Bibliographical Review on the Electron Microscopy of Spirochetes

Zensaku YOSHII

Department of Microbiology, Yamaguchi University School of Medicine, Ube, Japan (755)

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SUMMARY

Articles of the electron microscopy in spirochetology since the earlier days were collected and the list of them was compiled.

They were classified and arranged based on many items, such as kind of spirochete, organelle which is a main subject in the study, taxonomical study, immunological and serological studies, pathology (infection and infected tissues), and sampling method. They were also discussed from the historical viewpoint as well as the bibliographical considerations. This review must be useful in the looking of articles and would be convenient to young scholars for the quotation of them in spirochetal morphology.

INTRODUCTION

The history of electron microscope began in the first half of 1930's in Europe (1)(2). Applied studies in microbiology also started in the latter half of the same decade (3)(4). Regarding electron microscopy of spirochetes, the first description on the electron microscopic image of a spirochete might be published by Brüche and Haagen in 1939 (5). Since then, a large number of articles of the electron microscopy of spirochetes have been reported. They must have contributed on the morphological advancement in spirochetology, however, no review on them associated with a compiled list was published from the bibliographical and historical viewpoints.

The author compiled and reviewed them based on those items in order to provide much benefits to young scholars. The review must be a kind of a monument of seniors and is also considered to be worthy of publication.

COMPILATION OF THE LIST OF ARTICLES

Materials: All the articles entitled "Electron Microscopy of Spirochetes", "Electron Microscopic Studies of Spirochetes" and other related titles, or

papers contained electron micrographs of spirochetes, were collected and arranged.

Although there are many species, genera, families in the *Order Spirochaetales* (Bergey's Manual of Determinative Bacteriology, 7th and 8th Editions) (6) (7), some of them are still called with different names occaisionally. Besides, unidentified species or genera can also be seen actually in many articles. However, all the articles described under the title of any names of spirochetes were adopted in the present paper. Regarding *genus Saprospira*, it was thrown away from the *Order Spirochaetales* and set in a new group in Bergey's Manual of Determinative Bacteriology, 8th edition in 1974 (7). This edition must be based upon the proposal of Lewin's paper (8), however, the article of this genus was provisionally extracted in conformity to the custom in spirochetal taxonomy for a long period. Because adoption of this *genus Saprospira* would be beneficial in spirochetal taxonomy and comparative morphology in bacteriology.

Methods: Reprints of them were collected as many as possible in order to confirm the exactness of their descriptions, titles, books and magazines. In the case of unobtainable articles, they were copied from the original magazines. Some of them, which were by no means impossible to obtain, collection was then despaired and requoted from the references of some other articles. Abstract of publication at the academic meeting or article without electron micrograph was ommited in principle. All the articles were edited and listed alphabetically by author's name and given a file number to each article. This style of arrangement was effective for the classification in every item. Description of the list was followed by 3-line style; the first line was file number and authour's name, the second was title, the third was magazine or book (name, volume, pages and year). The list of all the articles collected is as follows:

THE LIST OF ARTICLES

1) Abe, S.:
Electron microscopic observation of syphilid.
I. The ultrastructure of *T. pallidum* (Nichols strain),
Bull. Pharm. Res. Inst. (Takatsuki), No. **60**, 1-6, 1966.

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Acta Path. Microbiol. Scand. Sect. B., **82**, 329–344, 1974.

256) Hovind-Hougen, K.:
The ultrastructure of cultivable treponemes.
2) *Treponema calligyrum, T. minutum* and *T. microdentium,*
Acta Path. Microbiol. Scand. Sect. B. **82**, 495–507, 1974.

257) Hovind-Hougen, K.:
The ultrastructure of cultivable treponemes.
3) *Treponema genitalis,*
Acta Path. Microbiol. Scand. Sect. B, **83**, 91–99, 1975.

258) Hovind-Hougen, K. Birch-Andersen, A., and Jensen, H-J.S.:
Ultrastructure of cells of *Treponema pertenue* obtained from experimentally infected hamsters,
Acta Path. Microbiol. Scand. Sect. B, **84**, 101–108, 1976.

259) Johnson, R.C., Ritzi, D.N., and Liuermore, B.P.:
Outer envelope of virulent *Treponema pallidum,*
Infect. Immun., **8**, 291–295, 1973.

260) Jones, A.M., Zeigler, J.A., and Jones, R.H.:
Experimental syphilis vaccines in rabbits.
I. Differential protection with an adjuvant spectrum,
Brit. J. Vener. Dis., **52**, 9–17, 1976.

261) Fitzgerald, T.J., Miller, J.N., and Sykes, J.A.:
Treponema pallidum (Nichols Strain) in tissue cultures: cellular attachment, entry, and survival,
Infect. Immun., **11**, 1133–1140, 1975.

262) Zeigler, J.A., Jones, R.H., Jones, A.M., and Kubica, K.M.:
Demonstration of extracellular material at the surface of pathogenic *T. pallidum* cells.
Brit. J. Vener. Dis., **52**, 1–8, 1976.

CLASSIFICATION OF THE ARTICLES

All the articles in the list above mentioned were classified based on the several items, such as the articles with unobtainable copies, description style (review paper), kind of spirochetes, taxonomy, immunology, physico-chemical effects, phage, pathology, life-history or life-cycle, and sampling methods.

1. Articles which were unable to obtain copies are as follows:
   12, 14, 17, 37, 42, 54, 57, 63, 67, 91, 95, 97, 98, 99, 100, 110, 111, 112, 116, 136, 138, 139, 152, 153, 156, 157, 159, 161, 163, 172, 173, 174, 175, 177, 180, 182, 183, 186, 193, 201, 202, 203, 217, 218, 228, 251.
   Total number of articles=46,
   The deficiency rate---46/262=17.5%
2. Description style of articles, particularly in review style. Reviews are as follows:
   16, 19, 24, 184, 211, 215, 243.
3. Classification of articles.
   A. Kind of spirochetes:
      i) genus Spirochaeta:
      26, 27, 28, 35, 44, 56, 77, 227, 254.
      ii) genus Cristispira:
      65, 184, 191.
      iii) genus Saprospira:
      46, 120, 121, 122, 123, 124.
      iv) genus Borrelia:
      3, 8, 16, 20, 21, 32, 33, 41, 59, 73, 74, 75, 76, 101, 103, 107, 108, 114, 129, 141, 175, 182, 185, 206, 211, 219, 220, 223, 243, 245.
      v) genus Treponema:
      1, 2, 6, 7, 10, 15, 16, 18, 25, 32, 54, 55, 60, 63, 64, 69, 70, 71, 72, 78, 79, 80, 81, 85, 86, 88, 92, 93, 94, 95, 102, 106, 108, 114, 115, 116, 117, 118, 119, 130, 135, 142, 143, 145, 146, 147, 148, 149, 150, 153, 154, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 182, 183, 184, 185, 186, 189, 190, 195, 196, 197, 204, 205, 206, 208, 209, 210, 215, 221, 223, 224, 228, 231, 233, 234, 235, 236, 237, 243, 246, 247, 253, 255, 256, 257, 258, 259, 260, 261, 262.

vi) genus Leptospira:

4, 5, 9, 12, 13, 14, 16, 17, 18, 19, 22, 23, 29, 33, 34, 36, 37, 38, 39, 40, 42, 43, 45, 47, 48, 49, 50, 51, 52, 53, 57, 58, 59, 61, 66, 67, 82, 83, 87, 89, 90, 91, 97, 98, 99, 100, 104, 105, 108, 109, 110, 111, 112, 113, 114, 131, 136, 137, 138, 139, 140, 151, 152, 155, 163, 176, 177, 178, 179, 180, 182, 185, 187, 188, 192, 193, 194, 195, 198, 199, 201, 202, 203, 206, 207, 213, 214, 216, 217, 218, 219, 222, 223, 226, 232, 238, 242, 244, 249, 251, 252.

vii) Oral Spirochetes:

24, 30, 31, 68, 84, 96, 125, 126, 127, 128, 132, 133, 134, 150, 200, 209, 212, 225, 229, 230, 239, 241, 250.

B. Taxonomical study:

16, 19, 128, 182, 195, 200.

C. Immunological and serological studies:

5, 19, 28, 45, 62, 135, 155, 176, 204, 242, 252.

D. Effects by anitibiotics, chemical and physical tretments:

5, 43, 58, 67, 76, 83, 93, 108, 112, 113, 145, 170, 174, 189, 228, 232, 244, 245.

E. Phage, Rhapidsome & Bacteriocin:

46, 120, 122, 123, 124, 188.

F. Pathological study:--- infected organ or tissue:

2, 3, 11, 24, 36, 37, 38, 39, 55, 80, 92, 126, 131, 137, 138, 139, 158, 159, 166, 167, 168, 169, 170, 171, 186, 192, 193, 201, 208, 210, 253, 261.

G. Life-cycle, granular form and cyst:

18, 31, 41, 48, 49, 61, 69, 70, 84, 90, 99, 105, 109, 133, 134, 168, 206, 216, 225, 227, 238, 249.

H. Axial filament:

25, 26, 27, 28, 30, 32, 44, 45, 50, 65, 69, 77, 79, 84, 86, 92, 94, 95, 96, 98, 107, 125, 126, 127, 132, 133, 140, 141, 146, 151, 158, 159, 187, 209, 212, 220, 221, 223, 222, 230, 236, 237, 239, 241, 245, 246, 247, 250.

4. Sampling Methods:

A. Shadowing:

6, 7, 13, 15, 16, 18, 22, 23, 26, 32, 33, 34, 43, 44, 46, 47, 48, 49, 50, 58, 62, 64, 66, 68, 69, 81, 82, 83, 102, 104, 105, 107, 108, 109, 113, 114, 117, 120, 121, 126, 133, 135, 140, 141, 142, 146, 147, 148, 154, 178, 181, 189, 196, 197, 198,

199, 206, 208, 213, 214, 218, 220, 221, 222, 223, 226, 227, 231, 232, 234, 242, 245, 246, 248, 249, 250, 252.

B. Sectioning:

1, 2, 3, 11, 22, 26, 29, 30, 31, 36, 37, 38, 39, 44, 51, 52, 53, 55, 60, 65, 71, 72, 79, 80, 82, 86, 92, 101, 102, 103, 104, 106, 107, 110, 115, 118, 121, 124, 135, 126, 127, 128, 131, 134, 137, 141, 151, 158, 159, 160, 162, 166, 167, 168, 169, 170, 171, 176, 185, 187, 191, 190, 192, 198, 199, 204, 205, 208, 209, 210, 232, 233, 240, 241, 242, 252, 253, 254, 255, 256, 257, 258, 260, 261.

C. Negative staining:

4, 5, 22, 26, 27, 28, 29, 30, 31, 35, 44, 45, 46, 77, 78, 80, 84, 85, 86, 92, 93, 94, 95, 96, 106, 109, 115, 122, 123, 127, 128, 132, 151, 160, 166, 167, 168, 176, 185, 186, 188, 190, 204, 205, 212, 225, 229, 230, 233, 235, 239, 240, 244, 247, 259.

Table 1. Chronological distribution of articles of each spirochetal group.

	Sp	Cr	Sap	B	T	L	Os	Total
1939						1		1
1942					3			3
1943				1	2	1	1	5
1944					1			1
1945				1				1
1946					2			2
1947	1			2	1	3		7
1948				1	1	1		3
1949				1		4		5
1950				2	2	3	1	8
1951				1	7			8
1952				2	3	3		8
1953				3	3	3		9
1954				1	5	3		9
1955				3	4	6		13
1956				3	5	6	1	15
1957				1	2	4		7
1958				1	2	11	1	15
1959								0
1960	1		1	1	1	5		9
1961				1	1	1		3
1962			1			4		5
1963		1	1		2	3	1	8
1964			2		1	2	4	9
1965		1	1	2	8	6	1	19
1966					8	12	1	21
1967	1				3	7		11
1968	1			1	7	4	3	16
1969	1			1	4	6	1	13
1970	1				4	1	1	7
1971	3				6		1	10
1972					7	1	2	10
1973				1	4	2	3	10
1974					4	2	2	8
1975					5			5
1976					3			3
Total	9	2	6	30	111	105	24	287*

Abbreviation: Sp···genus Spirochaeta, Cr···genus Cristispira
Sap···genus Saprospira, B···genus Borrelia
T···genus Treponema, L···genus Leptospira
OS···oral spirochetes.

*Exact total number is 262 in the list of articles, however, this number shows a surplus. It is the reason why some articles containing more than two kinds of spirochetes were counted as multiple number.

DISCUSSIONS AND CONSIDERATIONS

Past and Present on the Electron Microscopy of Spirochetes.

Arrangement and classification of articles in the list and table above mentioned show a transition of the study, that is, increase of observations following the advancement of electron microscope and the development of specimen preparation methods can be recognized. The rise and fall of number of articles were arranged and totalized in Table 1. There are two peaks of abundant number of articles; one is the period of 1955–1958 and the other is of 1965–1969, and both of them followed by newer sampling methods. The former followed by the spread of metallic shadowing (9,10) and the advance of ultrathin sectioning (11, 12, 13, 14, 15, 16), and the latter also by the establishment of negative staining technique (17).

In some spirochetes, such as leptospira and treponemata, their articles are more abundant than other groups. This phenomenon can be explained with two different reasons, one is the successful cultivation in the former and the other is a caustive microorganism of very important disease called syphilis in the latter, so that they must had been studied preferentially.

Regarding sampling methods, shadow-casting by Williams and Wyckoff (9,10) contributed better resolution as well as three dimensional status of spirochetal cell than simple mounting method with poor contrast. Ultrathin sectioning (11, 12, 13, 14, 15, 16) provided a lot of information on the intracellular organization of cell body.

For instance, cytoplasm with abundant ribosomes, nuclear site in which DNA-fibers were stored, location of so-called body fibers and other structural features, were exposured.

Negative staining technique (17) revealed ultrafine structures of some organella. Especially, macromolecular architecture of axial filament was demonstrated by some workers (18, 19). They opened the era of macromolecular morphology in spirochetology and much information has been provided in serveral other organella since then.

These observations were effective in the decision of the genus and differentiation of each spirochete, as well as the position of spirochetes among microbes. A long standing question, "Whether they are bacteria or protozoa?" (20), will be solved based on these results and a new definition of the spirochetes will be established in the near future.

Suggestion and Advice toward the Advancement of Spirochetal Morphology with Electron Microscope in the Future.

1) Fundamental construction of spirochetal cell must be established and

macromolecular architecture of every organelle should be more studied in connection with its function.

2) Some genera of spirochetes, such as *genus Spirochaeta*, *genus Cristispira* and *genus Borrelia*, should be more studied and information on them must be accumulated, because articles on these genera are relatively few in number than others.

3) Life-history and life-cycle of every genus of spirochetes must be studied more in details.

4) Pathogenesis of spirochetes (infection and infected tissues) should be more studied in parallel with light microscopy.

5) Analysis of chemical component and immunological properties of spirochetal cells should be studied in connection with electron microscopic morphology.

6) Contribution of electron microscopical information on the spirochetal anatomy can be expected to the comparative taxonomy of spirochetes and other related microorganisms.

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