Dr. Ewart

B. Atkinson

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[Proc: Royal Society 1878]

[*From the* Proceedings of the Royal Society, No. 188, 1878.]

"The Life-History of Bacterium termo and Micrococcus, with further Observations on Bacillus." By J. Cossar Ewart, M.D. Edin., University College, London. Communicated by Professor Huxley, Sec. R.S. Received June 20, 1878.

[Plate 10.]

While recently studying the phases through which the now familiar organism *Bacillus anthracis* passes, my attention was often directed to two still more familiar organisms, *Bacterium termo* and *Micrococcus.* Frequently from cultivations of *Bacillus* both rods, spores, and filaments disappeared, and in their place millions of *Micrococci* and the short-jointed rods of *B. termo* were found.* In the short rods of *B. termo,* which in the struggle for existence overcame the less active *Bacilli,* minute bright particles were often present. These exactly resembled the *Micrococci* in the field around and between them, and were evidently the remains of spores out of which the rods had just been developed. The presence of *Micrococcus*-like spores in the short rods

* This disappearance of the one and appearance of the others accounts for early investigators believing that there was a continuity of development between *Bacilli* and septic organisms.

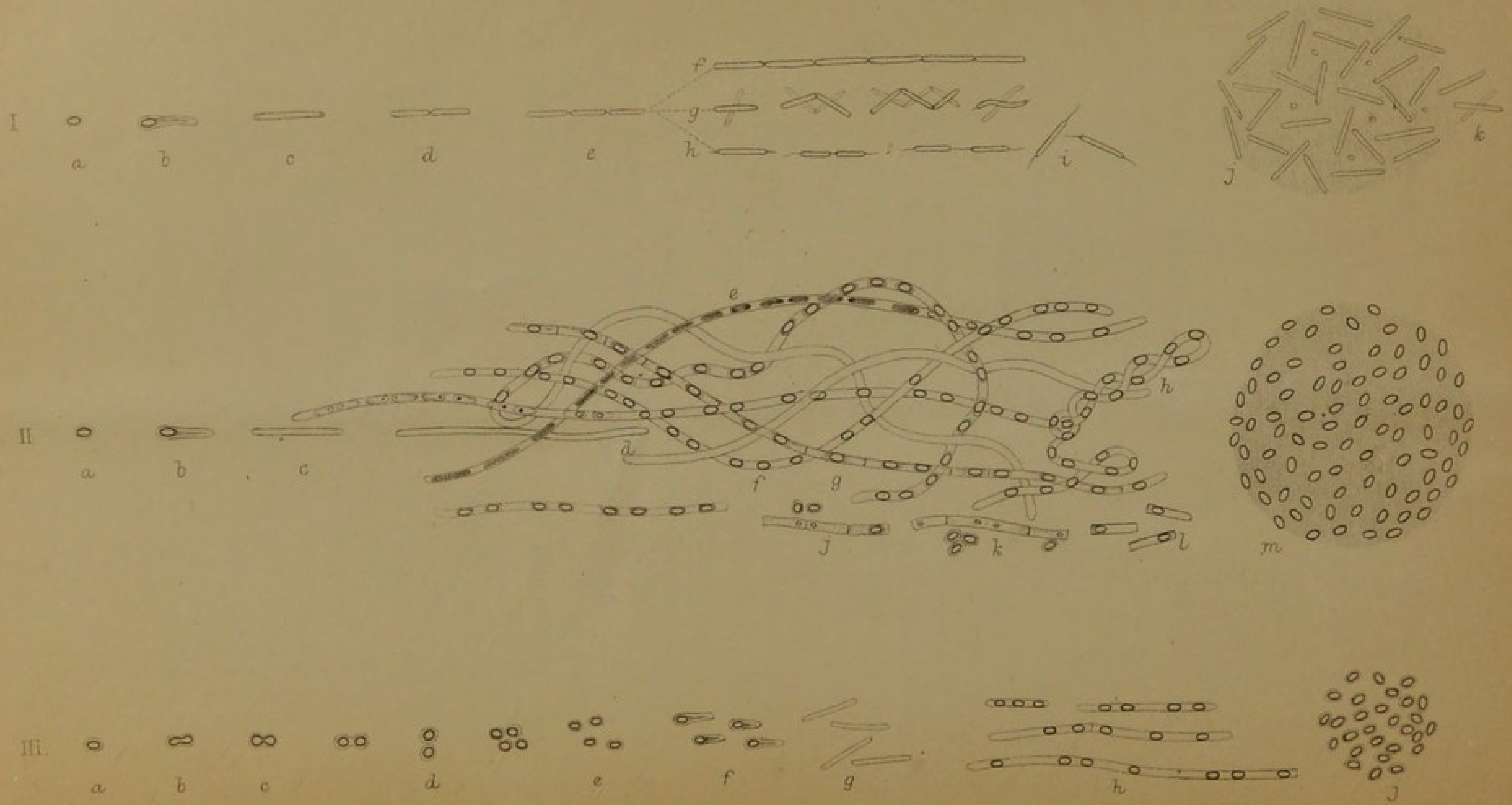
Effects of Desiccation and of different Temperatures on Bacterium termo and Micrococcus.

If the oil be removed by blotting paper from between the glass ring and the covering-glass of a preparation made as above described, or if the covering-glass be fractured without being displaced, the cultivation liquid rapidly evaporates, and the remains of what a few minutes before were active organisms are in great part left adhering to the under surface of the covering-glass. Preparations treated in this way may be either subjected to high or low temperatures; or, when protected by a glass cap, may be left in the ordinary atmosphere. The result of desiccation was ascertained by infecting flasks containing sterilized organic infusions. Such flasks infected with rods desiccated at 20° C. remain sterile, but flasks infected with desiccated spores soon teem with *Bacteria*, and flasks infected with desiccated *Micrococci* soon teem with round, oval, and dumbbell-shaped organisms, leading to the conclusion that desiccation destroys Bacterial rods; but that, though continued for weeks, it has no influence on spores or *Micrococci*. If *Micrococci* and the spores of *B. termo* are not destroyed by desiccation in a small protected atmosphere, it may be further inferred that they retain their viability when dried in the ordinary atmosphere, and, being extremely small and light, that after they are dry they will float about along with other solid particles in disturbed, and settle down in quiet, atmospheres, without undergoing any change until they find themselves in a medium which admits of their growth and development. In all probability desiccation destroys the oval and dumbbell-shaped forms of *Micrococcus*, the round spore-like forms only retaining their vitality. That spores exist in our ordinary atmosphere may be easily proved by placing sterile organic infusions in different parts of a building. In such a building as University College a considerable number of different organisms may be found. In cold rooms the infusions may remain sterile for a considerable time, but generally a scum soon appears on the surface, which, on examination, may contain, besides *Bacteria*, fungi, monads, and other low forms from both the animal and vegetable kingdom. However readily the unprotected rods of *Bacteria* are destroyed when desiccated at a temperature of 30° C., they are not destroyed in the substance of a spleen or kidney when the temperature is raised to 40° C. Those in the outer part of the dried portion of spleen or kidney are destroyed, but those in the centre are protected by the hard outer cake, not only because this hard shell tends to keep the heat from them, but especially because it tends to prevent complete desiccation.

Effects of Ebullition.

Along with Dr. Burdon Sanderson,* I have shown that the spores

* "Quart. Journ. Micro. Science," April, 1878.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of *B. anthracis* are destroyed when the fluid they are suspended in is kept for a few minutes at the point of ebullition. The same is true of *B. termo* and *Micrococcus*. On the other hand, when they are subjected to a temperature of 110° C. in a dry state they are not destroyed; they are rendered inactive, however, by a temperature of 120° C. The difference between the effects of moist and dry heat is probably owing to the gelatinous capsules of the spores and *Micrococci* giving way, and thus allowing the boiling fluid to come into direct contact with the unprotected central protoplasm.

Further Observations on Bacillus.

On the surface of sea water, containing the remains of seaweeds, Pedicellinæ and skeleton shrimps, brought from Roscoff by Mr. Geddes last April, a few patches of scum appeared which, on examination, were found to be made up of active and resting *Bacilli*, exactly resembling in size and form *Bacillus subtilis*. Day by day the small pellucid areas increased until the whole surface of the water (about 12 centimetres in diameter) was completely covered with a thick opaque scum, which, after remaining entire for seven days, gave way in the middle, and soon sank to the bottom, remaining there in a torn and broken condition.

The minute spots which first appeared were almost entirely made up of active *Bacilli*, rapidly multiplying by transverse fission. As the patches increased in size, the *Bacilli* either formed zooglœa, or lengthened into filaments. The process of lengthening was, as compared with *B. anthracis*, when cultivated on the warm stage, a remarkably slow one, and often the filaments which with the No. VIII Hartnack were apparently 3 or 4 centimetres in length, moved in a languid way amongst the active *Bacilli*. Having increased to about forty times the length of the original rod, the protoplasm divided and condensed into spores, the steps of the process being similar to those already described in *B. anthracis*.* The spores next escaped from the filaments, and either formed zooglœa, or germinated into another generation of rods. This increase of the rods by simple division, and by spore formation, continued until the whole of the surface of the water was covered by the scum.

On examination, six days after the scum sank to the bottom, an immense zooglœa was found made up of a relatively small number of quiescent rods, embedded in a thick transparent matrix (Series I, *j*). But of especial interest were a considerable number of large and small granular masses, made up of minute round particles, the smaller ones resembling the "cell families" of *Ascococcus*,† and the larger ones

* *Loc. cit.*

† Cohn, "Beit. zur Biol. der Pflanzen."

the peach-coloured granular disks figured by Lankester from old cultivations of *Bacterium rubescens*, in which "the nourishment had dwindled to its very smallest limit."

In the zöoglœa all the *Bacilli* were quiescent, and it is important to observe that in none of them was there any appearance of division; but around the edge (Series I, *k*) some were in active motion, whether entering or leaving the motile stage I do not know. The close resemblance of these masses, both in form and in time of appearance to the "macroplasts" of Lankester,* led me to watch their development. First, the spore divided into four sporules quite as in *Bacillus anthracis*,† but these sporules again divided forming a granular mass (Series IV, *d*, *e*), division and growth going on simultaneously till a large very finely granular sphere was produced. (Series IV, *g*.)

When one of the large spheres was broken up, round particles (Series IV, *h*) spread far and wide over the field. These particles, when placed in a fresh drop of sea water enlarged (Series IV, *i*) and germinated into rods. (Series IV, *j*.)

If then a single minute spore is thus capable of producing innumerable still more minute germs, and if these, as all experiment tends to show, resist desiccation at ordinary temperatures, Professor Huxley's dictum may unhesitatingly be repeated and endorsed, that, considering the lightness of *Bacterium* germs, and the wide diffusion of the organisms which produce them, it is impossible to conceive that they should not be suspended in the atmosphere in myriads.

Morphological Considerations.

Various investigators, notably Huxley and Lankester, have long ago asserted the Protean nature of *Bacteria*, and the accompanying plate (exclusively compiled from actual observation), is an attempt to summarise and define what we at present know of the phases through which three of these forms, *Bacillus*, *Bacterium*, and *Micrococcus* may pass. Such a diagrammatic representation may be the more useful, seeing that at present our knowledge is scattered through many papers.

Series I represents the most common phases through which *Bacillus* may pass. The spore germinates into a rod, this divides, the portions, if at rest, either falling apart or forming a jointed filament (*f*). The motile stage may be assumed, during which division also goes on (*g*), *zigzag forms being produced which have often been mistaken for Vibriones.* While division is going on, the development of the cilia is often beautifully seen with a high immersion. First, the cellulose

* "Quart. Journ. Micro. Science," vol. xvii, New Series, p. 27, Plate III.

† *Loc. cit.*

wall gives way, and the segments as they separate slowly draw out a thin viscous thread of protoplasm. One segment generally fixes itself to the cover glass, while the other wriggles about in all directions, moving and resting by turns, until the almost invisible thread gives way in the middle so as to form two cilia. Thus the first formed motile rods should only have one cilium, and such are occasionally seen, but are probably soon supplanted by their more active biciliate progeny. After being alternately at rest and in motion for an indefinite period, they may rise to the surface of the fluid to form a zooglœa (*j*) and after some time again become motile.

In Series II the spore immediately after germinating develops into a long filament or unbranched hypha, which interweaves with others to form a mycelium. The protoplasm of the filaments soon contracts into rows of " chlamydospores " (*f*) which either escape through the cellulose walls, or are set free by the disintegration of the hypha (*i j k*). When once free they may either form a zooglœa (*m*) or germinate immediately.

In Series III the spore divides into four sporules (*e*), which, while separating, move very energetically. This takes place when the nutritive fluid is becoming exhausted, the sporules not germinating until more nourishment is obtainable. If fresh pabulum be not added the sporules may continue dividing (Series IV) so as to form large finely granular spheres (*e f g*), compound masses resulting from the division of several adjacent spores. These disintegrate, setting free numerous small round particles (*h*) which in suitable media enlarge and germinate (*j*).

Leaving *Bacillus*, Series V shows the life-cycle of *Bacterium termo*. The round spore germinates into a short rod (*b*), which either divides into a chain (*e*), or into separate rods, with resting and motile stages alternating (*f g*), while the short rods may lengthen into delicate spore-bearing filaments (*h i*). The thorough correspondence of all this with Series I and II of *Bacillus* is very obvious and suggestive. Moreover, these spores (*j*) exactly resemble the *Micrococcus* represented below in Series VI.

Here the spore-like *Micrococcus* becomes dumbbell-shaped and divides into two (*c*), which again divide, and so on indefinitely, a preparation of *Micrococci* kept for three months having shown no tendency to germinate into rods. Resting and motile stages (*g*), chains (*f*), and zooglœa (*h*) are often observed.

EXPLANATION OF THE PLATE.

Series I—IV. Phases in Life-History of *Bacillus*.
" V. " " *Bacterium termo*.
" VI. " " *Micrococcus*.

LONDON:
HARRISON AND SONS, PRINTERS IN ORDINARY TO HER MAJESTY,
ST. MARTIN'S LANE.