ELECTRON MICROSCOPY OF STRUCTURE OF FLAGELLUM OF *VIBRIO COMMA*

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The development and use of the electron microscope has made it possible to observe structures which were invisible with the light microscope. Thus the structure of the bacterial flagella, whose diameter lies far below the resolving power of the light microscope, came to be observed, and recently the flagellar structure consisting of the flagellum (central filament or core) and the surrounding sheath has been observed in some bacteria (De Robertis and Franchi, 1951; van Iterson, 1953; Labaw, and Mosley, 1955; Braun, 1956, Gordon and Follett, 1962).

This paper reports on a sheath-like structure and its probable function of the flagellum of cholera vibrios.

MATERIALS AND METHODS

Strain used. *Vibrio comma*, the Inaba strain; El Tor vibrio, the Nobechi strain. The organisms had been maintained on the cooked meat media (Eiken Co., Japan). The cells, cultured on the brain heart infusion agar (Eiken Co., Japan.) for about 16 hr, or on the cooked meat media for about 6 hr, were washed with saline solution by centrifugation and treated as follows.

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 fluid with filter paper the specimen was dried at 37 C for 5 minutes and shadow-casted with the chromium metal at an angle of about 9 : 1. One percent osmium tetroxide solution prepared with a veronal-acetate buffer (pH 7.2) was used for the fixation of the cells.

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

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An Akashi Model TRS-50 E-1 instrument was used and the micrographs were taken at an initial magnification of 8 000 to 10 000× and enlarged photographically.

RESULTS

The membraneons structure as an appendage of the vibrio

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. 1, 2, 3 and 4. A similar membraneous structure was also observed on the vibrio which was cultured on the liquid media, the cooked meat media, as shown in Fig. 5. The membraneous structure was very thin and had a ribbon-like shape. The flagellum was situated likely to wrap the flagellum. The shape of the flagellum equipped with the membraneous structure was observed more dististinctly when shadow-casted with the metal after the staining with PTA. The membraneous structure became narrowed at the proximal and the distal portions of the flagellum. The membraneous structure seemed easily to contractile or to extend with the motion of the flagellum; and the width of the membraneous structure was sometimes more than 260 mμ. The spontaneous folding at a portion of the membraneous structure suggested how thin the membrane is, as shown in Fig. 6. The membraneous structure was too thin to cast the shadow. Therefore, the membraneous structure was not demonstrated by the shadow-casting method. However, the figure which suggests the presence of the membraneous structure was obtained as shown in Fig. 7.

Generally, the cultivation of the vibrios on the solid media was more suitable than on the liquid media for the detection of the membraneous structure. Especially, the brain heart infusion agar was better than the nutrient agar for this purpose. When the serial subcultures on the brain heart infusion agar resulted in the poor formation of the membraneous structure, the cultures should be again returned to the cooked meat media. The formation of the membraneous structure became also very poor when the vibrios had been serially subcultured on the broth or the peptone water.

The flagellum and its sheath

Two types of the flagella, the thick type and the fine one, were occasionally observed in a specimen as shown in Fig. 8. The amplitude (diameter) of the thick flagella was about 28±2 mμ and that of the fine was about 14±1 mμ. The figure suggested that the difference of the amplitude may be due to the existence or lacking of the sheath covering. It seemed to be that the vibrio is originally equipped with a flagellum which is covered with a sheath as shown in Fig. 9 and may sometimes lose the sheath as shown in Fig. 10. Partial swellings of the thick flagellum were sometimes observed as shown in Fig. 9. Two vibrio cells equipped respectively with each flagellum were shown in Fig. 11. One cell was equipped with a fine flagellum and the other

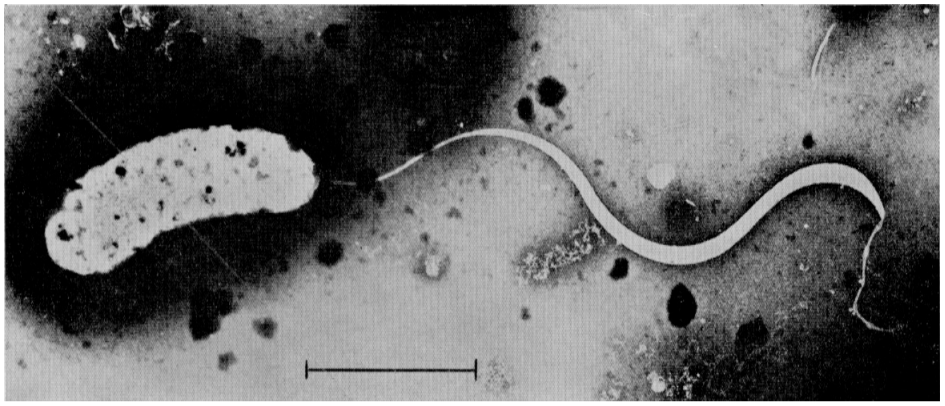

FIG. 1. Electron micrograph of *Vibrio comma*. Cultured on brain heart infusion agar. Stained negatively with PTA. The folded distal portion of membraneous structure is enlarged in Fig. 6. Bar of each illustration indicates 1 μ.

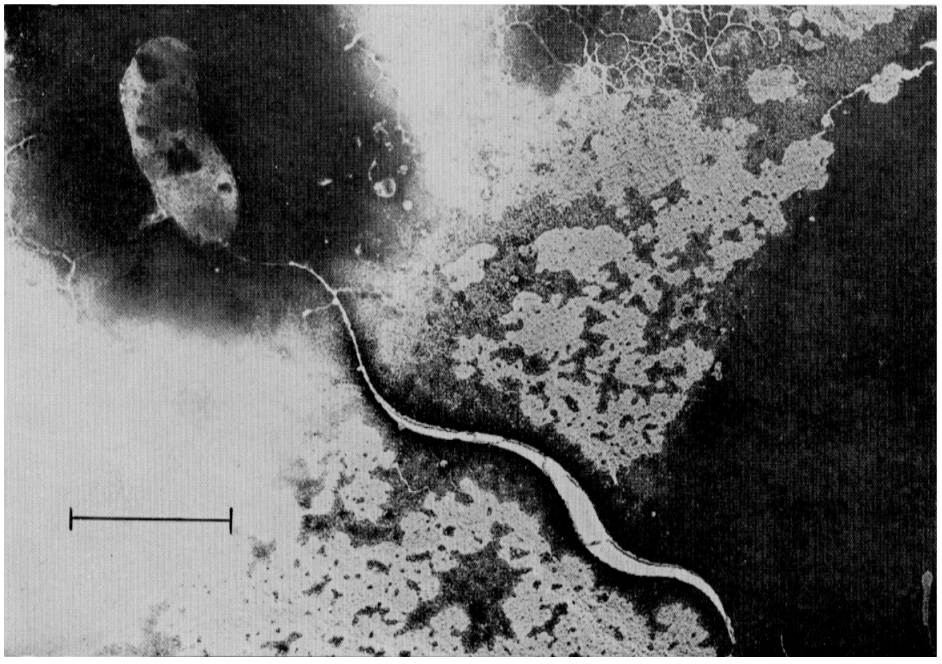

FIG. 2. Electron micrograph of El Tor vibrio. Cultured on brain heart infusion agar. Stained negatively with PTA and shadow-casted with chromium.

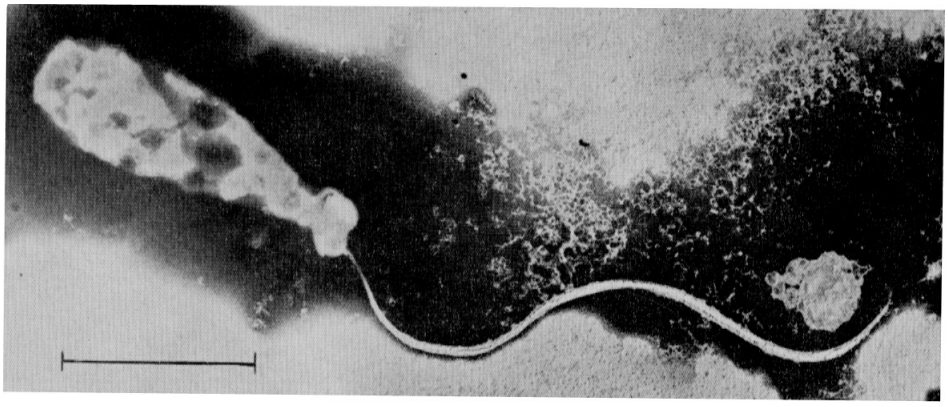

FIG. 3

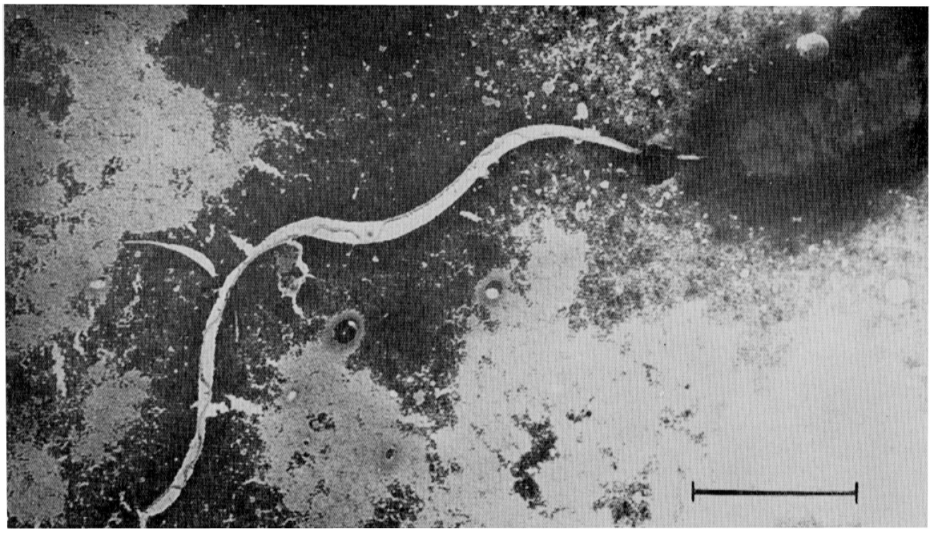

FIG. 4

FIG. 3 and FIG. 4. Electron micrograph of *Vibrio comma*. Culrured on brain heart infusion agar. Stained with PTA and shadow-casted with chromium.

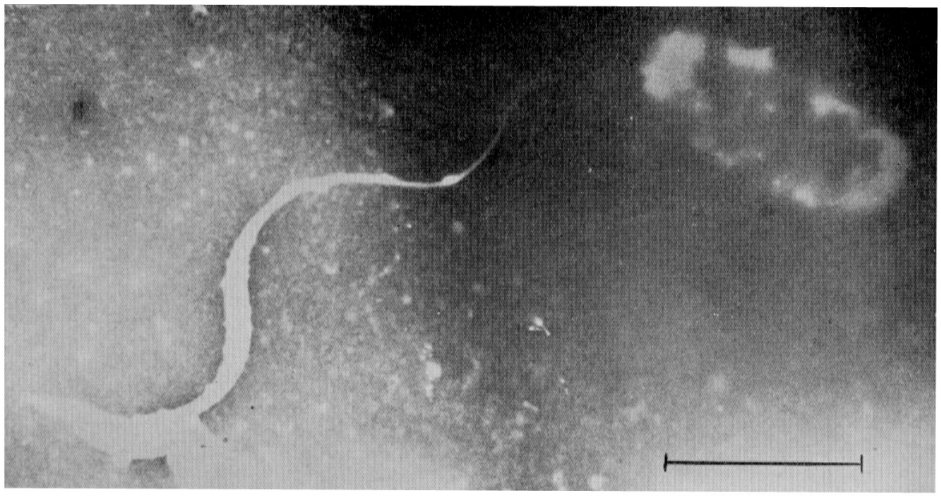

FIG. 5. Electron micrograph of *Vibrio comma*. Cultured on cooked meat media. Stained negatively with PTA.

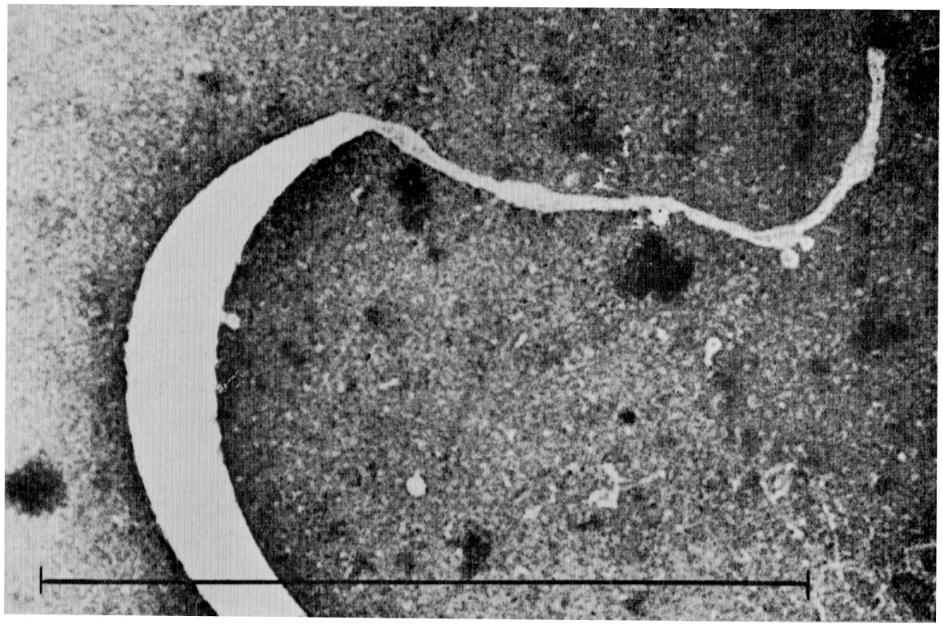

FIG. 6. Electron micrograph of *Vibrio comma*. Cultured on brain heart infusion agar. Stained negatively with PTA. Folded distal portion of membraneous structure is shown.

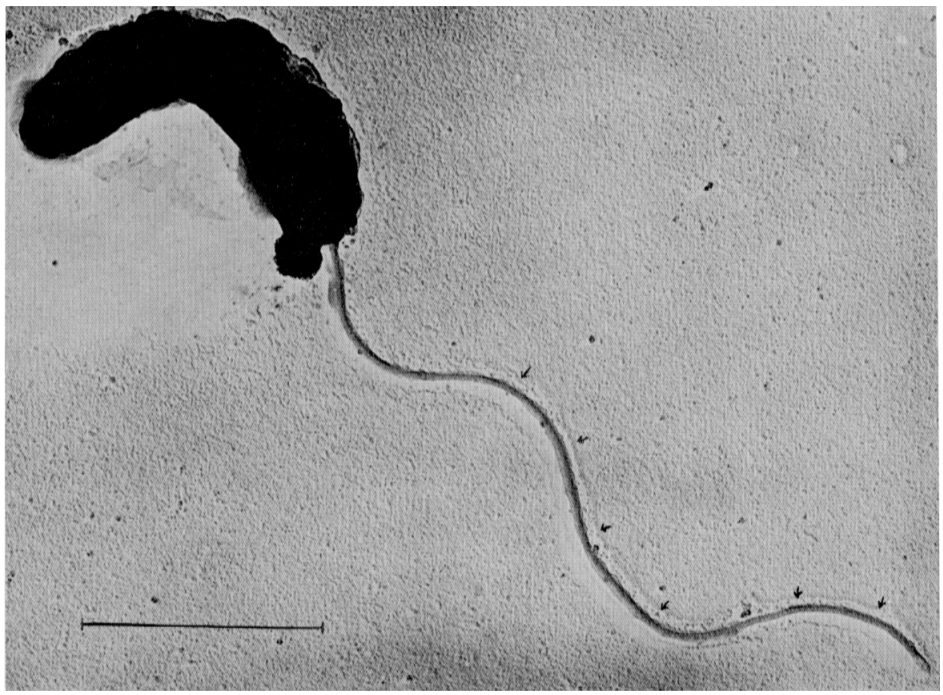

FIG. 7. Electron micrograph of *Vibrio comma*. Cultured on brain heart infusion agar. Shadow-casted with chromium. Arrows indicate possible existence of the membraneous structure.

with a thick one. Partially enlarged figure of these flagella was shown in Fig. 12. In this figure, it seemed to be that the thick flagellum was composed of a central filament and an outer layer. The amplitude of the central filament was about 14 mμ and coincided with that of the thin flagellum of the other cell. It seemed to be that the central filament may be the flagellum iteslf and the outer layer may be the sheath which surround the flagellum. No photograph which suggests distinct discontinuity of the sheath and the membraneous structure was obtained.

DISCUSSION

Most bacteriologists have been of the opinion that the bacterial flagella are the motor organs in spite of several unsolved fundamental questions. Several theories on the mechanism of the flagellar propulsion have been discussed (Weibull, 1960). It is admittedly, however, difficult to understand at first glance how the action of the tenuous flagella can propel rapidly the much larger and heavier bacteria 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). It is generally

FIG. 8. Electron micrograph of flagellar fragments of *Vibrio comma*. Cultured on brain heart infusion agar. Shadow-casted with chromium. Two types of flagella, sheathed and unsheathed, are shown.

believed that the flagellum has a helical shape and looks like a turning corkscrew when it moves slowly. The movement of *Vibrio comma* and El Tor vibrio, like other vibrios, are very rapid and are not restricted within one plane. In addition, rapid semisomersaults or even somersaults are often performed by these bacteria. However, it is difficult to explain how the action of the wire-shaped flagellum alone can perform such rapid and complicated movements. When rapid propulsion is required, the flagellum, if alone, is not necessarily an effective motor organ because of its shape. Generally, the torque for rapid propulsion may be produced more effectively by an oar-shaped organ than a rod-shaped one.

A membraneous structure connected with the flagellum or the flagellar sheath has been pointed out in some vibrios as shown in several figures. Similar structure has also been observed in *Vibrio parahaemolyticus* (unpublished). It is probable that the other aquatic vibrios may have the same structure. However, similar structure was not observed on *Pseudomanas aeruginosa*, *Salmonella typhosa* and the Reiter treponema. The membraneous structure seems to act as a propulsion organ. The flagellar propulsion of the vibrios may be strongly strengthend by the torque possibly produced by the action of the membraneous structure. The rapid semisomersaults or even the somersaults seem to be possible by the propulsive torque produced probably by this action.

It had been observed that some protozoa, as trichomonases or trypanosomas, have an analogous undulating membrane of which structure is alike but not identical with that of the vibrios. The analogy suggests that there may be a evolutional relationship between vibrios and protozoa in their ancestors.

Occasionally, the flagellum of *V. comma* and of El Tor vibrio appear

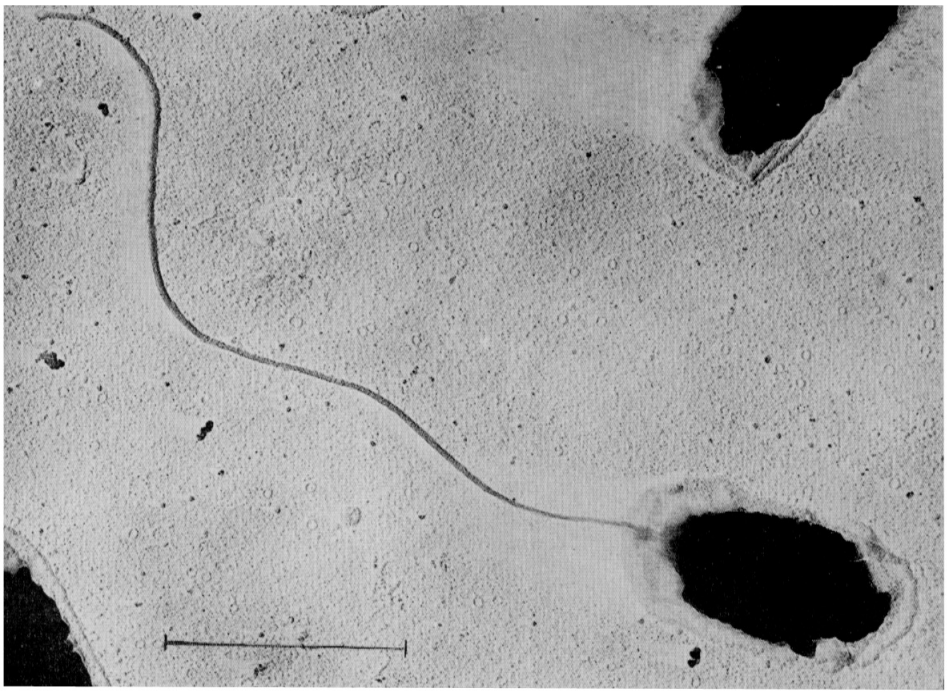

FIG. 9. Electron micrograph of *Vibrio comma*. Cultured on brain heart infusion agar. Shadow-casted with chromium.

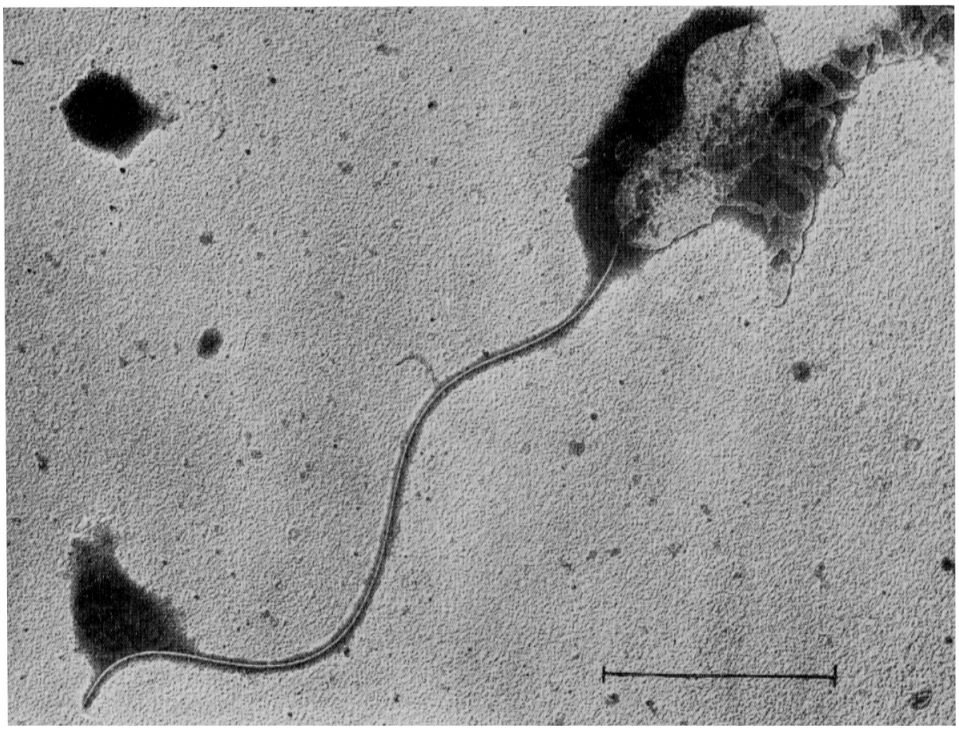

FIG. 10. Electron micrograph of *Vibrio comma*. Cultured on brain heart infusion agar. Stained negatively with PTA and shadow-casted with chromium.

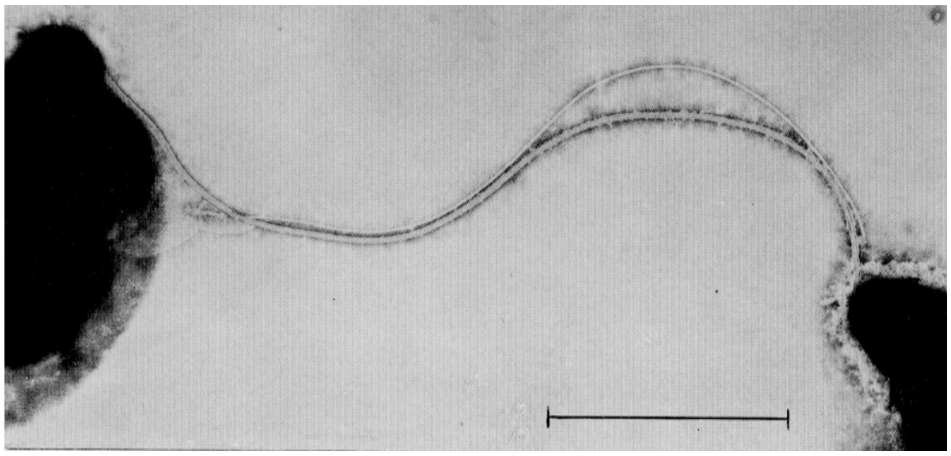

FIG. 11. Electron micrograph of *Vibrio comma*. Cultured on brain heart infusion agar. Stained negatively with PTA.

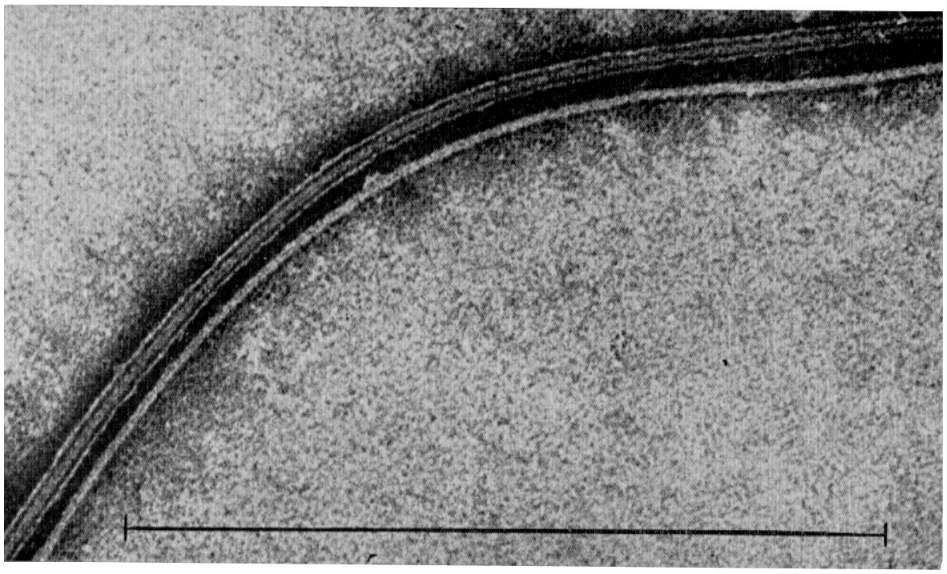

FIG. 12. Electron micrograph of flagella of *Vibrio comma*. Partially enlarged figure of Fig. 11.

resembling a stiff wire and its wavelength is fairly constant (Leifson, 1960). The formation of this type of the flagellar curvature may be due partly to distorsion during drying and partly to other extraneous factors. A sheath-like structure was found on the flagellum of *V. metchnikovii*, after the cell had been partly autolysed (van Iterson, 1953). *V. comma* and El Tor vibrio have also been found to have a sheath structure surrounding the flagellum. The

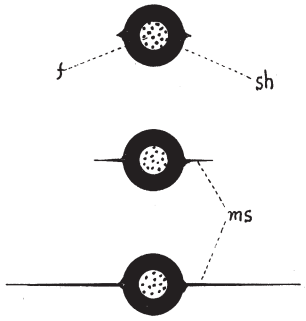

FIG. 13. Schema of transversed section of possible forms of vibrio flagellation: flagellum (f); sheath (sh); membraneous structure (ms).

flagellum which covered with the sheath resemble a serpentine cord which winds irregularly and do not resemble a stiff wire when stained negatively with PTA as shown in Fig. 4.

The membraneous structure and the sheath of the flagellum seemed to be continuous and inseparable. It seemed to be that the membraneous structure contracts so tightly that it disappears outwardly when the cell is in a complete resting state. Thus, we feel that the membraneous structure may be a variable projection of the flagellar sheath as illustrated schematically in Fig. 13. If so, we would propose to designate the appendage as "undulating membrane" which consist of the membraneous structure and the flagellar sheath. Further observations on the undulating membrane are expected.

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