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NOTES

Electron Microscopy of Particles Associated with a Bacteriocinogenic *Vibrio cholerae* Strain

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The existence in *Vibrio cholerae* (*Vibrio comma*) of bacteriocins (vibriocins) is controversial, and special techniques seem necessary for their demonstration (A. H. Wahba, Bull. World Health Organ. **33**:661, 1965). Electron microscopic studies of bacteriocin preparations (pyocins) from *Pseudomonas aeruginosa* (M. Kageyama, J. Biochem. **55**:49, 1964; S. Ishii, Y. Nishi, and F. Egami, J. Mol. Biol. **13**:428, 1965; D. E. Bradley, p. 115, *in* R. Uyeda [ed.], *Electron Microscopy 1966*, vol. 2, Maruzen Co., Ltd., Tokyo, 1966; T. B. Higerd, C. A. Baechler, and R. S. Berk, J. Bacteriol. **93**:1976, 1967) revealed particles with structural similarities to the tail of the coliphage T4. This note reports electron microscopic investigation of particles, resembling those of pyocins, which have been observed to be associated with one bacteriocinogenic strain of *V. cholerae* but have not been found with six nonbacteriocinogenic *V. cholerae* strains.

Cholera strain CRC 10425 obtained from J. C. Feeley, Division of Biologics Standards, National Institutes of Health, was isolated originally in Calcutta in 1964. This culture was bacteriocinogenic against 10 of 28 indicator *V. cholerae* strains tested. Bacteriocin activity was determined by a modification of the method of A. H. Wahba (Bull. World Health Organ. **33**:661, 1965) in which sensitive indicator strains were detected by zones of inhibition which developed around them when they were flooded as broth cultures over chloroform-killed stab colonies of the bacteriocinogen cultured on Trypticase Soy Agar (BBL) plates. The preparation of electron microscopic specimens involved cells grown for 18 hr at 37 C in Trypticase Soy Broth (*p*H 8) and washed three times in 0.2 M ammonium acetate (*p*H 6.7), negatively stained with 2% (w/v) phosphotungstic acid (*p*H 5.2), and observed with a Siemens Elmiskop IA electron microscope operating at 80 kv.

Size and morphology of the observed particles (Fig. 1) resemble those of the pyocins found in other bacteria (cited above) and, more particularly (Fig. 1a, b, and c), tail structures of certain cholera phages (J. F. Vieu, P. Nicolle, and J. Gallut, Proc. Cholera Res. Symp., U.S. Government Printing Office, Washngton, D.C., 1965; and *unpublished observations*). The sheaths of some particles (Fig. 1c) are shorter and appear fragmented; yet, they still surround the core. This morphology reflects closely the degraded sheath appearance of *p*-chloromercuribenzoate-treated pyocins reported by Higerd et al. Figures 1d, e, and f show empty and partly filled cylindrical particles which differ morphologically from those in Fig. 1a, b, and c. Distances between the visible segmentations are not uniform, possibly indicating breaks which occur during drying of particles originally 3,000 A or more in length.

These observations suggest that the particles in Fig. 1a, b, and c may be identical with tail portions of defective *V. cholerae* phages. One or both particle types may be responsible for vibriocin activity. Evidence that pyocins and the protein moiety of *P. aeruginosa* endotoxin are chemically and immunologically related (J. Y. Homma and N. Suzuke, Japan. J. Exptl. Med. **31**:209, 1961) provokes conjecture that similar relations could exist for vibriocins. Such hypotheses lend special interest to these particles, which should be investigated in relation to their possible role in the disease process.

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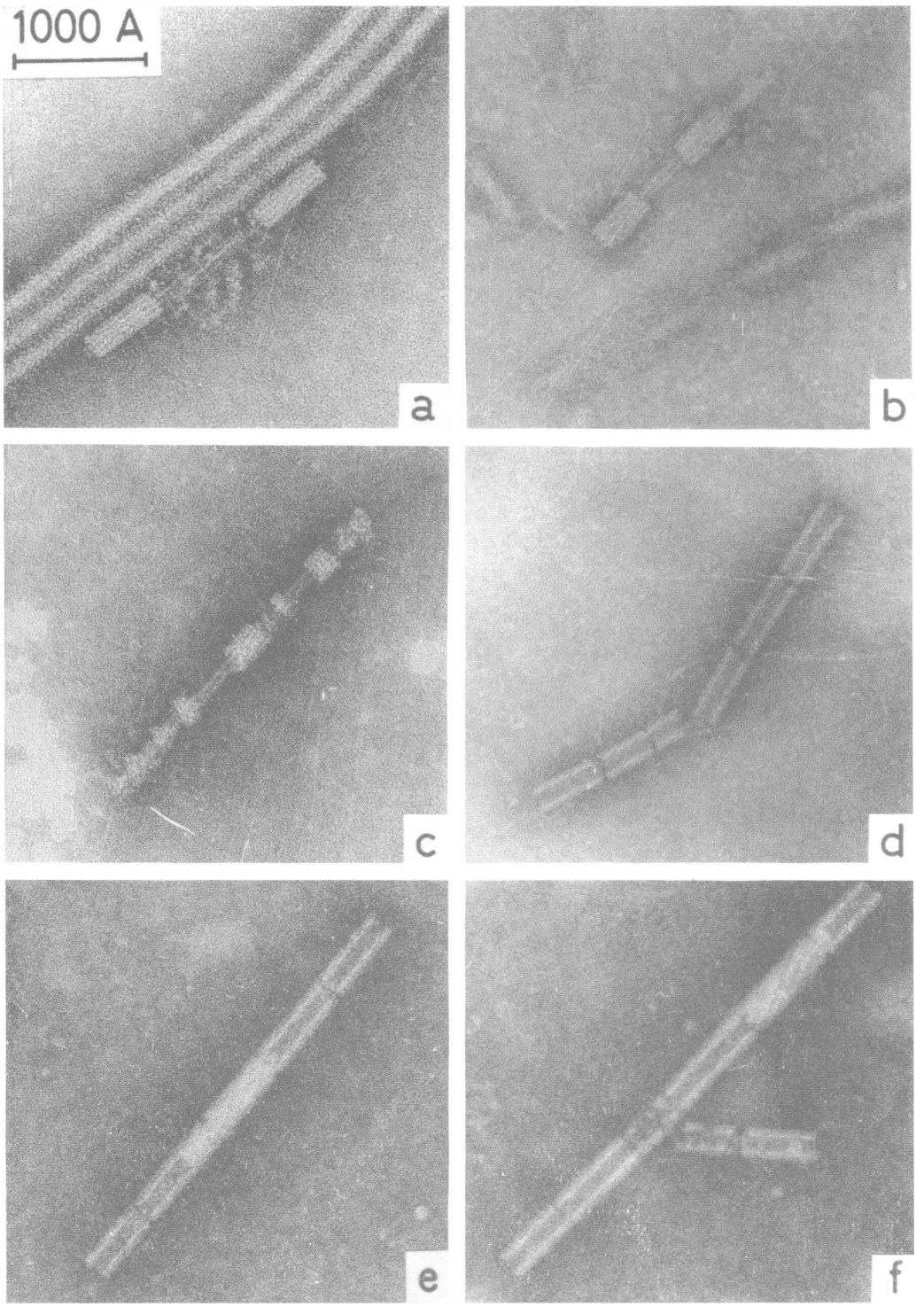

FIG. 1. *Particles associated with a bacteriocinogenic strain of Vibrio cholerae. Negatively stained with phosphotungstic acid.* × *200,000. (a, b, and c) Sheath diameter is about 220 A, core diameter about 100 A. (a and b) Two particles attached core-to-core, as frequently observed; flagellar portions lie nearby. (c) Particle with shorter sheaths that appear fragmented as compared to a and b. (d, e, and f) Particles which differ morphologically from a, b, and c; most are empty (d) or partly empty (e and f). Outer diameter of sheaths is 200 to 250 A, inner diameter about 100 A. Distances between segmentations vary between 400 and 1,300 A. The filled part in f shows a low-pitched helical structure. If one assumes that the particle is stained only on that side facing the carbon support, the helices are right-handed.*