Experiment XIX.

April 4th, 1878. Guinea-pig weighing $1\frac{1}{2}$ lb. Injected 4 centigrammes of cobra poison into leg.

4.1 P.M. Ligature applied immediately. Permanganate of potash applied immediately.
4.5 P.M. Twitching.
4.10 „ Dying.
4.13 „ Convulsion.
4.14 „ Dead.

Experiment XX.

April 4th, 1878. Guinea-pig weighing 1 lb.

3.45′ 20″ P.M. Injected $\frac{3}{4}$ gr. = 4 centigrammes of cobra poison under skin of leg. A ligature was applied round the leg in one minute, and in five minutes permanganate of potash was rubbed into an incision made over the site of injection.

3.52 P.M. Ligature cut.
3.53 „ Twitching violently; leg paralysed.
3.55 „
3.57 „ Dying.
3.58 „ Dead—less than 13 minutes.

VIII. "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.

led me to conclude not only that *B. termo* had a life-history similar to that of *Bacillus anthracis*, but also that Billroth was probably right in believing that *Micrococci* were the spores of ordinary *Bacteria*.

I have already proved * that *Bacillus anthracis*, like *B. subtilis*, and the *Bacillus* found by Dr. Klein on animals suffering from pneumo-enteritis (pig typhoid), is, though rarely, an active organism, the motile phase appearing at irregular intervals; that the protoplasm of the long filaments into which the rods lengthen divides into segments, which, either at once or after again dividing, condense into spores; that the spores before germinating may divide into sporules, and that the new rod is not formed, as stated by Dr. Koch,† out of the gelatinous-looking envelope, but out of the spore itself—the spore germinating, and pushing out a process from one of its ends, which carries along with it the thin capsule, converting it into the sheath of the rod.

Before attempting to compare the life-history of *Bacterium termo* with what we know of the life-history of *Bacillus anthracis*, it was necessary to have *B. termo* isolated from all other organisms. After many failures, I was fortunate enough to find a cultivation in which the rods of *B. termo* were alone visible. After keeping this cultivation under observation for some time, others were made by infecting fresh drops of *humor aqueus*, previously placed on absolutely clean covering-glasses, with as small a drop of the liquid as possible on the point of a needle. The covering-glasses were inverted over "cells" made by fixing glass rings to ordinary slides by means of Brunswick black, the cells having been carefully washed immediately before use with absolute alcohol. A thin layer of olive oil between the edge of the glass ring and the covering-glass prevented evaporation and the entrance of moisture and solid particles from the surrounding atmosphere.

In cultivations of *B. termo* prepared in this way, and kept at a temperature of 30° C., I made out that, under certain conditions, the rods, instead of undergoing fissiparous division, lengthened into filaments, in which in due time spores appeared. (Plate 10. Series V, *h*, *i*.) The filaments were shorter than those of *B. anthracis*, and they never showed any tendency to form a network or mycelium. The spores were extremely small, bright, almost spherical bodies. In from two to three days after their formation the spores escaped from the filaments, and either lay isolated near the centre or formed a zooglœa (Series VI, *h*) at the edge of the cultivation. Having been free for some time, they germinated into short slender rods. (Series V, *b*, *c*.) The young rods did not at once lengthen into another generation of spore-bearing filaments, but, becoming active, they multiplied by transverse fission. (Series V, *d*, *e*.) The filaments packed full of spores often resembled "*Micrococcus* chains," and only differed from filaments containing spores, which I

* "Quart. Journ. Micro. Science," April, 1878.

† "Beitrage zur Biologie der Pflanzen," II, 2, 1876.

lately found in scum from the surface of sea-water by being motionless. Those in the scum, notwithstanding their length and the number of spores in them, were moving freely about the field.

Having satisfied myself that, under certain conditions, the rods of *B. termo* lengthened into filaments, and that the spores formed in them when set free germinated into short rods, I next directed my attention to *Micrococcus*, in order, if possible, to make out whether it was a distinct organism, or whether it was simply a phase in the life-history of some common *Bacterium, e.g., Bacterium termo*. After making numerous preparations, I at last succeeded in getting a cultivation in which only *Micrococci* were present. The cultivation was made by adding to a drop of *humor aqueus* from a fresh ox's eye a minimal quantity of pus from a newly opened abscess on the point of a calcined needle. For three days there was no indication of organisms, but on the fourth, small moving particles were visible, which, when examined with a No. XII immersion (Hartnack), were seen to be either round, oval, or dumbbell-shaped, and often in groups of two and four. (Series VI, *g*.)

A long and careful study proved that the different forms were all phases of the same organism ; the oval forms became dumbbell-shaped, and then divided into two round bodies similar to, but smaller than, the sporules of *Bacillus anthracis*. The two round bodies moved actively about till they separated from each other, when each became dumbbell-shaped and divided as before. Being apparently in a suitable medium and at a favourable temperature, they were extremely active and increased so rapidly that they soon formed a large milky spot in the centre of the cultivation liquid. This cloud, when examined with a low power, was seen to dip well down into the fluid, thus corresponding to the plug of *Bacteria* found by Professor Tyndall in several of his test-tubes.*

Though kept under observation for three weeks, not one of the round or oval organisms present ever germinated into a rod. During the fourth week three fresh drops of *humor aqueus* were infected with the smallest possible number of *Micrococci* from the above preparation ; also several flasks containing a sterilized turnip infusion. Both in the aqueous humour preparations and in the turnip infusion they rapidly increased, but none of them germinated into rods. This experiment was again repeated with a like result. Hence, having failed to find *Micrococcus* developing into Bacterial rods, it may, in the meantime, be inferred that it is a distinct form: or just as *Torula* may be an arrested phase of some *Penicillium*-like organism, so may *Micrococcus* be the spore of a *Bacterium* which has either altogether lost its power to germinate, or can only do so under very peculiar conditions. That *Micrococcus* closely resembles *Torula* will be at once apparent.

* "Phil. Trans.," 1877.

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.

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 zooglœa 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.

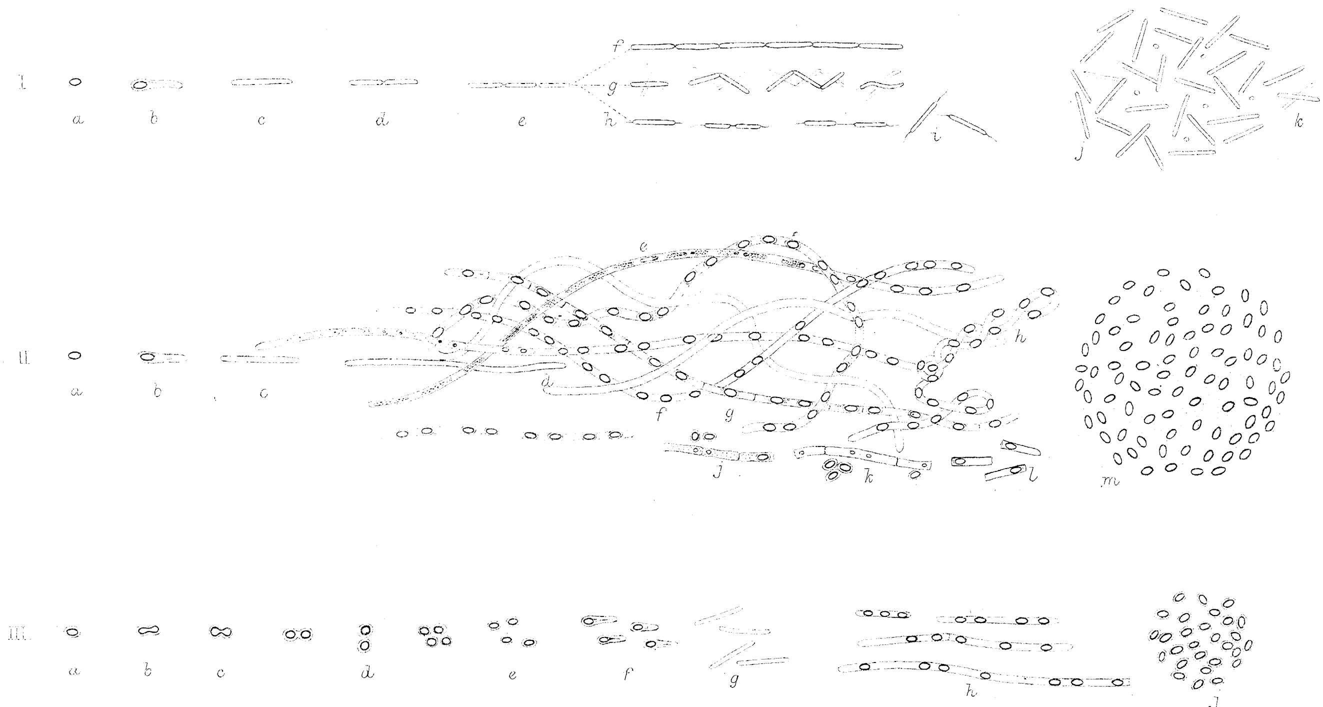
In Series III the spore divides into four sporules (*e*), which, while separating, move very energetically. This takes place when the nutritive fluid is becoming exhausted, the sporules not germinating until more nourishment is obtainable. If fresh pabulum be not added the sporules may continue dividing (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*).

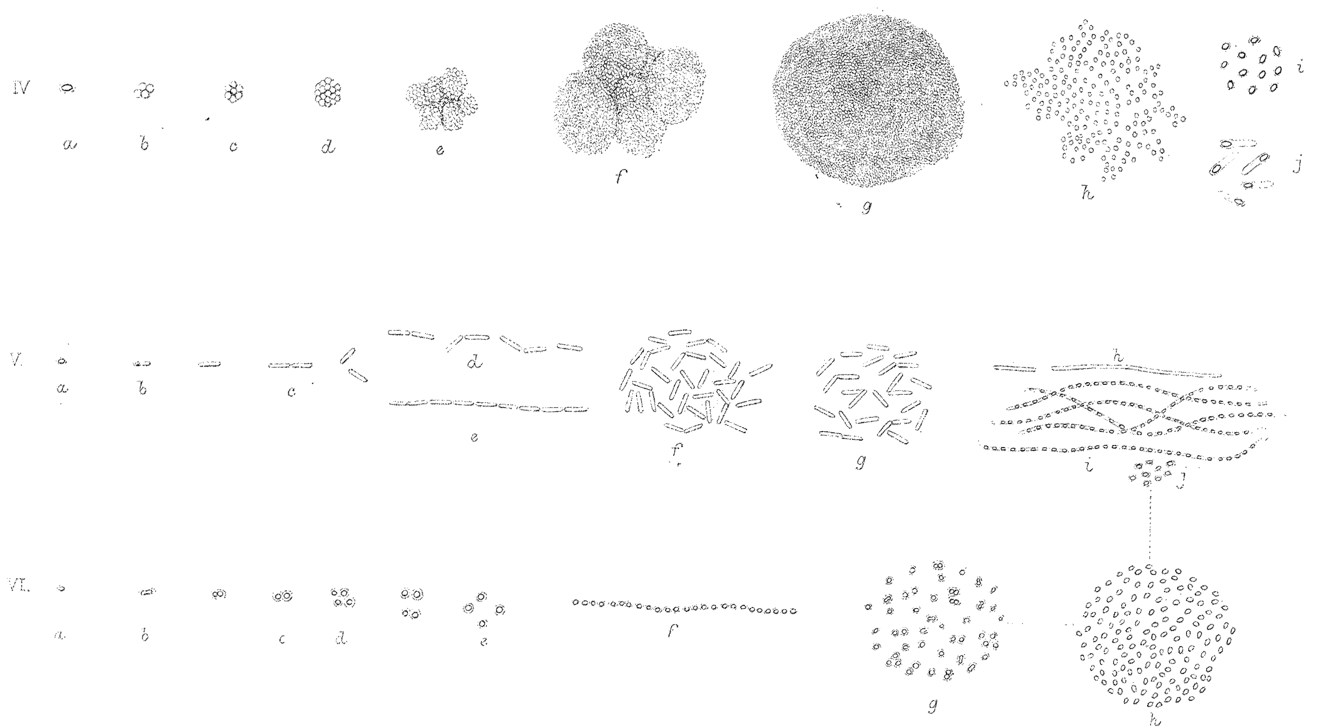
Leaving *Bacillus*, Series V shows the life-cycle of *Bacterium termo.* The round spore germinates into a short rod (*b*), which either divides into a chain (*e*), or into separate rods, with resting and motile stages alternating (*f g*), while the short rods may lengthen into delicate spore-bearing 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.*





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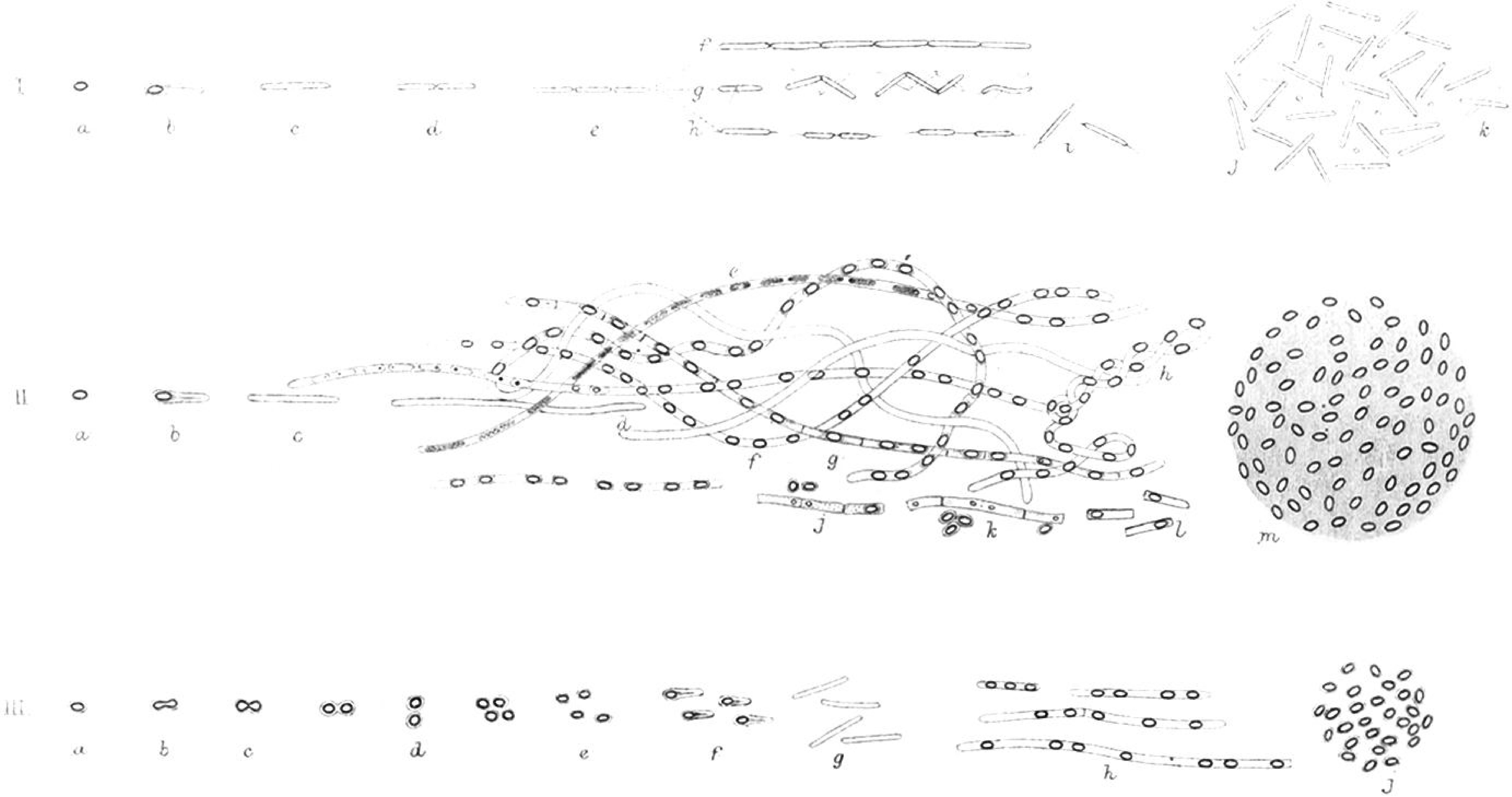

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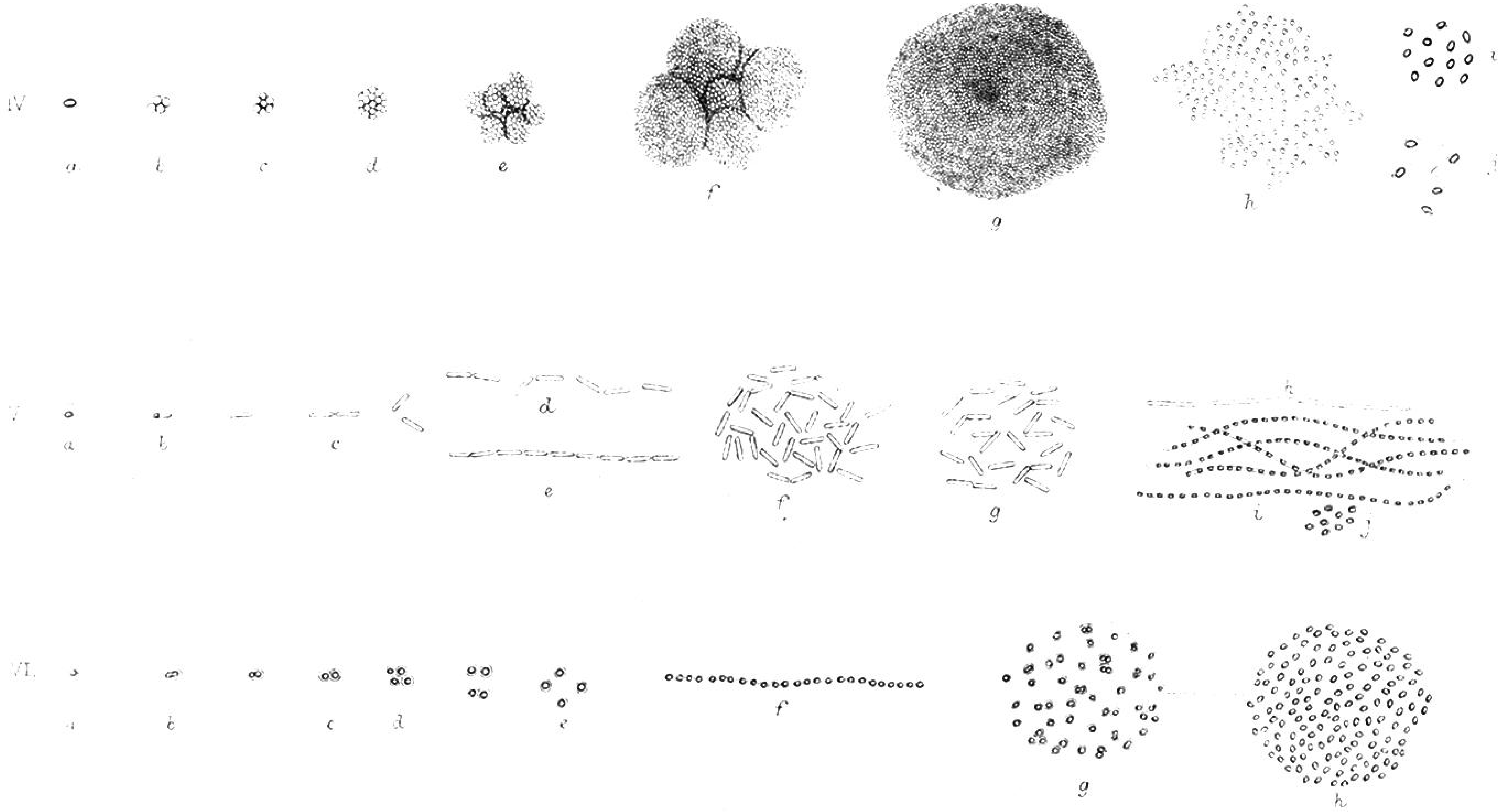
IV
a b c d e f g h i
V
a b c d e f g h k i j
VI.
a b c d e f g h