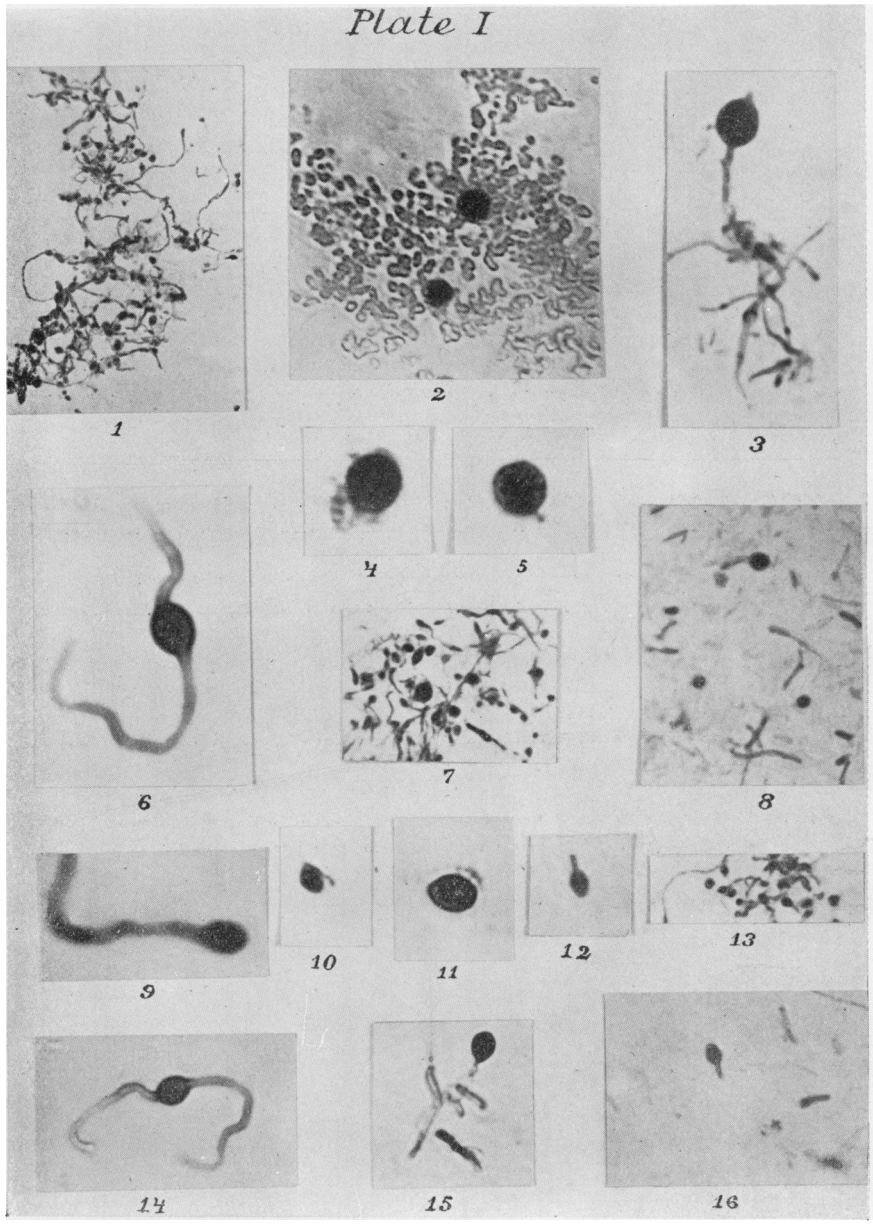
Figures 3, 9 and 15. The same bacterium with a chlamydospore developing near or in the end of a hypha. In the two latter the microörganisms have become more or less club-like.

Figures 6 and 14. Development of the spore in the middle of the hypha. The swelling of the thread, the thickening of the wall, and the gathering of the protoplasm to the spore are clearly seen.

Figures 10 and 11. Free spores, the former still with remnants of the thread adherent to the wall, as is mostly the case.

Figures 2, 4 and 5. Round bodies in a culture of *V. cholerae* on slanted agar three weeks old. In figure 2 the size of the bodies can be compared with that of the usual form of the microörganism. In figure 5 the body shows a definite granulation. The bodies are supposed to be resting cells.

Figures 8, 12 and 16. Pictures from a slanted agar culture of *Bact. typhi* three weeks old. In the two latter a chlamydospore is seen to develop at the end of a rod, giving to this a club-like shape. The next stage, with the rod shrinking away and the spore more or less liberated, can be observed on figure 8.



(Bergstrand: The Morphology of Bacteria.)