ELECTRON-MICROSCOPE STUDIES ON RELAPSING FEVER SPIROCHAETES

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(With Plate 16)

In December 1945, Lofgren & Soule described the structure of *Spirochaeta novyi*, as revealed by the electron microscope but, owing to the present emergency conditions, this work has only now come to our knowledge.

Five years ago we (Babudieri & Bocciarelli, 1943) published the results of similar research, but, owing to the war, this did not reach America.

Our results and conclusions do not completely agree with those of Lofgren & Soule, so that we have thought it desirable to summarize the results of our work.

Our work was carried out on one strain of *Sp. novyi* and one of *Sp. obermeieri*, maintained by passages in mice or cultivated on Illert medium. We have observed no important differences between the strains cultivated *in vivo* or *in vitro*.

Contrary to the procedure of the American workers, we fixed the spirochaetes with damp osmic acid to avoid alterations during the successive repeated centrifugings which are necessary to get rid of extraneous matter.

Lofgren & Soule stated that the ends of the spirochaetes were always sharply pointed, though some with rounded ends can be seen in their photographs. We, on the contrary, have always found two forms of spirochaetes, one with both ends sharply pointed (Pl. 16, fig. 1), the other with one end sharply pointed and the other rounded (P. 16, fig. 2). The proportion between the two types varied. In cultures, in the active phase of reproduction, those with both ends sharply pointed are more common, but when the reproduction activity is less, the two forms occur in nearly equal numbers.

We explain this difference by supposing that the typical form of the resting *Spironema* is that with a sharply pointed and a rounded end. In the reproductive process the spirochaete shows a central thinning (Pl. 16, fig. 3) which proceeds till complete separation into two bodies occurs. Of these two newly formed spirochaetes, one will evidently have a sharply pointed end (the newly formed one) and a rounded end (the existing one), while the ends of the other will be both sharply pointed. If the reproductive rhythm is very rapid the spirochaetes will not pass through the resting stage with a rounded end, and the numbers with both ends sharply pointed will be far greater than those with one end rounded and the other sharply pointed.

The protoplasm, according to the American workers as well as according to our own observations, has no notable characteristics, except some vacuoles (Pl. 16, fig. 4) and some granules, which in our opinion occur only in altered spirochaetes.

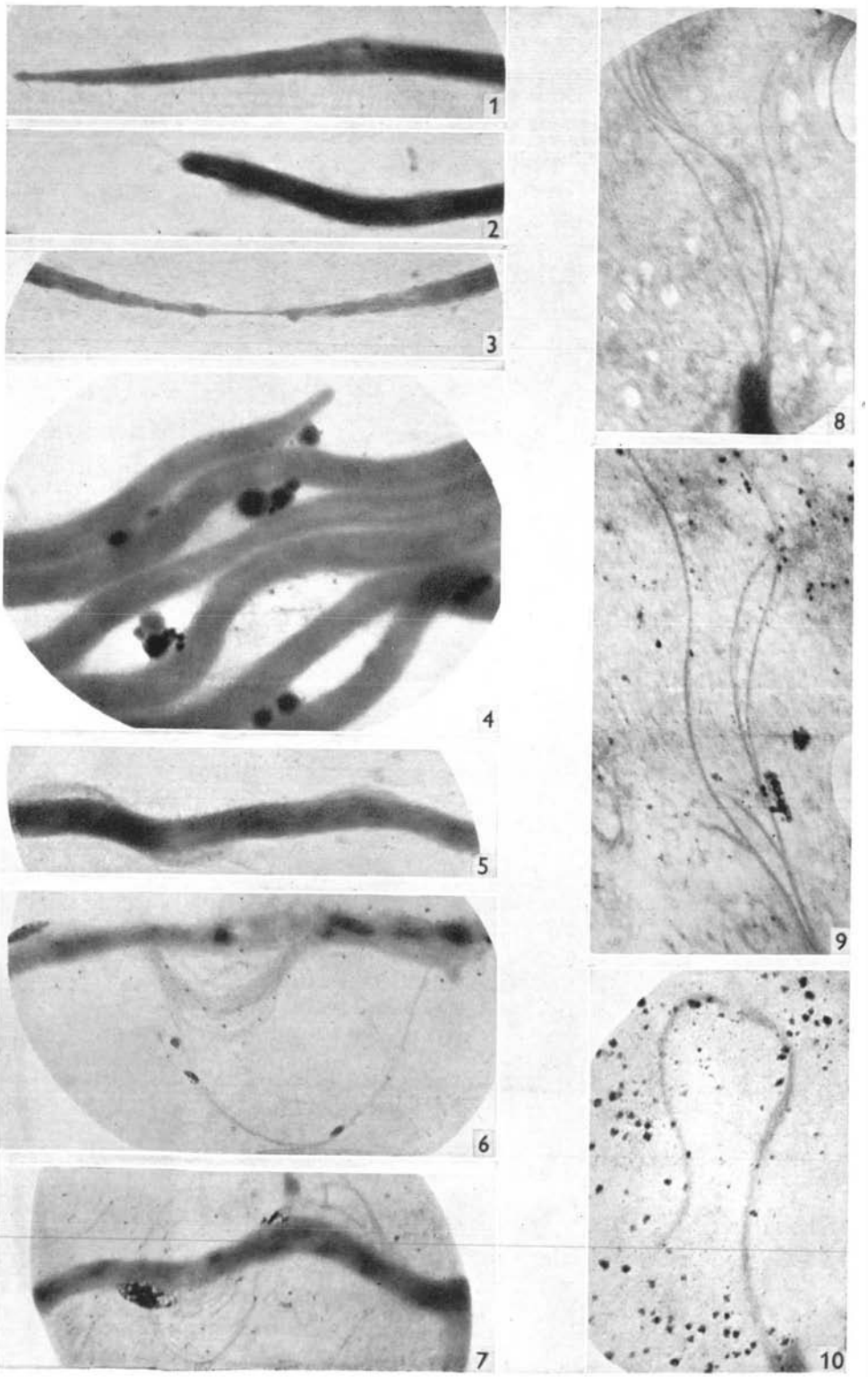
Neither we nor the American workers can establish the presence of an occasional axostyle. We have not seen a real cell wall either in spirochaetes undergoing division or in those in which a plasmophthisis had been artificially provoked. Lofgren & Soule admit its existence on the ground of the appearance of thin fibres in hydrolysed spirochaetes, which, they think, are the outcome of the disintegrated periplast, having a fibrillar constitution. As shown afterwards, we explain the nature of this fibre in a different way. In any case the existence of a peripheral membrane-like thickening of the protoplasm is very probable.

The American workers do not mention an undulating membrane. We, however, succeeded in bringing it into evidence very clearly (Pl. 16, fig. 5). It begins a short distance from the end and stretches the whole length of the body of the spirochaete. It is about 0·05–0·1 μ in breadth. If macerated, it shreds to form thin flagella-like fibres (Pl. 16, figs. 6, 7). Lofgren & Soule interpreted these fibres as coming from the periplast. In our opinion, they result, however, from the disintegration of the undulating membrane. They have really a sinuous course, like the membrane; they are never found at the same time on both borders of the spirochaetes, as would happen if they proceeded from the cell wall, and finally we can clearly observe in some cases the breaking into fibrillae of the undulating membrane.

Lofgren & Soule found, in hydrolysed spirochaetes, terminal filaments and flagella, and regarded them as artefacts produced by disintegration of the cell wall.

We have never seen real flagella in spirochaetes with both ends sharply pointed. On the other hand, in those having a rounded end we have noticed the presence of a tuft of 3–10 thin flagella, about 5 μ in length (Pl. 16, figs. 8, 9), or of a thicker filament of the

https://doi.org/10.1017/S0022172400036639 Published online by Cambridge University Press



https://doi.org/10.1017/S0022172400036639 Published online by Cambridge University Press

same length (Pl. 16, fig. 10). These formations always proceeded from a point near the rounded end.

It has not been possible to establish which is the normal form or whether the flagella arise from the disintegration of such a thick filament, or whether, on the contrary, this filament is the product of the agglutination of the flagella. In any case these formations cannot be regarded as artificial products.

Occasionally we observed extra spirochaetal granules and globules, as described by Lofgren & Soule, but we doubt if they are associated with the spirochaetes.

Our work leads us to characterize the genus *Spironema* as spirochaetes with large and irregular spirals, which are narrow and more regular in culture. An axial filament is absent. There is no real cell wall and an undulating membrane is present. In round-ended forms there is a tuft of flagella or a subterminal filament resembling them. Division is transverse. Disintegration occurs in bile-salts, and stopping of movement and slow disintegration by saponin.

The presence of flagella and of an undulating membrane bring the genus *Spironema* near *Trypanosoma*, as Schaudinn foresaw over 50 years ago.

The greatest difference between them is that *Spironema* divides transversely, while *Trypanosoma* divides longitudinally.

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EXPLANATION OF PLATE 16

All the micrographs are magnified × 20,000. For descriptions of figures see text.

(*MS. received for publication* 11. VIII. 48.—Ed.)