

Scanning Electron Microscopy on the Spiral Direction of the Cell Bodies of Some Spirochetes

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ABSTRACT

Three strains of spirochetes, *Borrelia duttonii*, *Treponema pallidum*, and *Treponema phagedenis*, were observed under a scanning electron microscope, and their spiral directions were described. The right-handed turn in *B. duttonii*, the left-handed turn in *T. pallidum* were evidenced. The appearance of both types of spiral direction in *T. phagedenis* was recognized and discussed.

Key Words: scanning electron microscopy ; spiral direction ; spirochetes

INTRODUCTION

Although the spirochetes have a helical cell body, their spiral directions have not been exactly determined even by an electron microscope, and their morphological features for that matter were not noticed in Bergey's Manual of Determinative Bacteriology¹⁾. Recently, the theoretical considerations and the fundamental conditions of experimental studies on spiral direction of the cell bodies of helical bacteria were described by Yoshii²⁾. Consequently, five serotype strains of the genus *Leptospira* were observed under scanning electron microscopy (SEM), and their cell bodies showed a right-handed turn without exception³⁾. Some researchers^{4,5)} also noticed on the spiral direction of spirochetal cell bodies. The present paper describes the spiral directions of three strains of spirochetes examined under SEM as a preliminary report.

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MATERIALS AND METHODS

Strains of spirochetes

1) *Borrelia duttonii* is the Kyoto University strain, which has been maintained through mouse passage by intraperitoneal inoculation in our laboratory for several years.

2) *Treponema pallidum* is the Nichols strain, which has been maintained through rabbit passage by testicle inoculation in our laboratory for several years.

3) *Treponema phagedenis* biotype Reiter (Reiter treponeme), which has been maintained *in vitro* in a culture medium⁶⁾ for ten years.

Specimen preparation for SEM

Spirochetal cells, harvested from the infected animals or culture media⁶⁾, were washed 3 times in saline solution by centrifugation at 3,300xg and treated by double fixation with 2% glutaraldehyde and 1% osmic acid. After fixation, the materials were washed again, dehydrated through the acetone series, and then dried with the critical point drying technique (CPD) by the small envelope method⁷⁾. The dried cells on the glass slide or filter paper were coated with Au-Pd alloy in an evaporator.

Scanning electron microscopy

The JSM-F7 type of SEM instrument was used at 13kV for the present study. More than 200 cells of each strain were usually observed under SEM with stereographic technique. The final decision on the spiral direction was made under the previously described criteria²⁾ after observing the photographs carefully printed.

Theoretical considerations and fundamental conditions for studies

The definition of spiral direction was based on the proposal of Kihara⁸⁾; the helix of a drill twist possesses the right-handed turn. As in the previous paper²⁾, the SEM instrument in our laboratory was preconfirmed to demonstrate the orthogonal image and not the mirror image.

OBSERVATIONS

Borrelia duttonii: The SEM image of *B. duttonii* is demonstrated in Fig. 1. The spiral direction of cell bodies of this spirochete always showed the right-handed turn.

Treponema pallidum: The SEM images of *T. pallidum* (Nichols strain) are displayed in Figs. 2-3. The spiral direction of cell bodies of this spirochete always showed the left-handed turn.

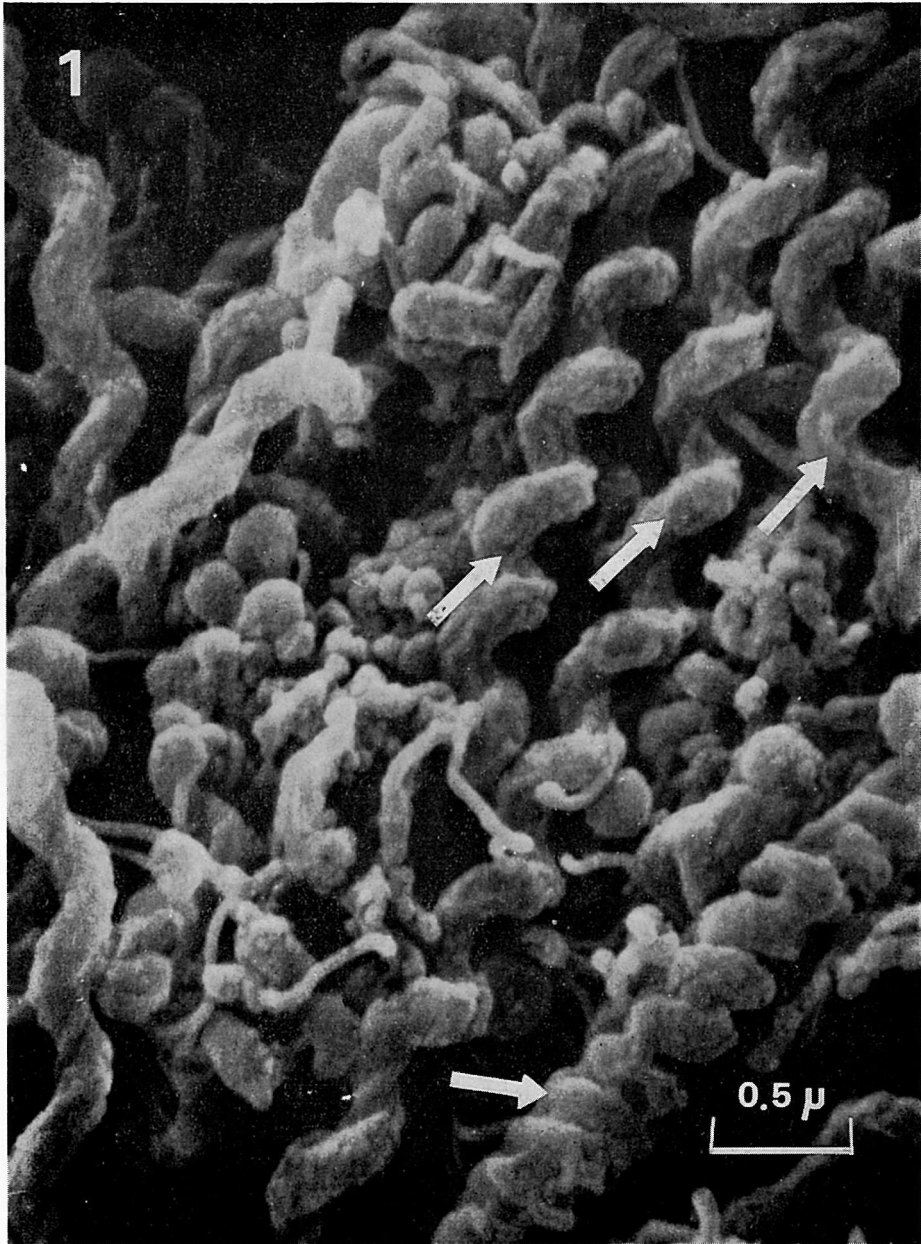


Fig. 1 *Borrelia duttonii*, showing the definite right-handed turn in each cell body (Arrows).
Magnification: $\times 38,000$

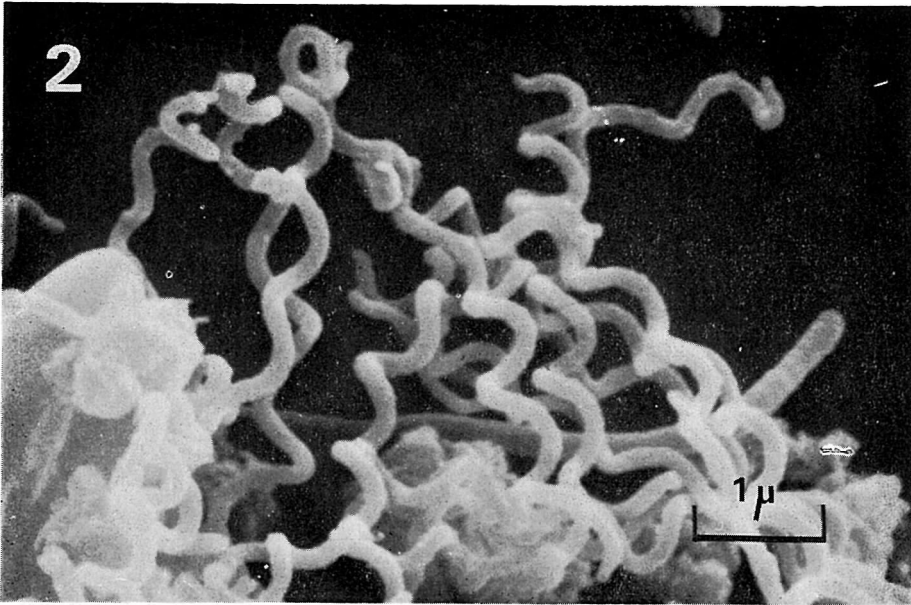


Fig. 2 *Treponema pallidum*, Nichols strain. Many cells with the left-handed turn can be seen. Magnification : $\times 17,500$

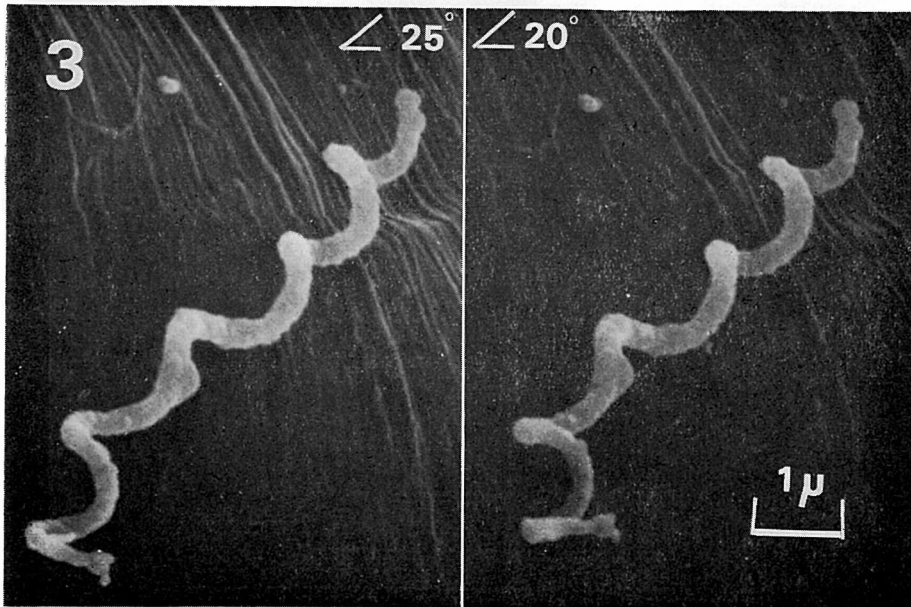


Fig. 3 *T. pallidum*, Nichols strain, illustrating two graphs of a cell with stereographic views from different angles. The left-handed turn of cell body can be seen definitely. Magnification : $\times 11,500$

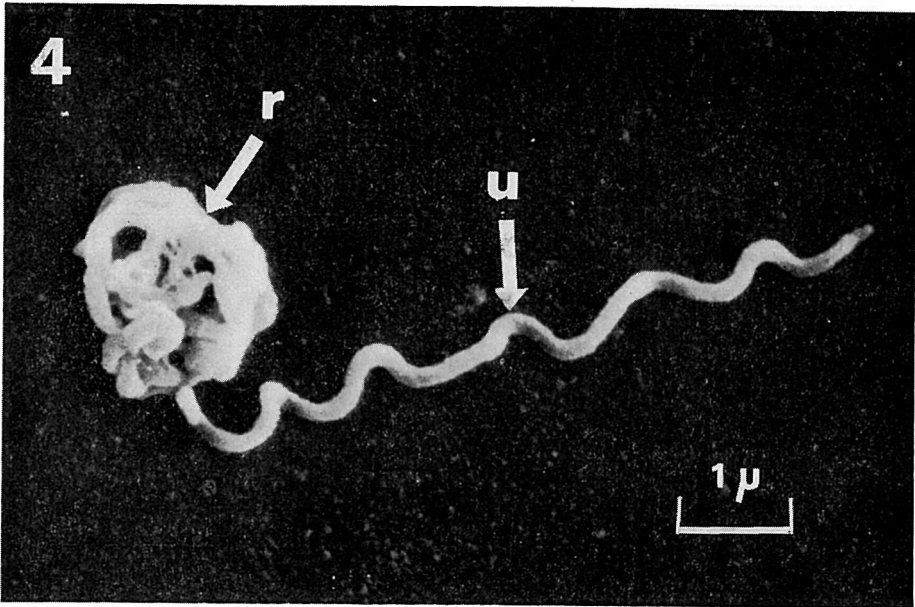


Fig. 4 *Treponema phagedenis* biotype Reiter. A rounded form (r) and a usual cell (u) can be seen. The latter shows the left-handed turn of cell body. Magnification: $\times 15,000$

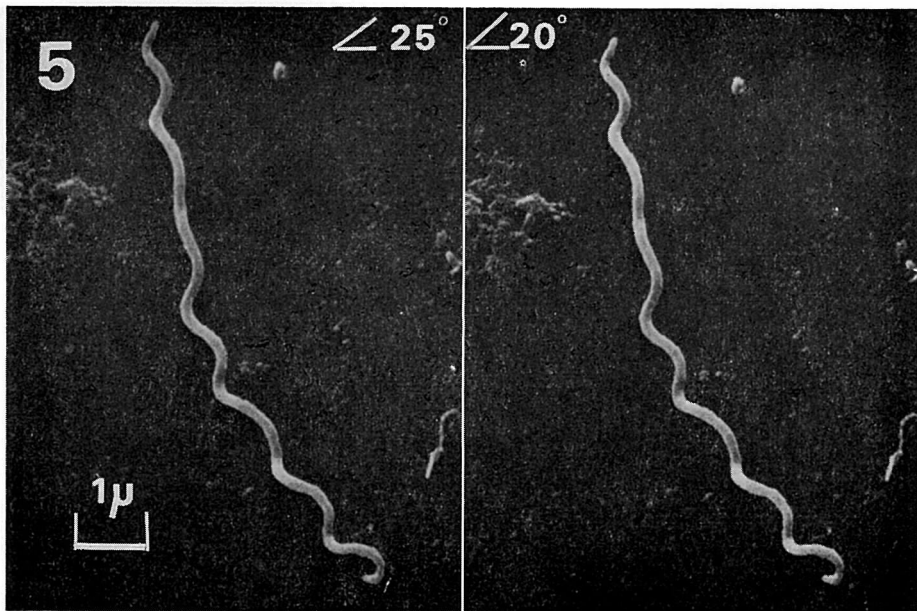


Fig. 5 *T. phagedenis* biotype Reiter, illustrating two graphs of a cell with stereographic views from different angles. The left-handed turn of cell body can be clearly seen. Magnification: $\times 9,000$

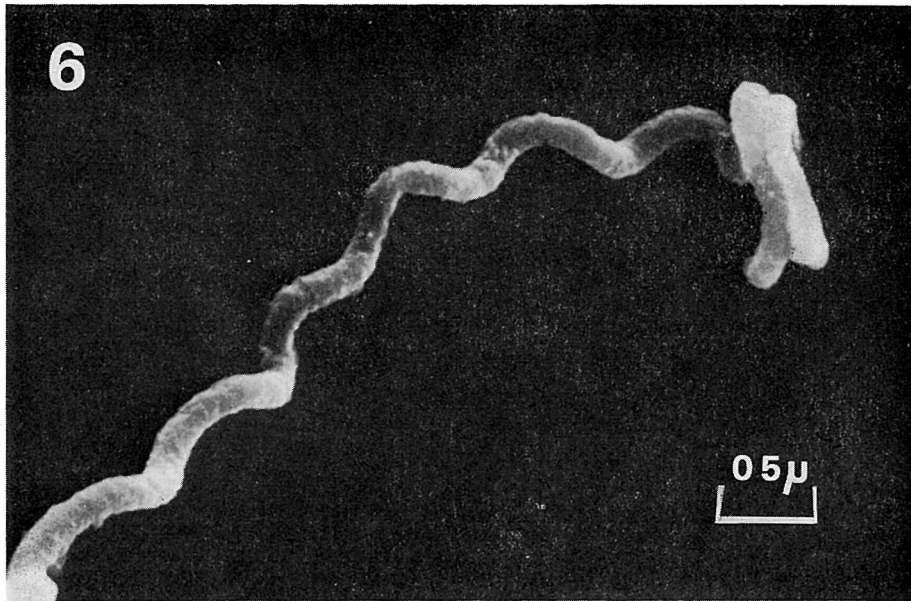


Fig. 6 *T. phagedenis* biotype Reiter, showing a cell body with the right-handed turn. Magnification : $\times 26,000$

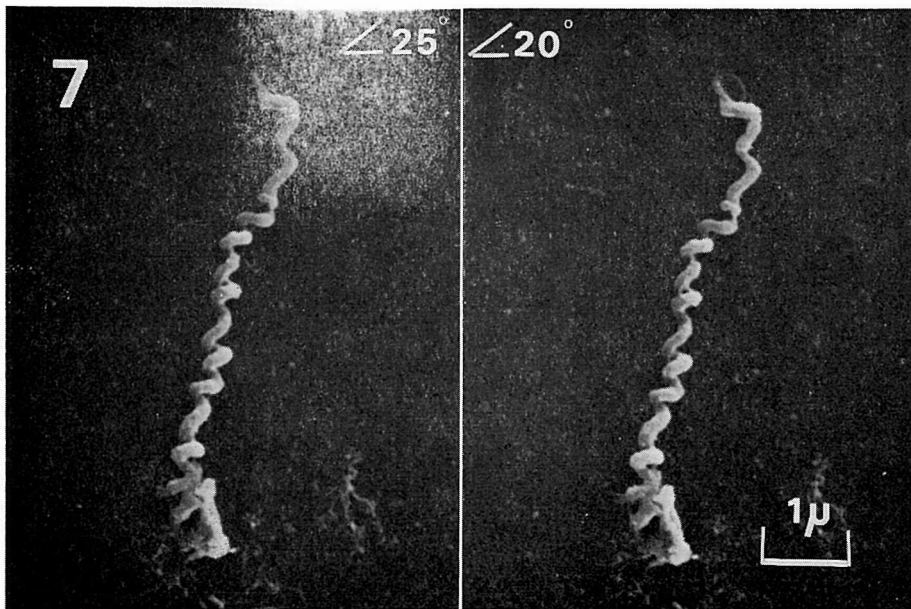


Fig. 7 *T. phagedenis* biotype Reiter, showing two graphs of a cell with stereographic views from different angles. The right-handed turn of cell body can be clearly seen. Magnification : $\times 11,000$

Treponema phagedenis: The SEM images of *T. phagedenis* biotype Reiter are demonstrated in Figs. 4-7. Two spiral directions were observed in this spirochete. Three fourth of all cell bodies showed the left-handed turn and one fourth of them showed the right-handed turn.

DISCUSSION

Under SEM only the right-handed turn of spiral direction was observed in the cell population of *B. duttonii* (Fig. 1) and only the left-handed turn was seen in *T. pallidum* (Figs. 2-3). Thus, each of these strains includes cell bodies with only one type of spiral direction. On the other hand, both the right-handed and the left-handed turn were recognized in *T. phagedenis* biotype Reiter (Figs. 4-7). The stock culture of the latter treponeme is regarded to be the mixture of two different types of spiral direction of cell body.

Two possibilities for the mixed population in the cell type in *T. phagedenis* can be assumed. One possibility is that two opposite types of cells had been living originally in the lesion of patient and both of them have been growing in the culture media. The other is that only one type of cells, either right- or left-handed turn, originally lived in the lesion of patient, and the other type appeared on mutation during the long term cultivation. The answer to this question awaits future work.

As information concerning the spiral direction of the cell bodies of spirochetes increases, the advancement of cytology, locomotive physiology and taxonomy of the spirochetology will be facilitated.

REFERENCES

- 1) Smibert, R.M., Canale-Parola, E., Kuhn, D.A., Felsenfeld, O. and Turner, L.H.: Part 5. The Spirochetes, In R.E. Buchanan and N.E. Gibbons (eds.), *Bergey's Manual of Determinative Bacteriology*, 8th ed., Williams & Wilkins Baltimore, 1974, pp. 167-195.
- 2) Yoshii, Z.: Fundamental considerations and experimental conditions on the study of the spiral direction of helical bacteria. *Bull. Yamaguchi Med. Sch.*, 25 : 115-127, 1978.
- 3) Yoshii, Z.: Studies on the spiral direction of the leptospiral cell body. *Proc. Jpn. Acad.*, 54 : Ser. B., 200-205, 1978.
- 4) Kayser, A. et Adrian, M.: Les Spirochetes: Sens de l' enroulement. *Ann. Microbiol. (Inst. Pasteur)*, 129A: 351-360, 1978.
- 5) Carleton, O., Charon, N.W., Allender, P. and O'Brien, S.: Helix handedness of *Leptospira interrogans* as determined by scanning electron microscopy. *J. Bacteriol.*, 137 : 1413-1416, 1979.
- 6) Brewer, J.H.: Clear liquid mediums for the aerobic cultivation of anaerobes. *JAMA.*, 115 : 598-600, 1940.
- 7) Yoshii, Z., Tanaka, S., Konishi, H., Ohkusa, A., Takamura, A., Kobayashi, M. and Iwasaki, A.: Studies on the specimen preparation methods for scanning electron microscopy. Part III. Drying method of microbial and free cell materials. 2. A contrivance in critical point

drying—Utilization of the small envelope of filter paper (in Japanese). *Yamaguchi Med. J.*, 26:197-203, 1977.

- 8) Kihara, H.: A proposal on the unification of the terms "right- and left-handedness" (in Japanese). *Heredity*, 29:2-4, 1975.