phosphate buffer, to minimize the phosphate content of the extracts. In experiments with 1 atm $N_2$, the free ammonia was isolated by the Conway procedure (Mortenson, Anal. Biochem. **2**:216, 1961) and analyzed with Nessler's reagent (Johnson, J. Biol. Chem. **137**:575, 1941).

In the closely related organism *C. butyricum*, Wolfe and O'Kane (J. Biol. Chem. **205**:755, 1953) showed that arsenate accelerated pyruvate metabolism and prevented the accumulation of acetyl phosphate from the phosphoroclastic reaction. The data in Table 1 illustrate the inhibitory effect of arsenate on $N_2$ fixation in extracts from *C. pasteurianum*. This result suggests a requirement for "high energy" phosphate in $N_2$ fixation. The addition of glucose to *C. butylicum* extracts prevents the accumulation of acetyl phosphate formed in the phosphoroclastic reaction. The glucose is converted rapidly to hexose phosphate (Koepsell, Johnson, and Meek, J. Biol. Chem. **154**:535, 1944). Table 1 shows the effect of added glucose on $N_2$ fixation. Acid-stable (1 N hydrochloric acid, 7 min, 100 C) ester phosphate, presumably hexose phosphate, was formed in the presence of added glucose. Tests with firefly luciferase assay showed that the *C. pasteurianum* extracts, with added pyruvate, produced adenosine triphosphate (ATP). In the presence on glucose, ATP apparently is utilized for hexose phosphorylation at the expense of $N_2$ fixation. Carnahan et al. reported an inhibitory effect on nitrogen fixation of ATP or acetyl phosphate added at molar concentrations which were optimal for pyruvate. However, ATP and acetyl phosphate do not accumulate to such levels in the reaction mixtures. Preliminary experiments in this laboratory also indicate that added acetyl phosphate inhibits pyruvate metabolism.

TABLE 1. *Nitrogen fixation by cell-free extracts from Clostridium pasteurianum*

| Compound added* | Nitrogen fixation as % of control |
|---|---|
| Arsenate ($10^{-2}$ M final concn) | 2–7 |
| Glucose (10 mg) | 7 |
| Glucose (35 mg) | 5 |
| Glucose (102 mg) | 0 |

* Each flask also contained cell-free extract (1 to 2 mg protein-N), 20 mg of sodium pyruvate, and 0.05 M phosphate buffer (pH 6.8; in the arsenate experiments, the corresponding arsenate buffer was added), in a final volume of 1 to 1.2 ml. The gas phase for incubation was either 1 atm of $N_2$ (control flasks, minus arsenate or glucose, average 70 $\mu$g N fixed) or 0.05 atm $N_2$ (63 atom % $N^{15}$) plus helium to 1 atm (control flasks fixed 0.1 to 0.6 atom % excess $N^{15}$). Incubation was at 32 C for 1 hr.

These experiments show the necessity for "high energy" phosphate for $N_2$ fixation by extracts of *C. pasteurianum*. Clarification of the relationship between the $H_2$-evolving and $N_2$-activating systems in the $N_2$-fixing enzyme complex may suggest how alternative sources of ATP and reducing power may be substituted for the phosphoroclastic system.

## CELL DIVISION IN A SPECIES OF *ERWINIA*

### VI. GROWTH OF CELLS FROM THE DIVISION END

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Cole and Hahn (Science **135**:722, 1962) published pictorial evidence, obtained using fluorescein isothiocyanate-labeled antibody, demonstrating that streptococcal cells grow from one end. This question has been debated for several years. Bisset and Pease (J. Gen. Microbiol. **16**:382, 1957) interpreted Pennington's work (J. Bacteriol. **59**:617, 1950) to indicate that cells grow

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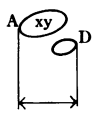
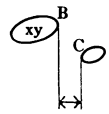
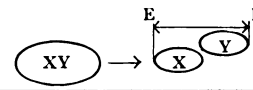
TABLE 1. *Measurement of growth and "fix" points on a cell before and after division*

| Time from zero | Distance A to D  | Distance B to C  | Increase in size of X and Y after division  | | | |
|---|---|---|---|---|---|---|
| | | | Distance for XY or E to F | Size of X | Size of Y | Total size increase (X + Y) |
| *min* | *μ* | *μ* | *μ* | *μ* | *μ* | *μ* |
| 214 | 6.5 | 0.0 | 5.5 | | | |
| 227 | 6.5 | 0.25 | 5.5 | 3.2 | 3.0 | 6.2 |
| 242 | 6.5 | 0.25 | 5.5 | 3.5 | 3.2 | 6.7 |
| 252 | 6.5 | 0.25 | 5.5 | 3.8 | 3.5 | 7.3 |
| 270 | 6.5 | 0.25 | 5.5 | 4.1 | 4.0 | 8.1 |
| 285 | 6.5 | 0.25 | 5.5 | 4.6 | 4.2 | 8.8 |
| 300 | 6.75 | 0.00 | 5.5 | 5.0 | 4.2 | 9.2 |

from one end. Further work (Hale and Bisset, J. Gen. Microbiol. **18**:688, 1958) appeared to substantiate this belief. Murray, Francombe, and Mayall (Can. J. Microbiol. **5**:641, 1959) also suggested that bacteria grow from one end. Williams (J. Bacteriol. **78**:374, 1959) published evidence to the contrary.

I have been interested in this problem because growth from one end would aid in formulating a working hypothesis regarding the problem of why bacterial cells divided in the approximate center.

My data (Table 1, Fig. 1–7) support the concept of growth from one end: the division end. These data were obtained in a manner different from that of other investigators. The technique consisted of growing cells of an *Erwinia* sp. in microculture on the elongating and defined medium of Grula (J. Bacteriol. **80**:375, 1960) and photographing them using phase microscopy (bright, medium-contrast objective). Fix points and measurements on a divided cell are given in Table 1.

Because bacteria grow from the division end, this end becomes the area of greatest metabolic activity, particularly for cell-wall synthesis. Considered from this point of view, the illustrations relating to "protoplasting" of growing *Escherichia coli* cells, presented by Lederberg and St. Clair (J. Bacteriol. **75**:143, 1958) become even more meaningful. Their evidence shows that penicillin causes wall damage either at one end (the growing end) of a cell or in the middle, where the septum for division is being synthesized.

Growth from one end could also mean that cells divide in the approximate middle because this is where the division "control center" was left after division and prior to growth from that point.

To obtain additional evidence on this latter point, cells of *Erwinia* were elongated by growth in the presence of D-serine (Grula and Grula, J. Bacteriol. **83**:981, 1962) and were then transferred to Nutrient Agar to allow division. Pictures of the division events are shown in Fig. 8–19. It should be noted that the filamentous cell did not divide in the middle. Also, it did not divide only at one end or fragment into many pieces, each 1.5 to 2 *μ* long ("normal" length of these cells). Further, several "latent" divisions in the main body of the filament occurred *after* new daughter cells had grown and divided. This pattern was seen many times, and results were duplicated using penicillin-produced filaments sparked to divide by pantoyl lactone (Grula and Grula, J. Bacteriol. **83**:981, 1962).

Therefore, a filamentous cell, unlike a normal cell, divides into several cells. Also, filamentous cells are polynucleate. Although not shown here, the nuclei of filamentous cells appear compact and spherical; when the filament begins to divide, the nuclear material becomes diffuse.

Although growth from one end offers an intriguing possibility for localization of division centers in the approximate center of a "normally"

dividing cell, it does not explain results obtained using filamentous cells. I, nevertheless, offer the theory and pictures for others who wish to study this aspect of bacterial growth and cell division.

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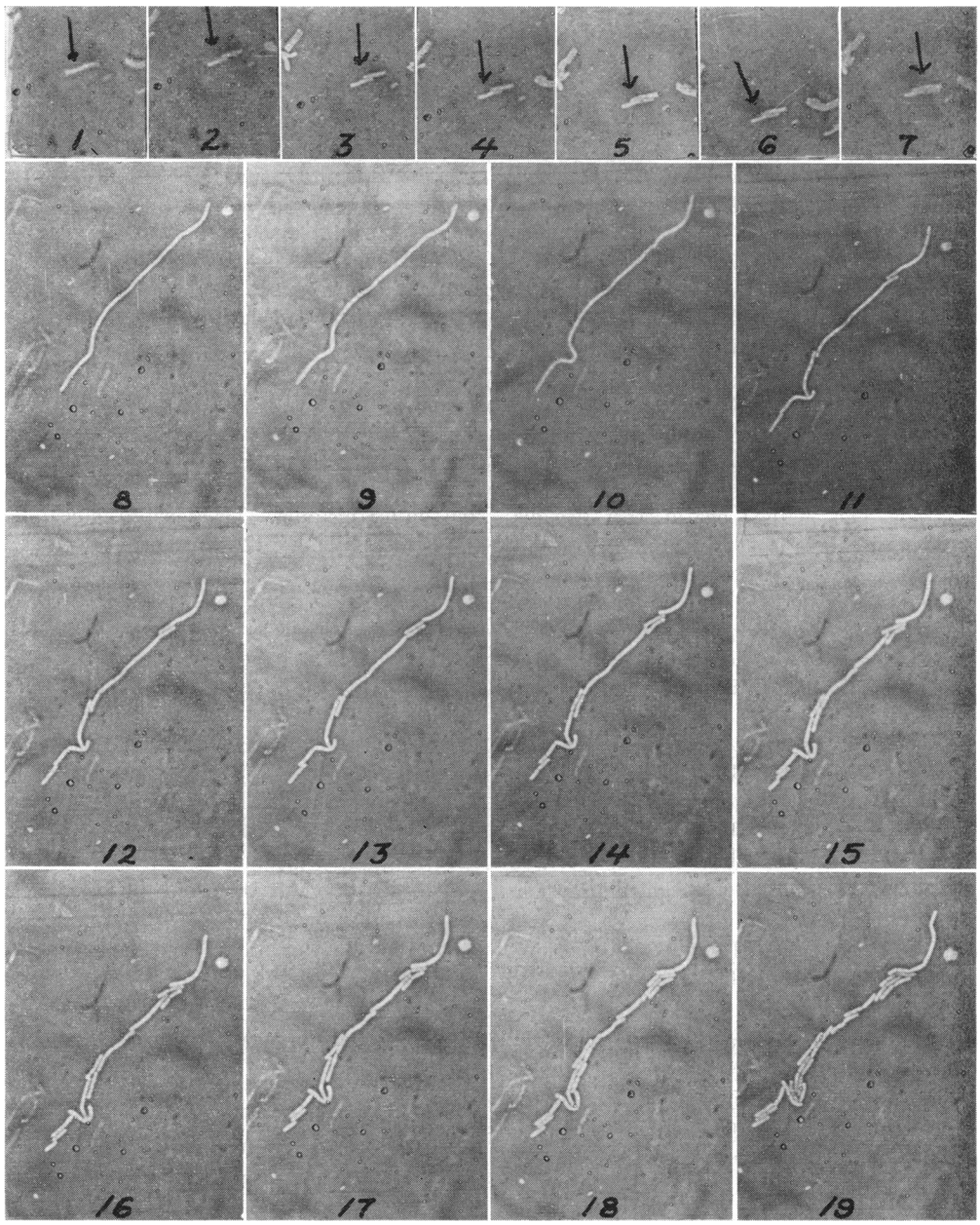


FIG. 1–7. *Division of one cell and subsequent growth. Time intervals from zero time (in min): 214, 227, 242, 252, 270, 285, and 300. All magnifications* × *1,000.*

FIG. 8–19. *Division events in a filamentous cell. Time intervals from zero time (in min): 0, 10, 30, 45, 53, 60, 70, 80, 87, 93, 104, and 135. All magnifications* × *1,000.*