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Mode of Extension of Cell Surface During Growth of *Spirillum volutans*

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The conclusion that diffuse intercalation of new wall into old occurs in *Spirillum anulus*, rather than formation of new wall at only one or a few growing points (M. A. Williams, J. Bacteriol. **78**:374, 1959), is based on an assumed fixed relationship of cytoplasmic inclusions to adjacent wall. In the present work, the immunofluorescence method of R. M. Cole and J. J. Hahn (Science **135**:722, 1962) offered a more direct approach to this problem.

S. volutans appeared to offer advantages because of its larger size and because it has been proposed as the type species of the genus (M. A. Williams, Intern. Bull. Bacteriol. Nomencl. Taxon. **9**:137, 1959). The Wells strain, ATCC 19554, was used (J. S. Wells, Jr., and N. R. Krieg, J. Bacteriol. **90**:817, 1965). A medium of the following composition (grams per liter) was employed: peptone (Difco), 10.0; succinic acid, 2.0; $(NH_4)_2SO_4$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; $FeCl_3 \cdot 6H_2O$, 0.002; and $MnSO_4 \cdot H_2O$, 0.002; *p*H, 6.8. After autoclaving, the atmosphere within the culture vessel was adjusted to contain 6% oxygen and 94% nitrogen. Cells grown for 24 hr at 30 C were centrifuged, suspended in 0.5 ml of growth medium, and exposed for 5 min to 0.2 ml of the globulin fraction of rabbit antiserum, previously prepared by immunization with whole-cell suspensions and conjugated with fluorescein isothiocyanate (W. B. Cherry, M. Goldman, and T. R. Carski, Public Health Serv. Publ. 729, 1960). Cells were then washed twice with grow medium and planted in 75 ml of medium (6% oxygen atmosphere) to yield 5×10^4 cells per milliliter. Growth was followed by hemocytometer count, the average generation time being ca. 4.2 hr. At intervals beginning with 0 hr, samples of growing culture were centrifuged, suspended in *p*H 7.6 growth medium, and mounted under cover slips. The wet mounts were partially dried to flatten the helical cells sufficiently for observation. Living cells only (as determined by observing the movement of the polar tufts of flagella) were photographed with a Leitz Ortholux microscope fitted with Heine condenser, Pv Apo 90×/1.15 objective, and Osram HBO mercury burner. Exposures were for 4 min on Plus X Pan film, with 8-min development in Ansco Hyfinol. A uniform decrease in cell fluorescence with increasing growth time (Fig. 1) indicated diffuse intercalation of new cell surface into old; in most cases, however, cells retained one bright polar region (arrows, Fig. 1). This correlated well with similar results obtained by E. H. Beachey and R. M. Cole (Bacteriol. Proc., p. 27, 1966) with *Escherichia coli*, who suggested that polar wall was also formed by diffuse intercalation but at a slower rate. Eventually, even the polar areas of *S. volutans* faded. Control cells, killed with Formalin after being labeled at 0 hr, retained their fluorescence indefinitely.

Chloramphenicol (5 μg/ml) was found to inhibit cell multiplication completely; the inhibited cells, still actively motile, retained their fluorescence for at least 19 hr (Fig. 2), although some diminishment did occur. Interestingly, the development of discrete gaps was not induced by the antibiotic, in contrast to results reported for *Salmonella typhosa* (R. M. Cole, Science **143**:820, 1964).

Specificity of labeling was indicated by (i) complete blocking of attachment of labeled antibodies by prior exposure of cells to unlabeled antibodies for 5 min, (ii) failure of labeled anti-*S. volutans* globulin to stain the large bacterium *S. anulus*, and (iii) failure of labeled normal globulin to stain *S. volutans*.

Although the surface antigens involved are stable to boiling for 1 hr, their exact nature is unknown. Since microcapsular antigens might be involved, and since the exact location of the antigen-antibody reaction is not certain, we have used the term "cell surface extension" rather than "cell wall extension"; i.e., whether or not the specific portion of the cell surface known as the mucopeptide layer grows by diffuse intercalation cannot yet be stated.

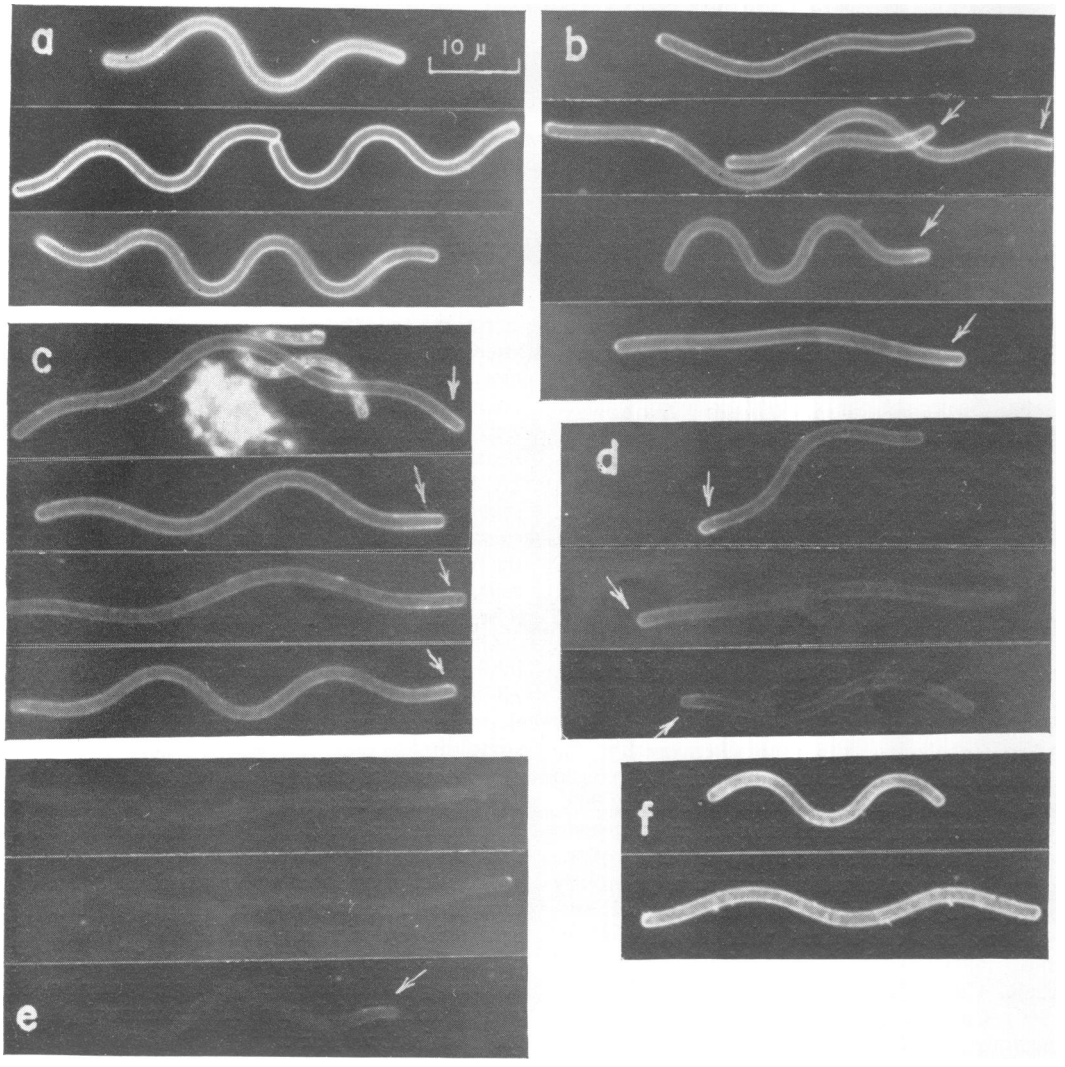

FIG. 1. *Living cells of Spirillum volutans labeled with fluorescent anti-S. volutans globulin, washed, and examined (a) immediately, (b) after growth for 1 to 2 hr, (c) after growth for 4 to 5 hr, (d) after growth for 6 to 7 hr, (e) after growth for 8 to 9 hr; (f) = control cells, killed with Formalin after labeling at 0 hr and incubated for 9 hr. Some cell debris and a partially autolyzed cell which have retained their initial brilliance are present at the top of (c). Arrows indicate the slower fading of polar regions.*

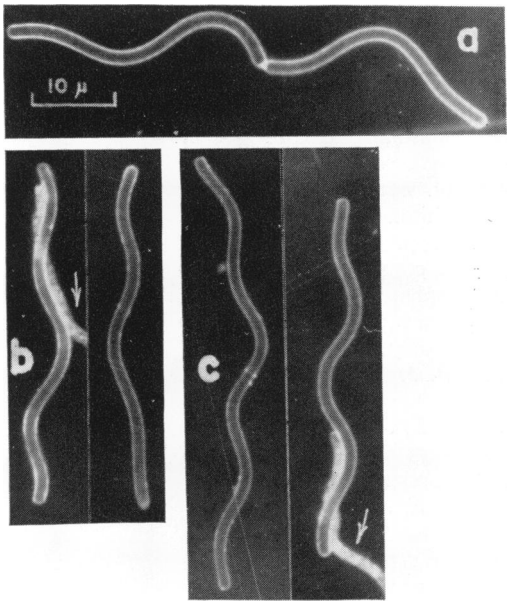

FIG. 2. *Living cells of Spirillum volutans labeled with fluorescent anti-S. volutans globulin, washed, and examined (a) immediately, (b) after 9.5 hr of incubation in chloramphenicol broth, and (c) after 19 hr of incubation in chloramphenicol broth. Arrows indicate partially autolyzed cells, which have retained their initial brilliance.*