# EVIDENCE FOR THE OCCURRENCE OF MITOSIS IN THE MICROCOCCI[1]

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Knaysi (1938, 1942, 1944) and Lewis (1941) have reviewed the literature on the existence of a nucleus in the *Coccaceae*. The first critical attempt to study the nucleus in the *Coccaceae* was made by Ellis (1902–1903). Using methods developed by Meyer (1912), he was able to detect one or two granules in the cell of *Sarcina ureae*. However, he was unable to observe similar granules in the cells of *Streptococcus pyogenes*.

Dobell (1911) stated that "the Coccus forms studied possess a single, centrally placed, spherical nucleus in each cell, which divides by a simple amitosis." This type of nuclear organization was found in forms belonging to the genera *Micrococcus* and *Sarcina*. Meyer (1912) also described structures he considered to be nuclear in various sarcinae.

Gutstein (1925) observed an eccentric granule having a diameter equal to one-fourth to one-third of that of the cell in the cells of *Staphylococcus*, *Streptococcus*, and *Pneumococcus*. Gutstein thought these granules consisted of lipoprotein. Schumacher (1926) studied a micrococcus and a gonococcus and claimed a nucleus for both of these organisms.

Petter (1933), Milovidov (1935), and Sassuchin (1935) applied the Feulgen technique to various sarcinae and reported that the reaction was restricted to discrete bodies of regular size and shape. Delaporte (1936, 1939) studied five strains of *Sarcina* and nine strains of *Micrococcus*; she observed a central structure which was not identifiable with any of the known reserve materials and which gave a positive nucleal reaction. In 1936 she interpreted this structure as a central body, but in her later work (1939) she considered it a granular nucleus. Stille (1937), by reducing the temperature of acid hydrolysis to 40 C, demonstrated localized, positive nucleal reactions with various bacteria, including *Sarcina*.

Piekarski (1938, 1939a,b) studied *Sarcina alba* and various staphylococci, among other bacteria, microchemically and with the ultraviolet and the electron microscopes. In *Sarcina alba* and the staphylococci studied he found a Feulgen-positive granule which he called a "nucleoid". The electron microscope failed to resolve any structure in the "nucleoid", which led Piekarski to believe that it was not made up of individual elements (chromosomes).

Robinow (1942) applied the Feulgen technique to *Sarcina lutea* and *Sarcina aurantiaca* in order to determine the number of granules present in each cell. He thought that many cells contained two so-called "chromatinic bodies", and stated that division of the chromatinic material appeared to be by longitudinal fission. The structure of the bodies appeared to him to correspond to the nuclear structures described in sarcinae by Dobell and Meyer.

Knaysi (1942) studied *Staphylococcus flavo-cyaneus*, observing that the resting cell of the organism is spherical or slightly ellipsoidal, and that it usually contains a single granule or, occasionally, two round or ellipsoidal granules. As the cell grows, it assumes the form of an elongated ellipsoid and contains either a single rod-like granule along its major axis or two elongated, kidney or crescent shaped granules lying in a direction perpendicular to the major axis. Knaysi believed that this kidney-like shape may be due to an orientation stress resulting in bending of the granules. Upon further development the cell may contain three or four granules, sometimes of the same form or of a combination of forms. On the basis of various tests Knaysi concluded that the intracellular structures noted were most probably made up of nucleoprotein.

In 1944, the same author, working with *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Neisseria gonorrhoeae*, could show no differentiated, intracellular structures. He concluded, on the basis of these later results, that bacteria contain nuclear material which, depending on conditions as yet unknown but probably related to environment and development, may be diffuse in the protoplasm, or may be partially or totally differentiated into a nucleus.

Knaysi and Mudd (1943) reinvestigated Knaysi's findings in *Staphylococcus flavo-cyaneus*, *Neisseria gonorrhoeae*, and *Neisseria meningitidis*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. In *Staphylococcus flavo-cyaneus* they noted that the cells may contain one or several granules which often appeared paired or constricted, suggesting possible division. On the basis of morphology, position, and solubility, they believed that the granules were identical with those previously studied cytologically and microchemically by Knaysi. These observations seemed to support Knaysi's view that they represent the cell nuclei, but in the cells of *Neisseria gonorrhoeae*, *Staphylococcus aureus*, and *Streptococcus pyogenes*, Knaysi and Mudd were unable to detect any structure.

Tulasne and Vendrely (1947) in an investigation of the exact localization of ribonucleic acid and desoxyribonucleic acid in bacterial cells saw well defined nuclei in gonococci treated with ribonuclease.

Bisset (1948a) studied long-chained and short-chained variants of *Streptococcus pyogenes*, *S. viridans*, and *S. faecalis*; *Staphylococcus aureus*, and *S. albus*; and *Sarcina lutea*. He declared that cocci of the long-chained strains were frequently divided by transverse cell walls into two cells, each containing a single chromatinic body; in the short-chained strains no transverse septum was formed and the cocci divided by constriction; they were unicellular and were described as usually containing a pair of chromatinic bodies. Analogous types were described as occurring in *S. aureus* and *S. albus*. The majority of both types of staphylococci resembled the long-chained strains in minute structure. In one of

the morphological forms the cells contained a well defined granule, central or slightly eccentric in position. Division of the granule preceded that of the coccus. Bisset regards this granule as a discrete nucleus. Morphologically this staphylococcus was similar to the strain of *S. lutea* examined although the *S. lutea* was arranged in the typical octad packets.

In 1950 Bisset discussed the spherical vegetative nucleus and states that the exact behavior of the nucleus of cocci at cell division has not been recorded. To this author, in those cocci which possess a spherical, vegetative nucleus, this structure appears to elongate with the cell in the course of division. He finds the vesicular type of nucleus in spores, microcysts, and resting stages of other bacteria as well as in active stages of certain cocci.

Previously Bisset (1948b) had studied the nucleus of the gram negative cocci. These organisms he describes as unicellular or as being divided in two by a transverse membrane. He considers the two-celled cocci as typical of *Neisseria*. The majority of gram negative cocci that he studied were unicellular, and he considered them to possess a spherical nucleus, which was usually slightly eccentric in position, and which he described as dividing by simple constriction. Dividing cells were described as containing one or more nuclei; whereas cells in occasional short filaments were thought to contain as many as five nuclei. These various observations have not yet been confirmed (see discussion for further consideration of Bisset's concepts).

Smith (1950) studied a strain of *S. aureus* by an improved staining technique for the nuclear chromatin of bacterial cells. Smith's method was based upon the earlier work of DeLamater *et al.* (1950). Smith noted a variety of chromatinic arrangements. The structures observed agreed with respect to apparent size, number, and distribution with the Feulgen positive bodies demonstrated for staphylococci by Knaysi. Occasional cells contained but a single, round granule of chromatin. The majority of cells, however, possessed a distinct pair of chromatinic structures associated with the dividing cell, and their morphology seemed to vary with the stage of division. In what might be an early phase of cellular division, two distinct half-moon basophilic bodies were noted, separated by a rather narrow nonbasophilic zone. Cells more advanced in the divisional process showed pairs of structures that were more condensed. These paired structures often appeared to be connected by a strand of chromatin.

Hunter, Mudd, and Woodburn (1950) in a study of sulfonamide-susceptible and sulfonamide-resistant strains of *S. aureus* could detect no internal structure with the light microscope other than that which has been observed by other investigators. The electron microscope failed to show any internal structure whatsoever.

Appelby (1939), using a technique of vital staining, studied a sarcina and a micrococcus. He observed that the majority of cells of the sarcina contained one round densely staining granule, generally located near the cell membrane. It appeared to divide by elongation and constriction in the middle, presenting a temporary dumb bell shaped form and resulting in two round granules in one cell. In the micrococcus each cell contained one granule, which was usually

round, but sometimes in the form of a short rod, either uniform in width or thickened at the ends.

Barnard (1930) photographed cells of *S. aureus* by ultraviolet light and obtained images which, according to his interpretation, suggest that bacteria contain a nucleus which undergoes mitotic division.

In support of the chromosome theory of the nucleus, Lindegren and Mellon (1932) described a diplococcus in which they thought the nucleus consisted of a single haploid chromosome which contained seven chromomeres. Later Lindegren (1942) described what he considered to be nuclear fusion and chromosomal elongation in *Micrococcus ochraceous*. Many of his figures suggest the interphase nuclei herein described.

Johnson and Gray (1949) studied species of rod shaped and coccoid cells of luminous bacteria. They noted that the nuclear material in the coccoid cells of *Photobacterium phosphoreum* appeared either as a darkly staining central body or as two or more units distributed in a manner suggestive of mitosis in higher organisms.

DeLamater (1952a,b) has presented preliminary observations on mitosis in *Micrococcus cryophilus* which is presented in detail here. Additional evidence for the occurrence of mitosis in other bacteria is presented elsewhere (DeLamater, 1951b, 1952c; DeLamater and Hunter, 1951; DeLamater and Mudd, 1951; DeLamater, Hunter, and Mudd, 1952).

## MATERIALS AND METHODS

The organism studied is *Micrococcus cryophilus*, isolated from frozen fresh pork sausage at the Agricultural Experimental Station in Beltsville, Maryland. A description of this organism has recently been published (McLean *et al.*, 1951).

The coccus was grown in Morton and Engley's (1945) broth at 20 C until a fairly uniform turbidity was evident. This required 24 to 48 hours. Then five-tenths ml of the broth suspension was spread evenly over the surface of a Morton and Engley's agar plate with a glass spreader. The plate was left open until dry and then incubated at 10 C until a thin film of growth developed. This incubation time varied somewhat with the amount of growth in the broth culture at the time of inoculation of the plate.

When the desired stage of growth had been reached, an agar block was cut out with a scalpel and the organisms fixed for two minutes in 2 per cent osmium tetroxide vapor while still on the agar. An impression film was made then on a coverslip and immediately (before drying could occur) hydrolyzed in 1 N HCl at 60 C for from 5 to 10 minutes, according to the staining procedure of DeLamater (1951a, 1952e), with the exception that no thionyl chloride was added to the thionine stain. The coverslip was left in a 0.25 per cent solution of thionine in alcohol for at least twelve hours, then rinsed in distilled water, and transferred to cold ethyl alcohol kept at approximately −50 C by packing in solid $CO_2$. Dehydration proceeded over a period of twelve hours in the cold alcohol. Following this treatment, the coverslip was transferred immediately from the cold

alcohol to ethyl alcohol at room temperature for 20 to 25 seconds, then rinsed several times in xylol, and mounted permanently on a glass slide with "clarite" mounting medium.

<div align="center">RESULTS</div>

A complete mitotic cycle, which appears to be the same as that which occurs in larger organisms, has been observed in this micrococcus. Each phase of the cycle has been clearly seen and photographed (see figures 1 to 32). An interpretative sketch of each photograph is likewise presented. The numbers of each correspond.

Prophase nuclei show the chromosomes contracting and thickening prior to becoming arranged on the equatorial plate (figure 32). In late prophase a single centriole appears and lies to one side of the condensed cluster of chromosomes (figures 1 to 2). The spindle becomes evident, extending between this centriole and the two clumped chromosomes. Subsequently the centriole divides and the two products migrate to opposite sides of the tightly contracted chromosomes to form typical metaphase spindles (figures 3 to 9, 13, 14, 21, 23, 27, 32).

The metaphase stage of the mitotic cycle is very characteristic. The chromosomes are seen as two darkly stained masses or granules, lying between the centrioles in typical spindles. Figures 5, 6, 7, and 9 show this especially well.

During anaphase the chromosomes begin to move apart (figures 10 to 13), and the doubling of the chromosomes can be seen. The daughter chromosomes move toward the poles at opposite sides of the cell. Figures 10 and 12 show this especially clearly. The centrioles and spindles are distinguishable, and the slight separation of the daughter chromosomes can be seen.

In later anaphase the chromosomes have moved farther apart and a distinct separation can be seen between the sets of daughter chromosomes. The spindle fibers and centrioles are still visible. In telophase the chromosomes mass together and are seen as darkly stained granules at each end of the cell. The centrioles are no longer visible. During this nuclear process the cell appears to elongate in the long axis of the dividing nuclear spindle.

Subsequently the formation of daughter cells occurs. A plate is formed across the cell between the separating clusters of chromosomes. This plate gradually extends outwards toward the cell walls while the cell wall appears to constrict somewhat to meet it with the subsequent formation of a complete partition and two individual daughter cells. This is essentially similar to what occurs in the onion root tip. Figures 16 to 20 illustrate this process. During the formation of the cross-wall the chromosomes are densely clumped.

As stated previously, the plate formation occurs at right angles to the long axis of the cell. During metaphase and anaphase the separation of the chromosomes also occurs at right angles to the long axis of the cell. During anaphase the axis of the cell changes; the somewhat flattened cell seen in metaphase and early anaphase rounds up and then elongates in the perpendicular direction which is the axis of the mitotic spindle. Thus what was formerly the short axis
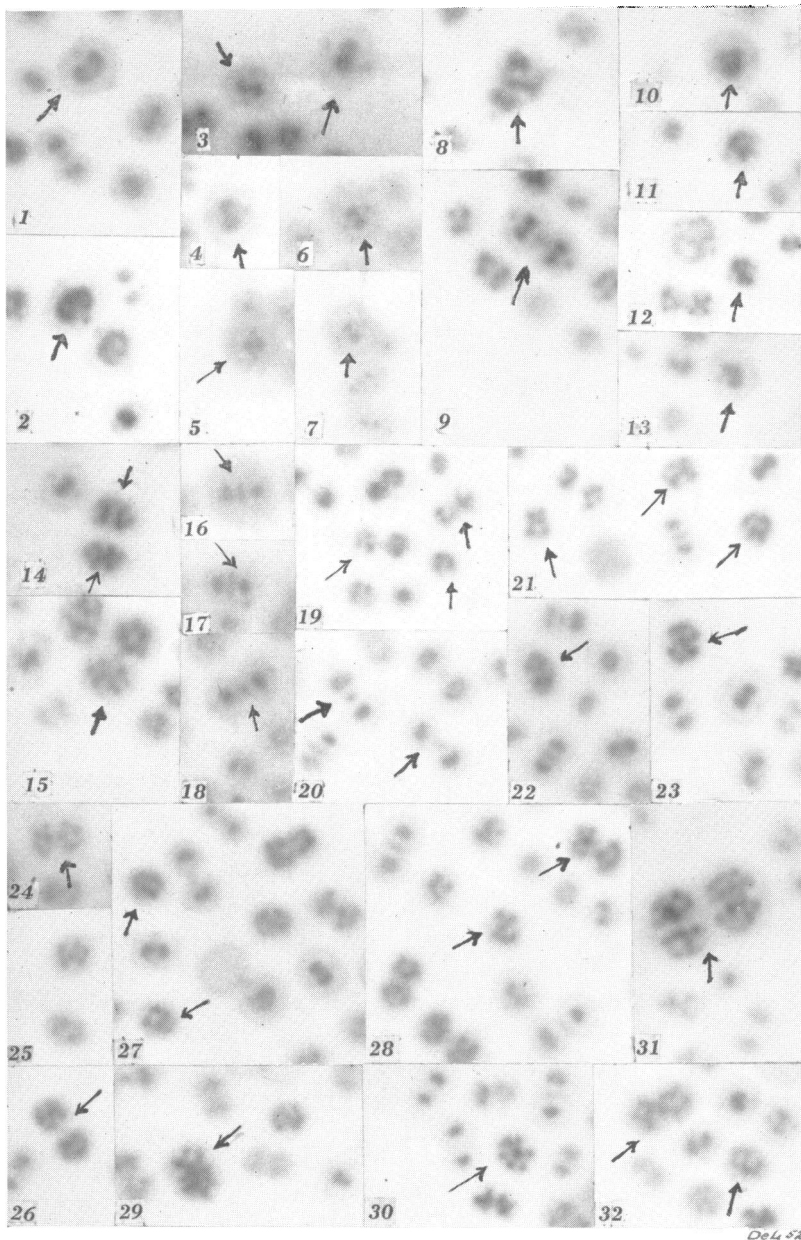
*Micrococcus cryophilus*—growth in Morton and Engley broth for 48 hours; growth on Morton and Engley agar for 5 hours at 10 C; fixation for 2 minutes in 2 per cent osmium tetroxide vapor; N HCl for 5 minutes at 60 C; 0.25 per cent thionine for 12 hours; ethyl alcohol at −50 C for 12 hours; ethyl alcohol at room temperature for 20 seconds; rinse in xylol. Photographs 4,450×.

*Figure 1.* Prophase; chromosomes contracting; centriole prior to division; one spindle evident.

*Figure 2.* Prophase, showing same structures as figure 1; earlier stage with chromosomes not as condensed.

*Figures 3 to 9.* Metaphase; chromosomes contracted and lined up on metaphase plate; two centrioles and complete spindle evident.

*Figures 10 to 13.* Anaphase; chromosomes beginning to pull apart toward poles; centrioles and spindles still distinguishable.

*Figure 14.* Telophase; early stage, showing further separation of chromosomes.

*Figure 15.* Telophase; later stage showing distinct separation of chromosomes; cell to right and above in interphase.
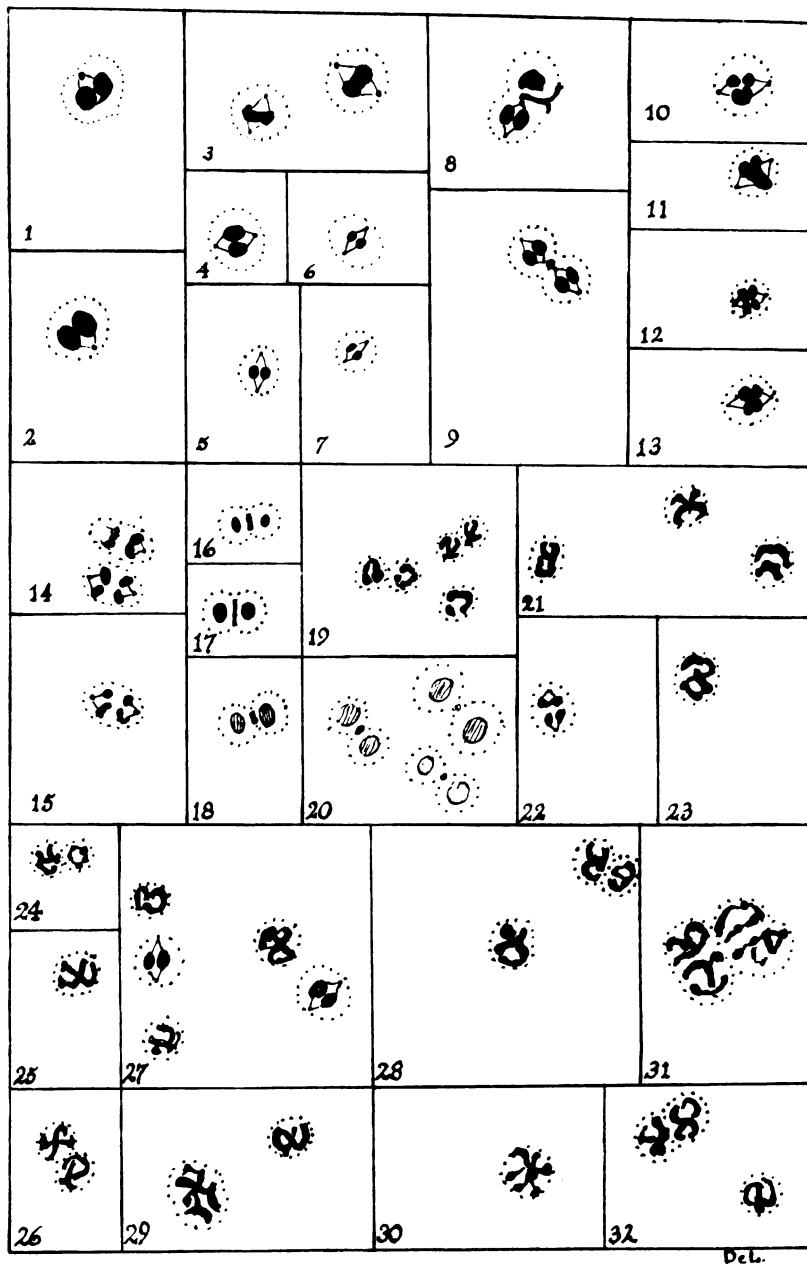
*Figures 16 to 18.* Telophase; very late stage with chromosomes massed together in darkly staining areas. Also shows the development of the cross-wall by means of cell plate with the subsequent formation of two individual daughter cells.

*Figures 19 to 20.* Interphase; early stage, showing chromosome masses beginning to loosen and elongate.

*Figures 21 to 23.* Interphase; later stage with chromosomes more thread-like and beaded. Cells in several other mitotic stages can also be seen.

*Figure 24.* Telophase; cells tipped so that the lower halves of the nuclei are not clearly visible.

*Figures 25 to 31.* Interphase; various stages showing the development of the chromosomes into beaded, thread-like filaments. Other mitotic figures also evident. Figure 30 especially illustrates the beaded nature of the chromosomes.

*Figure 32.* Interphase; very late stage, showing the chromosomes beginning to shorten and contract in preparation for prophase.

of the cell becomes the long axis. In no instance was a rotation of the mitotic figure, during anaphase, observed, or were configurations observed which could be so construed.

Following the formation of daughter cells, the compact masses of chromosomes seen in late telophase begin to loosen and the chromosomes elongate until finally they are seen as beaded, thread-like filaments. This stage closely resembles the interphase or vegetative nucleus of larger organisms. Progressive enlargement of the nuclei and the elongation of the chromosomes are seen in figures 21 to 28. Figures 30 and 31 illustrate particularly well the beaded nature of the chromosomal filaments in large interphase nuclei. The darkly staining central granule of the cell seen in figure 30 is probably a nucleolus.

During late interphase the chromosomes begin to shorten and thicken in early prophase. Figure 32 shows the chromosomal filaments already somewhat shortened and condensed. With further contraction of the chromosomes and the appearance of a centriole and spindle fibers, prophase is accomplished and the cycle is completed.

## DISCUSSION

The existence of a mitotic process in bacteria has long been suspected on *a priori* theoretical grounds. Studies on the variation and genetics of microorganisms have yielded results which prove the existence of genic elements in the bacterial cell (Lederberg, 1951). These results have led many workers to hypothesize the existence of some type of mitotic process operating in cell division. However, until recently no substantiating cytological evidence was available. The older nonspecific techniques of staining did not delineate structure within the bacterial nucleus. With the development of newer methods, darkly stained bodies, believed to be chromatinic material, were revealed. With the further development of methods (DeLamater, 1951a), further delineation of intrinsic detail of the bacterial nucleus has become possible. This method has permitted visualization of undistorted internal cell structure with the resultant observation of what appears to be a true mitotic process. The organism reported on here constitutes the third demonstration of a mitotic process in bacteria, the others being in *Bacillus megaterium* and *Caryophanon latum*. This is, however, the first example of mitosis in the cocci. On the basis of the observations on these three organisms, it is to be suspected that the mitotic process is general throughout the bacteria. Unpublished and as yet incomplete observations on three other organisms, including *Escherichia coli*, *Bacillus cereus*, and a second micrococcus, substantiate this view. Studies on these additional organisms will be presented elsewhere.

The nucleus and the divisional process observed in this micrococcus are comparable to those seen in larger organisms. The details of the process suggest a high order of development and differentiation. The chromosomes appear to undergo a process of elongation and contraction during the course of the cycle similar to that which occurs in larger nuclei. The appearance of centrioles and visible spindles, the doubling of the chromosomes, and the formation of a cell

plate similar to that which occurs in onion root tips, all indicate a degree of organization much higher than has previously been thought.

The establishment of the existence of a typical mitotic process in bacteria is of importance in the basic understanding of the genetics of bacterial variation and maintenance of phenotype as well as many other aspects of bacterial genetics. The substantiation of genetic evidence with cytological evidence will do much in furthering the study of bacterial genetics.

It seems likely that Bisset and Lindegren have both observed and recorded recognizable phases or stages in a mitotic process. The interpretations of these authors, however, neither agree with one another nor with that of the present writers.

Bisset (1952) has recently taken issue with the concepts and observations presented here. He considers that the staining procedure is too nonspecific to render a valid picture, and that the "spindle" observed and demonstrated here is in fact a misinterpretation of the cross-wall with the two condensed nuclei on either side. He further contends that the method of freezing dehydration must so disrupt the cell that all internal structure is disorganized.

Bisset has been answered in detail in the same journal (DeLamater, 1952*d*). Undoubtedly certain stages recorded here correspond to those drawn, but not photographed, by Bisset, but the explanations and interpretations which he proposes are not sufficiently comprehensive to explain away what is to be observed in these organisms, nor the photographic records which are presented.

## SUMMARY

What appears to be a typical mitotic process has been described in detail for the first time in *Micrococcus cryophilus*. This process is characterized by centrioles and visible spindles, by the elongation and contraction of the chromosomes during the course of the mitotic cycle, by the doubling of the chromosomes, and by the formation of a cell plate with the subsequent development of two individual daughter cells.

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