

# THE CYTOLOGY OF MICROCOCCUS CRYOPHILUS

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In a series of publications DeLamater has claimed that *Micrococcus cryophilus* is a unicellular coccus, the nuclei of which divide by a typical mitotic process. In the most recent of these (DeLamater and Woodburn, 1952) the existence of evidence (Bisset, 1950) indicating that many cocci are internally divided by cross-walls is admitted but is described as "unconfirmed". In view of its importance to the question of the validity of their interpretations, it is rather surprising that DeLamater and his collaborators have not chosen to confirm this point. However, Chance (1953) has demonstrated recently the development of strongly basophilic septa in cocci, which corroborates my suggestion that DeLamater's "metaphase spindle" consists of such a septum between two nuclear bodies.

The purpose of this paper is to provide photographic evidence of the multicellularity of *M. cryophilus*, and to indicate how the entire "mitotic cycle" is explicable in terms of this multicellular structure which DeLamater and his associates have failed to recognize.

## MATERIALS AND METHODS

A strain of *M. cryophilus* was obtained from the American Type Culture Collection through the kindness of Dr. S. T. Cowan. Two strains of micrococci of identical morphology were obtained from type cultures maintained in this laboratory and were used for comparison.

Cell walls were demonstrated by phosphomolybdic acid and methyl green according to the method of Hale (1953). The nuclear bodies and basophilic elements in the cytoplasm were demonstrated by mordanting with 10 per cent trichloroacetic acid for twenty minutes at room temperature and staining with 10 per cent giemsa solution (Gurr's R.66) for approximately ten minutes. A number of preparations were stained also by the method of Chance (1952), which stains both cell walls and nuclear bodies simultaneously, to some extent, and by DeLamater's technique. Unfixed water mounted preparations

were used in all cases except the last two which involve drying or dehydration in alcohol. The stained appearances obtained in *M. cryophilus* and in the other cocci were alike; and these descriptions may be assumed to apply equally to all these strains and to many others which have at various times been examined.

## RESULTS

Preparations from cultures at ages varying from two hours to four or more days were stained by Hale's method. It was obvious immediately that few or none of the cocci contained less than two cells and that many were four celled (figures 1 and 2). The cells were separated by thick cross-walls which stained with great clarity. In some cases the granules at the junction of cell wall and cross-wall, which constitute DeLamater's "centrioles" in both cocci and in other types of bacteria (Bisset, 1953), were very clearly visible and were equally clearly *not* centrioles (figure 5).

Similar, if less spectacular, evidence of multicellularity was obtainable by Chance's method, and here the appearance of a cross-wall between two nuclear bodies (or sometimes between two dividing pairs) which constitutes DeLamater's "metaphase spindle" was very obvious (figure 6). This method, however, suffers from the defect that a clear demonstration is given only of the more mature cross-walls, whereas the nuclear bodies often are aggregated in central masses and cannot readily be independently resolved.

Corresponding cultures mordanted with trichloroacetic acid and stained with giemsa revealed a variety of nuclear configurations corresponding to the cellular structure shown by the cell wall stains and indicating that each cell possessed one or two nuclei. The latter were presumably in the process of division (figures 3 and 4). Thus, each coccus contained from two to six or eight nuclear bodies, separated by cell walls. In water mounted preparations this cellular organization was quite distinct, but in those which were subjected to DeLamater's technique of transfer from aqueous

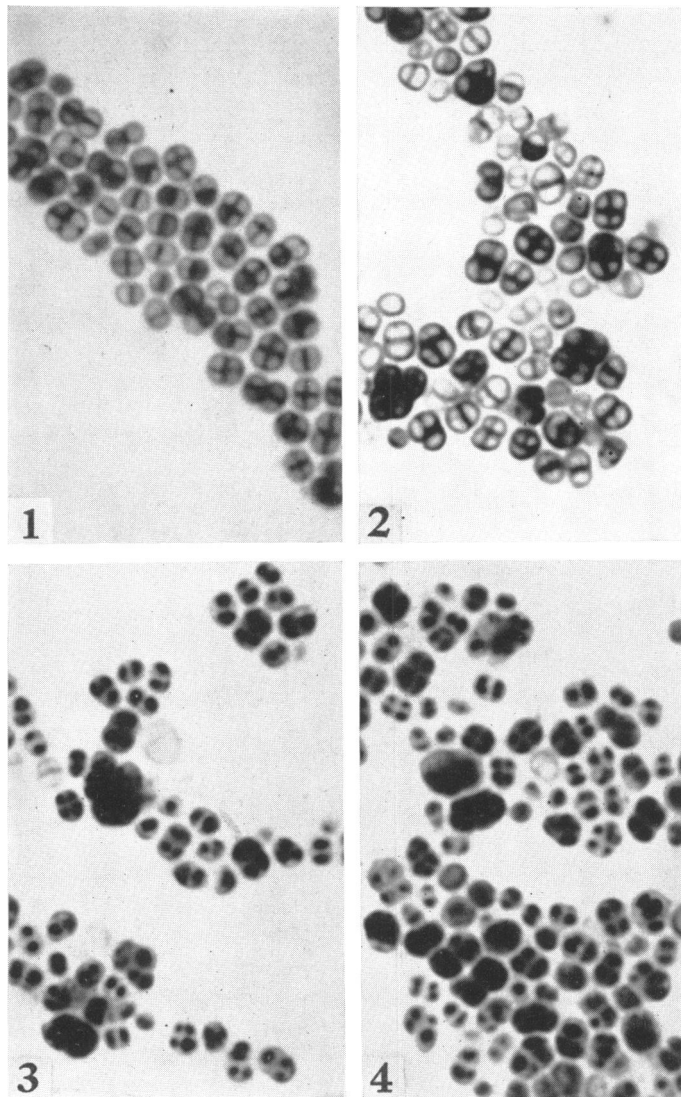


Figure 1. Cell walls of two and four celled cocci from 2 hr culture on nutrient agar. Hale's method,  $\times 2,400$ .

Figure 2. Cell walls of two and four celled cocci from 24 hr culture. The cocci are much less uniform in size and appearance. Hale's method,  $\times 2,400$ .

Figures 3 and 4. Nuclear bodies of cocci from 2 hr and 24 hr cultures, respectively. All the nuclear configurations claimed by DeLamater to represent mitotic figures can be observed; but comparison with figures 1 and 2 makes it apparent that this interpretation is not valid since each "figure" comprises the contents of two or four cells. Stained by trichloroacetic acid and giemsa,  $\times 2,400$ .

solution to absolute alcohol or acetone this was largely obscured, as may be observed in the illustrations of DeLamater and Woodburn (1952).

The appearances misinterpreted as mitotic figures were obtainable with equal or even greater facility if the material were transferred to alcohol at higher temperatures than that of solid carbon

dioxide, as recommended by DeLamater. Indeed, the only effect of the lower temperature was to reduce the speed of the reaction. Excellent "mitotic figures" were obtained by *boiling* the material in alcohol or xylol before or after staining. Similar results have been obtained by prolonged soaking in carbon tetrachloride or other organic

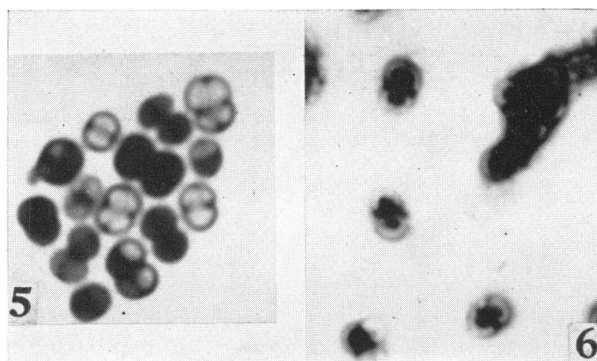
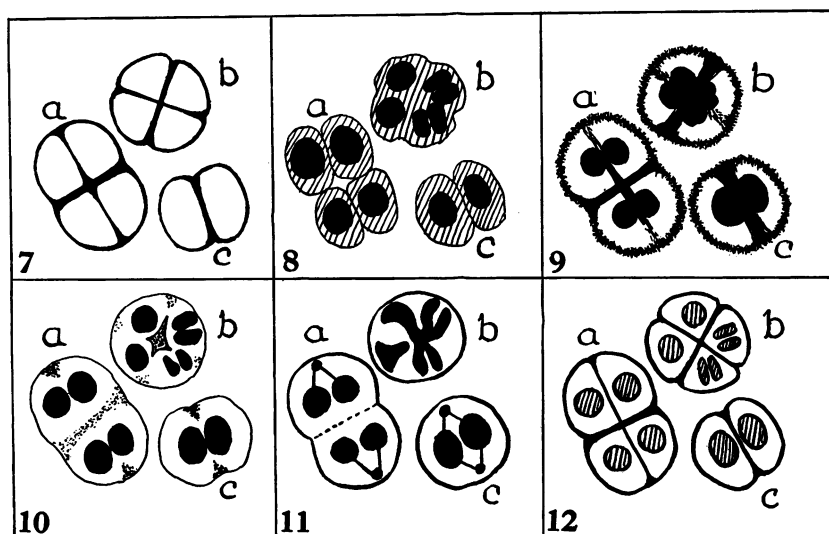


Figure 5. Cocci stained by Hale's method with Janus green. The granules at the junction of cell walls and cross-walls (DeLamater's "centrioles") are seen clearly.  $\times 3,200$ .

Figure 6. Cocci stained by Chance's method. Nuclear aggregates on each side of a mature septum (DeLamater's "metaphase spindles") are seen clearly.  $\times 3,200$ .



Figures 7-10. Comparison of appearances in a group of cocci by various staining methods. Figures 7 and 8 show the appearance of two and four celled cocci stained for cell walls and nuclear bodies, respectively, as described in this paper. Figure 9 shows the same group as seen by Chance's method; the nuclei are aggregated centrally, and only the mature cross-walls stain well. Figure 10 shows the appearance produced by DeLamater's technique.

Figure 11. DeLamater's interpretation of the same group (as in figure 10) in terms of "mitotic figures"; a—"telophase", b—"late interphase"; c—"metaphase".

Figure 12. Interpretation of the true cytological structure of such cocci from the evidence described in this paper.

solvents (Clark, *personal communication*; Bisset, 1952). These observations will be the subject of a further communication and accordingly are not described in detail at this time.

#### DISCUSSION

Correlation of the cell wall and nuclear preparations illustrated in this paper leaves no room for

doubt that *M. cryophilus* resembles the majority of large cocci in being multicellular. Each coccus contains two, four, or more cells, and each cell contains one or two nuclei.

Comparison of these photomicrographs with those claimed by DeLamater to illustrate "mitotic figures" in this organism renders it abundantly clear that DeLamater's interpretation is

entirely false since each of his "chromosomes" can be seen clearly to be the nucleus of a different cell, whereas his "centrioles" are the granules at the junction of cell wall and cross-wall as in his figures of "mitosis" in rod shaped bacteria (Bisset, 1953).

When making my earlier criticisms of his work (Bisset, 1951), I believed that DeLamater considered his "metaphase spindle" to be dividing in the same sense as the coccus in which it was contained and that he therefore regarded the nuclei as "centrioles". But the diagrams accompanying his more recent publication of the same photomicrographs (DeLamater and Woodburn, 1952) render it apparent that his "metaphase" is supposed to be dividing in a plane at right angles to the obvious plane of division of the coccus. This realization serves both to underline the incredibility of DeLamater's interpretation and to bring the explanation of the appearances in cocci and bacilli more closely into line.

In figures 7 to 12 the appearances given by a group of cocci, as stained by various methods, are compared; figures 7 and 8 show one two celled and two four celled cocci as they appear when stained by Hale's method for cell walls and by trichloroacetic acid-giemsa, respectively. Each cell is seen to contain one nuclear element, sometimes in process of an apparently amitotic division. Figure 9 shows the appearance produced by Chance's method. In my opinion it is the aggregation of material at the center of the coccus, probably caused by drying, which, in combination with the differentiating effect of the nigrosin surrounding the coccus, gives the impression of centrifugal growth of the septa as described by Chance (1953). Figures 10 and 11 show, respectively, the appearance of such cells stained and dehydrated by DeLamater's method and DeLamater's interpretation of these appearances as mitotic figures. The four celled cocci provide his "telophase" (a) and "late interphase" (b), whereas the two celled coccus (c) is DeLamater's "metaphase". Figure 12 shows the entire cellular structure of these cocci as interpreted from all available evidence.

In addition to these explanations of the appearances upon which DeLamater bases his interpretations, it is apparent that his claims in respect of the value of his technique in safeguarding the original appearances of the material are entirely unjustifiable since similar appearances can be obtained by dehydration in concen-

trated solvents at any temperature. The avoidance of the effect of transfer from water to absolute alcohol has long been one of the principal objects of classical dehydration techniques; and it is remarkable that DeLamater's claim to have avoided this effect by conducting the transfer at low temperatures should have been advanced in the complete absence of adequate controls of any kind.

The validity of DeLamater's parallel claim of improved clarity in his photomicrographs may be judged by comparison of his published illustrations of *M. cryophilus* with those in this paper.

#### SUMMARY

Cytological staining of *Micrococcus cryophilus* shows that each coccus contains two or four cells separated by strongly developed cross-walls. Each cell contains one or two nuclear bodies. This evidence invalidates the claims of DeLamater to demonstrate "mitotic spindles" in this organism which he claims to be unicellular. Explanations are given of the appearances upon which he bases those interpretations, and it is noted that appearances similar to those given by DeLamater's freezing alcohol technique may be produced by prolonged immersion or boiling of material in concentrated organic solvents.

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