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V. *A Soil Bacillus of the Type of* De Bary's B. Megatherium.

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Communicated by Professor H. Marshall Ward, *F.R.S.*

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[Plates 14–16.]

During the course of an investigation of the bacterial flora of certain garden soils in the vicinity of the Botanical Laboratory of Cambridge University, I succeeded, with the assistance of Professor Marshall Ward, in isolating a form which proved to be of rather exceptional interest on account of its large size and its marked predilection for acid saccharine culture-media.

The method pursued consisted in taking samples of soil, with the usual precautions against contamination, at a depth of about an inch below the surface, and using portions of these, shaken up with distilled water, for the inoculation of gelatine cultures in Petri-dishes, either directly or after preliminary heating to almost the boiling point. The latter means served to prevent the growth of moulds, while it became apparent early in the course of the investigation that the use of certain acid media served very effectually to isolate the organism in question from other bacteria.

Dilution cultures were made in Petri-dishes by the following method. Six dishes were thoroughly sterilized and allowed to cool, while as many tubes (including the infected tube), each containing about 10 centims. of gelatine, were raised to 30°–35° C., in a water-bath. As soon as the gelatine was completely melted the contents of the infected tube were poured into the first dish, the contents of the second tube were added to the first, shaken, and then poured into the second dish, and so on, until the 6 plates constituting the series were prepared, the first tube being used each time as the source of infection. It is evident that by this method the first dish, which we may designate as Plate A, would receive the greater part of the germs contained in the first tube, while enough would remain in the few drops left in that tube thoroughly to infect the gelatine from the second tube. As a matter of fact Plates A and B are usually overcrowded, and Plate F is liable to be blank, but the intermediate ones may be relied upon to produce a few well-isolated colonies.

In the initial cultures various acid media were used, such as malt-gelatine, yeast

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extract-saccharose-gelatine, peptone-gelatine, saccharose-gelatine, saccharose-agar, &c. The primary cultures in the three first mentioned produced nothing striking, but in two series of plates of gelatine containing 10 per cent. of saccharose, the results were very different.

In the preparation of these the original tube infected from the soil-sample had been heated to 99° C., for five minutes. The plates were prepared on October 22nd at 3.30 P.M., and kept at the ordinary temperature of the laboratory (15°–20° C.); examined at 2.30 P.M. on October 24th, Plate A in each series showed a few pin-point whitish colonies, which by the following day at noon appeared as pearly, translucent drops upon the surface of the gelatine in Plates A–C. In none of the plates were these colonies abundant, but the plates, owing to the acidity of the medium, showed no admixture of other forms, and these cultures remained pure for the full six weeks during which they were under observation. It was evident, therefore, that here was a bacterial organism which could be isolated from moulds by the simple method of heating the soil, and from the numerous other bacterial organisms found in the soil, by the use of acid media. The fact was also interesting that it grew luxuriantly on saccharine gelatine, but, as later experience proved, not on gelatine or agar containing no sugar or peptone. These facts were sufficient to arrest attention and to make a further study of the organism comparatively simple.

As noted above, the colonies on saccharose-gelatine plates occur in the form of large drops, 1–2 millims. in diameter, at the end of 3 days, and rising about 0·5 millim. above the surface, of a greyish-white colour to the naked eye, and yellowish or tawny under a $\frac{2}{3}$-in. objective, translucent and with a pearly lustre (Plate 14, fig. 2). In texture they are gelatinous and stringy and do not, at first, separate bodily from the gelatine. Later, however, in the course of a week, small colonies come away intact on a platinum needle, indicating a very slow liquefaction of the gelatine immediately beneath the colony. Eventually each colony comes to lie in a shallow pool of liquefied gelatine, while at the same time it gradually loses its well-defined contour, firm consistency, and drop-like form, and appears as a whitish flocculent mass in the liquid. At this later period it is usually found to consist almost entirely of spores.

Before proceeding to describe this organism in detail it will be well to note briefly its gross appearance in tube and plate cultures on this and other media. A streak culture on saccharose-gelatine shows active growth within 48 hours, and its character does not differ essentially from that on the plates except that it is usually flatter and more expanded, and liquefaction proceeds somewhat more rapidly. A stab culture on the same medium shows on the surface, in the course of a week or less, a yellowish-white, circular, flattened growth with fluted edges; very slight growth occurs in the stab. On peptone-gelatine, both plate and streak cultures differ markedly from those on saccharose-gelatine. The colonies on the plates are very much smaller, hardly exceeding the size of a pin-head even after a month's duration, and are opaque and whitish rather than translucent. Streak cultures grow readily

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and liquefy the gelatine rapidly, producing a deep spoon-shaped trough; if the tube is kept erect the flat ribbon-like growth slips down bodily into the liquid gelatine, at the base of the slope in which it lies coiled up and intact, until in the course of a week it disintegrates and forms spores (Plate 14, fig. 4). This firm character of the growth, both in plates and tubes, is characteristic. In stab cultures the growth is similar to that seen in saccharose-gelatine, but the smaller surface-colony soon sinks in and produces eventually a funnel-shaped cavity in which the organism lies in a flocculent mass (Plate 14, figs. 5 and 6).

Cultures on saccharose-agar are very striking. In plate and streak cultures, within a week the organism forms a flat, dense, opaque, expanded, yellowish-white or tawny growth (Plate 14, fig. 3); circular on the plates, of a dull lustre, slightly wrinkled or terraced, and measuring almost a centimetre in diameter (Plate 14, fig. 1). In consistency this growth is neither gelatinous nor viscous, but rather cheesy or waxy. On potato the organism grows rapidly, forming a whitish, slimy coating, resembling condensed milk or white paint, exceedingly viscous, and becoming wrinkled or granular and tawny with age (Plate 14, figs. 7 and 8).

These very dissimilar appearances on various culture media are striking, and certain of the differences might suggest that admixture of foreign germs had occurred; this is not the case, however, for not only do parallel cultures from the same soil-sample show these divergences, but the character of the growth shows the same differences in cross cultures from one medium to another. Thus the cheesy growth seen on agar becomes gelatinous when transferred to saccharose-gelatine, and the viscous slime on potato produces firm, small, circular colonies on peptone-gelatine, and *vice versâ*. The differences appear due in large measure, if not entirely, to the presence or absence of a gelatinous sheath investing the rods and always accompanying vigorous growth in the presence of carbohydrates, especially cane-sugar. This sheath is very evident in fresh cultures from soil, the prominent drop-like colonies being composed of capsuled rods and filaments imbedded in a mass of jelly and forming a beautiful zoogloœa (Plate 15, fig. 13). On potato the gelatinous sheath is also very evident, and gives to the growth its peculiarly viscous character, whereas on peptone-gelatine, and on other solid media devoid of carbohydrates, the sheath is much reduced or entirely wanting, and the growth is neither gelatinous nor viscous. Moreover the gelatinous sheath is a very evanescent character. It gradually disappears with repeated cultures even on saccharose-gelatine, so that, after two or three transfers, the colonies assume the form and size seen on peptone-gelatine; the same thing occurs in a primary culture (that is, one directly from the soil) if allowed to remain undisturbed for two or three weeks or until the gelatine beneath the colony is liquefied thoroughly; and finally, as might be inferred from the latter fact, dilution of the gelatine quickly brings about the solution and disappearance of the sheath.

This process may be observed very readily in hanging-drop cultures in 10 per cent. saccharose-gelatine; it happens only too frequently that the normal liquefaction of

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the gelatine by the growth of the organism is accentuated by the condensation of water on the cover-glass, and in such cases I have often seen a small zoogloea in the denser portion of the drop burst, as it were, and its component rods and chains stream out into the dilute portion of the drop, where they continue their growth without any sign of a firm gelatinous sheath (Plate 15, figs. 2 and 3). As comfirmatory of this fact it may be mentioned that the slight ring and deposit which this organism slowly produces in tubes of broth or saccharose-broth is found to consist of rods and chains the sheaths of which have almost or quite dissolved. It may therefore be concluded that while this organism is normally capsuled in fresh cultures and in certain media containing carbohydrates, and under such circumstances is gelatinous or viscous, this character gradually disappears as the culture ages, as it becomes dilute, or as the organism is subjected to repeated transfers in the same gelatine medium; and further, that on media containing no carbohydrates the gelatinous sheath is very much reduced, if not altogether wanting.

In liquid cultures, as stated above, the organism is not capsuled and growth is very slow. Thus in broth or saccharose-broth at ordinary temperatures, a slight ring is formed in the course of a week, and upon shaking the tube a very faint whitish deposit rises in a swirl from the bottom; it is only later and at higher temperatures, if at all, that there is any turbidity, and under no circumstances is there any trace of a persistent film on the surface. Cultures in milk slowly peptonize it, and in the course of ten days the cultures give an alkaline reaction; there is no coagulation at any period.

Active growth of the organism is dependent on the presence of oxygen. This was tested in a variety of ways. Tubes containing fresh cultures on potato were enclosed in larger tubes, tightly stoppered, and containing in a bulb at the bottom a solution of pyrogallic acid and potassium hydrate, whereby the oxygen was absorbed. Other tubes and plates of saccharose-gelatine, saccharose-agar, and other media, liquid as well as solid, on which the organism was known to grow, were infected and placed under a bell-jar sealed with wax to a ground-glass plate. This was connected with a water-pump and manometer, and the air was exhausted as completely as possible. Growth occurred to a slight extent in both cases, but it was very slow and weak as compared with the growth in duplicate cultures in the air. Both of these methods of absorbing or excluding oxygen are open to objections, and later the cultures were repeated in a more satisfactory manner. A hydrogen generator was connected on one side with a test-tube containing a bit of phosphorus immersed in water under a bell-jar; on the other, with a series of stoppered bottles containing respectively pyrogallic acid and potassium hydrate in solution, argentic nitrate in solution, and distilled water. With the latter was connected a stoppered jar containing tube-cultures, and attached to the aspirator and manometer. After exhausting the air from the jar the hydrogen was allowed to pass into and through it, and this process was repeated four times. The cultures, under these conditions, showed only a very slight growth at the end of ten

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days, while check cultures showed a fairly normal development. These cultures were made from rather old material, and later, when fresh material was again secured, the experiments were tried again with plates and tubes, the former placed under bell-jars and supplied with pure hydrogen as before, the latter enclosed in flasks containing a solution of pyrogallic acid which were then either exhausted for twelve hours continuously, and placed in the incubator at 32° C., or consecutively exhausted and supplied with pure hydrogen, as in the case of the plates. In two days all of the plate-cultures, whether in air or hydrogen, showed equally strong growth (*cf.* Plate 14, figs. 1 and 2). The tube-cultures in air, for some unexplained reason, showed only slight growth after six days, but the growth *in vacuo* or in hydrogen was manifestly still weaker. It is safe to conclude, then, that this organism is a facultative anaërobe, growing equally well *in vacuo*, or in pure hydrogen, as in air, provided only that the cultures are made with fresh vigorous material, and that they are given a suitable environment, such as is secured by the use of solid media containing carbohydrates or peptone, and a temperature of at least 20° C.

We may now pass to a consideration of the organism itself in detail.

Examination of fresh cultures shows that it is a straight or slightly curved bacillus, rod-shaped with rounded ends, and measures 1·2 μ to 1·5 μ in diameter. The length of the individual rods is very variable, but in fresh cultures from the soil or from spores sown in hanging drops, they measure 3·4 μ–7·7 μ. The rods rarely occur isolated, and that only in liquid or old cultures; usually they are united in straight chains or curved chaplets composed of many individuals (Plate 15, fig. 3). If the chain remains straight, fifty or more rods may be united to form it, but if, as generally happens, the chain becomes bent, the angle gradually becomes more and more acute, and presently the chain breaks at that point. This refers more particularly to growth in liquid or dilute cultures. In more solid media, such as 10 per cent. saccharose-gelatine, it seldom happens that even three rods form in a straight line; the result is the formation of a little colony composed of more or less isolated rods imbedded in a common mass of jelly. The occurrence of capsuled rods in gelatine cultures has already been mentioned (Plate 15, fig. 13). In case the gelatine becomes diluted, the jelly disappears and long filaments of naked rods are formed. Growth is intercalary as well as terminal, a septum forming in the middle of a rod each half of which thereupon grows to the original size of the parent rod, that is, in the case of vigorously vegetative rods. As the culture ages and the period of spore-formation approaches, intercallary growth gradually ceases, so that before the spores are actually formed the length of the rods is only about twice their width, or even less (Plate 16, fig. 3).

In describing the morphology of this bacillus, attention should be drawn to a marked difference in the habit of growth between surface colonies and colonies partially or wholly submerged in the gelatine. In hanging drops of peptone-gelatine, for example, a colony may frequently be observed to develop on the surface of the

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drop; in which case, instead of being rounded and gelatinous, it presents the appearance of a mycelial growth, which, under a high power, is seen to consist of long filaments arranged in parallel strands (Plate 15, figs. 9 and 10). Twenty-four hours later such a colony shows the appearance seen in fig. 11, at which time the filaments, while showing no evidence of septation except when stained, are breaking up into their component rods. In 48 hours the filaments are distinctly septate (Plate 16, fig. 1), and later they disintegrate completely and form spores as usual. Plate 15, fig. 12, shows a submerged gelatinous colony which, on coming to the surface of the gelatine, at once proceeds to deliquesce, as it were, and to produce the same twisted strands of parallel, septate filaments.

The formation and the germination of the spores can be followed without much difficulty in hanging-drop cultures in saccharose-gelatine, or, better still, in saccharose-broth. Throughout these cultures I used the form of cell originally devised by Marshall Ward,* with the single modification of the substitution of vaseline for gelatine as a means of sealing the cover-glass to the cell; this renders easier the removal and cleaning of the cover-glass in case a permanent preparation of the culture *in situ* is desired. For a like reason, I used, in all my later cultures, 5 per cent. saccharose-broth, in place of the saccharose-gelatine which I had used at first. The only disadvantage attending the use of a liquid-drop is that it is liable to become diluted through the condensation of water on the cover-glass, sometimes to such an extent as to cause it to run down the side of the cell. However, by allowing the heated slide bearing the cell to become perfectly cool before placing the cover-glass in position, and by applying the drop while the sterilized cover-glass is still decidedly warm, and at once placing the latter in position, the drop can usually be kept intact. A culture of this kind can be kept for ten days or two weeks, and if a preparation of it is desired, it is only necessary to remove the cover-glass, wipe off the vaseline carefully, dilute the culture with a drop of water in order to distribute the contents in a thin layer, dry, stain, and mount as usual.

To secure the early stages of germination, hanging-drop cultures in saccharose-gelatine or saccharose-broth were prepared from tube-cultures on saccharose-agar a week or ten days old. These could be relied upon to consist of practically nothing but spores. In some cases the sowing of spores only was ensured by transferring a bit of the culture to a tube of distilled water, heating it to 80° C., and pouring a few drops of this into a tube of saccharose-broth or other medium from which the drop cultures were then made. Usually, however, quite as good results were obtained by infecting the saccharose-broth directly, and not heating. Some difficulty was experienced in so timing the cultures that the germinating spores could be observed by daylight, for the time required for germination varies considerably according to the ripeness of the spores, the character of the medium in which they are sown, and the temperature. Moreover, the development subsequent to germination is rapid,

* 'Phil. Trans.,' B, 1892, vol. 183, p. 131.

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so that it frequently happens that spores sown late in the afternoon, and kept at the ordinary temperature of the laboratory, have, by the next morning, developed into chains consisting of several rods. The best results were obtained by starting the cultures early in the afternoon, keeping them at a low temperature (10°–12° C.) all night, and, in the morning, transferring them to the stage of a microscope enclosed in a warm chamber, which gave a temperature very slowly rising from 18° C. to 22° C. By this means were secured the various stages of germination in peptone-gelatine figured on Plate 15, figs. 4 and 5. In two hours after sowing the elliptical spore, surrounded by a delicate gelatinous sheath, begins to swell and assume a more nearly spherical shape. An hour later, the spore has become ovoid and the wall, at the more pointed end, is distinctly thinner. If at this stage the culture is placed at a low temperature, growth is checked, only to be renewed with greater activity the following morning as soon as the culture is placed in the warm chamber. It will now be observed that the spore has become pear-shaped, the thinner end having been pushed out, like the tip of a glove-finger, to form the first rod. In the course of an hour this growing tip is as long as the spore, and in about two hours the length has doubled. Thus far, there is no sign of septation; the inner wall of the spore has simply grown out into a non-septate germ-tube, nor are any thick sheaths visible in this peptone-gelatine culture.

The observations on this particular cell were interrupted at this point, but they were continued upon another cell of the same series—Plate 15, fig. 5. At 10.15 A.M., the primary rod still attached to the spore presented the appearance seen at (*a*). At 10.45 it had increased in length, and a very delicate septum was visible, dividing it into two equal parts (*b*); at 11.45 the septum was very distinct, and the wall of the rod was slightly contracted at that point (*d*); at 12.15 P.M. the whole rod had turned and was growing downward toward the lower surface of the gelatine drop (*e*); and at 1 P.M. the distal half of the rod had again divided (*f*). Shortly afterwards the fully formed young rods must have begun to separate, for at 3 o'clock a little colony of six rods had formed (*g*), and an hour later seven rods could be made out (*h*). What became of the spore-wall I was unable to determine. At 3 o'clock it was visible, still attached to the initial rod, but an hour later it had either been cast off and had disappeared, or lay below the plane in focus. Forty hours later a dense little colony of rods had formed (fig. 6) which, in the course of three days, had become transformed into spores (fig. 7). Fig. 8 shows the spores more highly magnified.

In such a culture as I have described it is manifestly very difficult, if not impossible, to form an accurate estimate of the rate of growth, since the rods do not remain in straight chains, but break apart and form a dense colony. In a drop of 5 per cent. saccharose-broth, however, the rods are not capsuled, and form long chains, thus admitting of direct measurements of growth. The method pursued was to place drop-cultures of pure spores at different degrees of temperature, allow them to germinate, and then take consecutive measurements of a single rod, or of a short

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chain, until its length had doubled. The time required for this process gives the average rate of growth. It is, of course, advisable to select for this purpose only young, vigorous rods, since, as already stated, growth becomes slower as the period of spore-formation approaches; in all cases, therefore, my observations were made, if possible, upon single, isolated rods, shortly after their emission from the spores.

On December 2nd, at 8 A.M., a number of hanging drop-cultures of pure spores were made. Most of them were at once placed in the incubator at 24° C., one was placed under the microscope in a warm chamber at 22¼° C., and one was kept at the ordinary temperature of the laboratory (about 20° C.). At 9.15 A.M., on the following day, most of the spores at 22¼° C. had germinated, and in most cases produced long, twisted chains; there were, however, plenty of isolated rods, the only difficulty being to select one which would remain in one plane long enough to allow of accurate and consecutive measurements, for, although no evidence had presented itself in any of my cultures hitherto to suggest that the organism with which I was dealing was other than non-motile, nevertheless the young rods in this liquid culture did not remain continuously either in the same plane or in the same position in the field.* This I attributed at the time to the action of diffusion currents set up by the gradual dilution of the drop by water condensing on the cover-glass. After many failures, and much loss of time, the following measurements of a pair of rods were obtained, 30½ hours after sowing the spores:—

TABLE I.

Time.	Temperature.	Length.	Increment.	Remarks.
P.M.	°C.	μ.	μ.	
2.35	22·25	17·4	...	
2.45	...	19·5	2·1	The rods separated.
2.49	...	...	...	Continued measurements of rod A.
2.51	22·5	8·9	...	
2.55	...	9·7	0·8	
3.03	22·5	13·0	3·3	
3.05	...	...	...	Septum formed.
3.15	...	15·2	2·2	
3.25	...	16·3	1·1	
3.35	...	17·4	1·1	
3.45	22·25	18·4	1·0	

Doubled in length at 3.39. DOUBLING PERIOD = 48 MINS.†

* The fact should be noted, in this connection, that the slight ring observable in broth-cultures was always formed at the highest point that the liquid touched on the walls of the tube. This might be taken to indicate a certain degree of motility on the part of the rods.

† By this expression, as originally used by MARSHALL WARD (*cf.* 'Roy. Soc. Proc.,' vol. 58, p. 139), is meant the time required for a rod or chain of rods to double its original length. Where growth is intercalary this represents very accurately the rate of growth.

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The spores kept at the normal temperature of the laboratory germinated much more slowly. When examined at 9 A.M. on December 3rd, 25 hours after sowing, they showed no sign of germination beyond a slight increase in size. The laboratory temperature was subject to marked fluctuation, running at night as low as 12° C., and this doubtless accounts for the retarded germination. As will be seen later, moreover, the minimum temperature of growth in this species is comparatively high, and the difference of a few degrees below the optimum has a very marked influence upon the growth of the rods. It was not until 11 A.M. on December 5th, 75 hours after sowing, that rods and chains were found which could be satisfactorily measured.

The following tables give the doubling period or rate of growth, first of a pair of isolated rods, and secondly of the terminal rod and terminal pair of rods of a fairly long chain.*

TABLE II.

Time.	Temperature.	Length.	Increment.	Remarks.
	°C.	μ.	μ.	
11.15 A.M.	19·75	14·6	...	
11.30	...	16·3	1·7	
11.45	20·25	17·4	1·1	Primary septum forming in each rod.
12.0 M.	...	19·7	2·3	
12.15 P.M.	20·5	20·8	1·1	Primary septa complete.
12.30	...	23·0	2·2	
12.45	20·5	24·7	1·7	
1.0	...	27·5	2·8	Chain consists of four fully formed rods.
1.15	...	29·2	1·7	Terminal rod dividing again.
1.30	20·5	31·0	1·8	Chain consists of five rods.

DOUBLING PERIOD = 2 HOURS.

* In order to eliminate as far as possible the personal element in these observations, the consecutive measurements were recorded only in terms of the micrometer divisions, and were worked out at the close of the observations.

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TABLE III.

Time.	Temperature.	Length.	Increment.	Remarks.
P.M.	°C.	μ.	μ.	
2.05	20·75	A. (Terminal rod) . . 10·3 B. (Terminal pair) . 20·6		
2.20	...	A. . . . . 11·2 B. . . . . 21·5	0·9 0·9	
2.35	20·75	A. . . . . 12·9 B. . . . . 24·0	1·7 2·5	Primary septum forming in each rod.
2.50	...	A. . . . . 13·7 B. . . . . 24·0	0·8 0·0	No measurable growth was observed in B at this period.
3.05	20·0	A. . . . . 15·4 B. . . . . 24·9	1·7 0·9	Secondary septa forming.
3.20	19·75	A. . . . . 16·3 B. . . . . 29·2	0·9 4·3	
3.35	19·75	A. . . . . 17·2 B. . . . . 30·9	0·9 1·7	
3.50	19·75	A. . . . . 18·9 B. . . . . 33·5	1·7 2·6	Incandescent burner lighted.
4.05	...	A. . . . . 20·6 B. . . . . 35·2	1·7 1·7	Each of the original rods has divided into four fully formed rods.
4.35	20·5	A. . . . . 24·0 B. . . . . 37·8	3·4 2·6	
4.50	21·0	A. . . . . 26·7 B. . . . . 40·3	2·7 2·5	
5.05	21·0	A. . . . . 27·5 B. . . . . 45·6	0·8 5·3	

DOUBLING PERIOD OF A. = 2 HOURS.
DOUBLING PERIOD OF B. = 2 HOURS 47 MINS.*

These tables and curves are not particularly striking, but they serve to show graphically (1) that the growth of this bacillus in the medium given is slow at these low temperatures, and (2) the effect upon the rate of growth of a difference of temperature amounting to only about 2 degrees (*cf.* Curves III–A and I). They also show a peculiar rhythmic growth, especially in the case of Curve II, which seems to be connected with cell-division, since it was observed that the appearance of a septum was preceded by a period of retarded growth, and that as soon as the septum was formed more rapid growth ensued. It is also interesting to note in Curves III–A and III–B the acceleration of growth which accompanied the slow rise of temperature caused by lighting an incandescent gas-burner shortly before four o'clock. From these facts it is evident that the rapidity of growth, and therefore, by

* The longer period required for doubling in the case of the pair of rods, as compared with the terminal rod, was due to irregularities which occurred in the rate of growth of the penultimate rod. At times the growth of this rod almost or quite ceased, therefore the doubling period of the terminal rod alone represents more accurately the rate of growth of the organism.

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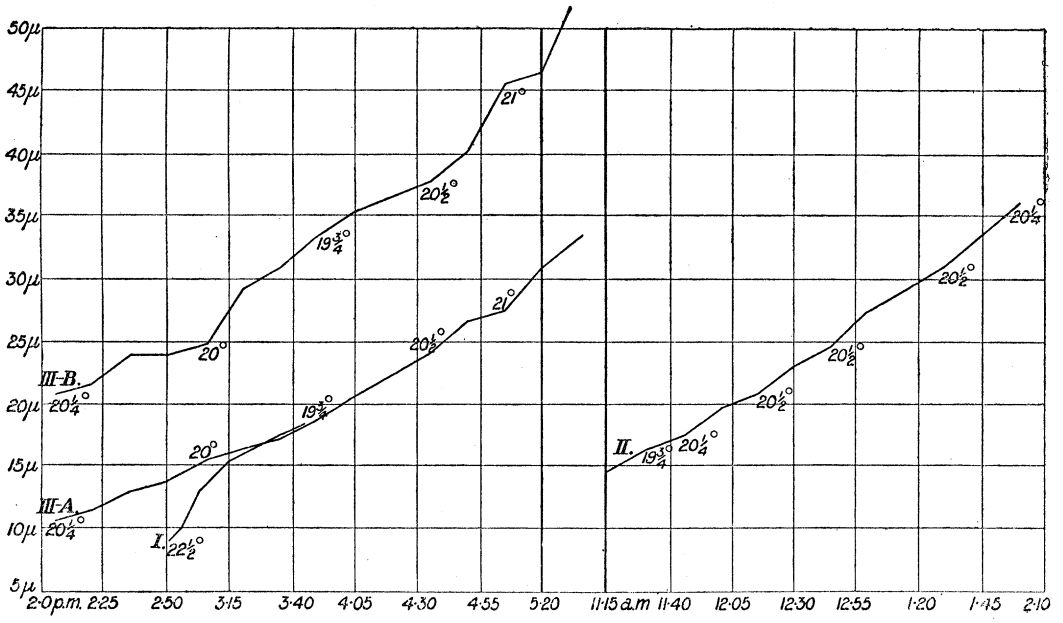
inference, the period required for spore-formation, is dependent upon temperature, and, furthermore, that the optimum is probably nearer 30° C. than 20° C. As to the minimum temperature at which growth occurs, the following facts are of interest, though they are not conclusive.



Growth Curves plotted from Tables I, II, and III.

It has been already stated that an effective means of delaying germination and growth consists in placing cultures at a temperature of 10° C.; this fact was repeatedly taken advantage of in my observations on germination. It was also very noticeable that with the approach of cold weather the organism developed much more slowly in cultures kept at the rather low temperature of the laboratory, and also that, under these conditions, instead of producing spores in great abundance in the course of a few days, the individual rods became swollen and transformed into most remarkable involution forms. During the early part of December, the exceptionally warm weather which had prevailed continuously, suddenly changed, and for two nights the ground froze to the depth of about an inch. Attempts to secure a fresh supply of the organism from the soil after this cold spell either failed entirely, or resulted in the very slow formation, on plates of saccharose-gelatine of pearly, drop-like colonies, identical in appearance with those obtained during warmer weather, but consisting almost entirely of involution forms. From such observations it seems safe to infer that the minimum temperature consistent with active growth is in the neighbourhood of 15° C., and that the most rapid growth takes place at a temperature not lower than 33° C. The spores themselves are very resistant to high temperatures, germinating quite as well after being subjected, in distilled water, to a temperature of 99° C. for five minutes, as when not heated.

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The formation of spores in drop-cultures proceeds with considerable rapidity under favourable conditions, as is seen by the following records :—Fresh spores from a slant culture on saccharose-agar, heated to 80° C., and sown in a drop of saccharose-gelatine at 19·5° C., produced ripe spores in 70½ hours; in saccharose-broth at 23° C., the time required was 64 hours; in saccharose-broth at 21° C.–23·5° C., 74 hours; on peptone-gelatine at 15° C., 93 hours. These figures, with the exception of the first set, are only approximate, since in most cases the spores were formed at night and the period had to be reckoned from the hour of sowing to the hour when the first observations were made in the morning.

The details of spore-formation are figured in Plate 16, fig. 3, *a*–*i*, and were obtained by making a dozen or more cell cultures in saccharose-broth, placing them in the incubator at 23° C., and making consecutive mountings of them at intervals of two hours or more. The cover slip was lifted, the vaseline carefully removed, and, after diluting the culture with a drop of water, it was set aside to dry. When perfectly dry it was passed through the flame and stained in hot carbol-fuchsin, aniline-gentian violet, or by GRAM'S method. By this means, all of the stages of spore-formation could be very satisfactorily followed. As already noted, the growth in length of the individual rods becomes gradually less as the period of spore-formation approaches, until, just before the spores begin to form, the length hardly exceeds the diameter, and the formation of the final septum is not accompanied generally by an actual separation of the parent rod into two distinct rods. This is seen very clearly in fig. 1, *i*, where, before staining, the chain appeared to be composed of cylindrical rods with rounded ends and of the normal length, each of which had produced two spores. Staining, however, brings out the true condition of things, and shows that complete separation has not taken place, and that each rod is very short and has produced but one spore. That separation between the ultimate rods sometimes occurs is seen by reference to fig. 3, g^1.

The first stage in spore-formation is the peripheral condensation of the previously homogeneous protoplasm (fig. 3, *a*–*c*). Although, in some cases, the peripheral distribution of the protoplasm is almost complete, in others it shows a tendency to collect in small masses in the corners of the rods (fig. 3, *c*). The peripheral layer, whether complete or not, now begins to collect gradually at the distal end of the rod (fig. 3, *d* and *e*), but portions of it are always left behind either as small masses on the wall or as a deposit on the septum (fig. 3, *d*). Occasionally the portion which is not to take part in the formation of the spore remains for a time distributed evenly in the interior of the rod (fig. 3, *e* and *f*), but eventually this too collects in the neighbourhood of the septum. If complete division of the rods has preceded spore-formation the remnant of the protoplasm collects in a small mass or layer at the proximal end, that is, the end next to the last septum, and the spore itself always forms at the distal end (fig. 3, g^1, g^2, and g^3). Gradually the bulk of the protoplasm collects at this end, becomes more dense, and finally forms an oval, highly refringent

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spore, measuring, in unstained specimens, 2–2·8 $\mu \times 0{\cdot}8$–1 μ. It is difficult to say how the spore is freed from the delicate investing rod. It seems to be rather by the dissolution of the wall than by its rupture, and in carefully stained preparations examined under a homogeneous immersion it is seen that the spore makes its escape at a point where the extremely delicate wall of the rod is devoid of the traces of protoplasmic matter which partially line it (fig. 3, *j*). At *k*, fig. 3, is seen a spore just issuing from what was evidently a pair of united rods; from one of the latter the spore has already issued and the wall of the rod, together with the septum, has almost disappeared; from a thin point in the wall of the other rod the spore is just issuing.

We must now pass to the consideration of an extremely interesting and rather surprising stage in the life-history of this organism. Up to the time when a liquid medium was substituted for gelatine in the case of the cell-cultures, there was, as has been said, little, if any evidence to show that the organism was other than non-motile. On November 26th a dozen cell-cultures of fresh spores were made in saccharose-broth, at 5 P.M., for the purpose of securing material illustrative of germination and spore-formation. These were placed for the night in an incubator at 23° C. On examining the cells the next morning at 9 o'clock every drop was found to contain numbers of rods, either isolated or united in chains of 2–10 individuals, and in appreciably active motion. The motility was confined to the isolated rods and short chains, and consisted of a rather rapid progression either forwards or backwards, accompanied by undulating motions of the rod or chain, and slow rotation about its long axis. The undulating and progressive motions of the isolated rods were continuous, steady, and fairly rapid, but gradually, as the rod divided and formed a chain of two united rods, the activity became less marked and was largely confined to the forward rod, which swam about dragging after it its almost quiescent companion. By the time three rods had formed, the progressive movements had become very slow, and when the chains of rods had increased to four, motility was in most cases visible only as a slow waving from side to side on the part of the terminal rod or an equally slow rotation of the chain on its long axis. Within an hour long chains had formed and there was no further sign of motility. I was utterly at a loss to reconcile these observations with what I knew of the organism so far, and I was driven to the conclusion that in preparing the cells I had used a contaminated broth. Nevertheless one important fact militated against this theory. After the cultures were made, examination showed that every drop contained from 20 to 100 or more spores; when examined the next morning very few spores were visible in any of the drops, while every one showed an abundance of motile rods. The whole experiment was at once repeated under as nearly as possible the same conditions, but without success, because the spores sown in the evening had, by the following morning, produced long chains. Subsequently I was enabled to substantiate my previous observations. On December 26th a plate-culture on saccharose-gelatine,

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prepared three days previously from a sample of soil, showed a number of colonies, perfectly normal in appearance, but consisting almost entirely of involution forms. Desiring to ascertain the ultimate fate of these peculiar forms and to see if they revived and grew further, I made hanging-drop cultures of them in saccharose-broth at 4 P.M. and placed them in the incubator at 23·5° C. On examining them at 9.30 the next morning most of them were found to have grown out to normal rods or very short chains, all of which showed the same progressive, undulating, and rotary movements which I had observed before in rods freshly developed from spores. Another series of cells prepared with involution forms, at 11.30 P.M., December 27th, and placed at 22·5° C., showed motile rods 10 hours later. In both of these series active motility became gradually less, until it ceased entirely, about 24 hours after the cultures were made. Finally a tube of broth inoculated with fresh spores on December 30th, at 5 P.M., and kept at 23° C., showed at 9 A.M. the next day a slight turbidity, which was found to consist of actively motile rods and chains. The latter retained their motility longer than I had ever observed before, chains of seven or even eight rods still exhibiting slight undulating and rotary motions at the end of 12 hours. These observations are important, since the fact of motility might very easily be overlooked, confined, as it is, to the youngest stages of the rods and to a comparatively brief period of time, and to special media.

In old cultures or in cultures made under unfavourable conditions, involution forms are produced in great numbers and astonishing varieties. Plate 16, fig. 6 shows a number of these forms from a culture six weeks old on saccharose-agar at 33°. The culture contains a few swollen but almost normal rods of the form usually preceding spore-formation, (*b*), but most of the rods and filaments have become irregularly swollen, often into long fusiform, inflated, or drum-stick bodies. In these altered filaments no septation can be seen; the contents are coarsely granular, and include, especially in the swollen portions, roundish bodies which stain very deeply with carbol-fuchsin. They show no blue colour with iodine, and are probably oil-drops. At *c*, *d*, and *e* are seen single and paired rods which have become immensely swollen, and bear no resemblance whatever to normal rods. At α is figured one of the drum-stick forms which shows two ripe spores, separated by a very delicate septum, at the end of the filamentous portion. Evidently these spores were formed while the conditions were still favourable and before the filaments became transformed into involution forms. That many, at least, of these forms are capable, under altered conditions, of reverting to their normal form and of continuing growth in a perfectly characteristic manner, was repeatedly proved by transferring them to suitable media. Similar involution forms were produced in plate-cultures on saccharose-gelatine, prepared directly from the soil during cold weather, and kept at 10·16° C. In these cases growth was extremely slow. Plates prepared on December 20th showed, in the course of three days, one or two minute translucent drops on the surface, but it was not until the 26th that growth was sufficiently advanced to admit of accurate

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examination. On that date one of the plates showed a number of the familiar domed pearly colonies characteristic of this species; one of these colonies was gelatinous and proved to be composed of normal, slightly capsuled rods. Most of the others, however, though precisely the same in appearance, were found to consist of swollen involution forms produced from single rods or very short chains; these colonies were not gelatinous, but slimy, and spread out over the surface of the gelatine when touched with a needle. Some of these involution forms are shown in fig. 4 of Plate 16, and more highly magnified in fig. 7. In some cases the granular contents have undergone partial plasmolysis and have collected in masses within the rods, in others this has not occurred. At *a*, fig. 4, is seen a pair of rods, one of which remains normal, while the other has become converted into a swollen involution form. Such cases were not uncommon. Drop-cultures of these forms were made in saccharose-broth at 4 P.M. on December 26th, and placed in a warm chamber at 22° C. The following morning at 9.30 many of the involution forms had become normal motile rods, and it was not difficult to follow the process. The large granules occupying the interior become gradually smaller, while, at the same time, if plasmolysis has not gone too far, the masses of protoplosm slowly fuse together. The coarse granulation becomes less and less apparent, and seems to be due to the flowing together of oil-drops; the rod contracts in width and increases in length, and at last presents the normal appearance of a cylindrical rod with rounded ends and hyaline contents, which thereupon moves away with increasingly active motions. The tube of saccharose-broth from which these drop-cultures were made was kept all night at the temperature of the laboratory, and when examined the next norning was found to be filled with the less advanced forms figured on Plate 16, fig. 5.

We are now in a position to summarise, in schedule form, our knowledge of this interesting organism, and to compare it with known species.

Habitat.

In clayey soil; uniformly in samples taken at a depth of $\frac{1}{2}$–1 inch below the surface.

Morphology.

Straight or slightly curved rods, measuring 3·4–7·7 μ × 1·2–1·5 μ, with rounded ends and hyaline or granular contents; provided with a firm gelatinous sheath on solid media containing carbohydrates; otherwise almost naked. Isolated, or in filaments composed of 2–50 or more individuals. Rods or short chains, developed freshly from spores or involution forms sown in liquid media, exhibit motility for a few hours. Produces, at one end of the rod, an oval, highly refringent spore, measuring 2–2·8 μ × 0·8–1 μ, and usually occupying an oblique position. Involution forms common.

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Growth.

Active only in acid media. Neutralizing the medium retards the growth.

Saccharose-gelatine at 15° *C.*

Plates.—Visible within 48 hours. In 3 days forms pearly, domed, translucent, circular colonies, 1–2 millims. in diameter and 0·5–1 millim. high. Gelatinous or viscous. Rods capsuled. Slowly liquefying the gelatine after the third day, and finally disintegrating and forming a slimy, whitish flocculence in the liquefied gelatine.

Streak.—Forms in 2–4 days a pearly, translucent growth all along the streak. Either at first rising above the surface, or immediately sinking into it. Not spreading. Slowly liquefies, forming a spoon-shaped trough, and finally slipping down and forming a flocculent, whitish deposit in the liquid gelatine.

Stab.—Forms in a week or less a whitish, almost flat, circular colony, 5–8 millims. in diameter and with fluted edges, which slowly liquefies, the gelatine forming a funnel-shaped cavity. Slight growth to full depth of stab, but no liquefaction below the surface.

Saccharose-agar at 15° *C.*

Plates.—In 2–3 days forms circular, flat, yellowish-white or tawny, opaque colonies, with a shiny surface, and attaining a diameter of 5 millims. to 1 centim. Later, wrinkled and terraced or zoned. Cheesy or waxy in consistency, and emitting a strong, fœtid odour exactly resembling melted glue.

Streak.—Rapid growth. Forms in 2–3 days a fine growth, 5–8 millims. broad; flat, shiny, pale yellowish-white, opaque, at first very finely wrinkled or marked with cross folds, later smooth, and becoming wrinkled at the edges. Collecting as a dirty-white deposit at the base of the slant. Forms spores in 4–6 days.

Saccharose-agar at 23° *C. and* 33° *C.*

At these temperatures growth is of the same general character, but much more rapid, large colonies in process of spore-formation occurring within 24 hours. The substitution of glucose for saccharose has no appreciable difference in effect. Neutralizing the agar retards the growth decidedly.

Peptone-gelatine at 15° *C.*

Plates.—Forms in 2 days very small, whitish, more or less opaque, non-gelatinous colonies, which liquefy the gelatine rapidly, giving to the surface, at first, a pitted or pock-marked appearance. Rods naked or provided with a very thin sheath. Liquefying power increased by cultivation.

Streak.—Growth rapid; forming in 24–48 hours a flat, whitish ribbon, which

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speedily liquefies the gelatine and slips down bodily in the form of a coil lying in the liquefied gelatine.

Stab.—Produces a small, circular, flat, and whitish growth on the surface, and a slight growth to the full depth of the stab. Liquefaction proceeds rapidly at the surface in the form of a funnel-shaped cavity, in which the organism forms eventually a whitish flocculence.

Peptone-agar at 23° C. and 32° C.

Plates.—Not differing essentially from the growth on saccharose-agar at these temperatures, but development more rapid. Spores formed in 22 hours. Colonies attaining a diameter of 5 millims.

Streak.—Forms in 2 days a thin, yellowish-white, opaque growth, spreading evenly on both sides of the streak. At first smooth and shiny, later becoming very finely wrinkled and marked with cross-folds.

Broth at 15° C.

In 4 days forms a very slight ring and a light, whitish deposit. No surface film and no turbidity.

Broth at 23° C. and 32° C.

In 24 hours after sowing spores the liquid is distinctly turbid and contains motile rods and filaments. In 3 days there is a slight ring, but no film or deposit. Later, a rather heavy, whitish, flocculent deposit forms. Much less growth in saccharose-broth at all temperatures. Slight ring and no turbidity in 3 days.

Potato at 15° C.

Growth visible in 24 hours as a moist, shiny, whitish film, which spreads rapidly. In 48 hours resembling condensed milk; extremely viscous. Later becoming thrown into wrinkles or anastomosing folds. In 1–2 weeks yellowish-white, slimy. In 2–3 weeks becoming tawny in colour, dull and waxy or granular in appearance, and cheesy in consistency.

Potato at 23° C. and 32° C.

Growth more rapid, becoming yellowish, wrinkled and cheesy in 4–7 days. Involution forms or spores are produced in 2–4 days.

Growth is much neater in habit in the presence of a 10 per cent. saccharose solution than when only distilled water is used to keep the potato moist.

Milk.

In 1–2 weeks the milk becomes transparent and of a rich reddish-brown colour. There is peptonisation but no coagulation. The culture gives a decided alkaline

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reaction. These effects are produced more rapidly at temperatures between 20° C. and 33° C. than at ordinary temperatures.

Requirements as to Oxygen.

Aërobic. Facultative anaërobic in various degrees, according to the character of the culture medium and the temperature.

Spore-formation.

Produces spores readily in all media at 15° C., 23° C., and 32° C., within 4 days. On peptone-agar plates, at 32° C., spores are formed in 22 hours; in saccharose-gelatine drops, at 19·5° C., in 70 hours; in saccharose-broth drops, at 23° C., in 64 hours. Each rod produces obliquely at one end a single, oval, refringent spore, 2–2·8 $\mu \times 0{\cdot}8$–1 μ, which germinates in a direction parallel to the longer axis.

Involution Forms.

Produced abundantly under conditions unfavourable to growth, as in old cultures, and in cultures near the maximum or minimum degrees of temperature.

Temperature.

Optimum temperature about 23°–25° C. At higher temperatures growth is more rapid, but there is a tendency for the rods to pass over into involution forms. The same tendency is observable at temperatures below 15° C. Ripe spores are resistant to a temperature of 100° C., maintained for 5 minutes.

Reaction to Stains.

Rods and spores stain deeply with hot carbol-fuchsin, aniline-gentian-violet, and by GRAM'S method. No blue colour with iodine.

Pathogenicity.

Non-pathogenic to guinea-pigs.*

Gas.

No sign of gas formation in saccharine solutions, or when submerged in gelatine and sugar media.

In very young cultures, six to eight hours at 35° C., it has been possible to obtain the proof of the existence of cilia by ERMENGEM'S silver method.† As shown in

* The pathogenicity of this species was kindly tested for me by Dr. PIGG, of the Pathological Laboratory, Camb.

† 'See MIGULA, 'Syst. d. Bakterien,' vol. 1, p. 105.

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fig. 14, Plate II., there are about eight to a dozen peritrichous cilia. These are extremely fugacious, and no evidence of their presence could be obtained in cultures older than twelve hours. These cilia afford still further evidence of the alliance of this bacillus to *B. Megatherium*.

Although we possess such full details regarding this species, I find great difficulty in placing it under any known species. Referring to EISENBERG's treatise, 'Bakteriologische Diagnostik,' it evidently falls into the Bacillus group of the non-pathogenic, liquefying bacteria which produce no pigment. Among the thirty-seven species therein described only three, or possibly four, are comparable with it. These are as follows:—*Bacillus aërophilus*, LIBORIUS: This species resembles mine in some respects morphologically, in its behaviour on potato and on gelatine streak-cultures, and in the formation of the spores, but its obligate aërobic character, colour, mode of liquefaction, and other details seem to mark it as different. *B. Megatherium*, DE BARY: This species approaches mine much more closely, and the character of its motility, mode of germination, aërobic nature and other characters do not distinguish it. It appears certain that the two are in any case very closely allied, but DE BARY'S form has never been sufficiently diagnosed for us to be certain. *B. tumescens*, ZOPF.: This species again seems to bear some resemblance to mine, but the diagnosis is insufficient and might apply equally well to a number of forms. The behaviour of my species in some respects constantly recalled that of the well-known *B. subtilis*, EHR., but the formation of a superficial film in liquid cultures, the germination of the spore at right angles to the longer axis, the more rapid movements, &c., essentially characteristic of that species, are never seen in mine. The descriptions and figures of *B. vulgatus* (FLÜGGE), MIG., and *B. mesentericus* (FLÜGGE), LEHM. and NEUM., as given in LEHMANN and NEUMANN'S 'Atlas und Grundriss der Bakteriologie,' show that these species, especially the latter, bear marked resemblances to mine. The behaviour of *B. mesentericus* on gelatine and agar plates, in streak and stab cultures and, above all, in old potato cultures, is very similar to that of the form which I have studied. It is only when we come to liquid cultures that we find a marked difference. In the case of the two species above mentioned, the first forms on the surface of broth, "ein festes, grauweisses Häutchen, welches sich beim Schütteln nicht zerteilen lässt," and the second is thus described, "Mässig getrübt, auf der Oberfläche ein Häutchen." In broth-cultures of my species there is no sign of a surface-film, either after long cultivation or in cultures fresh from the soil. Furthermore, my species forms, in streak cultures on agar, a dense, whitish deposit, completely filling the water of condensation at the base of the slope, while in similar cultures of *B. vulgatus* and *B. mesentericus* the water remains clear, and a firm membrane forms on its surface. This, of course, merely indicates a difference in the demands which the organisms make upon free oxygen. How far this is a constant and invariable specific mark we are not, as yet, in a position to state with certainty, but, for the present, we must conclude that my species is differentiated by this partially anaërobic character from either of the two

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species mentioned above. Finally, we must consider a species described by RUSSELL under the name *Bacillus granulosus.** I have not seen RUSSELL'S original description, but, as given by STERNBERG, it agrees very closely with that of my species. The morphology (measurements not given) is practically the same, and it behaves in a similar manner in both solid and liquid culture-media, but the motility is far less pronounced than in my species, and spores are formed in bodies resembling my involution forms, and not, as I infer from the description and figures, in the normal rods.

DE BARY found his type form in boiled cabbage, but it has also been found in water,† and it is noteworthy that the soil whence the present form was isolated had grown cabbages this year. The present species was accordingly sown in sterilised infusion of cabbage, where it formed a rapid growth of the typical motile rods, 1·5 μ thick, and collecting in flocks and in a white ring above. It also forms a dense white pasty growth on pieces of boiled cabbage. No SH_2 is evolved.

B. Megatherium is given by DE BARY as 2·5 μ in thickness, but several observers have found it much thinner, *e.g.*, SACCARDO‡ gives 1·37–2·5 μ, and LEHMANN and NEUMANN§ state that their measurements afford "ein sicherer Beweiss dass der Organismus durch die lange Kultur kleiner wird." They obtained it 0·6–08 μ only in thickness. This is not so astonishing now as it would have been a few years ago, since *B. subtilis* has been shown to vary from 0·8–1·2 μ in thickness,‖ and MIGULA¶ found *B. oxalaticus* to vary between 2·5 and 4 μ in thickness, while LEHMANN and NEUMANN** obtained it as thin as 0·8–1·6 μ, all of which merely goes to show that we can no more rely on the thickness of Bacteria than we can on that of Fungi, since KLEBS has shown how closely connected the diameters of hyphæ are with the food materials.††

The peculiar motility, the tendency to form the spores in the terminal parts of the rods, and the great tendency to produce involution forms are further points of agreement with *B. Megatherium.*

On the other hand the present form is less curved, and there are differences in the details of spore-formation and germination; the differences in size of the spores no doubt go with those of the size of the rods.

DE BARY does not describe a gelatinous sheath, but some of his figures suggest that a "capsule" is occasionally present.

* *Cf.* 'Sternberg Manual of Bacteriology,' 1892, p. 711.
† LUSTIG, 'Bakteriologische Diagnostik des Wassers,' p. 101.
‡ 'Sylloge Fungorum,' vol. 8, p. 972.
§ 'Bakt. Diagnostik,' p. 295.
‖ BUCHNER. ZOPH, 'Die Spaltpilze," p. 28.
¶ MIGULA. 'Arbeiten des Backt. Inst. d. Grossh. Hochschule zu Karlsruhe,' 1894, p. 1.
** *Loc. cit.*, p. 297.
†† KLEBS, 'Die Bedingungen der Fortpflanzung einiger Algen und Pilze.'

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LEHMANN and NEUMANN* have pointed out the remarkably close affinities which exist between *B. subtilis*, *B. Megatherium*, and the various forms of "potato bacilli," *B. mesentericus*, &c., and it is worthy of note that some of the latter form gelatinous sheaths when cultivated in media containing sugar.

The close resemblances between *B. subtilis*, *B. Megatherium*, and *B. mesentericus* in gelatine plate-cultures have been insisted on by LEHMANN and NEUMANN,† and it only remains to point out that the present form only differs in minute details from *B. Megatherium* when grown on peptone-gelatine; this refers to stab and streak cultures as well as plates.

On plates of peptone-agar the general resemblances to *B. Megatherium* are obvious, but whether the glue-like odour is a sufficient difference remains to be seen. The streak-cultures on peptone-agar are similar in colour, folds, and consistency to *B. subtilis* and *B. Megatherium*.

As regards broth cultures, it would seem that the marked folded pellicle formed by *B. subtilis* and *B. mesentericus* should distinguish them from *B. Megatherium* and the present species, which behave similarly in broth, forming a slight veil and hardly perceptible turbidity at most.

On potato *B. subtilis* is generally distinguished by its dull and powdery growth from *B. mesentericus*, which forms a somewhat shiny, slimy, and eventually folded expansion; and *B. Megatherium* resembles the former. It will be observed that the present form presents on potato the same yellowish, slimy, early growth as the above, and later becomes wrinkled and folded like *B. mesentericus*, and eventually powdery and mealy as *B. subtilis*.

B. Megatherium is said by LEHMANN and NEUMANN to coagulate milk with a feebly alkaline reaction, and *B. subtilis* and *B. mesentericus* similarly; with the exception that no obvious coagulum was observed, the present species behaves somewhat in the same manner.

LEHMANN and NEUMANN also found that *B. Megatherium*, *B. subtilis*, and *B. mesentericus* are all facultative anaërobes, again agreeing with the present species. All colour by GRAM'S method, and all agree in forming no gas in sugar solutions.

In view of these facts it seems clear that the form which is the subject of these notes bears resemblances to the group of common soil bacilli, *B. Megatherium*, and to *Bacillus granulosus*, RUSS., but its precise systematic position remains to be determined by the future investigation of related species. The objection that DE BARY described his *B. Megatherium*‡ as 2·5 μ thick can be met by the reply that other observers have found it very variable in diameter.

In order to avoid the inconvenience of referring to this form as a mere variety of

* *Loc. cit.*, pp. 280, 295-6, 297–301.

† *Loc. cit.*, p. 295.

‡ 'Morph. and Biol. of Fungi, &c.,' p. 463. (There is a misprint in the size.)

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its group, it may be advisable to give it a distinctive name. Its habitat suggests the specific name *hortulensis*, and it may therefore be called *B. hortulensis* (STURG.).

In conclusion, I desire to record my great indebtedness to Professor MARSHALL WARD, to whose constant advice and assistance these notes owe any degree of value which they may be found to possess.

DESCRIPTION OF PLATES.

PLATE 14.

Fig. 1. Plate-culture on peptone-agar, four days old. Grown in air, at 23° C. About two-thirds natural size.

Fig. 2. Plate-culture on yeast-extract-saccharose-gelatine, four days old. Grown in hydrogen at 20° C. About two-thirds natural size.

Fig. 3. Streak-culture on saccharose-agar, twelve days old. Grown in air at 17° C. Natural size.

Fig. 4. Streak-culture on peptone-gelatine, four days old. Grown in air at 17° C. Tube kept upright. Natural size.

Fig. 5. Stab-culture in peptone-gelatine, four days old. Grown in air at 17° C. Natural size.

Fig. 6. The same, twelve days old.

Fig. 7. Streak-culture on potato, moistened with a 10 per cent. saccharose solution; one week old. Grown in air at 17° C. Slightly reduced.

Fig. 8. Streak-culture on potato, moistened with distilled water; three weeks old. Grown in air at 17° C. About natural size.

PLATE 15.

Fig. 1. Normal gelatinous colony on saccharose-gelatine in hanging-drop. Two days old. × 154.

Fig. 2. Colonies from a similar culture, in which the gelatine has become diluted with water. Two days old. × 154.

Fig. 3. Rods from the thinner portion of the colonies shown in fig. 2. × 650.

Fig. 4. Ripe spores sown in a hanging-drop of peptone-gelatine at noon, November 18. Temperature 17° C., *a* drawn at 2.30 P.M., *b* at 4.0; *c* at 9.30 A.M., November 19, after being kept all night at 10° C.; *d* at 11.30 A.M.

Fig. 5. A similar series: *a* drawn November 19, at 10.15 A.M.: *b* at 10.45; *c* at 11.15; *d* at 11.45; *e* at 12.15 P.M.; *f* at 1.0; *g* at 3.0; *h* at 4.0.

Fig. 6. Colony produced from the series shown in fig. 5, three days after the sowing of the spores. × 440.

Fig. 7. The same colony three days later; consisting entirely of spores. × 440.

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PLATE 16.

Fig. 1. The colony figured on Plate 14, fig. 11, drawn November 28. × 440. The filaments forming the strands now show septation, and in places are breaking up into their component rods.

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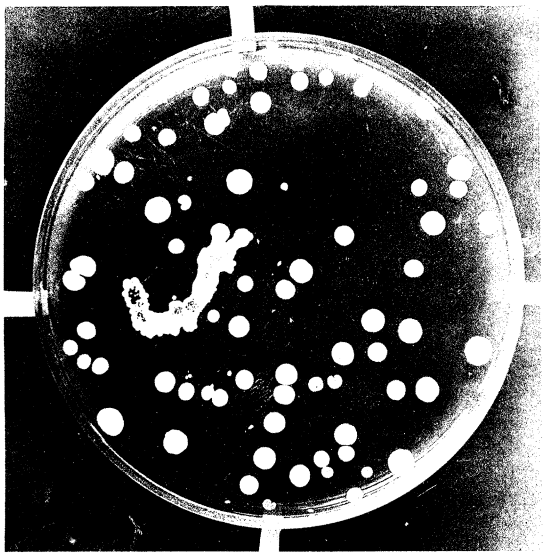

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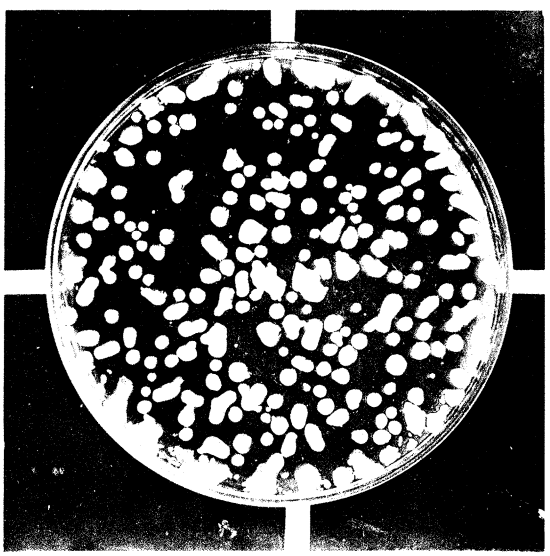

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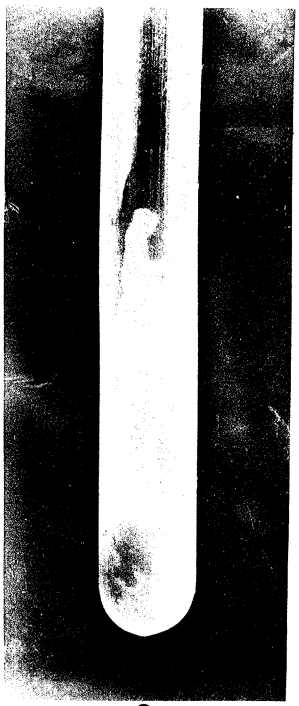

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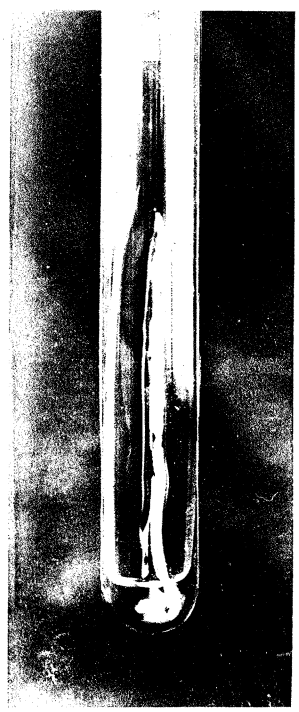

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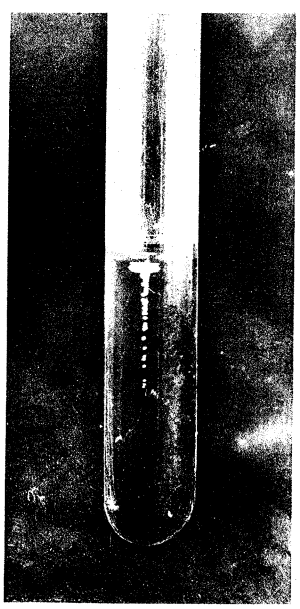

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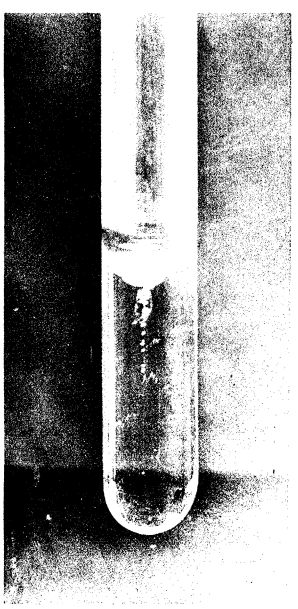

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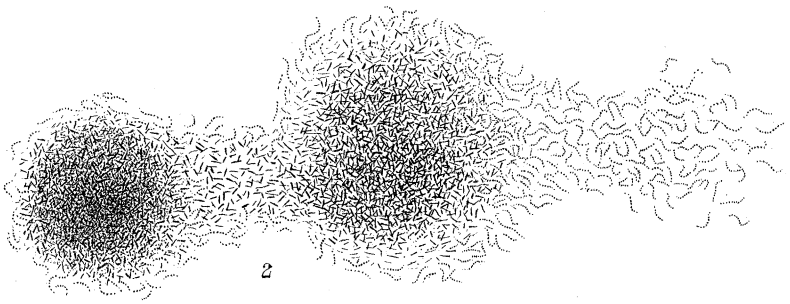
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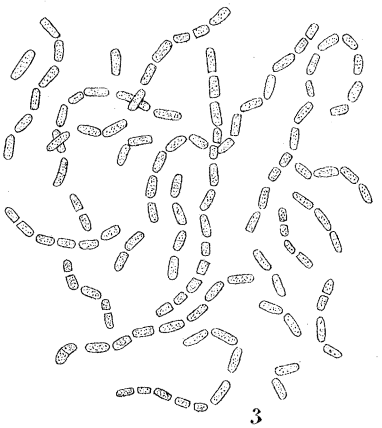
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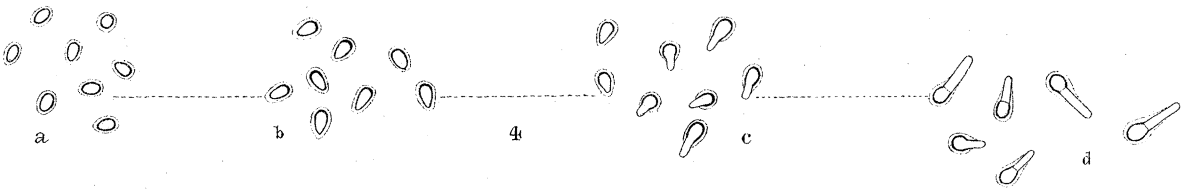

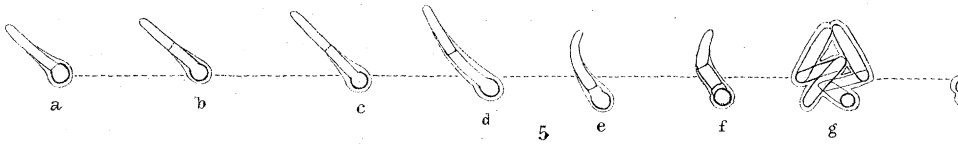

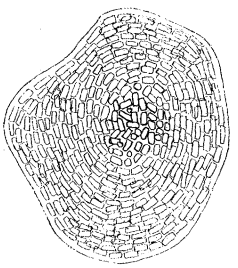
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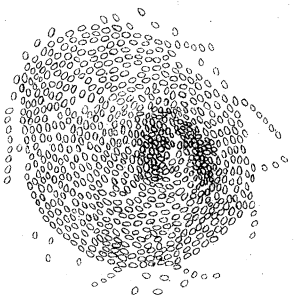
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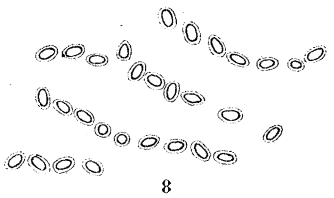
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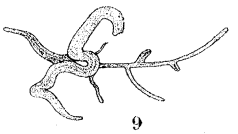
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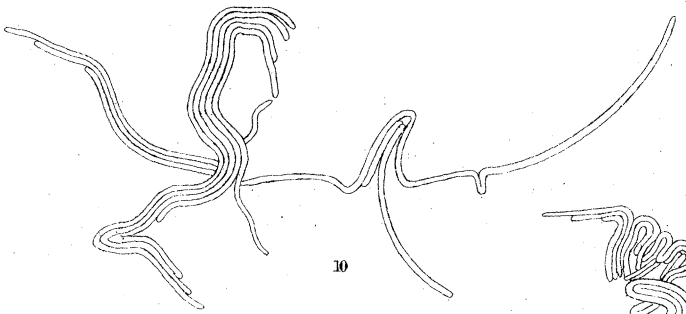
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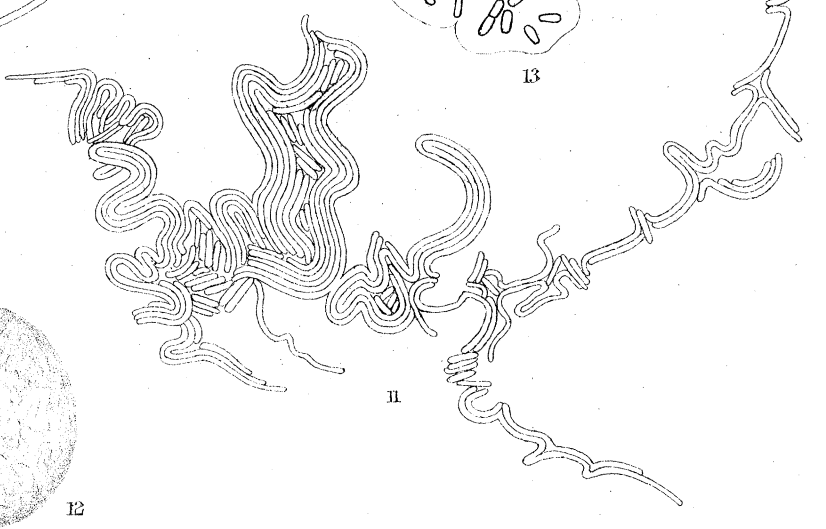
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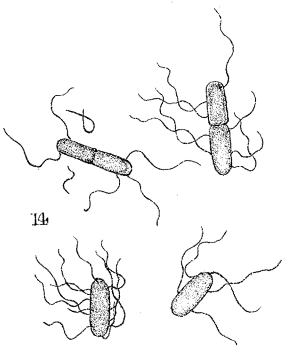
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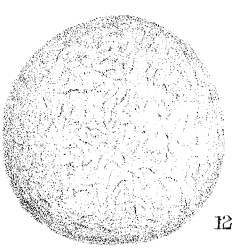
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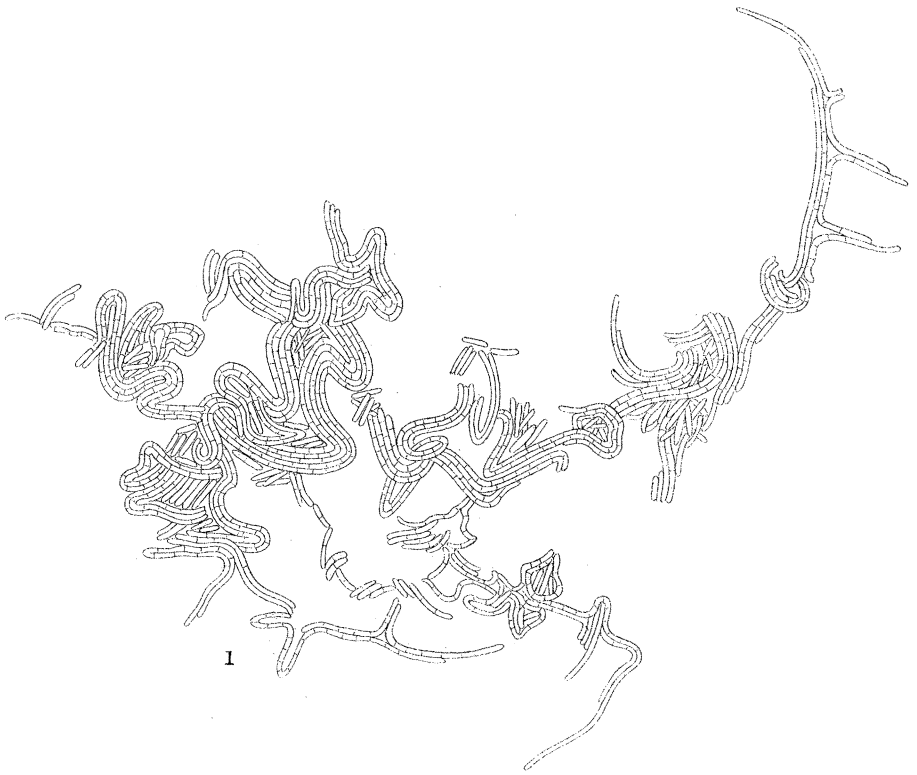

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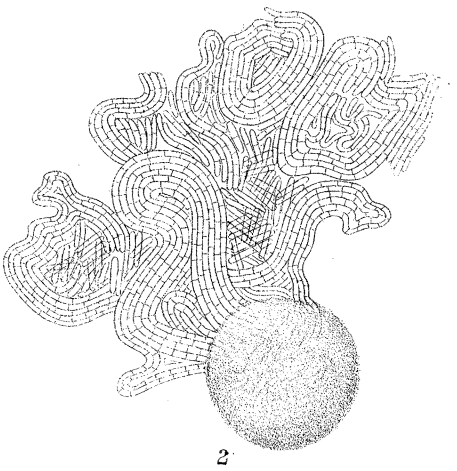

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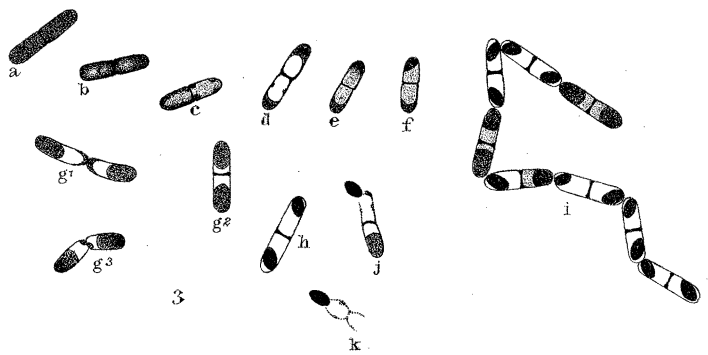

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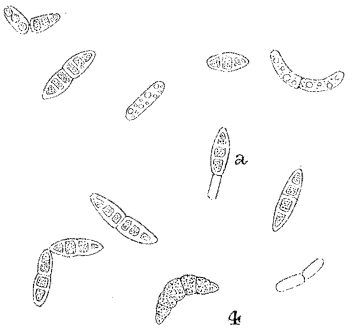

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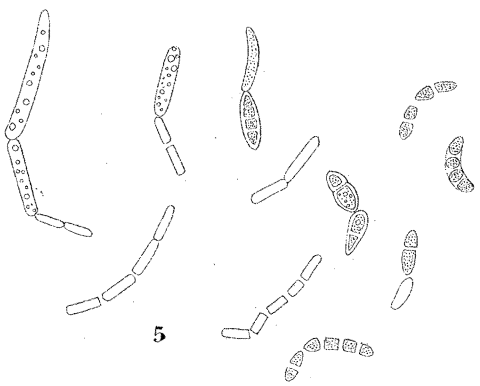

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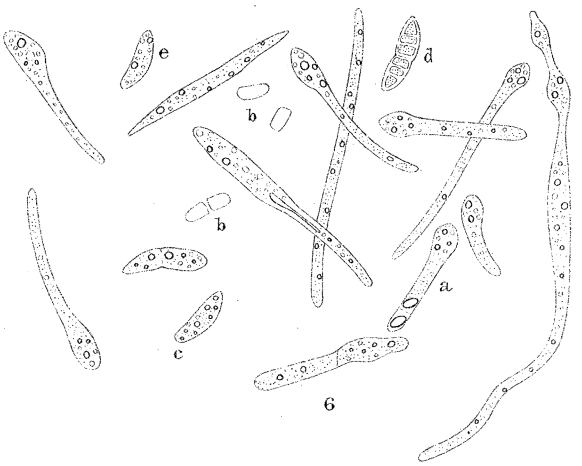

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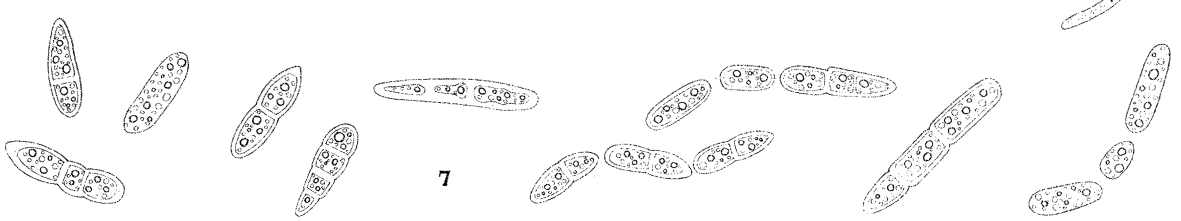

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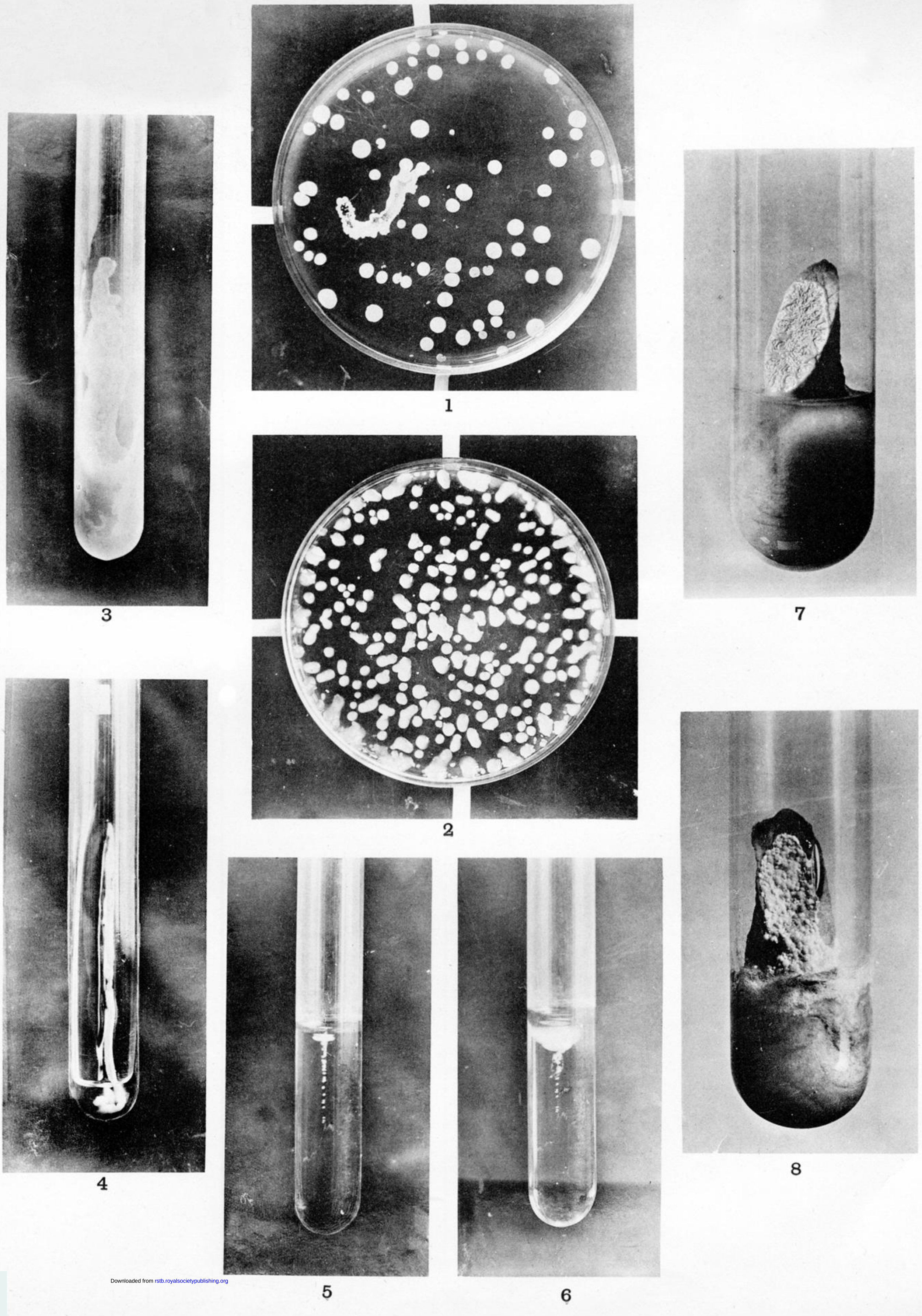

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PLATE 14.

Fig. 1. Plate-culture on peptone-agar, four days old. Grown in air, at 23° C. About two-thirds natural size.

Fig. 2. Plate-culture on yeast-extract-saccharose-gelatine, four days old. Grown in hydrogen at 20° C. About two-thirds natural size.

Fig. 3. Streak-culture on saccharose-agar, twelve days old. Grown in air at 17° C. Natural size.

Fig. 4. Streak-culture on peptone-gelatine, four days old. Grown in air at 17° C. Tube kept upright. Natural size.

Fig. 5. Stab-culture in peptone-gelatine, four days old. Grown in air at 17° C. Natural size.

Fig. 6. The same, twelve days old.

Fig. 7. Streak-culture on potato, moistened with a 10 per cent. saccharose solution; one week old. Grown in air at 17° C. Slightly reduced.

Fig. 8. Streak-culture on potato, moistened with distilled water; three weeks old. Grown in air at 17° C. About natural size.

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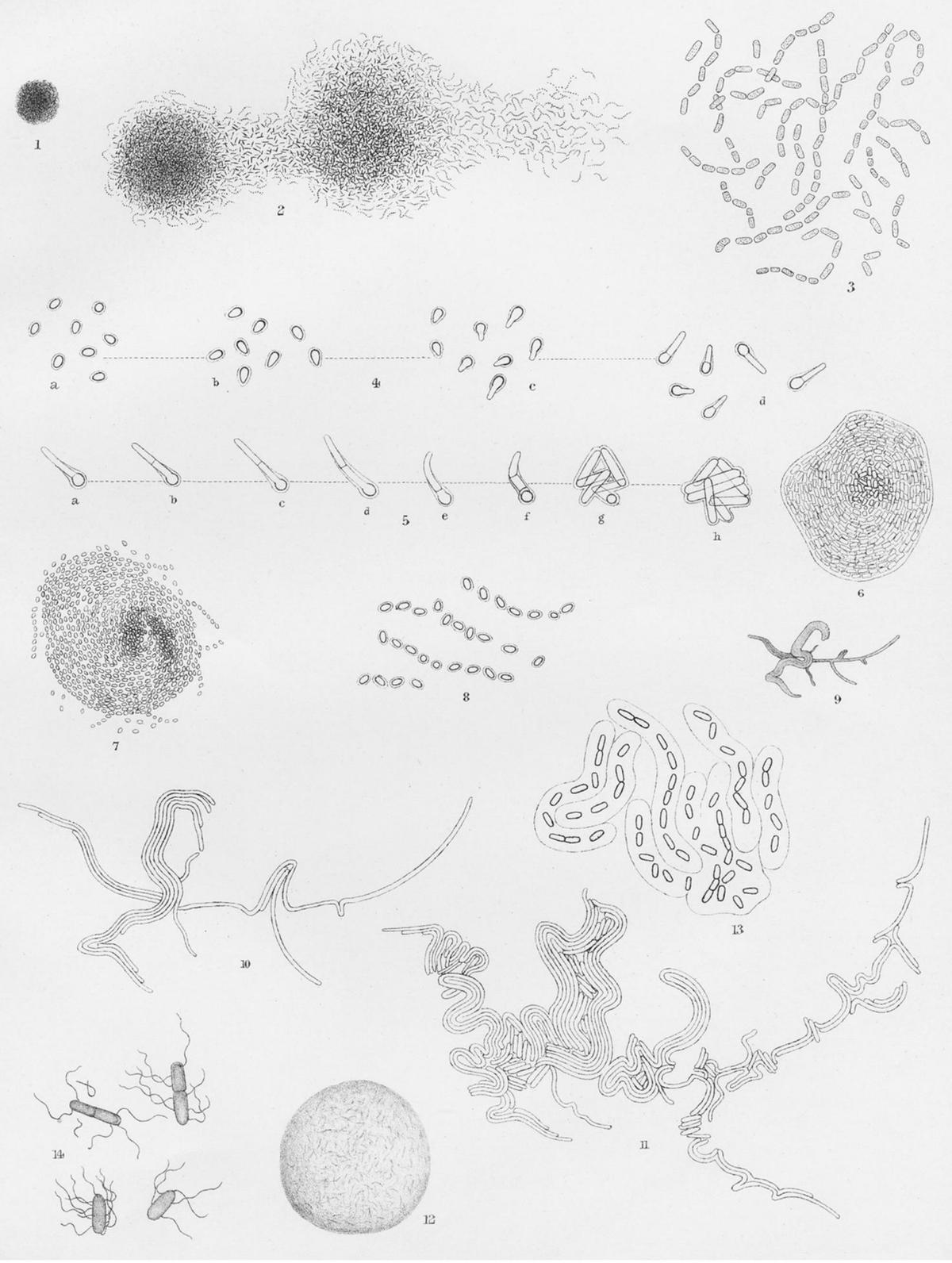

PLATE 15.

Fig. 1. Normal gelatinous colony on saccharose-gelatine in hanging-drop. Two days old. × 154.

Fig. 2. Colonies from a similar culture, in which the gelatine has become diluted with water. Two days old. × 154.

Fig. 3. Rods from the thinner portion of the colonies shown in fig. 2. × 650.

Fig. 4. Ripe spores sown in a hanging-drop of peptone-gelatine at noon, November 18. Temperature 17° C., *a* drawn at 2.30 P.M., *b* at 4.0; *c* at 9.30 A.M., November 19, after being kept all night at 10° C.; *d* at 11.30 A.M.

Fig. 5. A similar series: *a* drawn November 19, at 10.15 A.M.: *b* at 10.45; *c* at 11.15; *d* at 11.45; *e* at 12.15 P.M.; *f* at 1.0; *g* at 3.0; *h* at 4.0.

Fig. 6. Colony produced from the series shown in fig. 5, three days after the sowing of the spores. × 440.

Fig. 7. The same colony three days later; consisting entirely of spores. × 440.

Fig. 8. Spores from the same colony. × 1100.

Fig. 9. Non-gelatinous surface-colony produced on November 26, two days after sowing ripe spores in a hanging-drop of peptone-gelatine at 17° C. × 110.

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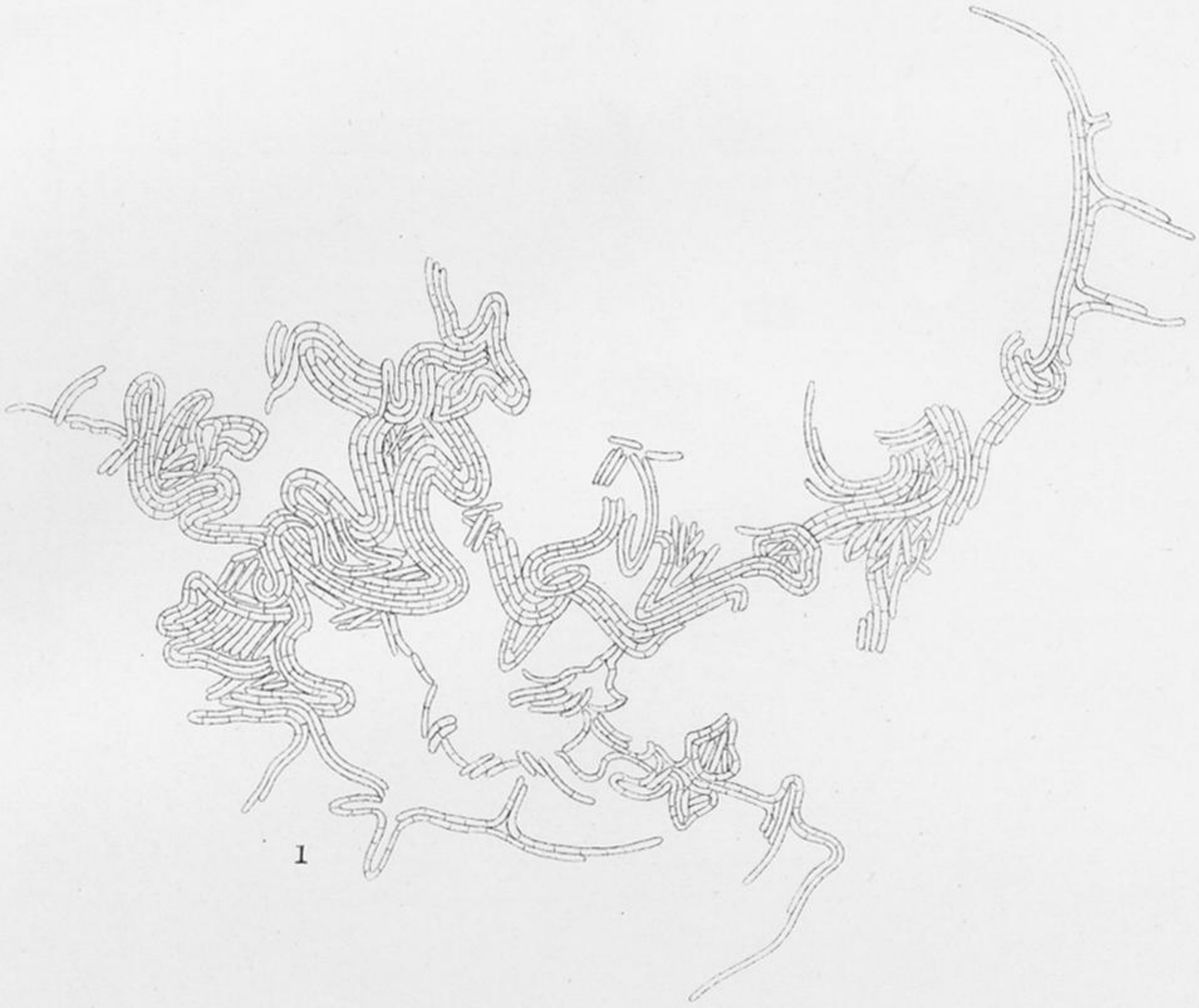

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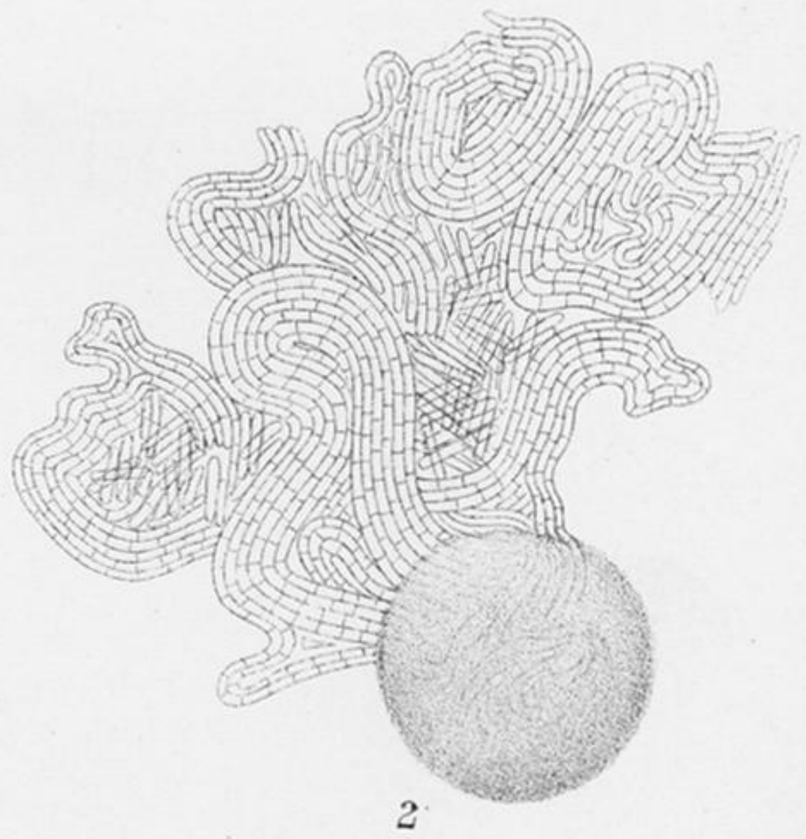

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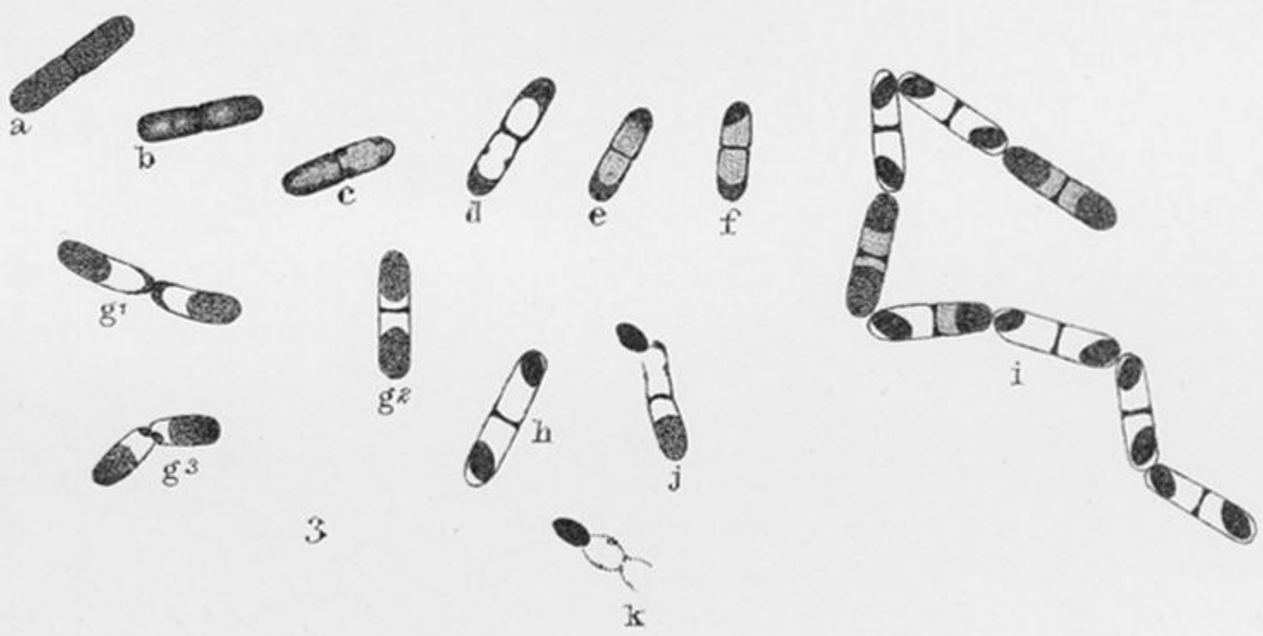

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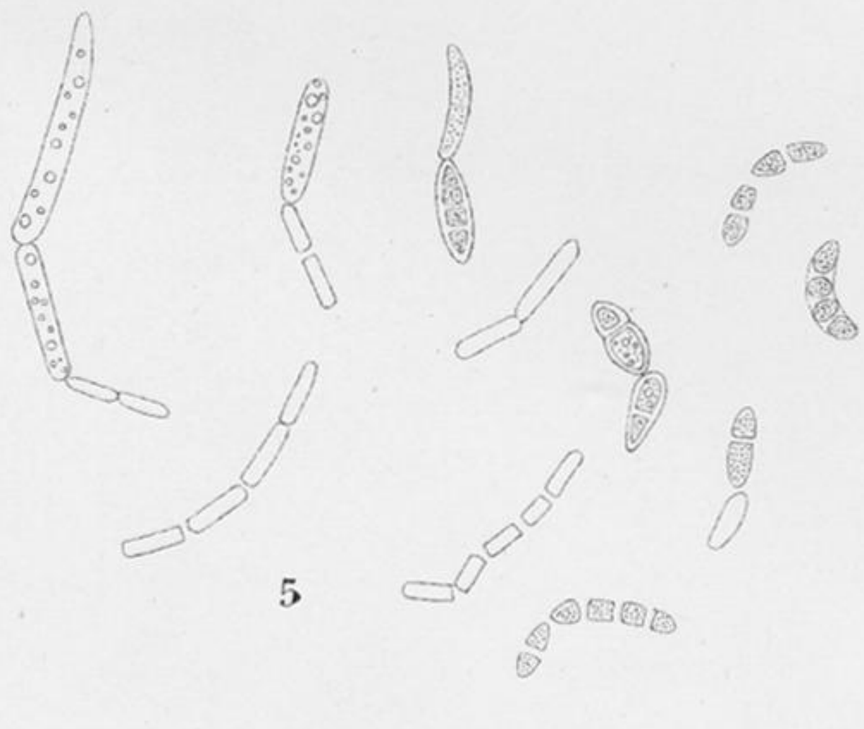

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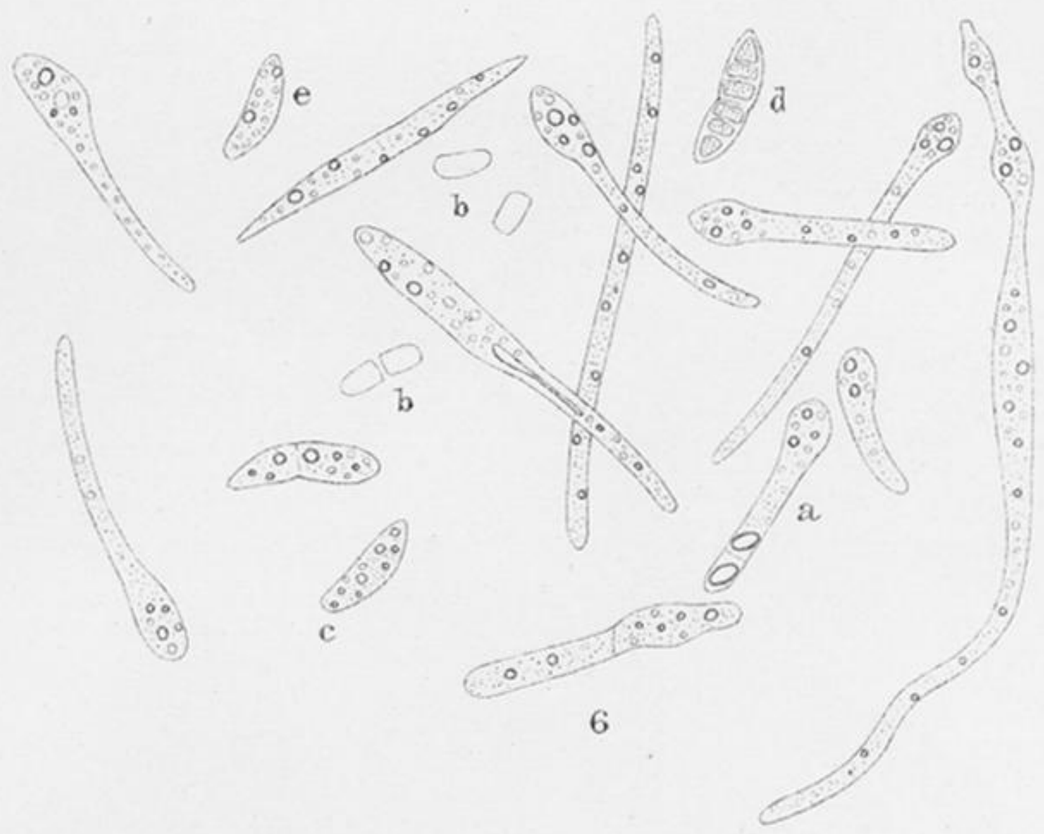

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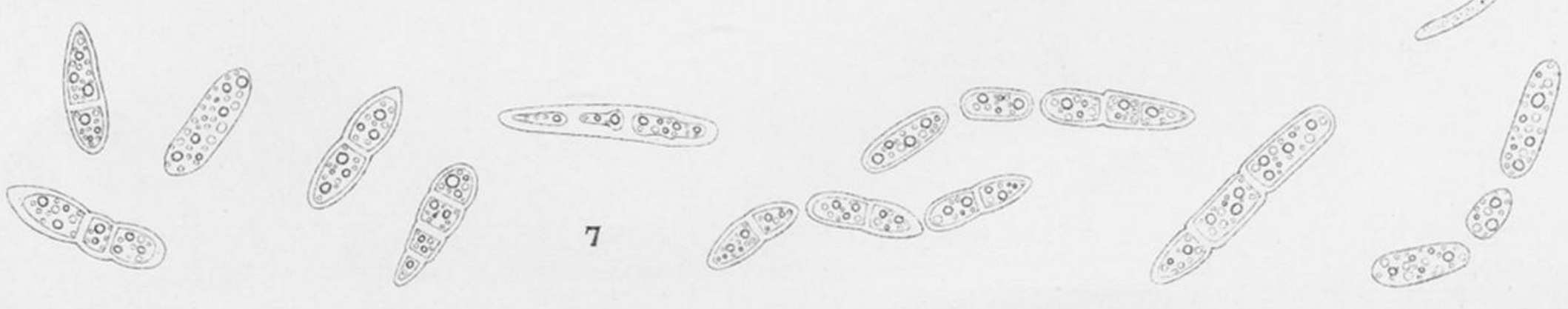

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