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Influence of *p*H on *Proteus* Flagella

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When the flagella of *Proteus* species are exposed to an acid environment above *p*H 5, they become curly and their wavelength is reduced to half of the normal value. This effect, called "biplicity" or "doubling phenomenon" by Pijper (Nature **175**:214, 1955), was first demonstrated by Leifson, Carhart, and Fulton (J. Bacteriol. **69**:73, 1955), who examined stained preparations with a light microscope. These curly flagella and the stages of their development have not previously been examined in an electron microscope with any bacteria; such observations with *P. mirabilis* are reported here.

A suspension of *P. mirabilis* from a motility-tube culture was inoculated uniformly but sparsely onto the surface of a plate of Heart Infusion Agar (*see* Hoeniger, J. Gen. Microbiol. **39**: 195, 1965) which had been adjusted to values between *p*H 5.0 and 7.4 with McIlvaine's buffer; the cultures were incubated at 30 C. The bacteria were floated off the agar with saline adjusted to the same *p*H as the plating medium, and were then fixed with formalin. After the cells had been washed, they were negatively stained with 1% potassium phosphotungstate (Thornley and Horne, J. Gen. Microbiol. **28**:51, 1962) containing 0.01 to 0.02% bovine serum albumin (which promotes spreading of the phosphotungstate), and were viewed in an electron microscope.

When organisms from the more acid media were examined, many were found to have flagella with two distinctly different types of curvature: "normal" with a wavelength of 2.13 ± 0.14 μ, and "curly" with a wavelength of 1.01 ± 0.1 μ. These values for mean and standard deviation agree well with those for *P. mirabilis* published by Leifson et al. A bacterium from the *p*H 5.4 medium is shown in Fig. 1; two of the eight flagella arising near the end of this short rod are seen to be curly, and the remainder have normal curvature.

The proportion of curly flagella varied with *p*H of the growth medium as follows: over the range *p*H 6.0 to 7.4, no flagella were curly; at *p*H 5.8, 0 to 5% were curly; at *p*H 5.6, 10 to 20%; at *p*H 5.4, 25 to 75%; at *p*H 5.2, 80 to 100%; and at *p*H 5.0, 100%. It was not, however, necessary to cultivate the bacteria on acid media to elicit curliness; the same effect was obtained by growing the organisms on a normal medium (Heart Infusion Agar, *p*H 7.4) and removing them in buffered saline adjusted over the same *p*H range of 5.0 to 7.4. Such an observation was also made by Leifson et al., although in both types of experiment they found the effective *p*H range to be 6.2 to 7.0 instead of 5.0 to 5.8. This discrepancy may simply be a reflection of interstrain differences.

Stages in bacterial differentiation and flagellar development were examined with cultures growing at *p*H 5.4. The bacteria changed from coccoid to rod-shaped, and then, after 6 to 7 hr of incubation, to elongated forms; thus, the well-known *Proteus* swarmers developed just as they do in normal cultures (Hoeniger, J Gen. Microbiol. **39**:195, 1965). During the incubation, organisms from cultures of each age developed a large proportion of flagella with a curly shape. Figure 2 shows the end of a 2-hr rod with new flagella of various lengths, mainly curly. The whole organism had 38 flagella ranging from 0.3 to 2.6 μ, with a mean length of 1.3 μ; the latter value falls in the range for 1.5-hr cells from a normal medium, i.e., 1.54 ± 0.22 μ (Hoeniger, J. Gen. Microbiol. **39**:195, 1965). Figure 3 shows the tip of a swarmer equipped with numerous curly and some normal flagella; the organism itself was 12 μ long and had ca. 800 flagella ranging from 0.6 to 7.6 μ, the mean length being 3.9 μ. For comparable swarmers developing on normal medium, the flagellar length was 3.84 ± 0.32 μ (Hoeniger, J. Gen. Microbiol. **39**:195, 1965).

The influence of acidity on motility *per se* was determined only for the swarmers. The bacteria were rinsed off the medium in broth buffered at the same *p*H as the growth medium. Those developing on media at *p*H 5.6 to 7.4 all moved in the normal, slow but sinuous, translatory manner; as already stated, on such organisms 20% or fewer of the flagella were curly. At *p*H 5.4, swarmers (like the organism in Fig. 3) either rotated or moved normally, 25 to 75% of their flagella being curly. Those developing at *p*H 5.2 (with 80 to 100% of their flagella curly) mostly rotated, but occasionally translated in a lopsided, circular path. By contrast, swarmers developing at *p*H 5.0 were mainly nonmotile, though a few

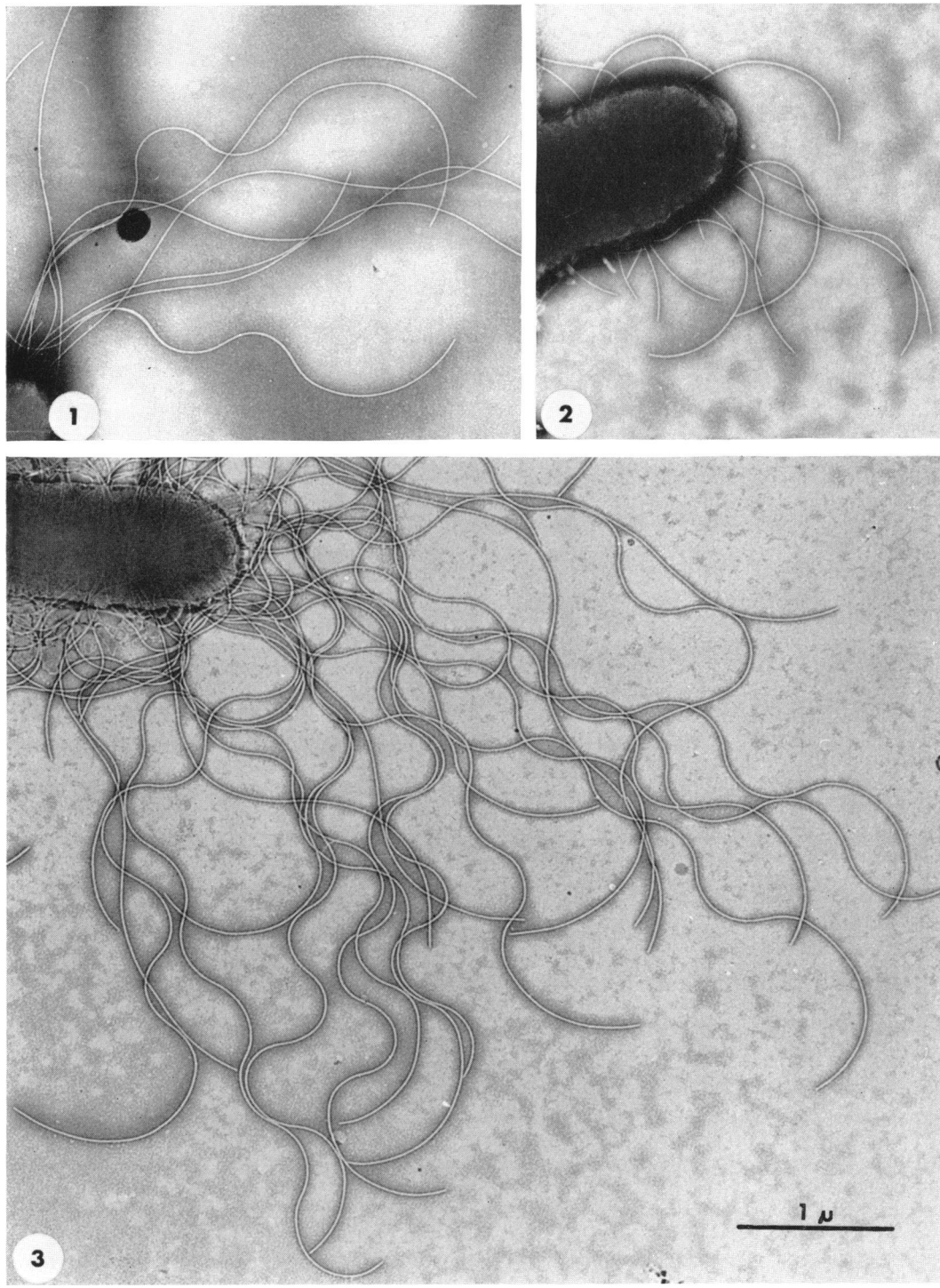

FIG. 1. *Curly and normal flagella near the end of a rod of Proteus mirabilis growing on pH 5.4 agar.* ×*25,000.*

FIG. 2. *Rod from a 2-hr culture showing early stages in development of curly flagella.* ×*25,000.*

FIG. 3. *End of a swarmer bearing numerous curly and some normal flagella; from a 6.5-hr plate.* ×*25,000.*

rotated or moved in a lopsided manner; such organisms had only curly flagella.

Apparently, the curly flagella of *P. mirabilis* are much less effective organelles of motility than are normal flagella. In this respect, they resemble the curly flagella of *Salmonella typhimurium* synthesized in the presence of the amino acid analogue, *p*-fluorophenylalanine (Kerridge, J. Gen. Microbiol. **23**:519, 1960), and those of the mutant of *S. typhimurium* studied by Iino (J. Gen. Microbiol. **27**:167, 1962). Lowering the *p*H as far as 5.0 does not appear to affect the synthesis of flagellar protein to any great extent, though it probably alters the conformation of the strands of flagellin molecules within the flagellum in some way as yet unknown. This problem is being investigated further at the level of flagellar ultrastructure.

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