On the life-history of spirillum / by Patrick Geddes and J. Cossar Ewart ; communicated by Professor Huxley.

12. Spirillum

by

P. Geddes & J. B. Ed

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"On the Life-History of Spirillum." By PATRICK GEDDES and J. COSSAR EWART, M.D. Edin., University College, London. Communicated by Professor HUXLEY, Sec. R.S. Received June 20, 1878.

[PLATES 11, 12.]

Notwithstanding the numerous and fruitful researches which have been recently made into the life-history of *Bacteria*, our knowledge of the common and interesting curved and spiral forms—the *Vibrio** and *Spirillum* of Ehrenberg—has made little or no advance since his time, neither embryonic nor reproductive forms having ever been observed; while even the zoogloea phase, so characteristic of *Bacterium* and *Bacillus*, has only once been mentioned,† and then in a different form.

A fresh-water aquarium, which has been stagnating since last summer in the Physiological Laboratory of University College, contained in winter vast numbers of ordinary motile *Spirillum*. On recently re-examining the water, one zoogloea film after another having in the meantime formed on the surface, thickened, broken, and sunk, we found that these motile forms had almost disappeared, while the films consisted almost entirely of resting *Spirillum* in a gelatinous-looking matrix, similar to that of *Bacterium* and *Bacillus*. Among these were two or three apparently distinct kinds of filaments, some resting and colourless, others motile, and filled with highly refracting bright yellowish-brown spheres. Such a field is represented in fig. 1.

The gloea had, even to the naked eye, a brownish tint, which under the microscope was distinctly traceable to the *Spirilla*, the matrix being also faintly tinged. When a fragment was mounted, water very rapidly revived the perfectly quiescent organisms at the torn edges. These, after some efforts, succeeded in disengaging themselves, and in a few seconds were darting to and fro, fully motile. The rapidity and extent of this change from the resting to the motile condition, which we have witnessed again and again, was of extraordinary interest and beauty. When crowded into a narrow space, the motile *Spirilla* showed their bright brown madder tint very intensely—a single one, unless very carefully examined, appearing colourless (fig. 1).

The resting *Spirilla* are of very various shapes, some singly bent, others slightly curved at one or both ends, and others coiled like corkscrews of two or more turns (fig. 1). We were much astonished

* We are very strongly of the opinion that the forms described by various authors as *Vibrio* are merely either—(1.) Zigzag dividing *Bacillus*; (2.) Slightly waved *Bacillus*; or (3.) Undeveloped *Spirillum*, and hence that *Vibrio* should no longer be used as a generic term.

† Lankester, "Quart. Journ. Micr. Sci.," vol. xiii, p. 424.

to note that amongst these lay short, comma-shaped forms (fig. 11), the head of the "comma" exactly resembling in size and colour the yellowish-brown spheres contained in the long motile filaments above mentioned. Every possible gradation in size and curvature existing between the smallest comma and the longest spiral, the explanation at once suggested itself that here we had the germination of spores into *Spirilla*. The adult resting forms might often easily be mistaken for germinating spores; but, by careful focussing, the apparent spore is resolved into a mere twist or upturned end, and we have therefore specially studied and carefully figured such possible sources of fallacy (fig. 12).

The spore-bearing hyphæ, to the study of which we were thus led, may be next described. They were of enormous length and constantly in motion, the shorter progressing with a strange, unsteady, forward movement; the longer, like intertwisting snakes, convoluting into loops, knots, and spirals, to form a motile mycelium (fig. 1). After remaining entire for a considerable time they break up into longer and shorter, still motile, segments which may again divide (fig. 7). We have repeatedly observed a filament, as it crept along, sowing its own spores, which either escaped at the end or through the walls, leaving slightly smaller clear vacuoles in the cellulose, to show where they had lain (fig. 7*a*). In all other cases in the vegetable kingdom, so far as we know, save two other Bacterial forms,* the spores or seeds may have the means of active or passive locomotion, but the parent organism, at least, is always quiescent; here the reverse holds good, but the same end is gained—the parent being locomotive and the spores quiescent.

The development of the filaments and their relation to the resting and motile spirals we next endeavoured to ascertain. In various preparations, especially in those kept at a temperature of 25° C., we found some long irregularly-curved *Spirilla* (fig. 2), and associated with these, and often scarcely distinguishable from them, were delicate wavy filaments, which were slowly changing their form (fig. 3), sometimes straightening, sometimes looping, but often recurring to the *Vibrio*-like type (fig. 3). These increased in length and thickness, and became motionless (fig. 4*b*), and their protoplasm gradually condensed into clear round spheres, which were at first almost colourless, but became tinged with yellow, which deepened into the characteristic brown, the filament then returning to the motile condition. The young spores were at first arranged with some regularity (fig. 4*c*), but this disappeared as they ripened and divided. The process of division is very remarkable, dumbbell-shaped, triradiate, and budding masses occurring together in the same filament, the resulting spores being

* (1.) *Bacterium rubescens.* Lankester, "Quart. Journ. Micr. Sci.," vol. xvi.
(2.) *Bacillus* from sea water described by one of us in the preceding paper.

often unequal in size (fig. 6). Such irregularity of division very often also takes place outside the filaments (fig. 14).

When naturally sown in the glœa, in the way above described, the spores encapsulate themselves, and very often, if not always, divide into two or more sporules (fig. 8*a*). The capsules may remain isolated or also divide, forming patches of two or three (fig. 8*b*), or along with others, uniting into large irregularly cohering masses (fig. 13*c*). Sometimes, also, many may be enclosed in a definite cellulose envelope (fig. 8*d*); this last perhaps arising, like a colony of *Glœocapsa*, from the division of a single spore. When old, the capsules may lose their pale blue colour, and even become suffused with dingy brown.

The sporules may either desert the capsules (fig. 8*e*), leaving vacuoles, as in the ripe filaments, such empty capsules being found in great numbers; or may cause them to become actively motile, their motion being either corkscrew-like or direct without revolution; in the former case resembling a *Spirillum*, in the latter a monad. On escaping from the capsule, the sporules appear generally to rise to the surface, forming large dark granular masses (fig. 10).

A nutritive fluid, prepared by boiling a piece of zooglœa in some water taken fron the aquarium, was inoculated with these surface sporules and placed on the warm stage. Twenty-four hours after, many of the brown sporules were sending out a small curved hypha, at first slightly tinged, while later, distinct "commas," and even young *Spirilla*, were developed.

It is of great interest that we occasionally found a large, very finely granular sphere of the characteristic colour (fig. 9), while smaller and coarser masses were more abundant (fig. 9*b*), affording a transition to the common sporule cyst. These correspond to the smaller "macroplasts" figured by Lankester,* in his *Bacterium rubescens*, and to the finely granular colourless spheres, figured by one of us in the preceding paper† from a species of *Bacillus*. These probably result, as in *Bacillus*, from the long-continued subdivision of a single spore or sporule.

The division of the spores inside and outside the filaments is of great interest, and goes far to prove the unspecialised and Protean nature of Bacterial forms, almost all possible modes of division being found in the same field. Some divide transversely into two equal parts, others bud like *Torulæ*, others again lengthen into rod-like and *Vibrio*-like forms, and then break up into three or four portions (figs. 13 and 14).

The life-history of *Spirillum*, so far as we at present know, may be thus summarised. The well-known motile corkscrew may alternate

* "Quart. Journ. Micr. Sci.," vol. xvi, Plate 3.

† Plate 10, Series IV, *e f g*.

between the active and resting states, and ultimately lengthen out into a small filament, which loses its definite twist and may freely bend or straighten. This thread grows into a much larger and longer motionless filament, in which spores appear. These rapidly divide and acquire a bright brown colour, the filament reassuming the motile condition, and sooner or later breaking up. The freed spores encyst and divide, forming capsules, which after a period of quiescence themselves become motile, the sporules contained in them escape and germinate into "commas," which become *Vibrio*-like, and soon grow into the common motile *Spirillum*.

The resemblance of all this to the life-history of *Bacterium termo* and *Bacillus* described in the preceding paper is at once apparent. Not only is there the same alternation of a resting with a motile phase, but there is a lengthening into filaments the protoplasm of which condenses into spores which divide and germinate. Moreover, there are also moving filaments, and finely granular spheres, while the resemblance to *B. rubescens* is even more striking. That the deeply coloured spherules, figured by Lankester in the filaments and capsules, and described as "loculi," as well as the so called "sulphur-granules" of Cohn,* correspond to our "spores" is extremely probable, although their germination has not yet been observed.

EXPLANATION OF PLATES.

Plate 11.

Figure 1. The left half of the drawing represents a portion of a large glœa film of resting *Spirillum*, containing also (1) a motile mycelium of spore-bearing filaments, (2) many spore capsules, grouped and isolated. On the left are free active *Spirilla*, which have disengaged themselves from the film; at (*a*) they are crowded, showing a distinct brown colour; at (*b*), where the liquid is drying up, is shown the intertwisting of the longer spirals, which not unfrequently occur. Many motile spore capsules are shown, also a filament breaking up.

Figure 2. *Spirilla* beginning to lengthen into filaments.

Figure 3. Young filaments still freely motile.

Figure 4. (*a*.) Similar young motile filaments.
(*b*.) More fully developed filaments, no longer motile.
(*c*.) A filament, of which the protoplasm is condensing into round faintly-coloured spores.

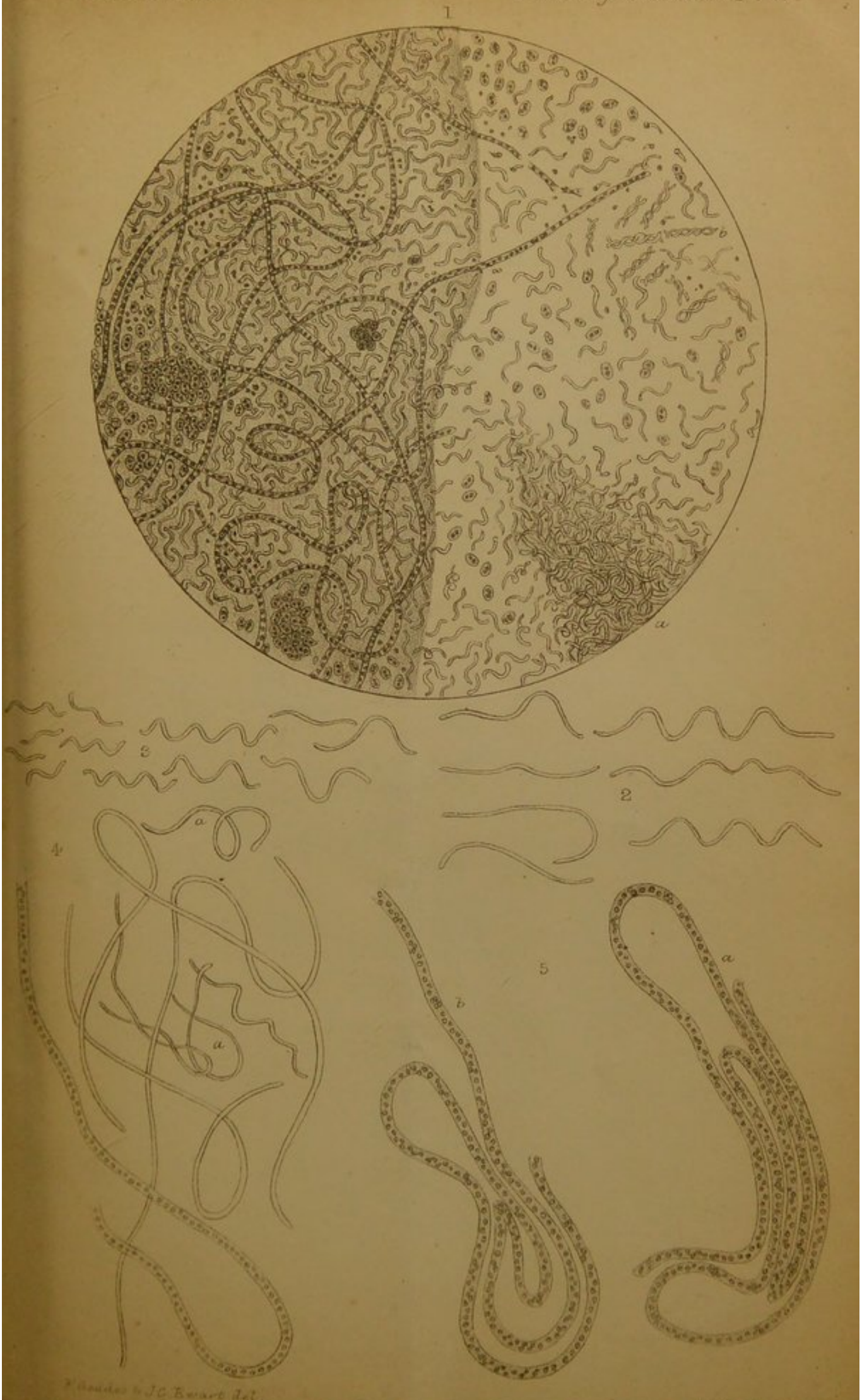
Figure 5. Two successive drawings of a ripe motile filament.

Plate 12.

Figure 6. Part of a mature motile filament, showing the irregular size and arrangement of the budding and dividing spores, also empty spaces whence spores have been dropped (*a*).

Figure 7. Successive sketches of a filament breaking up. (*a*.) Spaces occupied by spores.

* "Beiträge zur Biol. d. Pfl.," 3, 1875.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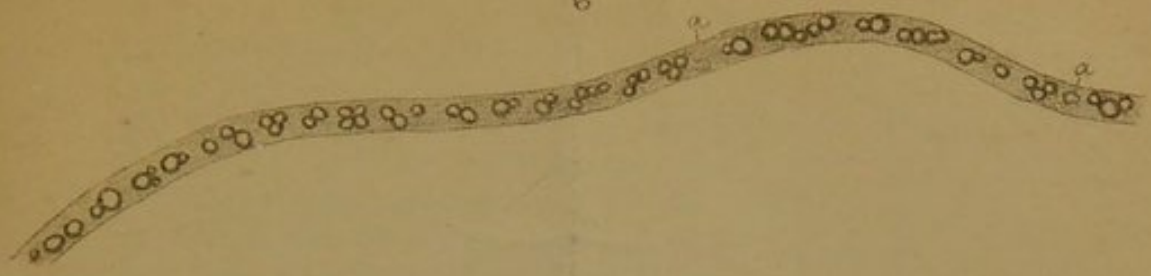

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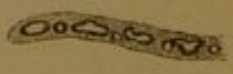

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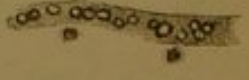

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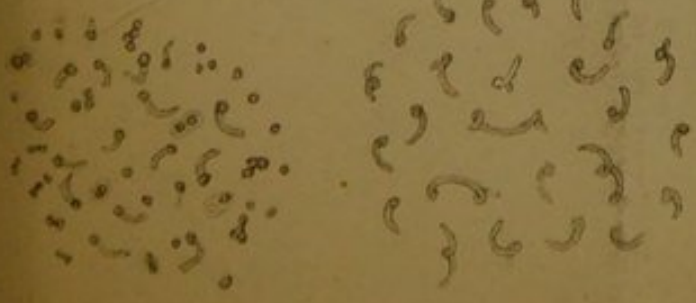

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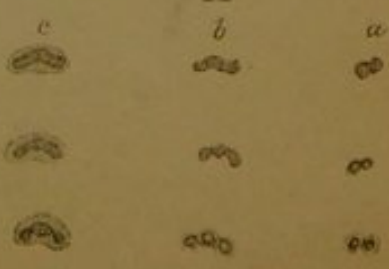

Figure 8. (*a*.) Spores encapsulating and dividing.
(*b*.) A small group of capsules.
(*c*.) A large group of capsules.
(*d*.) A group of capsules surrounded by a common envelope.
(*e*.) Sporules escaping from their capsules.
(*f*.) Large empty capsules.
(*g*.) Phases undergone by a single capsule.

Figure 9. (*a*.) Large finely granular brown sphere.
(*b*.) Smaller coarsely granular spheres.

Figure 10. Patch of naked spores from surface of liquid.

Figure 11. Spores germinating into *Spirilla*, at first comma-shaped.

Figure 12. *Spirilla* resembling germinating spores.

Figure 13. Successive drawings of a portion of same filament, showing extreme irregularity of division of spores.

Figure 14. Irregular division outside filament—(*a*) and (*b*), naked; (*c*) within capsule.

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