

## ELECTRON MICROSCOPY OF FLAGELLATION OF *VIBRIO PARAHAEMOLYTICUS*

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The structure of the bacterial flagellum of which diameter lies far below the resolving power of the light microscope came to be observed by the electron microscope. Recently the flagellar structure consisting of the flagellum (central filament or core) and the surrounding sheath has been demonstrated in some bacteria (De Robertis and Franchi, 1951; van Iterson, 1953; Labow, and Mosley, 1955; Braun, 1956; Gordon, and Follett, 1962; Glauert, Kerridge, and Horne, 1963).

*Vibrio comma* and El Tor vibrio which cultured on the brain heart infusion agar or on the cooked meat media were found to have an appendage, undulating membrane, which was fairly observed by the negative staining method using the phosphotungstic acid (Ogasawara, and Kuno, 1963).

This paper reports on the existence of a similar appendage of *Vibrio parahaemolyticus* which could be observed by the negative staining method (Brenner and Horne, 1959).

### MATERIALS AND METHODS

*Strain used.* The No. 5 strain of *Vibrio parahaemolyticus* which belongs to the serotype O2. The strain was isolated from a food poisoning case about one year ago at Nagoya.

*Preparation of specimens.* The organism had been maintained on the cooked meat media (Eiken Co., Japan). The cells, cultured on the brain heart infusion agar (Eiken Co.) for about 16 hr, or on the cooked meat media for about 6 hr, were treated as the following method 1 or method 2.

1. Metal shadow-casting method. The cells were floated on the surface of a droplet of the fixing solution on the formvar-coated 100 mesh copper grid. After removing the excess of the fluid with filter paper the cells were dried at 37°C for several minutes and shadow-casted with the chromium metal at an angle of about 9:1. An 1 percent osmium tetroxide solution prepared with a veronal-acetate buffer (pH 7.2) was used for the fixation of the cells.

2. Negative contrast-staining method. The cells were floated on the surface of a droplet of PTA (2 percent phosphotungstic acid solution adjusted the pH to 7.0 with KOH, Brenner and Horne, 1959) on the formvar grid. The excess of the fluid was removed with filter paper. After inactivation by irradiation of the ultraviolet lamp for 3 minutes, the cells were shadow-casted with the metal. The metalshadow-casting procedure was omitted in some cases.

An Akashi Model TRS-50 E-1 instrument was used. The specimens prepared by the method 1 or method 2 were examined in the instrument. The micrographs were taken at an initial magnification of 8 000 to 10 000 $\times$  and enlarged photographically.

#### RESULTS

The vibrios appeared usually as straight, or slightly curved rods which vary from 1.7  $\mu$  to 2.6  $\mu$  in length and from 0.6  $\mu$  to 1.0  $\mu$  in width when stained negatively with PTA. The cell was equipped with a flagellum which swelled here and there as shown in Fig. 1.

##### *The membraneous structure as an appendage of the flagellum*

It was observed that the flagellum of the vibrio cultured on the brain heart infusion agar was equipped with a membraneous structure which was stained negatively with PTA and was transparent for the electron as shown in Fig. 2,

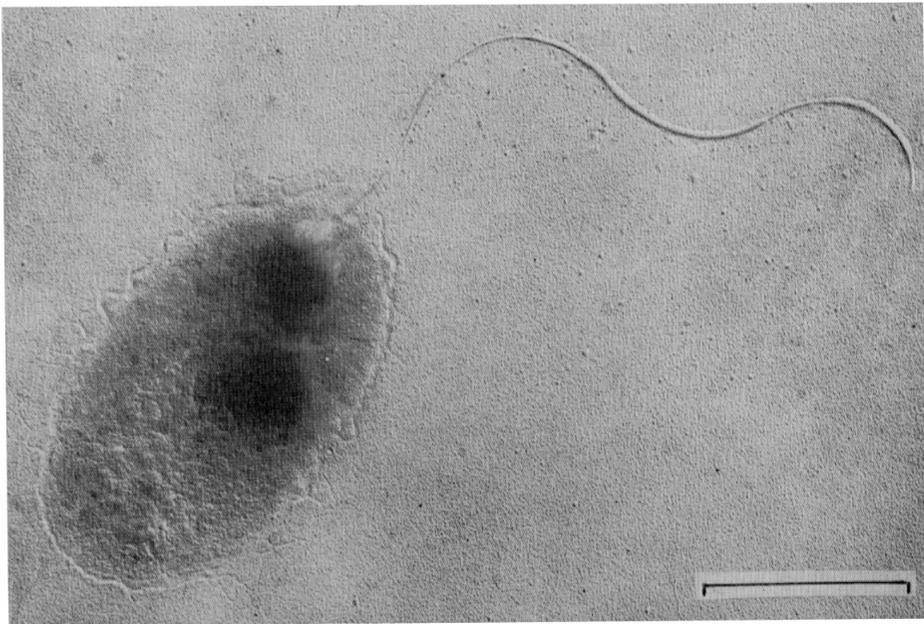


FIG. 1. Electron micrograph of *V. parahaemolyticus*. Cultured on brain heart infusion agar. Shadow-casted with chromium. Bar of each fig. represents 1  $\mu$ .

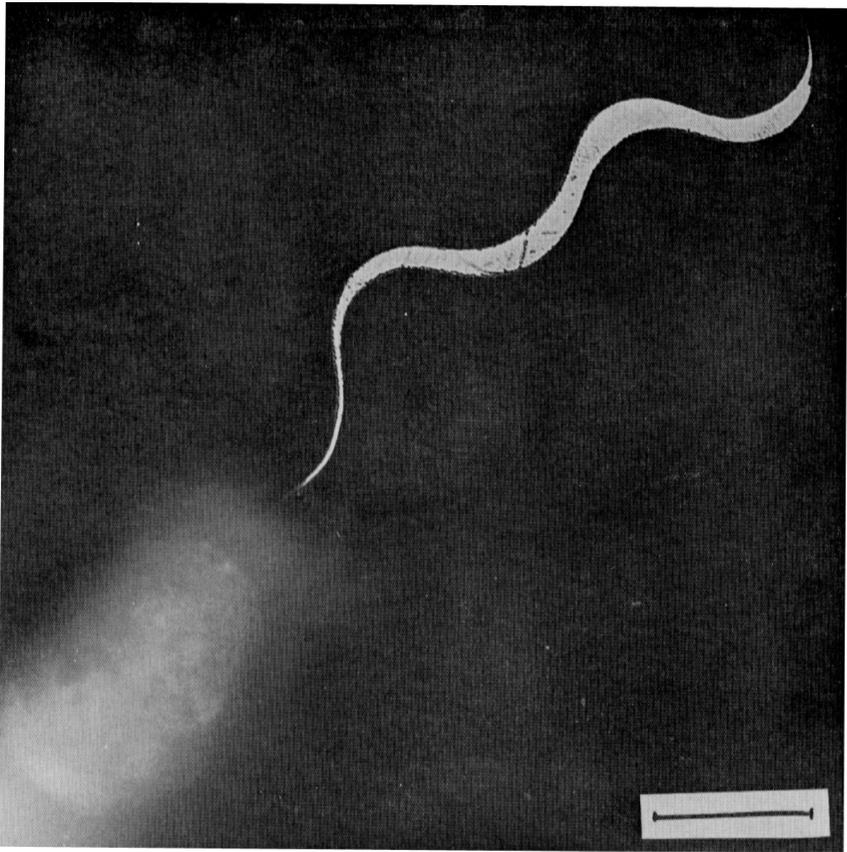


FIG. 2. Electron micrograph of *V. parahaemolyticus*. Cultured on brain heart infusion agar. Stained negatively with PTA and shadow-casted with chromium.

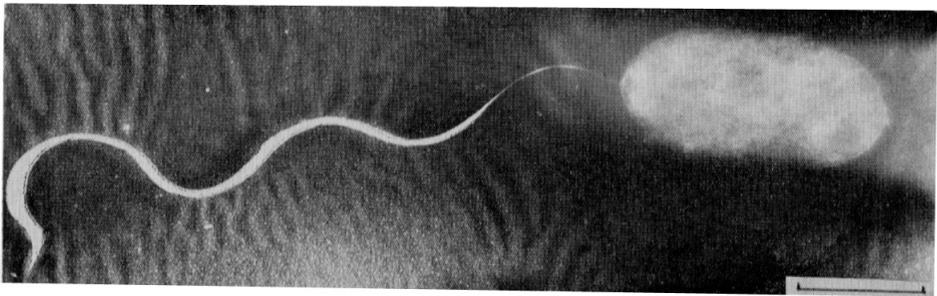


FIG. 3. Electron micrograph of *V. parahaemolyticus*. Cultured on brain heart infusion agar. Stained negatively with PTA and shadow-casted with chromium.

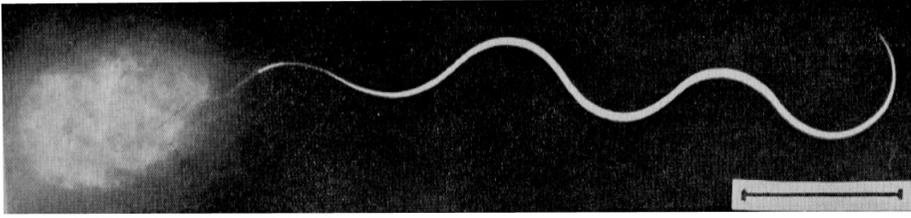


FIG. 4. Electron micrograph of *V. parahaemolyticus*. Cultured on brain heart infusion agar. Stained negatively with PTA and shadow-casted with chromium.

3,4. The thickness of the membranous structure was very thin and became narrowed at the proximal and the distal portion of the flagellum. The membranous structure was contractile and seemed to change the width according to the flagellar movement. The width of the membranous structure was sometimes more than  $260\text{ m}\mu$ .

*The covering of the flagellum.*

Many micrographs taken by us suggested that the flagellum of the vibrio

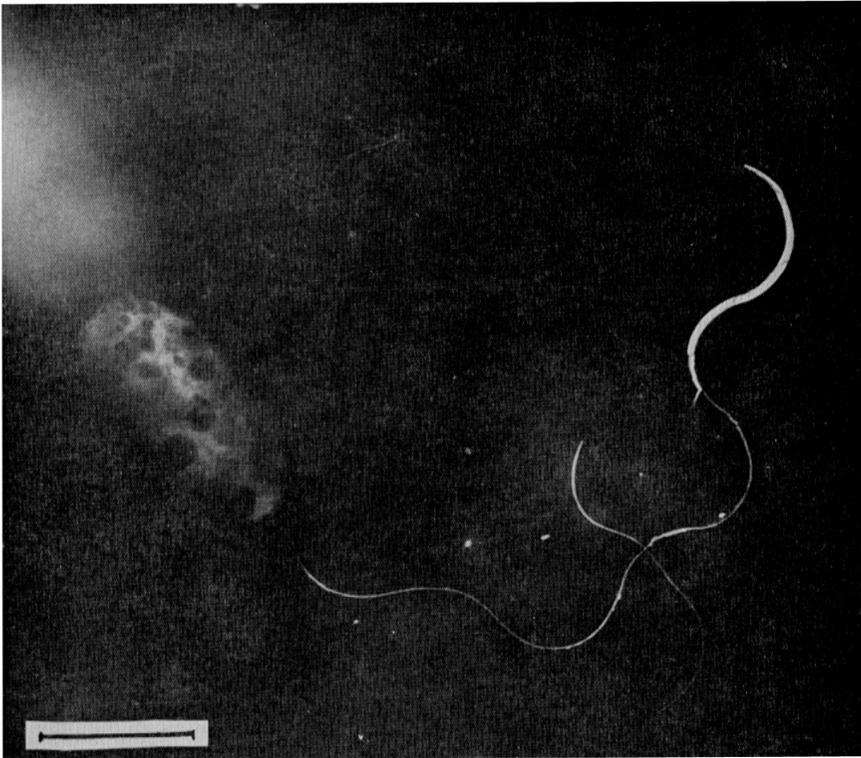


FIG. 5. Electron micrograph of *V. parahaemolyticus*. Cultured on brain heart infusion agar. Stained negatively with PTA. Distal portion of flagellum is covered with sheath.



FIG. 6. Electron micrograph of *V. parahaemolyticus*. Cultured on brain heart infusion agar. Shadow-casted with chromium. Stiff wire-like flagellar segments are shown.

is originally covered with a sheath or a membranous structure. The amplitude of the flagellum which was separated from the sheath or the membranous structure was about  $14 \pm 1 \text{ m}\mu$  as shown in Fig. 5, 6. The wavelength of the naked flagella was fairly constant and was estimated as  $1.9 \mu$ . The width of the sheath was not necessarily uniform and swelled at several portions as shown in Fig. 1. Fig. 7 indicated an expanded flagellar structure which seemed to be covered almost completely with the sheath. Distinct boundary between flagellar sheath and the membranous structure was not observed.

*The piliform appendages of V. parahaemolyticus*

The piliform appendages (fimbriae) growing from the surface layer of the vibrio were shown in Fig. 8. They were more fine and more irregularly bent than the flagellum and have a diameter of about 7 to  $8 \text{ m}\mu$  when shadowcasted. However, they looked like tree branches when stained negatively with PTA as shown in Fig. 9.

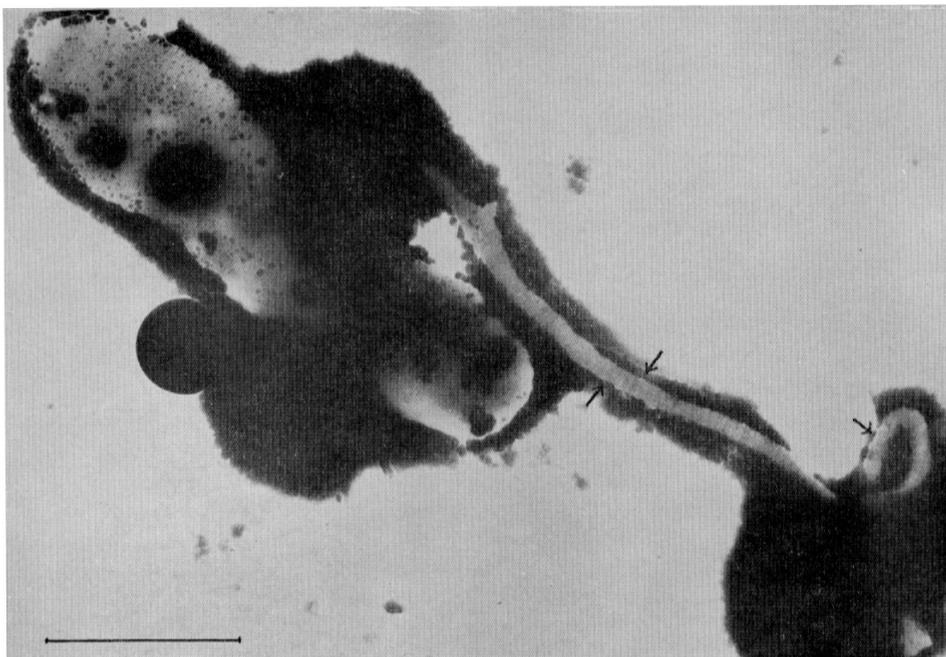


FIG. 7. Electron micrograph of *V. parahaemolyticus*. Cultured on cooked meat media. Stained negatively with PTA. Fixation was omitted. Arrows indicate a flagellum. Flagellar amplitude exceeds 120 m $\mu$ .

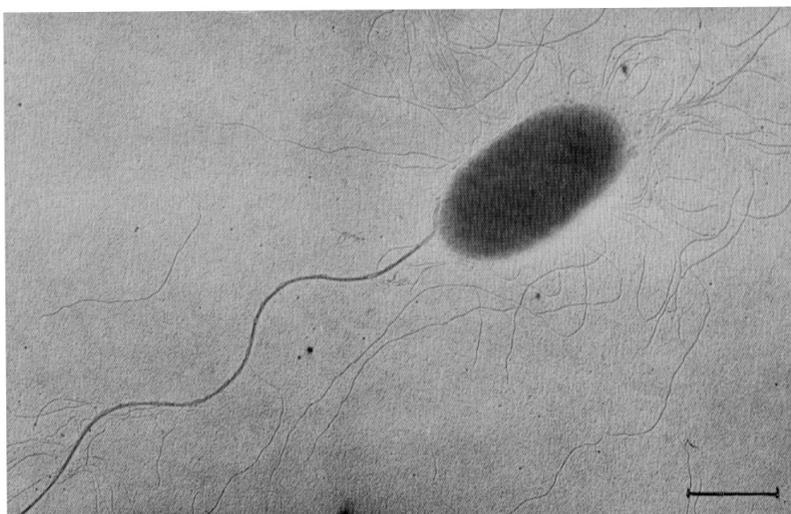


FIG. 8. Electron micrograph of *V. parahaemolyticus*. Cultured on brain heart infusion agar. Shadow-casted with chromium.

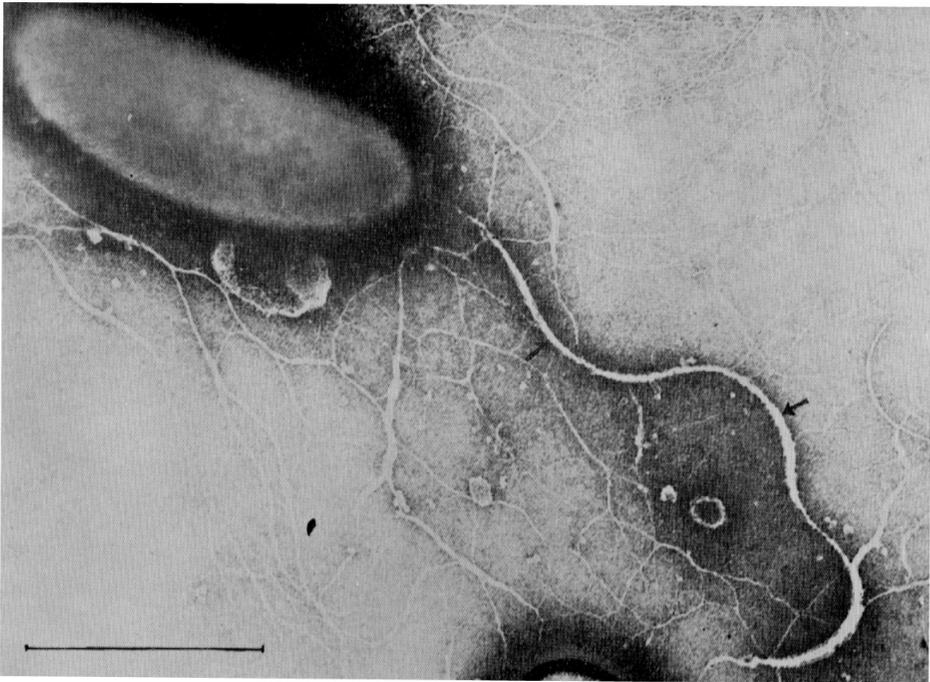


FIG. 9. Electron micrograph of *V. parahaemolyticus*. Cultured on brain heart infusion agar. Stained negatively with PTA. Fixation was omitted. Arrows indicate a flagellum.

#### DISCUSSION

Most bacteriologists are of the opinion that the bacterial flagella are the motor organs although several questions on the mechanism of flagellar propulsion remain unsolved (Weibull, 1960). It is admittedly, however, difficult to understand how the action of the tenuous flagella can propel rapidly the much larger and heavier bacterial soma through a medium of the viscosity of water, and one eminent British physicist, on seeing some of the first electron micrographs of flagella, declared it to be impossible (Robinow, 1960). Generally, bacterial flagellum has a helical shape and is believed to behave like a turning corkscrew when it moves. The movements of *V. parahaemolyticus*, like other aquatic vibrios, are very rapid and are not restricted within one plane. In addition, rapid semisomersaults or somersaults are often performed by the vibrio. However, it is difficult to explain how the actions of the stiff wire-shaped fine flagellum alone can perform such rapid and complicated movements. If rapid propulsion is required, the flagellum is not necessarily an effective motor organ because of its fine amplitude. Generally, the torque for rapid propulsion may be produced more effectively by an oarshaped organ than a rod-shaped one.

A membranous structure connected with the flagellum or the flagellar

sheath has been demonstrated in the vibrio as shown in several figures. Similar appendage was also observed in *V. comma* and El Tor vibrio (Ogasawara and Kuno, 1963). The membranous structure seemed to be a propulsion organ. The flagellar propulsion of *V. parahaemolyticus* may be strongly strengthened by the torque produced possibly by the undulating movement of the membranous structure. The rapid semisomersaults or even the somersaults seem to be possible by the propulsive torque produced by this action. The action of the membranous structure may be performed passively by the flagellar movement.

Occasionally, the flagellum of *V. parahaemolyticus* appeared resembling a stiff wire having an amplitude of about 14  $m\mu$ , and its wavelength was fairly constant when shadow-casted with the metal. The formation of this type of flagellar curvature may be due partly to distorsion during drying and partly to other extraneous factors in course of the preparation of the specimen. We feel that the central filament (De Robertis and Franchi, 1951) may be no more than the naked flagellum itself on account of its size and structure.

A sheath-like structure was found on the flagellum of *Vibrio metchnikovii*, after the cells had been partly autolysed (van Iterson, 1953) and also on the flagellum of *V. comma* and of El Tor vibrio (Ogasawara and Kuno, 1963). The flagellum which was covered with the membranous structure resemble a serpentine cord as shown in Fig. 2.

The membranous structure and the sheath of the flagellum seemed to be continuous and inseparable. It seemed to be that the membranous structure contracts so tightly that it disappears outwardly when the cell is in a nonmotile state.

That is to say that the membranous structure may be a developed projection of the flagellar sheath at moving. The relation between the membranous structure and the flagellar sheath may be illustrated schematically in Fig. 10. Thus, we would propose to designate the appendage as "undulating membrane" which is composed of the membranous structure and the flagellar sheath.

The bacteria equipped with the flagellation may be divided at least to two groups: the cells of one group have the undulating membrane and the

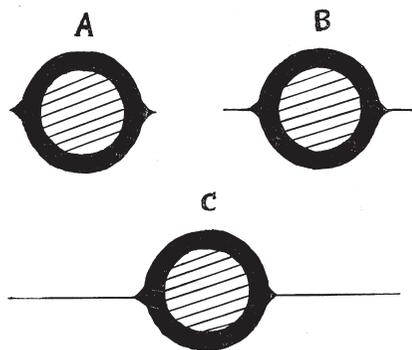


FIG. 10. Schematic illustration of flagellation of *Vibrio parahaemolyticus*.

Transverse sections of flagellation; flagellum (stripes), undulating membrane (black). Non-motile state (A), States of flagellar propulsion (B, C).

others have not the membrane. The flagellar movement of the former may be more rapid and more complicated than that of the latter.

Bacterial pili (fimbriae) are only visible in the electron microscope. It has generally been observed that the pili of different species of bacteria have respectively an uniform diameter when shadow-casted. The piliform appendages of *V. parahaemolyticus* had also an uniform diameter of about 7 to 8 m $\mu$  when shadow-casted. However, if the specimen was stained negatively with PTA, the piliform appendages looked like tree branches of which diameter was not uniform as shown in Fig. 9. These observations suggest that the piliform appendages of the vibrio may be no more than the secretion from the surface layers of the cell as shown in Fig. 1.

Further studies on the undulating membrane and the piliform appendage of *V. parahaemolyticus* are waiting.

#### SUMMARY

*Vibrio parahaemolyticus* (serotype O 2; No. 5 strain) which cultured on the brain heart infusion agar or on the cooked meat media was found to have a new flagellar appendage, designated as undulating membrane, when stained negatively with the phosphotungstic acid. The electron transparent undulating membrane had narrowed at the proximal and the distal portions of the flagellum. The thickness of the undulating membrane was too thin to cast the shadow. The undulating membrane seemed to be contractile and to change the shape according to the flagellar movements.

The amplitude (diameter) of the flagellum was about 14 m $\mu$ . The apparently thicker flagellum was covered with a sheath which seemed to be variable and to develop the membraneous projection at movements.

It was discussed on the relation between the vibrio movements and the flagellar appendage which is composed of the flagellar sheath with the membraneous structure.

Bacterial piliform appendages (fimbriae) were found on the surface of *V. parahaemolyticus*. The shadow-casted shape and the size of the piliform appendages were not coincided with that which stained negatively with the phosphotungstic acid.

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