CELL ELONGATION AND DIVISION IN *SPIRILLUM ANULUS*[1]

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Received for publication February 28, 1959

The hypothesis that the main growth of the cell envelope of bacteria is from a growing point at one pole in unicellular bacteria and from the points of junction of cell wall and cross-wall in septate bacteria has been advanced by Bisset (1951, 1953, 1955, 1956). A corollary of this hypothesis is the consideration that the growth of the daughter cell from the mother cell is by a process resembling budding in that the mother cell retains most of its cell wall and mature flagella, whereas the bud has a newly grown cell wall and flagella in an early stage of development. The experimental evidence for this hypothesis is based on the division and arrangement of flagella in growing and dividing cells as well as the fact that at the putative growing point the cell walls appear thinner and less well developed than over the remainder of the cell.

Stocker (1956), on the other hand, has advanced the hypothesis that cell growth and elongation occur diffusely by the intercalation of the cell wall along its length. Stocker's hypothesis is based on the investigation of the unilinear transmission of motility in nonmotile strains of Salmonella by transduction.

The two hypotheses appear to be diametrically opposed although the possibility exists that both types of cell growth may occur among the various types of bacteria. It is highly improbable, however, that both types should occur in species of the same genus; species of the genus *Salmonella* were among the experimental organisms used by Bisset and co-workers. Since their experimental data are based on evidence found in fixed cells (flagellar stain preparations or in electron micrographs) and those of Stocker are derived from genetical considerations, it was believed that more concrete evidence of cell growth and elongation could be obtained from observations of these processes in living cells.

MATERIAL AND METHODS

Spirillum anulus (Williams and Rittenberg, 1957) was chosen as the experimental organism because this organism grows very slowly and motility is sluggish in early cells, permitting continuous observations of the same group of cells over an extended period of time. The organism was grown in broth of the following composition: peptone, 5.0 g; beef extract, 3.0 g; yeast autolysate (Basamin, Anheuser-Busch, Inc., St. Louis, Missouri), 3.0 g; distilled water, 1 L. Broth tubes were inoculated with 0.1 ml of a 7-day broth culture and incubated at 30 C. The tubes were centrifuged at 2-hr intervals, the supernatant fluid discarded by decanting, and the sedimented cells shaken vigorously in the fluid remaining in the tubes so that a representative sample of the cells could be obtained. Samples were removed with a Pasteur pipette and one drop of the cells was placed in a drop of broth on a sterile glass slide. The preparation was covered with a number 0 cover slip and excess fluid removed with filter paper to increase visibility and reduce motility of the cells. The resulting preparation was ringed with immersion oil to prevent drying of the wet mount preparation during the observations.

The observations were made with the phase attachment of the Leitz Ortholux microscope, equipped with an oil immersion phase objective (90×), N.A. 1.10, and compensating oculars (12×). The phase condenser of the Leitz phase attachment permits the transition from bright field to phase contrast to dark field by the adjustment of the height of the mirror body in the condenser. Both phase contrast and dark-field photographs were made on Royal Pan sheet film with the Leitz Makam camera. The green filter, supplied with the Leitz phase equipment, was used in photography.

[1] A summary of this work was presented at the 59th Meeting of the Society of American Bacteriologists, St. Louis, Missouri, May 10 to 14, 1959.

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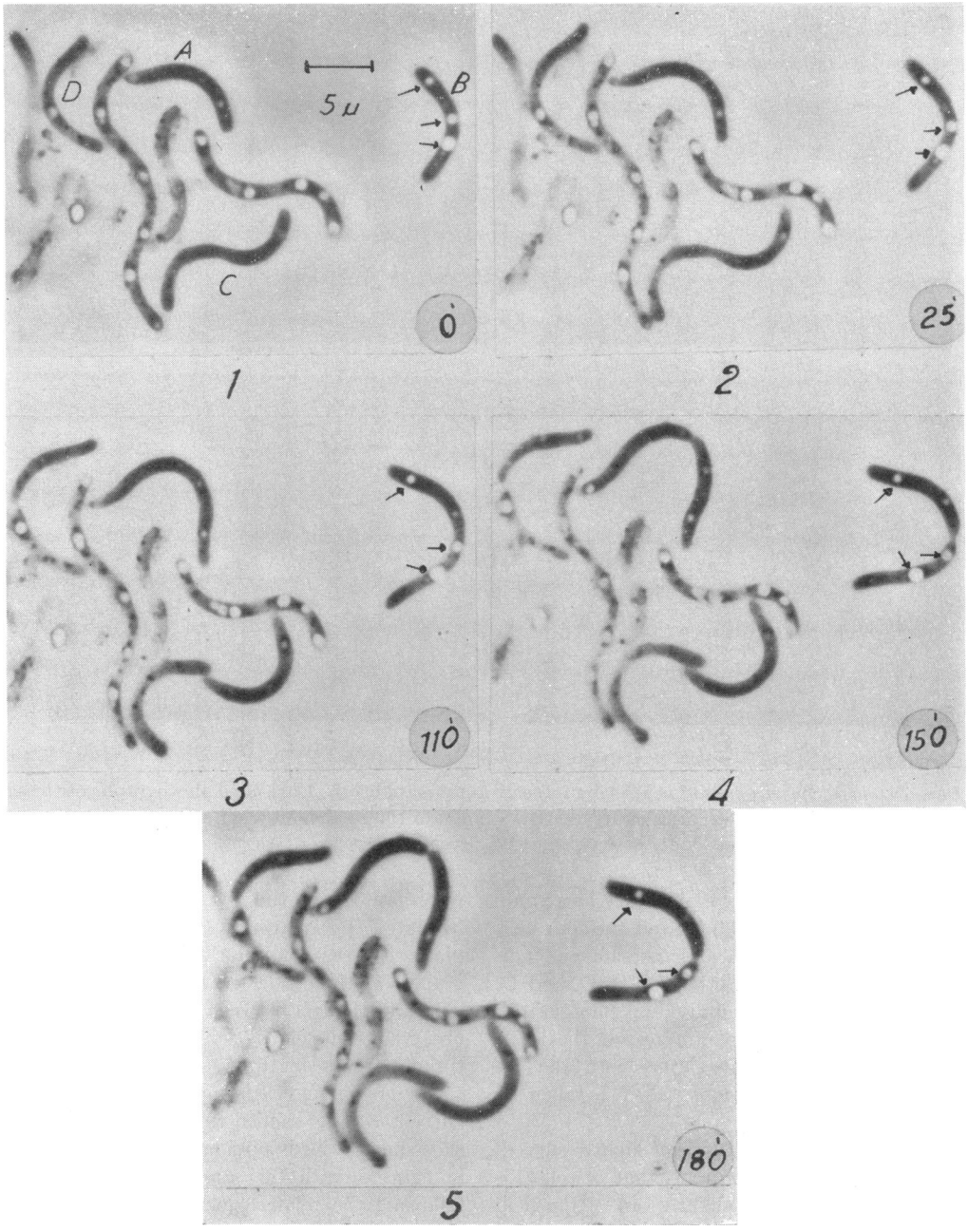
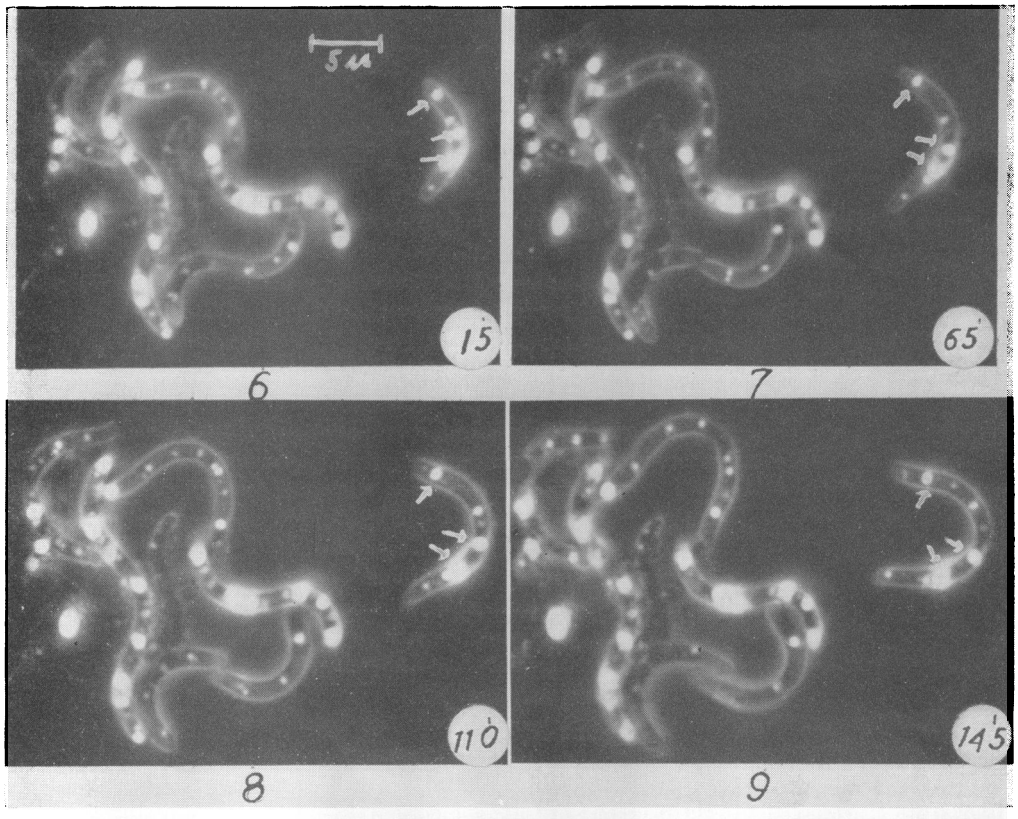


Figures 1 to 5. Spirillum anulus growing in yeast autolysate broth. The cells showing growth and division are indicated in figure 1 by the letters A, B, C and D. The arrows in figures 2 to 5 show the inclusions of cell B used as markers. The time of the observation at which the photographs were made is shown in the lower right corner. Phase contrast photography.

RESULTS AND DISCUSSION

Cell division was not observed in the living cells of *S. anulus* before the 6th hr of growth and then it was observed in only an occasional cell. By the 14th hr, however, cell division was occurring in a small proportion of the cells in a regular manner. Cell growth, elongation, and division are shown to occur in four of a group of



Figures 6 to 9. Spirillum anulus growing in yeast autolysate broth. Dark-field photographs of the same group of cells as in the preceding figures. Arrows indicate the inclusions used as markers in the phase contrast photographs.

cells taken from a 14-hr broth culture. This group of cells was observed for 3 hr and the four cells which underwent growth and division are indicated in figure 1 by the letters A, B, C, and D. The times at which the individual photographs were taken are shown, in minutes, at the lower right corner of the photographs with the beginning of the observations (14 hr) taken as zero time.

Cells A and B show the form of slightly curved, comma-shaped cells in figure 1; that of cell C is S-shaped. Cell D, being slightly out of the field of vision in the photographs, will not be discussed although growth and elongation occurred in this cell in the same manner as in the others. Cell B is of primary importance and will be used in the discussion of growth and elongation. Three inclusions are plainly visible in this cell in the phase contrast series of photographs (figures 1 through 5) although the dark-field series (figures 6 through 9) show that more than three inclusions were present in the cell. These inclusions are indicated by markers (arrows) in both the phase contrast and dark-field series of photographs. By following the relative increase of the length of the cell between the markers, it can be observed that elongation of the cell occurred throughout its length.

The change in cell form from that of a slightly curved, comma-shaped cell to that of a horseshoe-shaped cell is obvious by comparing cell B in figure 1 with the same cell in figure 5. By following the relative increase in the length of the cell between the inclusions (used as markers) at each pole of the cell, it may be observed that the greater amount of cell elongation occurred at the center of the cell. No evidence of budding from one or both poles was observed.

The processes of cell division can be followed more clearly in cell C, which was constricted at the time the observations were begun. The incipient constriction is obvious in both phase contrast

(figures 1 and 2) and in the dark-field photographs (figure 6). The completion of the constriction is evident in figure 7, 65 min after the observations were first made. The increase in cell length of the two sister cells of C can be observed in figures 3 through 9. Cell division was completed in cells A, B, and D at the end of 150 min.

In the dark-field series of photographs (figures 6 through 9), in which the cell wall is more clearly shown, it can be seen that no septum is present in the cells until constriction is completed. There is no evidence of septa in any of the cells, including those in which cell division did not occur, except after constriction. From this absence of septa in the cells, it can be concluded that *S. anulus* is unicellular. The unicellular nature of species of *Spirillum* is also shown in the electron micrographs of autolyzed cells of this genus by van Iterson (1953) and in the ultrathin sections of *Spirillum serpens* by Chapman and Kroll (1957). From the data shown for the growth and elongation of living cells of *S. anulus*, it can be concluded that growth in this unicellular organism does not occur by budding, as has been postulated to occur in other unicellular bacteria by Bisset (1951, 1953, 1956).

Bisset's theories with regard to the growth of cell envelopes in bacteria appear to be based on the consideration that these cell envelopes are dead rather than living structures. The adoption of such a hypothesis by Bisset has made it necessary for him to postulate some type of growing point in bacterial cells in order to account for cell growth and elongation. The data given for *S. anulus* cannot be used to determine whether cell envelopes are dead or living but, according to Bisset (1953, p. 18) it is "logical to assume that a rigid yet dead structure, such as a cell wall . . . could not grow all over its surface, but could approximate to doing so only by constantly tearing and reforming, of which there is no evidence." In the dark-field series of photographs (figures 6 through 9) the cell wall of *S. anulus* can be observed clearly; there is no evidence of any process of "tearing and reforming" of the cell wall in these photographs. Since there is no evidence of growth from one pole only, the logical assumption is that the cell walls of *S. anulus* are living structures. As stated above, the pictorial evidence of the growth and elongation of the cells of *S. anulus* provide no direct evidence on this matter.

From a consideration of the data shown to occur in growing cells of *S. anulus*, it is concluded that growth and elongation of the cells occur diffusely along the entire length of the cell, by intercalation of the cell wall, as postulated by Stocker (1956).

SUMMARY

Cell growth, elongation, and division were observed to occur in living cells of *Spirillum anulus*, as observed by phase contrast and dark-field microscopy. By the use of the inclusions of the cells as markers, it has been shown that growth and elongation of the cells occur diffusely, by the intercalation of the cell wall, as postulated by Stocker in 1956. No evidence of budding was observed in the unicellular *S. anulus* to substantiate Bisset's theory of a growing point in such organisms.

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