Fourth Report to the Royal Society Water Research Committee.

By Percy F. Frankland, Ph.D., B.Sc., F.R.S., Professor of Chemistry in Mason College, Birmingham, and H. Marshall Ward, D.Sc., F.R.S., F.L.S., F.R.H.S., Professor of Botany, Royal Indian Engineering College, Cooper's Hill. Presented to the President and Council, March 14, 1895.

"On the Biology of *Bacillus ramosus* (Fraenkel), a Schizomycete of the River Thames." By H. Marshall Ward, D.Sc., F.R.S., F.L.S., F.R.H.S., Professor of Botany, Cooper's Hill.

Introductory.

Morphology and Classification.

During the progress of my investigation of the bacterial flora of the River Thames, I have frequently isolated from the water a schizomycete, which turns out to be in many respects one of the most interesting forms yet met with, and probably one of the most instructive species yet known to science.

It occurs at all seasons, more or less, but oftenest during the autumn and winter months, and is found on the isolation plates as white colonies, becoming yellowish with age, which rapidly develop into large membranous growths, so like the mycelium of a fine mould that it is quite conceivable, or even probable in some cases, observers might pass it by as a small mucor or other fungus, not belonging to the schizomycetes at all.

It appears on the plates at all times of the year, though it seems to be commoner in the river water taken in autumn and winter than in the samples of spring and summer.

It attracted my attention at an early period in the investigation, owing to its rapid growth, the relatively large size of the filaments and cells, and especially the facility with which it can be made to develop spores. Moreover, it presents certain superficial, though not unimportant, resemblances in some of its characters to cultures of *Bacillus anthracis*, from which species, however, it is decidedly distinct, as evinced by its larger size and other pronounced characters.

I have now, after long and careful studies of its behaviour on different media, and in various circumstances, little hesitation in referring this species to Fraenkel's *Bacillus ramosus*, the form commonly known as the *Wurzel-bacillus* in Germany, but which passes under various names, and concerning which there is still a good deal of confusion in the various text-books and bacterial floras which mention it.

I shall discuss these matters and the synonymy at the end of the present section of the paper; but since I have succeeded in following out the life-history of this species in a singularly complete manner, and find the organism a remarkably typical and instructive one, it has seemed worth while to give in detail all the facts which have come under my observation, and especially to call the reader's attention to the fact that it runs through its entire life-history, from spore to spore-formation, in from thirty to sixty hours at ordinary temperatures,* and that I have been enabled to follow the course of this life-history by continuous observations under powers (1/12th and 1/20th oil-immersions) much higher than have commonly been successfully employed for such observations.

Bacteriological Cultures.

In the preliminary plate-cultures in 10 per cent. gelatine, made to isolate it from the Thames water, the colonies look so like the mycelium of a fine white mould fungus, that, as said, it is quite conceivable they might be overlooked or neglected by observers not sufficiently on their guard concerning such deceptive forms. In fig. 1 is represented one of these colonies, natural size, as seen on the second day, at 15° C. From a darker centre, which already shows signs of softening and liquefying the gelatine, wavy strands, of varying diameters, radiate outwards, and break up into finer and finer strands or filaments, until they fade away imperceptibly at the margins. The whole of this circular colony has a milky-white, somewhat opaque appearance, especially in the denser centre, which may have a yellowish tinge now or a little later; as we approach the indistinct margins, the opacity gradually gives way to a translucency, and eventually transparency, which prevent sharp demarcation from the gelatine around.

In a recorded series of observations of such colonies, at 15° C., I found they were first visible to the unaided eye in about forty to forty-eight hours; in seventy-two hours the average diameter was 25 mm.; in ninety-six hours about 50 mm.; and in six days (164 hours) a single colony covered nearly half the area of a Petri dish.

* The ease with which it can be obtained and grown suggests that this species would be a much better type for teaching purposes than the smaller *B. subtilis* commonly used.

Examined under a low power ($\frac{1}{3}$-in. objective), the radiating strands are found to consist of closely woven and interwoven, wavy, and coiled tresses of filaments, with a distinct yellowish colour such as protoplasmic structures usually present by transmitted light. These tresses (fig. 2) break up into finer and finer strands, as said, towards the margin, and finally into single filaments.

The coarser tresses may consist of twenty to thirty closely twisted or woven filaments, and are perfectly evident even to the unaided eye in older (*e.g.*, six days) colonies, the margins of which, as shown in fig. 3, remind one of a complex river-system as depicted on a map, or even of the yellowish, radiately-veined plasmodium of some myxomycete.

I suppose this radiating meshwork of yellowish-white strands was one character that suggested the resemblance to a network of fine rootlets, creeping on a flat substratum, and so gave origin to the German name (*Wurzel-bacillus*) for this species.

As we shall see subsequently, the individual filaments, twisted and woven to form the tresses and meshwork referred to, are the very long, wavy, coiled, or straight cell-series of the schizomycete.

The liquefaction of the gelatine at 15° C. commences only slowly about the second day, when the centre of the colony is soft beneath the dense membrane of tresses; it gradually progresses day by day, until, about the fourth day, the plate can no longer be upturned without liquid oozing from below. In about a week most of the colonies are floating on the fluid mass of liquefied gelatine. The liquid is slimy, and never seems to become completely watery, as is the case with some species. At higher temperatures, such as 20° and 25° C., the liquefaction is more rapid, in proportion to the quicker development and growth generally.

Colonies submerged deep down in the solid gelatine of a tube grow also, though more slowly than those at the surface; this is enough to show that the organism is partially anaërobic, though only to a very slight extent. The stab-cultures to be described demonstrate the same fact. The ultimate filaments themselves are long, straight, wavy, or spirally-coiled cylinders, averaging about 1·75 μ in diameter, and regularly divided into segments by transverse septa, at intervals of from 3 to 5 or 6 μ. It depends on the stage of development and other factors how far these segments are isolated from one another. In some cases, especially in the young colonies, the cylindrical filaments are merely septate; in others they are indented at the septa, and we may then speak of the filaments as more properly segmented; while in yet other cases the segments are so nearly isolated —evidently by more or less complete fission of the septa—that it is almost necessary to regard the filaments as long chains of segments end to end.

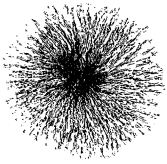


Fig. 1.

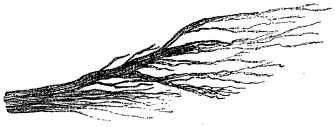


Fig. 2.

Fig. 3.

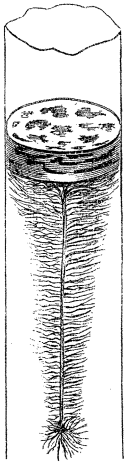


Fig. 6.

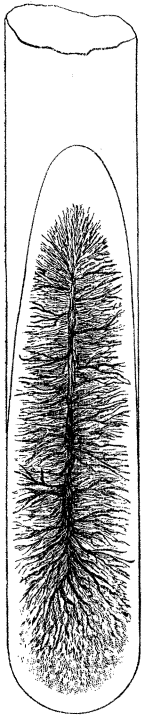


Fig. 4.

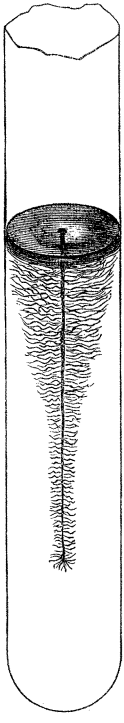


Fig. 5.

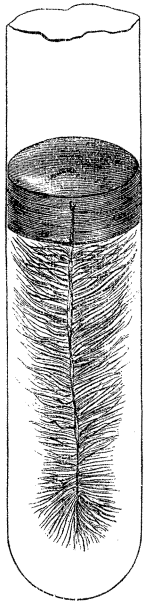


Fig. 7.

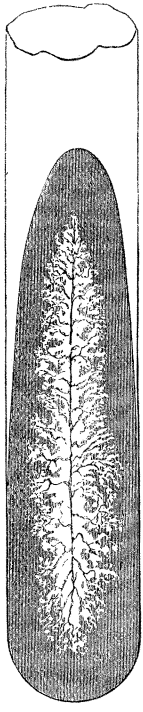


Fig. 8.

It hardly needs pointing out that these are merely so many stages in the progressive segmentation into *bacilli*, each bacillus being therefore a cylindrical rodlet, from 3 to 5 or 6 μ long by about 1·75 μ broad, and with rounded ends (see figs. 9—13).

Before this complete segmentation into bacilli, however, which only occurs, as a rule, very late in the life-history, the septate filaments often break across here and there at a septum, and so we have shorter filaments, each with twenty, fifty, or a hundred or more septa.

As I shall have to recur to these matters when tracing the development, however, no more need be said here further than that filaments and rodlets alike are usually quiescent, even in the liquefied gelatine,* and no cilia can be detected at any time; for the very slow growth-movements to be described later would hardly come under the head of motility in the sense ordinarily used in bacteriology.

Streak-cultures on gelatine at 15° C. result in very pretty and characteristic growths. A rapid extension of the tresses and filaments above described is effected from the streak all over the surface of the gelatine, so that in three or four days the white mycelium-like membrane covers nearly the whole area (fig. 4), and presents some resemblance to the diagram of the digestive and blood systems of certain worms. Meanwhile the gelatine begins to soften and liquefy, and on the fifth day the film, more or less broken up, is floating on the liquid. In ten days nearly all the gelatine is liquefied, and flocks of broken film float on and in it. The liquid does not show any noticeable turbidity at any time.

Stab-cultures in 10 per cent. gelatine at 15° C. are even more beautiful. From the white axis formed along the line of puncture, radiating silky-looking filaments branch out horizontally towards the walls of the tubes, and by the second or third day the culture looks so like a root, with its silky root-hairs radiating around, that this stage would seem best to justify the German name *Wurzel-bacillus*: it resembles strongly some cultures of *Bacillus anthracis*. On the fourth day liquefaction of the gelatine has definitely begun at the top, and the "root-hair system" now looks more like an inverted fir-tree (to use Fraenkel's simile), and this resemblance becomes more and more perfect as the culture gets older (see figs. 5, 6, and 7).

The liquefaction of the gelatine progresses slowly from above downwards, and the felted yellowish-white membrane floating on the top gradually breaks into flocculent patches. Even after three weeks the liquefaction has only extended about one-eighth of the distance from the top of the gelatine, but the fir-tree resemblance is

* A slow movement of isolated segments breaking off at high temperatures (about 35° C.) must be regarded as abnormal, as will be seen later on.

more pronounced on inverting the culture, owing to the depression of the longer lower branches (fig. 7).

At 20° C. the development is similar, but the "root-hair" or "inverted fir-tree" system and liquefaction proceed more rapidly.

Cultivated as a streak on agar at 20° C. the development is somewhat like that of the streak-cultures on gelatine, except that the widening streak and fimbriated offshoots which it gives off at right angles (fig. 8) are of a more opaque, granulated, chalky-white appearance, presenting resemblances to cotton-wool rather than to a silk texture. About the third day this granulated, chalky, mould-like growth has begun to develop spores in abundance, and as these ripen and the filaments break up into segments, the whole assumes a white pasty consistency.

Cultivated on potato at 20° C., this schizomycete grows rapidly at first, and in twenty-four hours looks like a dry, white mould spreading over the surface. After about forty-eight hours the surface of the thickish membrane formed becomes still dryer and duller in appearance, and by the third day the mass looks like a slightly wrinkled, powdery, rather thick, drying up pasty layer, white tinged with yellow or grey. In this stage further extension ceases and spores are forming, and after five or six days magnificent crops of well-ripened spores are to be obtained from these potato-cultures.

In broth at 20° C., abundant flocculent growths are evident in twenty to twenty-four hours, but no general turbidity. In forty-eight hours a thick, dull-white, mould-like membrane is formed on the surface, and flocculent cotton-wool-like masses have developed below, and occasional flocks of the same kind float in the otherwise clear broth. The floating membrane thickens rapidly, but the submerged flocks do not develop so quickly, evidently owing to the want of oxygen below the surface. As the membrane breaks up, flocks fall through the liquid and increase the cottony deposit, but otherwise no further change occurs up to the sixth or seventh day, when all growth ceases. The liquid remains clear throughout, evidently because the filaments do not break up into motile bacilli. All attempts to find cilia—by Loeffler's method as well as by the improved form of it given by Alfred Fischer*—have failed, and there can be little doubt that, like anthrax and some other forms, no such organs are present, common as they may be in the group of schizomycetes generally.†

Cultivated in milk at 20° C., no changes are visible at first, but it grows slowly and gradually dissolves the casein, with an alkaline reaction. The liquor becomes yellow, and copious networks of filaments are to be found in the cream above.

* See Pringsheim's Jahrb., 1894, p. 187.

† See Fischer, *loc. cit.*

In a 3 per cent. solution of glucose, to which a little broth and Liebig's extract (mere traces) are added, the cultures at 20° C. show rapid growth in twenty-four hours as white cotton-wool-like flocks, resembling those formed in broth.

Further research has shown that it grows readily in and on all ordinary media, at all temperatures from 12—15° C. up to 27—30° C., the optimum being near 25° C. But it is also capable of slow growth at temperatures below 7° or 8° C., and even above 38—39° C. for a short time. It is not strictly aërobic, but nearly so, and yields no pigment or other pronounced excretion.

I owe to the kindness of Dr. W. S. Lazarus-Barlow, of the Pathological Laboratory, Cambridge, the following proof that this organism is not pathogenic:—

"The question whether the bacillus were pathogenic or non-pathogenic was investigated on mice and on guinea-pigs. Beef-broth-cultures of ages varying from one to seventy-eight days, and in quantities of 2 c.c. in the case of the mice and 20 c.c. in the case of guinea-pigs, were used. The mice were inoculated sub-cutaneously, and the guinea-pigs intra-peritoneally. In no animal was any deviation from the normal observed except such as were directly and solely attributable to the introduction of the aseptic needle of the syringe. The bacillus is therefore non-pathogenic. This conclusion is further supported by the fact that the bacillus grows better at the room-temperature than at the body-temperature."

I have to record my thanks not only to Dr. Lazarus-Barlow for the trouble he took in the above investigation, but also to Professor Roy for permitting the investigation in his laboratory.

Characters of *Bacillus ramosus* (Fraenkel).

Habitat	Thames water, especially in autumn and winter, but also in summer.
Morphology	As long, straight, or curved filaments, often twisted into tresses; subsequently breaking up into segments (bacilli). Diameter of filaments = 1·75 μ, length 20, 50 to 100 μ or more. Bacilli 3 to 6 μ × 1·75 μ, with rounded ends, often in long chains. Forms spores = 1·75 to 2 μ × 1·5 μ.
Movements	Not motile, but the growing filaments exhibit slight nutation movements.
Colonies on gelatine plates at 15° C.	Appear in two days as circular, ill-defined, mould-like, white, rapidly-growing membranes. In three days may attain 25 mm. diameter; in ninety-six hours = 50 mm.; in six days 75 mm. or more. Mycelial-like membrane radiately veined with thicker and thinner strands of woven filaments, like a root-system or myxomycete plasmodium: slowly liquefies from about the third day.

Characters of *Bacillus ramosus* (Fraenkel)—*continued.*

Streak-cultures on gelatine at 15° C.	Spreads rapidly as a white, liquefying membrane, consisting of irregularly radiating off-shoots from streak.
Stab-cultures in gelatine at 15° C.	Fine white colonies along the line of puncture, giving off white, silky, radiating, and branched filaments, the whole looking like a root with root-hairs, and later like an inverted fir-tree. Liquefaction slow from above downwards.
Streak-cultures on agar at 20° C.	Growth much the same as on gelatine, but more opaque and chalky white, granulated. In three days develops spores, and the filaments break up into a pasty mass.
On potato at 20° C...	Spreads as a dull, pasty, white thick layer. In about three days becomes wrinkled and powdery, owing to breaking up of filaments and development of spores.
In broth at 20° C....	Rapidly develops a white, superficial, dull, mould-like membrane, and flocks of deposit like cotton wool below. Does not give rise to any general turbidity.
In 3 per cent. glucose solution at 20° C.	Rapid growth in twenty-four hours as white flocks, like those in broth, but feebler. Tends to form a slight ring, but no veil. No trace of fermentation. In ten days there are spores, but not numerous, and many segments have none.
In milk at 20° C.....	No visible signs in forty-eight hours, but thence onwards the casein is slowly dissolved from above downwards without precipitation, leaving a brown-yellow liquor below the cream, in which copious networks of filaments are developed. The reaction is strongly alkaline.
Requirements as to air	Aërobic, but partially anaërobic also.
Temperature.....	Grows best about 25° C., but will grow at any temperature between 6—12° C. and 38—39° C. The spores may be heated to 100° C. for a minute, but are killed by 2—5 minutes boiling. They withstand drying at 80° C., and germinate normally after it; they may also be kept at 60° C. for twenty-four hours without apparent injury.
Pigment	None.
Pathogenicity.....	Non-pathogenic for mice or guinea-pigs.

Classification.

It is obvious from the foregoing researches that this schizomycete comes into the group of true bacillar forms. If we adopt the very exhaustive classification attempted by Saccardo,* it goes into the

* 'Sylloge Fungorum,' 1889, vol. 8, p. 924. For an examination of this and other systems of classification see 'Annals of Botany,' vol. 6, 1892, p. 103.

second of his three sub-families—the *Baculogenæ*—though it is somewhat difficult to accept his statement that the bacillar segment, and not the filament, is the primary form. Be this as it may, however, this Thames schizomycete must be excluded from the *Trichogenæ*, Saccardo's first sub-family, embracing the genera *Crenothrix*, *Cladothrix*, *Beggiatoa*, and their allies, as well as from his third group—the *Coccogenæ*—which includes *Micrococcus*, *Sarcina*, *Streptococcus*, &c., &c.

Among the *Baculogenæ*—which includes the genera *Bacillus*, *Clostridium*, *Vibrio*, *Bacterium*, and their allies—it clearly comes under the *Endosporeæ*, a group embracing those genera which produce definite spores in the rods—*Pasteuria*, *Thiodictyon*, *Mantegazzia*, *Bacillus*, *Pasteurella*, *Clostridium*, *Cornilia*, *Vibrio*, *Spirillum*, and *Spiromonas*.

Pasteuria is excluded by its mode of longitudinal division. *Thiodictyon* by its cœnobial colonies. *Spirillum* and *Spiromonas* by their regular corkscrew-like twisting. *Cornilia* and *Vibrio* by their peculiar spore-formation and other characters; whence we are reduced to the true bacilleæ (*Eubacilleæ*).

This sub-group includes the genera *Mantegazzia*, with fusiform rodlets; *Bacillus*, with cylindrical rodlets; and *Pasteurella* and *Clostridium*, each of which has peculiarities of spore-formation different from those described.

This brings us to the genus *Bacillus* proper.

The further subdivision of this genus is still very unsatisfactory. Saccardo adopts a series of sections based on the habitats, whether anthrophobic, zoobic, pyogenic, zymogenic, saprophytic, and endophytic, subdividing further, according to the organs the pathogenic forms occur in, the behaviour towards gelatine, whether they form pigments, and so on.

Accepting this provisionally, the species in question comes under the saprophytic section. Here we find forms peculiar to the surface of the human body, or to the blood of cadavers, &c., and others especially characteristic of putrefaction, or stagnant water, and so on.

Clearly the present species is a *Bacillus* found in water, not necessarily stagnant, however, and saprophytic. It is, moreover, aërobic, achroic, and liquefies gelatine at ordinary temperatures, as we have seen.

If we now inquire what species this form belongs to, there are several decided and well-marked characters to guide us. There are not many schizomycetes known which are so persistently filamentous, and form such large, mycelium-like colonies on gelatine, and whose cells measure over 1·5 μ in thickness; which, moreover, easily form large, oval spores, and, finally, have such a characteristic stab-culture as this one.

Taking the large size of the bacillar segments as our primary clue, the vast majority of known bacillar or filamentous schizomycetes rarely exceed 0·5 μ in diameter, and very few surpass 1 μ.

Of these, we may at once dismiss Van Tieghem's giant *B. crassus*, 4 μ in diameter, and the thickest form known,* and even De Bary's *B. megaterium*, 2·5 μ in diameter, is much thicker than the form I am discussing.

A small number of species, such as *B. Brassica*, *B. tumescens*, *B. Zopfii*, *B. Mallei*, *B. ascoformans*, *B. indigogenus*, approach our form in dimensions, but their other characters at once separate them.

B. anthracis presents several suggestive resemblances to the present schizomycete, but it is thinner and smaller altogether, and its spores, in addition to being smaller, germinate differently, and have different temperature requirements. Moreover, though the stab-cultures of *B. anthracis* resemble those of the Thames form, the gelatine plate cultures are different. The spores of the former are also much more sensitive to light, and the Thames species is not pathogenic. Maschek's "*Bäumchen-bacillus*," though it forms a dendroid stab-culture, differs in almost all its other characters, and ferments saccharine solutions.

As regards the tendency to form mycelium-like colonies on the plates, several species found in water and elsewhere resemble the Thames species, *e.g.*, *B. radiatus*, *B. muscoides*, *B. polypiformis*, *B. mycoides*, *B. ramosus*, *B. implexus*.†

Of these, we may rapidly dismiss *B. muscoides*, *B. polypiformis*, and *B. radiatus*, since they are strictly anaërobic, and will not grow under ordinary conditions, to say nothing of their many other specific differences.

B. implexus is too thin, and Zimmermann's curt description of the colonies suffices to show that it is totally unlike the Thames form. *B. mycoides* is also too thin, but it forms oval spores, and the plate-cultures resemble those of the form in question in many particulars. The potato-cultures differ, however. More information is wanted about this species, which is said to be very common in soil and water.

There remains *B. ramosus*. I understand by this specific name the form described by Eisenberg‡ under that name, and known in Germany as the "*Wurzel-bacillus*." Crookshank§ gives the latter under the name *B. figurans*, and as a synonym for Flügge's *B. mycoides*, but Eisenberg gives quite a different description for the latter, and quotes Flügge and Zimmermann in support of his statements.

* Quoted by De Bary, 'Lectures on Bacteria,' p. 3. Of course I leave out of account the genera *Crenothrix*, *Beggiatoa*, &c.

† See Lustig, 'Diagnostik der Bakterien des Wassers,' 1893, p. 82.

‡ *Loc. cit.*, p. 126.

§ 'Manual of Bacteriology,' 1887, p. 311.

Saccardo* gives under *B. Praussnitzii*, a synonym *B. ramosus liquefaciens* of Flügge; whether this is the same I cannot determine from the very short and vague description.

Macé† gives *B. radicosus* as the "wurzel-bacillus," but his description is too vague to enable me to determine whether it is the *B. ramosus* of Eisenberg; he does not intend it for Flügge's *B. mycoides*, however, since he describes that separately. Zimmermann also gives it as *B. radicosus* and Tataroff as *B. radiciformis.*‡

The Franklands,§ who found the same, or a very similar, form common in the Thames and Lea, regard their species as the *B. ramosus* of Eisenberg and Fraenkel, and as the "wurzel-bacillus," and Lustig‖ gives the descriptions separately, but remarks on their probable identity.

I have little doubt that the species I have isolated from the Thames is Eisenberg's and Lustig's *B. ramosus;* that it is the "wurzel-bacillus" of Fraenkel and Eisenberg; and that it is identical with that found by the Franklands, though the figures given by the latter are not clear enough to identify it by.

In the size of the rodlets and filaments, mycelium-like plate-colonies, root-like stab-cultures, behaviour on agar and potatoes, characters of the spores, and, indeed, in almost every detail of which I can get information, my Thames form agrees with Fraenkel's *B. ramosus*, the "wurzel-bacillus" of the Germans. What the forms described by Macé, Crookshank, Saccardo, and Flügge may be is not clear, and probably some confusion exists here.

It seems pertinent to remark here that many bacteriologists are not sufficiently careful in all cases to look up the synonyms of the forms they describe, though this precaution is really more necessary in the deplorable state of their literature than probably in any other department of biology.

Germination of Spores.

As the mass cultures prove, the spores germinate readily at all ordinary temperatures, whence was to be inferred that no extraordinary difficulty ought to be incurred in observing the process, provided I could succeed in isolating a single spore under a sufficiently high power in a hanging drop, and such turned out to be the case.

The methods and apparatus employed were similar to those I had

* *Loc. cit.*, p. 989 (No. 202).

† *Loc. cit.*, p. 610.

‡ Zimmermann, 'Die Bakt. unserer Trink-und Nutz-wässer,' p. 30. Tataroff, Dorpat, 1891.

§ *Loc. cit.*, p. 388.

‖ *Loc. cit.*, pp. 96, 97.

used previously in isolating the yeasts and bacteria of the "ginger-beer plant."*

A small drop of nutrient gelatine or of broth, properly infected with ripe spores from an agar tube, was placed on the under surface of a sterilised cover-slip, and the latter luted by means of sterile gelatine, so as to serve as the lid to one of the glass cultivating chambers, properly sterilised, the floor of which consisted of a glass slip, luted by means of melted paraffin. Over the floor of the chamber was spread a layer of sterilised distilled water, and the tubular arms were plugged with cotton-wool saturated with water, all properly sterilised. The hanging drop, selected by examining a number and choosing that which appeared to contain only one, or at most a very few spores, separated by a sufficient distance, had to be sufficiently flat to enable the lens to penetrate through its depth.

Methods.

As regards my own practice in making these cell-cultures, the following note may be of use to other students; though of course different workers may operate differently.

I first sterilise the plugged cells in the hot-air steriliser. When these are nearly cool enough to han lle, I heat the quartz or glass floor of the cell between two plates of talc (about 3 × 6 inches) held in a large bunsen flame, and allow it to cool slightly. The cell is then placed in position on its floor, and a small block of high-melting paraffin—cooled after sterilising—is placed just outside the cell. The temperature is still high enough to melt this, and the liquid runs in by capillarity, and solidifies as the whole cools, cementing the cell to its floor.

When the culture is to be made I take such a prepared cell—several can be prepared and kept in sterile glass-covered dishes—and have ready the following: cover slips, a tube of infected gelatine (or other medium), platinum loops, sterile stiff gelatine, forceps, sterile water, and any convenient rest, such as a small ring of brass.

First, the necessary water is placed in the plugged cell, care being taken to wet the cotton plugs and that some water shall be retained on the inner side of each, whether a layer is spread over the floor or not.

Second, a cover-slip is sterilised between the talc-plates, and while it is cooling the platinum loops are held in the flame and set aside to cool.

Third, the cool sterile cover-slip is removed with the forceps, and laid on the brass ring support. Then the drop is quickly put on the centre of the slip, and the latter inverted on to the cell, so that the drop hangs over the centre.

* See 'Phil. Trans.,' vol. 183, 1892, B, p. 125.

Fourth, the slip with its hanging drop is now cemented as a roof to the cell by running melted sterile gelatine between it and the latter.

A little practice enables one to prepare such a culture in a few minutes, and very rarely need one go wrong if care is taken. The chief difficulties are with fluid drops, or very dilute gelatine, since they are apt to spread and run over the glass, especially when the air is moist and condenses quickly on the glass surface; in these cases, however, a little experience enables one to avoid letting the cover-slip get too cold before the drop is attached—though, of course the opposite danger has to be guarded against.

Having isolated a single spore, suspended in the drop of nutrient medium beneath the objective—the observations were made with Swift's 1/20th and Zeiss' 1/12th oil immersion, and with Zeiss' E, occ. 4—a drawing of the freshly sown spore was at once made. The culture was then left, with a bell-jar, darkened with black-paper over the whole, at a temperature of 15° to 20° C,* and further observations and drawings made at intervals. Naturally there were many failures, especially with the high-power immersion lens, and the following successful series were only obtained at intervals from cultures in which the thickness of the cover-slip, and of the hanging drop, the sufficient isolation of the spore, and the normal germination and further progress were suitable, and where no sudden changes of temperature interfered to check the growth, dry up the gelatine, or cause inconvenient condensations of moisture in the chamber, the relatively large size of which has again proved advantageous owing to the abundant supply of oxygen it ensures.

Under the conditions referred to, the spore without materially changing its ovoid shape begins to swell somewhat rapidly, and in from one to two hours has increased its dimensions from about $1{\cdot}5 \times 2\,\mu$ to $2 \times 2{\cdot}5\,\mu$ or more. As it does this the brilliant oil-drop-like contents become duller and more hyaline—like ground glass—and the sharply marked, almost black membrane, gradually loses some of the firmness of its contour, until it appears as a thinner limiting membrane. At the same time it becomes surrounded by an almost imperceptible pale halo-like investment which appears to be derived from the deliquescence of its most external layers, probably into a soft, transparent, swollen jelly. (*Cf.* figs. 9—12.)

In the course of the next one to two hours or so, the spore appears to be elongating. Close observation shows that this is due to a thinning out of the membrane at one of the ends, and soon afterwards the thinned out wall gives way, and the pale, hyaline, apparently homogeneous protoplasm, enveloped in an exceedingly

* These temperatures are somewhat low, and later results were got at 25—26° C. and higher.

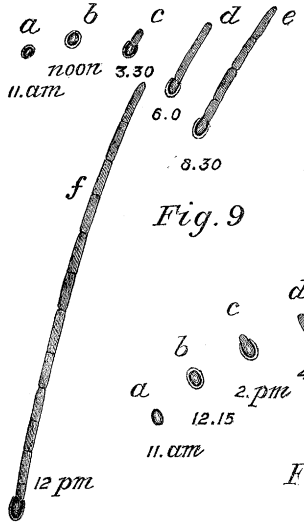


Fig. 9

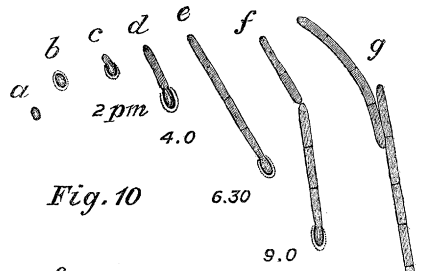


Fig. 10

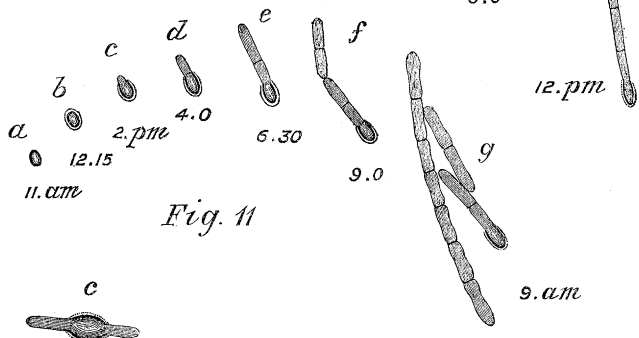


Fig. 11

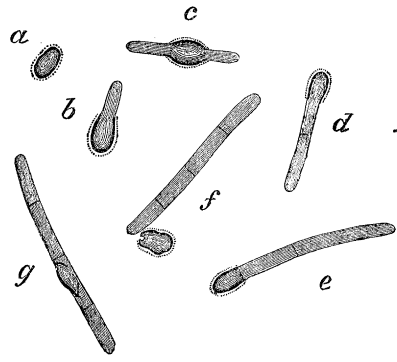
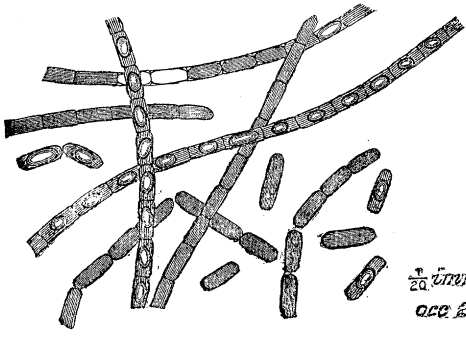


Fig. 12

Fig. 17



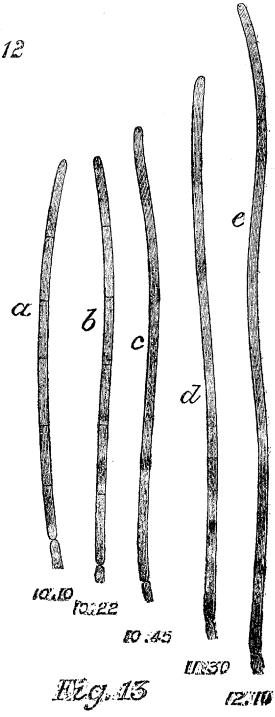


Fig. 13

tenuous membrane, pushes its way out and grows as a blunt rod, about 1·75 μ broad, with rounded apex, in the direction of the longer axis of the spore. (Figs. 9 *c*, 10 *c*, 11 *c*.)

In about four or five hours from the beginning of germination this straight rodlet has attained a length equal to twice that of the spore, and two or three hours later it has a length of approximately four times that of the spore (figs. 9 *e*, 10 *d*, 11 *e*), the membrane of which is still observable usually as a cap at the proximal rounded end of the rod. (Same figures and fig. 12 *b*, *d*, *e*.)

The above is by far the commonest mode of germination, but in some cases this normal condition of affairs is so far modified that both ends of the spore are softened, and each gives rise to a germinal rodlet (fig. 12 *c* and *g*) in which case the remains of the spore-membrane may be found either encircling the germinal rodlet, much as a napkin ring does a rolled up serviette (fig. 12 *c*), or ruptured at one side and merely adhering to the rodlet as in fig. 12 *g*. Occasionally, rodlets which have germinated out in the normal mode are found with the collapsed membrane lying loosely at one end, evidently having been thrown off, as in fig. 12 *f*; this seems to occur rather frequently in the later stages of germination in broth-cultures. All these phenomena point to the elastic nature of the thin, but tough, spore membrane.

When the germinal rod or filament has attained a length equal to about four or five times that of the swollen spore, the first division wall is usually seen in the centre (figs. 11 *e*, 12 *d*, and *e*). Whether the case illustrated in fig. 10 *d* is really an exception to this rule, or whether the apparent septum closer to the spore was really the rim of the burst spore (*cf.* fig 12 *e*), I cannot be certain; from the fact that I could not trace it in the next stage (fig. 10 *e*) it seems likely that the latter supposition is the correct one, and in any case the rule is that the first transverse septum divides the whole germinal filament into two cells approximately, but not necessarily exactly, equal in length, and measuring about 3 to 5 μ long by 1·75 μ broad.

The germination now rapidly proceeds by the growth in length of the stiff and nearly straight filament along its whole course, and in about six to eight hours from the commencement of the swelling of the spore, the filament is from 8 to 10 times as long as the spore (figs. 9 *e*, 10 *e*, 11 *f*), and each of the two cells into which it was segmented by the first transverse septum has been again bisected by a septum, thus cutting the filament into four segments (figs. as before).

The growth is therefore not merely terminal, but intercalary along the whole filament. I have spoken of the latter as being stiff and nearly straight; the qualifying word is necessary because close observation of broth cultures shows that the rounded apex performs

very slow and very slight movements, due to almost imperceptible curvatures of the filament. These can be traced by watching the growth for a few minutes under the eye-piece micrometer, and are interesting as indications of slight movements of nutation. As I shall have to speak of these, and the measurements I have made of them and of the rate of growth, when describing the behaviour of the older filaments, however, they may be passed over here simply with the remark that they do occur.

Even at this early stage, or a little later, the filaments, especially in broth cultures, may begin to break across, generally, but not always, at the oldest septum (figs. 11 *e*, and 10 *f*), though this process is by no means the common or normal one in rapidly growing cultures (fig. 9 *f*).

Sooner or later, however, some amount of fission does occur, and the further behaviour of the two or more broken filaments is interesting, as throwing light on the formation of the tresses and strands, and their false branches, which we meet with later.

Fig. 11 *f* and *g* is an instructive case in point. As the first stage shows, the filament broke at the oldest septum when little more than eight times as long as the spore, and consisting of four segments, and when only ten hours old. The lowermost two-celled segment, proximal to the spore, then slipped its now rounded tip to the right, and continued growing; the uppermost, free and distal, two-celled segment also grew so that both ends travelled further apart, and in such a way that the tip of the proximal segment slipped along its right side. Further divisions followed on growth in both cases, and the right hand (proximal) segment again broke during the night at its oldest segment, when it had divided into four cells, the ends again slipping over one another, so that at nine o'clock next morning (twelve hours later) the state of affairs was as in fig. 11 *g*. This was a gelatine culture, not growing very rapidly, but I shall have to revert to these phenomena later in discussing the process of growth in broth cultures.

It is already intelligible, however, that this slipping of the broken filaments one over the other, each portion growing independently soon gives rise to strands or tresses compounded of numerous filaments, the free ends of which stand off as false branches (the same thing is beginning in fig. 10 *f* and *g*), and so the initiation of a colony, with offshoots radiating in all directions into the surrounding medium, is established.

From the fact that well isolated spores, freely suspended in a drop of broth, give rise to filaments which may grow to many hundred times the length of the spore before any breaking across occurs, and that such breakage is very apt to occur when one such long filament eventually abuts on another, or on some obstacle which bends the

filament, I am strongly inclined to refer the breakage in the above cases to mechanical causes, *e.g.*, the resistance offered by the gelatine. Indeed, I have watched a long filament in broth, thus abutting on another, and seen it curve until bent into more than half a circle (evidently owing to intercalary growth between the two relatively fixed ends) and then suddenly snap at one of the septa, and the two freed ends dart past each other owing to the elasticity of the filament which had given way. At the same time, there is a period when the (much older) filaments are peculiarly apt to break up into shorter segments, preliminary to spore formation, and this must be due to other causes than mere fracture due to mechanical pressures or tensions, indeed, even in the above cases I do not suppose that the mechanical strains do more than determine the sudden rupture at a septum already prepared to give way, but which probably would not yet have done so had the filament been able to continue its onward growth in a more or less straight line.

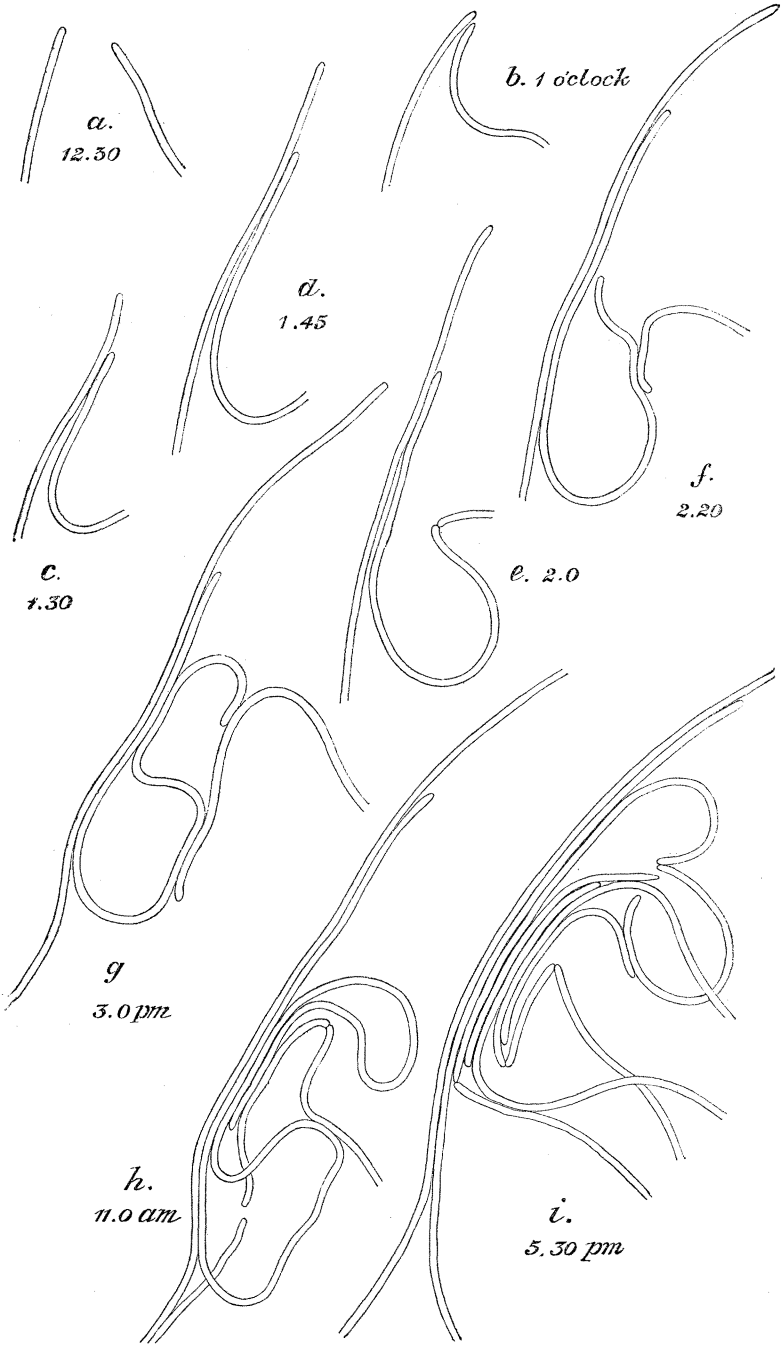
Formation of Tresses.

I have already described one mode of formation of the strands or tresses composed of numerous filaments lying parallel to one another, by means of the sliding growth of the two pieces of a ruptured filament one over the other.

Perhaps an even more common process is the modification of this figured in fig. 14 *a* to *i*, a series followed under the microscope (E occ. 4) on a broth culture at 17·5° to 19·5° C. The culture was the same as that from which figs. 9 and 13 were obtained, and, in fact just as fig. 13 represents particulars of the growth of one of the filaments developed from the spore of fig. 9 (and traced in that figure for the first thirteen hours of germination) followed from the twenty-fourth to the twenty-sixth hour from germination, so this fig. 14 takes up the further history of the same filament from twenty minutes later through another four hours; for the left-hand filament in fig. 14 *a* is the upper end of the filament *e*, fig. 13.

We left this at 12.10 P.M., having traced its growth and nutations during the preceding two hours. At 12.30 the distal end of another filament, segmented off lower down, was seen to be curving over towards the one referred to, and half-an-hour later (fig. 14 *b*) the tips of both were in contact and growing up alongside one another. I was strongly inclined to suspect the existence of a slight attraction between them from the relatively strong curvatures towards each other which they exhibited previous to contact, but could not be sure, and thought possibly the phenomenon was of the nature of a merely physical action. During the next hour (fig. 14 *c*, *d*, *e*) a longer and longer stretch of the growing, curved, right-hand filament laid itself

Fig. 14.



close to the rapidly elongating left-hand one, and then an interesting and instructive phenomenon ensued.

The long, curved, right-hand filament suddenly snapped at one of its septa, and shortly after the drawing (*e*) was made the two ends began to glide one over the other, and at 2.20 had assumed the positions shown in fig. 14 *f*. The rapid growth of the extreme right-hand filament caused it to curve strongly, and this marked curvature increased, until at 3 P.M. the large curve looked as if the filament was pressing the S-shaped one, trapped between it and the left-hand spring-like filament, elastically against the latter. (Fig. 14 *g*).

At any rate, the free end of this spring-like right-hand filament slipped soon after over the lower half of the S to the left, while its upper part went on, so to speak, pressing the upper limb of the S also to the left, until it had trapped it close up against the back of the S (fig. 14 *h*), and then it also snapped.

Comparison of the figures will convince us that the series of contiguous parallel filaments making up the middle portion of fig. 14 *i* are brought together by these repeated doublings up of the snapped filaments, pressing up close to one another. The series also shows pretty clearly how pronounced is the intercalary growth, *e.g.*, along the curved upper and lower line of the S-shaped piece in figs. *g*, *h*, and *i*.

The process may be summarised as consisting in (1) the looping of filaments, which go on elongating by intercalary growth, the two limbs of the loop being doubled one on to the other, possibly by pressure of other filaments; (2) the snapping of the same when the doubling up becomes very sharp; and (3) the straightening out of the broken pieces side by side, and further parallel growth in close contiguity as a strand or tress.

The case followed is a relatively simple one. In larger and older colonies, and in cultures where many colonies are growing together, the tresses may consist of scores and even hundreds of such parallel filaments. Moreover, they are not necessarily arranged in the flat, ribbon-like manner depicted in these young and small cultures growing in a thin layer of broth, but may be thick and of various sections.

It will also be understood how—quite apart from any question of mutual attraction between filament and filament—the fact of the two terminal stretches of a long and otherwise free loop being relatively fixed as they grow along the sides of such a strand, may force the loop to make coils of various kinds during the elongation due to intercalary growth, and it is thus I explain the extremely common occurrence of these coils in large cultures.

Development of Spores.

Perhaps the most interesting—as it certainly has been one of the most difficult—series of observations I have made on this schizo-

mycete, has been the tracing of the successive stages of development of the spores under high-power objectives; and here again I have had the satisfaction of tracing the whole process in one of the filaments of the same series as those figured in figs. 9, 13, and 14.

The strand drawn in fig. 14 *i*, was left at 5.30 P.M. at a stage too complex to follow further, and when its age—reckoned from the swelling of the spore which produced the filaments—was 30½ hours. At 9 o'clock next morning these filaments showed evidence of proceeding to the development of spores, and before night every filament had a completely developed spore in each of its numerous segments, or if any of the segments remained barren they must have been very few indeed.

I had already traced the development of the spores, but it was obviously an interesting task to do this in one of these filaments which I had kept so continuously under observation, and the following description refers to a portion developed during the night from the further growth of one of the loose filaments in fig. 14 *i*.

To do this I exchanged the objective (E, occ. 4) for a 1/12th oil immersion, and at 10.30 A.M. made the notes of the first changes which initiate the formation of the endogenous spores. This culture which, as we have seen, was in broth at 20° C.* (occasionally lower), was now aged 47½ hours from the moment of drawing the spores (fig. 9 *a*).

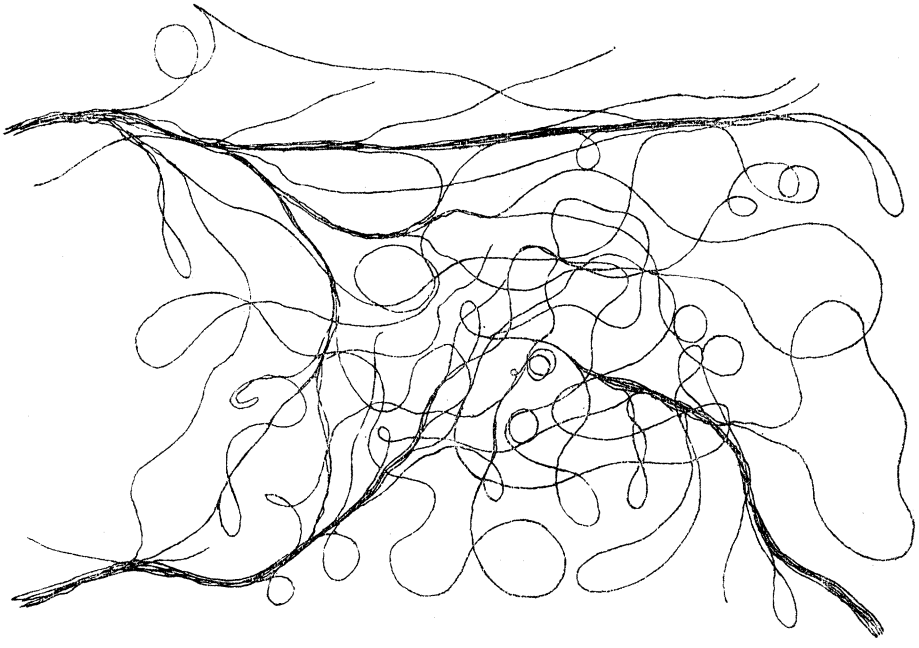
The first indication of spore-formation consists in the appearance of brilliant points in the cells which have now ceased to grow or divide. There may be one or two or more of these bright spots in each cell, and the septa show signs which seem to precede a tendency to split. They look swollen and bright, but I am not sure whether this is really due to such a change in them, or whether it is owing to a slight contraction of the protoplasm from contact with the walls.

During the course of the next three or four hours the bright, oily-looking globules† gradually enlarge, and, where more than one occur in a cell, some may run together into one bright mass, which slowly balls itself together in the centre of the cell, and, in the case described, there was one such mass definitely established as an oval body in each segment at 3.30 P.M. In the course of the next hour this bright, oval body had increased in size, apparently at the expense of the cell-protoplasm in which it lay, and constituted a definite spore. This slowly acquired a more and more pronounced spore-membrane towards

* This, like all the temperatures in this part of the work, refers to the air temperature: a point of some importance later on.

† They are not oil, because they stain with methylene blue and other dyes; it is more likely that they are the chromatin granules referred to in Hueppe, 'Methoden der Bakt.-forsch.,' 5th edit., 1891, p. 154.

FIG. 15.



evening, and by 10 P.M., or thereabouts, was completed in all respects as a ripe spore like that started with sixty-nine hours previously.

A still better series of stages observed in the development of the spore is shown in fig. 18. The filaments of a very vigorous culture in broth at 20° C. were passing over to the development of spores in nineteen to twenty hours after sowing, and at 10.55 A.M. the cells, about twice as long as broad, had their protoplasm studded with the minute brilliant points already referred to (fig. 18 *a*). These bright masses stain deeply with methylene blue, as said, and are probably of the nature of the chromatin granules referred to in works on bacteriology.* They also slowly change their positions in the protoplasm, and at 11.40 A.M. had changed in numbers, positions, and sizes, as shown in fig. 18 *b*. These changes slowly proceeded, in the case under observation, until all the bright substance was aggregated near the centre of the protoplasm into a more or less definite oval body (fig. 18 *c*) which is evidently the incipient spore. This was at 9 P.M. During the night these masses had each become larger, more defined, and had clothed itself with a distinct cell wall (fig. 18 *d*). Each cell now contained one distinct oval spore, and was more cut off from its neighbour by a tendency to rounding

* See Hueppe, 'Methoden,' *loc. cit.*

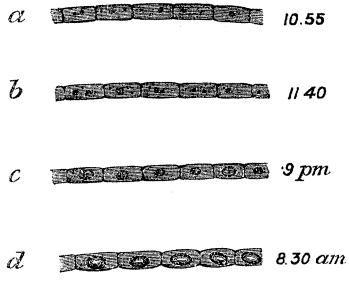


Fig. 18.

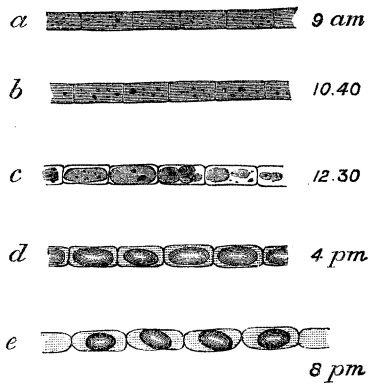


Fig. 19.

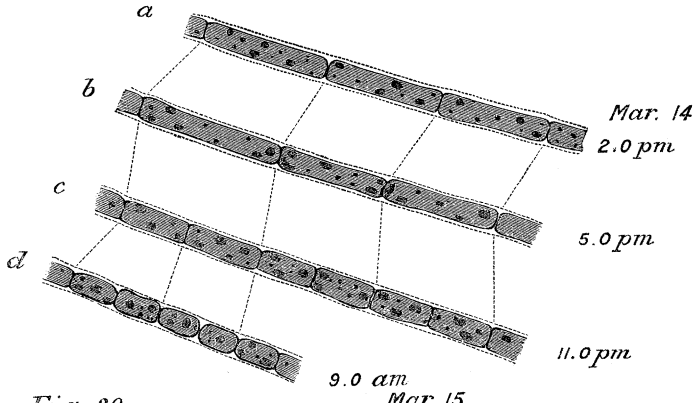


Fig. 20.

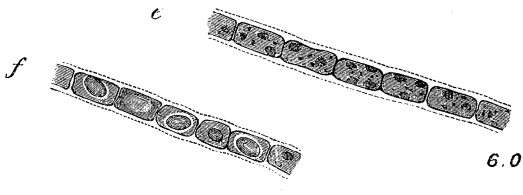


Fig. 21.

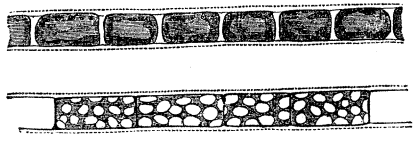


Fig. 22.

off. The ripe spore does not contain all the cell-contents, but lies embedded in a matrix of something remaining over from the cell-protoplasm. This might also be inferred from the double staining of which such preparations are capable.

In this case, therefore, the whole life-history—from spore to spore—was gone through in a little over forty hours.

Another equally interesting and rapid case of spore-formation is figured in fig. 19, where the changes in the granules and the gradual isolation of the spore mother-cells are again evident traced under a still higher power (1/20th oil immersion).

In fig. 20 I have shown a series in a case which presents some points of extreme interest, which, it seems to me, would be well worth special study.

A culture which had been exposed to blue light, and was much retarded, began to show signs of spore-formation at 2 P.M.—the filaments being then sixty-four hours from sowing. It was now in ordinary daylight, but I have no records of the details of temperature.

The peculiarities observable are (1) the very late appearance of the bright granules; (2) that they were first developed in cells much longer than usual; (3) their slow changes of position during several hours (fig. 20 *a*—*c*); (4) the subsequent division of the cells to the short form characteristic of spore-mother-cells (fig. 20 *c*, *d*); and (5) the incomplete character of the spores when they did at last appear more definitely in some of the cells.

I cannot avoid the conclusion that the blue light had something to do with this retardation and hindrance of spore-formation—an observation quite in accordance with some earlier ones on the development of spores in fungi*—and, as said, believe the subject well worth further study.

That the bodies above described are true spores is proved by their behaviour towards staining and other reagents, their resistance to high temperatures,† and above all by their germination which, as we have seen, I have observed and traced stage by stage.

They refuse to stain by all the ordinary methods for staining the bacilli, but stand out from the coloured protoplasm in such cases like brilliant colourless oil drops; on the other hand they stain fairly easily by the usual methods for spores, and especially with aniline-fuchsin or anilin-methyl violet, after being passed 10 times through the flame.

The ripe spores from potato- or agar-cultures germinated readily

* See 'Bot. Zeitung,' 1885, p. 6, where L. Klein confirms Rindfleisch in the observation that the development of the conidia of *Botrytis* is inhibited by blue light and by ordinary daylight, but not by red light.

† See pp. 350 and 351 for more detailed proofs of this.

in broth in which they were heated to 80° C. for two hours, and somewhat more slowly in the same medium in which they were boiled for a minute; but after five minutes boiling they appeared to be all killed, since none germinated out in six days at 20° C.

Measurements of Growth of the Rods and Filaments.

As will be evident from what has been said above concerning the growth of the germinal filaments, they elongate in nearly a straight line so long as they are free to do so and meet with no mechanical obstruction. This fact, and the obviously rapid growth, led me to a method for measuring the rate of elongation; and not only did I succeed in doing this efficiently, but these growth measurements carried me on much further and to some unexpected and interesting results in another connection.

On placing the eye-piece micrometer so that its vertical division crossed the long axis of a filament shorter than the scale, it was easy to observe the gradual extension of the filament as its ends passed over the divisions. The value of each division was determined beforehand, by examining a stage-micrometer with the same combination as I used for the measurements.

Having selected a nearly straight filament which extended over twenty-seven of the fifty divisions on the scale, and having determined that each division was equal to 3 μ for the power—Zeiss E occ. 2—employed, it was evident the filament was 81 μ long.

It was growing in broth at 16° C. when put under observation at 10.10 A.M., and was watched for two hours, during which period the thermometer rose from 18° C. to 20° C.* The microscope stood under a shaded bell-jar at a south window, and the day was cloudy and dull.

At 10.22 A.M.—*i.e.*, twelve minutes after measuring the filament—it had elongated so as to cover thirty divisions instead of twenty-seven. In other words it had grown 9 μ longer (fig. 13 *a*, *b*) and had slightly altered its slight curvature. At 10.45 it had grown another 12 μ (fig. 13 *c*); at 11.30 it was longer by 18 μ, and at 12.10 its elongation amounted to 24 μ further.

That is to say, in the interval from 10.10 A.M. to 12.10 P.M. (two hours) the total growth in length of the filament amounted to 63 μ. During the first twelve minutes the rate of growth was 0·75 μ per minute; during the next twenty-three minutes the growth was at the rate of nearly 0·5 μ per minute; during the next forty-five minutes it was at the rate of 0·4 μ per minute; and during the last forty minutes at the rate of a little over 0·5 μ per minute.

These facts may be conveniently tabulated as follows:—

* Air temperatures throughout, except where specially given as otherwise.

Time.	Length.	Interval.	Growth.	Approximate rate per minute.
	μ.	mins.	μ.	μ.
10.10 A.M.	81	—	—	—
10.22 „	90	12	9	0·75
10.45 „	102	23	12	0·5
11.30 „	120	45	18	0·4
12.10 P.M.	144	40	24	0·6

It is obvious that the growth is moderately rapid (the length would be doubled in about $2\frac{1}{2}$ hours at same rate), but it seems to vary from time to time. An elongation of 63 μ in 120 minutes would give nearly 0·3 μ per minute at constant rate, whereas the rate varies considerably on either side of that.

These variations could not obviously be attributed to variations in temperature, for the thermometer was steadily rising the whole time, nor did I think they could be due to the measurements, for although the slight nutations do occasionally interfere with the strict accuracy of these, the disturbance can hardly be imagined to be so great as these variations imply, and repeated experience convinces me that this is not the explanation at all.

As already stated, the microscope was placed at a south window, under a bell-jar surrounded with black paper, and, except during the short periods necessary for drawing and recording, very little light could reach the object. The day was dull and rainy, but with somewhat brighter intervals. During the whole of the two hours the filament was describing the slight writhing movements which I regard as nutation curvatures, and the extent of which can be estimated by the drawings in fig. 13.

But another idea strikes one in connection with these measurements. If we take the germinating filaments, and draw them vertically to scale, on sectional paper, at any rate as regard the *lengths* attained during the various periods of growth, it is obvious that if their lengths are arranged as vertical lines (ordinates) on a base line divided into periods corresponding to the times (abscissæ) then the curve joining the tips of the filaments is *the curve of growth*, and clearly we may substitute mere vertical lines (ordinates) for the detailed drawing of the filaments themselves.

For instance, the curve of growth is got by straightening out the filaments of fig. 24, where all are drawn to scale at the indicated hours, and joining the tips—or, what amounts to the same thing, by joining the upper ends of ordinates of equal lengths erected on a base line.

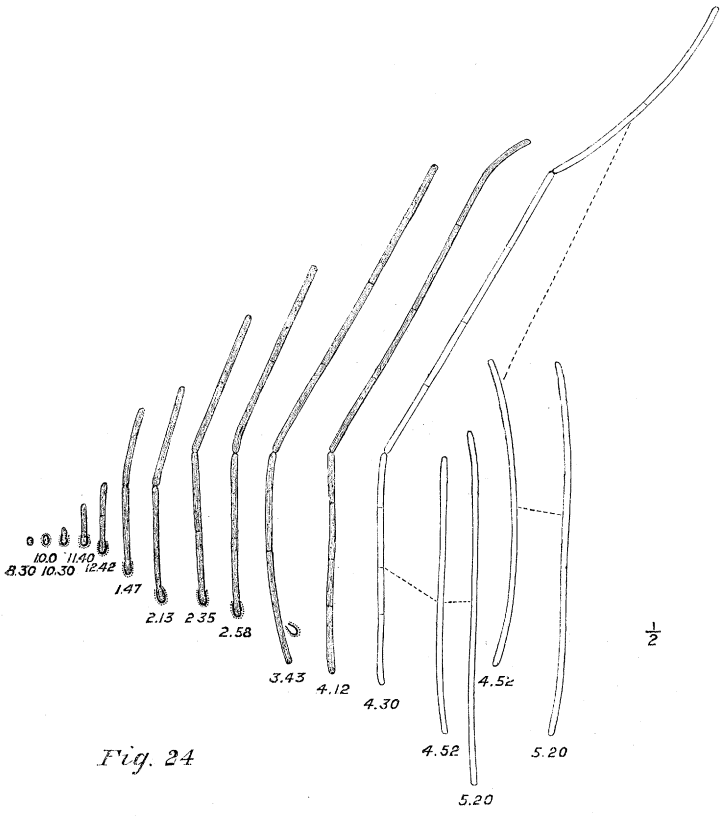


Fig. 24

In the further observations on growth, I availed myself largely of this idea and its consequences, with results of considerable interest and importance.

The following measurements of the rate of growth of the germinating filament (as contrasted with the longer older filament) were made. The spore was sown in 3 per cent. glucose solution (to which a little broth was added) shortly after 4 P.M.: the temperature was 20°—21° C. throughout.

From 5 P.M. to 6.5 P.M. the spore had swollen and elongated to 3 μ, and the measurements began at 8.10 P.M., when the germinal filament, still in the spore, measured 6 μ.

At 8.40 P.M. the young rodlet had elongated to 9 μ—*i.e.*, it had grown 3 μ in thirty minutes. At 9.45 it had grown another 9 μ. From 9.45 to 11.0 P.M. it grew 12 μ. From 11.0 to 11.45 it had grown 6 μ.

The measurements were then discontinued till 10.35 next morning, when 45 μ additional had been added.

The total growth in length from 8.10 P.M., when the length recorded was 6 μ, to 10.35 next morning, when the length was 81 μ, therefore, amounted to 75 μ in fourteen hours twenty-five minutes. This gives an average growth of about 0·086 μ per minute, but the rate varied considerably from time to time. Thus during the first thirty minutes (from 8.10 to 8.40 P.M.) it was about 0·33 μ per minute. During the next sixty-five minutes it averaged only about half that amount, a fact which puzzled me exceedingly, and which I thought might possibly be explained by the germinal rod being engaged in preparation for its first segmentation; for the first septum was visible shortly afterwards. But during the next period, of ninety-five minutes, the rate was apparently only about 0·12 μ per minute; and during the next forty-five minutes, almost the same average rate was observed, whence I came to think the explanation temporarily entertained may have to be abandoned. Then came the break in the observations. During the night—11.45 P.M. to 10.35 A.M. = 650 minutes—the total growth was 45 μ, *i.e.*, about 0·66 μ per minute on the average.

From the general slowness of growth of this specimen, I was more inclined to suppose that the medium (3 per cent. glucose solution) was less adapted for the needs of the organism than broth is, a conclusion fully borne out by tube-cultures, and subsequent experience.

Spores sown in broth at 15° C. at 3 P.M., had given rise to vigorously growing plants next morning, the long filaments of which were already breaking up as described on p. 279. One of these partial filaments was fixed under the micrometer scale, each division of which corresponded to 3 μ, with the combination (E/4) used. The filament was quite straight, and its ends were covered by the 11th and 38th divisions respectively when the observations began. During the whole period the temperature was 15° to 16° C. and did not fall or rise outside those limits. Moreover, I kept the microscope at a north window, and by means of cardboard screens and by turning the mirror between the observations, prevented any access of direct light such as might possibly be suspected of inhibiting the growth.

At 10.18 the filament was straight and measured 27 divisions of the scale in length—*i.e.*, it was 81 μ long, and the following shows the growths at successive intervals.

Time.	Length.	Interval.	Growth.	Approximate average rate per minute.
	μ.	mins.	μ.	μ.
10.18 A.M.	81·0	—	—	—
10.36 „	85·5	18	4·5	0·25
10.49 „	98·5	13	3·0	0·25
11.2 „	94·5	13	6·0	0·5
11.15 „	96·0	13	1·5	0·12
11.30 „	98·5	15	2·5	0·17
11.42 „	100·5	12	2·0	0·17
12.6 P.M.	105·0	24	4·5	0·2
12.25 „	109·5	19	4·5	0·5
12.42 „	112·0	17	2·5	0·12
12.56 „	114·0	14	2·0	0·14
2·0 „	120·0	64	6·0	0·1
3.0 „	126·0	60	6·0	0·1
4.0 „	135·0	60	9·0	0·15

It may seem a remarkable coincidence that this partial filament was the same length as that measured previously, but the explanation is very simple, though I did not discover it till later: it is that the long filaments tend to begin breaking up into isolated segments of this length (each consisting of several cells) very early under certain circumstances.

I have given the measurements taken at 2, 3, and 4 P.M., but these are far less useful than those preceding them, for two reasons: (1) the intervals are too long, and (2) the filament underwent considerable curvatures from about 2 o'clock onwards, so that I cannot insist on the accuracy of the measurements so strongly as on those made previously.

If, now, we examine these results, it is clear that the filament grows more slowly at 15° C. than it does at 20° C., as was to be expected. It is also evident that considerable variations in the rate of growth occur during the whole period, and since I could not refer these to the action of any external causes, it seemed necessary to assume that we have here a case of periodicity due to internal causes of growth. Thus, there was an acceleration between 10.49 and 11.2, and another between 12.6 and 12.25. I have as yet failed to correlate these with any observed phenomenon, but it is clearly a question worth asking, whether the slower periods intervening were not perhaps the periods during which new septa were put into the filaments—*i.e.*, period of cell-division. I was strongly inclined to think that is the case, though I had hitherto been unable to actually satisfy myself by directly observing the phenomenon. It is certain, however, that the filament at the beginning of the observations had fewer septa than at the end: for at 10.18 there were only 16 septa, whereas

at 4 o'clock there were certainly 23, and I believe more, perhaps 25 or 26, but those most recently formed are very difficult to see with the powers employed for measurements.

The whole period of growth under observation was (from 10.18 A.M. to 4 P.M.) six hours all but eighteen minutes, *i.e.*, 342 minutes, and during that period the total elongation (from 81 μ to 135 μ) amounted to 54 μ, and taking the average length of the growing segments at 5 μ, this would agree very well with the above, and, so far as it goes, is evidence in favour of the view I have supposed probable.

Having regard to the paucity of exact measurements after 12.56 in the above series, it is scarcely of value to note that the average rate of increment over the whole period, at 15° C., seems to be about 0·15 μ per minute; and the less useful since we have seen that there are such marked periods of slower and more rapid growth. Nevertheless, this would amount to a good deal in, say, sixty hours; if the germinal filament from a spore 2 μ long continued to grow only at that rate, the filament produced would be 600 μ long, and would be segmented into from 100 to 200 bacillar segments. But this is far less than actually occurs, as we shall see later.

In the following case the spore was sown, in dilute broth, a little after 10 A.M., and was put under observation by 11 A.M. The temperature was then 16° C.; but it fell to 15° by 4 P.M., and slowly down to 12° C. by 7 P.M. The spore was germinating at 12.10 P.M., when it measured 3 μ.

Time.	Length.	Interval.	Growth.	Approximate rate per minute.
	μ.	h. m.	μ.	μ.
12.10 P.M.	3·0	—	—	—
4.30 ,,	10·0	4 20	7·0	0·03
5.40 ,,	13·5	1 10	3·5	0·05
5.54 ,,	15·0	0 14	2·5	0·18
6.12 ,,	17·0	0 18	2·0	0·11
6.27 ,,	18·0	0 15	1·0	0·07
6.45 ,,	19·0	0 18	1·0	0·06
7.5 ,,	22·5	0 20	3·5	0·17

This gives a total growth of 19·5 μ in six hours and fifty-five minutes, with an average growth of 0·09 μ per minute if the rate were constant.

I was unable to distinguish any trace of a second septum up to 6.45, but at 7.5 the rod showed a perfectly sharp median septum, and two extremely faint secondary septa—one on each side.

I thought there could be little doubt that the sharp fall in the rate of growth at 6.27 and 6.45 was associated with the process of cell-

division which initiated these new septa, because the fall in temperature seemed probably too gradual and slow to account for it. No doubt these septa were already present at 6.45, and the increased rate of growth followed on their inception, but, with the power employed, they would be invisible for some time, as experience had shown me in other cases.

The following series were traced on a segment broken off from one of the filaments of the same culture as the last, and is therefore practically a continuation of that series into the night. It clearly bears out the same conclusion, that the period of cell-division entails more or less cessation of growth. In this case the temperature was 12° C. throughout the whole period during which the rod was kept under observation, and the whole growth took place in the dark, consequently the variations noticed cannot have been brought about by recognisable changes in the environment, but must be referred to internal causes.

Time.	Length.	Interval.	Growth.	Approximate rate per minute.
	μ.	h. m.	μ.	μ.
7.8 P.M.	5·0 (rod	with no trace of	septum).	—
7.34 „	14·0	0 26	7·0	0·27
8.39 „	22·5	1 5	6·5	0·10
9.10 „	25·5	0 31	3·0	0·09
9.28 „	27·0	0 18	1·5	0·08
9.55 „	30·0	0 27	3·0	0·11
10.23 „	34·0	0 28	4·0	0·14
10.48 „	38·0	0 25	4·0	0·16

In this case no septum was visible at 7.8 P.M., but there was one at 7.34 P.M., and the rod consisted of two cells. At 8.39 to 9.28 we have another period of slower growth, and during this interval each cell put in another septum; then it increased again, up to 10.48 P.M., when the observations ceased for the night.

At 8.30 on the following morning the filament measured 144 μ, having grown 106 μ during the nine hours and forty-two minutes, which would give 0·18 μ per minute as the approximate average if the rate were constant. This seems a somewhat high rate for the temperature (12° C.), and suggests a question which I afterwards tested; the question is, does the average rate of growth, other things being equal, increase during the dark hours of the night?

The following series, however, drew my attention to some questions of temperature more definitely than hitherto, and led, as we shall see, to some interesting results in this connection.

A spore, sown soon after 10 A.M. in dilute broth, was kept entirely

in the dark throughout the whole period of the experiment, except at the short intervals (one to two minutes each) necessary for recording the growth.

The temperature at 10—11 A.M. was 16° C., but it rose to 20—21° C. at 2 P.M., and remained at that until about 7 P.M., when it fell until it was again 16° C. at 8.30 P.M., and stayed so through the night.

The spore germinated vigorously, and the germinal rodlet was 10 μ long by 2 P.M. The following table shows its further behaviour:—

Time.	Length.	Interval.	Growth.	Approximate rate per minute.
	μ.	h. m.	μ.	μ.
11 A.M.	2 (spore)	—	—	—
2 P.M.	10·0	3 0	8·0	0·44
4.45 ,,	57·5	2 45	47·5	0·3
5.25 ,,	77·5	0 40	20·0	0·5
5.42 ,,	102·5	0 17	25·0	1·5
5.52 ,,	115·0	0 10	12·5	1·25
6.10 ,,	125·0	0 18	10·0	0·55
6.25 ,,	145·0	0 15	10·0	0·7
6.43 ,,	160·0	0 18	15·0	0·8
7.2 ,,	180·0	0 19	20·0	1·0
7.15 ,,	190·0	0 13	10·0	0·8
7.36 ,,	210·0	0 21	20·0	1·0
8.36 ,,	225·0	0 60	15·0	0·25
9.10 ,,	240·0	0 34	15·0	0·44
9.27 ,,	250·0	0 17	10·0	0·6

On scrutinising this table and drawing the curve representing the increments, it is evident that we have the effects of the higher temperature expressing themselves in conjunction with those (if there are any) due to absence of light.

The characters of the culture itself were all those of a superbly vigorous organism, and the crop of spores developed at the end of its growth—the spores were ripe in 72 hours from sowing—was a remarkably fine one.

As we see, in the period of growth recorded from 2 P.M. to 9.27 P.M., the rodlet elongated from 10 to 240 μ. In other words, it grew 230 μ, which is 115 times the length of the spore, in $7\frac{1}{2}$ hours. This would give an average growth of about 0·5 μ per minute throughout the period referred to.

On comparing these results with what were obtained during growth at the same temperature in ordinary daylight, I could not see how to escape the conclusion that the rapid rise of the curve is chiefly due to the absence of the inhibitory effect of the light.*

* Unless—a question which arose later—the temperature *inside* the culture-chamber is very different from that recorded by the thermometer standing, as here, outside the system.

Moreover, the same conclusion forced itself on one if other curves are compared: the general slope in each case is undoubtedly largely due to the lower temperature, but the decided rise in some cases about 6 P.M., when the daylight failed, took place *in spite of the falling temperature*, and the generally steeper ascent of others at this low temperature might be due to the cessation of light-action—at least I could see no other way of explaining it at the time.

It is clear from the foregoing results that the growth of the rods and filaments of this bacillus *can* be measured by the methods devised, and that the undertaking presents no particular difficulties so far, beyond those incident to all close and patient microscopic investigation.

But, beyond the fact that growth occurs at various rates, and can be thus measured, the results thus far give us far too little information to be of the value I anticipated from the application of the method. They simply raise a number of questions as to the action of various factors in influencing the course of growth and inducing the variations in the rate of growth which undoubtedly occur.

Such factors are (1) internal factors, such as the age of the filament, the process of cell-division, and possibly the vigour of the spore itself; and (2) external factors, such as temperature, light of various kinds, and the food-materials, &c.

Growth and Cell-division.

I made the following observations with a view to obtain more information concerning the question raised as regards the connection between growth and cell-division. The chief difficulty connected with them was, as might be expected, that very high powers have necessarily to be employed, and all the troubles of thin cover-slips, minute and not too deep drops, and so forth, arise.

I selected the tip of a filament which had been growing vigorously all the afternoon and evening, and which was in all about 600 μ long, and traced its behaviour under the 1/12th oil immersion. The piece I chose was the terminal portion from the tip to the first visible septum. Its length was measured as exactly as possible, and when the septum was under the division 0 of the scale the tip was exactly under the 14th division.

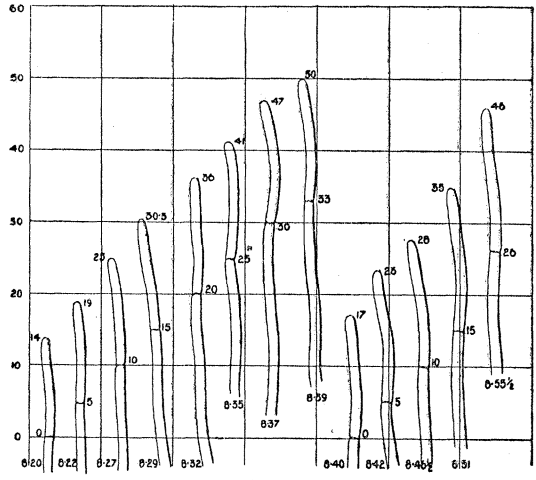
I had previously determined that six divisions on the scale used, each representing 4·5 μ with my ordinary measuring combination, are equal to fifteen divisions by this power, and therefore I was measuring nearly 2 μ per division (more exactly $= 1{\cdot}82\ \mu$).

At 8.20 P.M. the segment in question measured (under the 1/12th immersion) fourteen divisions, *i.e.*, $14 \times 1{\cdot}82 = 25{\cdot}48\ \mu$, and I started

the observations. The scale consisted of fifty divisions, and I started with the septum at 0 and the tip at 14. The following table gives the positions of this septum and tip at successive intervals as (1) the growth of the terminal segment carried tip and septum further apart, and (2) the elongation of the rest of the filament as a whole pushed the entire segment forward over the scale.

It will be noticed that to do this I observed the successive periods during which the filament pushed the septum over five divisions, as I found that the easiest plan, and then recorded the position of the tip at the time (see fig. 26).

FIG. 26.



Time.	Division of scale over septum.	Division of scale over tip.	Growth of segment	Interval.	Rate.
			μ.	mins.	*μ*.
8.20 P.M.	0	14·0	—	—	—
8.22 „	5	19·0	—	—	—
8.27 „	10	25·0	1·82	7	0·26
8.29 „	15	30·3	—	—	—
8.32 „	20	36·0	1·82	5	0·36
8.35 „	25	41·0	—	—	—
8.37 „	30	47·0	1·82	5	0·36
8.40 „	0*	17·0	—	—	—
8.42 „	5	23·0	1·82	5	0·36
8.46½ „	10	28·0	1·82	4½	0·4
8.51 „	15	35·0	3·64	4½	0·8
8.55½ „	26	46·0	—	—	—

* *i.e.*, I brought the septum back again so as to lie under the division 0 on the scale.

Here we see the segment elongated from fourteen divisions to twenty divisions, *i.e.*, it grew six divisions ($= 6 \times 1{\cdot}82 = 10{\cdot}92\ \mu$) in the interval from 8.20 to 8.51—*i.e.*, in thirty-one minutes—giving an average rate of growth of about $0{\cdot}35\ \mu$ per minute, though the rate of growth varies from time to time.

Now the whole filament was 600 divisions long ($= 1{,}092\ \mu$), and consisted of at least forty such segments,* and if each of them was growing at anything like this rate, no wonder the filament pushed this segment forward so quickly, for it would be elongating as a whole at the rate of $14\ \mu$ per minute.

To gain further information in this connection, I exchanged the objective for the combination I usually employ for measuring, and measured the growth of about a third of the whole filament (including the part here concerned) during the seven minutes from 9 to 9.7 P.M., *i.e.*, beginning $4\frac{1}{2}$ minutes after the last measurement.

At 9 P.M. the piece observed was $210\ \mu$ long, and at 9.7 P.M. it had elongated to $250\ \mu$, giving a growth of $40\ \mu$, which is at the rate of $5{\cdot}7\ \mu$ per minute. If this was going on through the rest of the length, the filament as a whole would be growing at a rate considerably in excess of my estimate.

Now if we look at the distance through which the measured segment was pushed during the thirty-one minutes' period given above, we find it amounts to forty-five divisions ($= 45 \times 1{\cdot}82 = 81{\cdot}90\ \mu$), so that even such numbers as I have proposed need not seem extravagant, and indeed I have reason to know they are much below the real ones in many cases.

One of the most interesting cases of rapid growth I have seen is the following.

Spores sown in normal gelatine at 22° C. had germinated out to filaments 80—100 μ long in $5\frac{1}{2}$ hours in the dark, and were then put into the dark Sachs' box† at 28° C. The temperature was then slowly raised, so that in two hours it had risen to 34° C., half an hour later to 38° C., and in the next quarter of an hour to 39° C. By chance I happened now to catch a broken-off segment which was growing at the maximum rate that precedes death at these high temperatures. The measurements were as follows:—

* Almost certainly more than 40, but I could not determine accurately because the last formed septa are not sufficiently distinct.

† See p. 394 for description of this box and the method of using it.

Time.	Length.	Interval.	Growth.	Rate.	Temperature.
	μ.	mins.	μ.	μ.	°C.
5.12 P.M.	139·5	—	—	—	39·0
5.14 „	153·0	2	13·5	6·7	39·1
5.15½ „	175·5	1½	22·5	15·0	39·25
5.17 „	198·0	1½	22·5	15·0	39·2

Here we see growth going on at the enormous rate, hardly measurable, of 15 μ per minute. In such cases the growing tip is seen moving almost like an *Oscillatoria.*

Then the filament suddenly contracted and broke up, and in a short time presented the granular appearance of dead cells.

The following measurements under the 1/12th immersion were made on a germinal filament which had emerged from the spore during the night. The culture in broth was exposed to the daylight the whole time,* but the sun was obscured by haze and clouds. The temperature was rising slowly from 18° to 21° C., also during the whole period observed.

Each division of the micrometer scale was in this case again equal to 1·82 μ, and the measurements were made as 1, 2, 3, or 4 divisions were travelled over.

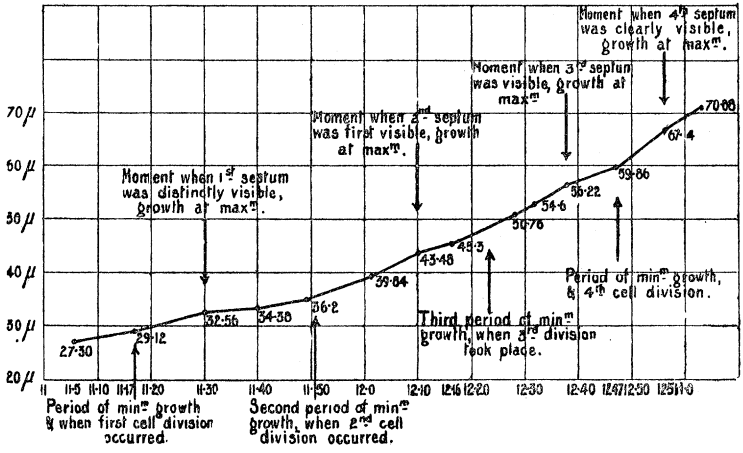
Time.	Length.	Interval.	Growth.	Rate.	Temperature of air.
	μ.	mins.	μ.	μ.	°C.
11.5 A.M.	27·30	—	—	—	18·0
11.17 „	29·12	12	1·82	0·15	18·0
11.30 „	32·56	13	3·64	0·28	18·0
11.40 „	34·38	10	1·82	0·18	18·5
11.49 „	36·20	9	1·82	0·20	18·5
12.1 P.M.	39·84	20	3·64	0·18	18·5
12.10 „	43·48	9	3·64	0·40	19·0
12.16 „	45·30	6	1·82	0·30	19·0
12.28 „	50·76	12	5·46	0·42	19·0
12.38 „	56·22	10	5·46	0·54	19·5
12.47 „	59·86	9	3·64	0·18	20·0
12.56 „	67·14	9	7·28	0·81	20·5
1.2 „	70·88	6	3·64	0·60	21·0
1.5 „	74·52	3	3·64	1·21	21·0

Here we have a total growth of the young filament amounting to 47·22 μ in 120 minutes, which would give an average rate of nearly

* It should be noted that in these high-power observations a bright illumination has of course to be employed.

0·4 μ per minute if constant. But obviously the rate was not constant, as the table and curve show (see fig. 27).

Fig. 27.



I made the following observations in addition to see if the variations were connected with cell-division as suspected. Up to 11.17 no septum was really visible* in the young filament, but a distinct median one was seen at 11.30 dividing the filament into a proximal and a distal half. By 12.10 a second septum was clearly visible bisecting the distal half, but none was as yet visible in the proximal half to which the spore membrane still clung. At 12.38 the proximal segment was also divided by a visible median septum. At 12.47 I measured both the primary segments and found the proximal, recently divided, one shorter than the distal one, in the ratio of 15 to 18; that is to say, the whole filament measured 59·86 μ, as seen, but that part of it to the proximal side of the first septum was only 27 μ, that to the distal 33 μ in length; so that already the symmetry of the filament was disturbed, and further measurements confirmed this.

At 12.56 the proximal segment had one septum, now very distinct, and measured 30·5 μ, whereas the distal one was by this time provided with two visible septa, and measured nearly 37 μ; at 1.5 P.M. the proximal one still had but one septum visible, and measured 32·5 μ, whereas the distal one, with its two visible septa, measured 42·5 μ.

Hence we see the two primary segments resulting from the first division of the germinal filament grow and (so far as *visible* segments

* Probably thin septa were present, but they were not *visible* in the living and rapidly growing filament.

show) divide at different rates from the first. I am disposed to regard the slower growth of the proximal segment, in part at any rate, to its being more especially concerned with the absorption of food-materials from the spore; though the fact that it is still behind-hand, even after escaping from the spore-membrane, may indicate a deeper meaning—possibly that differences between basal and apical regions are more strongly defined in these organisms than we suppose.

But another point must be considered before the curve can be understood, and for this purpose it seems necessary to introduce a simple nomenclature for the divisions and segments.

We may term the first septum, which divides the whole germinal filament into its first two segments, the primary septum; thus the primary septum was first *visible* at 11.30, dividing the filament into a proximal and a distal primary segment. At 12.10 the longer *distal* primary segment showed a further division by a *secondary* septum into two *secondary* segments; but the corresponding secondary septum in the *proximal* primary segment was not visible until 12.38.

At 12.56 the distal secondary segment of the primary distal segment had a *tertiary* septum plainly visible, and the filament as a whole, therefore, consisted at this hour of five visible portions, two belonging to the primary proximal segment, and three belonging to the primary distal segment.

On turning to the curve of growth (fig. 27), it may now be possible to understand its principal features if we first accept as a fact that the period when a septum is first *distinctly visible* in these brilliant living cells is some time *after the moment of actual cell-division*. This, I think, must be accepted, because I find stained preparations of such filaments show many more septa to be actually present than can be seen in the living filaments, owing to the extremely high refrangibility of the protoplasm obscuring the view of the most recently formed and still tenuous walls.

In the curve referred to, as I understand it, its general form, with a higher and higher rate of ascent as time goes on, is due to the total increasing elongation of all the segments simultaneously—aided in this case by the slight continuous rise of temperature.

But, although the general sweep of the curve is such, there are clear indications of smaller curves, convex also to the abscissæ, on which points of maximum rate of growth are seen at 11.30, 12.10, 12.38, and 12.56, and points of minimum rate in each case about mid-way between these times.

Now, it was at just these periods—the intervals between which are approximately 40, 30, and 20 minutes respectively—that the new cell-walls were first perceptible, and it seems almost certain that the intervening periods of slowest rate of growth, viz., about 11.15, 11.50, 12.20, and 12.40 respectively, were the approximate moments

of insertion of the septa, and, consequently, moments when elongation would be going on with least rapidity, because the cell-contents were too busy with the act of division to be then growing at quickest rates.

In any case, it is obvious that the growth of even these young filaments is a complicated phenomenon, and that the following points at least have to be regarded in considering it.

(1) There is the total elongation of the filament as a whole, and (2) the growth of the individual segments or cells; (3) the growth of any one segment or cell is not necessarily going on at exactly the same rate as a neighbouring one: this depends on the position and on the age of the cell concerned; (4) there is evidence to show that the rate of growth of any cell or segment, and consequently that of the filament, as a whole, is profoundly affected by several factors, such as temperature, the nature of the food-medium, the presence of other filaments which may aid the given one (at certain stages) by increasing the peptonisation in the immediate neighbourhood—though we must believe that in other stages these neighbouring filaments retard it by acting as competitors for the available food; and, lastly, possibly light, some rays of which (perhaps blue) may retard the growth, while others (possibly the red and infra-red) may accelerate it.

The following observations with the 1/12th immersion were made on segments of a two-days' growth in broth, with a trace of gelatine, in bright light—not direct sunlight—at 17° to 18° C.

A terminal segment showing three distinct septa, but already composed of at least eight cells—the other four (secondary) septa being very difficult to observe—was 54·6 μ long at 9.5 A.M. The growths are shown in the following table, and, as before, we see that considerable irregularity is observable in the rate from time to time:—

Time.	Length.	Interval.	Growth.	Rate.
	μ.	mins.	μ.	μ.
9.5 A.M.	54·6	—	—	—
9.12 „	56·42	7	1·82	0·26
9.23 „	58·24	11	1·82	0·17
9.31 „	60·06	8	1·82	0·22
9.38 „	60·97	7	0·91	0·13
9.40 „	61·88	2	0·91	0·45

The segment was being pushed forward by the rest of the filament at the rate of about 1 μ per minute—36·4 μ in 34 minutes actually measured—and was slightly undulating the whole time. In fact the movement forwards, a scarcely perceptible series of jerks, reminded one of the movements of the hands of a watch.

At 9.45 another segment, also a terminal one, was selected for observation, and behaved as follows:—

Time.	Length.	Interval.	Growth.	Rate.
	μ.	mins.	μ.	μ.
9.45 A.M.	18·2	—	—	—
9.50 „	18·11	5	0·91	0·18
10.5 „	20·02	10	0·91	0·09
10.20 „	20·93	15	0·91	0·06
10.50 „	33·67	30	12·74	0·42

Here, again, the extraordinarily irregular growth comes out clearly, looking as if the segment, having gradually slowed down its rate of growth to a minimum from 9.45 to 10.20, suddenly began to grow at a rapid rate again.

The following measurements were made on a terminal segment, of the same culture, measuring 18·2 μ, and showing one distinct septum only, though each cell was almost certainly divided by a median septum during the period.

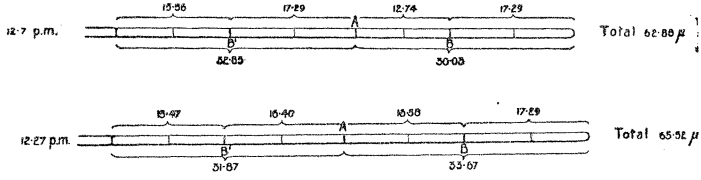
Time.	Length.	Interval.	Growth.	Rate.
	μ	mins.	μ.	μ.
11.0 A.M.	18·2	—	—	—
11.20 „	18·2	20	0	0
11.35 „	20·02	15	1·82	0·12
11.55 „	20·02	20	0	0

At the end of the period I assured myself that the segment contained one very distinct septum dividing it into two parts, or secondary segments; the apical one measuring not quite 11 μ (my numbers give 10·92), and the other a little over 9 μ (9·10), and each of these had an extremely faint median septum in it. According to this, the segment 20 μ long consisted of four cells each, more or less, 5 μ long, and we must infer that they were growing at slightly different rates part of the time.

Another terminal segment of the same culture was watched under the 1/12th immersion. It measured 62·88 μ at 12.7 (P.M.) and consisted of eight visible cells, and probably each of these with a septum. As near as I could measure, the primary septa, which were very distinct, stood as follows: One (which I will call A) stood at 30·03 μ from the apex, dividing the whole segment selected into a (fig. 28) distal (apical) moiety measuring almost exactly 30 μ, and a proximal

moiety measuring 31·85 μ. Each of these portions was again sub-divided by a septum; the distal one, by a septum B, into a distal moiety just over 17 μ in length, and a proximal moiety not quite 13 μ long; the proximal one, by a septum B', into a distal moiety measuring just over 17 μ, and a proximal moiety slightly more than 15·5 μ. Close observation showed that each of these was again sub-divided by a faint, approximately median septum, into two cells.

FIG. 28.



Now it is perfectly obvious from this that the various subsidiary segments or cells, measured at any period, are not of exactly the same length.

At 12.27 I again measured, as exactly as possible, the relative positions on the scale of the above septa to see if I could determine the relative rates of growth of the various segments in the intervening twenty minutes.

The septum A had moved backwards nearly 3 μ on the scale, and the apex and base of the whole segment were driven apart about this distance. This suggests that the growth (intercalary) had occurred chiefly in the distal part of the original segment, and such was the case.

The distance between the septum B and the apex was not measurably altered, *i.e.*, the part to the distal side still measured 17·29 μ; but the distance between B and A had increased from 12·74 to 16·38 μ. On the proximal side of the septum A no appreciable changes had occurred. It is true, my measurements, as multiplied out, are a trifle different from those first made, but the differences may be neglected, as of course I could not measure to the decimals concerned.

What does come out, and very clearly, is that during the interval named the growth was entirely in one only of the four cells, and not in all of them. It is obvious that such phenomena complicate the question of the growth of the entire filament still more, and no doubt some of the minute variations observed are due to such events as these.

I made the following measurements under the 1/20th immersion, on a terminal segment 36 μ long, in the sister culture to the last one,

standing side by side with it. The temperature was rather low, 15° C., and the observations soon came to an end owing to the close-focussing objective cracking the very thin cover slip. I made many attempts to measure growths for longer periods under this lens, but the failures were so frequent owing to the extremely thin cover slips and hanging drops necessary for so high a power, and the difficulties of illumination, that I had to abandon them.

The value of each division of the micrometer scale was found to be from 1·20 μ to 1·25 μ, but here again I found it hopeless to attain to greater exactitude of measurement. However, the following attempt is at least interesting.

Time.	Length.	Interval.	Growth.	Rate.
	μ.	mins.	μ.	μ.
9.17 A.M.	36·0	—	—	—
9.19½ ,,	37·8	2½	1·8	0·7
9.22 ,,	38·4	2½	0·6	0·24

On comparing the figures with other measurements at the same temperatures, moreover, they agree very well; so that it may yet be possible to carry out measurements with this power.

It should be noticed—as a point of importance in what follows—that these minute variations observed with the highest powers are not traceable (and probably neutralise one another along the filaments) when longer stretches are measured with the lower powers.

After considerably greater experience with these curves, I am able to sum up the meaning of these experiments more clearly.

1. They give evidence that the growth of the filament as a whole is intercalary, and due to increase in length and division of all its cells, along the entire course of the filament.

2. The different rates in the general growth observed are due partly to differences in temperature, partly to differences in age of the portions observed, partly to differences in the food-medium in which the organism is growing, and partly to other causes.

3. The small variations in rate of growth, especially those traced under high powers, are due partly to small and unrecorded variations in temperature, *e.g.*, cooling of the thin cover-slip when the bell-jar was lifted (as in the experiment on p. 306), and partly to the causes assigned on p. 302, namely, pauses during the intercalation of the new segment walls, and, no doubt, to some extent, to curvatures in the filaments, and want of practice on my part in recording the observations so accurately as I learned to do later on.

Comparative Measurements.

The foregoing results led to the attempts—now to be described—to grow two filaments side by side, one in the light and the other in the dark, to see if the action of light could be detected by any change in the growth curve.

Before passing to these experiments, I obtained satisfactory evidence that two cultures, side by side and under the *same* conditions, behave similarly.*

On February 18th I started the following comparative cultures, to see how far I could test the action of daylight rendered so probable by some of the foregoing results.

Spores were sown in broth at 9 A.M. in two hanging drop cultures, each with a layer of water at the bottom to prevent rapid changes of temperature or drying up. The two cultures were then put under a dark bell-jar, covered with foil and brown paper, at 18° C., rising to 21° C., close to the south window where I intended to start the experiment. The cultures were left thus so that the spores should have time to germinate out normally, which they began to do about noon; they were left undisturbed till 2 P.M.

Meanwhile, I had selected two similar bell-jars and placed these a the window, each over a microscope provided with a thermometer; one bell-jar was darkened with tin foil and brown paper, the other not. The thermometers were examined, and readings taken from time to time. The differences observed at first, and due to the shading action of the covering, began to get less and less as the bell-jars, table, and microscopes, were warmed by the sun, which was bright and hot; and by noon the temperatures were very nearly the same in each jar.

At 12.30 the thermometer under the light jar indicated a temperature of 25·5° C.; that under the darkened one =24·6° C.,† and this difference of nearly 1° C. was maintained till about 1.30, the readings being as follows:—

	12.30 P.M.	12.45 P.M.	1.0 P.M.	1.15 P.M.	1.30 P.M.
	°C.	°C.	°C.	°C.	°C.
Dark ...	25·5	26·0	27·0	27·5	28·0
Light...	24·6	25·5	26·0	27·0	27·5

About 2 P.M. the sun began to sink behind trees and the roof of a building facing the window, and now it was possible to use the bright light from the sky without danger of direct insolation.

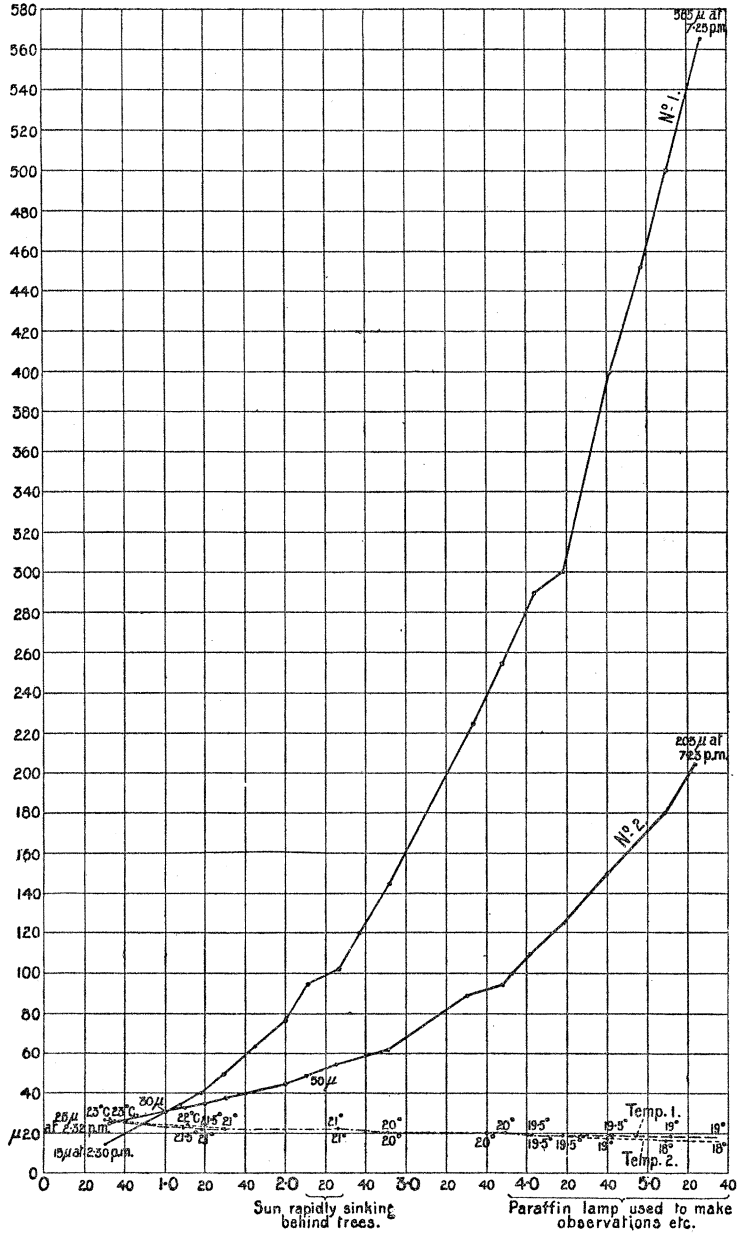
* Better proofs of this were obtained later, however, and are given at pp. 359—361.

† These are all air temperatures, unless the contrary is specially stated.

At 2.20 P.M. the thermometer in both bell-jars stood at 23° C., and, as the curve of temperature shows, they were so nearly equal throughout the experiment that I had little hesitation in concluding that the cultures did not vary much from the same temperature at any time; because I thought it could hardly be imagined that the hanging drops in these damp cells rapidly vary with the temperature of the environment, and still less so the filaments growing in them.

At 2.30 the observations were begun by noting the temperature, and measuring the rod in each case. As the following tabular record shows, the difference in time between the two notes rarely exceeded a couple of minutes, whence the two series are closely comparable in all respects.

It was not until much later that the question arose—or, rather, acquired the great importance I now attach to it—how far the culture in the light could avail itself of or be affected by the infra-red rays reflected from the mirrors, and so complicate the matter of temperature effects in these experiments. This matter is of pregnant importance in its bearing on all physiological experiments of this kind, however.


580
560
540
520
500
480
460
440
420
400
380
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
μ20
0
585 μ at 7·25 p.m.
No. 1.
205 μ at 7·25 p.m.
No. 2.
50 μ
30 μ
25 μ at 2·32 p.m.
15 μ at 2·50 p.m.
23°C.
23°C.
22°C.
21·5°
21·5°
21°
21°
20°
20°
20°
20°
19·5°
19·5°
19·5°
19·5°
19°
19°
18°
18°
Temp. 1.
Temp. 2.
0 20 40 1·0 20 40 2·0 20 40 3·0 20 40 4·0 20 40 5·0 20 40
Sun rapidly sinking behind trees.
Paraffin lamp used to make observations etc.

Culture in dark.						Culture in light.					
Time.	Length.	Interval.	Growth.	Approximate rate per minute.	Temperature.	Time.	Length.	Interval.	Growth.	Approximate rate per minute.	Temperature.
P.M.	μ.	mins.	μ.	μ.	°C.	P.M.	μ.	mins.	μ.	μ.	°C.
2.30	15·0	—	—	—	23·0	2·32	25·0	—	—	—	23·0
3.8	35·0	38	20	0·5	21·5	3·10	32·5	38	7·5	0·2	22·0
3.19	40·0	11	5	0·45	21·0	3·20	35·0	10	2·5	0·25	21·5
3.29	50·0	10	10	1·0	,,	3·30	38·0	10	3·0	0·3	21·0
3.45	65·0	16	15	0·95	,,	3·40	40·0	10	2·0	0·2	,,
4.0	78·0	15	13	0·86	,,	4·0	44·0	20	4·0	0·2	,,
* 4.11	96·0	11	18	1·6	,,	4·10	49·0	10	5·0	0·5	,,
* 4.26	102·0	15	6	0·4	,,	4·25	55·0	15	6·0	0·4	,,
* 4.52	145·0	26	43	1·6	20·0	4·50	62·0	25	7·0	0·28	20·0
5.32	225·0	40	80	2·0	,,	5·30	90·0	40	28·0	0·7	,,
†5.48	255·0	16	30	1·87	,,	†5·47	95·0	17	5·0	0·3	,,
6.3	290·0	15	35	2·3	19·5	6·1	110·0	14	15·0	1·0	19·5
6.19	300·0	16	10	0·6	,,	6·18	125·0	17	15·0	0·88	,,
6.41	400·0	22	100	4 5	,,	6·40	150·0	22	25 0	1·1	19·0
7.10	500·0	29	100	3·4	19·0	7·8	180·0	28	30·0	1·0	18·0
7.25	565·0	15	65	4·3	,,	7·23	205·0	15	25·0	1·66	18·0
10.45	—	—	—	—	18·0	10·45	—	—	—	—	18·0

Curves 1 and 2.

* Sun sinking fast behind trees, and light beginning to fade.

† For these (5.47 and 5.48 P.M.) and succeeding observations I had to use artificial light; I employed a paraffin lamp, about 2 feet from microscope, but the time occupied in making the observations was probably too short for perceptible effects.

The doubling periods* calculated on the curves were as follows :—

1. Dark culture.

				Approximate (air) temps.
15— 30 μ	= 2.30 to 3	P.M. =	30 minutes at	23·0 —21·75° C.
30— 60	= 3.0 „ 3.40	=	40 „ „	21·75—21
60—120	= 3.40 „ 4.37	=	57 „ „	21 —20·5
120—240	= 4.37 „ 5.40	=	63 „ „	20·5 —20
240—480	= 5·40 „ 7.4	=	104 „ „	20 —19

Now my constant temperature cultures in broth in the dark at 23—21·75° run from 32 minutes at 23—24° C. to 63 (at 20—21°) and at 18—19° C. the extremes are 67—78 minutes; the range is a wide one—*i.e.*, 32—78—but it may be regarded as including the above cases, or very nearly so, and perhaps, therefore, the air-temperatures given did not depart far from those of the culture cells.

2. The culture in the light for the same date has the following doubling periods :—

			Approximate (air) temps.
25— 50 μ	= 2.32—4.12 =	100 minutes at	23 —21° C.
50—100	= 4.12—5.53 =	101 „ „	21 —19·5
100—200	= 5.53—7.20 =	97 „ „	19·5—18

And there is nothing comparable as regards time with such long periods anywhere in my broth cultures at constant temperatures, in the dark.

Unless, therefore, we assume that the air-temperatures given depart very widely, in different ways, from the real temperatures of the culture-drops, we must assume that the lower growth in the exposed culture was due to inhibition by the light rays.

Probably the most startling fact which comes out on examining these results is the extremely rapid growth of the dark culture from 6.41 onwards. To explain this it is necessary to point out one or two things not obvious at all. In the first place, as the filament grows longer there are more and more cells formed by the repeated bi-partition—*i.e.*, there are more and more elongated segments in the chain, and therefore a greater elongation of the whole as the sum of the growths of the individual cells becomes greater. Then, secondly, the vigour of the entire cell-series evidently increases as the feeding and other powers—*e.g.*, peptonising activity, &c.—increase.

Consequently, after a certain period these strongly developing filaments which have not been exposed to any disturbing agent, grow at

* The doubling period is the time required for a given length of filament to double this length (see p. 403).

rates which are really enormous if we regard the relations of size. This is the more marvellous when one notices that this rapidity of growth was increasing *although the temperature was falling the whole time.*

This last is an important point, for if we now turn to the culture in the light, we find nothing like such a general increase in the power of development. Nevertheless, the filament clearly began to grow much more rapidly after sun-down (see 5.47 and 6.1 P.M), and was evidently tending towards some such maximum at 7.23.

Now, why did not this filament begin to get up its maximum of growth as soon as the other? It was a *longer* and more vigorous specimen to begin with, for I purposely chose a germinal filament 25 μ long, whereas that in the dark was only 15 μ long—and unless some inhibiting agent was keeping it back, it seems incredible that its general curve of growth should ascend so slowly before sun-down.

But it seemed clear that the inhibition cannot be due to the temperature, because that was falling too slowly to produce such an effect; and, besides, it falls practically equally in both cases, so that if it checked the growth of this culture, one asks why the lowering of the temperature did not check it in the dark?

If, then, the growth is accelerated after sun-down, *in spite of a fall of temperature*—as we see to be the case—the logical conclusion seemed to be that the acceleration is due to the withdrawal of the inhibiting rays of light, and on the hypothesis that this is the case the curves are intelligible.

Germination and Growth behind Glass Screens.

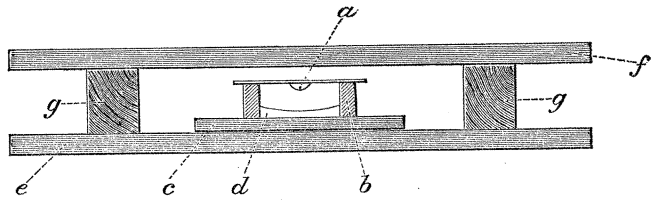
In view of the encouraging results of which the above is an illustration, it appeared worth while to further test the action of light on the germination and growth of this schizomycete, and this I did with the following measure of success.

The method employed was as follows:—A quantity of dilute broth in a test-tube was infected with the ripe spores to such an extent that the smallest drop I could manipulate with a platinum loop contained on the average at least about twelve and not more than about twenty-five spores.

I then made a number of hanging-drop cultures in the usual manner, on thin, sterilised cover-slips, each being at once luted to the moist chamber by means of sterile gelatine.

Each moist chamber was then placed on a piece of glass, plain or coloured, about $3\frac{1}{2}$ inches square, and covered by a similar square of the same glass, supported just above the cover-slip from which the drop was suspended, by means of a suitable wooden block.

The following diagram gives the sectional view.



a = hanging drop, suspended from cover slip forming roof of moist chamber; *b* = glass cell in section; *c* = glass slide forming floor of moist chamber; *d* = layer of water to prevent hanging drop drying up; *e* = lower coloured or other glass screen; *f* = upper ditto, supported on wooden blocks shown in section at *gg*.

The glass screens were made sufficiently large to ensure that no light could possibly reach the moist-chamber that had not previously traversed them.

All being thus ready, the moist-chambers were placed in position, and the upper screen removed while I focussed the contents of the hanging-drop, and arranged so that the mirror of the microscope threw the light directly up, through the lower screen. The tube of the microscope was then drawn up so as to raise the objective sufficiently to allow of the upper screen being placed in position.

All the microscopes thus loaded were placed side by side at a south window, and illuminated exactly alike, and for equal periods; maximum and minimum thermometers being placed in position by them.

At stated intervals I examined all the cultures, and measured the growth, &c., of the filaments as follows:—In every case a number of filaments—usually five to ten—were measured, and an estimate formed of the commonest length that prevailed. Then I sought throughout the drop for the *longest* and for the *shortest* filament I could find.

In this way I was enabled to obtain a fair estimate of the amount of increment going on in each culture, as compared with a standard culture kept in the dark; for numerous trials had shown me that the spores germinate so freely, and the germinal rodlets in any one drop grow so evenly during the early periods, that the method may be trusted at least up to the point I push it to.

The following experimental series are typical, and serve very well to bring out the instructive results obtained by these comparative cultures.

To test the relative effects of strong blue and red light on the growth of the germinal filaments, two cultures were made in glass cells, as usual, and placed behind blue and red glass screens respectively.

The spores were sown at 10 A.M. and placed in the light over mirrors at a south window. The temperature was fairly steady at 16° C. The light was chiefly reflected from brightly illuminated clouds, but intermittent sunshine touched the mirrors from 11.35 A.M. to 1 P.M., at intervals.

At 4 P.M.—*i.e.*, six hours after sowing, the spores behind the red screens had germinated to rodlets about 10 μ long, whereas no trace of germination could be observed behind the blue glass. At 8 P.M. the temperature having only fallen half a degree, the average length of the filaments behind the red glass was 125 to 135 μ, but none of the spores behind the blue glass had germinated out.

Next morning at 10 A.M. the filaments in the red light were 750 to 1,000 μ long, as near as I could measure them, but all the spores in the blue were dead, as evinced by their not germinating during the day at 14—18° C., nor during the two further days the culture was kept.

This experiment, and several others which confirmed it in every respect, shows clearly that in bright sun-light the spores are killed behind blue screens, whereas the same exposure behind red screens leads to no injury whatever—confirming the numerous similar results with macroscopic cultures.

On February 13 a bright, clear blue sky with strong sunshine prevailed all the morning, the afternoon being warm and with brilliant sunshine, obscured occasionally by clouds. I availed myself of the brilliant light reflected from the mirrors, but *only allowed direct sunshine* (reflected from the mirrors) *to play on the objects for a quarter of an hour* at the beginning of the exposure.

The spores had of course all been sown at the same time, so that each culture began under equal conditions. Six sowings were made, each containing about 20—30 spores in the hanging drop of dilute broth. One was kept under a darkened bell-jar, one behind ordinary glass, and the others behind red, blue, green, and pale olive glass respectively.* The exposures began at 11 A.M. at 16° C.; the temperature rose somewhat rapidly to 18° C., and reached 20—21° C. before 2 P.M., and remained at that till after 4 P.M., when it slowly sunk to 16° C. at 8 P.M., and remained at that through the night.

At 2 P.M. all had begun to germinate, and by 5 P.M. perceptible growth of the filaments could be observed and measured, and so on later. I carried out the measurements as follows, each under the same scale, every division of which (with the power Zeiss D/4 used) corresponded to 5 μ.

In each drop I sought for the *longest* and the *shortest* filament or

* I owe sincere thanks to Mr. Walker of the Normal School, South Kensington, and to Professor Rücker for much kindness in testing the diathermancy, transparency, and other properties of glass screens for me.

rodlet, a comparatively easy task with the small drops used and with this power, and noted the *commonest* average length which prevailed among the little plantlets developed. The following summary will give the best idea of the results. (Table, p. 315).

The averages were got by taking the sum of all the lengths measured, and dividing by the number of rods measured. This last number is placed in brackets next each average.

These results seemed to show very clearly that (1) the differences in the rapidity of growth are very large, and (2) they are not to be referred directly to the changes in temperature.

They also show that the growth in total darkness under the given conditions is much more rapid than the growth in any kind of light experimented with, except the red. It is *eventually* more rapid than in the red light, but not so *at first*, and I attributed the difference here to the fact that so long as the light was acting the dark heat rays in the red can make themselves felt to such an extent that the young filaments grow more actively during this period; when the light fails, however, between 6 and 7 P.M., the curve in the red falls—the effect of a falling thermometer, in fact.

In the present case the blue light seemed to be distinctly a retarding agent throughout, but it was not sufficiently *intense* to exercise its full effect, and the screen must be regarded as having acted more as a shade to the relatively low intensity of light employed than as a transmitter of any large proportion of the presumably injurious rays.

With the ordinary glass and the pale olive (which really transmits almost as much light) the retarding effects of the light seemed very evident. I attributed the more effective retardation behind the pale olive to the fact that it transmits about as strong a light as the ordinary glass, but with *relatively* more blue in its composition. The effects with green glass screens are often puzzling, as I have found in other cases. On the whole it seemed to act like a shade to the *intensity* of the light, but what does traverse has a lot of blue in it. It lets the dark heat rays through, and consequently the fall about 7 P.M. is like that of a fall of temperature.

In both cases—that of the red and that of the green—it will be noted that the fall of the curve would be aided by the fact that the temperature was also falling slowly all the time, and this latter fact also probably prevented the other curves (blue, ordinary, and pale olive) from ascending more rapidly from 7 P.M. onwards.

Without pressing this explanation too far, it seemed not unlikely that it was approximately correct, though the matter is undoubtedly a complex one.

In any case the above results seemed to show very clearly that whereas the mean growth of the filaments, in darkness, or behind

Relative Growths of Germinating Filaments in Darkness and in Various Coloured Lights.

Treatment of culture.	Lengths at 2 P.M. in μ.			Lengths at 5 P.M. in μ.			Lengths at 7 P.M. in μ.			Lengths at 9.30 P.M. in μ.		
	Average.	Extremes.	Mean.	Average.	Extremes.	Mean.	Average.	Extremes.	Mean.	Average.	Extremes.	Mean.
Dark.....	6—10	6—10	8·0	60·0 (5)*	55—75	65·0	Not taken†	125—175	150·0	346·0 (4)	200—450	325·0
Ordinary glass	5·0 (5)	4—5	4·5	28·0 (5)	25—30	27·5	55·0 (11)	20—85	52·5	102·0 (10)	50—130	90·0
Red.....	13·2 (5)	12—15	13·5	108·75 (4)	60—150	105·0	244·0 (11)	100—350	225·0	270·7 (7)	125—450	287·5
Blue.....	5·0 (4)	4—5	4·5	60·6 (4)	45—80	62·5	144·5 (10)	65—185	125·0	233·3 (9)	75—340	207·5
Green.....	9·0 (4)	8—10	9·0	55·0 (6)	35—80	57·5	101·87 (8)	60—175	117·5	110·0 (7)	60—200	130·0
Pale olive....	3·0 (4)	3—3·5	3·25	17·5 (4)	15—25	20·0	36·6 (11)	15—50	32·5	55·5 (10)	30—70	50·0

* The numbers in brackets in these columns give the number of measurements from which the average is taken.

† The average could not be taken here because a single filament was under observation at the time to obtain periodic records of its growth: hence the slide could not be searched all over.

red glass, which cuts out all the blue rays, at the temperature given, in $10\frac{1}{2}$ hours, amounts to from 144 to more than 160 times the length of the spore, it is only 25 to 45 times its length in the same period if exposed for six or seven hours of the time to light passing through glasses (pale olive or ordinary) which transmit most or all of the blue rays. Under the same conditions spores behind glasses (blue and green) which act partly as shades which reduce the intensity of the light (and in so far favour growth), and partly as screens which so alter the composition of what light is transmitted that it consists of *relatively* large quantities of blue, show decided retardation of the growth—which in the given period amounted to from 65 to 104 times the length of the spore—act as might be expected, and the amount of growth is somewhere between the extremes.

In the following experimental series these facts seemed to come out still more prominently, principally perhaps because the temperature was somewhat higher and the insolation longer.

The arrangements were exactly as before, but the culture under pale olive glass had to be discarded, owing to partial drying up of the drop, because the gelatine luting was incomplete.

The spores were sown at 11 A.M., and the exposure began at 12 noon, the temperature being 16° C. The sun was bright and hot, and was directed on to each culture at the commencement for five minutes. An hour later, *i.e.*, at a period when the swelling spores would be just on the point of germinating, another five minutes' insolation was allowed to each, and so on through the afternoon, *i.e.*, five minutes at 2, 3, and 4 P.M. respectively.

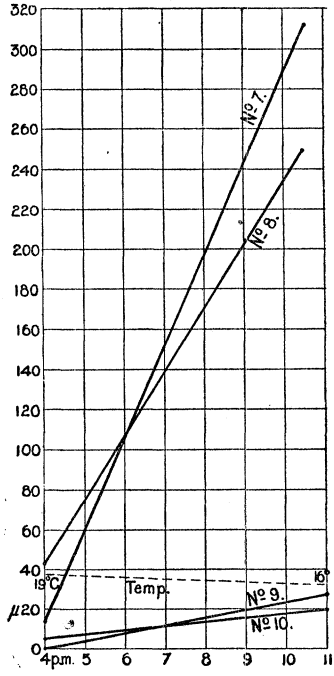
For the rest of the period the mirrors were carefully arranged, so that only bright light from the blue sky or from brilliantly illuminated clouds was allowed to fall on them.

The temperature steadily rose to 18—19° C. about 1 P.M. to 21—22° C. at 2 P.M., and to 23—24° C. between 2 and 3 P.M. At 4 P.M. it had fallen to 19° C., and so down to 16° C. at 10 P.M., at which it remained through the night.

The first measurements were taken at 4 P.M., *i.e.*, after the spores had been sown five hours. As before, I examined each culture in the order of its making, so that although the hours given in the table are only the approximate mean times of the periods (about half an hour) occupied in examining the whole series, they give the correct interval in each case, whence no allowance need be made for the minute increments of elongation during those periods (see Curves 7—10).

Relative Growths of Germinating Filaments in Darkness and in various Coloured Lights.

Treatment of culture.	Length at 4 P.M. in μ.			Length at 10.30 P.M. in μ.			Commonest length observed.
	Average.	Extremes.	Mean.	Average.	Extremes.	Mean.	
Dark ..	13·2(4)	10—16	14·0	296·85(8)	225—400	312·5	275
Ordinary glass	4·5(4) But most germinated out.	4—5 have not yet	4·5	21·87(8) Many spores still not germinated out.	5—35	20·0	6
Red ..	43·75(4)	30—55	42·5	286·25(8)	100—400	250·0	300
Blue ..	All spores swollen only, not yet germinated out			28·86(11)	7·5—50	28·75	30
Green ..	20·0(4)	15—25	20·0	334·3(8)	300—450	375·0	250



On comparing this table and curves with those last discussed, we find the following facts come out :—

The retarding action behind the blue and ordinary glass screens is much more marked, simply, no doubt, because the insolation was more intense. During the whole period (from 12 noon to 10.30 P.M.) of $10\frac{1}{2}$ hours, although the time of exposure to light was shorter by

an hour at least, the filaments developed from the spores in darkness or behind the powerful shades (green and red glass) grew to from 125 to 187 times the length of the spore; those behind the blue and ordinary glass only grew to 10 to 15 times the length of the spore.

Moreover, it is interesting to observe that, so far as it goes (and I do not wish to press it unduly, because a larger number of measurements will be required to decide such a point) the red glass *again* acted as an incubator during the early stages, which I attribute to its acting as a trap for the dark heat-rays; and the more pronounced retarding action of the blue glass so long as the stronger light could penetrate it, fell somewhat after six or seven o'clock, when the darkness set in.

As before, I regarded the green as acting much like the red: it cuts off much of the *intensity* of the light, and in so far is a shade, while it transmits a light of such composition that the dark heat rays are relatively in excess, and still some blue passes. Nevertheless, there is something puzzling about the behaviour of this green glass which I cannot as yet explain. The same uncertainty of its action prevails in other cases, as I have already stated.

To test still further the action of various coloured screens, I repeated the experiments with glass screens, taking a series of comparative thermometrical readings at the same time. The days chosen (February 18—19) were very bright and clear, and great care was taken to obtain the light used from the clear blue sky, to the east of the sun, so as not to run any risk of direct insolation at unexpected periods.

Moreover, before beginning the experimental exposures, I made a series of observations by placing the bulbs of thermometers between the glass screens exactly in the place occupied by the moist culture-chambers, and exposed them under all conditions.

When the thermometer lying on the table under the south window used for exposures, registered 25° C. in the sun, I found that the one with its bulb over the mirror of the microscope registered 27° C., when the light reflected from the blue sky at noon passed through the blue glass: on then reflecting the sun's image on to the same bulb between the blue glasses, the temperature, of course, rapidly rose, and was at 32° C. ten minutes later.

On repeating this with the bulb of a thermometer between the red glasses, the temperature rose to 33° C. in the same time (10 minutes), and rapidly fell to 28° C. when the mirror reflected only light from the blue sky to the east of the sun.

With the orange glasses, the temperature shot up to 33° C. in the sun's image, and fell to 27° C. in 10 minutes on turning the mirror as before; and with the green glasses, the temperature rose to 30° C. in the sun, and fell to 27° C. in the rays from the blue sky.

It was evident that, provided I kept to the reflected rays from the blue sky near the sun, the heating effect was about 2—3° higher than in the open air beside the microscope, and that the cultures between red glasses were exposed to at least about 1° or 2° higher than those between the blue, orange, or green glasses, and these facts must be carefully borne in mind when discussing the results.

The hanging drops, each containing about 25 spores, were made at 11.30 A.M., from broth, infected at 9 A.M., and kept at 15° C., so that the spores were already swelling and ready to begin germinating when the cultures were exposed at 12.30 noon.

As soon as the cultures were placed in position on the microscopes, I turned each mirror so as to powerfully insolate each culture for exactly five minutes; that was all the direct sunlight they were allowed during the whole period, except such rays as struck the top glass obliquely, and the heating effect of which must be taken into account in what follows.

The results of the four examinations I made of these cultures at about 2.45, 4.45, 7.0, and 10.46 P.M. respectively, showed considerably more growth in the red and orange than in the blue and green.

The culture in diffuse light was an accident, and not part of the planned series. It stood in a shaded part of the room, and was not only at a higher temperature during the hours of exposure, but was considerably more advanced to start with. It cannot, therefore, be compared with the others, and must be regarded as merely of passing interest: its curve fell rapidly after 7 P.M., possibly owing to the fall of temperature.

Two hanging drop cultures of broth with a trace of gelatine, of equal age—spores sown at 10—12° C. over night—were placed on microscopes in the south window arranged as follows:—The cells had a thin glass floor.

One had a sheet of red glass below, and a similar sheet above; the other a sheet of blue glass below, and screens of the same in front, so that all light direct from the sky had to traverse the glasses before reaching the drops. The only difference between the two arrangements (apart from the colour of the glasses) was that the first one had its second sheet of red glass close over the drop This difference was necessary owing to the microscopes being of different patterns.

Unfortunately the morning turned out cloudy, with rain at intervals, and occasional short breaks of sunshine; a high wind kept the temperature down to about 17—18° C., rising now and then to 19—20° C.

It will also be noted that I here exposed, not *spores*, but rodlets already strongly germinated out. When the red culture was started, at 10.45 A.M., the rodlet selected (to be kept under observa-

tion the whole time) was 30 μ long; its growth in length was recorded every 15 to 30 minutes or so, and the temperature as shown by a thermometer by the side of the cultures noted.

After allowing the growth to go on for $1\frac{1}{4}$ hours in the red, I changed the glasses, and put the blue ones here and the red ones on the other culture; after growing thus for $2\frac{1}{2}$ hours, the exchange to the original glasses was made again.

The blue culture was started three-quarters of an hour later than the red, a rodlet of almost the same length (31·5 μ) being selected for observation.

After being half an hour under the blue glasses, this was changed to red, and allowed to grow in red light for $2\frac{1}{2}$ hours.

The result was not sufficiently decisive to encourage my drawing any conclusion. It is true the rod growing under red glass seemed to have got a good start, so that its ultimate sojourn in blue light was less injurious than might, perhaps, have been expected; while the shorter stay of the other in blue light appeared nevertheless long enough to have affected it seriously. But meanwhile other factors complicated the matter, and I discarded this line of experiment. Before passing to the further developments, however, it will be well to see what the foregoing results amount to.

1. It is clear that growth occurs with increasing rapidity in suitable food materials and under suitable conditions, and is evidently intercalary. Since it comes to an end eventually, the curve must be that of a long drawn out $\int$.

2. The measurements show, also, that the rate of growth may vary owing to the action of certain factors, partly external and partly internal; and these variations may be local in a filament, or general.

3. The experiments show that the growth is slower on exposure to sunlight (and may even be stopped altogether) passing through certain screens, whereas it is not retarded perceptibly in light through other screens.

4. The results obtained so far, however, do not decide with certainty how far the differences with *growing filaments* depend on other factors than the light; though they are pretty definite as regards the *spores*, exposed in a resting condition. They even suggest that there may be differences in the two cases—*e.g.*, that the actively growing filaments may have some power of overcoming the detrimental action of rays which seriously injure, or even kill, the spores in a state of rest.

5. It may be added that there is no question that exposure to *direct* sunlight kills both spores and filaments; numerous trials have proved that. The question under investigation is rather how far lower intensities of light are efficacious in this respect. So far the measurements did not satisfactorily prove conclusively that active

growth once in full swing is retarded, during the short period I am able to measure it, by a low intensity of light independent of temperature.

Criticism of the foregoing Results, and Experiments on Temperatures.

The more I became familiarised with the methods which had given the foregoing results, the more evident it became that, instructive and valuable as the facts may be—and there can be no question as to their decisive confirmation of the conclusions of previous observers, as well as myself, that direct sunshine kills so long as the blue rays are not filtered out—they are not altogether satisfactory. The variations due to internal causes are so minute that, real though they are, they do not seriously affect the measurements of growth, provided I compare similar rods or long filaments during the same periods, and it is obvious that if I use cultures in the same food-material, and prepared alike, there can be no serious drawback in the method so far as *comparative* results go.

But over and over again I was impressed by the dangers possible as regards the screens employed. It is obvious that with an organism like this, very sensitive* to changes of temperature during its growth, differences of a few degrees at the critical periods might easily lead one into errors which, if neglected, would vitiate many of the conclusions entirely.

The matter is a serious one, for it must be remembered that biologists, and especially botanists, have long been in the habit of using coloured screens, and if these agents are responsible for changes of temperature not recorded in the observations, we are in danger of making fundamental errors.

The suspicion that the temperatures, as recorded merely by thermometers in the air beside the microscopes, might be less trustworthy guides to the changes of temperature going on in the hanging drop-cultures in the glass cells than had been hitherto assumed, now forced itself upon me; and with that, of course, the idea that I might be running risks of confusing the effects of high or low temperatures with those of light, became painfully disturbing.

In any case, it was obviously necessary to institute a series of experiments to test these notions, and it was well I did so, for the results, although not wholly unexpected, were more startling than I care to dwell on now.†

* It was not till a much later period that I found how extremely sensitive it is to temperature changes, as will be seen subsequently.

† It scarcely needs mention that the question here concerned is not the physical one whether two different screens transmit heat-rays in different proportions, but the physiological one how far the differences affect the organism experimented on. It can obviously be decided only by patient comparative investigations.

I select the following series of observations in illustration from a large number which I made for my own guidance: it does not seem necessary to publish all the notes, but as many readers may be unacquainted with the magnitude of the dangers referred to, I retain one or two experiments of a somewhat obviously elementary nature.

In order to understand the behaviour of the thermometer as an indication of what was going on in the culture-chambers, it was clearly necessary that I should make a series of blind experiments in which the bulbs of the thermometers were as carefully placed in the relative proper positions as possible.

The following series refer to the sort of temperature changes to be expected in the laboratory, near a south window, in spring.

Series 1 (*an extreme Case*).

Changes of temperature as indicated by thermometers (A) lying on table beside microscopes; (B) hanging in glass bell-jar exposed to light; (C) hanging in darkened bell-jar; (D) on a stool in the shade and much nearer the hot-water pipes which warmed the room.

Time.	Therm. on table.	Therm. in lighted bell.	Therm. in dark bell.	Therm. on stool in shade.
	° C.	° C.	° C.	° C.
8.30 A.M.	11·0	14	14·0	13·5
10.0 „	15·0	17	16·0	16·5
10.30 „	16·25	22 (sun on bulb; fell to 19 when off)	17·5	17·0
11.0 „	17·25	19	18·0	17·25
11 30 „	17·25	22 } sun on	20·0 }	17·25
12 noon	22·5 } sun	25 }	25·0 }	19·75
12.40 P.M.	30·0 } on			
1.0 „	30·0 } bulb	—	—	—

Obviously this kind of thing would not do for the experiments contemplated, and the first thing I set myself to overcome was the variations due to direct insolation, for it was found that the temperature of the air in the room used is remarkably constant, from the regular action of the heating apparatus, and the rise in temperature during the day and the fall during the night are comparatively small and slow, provided the nights are not frosty and that the venetian blinds are drawn in the evening.

Series 2 (*Average Cases*).

Thermometer readings when the bulb is placed between blue and red glasses, over the mirror of the microscope and in the position of the hanging drop.

(A.) Bulb not Blackened.

Time.	Blue.	Red.	Remarks.
	°C.	°C.	
11.15 A.M.	17·5	17·5	Sun not yet on window.
12.25 noon	23·5	23·0	Sun on glasses.
12.40 P.M.	21·0	21·0	Top glass shaded by cardboard.
1.0 „	25·0	23·0	Top shaded, but mirror in full sun. Hot glare, but hazy.
1.10 „	27·5	28·0	Full exposure to sun above and below.
2.0 „	21·0	21·0	
2.20 „	20·5	20·0	
2.40 „	20·0	19·5	
3.15 „	19·5	19·0	Sun off; full exposure to light from sky only.
3.45 „	18·75	18·5	
4.5 „	18·5	18·0	
4.40 „	18·25	18·0	
5.30 „	17·75	16·5	

(B.) Bulb Blackened.

Time.	Blue.	Red.	Remarks.
	°C.	°C.	
10.0 A.M.	15·0	15·0	Temperature of air at time of beginning.
10.45 „	17·0	16·75	Sun not yet on window. Plane mirrors and ordinary light.
11.0 „	17·0	16·75	
11.45 „	18·75	18·5	
11.55 „	25·0	23·5	Result of turning plane mirror to full sun (which also glares on top).
12.45 P.M.	24·5	24·0	Sun full on (very hazy sun all day).
1.0 „	22·75	22·5	Light with plane mirror from sky east of sun.
1.45 „	22·0	21·5	
1.50 „	21·0	20·25	Sun sinking behind roof of building to south.
1.56 „	20·0	19·5	
2.35 „	18·5	17·5	
3.0 „	18·0	17·25	
3.40 „	18·0	17·5	
3.50 „	18·0	17·5	
4.10 „	17·75	17·0	

These examples will suffice to show how far the screens differ as regards transmission of heat rays.

The following series is interesting as showing that the thermometer hanging in the glass bell-jar, with its bulb close to the culture-chamber, may undergo considerable variations without any corresponding reaction in the growth curvatures.

The experiment was designed to see if the light of a paraffin lamp would be sufficiently active to affect the growth; but the results showed that—for short periods, at any rate—this is not the case. As is well known, however, the flame of a paraffin lamp is very hot, and the possibility arises that the rise of temperature may mask the effect, if the paraffin light has any.

I give the results in any case, though they prove little by themselves: they only show that, either (1) the organism does not *rapidly** react to any great extent to quick, small, but considerable, variations of the temperature immediately around the culture-chamber, or (2) the inhibiting action of the light is sufficient to just undo or mask that effect.

The plan was as follows. A culture drop was prepared as usual, and germination allowed to proceed in the dark for several hours.

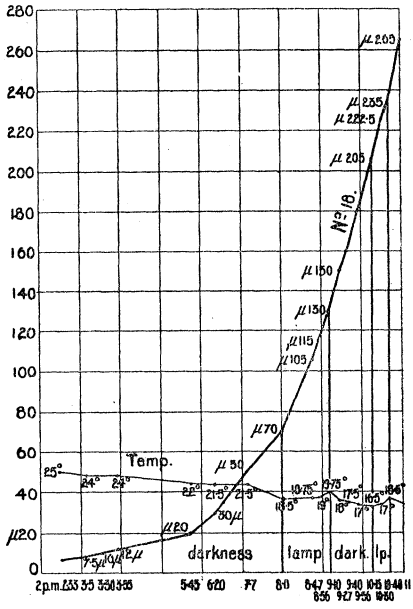
At 12 noon, a germinal filament 5 μ long was selected for observation. The temperature of the interior of the darkened bell-jar, as shown by a thermometer hanging with its bulb close to the culture, was 24° C. The reading and measurement being taken, I left the whole system undisturbed till after 2 o'clock, except to take the temperature readings given in the following tabular *résumé*:—

Time.		Temp.
12 noon	.....	24° C.
12.18 P.M.	.....	25
12.40 „	.....	28
1.0 „	.....	28
1.45 „	.....	27·5

At 2.33 the sun had passed off the window (and it was the direct insolation playing on the wrappings which so raised the temperature of the interior of the bell-jar), and the temperature at once fell to 25° C., and measurements were at once begun, as shown in the following table (see Curve 18).

	Time.	Length.	Interval.	Growth.	Rate.	Temp.
		μ.	mins.	μ.	μ.	° C.
	2.33 P.M.	6	—	—	—	25
	3.5 „	7·5	32	1·5	0·05	24
	3.30 „	10·0	25	2·5	0·1	24
	3.55 „	12·0	25	2·0	0·08	24
	5.45 „	20·0	110	8·0	0·07	22
	6.20 „	30·0	35	10·0	0·3	21·5
	7.7 „	50·0	47	20·0	0·42	21·5
In light of lamp	8.0 „	70·0	53	20·0	0·38	18·5
	8.47 „	105·0	47	35·0	0·74	18·75
	8.56 „	115·0	9	10·0	1·1	19
	9.10 „	130·0	14	15·0	1·0	19·75
	9.27 „	150·0	17	20·0	1·18	18
	9.40 „	160·0	13	10·0	0·77	17·5
	9.56 „	180·0	16	20·0	1·25	17
In light of lamp	10.15 „	205·0	19	25·0	1·66	16·5
	10.30 „	222·5	15	17·5	1·16	17·0
	10.40 „	235·0	10	12·5	1·25	18·5
	11.0 „	265·0	20	30·0	1·5	17

* It does react, however, as the curves show; but the result is not very great in short periods.



It will be noted that the evident rise of temperature from 8·47° to 9·10° was responded to by increased growth.

The following gives an idea of the differences in the curves observed on a hazy day, when the sun was very hot, but could be looked

Light.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
9.53 A.M.	8·0	—	—	—	17·0
10.12 ,,	—	—	—	—	17·5
10.15 ,,	—	—	—	—	18·0
10.25 ,,	—	—	—	—	18·0
10.55 ,,	10·0	62	2·0	0·03	18·0
11.17 ,,	—	—	—	—	18·0
12.28 P.M.	12·0	95	2·0	0·02	24·0
12.42 ,,	26·0	14	14·0	1·0	23·0
1.6 ,,	35·0	24	9·0	0·37	23·0
1.58 ,,	80·0	52	45·0	0·96	22·5
2.20 ,,	105·0	22	25·0	1·1	21·75
2.40 ,,	125·0	20	20·0	1·0	21·0
3.9 ,,	155·0	29	30·0	1·0	20·5
3.40 ,,	167·5	31	12·5	0·4	20·0
4.1 ,,	185·0	21	17·5	0·8	19·5
4.36 ,,	205·0	35	20·0	0·6	19·5
5.30 ,,	230·0	54	25·0	0·46	18·0
6.15 ,,	250·0	45	20·0	0·44	18·0

Dark.

Time.	Length.	Interval.	Growth.	Rate.	Temperature.
	μ.	mins.	μ.	μ.	° C.
9.50 A.M.	8·0	—	—	—	17·0
10.5 „	9·0	15	1·0	0·07	17·0
10.25 „	—	—	—	—	17·5
10.54 „	10·0	49	1·0	0·02	18·0
11.17 „	—	—	—	—	18·0
12.29 P.M.	12·0	35	2·0	0·06	20·0
12.50 „	20·0	21	8·0	0·38	20·75
1.7 „	27·5	17	7·5	0·44	21·0
2.0 „	45·0	53	17·5	0·33	21·0
2.21 „	55·0	21	10·0	0·5	20·75
2.42 „	70·0	21	15·0	0·7	20·5

at without protecting the eyes. One culture was under an ordinary bell-jar exposed to light, the other under a similar bell darkened with black paper, the thermometer bulbs being placed in the cavities of dummy cells, arranged as the culture cells in each case.

It would have been possible here to suppose that the light actually favoured the growth if other facts were not known.

By an accident, this filament was lost, and another (in the same cell) had to be selected for the continuation of the observations, which were resumed without any serious interference with the conditions at 3.13. The following are the results:—

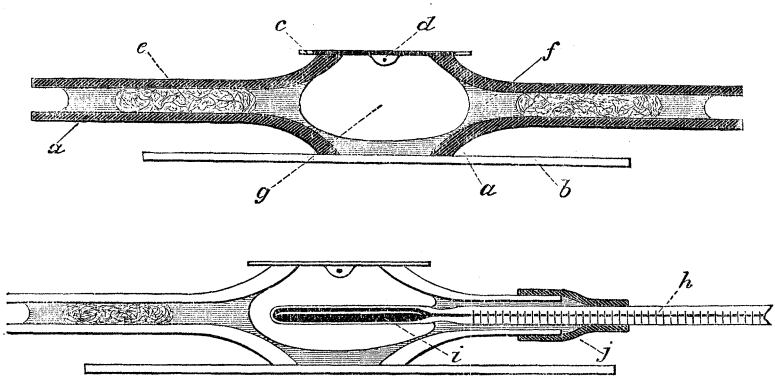
Dark.

Time.	Length.	Interval.	Growth.	Rate.	Temperature.
	μ.	mins.	μ.	μ.	° C.
3.13 P.M.	55	—	—	—	20·0
3.43 „	75	33	20	0·6	20·0
4.3 „	90	20	15	0·75	19·5
4.37 „	105	34	15	0·44	19·0
5.32 „	160	55	55	1·0	18·0

Here it is probable the rise of temperature in the lighted cell was responsible for the enhanced growth; and it may be concluded that if the light of this hazy day had any inhibitory effect, it was more than covered by the stimulus given to the organism by the heat-rays. It is by no means to be concluded with certainty that no inhibition occurred—only that none could be observed during the comparatively short period in which measurements are possible owing to the complication referred to.

The following set of experimental observations were made to test more accurately the heating of the cells, and the rise of temperature in the cultures, as compared with the temperature of the thermometer merely between the glasses, and that in the air close to the culture.

One of my moist chambers was prepared exactly as for a culture, except that one of the two arms was cut short so that the bulb of a thermometer could be inserted into the cavity as shown in the accompanying figure.



a = cavity of the glass cell (moist chamber) the atmosphere in which is kept saturated by water-vapour which evaporates from the layer of water b and wet cotton-wool plugs c and c'. d the hanging-drop suspended from the cover-slip. The latter is luted to the glass-cell by stiff gelatine; while the floor of the cell is formed by a glass slide (e) cemented by paraffin melting between 55° and 60° C.

At c' the second open arm of the glass cell has been cut short to receive the bulb of the thermometer f (the bulb of which may be blackened). g and g' are the coloured glasses, so placed that all the light reaching the hanging-drop d must pass through them.

Having arranged such a cell over the mirror of the microscope, and loaded another microscope with an exactly similar thermometer whose bulb (blackened or not as necessary) is merely suspended between glasses similar to g and g', I proceeded to compare the temperatures on a hot bright morning, at a south window, with those registered by thermometers hanging from the microscopes, and lying on the table at the base.

In the following table, the records of the thermometers outside the microscopes are given in the first column; those whose bulb was merely supported between the coloured glasses in the second (under the heading "dummy"); and that whose bulb was in the culture cell under the third column. The bulbs in second and third columns were blackened, the others not.

The coloured glasses used were the ruby ones marked in table. The thermometers in the air were exposed to the sun, and so the temperatures given are not really those of the air, but they serve my purpose for this preliminary experiment, which is only intended to give an instance of the enormous variations which may be got if the observer is not on his guard against insolation.

Time.	Air.	Dummy.	Cell.	Remarks.
	°C.	°C.	°C.	
10.50 A.M.	17·5	17·0	16·5	Concave mirror, and light from blue sky to west of rising sun.
11.0 „	18·0	17·25	16·75	
11.1 „	12·0—13·0	16·5	16·0	Opened window and let in cold wind.
11.2 „	10·5—11·0	15·5	14·5	
11.15 „	13·5—14·5	19·0	18·0	Open window. Full glare of sun on coloured glasses and mirrors, through the window glass.
11.18 „	15—16	22·0	19·0	
11.24 „	16·5	31·0	30·0	Same arrangement, but sun's glare with no glass of window intervening.
11.26 „	16·5—17·5	35·5	38·0	
11.30 „	18·5	45·0	44·0	Most intense direct glare from sun I could get, with no glass intervening.
11.32 „	18·5—19·0	45·5	45·0	
11.33 „	19·0	47·0	46·0	
11.34 „	19·5—20·0	47·5	45·0	Arrangement same, but shaded sun from top coloured glass.
11.37 „	20·0	48·0	45·0	
11.38 „	20·0	48·0	44·0	
11.40 „	20·0	40·5	35·0	
11.41 „	19·0	35·5	30·5	Upper red glass exposed to direct sun. Mirrors taking light from blue sky to east of sun.
11.42 „	18·0	32 5	29·0	
11.44 „	19·0—19·5	30·5	27·5	
11.45 „	19·5	30·0	27·5	

On looking at these results, one or two interesting facts of considerable importance to the investigation come out and serve as a basis for great caution in exposures of this kind.

In the first place it is clear that direct insolation causes such enormous rises of temperature in the cells, that no results could be expected sufficiently definite to be of use unless these could be controlled. Secondly, it is equally clear that the variations of temperature in such a south window are too sudden and great to be neglected as serious sources of error; and, thirdly, the ordinary mode of recording the temperature by means of thermometers merely hanging near the cultures obviously gives very imperfect results, and cannot be trusted for any such purposes as these.

On the other hand, the records as given by the blackened-bulb thermometer in the dummy and model cells (especially the latter) may be taken as true indications of the temperature in the culture-drops, and it is instructive to observe that the culture-cells respond *slowly* to alterations in the temperature of the air, but *very rapidly* to

changes in the intensity of the sun-light when direct on the mirror.

That the sluggishness in responding to the air-temperature is due to the protection of the glass and the layer of water will be accepted at once, I take it, and it will be equally obvious that the sharp rise in direct sunlight is owing to the dark heat rays, reflected from the mirror, passing easily through the red glass and water layer and being absorbed direct.

On the whole, then, we may conclude that so long as the culture-cells are protected from the *direct* rays of the sun, the temperature of their interior does not quickly vary, though it may be somewhat higher than that of a thermometer outside.

By keeping a thermometer, with blackened bulb, in such a culture-chamber side by side with the growth culture under examination, however, it may be accepted that the temperature of the growing schizomycete in the hanging drop is very accurately known.

Three culture chambers, with hanging drops of broth, were prepared over-night, the only difference introduced being that I employed thin *quartz* instead of glass for the floors. The spores germinated out slowly, at 10—12° C. during the night, and the filaments began growing more and more quickly as the temperature rose in the morning.

By 10 A.M. I had prepared a control or "dummy" cell, with the blackened bulb of a thermometer in it, as described on p. 327, and placed this beside two of the cultures, all in position on their microscopes, at the south window. One culture was arranged for exposure to light, and a thermometer (blackened bulb) hung to its microscope. The other culture was arranged in exactly the same way, but kept darkened by a dome of thick brown paper.

The third culture was placed in a cooler room at a north window, for growth at a lower temperature. At both windows ordinary thermometers were also placed to give the air temperatures in the usual way.

Unfortunately the day turned out dull and cloudy, with rain occasionally, but now and then the sun shone brightly at intervals during the morning.

The following tables give the results. Small thick cardboard slips were arranged as shutters to prevent the direct sun-light from falling on the mirrors or thermometers, &c., but with as little shutting off of light as possible.

Dark.

Time.	Length.	Interval.	Growth.	Rate.	Temperature of air.
	μ.	mins.	μ.	μ.	° C.
10.46 A.M.	43·5	—	—	—	17·5
11.1 „	47·85	15	4·35	0·29	17·5
11.16 „	53·50	15	5·65	0·38	17·5
11.31 „	58·72	15	10·87	0·72	17·5
11.46 „	67·42	15	8·70	0·58	17·0
12.12 P.M.	82·65	26	15·23	0·58	17·5
12.31 „	95·70	19	13·05	0·70	17·5
12.54 „	115·27	23	19·57	0·85	16·5
2.2 „	201·40	68	86·13	1·26	21·0*
2.26 „	238·25	24	36·85	1·53	19·0

* At 2.2 P.M. I turned the light from the cloudy sky on, taking care to shade from any danger of direct sun, of which, indeed, there was none now.

Total growth $=194\ \mu$ in three hours forty minutes, which at constant rate $=0{\cdot}88\ \mu$ per minute.

Growth to 12.54 P.M. $=71{\cdot}77\ \mu$ in 128 minutes, which at constant rate $=0{\cdot}56\ \mu$ per minute.

Growth afterwards $=123\ \mu$ in ninety-two minutes, which gives an average of $1{\cdot}3\ \mu$ per minute.

Light.

Time.	Length.	Interval.	Growth.	Rate.	Temperature of air.	Temperature "in dummy" cell.
	μ.	mins.	μ.	μ.	° C.	° C.
10.45 A.M.	73·95	—	—	—	17·0	17·0
11.0 „	80·91	15	9·96	0·66	17·5	17·5
11.15 „	89·17	15	8·26	0·55	17·0	17·5
11.30 „	97·87	15	8·70	0·58	16·0	17·0
11.45 „	108·75	15	10·85	0·72	16·5	17·5
12.6 P.M.	121·80	21	13·05	0·62	17·0	18·5
12.30 „	138·20	24	16·40	0·68	*20·0	18·5
12.52 „	154·42	22	16·22	0·74	15·0	17·0
2.0 „	217·50	68	63·08	0·92	20·0	20·0
2.30 „	261·0	30	43·50	1·45	20·0	21·0

* The sun was accidentally allowed to catch the bulb: on shading it, it fell to 17° C. in one minute.

Total growth $=187\ \mu$ in three hours forty-five minutes, which at constant rate $=0{\cdot}83\ \mu$ per minute.

Growth to 12.52 P.M. $=80{\cdot}5\ \mu$ in 127 minutes, giving an average of $0{\cdot}63\ \mu$ per minute.

Growth afterwards $=106{\cdot}5\ \mu$ in ninety-eight minutes, giving an average of $1{\cdot}08\ \mu$ per minute.

Third Culture, at Lower Temperature at North Window.

Time.	Length.	Interval.	Growth.	Rate.	Temperature of air (bulb not blackened).
	μ.	mins.	μ.	μ.	° C.
9.50 A.M.	33	—	—	—	12·5
10.25 „	42	35	9	0·26	12·5
10.40 „	45	15	3	0·20	13·0
10.53 „	49	13	4	0·30	14·0
11.18 „	54	25	5	0·20	14·5
11.32 „	60	14	6	0·40	14·5
11.48 „	64	16	4	0·25	14·5
12.14 P.M.	72	26	8	0·30	14·5
12.36 „	81	22	9	0·40	14·5
12.58 „	88	22	7	0·30	14·5

Total growth $=55$ μ in three hours eight minutes, which at constant rate $=0{\cdot}29$ μ per minute.

The scrutiny of these tables can leave little doubt that the general shape of the curves is due to the temperature, and the curve at the lower temperature is hardly useful for any other purposes.

As regards the other two curves, however, the sharper ascent of the curve of the dark culture from 1 o'clock to 2.30 requires explanation, and, unfortunately, the data do not quite suffice for it. Of course, the suspicion arises that it may be due to the inhibiting action of light so retarding the growth in the one case, that the darkened filament—although a younger, *and therefore more feebly growing* specimen to begin with—is gradually making way and more than catching it up.

The following comparative observations were made with the dummy cells, between $CuSO_4$ and bichromate screens, top and bottom, on a bright day in March.

Here, again, it is evident that the observer must be very carefully on his guard when using these screens, of glass bottles containing liquids which filter out different rays, of different heating power; and I would also call attention to the matter of the *heating up* of these screens in the sun and their power of slowly radiating their heat later on.

Time.	Temperature of cell in Blue.	Orange.
10·0 A.M.	14·5°	15·0°
11·30 „	21·0	22·0
12·15 P.M.....	19·0	20·0
12·30 „	21·0	22·0

(On exposing the dummy cells to the full glare of the sun the temperatures ran up to 25° (blue) and 32° (orange), and on shading they fell more slowly to 22° (blue) and 23° (orange).)

1 P.M.	19·0°	20·0°
2 „	22·0	23·0

(On full exposure the blue at once ran up to 25° C., and returned on shading to 23·5°; the orange ran up to 33° C., and returned to 24·5°.)

5 P.M.	17·0°	16·0°
10 „	12·5	13·5
9 A.M. next morning.	13·0	13·5

The following series were made with the screens below the cells, the thin glass roofs would therefore be exposed to radiation, &c., thus accounting for the more rapid changes—in part at any rate. This will probably suffice for calling attention to this point, which must also be carefully borne in mind. I shall have opportunities of referring to these and other matters of the kind subsequently; but for the present this and the foregoing illustrations of the dangers may suffice.

Time.	Remarks.	Temperature of dummy cell behind screen.	
		$CuSO_4$.	K. bichrom.
		° C.	° C.
12·0 noon	Full sun, top and bottom	34·0	31·0
12.15 P.M.	Heavy clouds, otherwise same	23·0	21·0
12.30 „	„ „ „ „	20·5	19·0
12.35 „	„ „ „ „	19·0	18·0
1.0 „	Full sun. Removed top screen	29·0	34·0
1.5 „	Heavy clouds, otherwise same	23·0	23·5
1.10 „	„ „ „ „	21·0	21·0
2.45 „	„ „ „ „	16·0	16·25
5.0 „	Raining „ „ „	16·5	16·5
Next day—			
9.0 A.M.	Sun coming up „ „	14·5	15·0
1.0 P.M.	Dull, rainy „ „	15·5	16·0
Next day—			
9.0 A.M.	Sun not up, blue sky	17·25	17·5
9.15 „	„ „ „	17·0	17·0
9.30 „	„ „ hazy	17·0	17·25
10.30 „	Sun coming, but hazy.....	17·75	18·5
12.30 P.M.	Clouds and blue sky, sunny	18·5	20·5
1.0 „	Clouds and bright sun, but not on	21·5	21·0
4.15 „	Cloudy, blue sky.....	20·25	19·25
Next day—			
11.30 A.M.	Cloudy, bright blue intervals	16·0	17·0
12.15 P.M.	Bright hot sun and clouds.....	21·0	21·0
1.0 „	„ „ „	19·0	20·0

Before leaving this part of the subject, there is one other criticism to be taken into account. Experiments show that great care must be exercised with respect to the cooling effects of radiation from the thin coverslips supporting the hanging drops. I shall have occasion to illustrate this as we proceed; it is not so much that one does not recognise a danger of this kind, as that one does not know the magnitude of its effects until experiments have been made to determine them, that makes it worth while to note it particularly.

As we shall see, the danger does not depend entirely on the direct effects of the fall of temperature on the organism in the drop, but also on the dilution of the drop by condensation of water, and sometimes making it spread over the surface of the glass, and so on. These, and difficulties of similar kind, have given much trouble, and sometimes resulted in the ruin of experiments which promised useful results.

Measurements of Growth in Light and Dark, under Known Conditions as to Temperature.

I now proceeded to make experimental cultures with due regard to the information previously obtained, and especially taking care to check the temperatures by keeping control cells—which I now have expressly made with small, black-bulb thermometers in them. Moreover, as time went on, and experience increased, I discarded the use of glass as much as possible, and eventually succeeded in getting rid of it altogether; for the whole tendency of experience in these researches has been to show that the more glass the light rays have to traverse, the more difficult it is to trace their effect.

At first, however, I had to be content with using open windows and quartz floors to the cells, the light rays being reflected from the glass mirror of the microscope and passed through glass or other screens, &c. Subsequent developments will be described as I proceed.

In the first set of examples of experiments it will be noted that the exposures are from the beginning—*i.e.*, it is the *spores* themselves which are submitted to the light-action; subsequently, I give experiments where the spores are first allowed to germinate out, and it is the actively growing *rods* or *filaments* which are exposed during post-germinal life.

Experiments with Spores.

Spores were sown as usual at 9 A.M., in broth drops, in cells with quartz floors, and the two cultures kept in the dark at 15° C. while further preparations proceeded.

By 10.15 A.M. four microscopes were fitted each with two flat

bottle-screens, filled with saturated alum solution, and a pair of coloured glasses, arranged as follows :—

On one microscope-stage lay an alum-screen over the mirror, and on this transparent screen a blue glass, then the culture chamber. Over the culture came another blue glass, and, lastly, an alum-screen.

Thus all light which passed into the chamber, whether reflected up from the mirror or direct from above, must traverse the alum-screen as well as the blue glass.

By the side of this stood a second microscope loaded in exactly the same way, except that a "dummy" cell with its (blackened bulb) thermometer replaced the true culture cell.

The third microscope was exactly as the first, but with red glasses in place of blue; and by its side an exactly similar control with a "dummy" cell.

Each pair of microscopes was further provided with a suspended thermometer, with bulb blackened, to give the air temperature.

All four microscopes stood side by side at the south window, and each was supplied with a narrow vertical shutter just broad enough to cast a band of shade across the mirror and loads, and so keep direct rays from the cells.

The day was brilliantly sunny, with deep-blue sky, and very few clouds till 2 P.M. Then heavy white clouds kept coming up, but the sunny intervals were numerous.

The following were the thermometric observations, the cultures (mirrors and screens) being illuminated *only by light from the sky*—never direct from the sun in the case of the cultures, and only occasionally and for short periods to get experimental information from the "dummy" cells.

From this table we observe (1) that the culture-cells were never allowed to exceed *at most* 25° to 26° C—indeed the cultures themselves never went so high, because the sun was not allowed to shine directly on either the mirrors or the top glasses; and (2) that, on the whole, the temperature of the cell between the red screens was about 0·5° to 1·0° higher than that of the cell between the blue screens.

Time.	Blue.		Red.	
	Therm. in air.	Therm. in dummy.	Therm. in air.	Therm. in dummy.
	°C.	°C.	°C.	°C.
*10.15 A.M.	17·5	18·75	17·5	19·0
10.30 „	19·5 Ray of sunlight	18·5	16·0	18·75
†11.25 „	18·0	18·0	23·5 Full sun on for a minute	21·0
12 (noon)	20·0	24·5	21·0	24·5
12.30 P.M.	21·0 Sun on top glass	26·0	21·0 Sun on top glass	27·0
12.40 „	23·0	24·0	24·0	26·0
12.50 „	24·0 Brilliant light from sky near sun, not direct solar rays	24·0	21·75 Brilliant light from sky near sun, but no direct sun	25·0
‡ 1.50 „	24·0	26·0	21·0	27·0
2.25 „	19·5 Cloud over sun	22·5	18·0 Cloud over sun	22·5
2.30 „	19·5	22·5	18·5	22·5
2.38 „	19·0	22·0	18·5	23·0
3.0 „	19·5	20·0	18·0	20·0
4.15 „	19·0	17·0	18·0	16·0
5.15 „	18·0	16·0	18·0	16·0
9.0 „	16·0	14·0	16·0	14·0

I examined the culture-drops at 12.30, 3 o'clock, 5.15 P.M., 9 P.M., and 11 P.M., taking note of the extremes and of the commonest lengths of the germinal filaments developed, with the following results (Curves 28 and 29, p. 337) :—

That the light and not the temperature was the effective agent in delaying the germination of the spores between the blue glasses hardly admits of any doubt after this experiment, the most astounding thing about the matter being the prolonged action of the inhibition effect; for it was permissible to expect that when once the germination had begun, the plantlets would rapidly gain strength during the dark hours of the night. Such was not the case, however, and this fact certainly seems to support the idea that the action is on something—whether substance or machinery—in the spore itself, so that the plant produced is really a weakened organism incapable of assimilating the nutritive substances of the food-medium with anything like the normal vigour.

Later on, it is true, the retarded blue culture did slowly recover

* Opened window for three minutes = all fell 5 to 6° C., but rose on closing again.

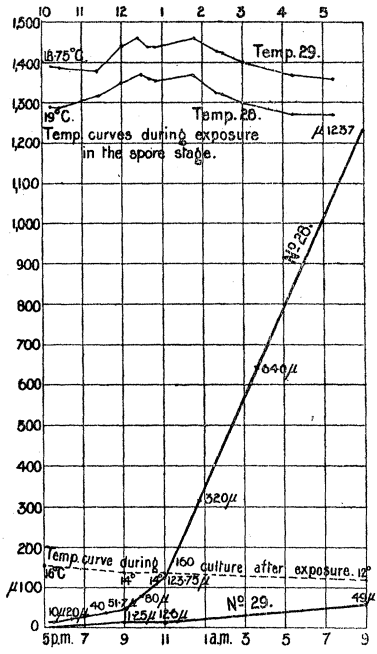
† On removing shutters from both dummies and turning mirror to sun, the readings during consecutive minutes = 19, 21, 25, 28, 31 (for blue), and 24, 27, 31, 35, 38 (for red); they fell as rapidly on replacing shutter and taking light from blue sky only.

‡ On exposing full as possible to sun the red dummy rose to 40—41·5° in a few minutes, the blue dummy to 39—40°. They fell as quickly on replacing. When fully shaded from all sunlight, both fell, in five minutes, to 22·5°.

Germination behind Coloured Screens.

Time.	Blue.				Red.			
	Average.	Extremes.	Mean.	Commonest lengths.	Average.	Extremes.	Mean.	Commonest lengths.
	μ.	μ.	μ.	μ.	μ.	μ.	μ.	μ.
12. 30 P.M.		Spores swollen only.				Spores swollen only.		
3 ,,		No germination.				Germination commencing.		
5. 15 ,,		None germinated out.			10 (2)	9—11·25	10	9—11·25
9 ,,	10·4 (5)	9—13·5	11·25	9	51·7 (14)	13·5—90	51·7	45—55
11 ,,	10·5 (13)	9—16·25	12·6	9—10	112·8 (12)	22·5—225	123·75	112·5
9 A.M.	38·82 (10)	9—90	49	40	—	675·0—1800	1237	—

Curves 28 and 29.



and eventually grew into normal and rapidly-spreading filaments; but the effects of the retardation and weakness were evident in the poverty and lateness of its spore-formation.

I repeated the foregoing experiment the next day, which was also sunny and with a blue sky with passing white clouds, under exactly the same conditions, excepting that the general temperature was lower throughout and the sky less clear. The utmost care was taken in the arrangements of the shutters, positions of microscope mirrors, &c., and no direct sunlight touched any of the apparatus. The thermometer readings were as follows—all with bulbs blackened:—

The readings show (1) that the temperature varied very slightly, and (2) that of the red culture was a trifle higher than that of the blue.

The spores, which had been sown at 10 A.M., and exposed at 10.30, were examined from time to time as before; and, also as before, germination began in the red culture long before it did in the blue.

After exposure for the day, I placed the cultures from 11 P.M. near an open window, with a pair of maximum and minimum thermometers by their side, in order to check the growth during the night, because there was a danger of the red one forming filaments

Time.	Blue.		Red.	
	Air.	Cell.	Air.	Cell.
	° C.	° C.	° C.	° C.
10.33 A.M.	16·0	14·0	16·0	14·0
11.30 „	18·0	16·0	18·0	16·0
11.45 „	16·5	16·0	18·0	17·0
12 (noon)	16·0	15·0	17·0	16·0
12.15 P.M.	17·0	15·0	18·0	15·5
12.30 „	18·0	16·5	19·5	16·5
12.45 „	19·0	17·0	19·0	17·0
1.0 „	18·25	17·0	19·0	17·0
2.0 „	18·5	16·0	17·75	16·0
3.0 „	19·0	17·5	19·0	17·5
3.30 „	19·0	17·0	18·0	17·5
6.0 „	16·0	15·0	16·75	16·0
9.0 „	15·0	13·0	15·5	14·0
11.0 „	14·0	12·5	14·5	13·5
9.0 A.M.	14·0	7·0	14·0	7·0
	Changed red glass for blue, and *vice versâ*.			
10.15 A.M.	14·25	13·0	14·0	12·5
11.15 „	16·0	16·0	15·75	14·5
1.0 P.M.	19·5	18·0	19·5	18·0
2.0 „	18·5	18·75	20·0	18·75
5.0 „	18·0	15·75	19·0	16·25

longer than I could measure next morning. The device was successful, as the curve shows.

Then, having measured both cultures at 9 A.M. next morning, I placed the one hitherto between *red* glasses between the *blue* glasses, and *vice versâ*, to see if, during the second day's exposure, there would be any appreciable changes in the curves. The results are given in the accompanying table and curves (Curves 30 and 31):—

As before, the germination in the blue was markedly retarded, as shown by the measurements up to 11 P.M. Both grew much more slowly during the night (when the temperature fell to 7° C.) than would have been the case at 12—14° C. (see preceding curve); and both began to grow quickly after 9 A.M., in accordance with the rapid rise of temperature from 9 to 11 A.M.

But the most interesting result is shown in the growth from 11 A.M. onwards, when the light began to increase in intensity, for the culture hitherto under blue glass, but now under *red*, began to grow much more rapidly, so that its curve offered to cross that of the now *retarded* culture previously in the red and now in the *blue*.

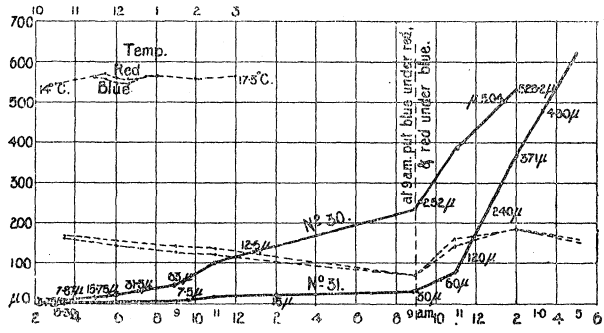
It seemed almost impossible to deny that this must be proof of the light-action.

Spores were sown at 10 A.M. in water, with one drop of broth per

Germination behind Coloured Screens.

Time.	Blue.				Red.			Commonest lengths.
	Average.	Extremes.	Mean.	Commonest lengths.	Average.	Extremes.	Mean.	
	μ.	μ.	μ.	μ.	μ.	μ.	μ.	μ.
2.0 P.M.	No traces of	germination.	—	—	Spores swelling a	nd beginning to g	erminate.	—
3.30 „	3·75 (10)	3·0 — 4·5	3·75	3—4	7·65 (10)	6·75— 9·0	7·87	7—8
6.0 „	4·5 (10)	4·0 — 5·0	4·5	4·5	20·14 (20)	13·5 — 29·25	21·3	22·5
9.0 „	6·0 (11)	5·0 — 8·0	6·5	5·5	51·75 (10)	22·5 — 81·0	51·7	45·0
11.0 „	10·4 (12)	6·75—15·0	10·8	11·25	96·7 (15)	45·0 —157·5	101·25	100·0
9.0 A.M.	40·0 (11)	9·0—54·0	31·0	45·0	210·0 (8)	112·5 —360·0	236·0	160—180
Changed red glass for blue, and *vice versâ*.								
11.0 A.M.	86·7 (13)	40·5—121·0	80·5	90·0	374·0 (13)	245·0 —540·0	392·0	360·0
2.0 P.M.	324·0 (10)	202·5—540·0	371·0	290·0	466·5 (8)	247·5 —810·0	528·2	450·0
5.0 „	652·5 (5)	337·0—900·0	618·0	675·0	—	—	—	—

Curves 30 and 31.



100 c.c., and exposed forthwith, one behind $CuSO_4$, the other behind K. bichromate.

No direct sunlight was allowed to fall on the cultures, cardboard shutters being used, and the mirrors took the light from the sky only. The day was hot and sunny, with heavy clouds occasionally and dull rainy afternoon, the following being the temperature, &c., records.

The cultures were examined at 2 P.M., 5 P.M., 10 P.M., and 9 A.M. next morning, with results as given below:—

Time.	Bichromate. t = cell.	$CuSO_4$. t = cell.
	° C.	° C.
10.0 A.M.	15·0	14·5
11.30 „	22·0	21·0
12.15 P.M.	20·0	19·0
At 12.30 turned full sunlight on and rose at once } to	32·0	25·0
Falling in 5 minutes, on readjusting shutters, &c. } to	25·0	22·0
1.0 P.M.	20·0	19·0
2.0 „	23·0	22·0
On turning full sunlight on, runs up at once } to	30·0	25·0
Falls in 5 minutes, on readjusting to	24·5	23·5
5.0 P.M.	16·0	17·0
10.0 „	13·5	12·5
9.0 P.M.	13·5	13·0

No germination had occurred in either culture at 2 P.M., nor even at 5 P.M.; but at 10 P.M. both had germinated, and the condition of affairs was as follows:—

In the blue culture the longest rods were 17·6 μ, and the shortest 8·8 μ, giving a mean of 13·2, which was about the average length.

In the red culture, however, I found filaments 44 μ, 76 μ, 110 μ, and even 150 μ in length. Mean of extremes = 97 μ.

On leaving the cultures through the night, at about 12—13° C., and examining at 9 A.M. next day, the one over bichromate had filaments from 220 μ to 660 μ long, whereas none in the blue light were more than 88 μ to 130 μ long.

Since the variations in temperature (N.B.—The rapid and extreme variations only refer to the control cells, and were made for experimental purposes) only concern the exposure of the, as yet, ungerminated spores, we can hardly err in referring the retardation of the spores in the blue-light to the inhibitory action of the light rays.

At 7 A.M. I sowed spores in gelatine-broth, and exposed forthwith at an east window. The cells had a quartz floor, and a plate of glass covered with tin-foil was put over them to keep the sun off the top glass. One was over red glass, the other over blue, and neither received any but rays reflected through these glasses from the microscope mirrors.

Each had a dummy cell by its side, arranged similarly, the temperatures of which ran as follows:—

Time.	Blue. ° C.	Red. ° C.
7.0 A.M.	17·5	17·5
9.15 „	20	20
9.45 „	22·5	22·5
10.20 „	24	24
12 noon	23·5	22
3.45 P.M.	20·5	20·5

The day was bright, and every care was taken to keep the direct sun's rays off by means of shutters, &c.

At 4 P.M. both cultures were removed to the south window, where the temperature was 21° C., and kept so till near 7 P.M., at which hour it had fallen to 20° C. It slowly fell during the night, as shown by curve, to 19—21°.

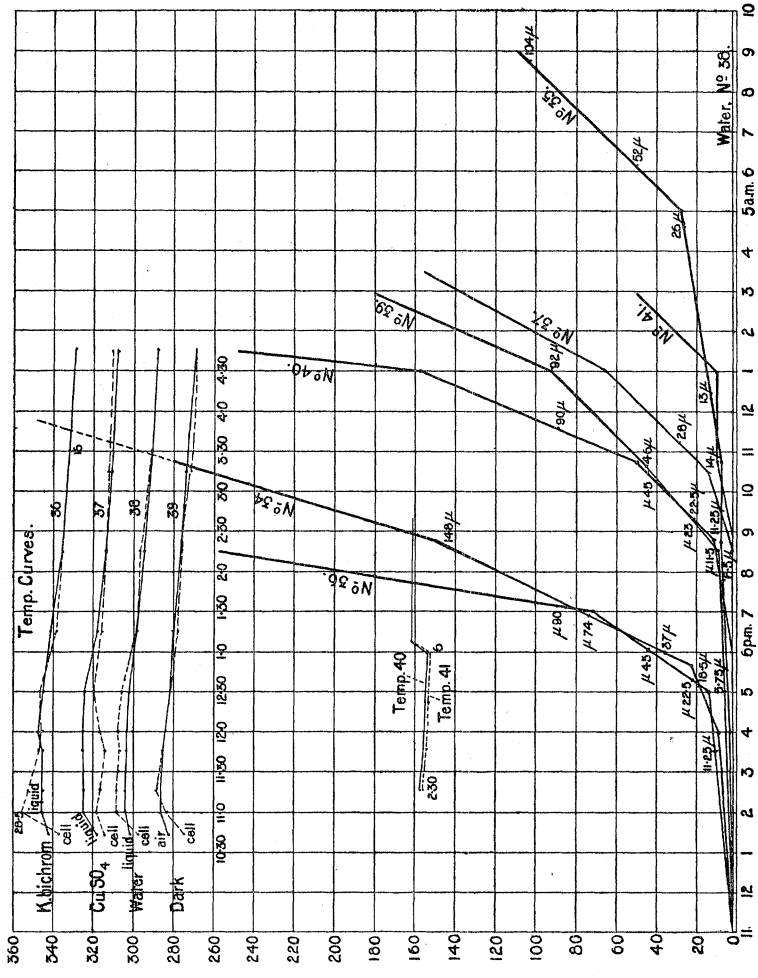
The table on the next page gives the results (see also Curves 34 and 35, p. 343):—

Here, again, the results seem definitely positive, and only to be accounted for as due to the light rays, and one must conclude that—with resting spores at any rate—the above exposure to reflected light through blue glass causes very pronounced retardation, suggesting that a little longer exposure (or a somewhat more intense light) would have inhibited the germination entirely.

Time.	Red.				Blue.			
	Average.	Extremes.	Mean.	Commonest.	Average.	Extremes.	Mean.	Commonest.
	μ.	μ.	μ.	μ.	μ.	μ.	μ.	μ.
7 A.M.			Sown.				Sown.	
12 noon			Swelling.				--	
4 P.M.	9 (5)	4·5—14	9·25	9—10	2·5 (5)	2—4·5	3·25	2—3
5.40 „	23·4 (7)	9—35	22	20—25	6·4 (6)	4·5—5	4·75	4—5
8.45 „	150 (4)	100—200	150	150	7 (4)	5—9	7	7
10.45 „	300 (5)	200—400	300	300	8·5 (4)	6—10	8	8
5 A.M.	Too long to measure, over 1000 μ.				20 (8)	10—45	27·5	20
11 „	Dense meshwork of filaments several thousand μ long.				200 (6)	80—600	340	150*

Curves 34 and 35.

* There was one filament about 500 to 600 μ long, and this brought up the average, but the majority were not above 150 μ or so.



Effect of Light from Blue Sky on Spores.

I now made a new departure. Starting from the fear lest any heating up of the bichromate, &c., screens, and the passage of the solar rays through so many glass layers, &c., might affect the results, I arranged screens and cultures as follows:—

Three large circular crystallisation dishes, 12 in. wide by 4 in. deep, were taken; they were of thin clear glass, with vertical walls and flat floor. These were supported, bottom down, each on four corks just large enough to clear the cover-slip roofs of my cells, with their hanging drops.

One dish was filled to a depth of 3 in. with clear water; one with $2\frac{1}{2}$ in. of $CuSO_4Am$, and the other with $2\frac{1}{2}$ in. K. bichromate.

Under each of the two latter I placed a plane mirror, flat, on which I laid the culture cell (quartz floor), and one of the "dummy cells" with its black-bulb thermometer. Under the water dish I placed a culture and its "dummy" control on a piece of slate; and by the side of this a culture and its dummy under a bell-jar darkened with foil and brown paper. Under the bell-jar I also hung a thermometer in the air. All four pieces of apparatus were on a large box outside the laboratory and looking east and north.

The day opened with a clear blue sky and light wind, but a good deal of white cloud (cumulus) came over after 10 A.M., and there was some haze about 1 P.M. After 2 P.M. a magnificent clear blue sky. The sun was on the box until noon, but it was often covered by white clouds, especially from 11.45 to 12.15 or so.

The following table (p. 345) gives the temperatures.

The sowings were made in broth-gelatine drops about 10.30 A.M., and were at once exposed as described. The dishes were so large that no side light could get in, and I was certain that only the light from the sky, after traversing the liquid, glass-bottom of dish, and thin cover-slip, reached the drop.

Each dish had a thermometer in the liquid, and care was taken* to avoid any complications arising from the cusp of light reflected from the vertical walls.

In order to meet the objection that the orange and blue liquids and cultures varied so much in temperature during the first $1\frac{1}{2}$ hours, I made two new sowings at 2.30, and put one under the blue and the other under the orange dish, exactly as before, and left them there till 6 P.M., so that they got $3\frac{1}{2}$ hours' exposure to the open sky only—no direct sun. The retardation in the blue is, nevertheless, nearly as marked as in the other cultures.

* After my attention was directed to the danger by noticing the high temperature to which the bichromate dummy cell was running at 11 A.M.; only the *dummy* was being touched by the cusp, however, so that the temperature recorded for the actual culture cell at this moment is too high.

Temperatures.

Time.	Dark culture.		Water.		$CuSO_4$ Am.		K. Bichromate.	
	Air.	Cell.	Liquid.	Cell.	Liquid.	Cell.	Liquid.	Cell.
	° C.	° C.	° C.	° C.	° C.	° C.	° C.	° C.
10.40 A.M.	21·0	17·5	20·5	19·0	19·0	17·5	20·5	19·0
11.0 „	23·0	21·5	22·0	24·0	22·0	19·0	22·5	28·5
11.15 „	23·0	23·5	22·0	24·0	22·0	18·25	22·5	27·0
11.45 „	22·0	22·0	21·5	23·0	22·5	17·5	22·5	23·5
12 noon	21·5	21·5	21·5	23·5	22·5	18·5	23·5	23·0
12.30 P.M.	20·5	20·5	21·0	22·5	22·0	20·0	22·5	23·0
1.15 „	20·0	19·5	19·5	19·5	19·0	18·0	20·5	19·0
2.15 „	18·5	18·5	18·0	18·5	17·0	17·0	18·0	18·0
3.30 „	17·0	16·0	16·0	16·0	15·0	15·5	16·0	16·0
4.0 „	—	21·0	—	21·0	—	21·0	—	21·0
10.30 „	—	20·5	—	20·5	—	20·5	—	20·5
1.0 A.M.	—	19·5	—	19·5	—	19·5	—	19·5
Temperature of 2.30 Cultures.								
2.30 „	—	—	—	—	17·0	17·0	18·0	18·0
3.30 „	—	—	—	—	15·0	15·5	16·0	16·0
4.45 „	—	—	—	—	14·0	15·0	15·0	15·0
6 0 „	—	—	—	—	13·0	13·25	14·5	14·0
8.45 „	—	—	—	—	—	21·0	—	21·0
10.45 „	—	—	—	—	—	20·5	—	20·5
1.0 „	—	—	—	—	—	19·5	—	19·5

At 3.30 all the four cultures were brought into the laboratory and placed side by side under a dark cover, and allowed to grow through the night at 21° C., falling to 20·5° at 10.30 P.M., and to 19·5° at 1 A.M., where it remained until 9 A.M., when the last observations were made.

The two 2.30 cultures were brought in at 6 P.M., and remained side by side with the others through the night.

If we examine these results (Curves 36—41, p. 343), the striking effects of the bichromate come out more clearly than ever. It is an undeniable fact that the spores germinate more quickly, produce stronger and more rapidly developing filaments, and sporify more early after exposure to the light coming through the orange screens than when left in the dark or behind any of the other screens for the same period.

Why is this? The reply first suggested is, because the bichromate lets so much of the heat rays through that the spores are raised to a higher temperature. But the same acceleration of germination is seen if the bichromate and $CuSO_4$ screens are not exposed to the sun, but only to light from the blue sky, and when the temperature of the cells behind them hardly differs—at any rate not more than

Time.	Red.				Blue.			
	Average.	Extremes.	Mean.	Commonest.	Average.	Extremes.	Mean.	Commonest.
	μ.	μ.	μ.	μ.	μ.	μ.	μ.	μ.
3.30 P.M.*	11·25 (6)	9—13·5	11·25	9—10	No germination.			
5.0 ,,	16·0 (10)	9—18	14·5	9—12	,,			
7.0 ,,	90·45 (10)	31·5—112·5	72·0	90	,,			
8.30 ,,	258·75 (10)	157—360	258·0	225—250	,,			
10.30 ,,	1000·0 (10)	900—1150	1025·0	950	9·1 (10)	4·5—23·75	14·0	Most are still ungerminated to 5 μ.
1.0 A.M.	Immeasurable; dense coils of filaments, certainly 2000 μ and more long				33·0 (16)	9—121·5	65·25	12—15
9.0 ,,	,,	,,	,,		Cannot measure, but most = about 450 to 900 μ.			
8.45 P.M.†	10·6 (6)	9—13·5	11·25	9—10	Not yet germinated.			
10.45 ,,	45·0 (14)	27—72	49·5	35—40	10·0 (2)	4·5—15 75	10·0	Could only find two germinated.
1.0 A.M.	160·0 (10)	45—270	157·5	150—160	10·8 (7)	6·75—13·5	10·0	9
9.0 ,,	Immeasurable; *i.e.*, filaments 1000 μ or more				—	—	—	Could only estimate 450—900

* Sown at 10.30 A.M.

† Sown at 2.30 P.M.

a degree. Moreover, the temperature of the water-cell also rose considerably above that of the $CuSO_4$ cell. Why, then, did the spores exposed to light through water not germinate out more rapidly? Those in the *dark* did! though not so rapidly as those behind bichromate.

It seems to me the only possible explanation is that behind the bichromate screen we have the effects of a higher temperature—due to the transmitted infra-red rays—superposed on the beneficial effects of the absence of the inhibiting blue rays, which the bichromate cuts off.

I think the absorbed infra-red rays must be effective in some way, or else the dark culture ought to have germinated as readily. If this is so, it raises an extremely interesting and important question as to the sources of energy available from without to these organisms, and may possibly help to throw light on many obscure points connected with the extraordinary capacity for work which they show.

But we cannot explain the striking difference between the water and the bichromate action as merely due to temperature. Indeed none of the results would accord with that, for it was merely a ques-

Water.				Dark.			
Average.	Extremes.	Mean.	Commonest.	Average.	Extremes.	Mean.	Commonest.
μ.	μ.	μ.	μ.	μ.	μ.	μ.	μ.
No germination				No germination.			
	,,			5·6 (10)	4·5—6·75	5·75	4·5
	,,			6·6 (10)	5—9	7·0	5·0
	,,			9·9 (10)	5—13·5	9·25	9·0
	,,			31·5 (11)	18—67·5	42·75	22·5
	,,			72·0 (12)	36—112	74·0	45—50
None germinated—all dead.				Dense meshwork of filaments over 1000 μ long.			

Curves 36—41.

tion of the rise in temperature between 10.40 and 12.30, before germination had begun be it remembered, and therefore acting on spores which will bear a high temperature, *e.g.*, 60° C. for some hours. The order of germination ought to have been (1) bichromate, (2) water, (3) dark, and (4) $CuSO_4$ culture, whereas it was (1) bichromate, (2) dark, (3) $CuSO_4$, and the spores behind water did not germinate at all, but were there lying dead.

In order to settle the doubt as to temperature, or sudden slight alterations of temperature, being the cause of the retardation and death of the spores, I repeated the experiment on the following day, exactly as before, excepting with the single modification that the table was so placed that *no direct sunshine whatever* was allowed to fall on the apparatus or spores; they received no light except that from the blue sky and white cumulus clouds passing through the liquids and glass.

The results, summarised in the table and curves (pp. 350, 351, and 353), show that my conclusion was right; the effect is solely an effect of the light passing through the screens.

The accompanying table (p. 349) gives the temperatures, during exposure, of the liquids, the air in the dark bell-jar, and the cells. At 4 P.M. the cells were all brought into the laboratory and kept under a dark jar. The temperatures during the night and following day are also recorded. The differences in temperature are so slight that the spores may be regarded as exposed to the same conditions as regards heat. Nevertheless the red and dark cultures germinated out five or six hours before the blue, and were much stronger and more rapid in growth, while the spores under water were killed, and all this with only five hours' exposure to diffuse light from a blue sky, which was, moreover, slightly hazy for a considerable part of the time, and never entirely free from white clouds.

This experiment seems conclusive. Moreover, it is instructive in other respects. It shows, for instance, that when the dark heat rays (transmitted by the bichromate the day before) are absent, the bichromate culture germinates out and grows like that in the dark, and that these rays are not necessary for the bactericidal effect through water. That the culture behind the $CuSO_4$ germinated out at all is no doubt due to the thick layer of strong $CuSO_4$ diminishing the quantity and intensity of the light; it is pretty certainly a mere matter of length of exposure.

It seems to me the importance of these results can scarcely be over-estimated in their bearing on the question of the death of bacteria in water. As the sun shines for a longer time and with more intensity on the river day by day with the advance of spring to summer the spores will be more and more retarded, and even those which escape actual death with the advent of the night are again

Temperatures.

Time.	Water.		Dark.		Red.		Blue.	
	Cell.	Liquid.	Cell.	Air.	Cell.	Liquid.	Cell.	Liquid.
	° C.	° C.	° C.	° C.	° C.	° C.	° C.	° C.
11.0 A.M.	16·5	16·0	15·5	16·5	15·75	16·0	16·25	15·0
11.30 „	17·0	17·0	15·5	16·5	15·5	15·5	17·0	17·0
12.0 noon	17·5	17·0	16·0	17·0	17·0	18·0	15·75	16·0
1.0 P.M.	17·0	16·0	16·0	18·0	17·0	18·0	16·5	17·0
2.0 „	17·0	15·0	16·5	17·0	17·0	17·5	16·0	16·5
2.30 „	17·0	15·0	16·5	17·0	17·0	17·0	16·0	16·0
4.0 „	16·5	14·0	16·0	16·25	16·0	15·5	15·0	15·5
4.15 „	21·5	—	21·5	—	21·5	—	21·5	—
5.45 „	21·5	—	21·5	—	21·5	—	21·5	—
7.0 „	21 0	—	21·0	—	21·0	—	21·0	—
8.45 „	20·0	—	20·0	—	20·0	—	20·0	—
11.30 „	19·0	—	19·0	—	19·0	—	19·0	—
2.0 A.M.	18·0	—	18·0	—	18·0	—	18·0	—
6.30 „	16·0	—	16·0	—	16·0	—	16·0	—
10.0 „	17·0	—	17·0	—	17·0	—	17·0	—
2.0 P.M.	19·0	—	19·0	—	19·0	—	19·0	—
7.0 „	19·0	—	19·0	—	19·0	—	19·0	—

exposed a few hours later as the early dawn brings the light on them once more. So that even if it were true that the bacillus is safe as soon as it has germinated, it would still be the case that only those spores which lurk in the shadows, or are rolled by the river into unexposed places, could recover.

We shall see later on, however, that this action of light on the spores in water is favoured by the low temperatures to which the river organisms are usually subjected for subsequent experiments show very clearly that whatever resisting action they may be supposed to have is put in abeyance when the temperature is too low for active and rapid growth.

Time.	Water.				Dark.			
	Average.	Extremes.	Mean.	Commonest.	Average.	Extremes.	Mean.	Commonest.
	μ.	μ.	μ.	μ.	μ.	μ.	μ.	μ.
5.45 P.M.	None germinated.				None germinated.			
7.0 „	„		„		Germination beginning.			
8.45 „	„		„		8·2 (10)	4·5— 11·25	7·9	6— 7
11.30 „	„		„		40·5 (10)	27·0— 58·5	42·75	36
2.0 A.M.	„		„		158·85 (10)	90·0—220·0	155·0	45— 90
6.30 „	„		„		528·75 (4)	200·0—900·0	550 0	450—600
10.0 „	„		„		Immeasurably long coils, &c.			
2.0 P.M.	„		„		„	„	„	
7.0 „	„		„		„	„	„	

To test the germinating power of the spores after baking, or, rather, drying at high temperatures, I proceeded as follows :—

A number of cover-slip preparations were made in the ordinary way, with spores distributed in distilled sterile water, and these were placed, infected side upwards, in Petri dishes, and exposed to 80° C. in the hot air sterilizer, and allowed to cool slowly (two hours) to 30° C.

The results show clearly that the germinating power of these spores dried at high temperatures is by no means destroyed by the high temperatures to which they are subjected.

In the series selected the spores were distributed on the slips at 10 A.M., and at once put into the hot-air steriliser at 80° C. At 12 noon they had cooled to just below 30° C., and each slip received a drop of broth-gelatine, and was arranged as the roof to a cell, the culture being now a hanging drop.

I take three of them, labelled 1, 2, and D. Nos. 1 and 2 were at once put into the incubator at 21° C.; this was 12.15 P.M. D was kept dry in diffuse light at 20° till 4 P.M., and then put in as the others.

The following table (p. 352) summarises the result.

All things considered, and especially the difficulties of measuring the averages of the more advanced cultures, these three curves agree very well, and show clearly that neither the germination nor the subsequent growth of the bacillus is in the least hurt by the heating and drying of the spores. Indeed, it is difficult not to suspect that the vigour of the organism is *improved* rather than injured by the treatment.*

Experiments with Growing Filaments.

Three broth-drop cultures were started; and placed at 20—22° C.

Red.				Blue.			
Average.	Extremes.	Mean.	Commonest.	Average.	Extremes.	Mean.	Commonest.
μ.	μ.	μ.	μ.	μ.	μ.	μ.	μ.
None germinated.				None germinated.			
Germination beginning.				,,	,,		
6·5 (10)	4·5— 9	6·25	5— 6	,,	,,		
25·65 (10)	13·5— 36	24·75	25	,,	,,		
111·6 (10)	59·0—157	108·0	45— 90	,,	,,		
823·0 (3)	675·0—900	787·5	800—900	10·5 (10)	4·5— 45	24·75	5
Immeasurably long coils.				60·0 (10)	15—112	63·5	25—45
,,	,,	,,		225·0 (10)	90—450	270·0	200
,,	,,	,,		Too long to measure.			

Curves 42—45.

in the dark at 9 A.M., where they remained until 2 P.M., when germination had begun in all.

They were then distributed as follows:—One remained in the dark under the bell-jar covered with foil and paper; a second was over a bottle screen of water, tinged slightly blue with $CuSO_4$, and arranged so that no direct rays reached it—the top being shaded by a thick card; while the third was exactly as the second, except that its screen was of K. bichromate. The two cultures in the light had each a dummy cell and thermometer rigged up exactly in the same way, and these and thermometers by their side and in the bell-jar gave the temperatures of the air and cultures.

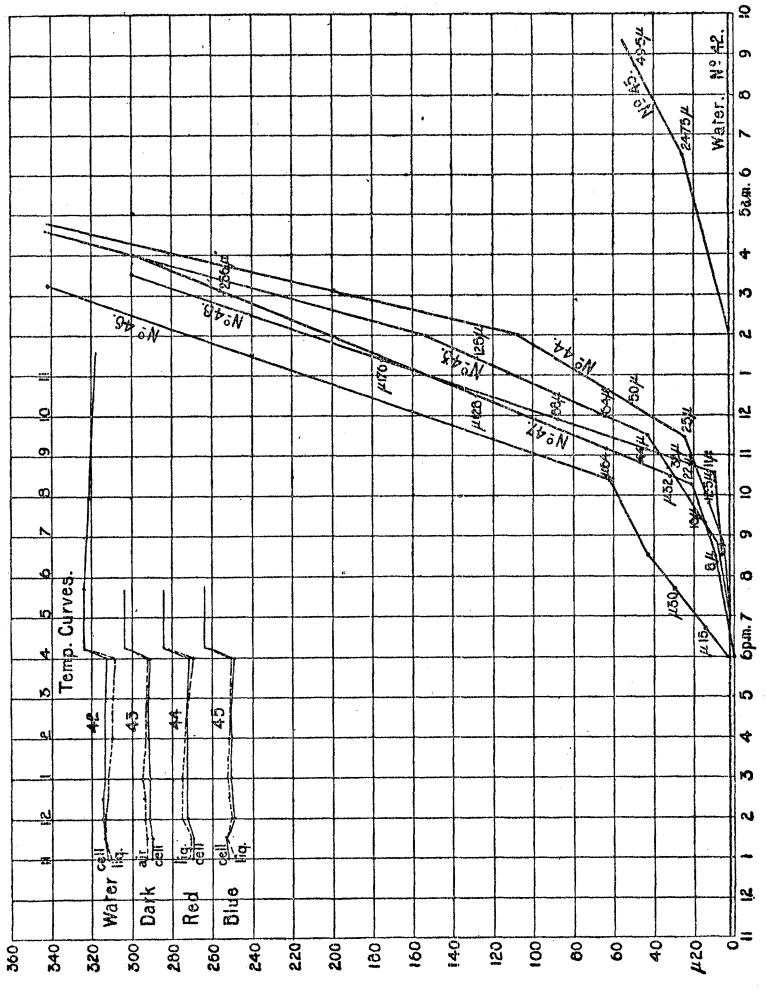
The day was a brilliantly hot and sunny one, but with light cumulus clouds floating slowly in the blue.

At 2 P.M. I selected a germinated rodlet, well out, under each microscope, *and kept it under observation the whole period.* This was made feasible by placing the screens below the stage of the microscope—the upper surface of the light cultures being shaded by

* Brefeld found that such was the case when the spores of *B. subtilis* were heated to boiling ('Unters. ü. Schimmelpilze,' B. IV, 1881, p. 51).

Time.	Culture 1.			Culture 2.			Culture D.		
	Average.	Extreme.	Mean.	Average.	Extreme.	Mean.	Average.	Extreme.	Mean.
	μ.	μ.	μ.	μ.	μ.	μ.	μ.	μ.	μ.
6.15 P.M.	4·25 (4)	2—6	4·0	7·4 (5)	2—13	7·5	—	—	—
8.30 „	8·3 (7)	3—16	8 5	43·0 (3)	25—60	42·5	5—6	—	5—6
10.15 „	19·0 (6)	10—32	21·0	59 0 (8)	27—95	61·0	—	—	—
10.30 „	—	—	—	—	—	—	9·0 (3)	6—12	9·0
3.30 A.M.	—	—	—	—	—	—	279·0 (5)	200—400	300·0
4.0 „	308·0 (5)	100—500	300·0	364·0 (5)	100—670	385·0	—	—	—

Curves 46—48.



cardboard—and they were sufficiently transparent to allow of direct vision and measurements of the growing filaments.

The following tables show the results, with the temperatures, &c.

Culture over Water-screen (+ trace of $CuSO_4$).

Time.	Length.	Interval.	Growth.	Rate.	Temperature.	
					Air.	Cell.
	μ.	mins.	μ.	μ.	° C.	° C.
2.25 P.M.	8·8	—	—	—	25·0	26·0
2.45 „	9·24	20	0·44	0·02	25·0	28·0
2.55 „	13·2	10	3·96	0·4	25·0	29·0
3.10 „	19·8	15	6·6	0·4	25·0	29·0
3.25 „	24·2	15	4·4	0·3	15·0	21·5
3.50 „	26·4	25	2·2	0·09	13·0	18·0
4.25 „	39·6	35	13·2	0·38	20·25	18·25
4.40 „	48·4	15	8·8	0·6	20·0	19·0
5.5 „	70·4	25	22·0	0·9	19·5	19·0
5.30 „	96·8	25	26·4	1·6	19·0	19·0
5.45 „	114·4	15	17·6	1·2	19·0	18·5
6.0 „	136·4	15	22·0	1·5	18·5	18·25
6.20 „	171·6	20	35·2	1·7	18·25	18·0

This rodlet therefore grew 162·8 μ in three hours fifty-five minutes, which at constant rate, would give 0·69 μ per minute—*i.e.*, 7·8 per cent. of the original length.

The filament doubled its length in the following periods:—

1. 8·8—17·6 μ in 32 minutes at 26—29° C.
2. 17·6—35·2 „ 42 „ 29—18·25° C.
3. 35·2—70·4 „ 52 „ 18·25—19° C.
4. 70·4—141 „ 58 „ 19—18·25° C.

Culture over Screen of K. Bichromate.

Time.	Length.	Interval.	Growth.	Rate.	Temperature.	
					Air.	Cell.
	μ.	mins.	μ.	μ.	° C.	° C.
2.17 P.M.	9·2	—	—	—	24·0	26·0
2.35 „	14·46	18	5·26	0·3	24·0	27·0
2.55 „	27·6	20	13·14	0·65	24·0	27·5
3.10 „	41·4	15	13·8	0·9	24·0	27·5
3.25 „	55·2	15	13·8	0·9	14·5	21·0
3.50 „	73·6	25	18·4	0·74	12·0	17·5
4.25 „	92·0	35	18·4	0·52	20·5	18·0
4.40 „	112·7	15	10·7	0·7	20·5	18·0
5.5 „	144·6	25	31·9	1·27	19·5	18·0
5.30 „	179·4	25	34·8	1·4	19·0	18·0
5.45 „	216·2	15	36·8	2·4	18·6	18·0
6.0 „	246·1	15	29·9	2·0	18·5	17·5
6.10 „	257·6	10	11·5	1·15	18·5	17·5

The rodlet therefore grew 248·4 μ in three hours fifty-three minutes, which gives over 1 μ per minute at constant rate—*i.e.*, more than 10·8 per cent. of the original length.

The doubling periods =

1. 9·2—18·5 μ in 25 minutes at 26—27·5° C.
2. 18·5—37·8 „ 23 „ 27·5—constant
3. 37 —74 „ 47 „ 27·5—17·5° C.
4. 74 —148 „ 75 „ 17·5—18° C.

Culture in Dark.

Time.	Length.	Interval.	Growth.	Rate.	Temperature of air.
	μ.	mins.	μ.	μ.	° C.
2.30 P.M.	6·6	—	—	—	23·0
2.45 „	11·0	15	4·4	0·3	23·5
2.58 „	11·44	13	0·44	0·03	24·0
3.10 „	13·2	12	1·76	0·15	24·0
3.25 „	13·2	15	0·0	0·0	23·5
3.50 „	15·4	25	2·2	0·09	23·0
4.25 „	15·4	35	0·0	0·0	23·0
4.40 „	17·6	15	2·2	0·12	22·0
5.5 „	19·8	35	2·2	0·06	21·5
5.30 „	22·44	25	2·64	0·1	21·0
5.45 „	26·4	15	3·96	0·26	21·0
6.0 „	28·6	15	2·2	0·12	20·75
6.30 „	35·2	30	6·6	0·22	20·5
7.0 „	48·4	30	13·2	0·44	20·5
8.0 „	79·2	60	30·8	0·5	20·0
10.0 „	176·0	120	96·8	0·8	19·25

Doubling periods =

1. 6·6— 13·2 μ in 40 minutes at 23—24° C.
2. 13·2— 26·4 „ 95 „ 24—21° C.
3. 26·4— 52·8 „ 86 „ 21—20° C.
4. 52·8—105·6 „ 82 „ 20° C.

I am unable to explain the slow rate of growth except on the hypothesis that the heat was rapidly radiated from the thin glasses on lifting the bell, and so kept the culture back.

Another possible—perhaps more probable—explanation is that the screens absorb (and subsequently radiate) heat from the solar rays; whereas the bell-jar did not do this to the same extent. The temperatures in the dark being, therefore, lower throughout all the early stages of the observation (only the air-temperature could be obtained owing to difficulties of manipulation) explains the effect.

Comparisons of the tables and curves of the two light cultures

bring out clearly the retarding action of the light, especially on noting that the temperature of the retarded culture was slightly higher, rather than lower, than the other.

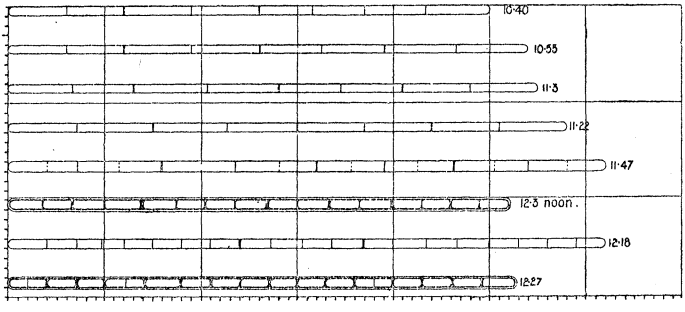
Effects of Intense Insolation.

Spores sown in a broth drop had germinated and developed good filaments in the dark at 20—22° C.; the culture was then put on a cool slab over-night, and kept slowly growing at 10—12° C.

At 10 A.M. the twenty-four hours culture had fine strong filaments when put at the south window under the 1/12th immersion, with Zeiss' screw micrometer eye-piece, for exact measurements of the growths. Each division of the scale gave exactly 2 μ.

The segment selected was an isolated one measuring just 50 μ long, and consisting of four distinct segments, each again, but less obviously, divided by a median or nearly median septum into two cells.

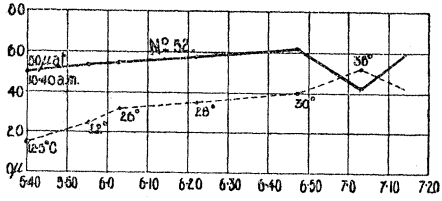
The diagrams below give the successive growths of these segments and cells, as observed from 10.40 to 12.27 under the power named, and fully bear out my previous observations that the growth is not perfectly uniform over the filament.



The following table summarises the total growth of the filament as a whole.

Time.	Length.	Interval.	Growth.	Rate.	Temp. of cell.
	μ.	mins.	μ.	μ.	° C.
10. 40 A.M.	50	—	—	—	12·5
10. 55 „	54	15	4	0·27	22·0
11. 3 „	55	8	1	0·12	26·0
11. 22 „	58	19	3	0 16	28·0
11. 47 „	62	25	4	0·16	30·0
12. 3 P.M.	52	Sharp	contraction of	whole.	36·0
12. 18 „	62	Had sl	owly regained	length.	26·0
12. 27 „	52	Perm	anently contr	acted.	36·0

Curve 52.



Here it must be noted that I was observing the filament in bright light, at relatively high temperatures; I attributed the slow growth from 10.55 to 11.47 to these factors.

The diagrams, which are drawn carefully to scale, give the relative elongations of the cells very accurately, and the reader is referred to them for details; the conclusion to be drawn from them is that from 10.40 to 10.55 the growth was entirely confined to the two right-hand segments, and then it occurred in the segments to the left end.

At 11.47 each of the right cells became divided, almost, if not quite, simultaneously, so that the filament now consisted of sixteen cells each from 3 to 4 μ long.

At 11.47 I was taking the light almost direct from the sun, and inadvertently allowed the cell to be more brilliantly illuminated than usual, and the reflected solar image in fact gradually got on to the culture-drop. On examining at 12.3 I found a most unexpected result—the whole filament had contracted from 62 μ (its length at 11.47) to 52 μ, and each of the 16 short cells composing it was now seen to be sharply contracted and lying distinct from its neighbour, from which it was separated by a broad cell wall. The whole was, in fact, now a series of cells inclosed in a distinct swollen sheath.

Still more remarkable was the fact that on again shading the culture, which at once brought the temperature down from 36 to 26° C., these cells at once closed up again, and the whole series once more slowly lengthened out, so that at 12.18 it was again 62 μ long. But no more growth occurred, though more septa were formed and the positions of others altered. On again turning the sun on, I saw these cells once more and *suddenly* contract away from one another, and clearly lie as contracted blocks of protoplasm in a distinct sheath.

Again they closed up, but more slowly, on shading the culture, and in ten minutes all the segments were marked with fine granular reticulations.

The cells were now dead, and we may conclude that the insolated protoplasm is killed by 5—10 minutes direct insolation at 35—36° C.

From subsequent experiments at these temperatures in the dark it may perhaps be concluded that the death here resulted from the high temperature, quite apart from any possible light action, and this

in spite of the fact that not all the cells were killed by the insolation (see below).

For my own part, I regard it as more probable that the high temperature merely *helped* the light action, for if the death was due to the high temperature, why did not all the cells die? That some escaped is intelligible if they were sheltered behind others—a by no means improbable event, for the culture was an advanced one, be it noted—whereas it is not easily explained on any assumption of heating or of poisoning.

It will be useful again to summarise the results of the foregoing section, and the suggestions they give rise to, before proceeding further.

1. So far as the spores exposed in the ripe resting condition are concerned, it may be regarded as proved that the blue-violet rays can retard or kill them, apart from any temperature effect, and it is shown that they are extremely resistant to high temperatures.

2. As regards actively growing bacilli, the evidence goes to show that the light action affects them also, by retarding their growth, and even eventually killing them, but, owing to certain difficulties, it is not so clearly shown quantitatively how far the effect is due to light alone, because the retarding effects are not observable *within* the short period during which measurements are taken.

3. In attempting to trace the effects of light (of moderate intensity only) it is so difficult to keep two growing filaments exactly at the same temperature under different conditions of illumination, that the quantitative results cannot be insisted upon too much in detail, for there is always the suspicion that even a difference of less than one degree of temperature may affect the rate of growth, and therefore alter the steepness or otherwise of the curve.

4. Some results suggest the possibility that the organism may even make use of rays at the red end of the spectrum in combatting the effect of those at the blue end.

5. The further possibility is suggested that the difference of behaviour between the spores and the filaments may simply depend on the relative activity of the protoplasm—that when the latter is comparatively inactive, as in the resting spore, it is incapable of resistance to the light action, but that, when it is actively engaged in metabolism and growth, it can resist the action if the temperature is favourable, and the more so the nearer the optimum the temperature is. On the other hand, extremes of temperature may favour the light-action.

6. Yet another hypothetical step may be taken. It is possible that the spores succumb so readily simply because a dangerously unstable supply of easily oxidisable materials is there, ready to be destroyed by the light-action; whereas, it is conceivable, these food-

materials are not present in the active cells of the growing filaments, for it is by no means improbable that the active protoplasm contains totally different compounds from the reserves in the inactive spore. Some such view is rather supported by the retarding action of the light on spore-formation.

7. It must not be forgotten that, in the majority of these experiments, the intensity of light employed is very low, and that the question investigated is not whether direct sunshine kills the organism (for there is no doubt on that head), but how far, and in what manner, ordinary daylight is effective.

Experiments without Glass and at known Temperatures.

As already mentioned, I contemplated avoiding glass altogether, in order to test the suspicion that some of the effects observed might be due to differences in diathermancy, &c.; this was done by employing thin quartz plates for all screens, floors of the cells, and even as cover-slips in some cases (though optical difficulties occurred here), and metallic surfaces for reflection.

The new apparatus took some time to get together, but I had some of the flat glass bottle-screens ground out by Mr. Hilger, and fitted with parallel plates of quartz, and used quartz floors to the cells, and even thin quartz cover-slips where necessary; then I replaced the mirror of each of the microscopes with one silvered in front, and was now in a position to repeat the foregoing observations with matters so arranged that none of the incident light could be absorbed by glass.

This is, perhaps, the best place to give the proofs that cultures standing side by side, and treated similarly in all respects, give growth curves so closely alike that we assume that the organism behaves similarly whenever subjected to the same conditions.

The following experiments show that the curves are alike in all essential respects, within certain limits of errors of measurement, when exposed to like conditions.

Comparative Measurements under like Conditions.

In order further to test the method of measurement, I made two cultures of spores sown in 10 per cent. gelatine and traces of broth and glucose, and kept at 22° C. in the dark incubator from 9 A.M. to 4 P.M. Germination had begun by 1 P.M., and at 4 P.M. the cultures were placed at 22° C. under microscopes, exactly alike, in diffuse light, the mirrors turned away in the intervals. A control thermometer cell was employed, and in each case a sturdy-looking filament selected of exactly the same length, as nearly as I could measure. Care was exercised to ensure that each scale measured evenly, and the measurements were made at various intervals, as follows:—

Time.	Lengths. A. μ.	Lengths. B. μ.	Temp. °C.
4.0 P.M.	36·0	36·0	22·0
4.10 „	42·75	43·83	22·0
4.32 „	54·0	54·5	22·0
5.5 „	76·5	77·0	22·0
5.35 „	99·0	100·0	21·75
5.55 „	119·25	121·5	21·75
6.5 „	130·5	135·0	21·5
6.30 „	157·5	166·5	21·5
6.45 „	182·25	195·75	21·0
7.0 „	204·75	222·75	21·0

It must be allowed, from the results, that the measurements attain a high degree of accuracy, but they suggest, at the same time, that the problem of detecting the exact effect of slight differences of illumination is extremely difficult for direct solution in this way.

After standing all night at 20° C., these two nearly equal cultures were exposed from 9 A.M. to 8 P.M. to a very blue sky, with passing white clouds, on July 4th, A over water, B over bichromate screen—quartz, silver mirror, shades, &c., as before—to see if spore-formation would be affected differently by the different lights. At 8 P.M., July 4, the final segmentations prior to spore-formation were visibly commencing in B, but neither showed spores as yet.

The following are the temperatures for the day, July 4:—

Time.	Temperatures. A. °C.	Temperatures. B. °C.
9.0 A.M.	17·5	17·5
10.0 „	18·5	18·5
11.0 „	20·25	20·0
11.45 „	22·0	22·0
12.30 P.M.	24·5	24·5

From 12.30 to 2.30 they were brought into the north laboratory, to avoid further rise of temperature; the temperature remained at 23°. At 2.30 they were returned to the south window.

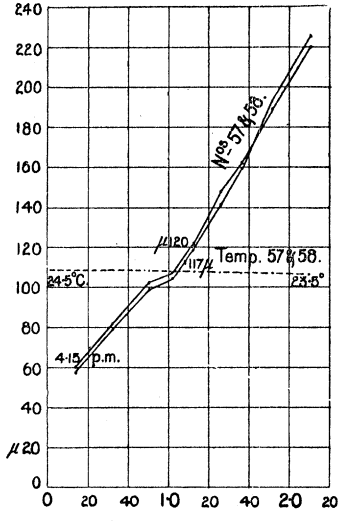
	°C.	°C.
2.30 P.M.	23·5	23·5
3.0 „	22·0	22·0
7.0 „	21·0	21·0
8.0 „	21·0	21·0

Owing to partial drying up, I had to abandon the cultures, but it was clear that B was far in advance of A in respect of spore-forma-

tion. I fear that a new difficulty arises here—that of ensuring that each culture-drop shall contain the same amount of food-materials, if it is to last a long time; of course one does one's best to have the drops of equal size, and the drying up was here due to the different water contents of the cotton-wool in the arms of the cells.

In the following case the experiment was made in the north laboratory, fully exposed to the overcast sky, the day hot and sultry. The spores were sown in weak gelatine, as before, at 9 A.M., and were in the dark at 22° C. till 2 P.M. Germination was established at 2.30 and at 4.15; the measurements were begun on two filaments almost exactly the same length. The results testify strongly to the accuracy of the measurements and the equality of growth under like conditions (Curves 57 and 58).

Time.	Lengths. A. μ.	Lengths. B. μ.	Temp. ° C.
4.15 P.M.	58·5	60·0	24·5
4.21 „	67·0	68·0	24·5
4.33 „	80·0	82·0	24·5
4.50 „	99·0	102·0	24·5
5.1 „	105·75	108 0	24·25
5.12 „	119·25	121·5	24·25
5.26 „	141·75	148·5	24
5.37 „	160	162	24
5.52 „	193·5	189	23·75
6.10 „	225	220	23·5



Note particularly the fall in the curve at 4.50 to 5.1, when the temperature began to fall; it shows how very sensitive to temperature-changes the *growing* filaments are, and how extremely difficult it is to eliminate this source of error.

Spores sown at 8.30 A.M., July 7, were kept at 22° C. in dark till 2.45 P.M., and then the selected rodlets treated exactly alike at the north window. Dull, cloudy, and eventually raining.

Time.	Lengths. A. μ.	Lengths. B. μ.	Temp. ° C.
2.45 P.M.	31·5	31·5	21·5
2.54 „	36·0	35·0	21·5
3.36 „	62·75	61·0	21·25
4.25 „	99·0	96·75	20·75
4.48 „	121·5	120·0	21·0
5.16 „	160·5	154·0	20·0
5.43 „	202·5	189·0	19·5

I now pass to the comparative cultures in light and dark, premising that each pair of cultures must be compared by itself, the difficulties of observation and preparation being so great that one cannot possibly push the method further.

As before, I begin with the exposures of ungerminated spores, and pass afterwards to exposures of already germinated rodlets and filaments in active growth.

On June 23 spores were sown in dilute broth-gelatine at 10 A.M., and exposed at once. The quartz cells, shades, and screens as before. The temperature at 10.30 was 19·5°, slowly falling at the open window to 19° C. at 12 noon, and exactly alike under both screens. The sky was overcast, with traces of hazy blue now and again, and a cool wind was blowing; no rain fell, but the afternoon was very dull. The following are the results, germination having begun soon after 12 noon.

June 23. Time.	Temperature.	Water. Average.	Water. Extreme.	Water. Mean.	Water. Commonest.
	° C.	μ.	μ.	μ.	μ.
2.0 P.M.	19·0	4·5—5·0	—	—	—
4.30 „	17·25	6·0	—	—	—
8.15 „	16·0	16·0 (8)	9—22·5	15·75	15·

June 23. Time.	Temperature.	K. Bichromate.			
		Average.	Extreme.	Mean.	Commonest.
	° C.	μ.	μ.	μ.	μ.
2.0 P.M.	19·0	5	—	—	—
4.30 „	17·5	9	6—12	9	8 or 9
8.15 „	16·0	47 (11)	13·5—76·5	45	50

The results are not so conclusive as in previous cases with brighter light, but, so far as they go, they bear out the inference already drawn, viz., that the light does inhibit the germination of the spores even though it is very feeble so far as blue rays are concerned.

The following experiment, carried out on June 30, a very hot day, with a slight haze but no clouds in the blue sky, bears out the foregoing conclusion that the action is due to intense destructive metabolism at high temperatures (see p. 375).

Spores were sown and exposed at 7 A.M., a more dilute gelatine, 5 per cent., with only traces of broth, being used. Control cultures in the dark at 24° C. showed that germination and growth can go on quite well in this medium, and filaments of 200 to 250 μ were developed by 3 P.M. from these controls put into the incubator at 7 A.M.

The temperatures reached were occasionally rather high, as the following table shows, but the exposed cultures were subjected to exactly the same influences, except as regards the light (never direct from the sun) filtered through the screens. The arrangement was exactly as before.

	Temperatures.	
Time.	Water. ° C.	Bichromate. ° C.
7 A.M.	18·0	18·0
8·30 „	18·5	18·5
11 „	20·5	20·5
12 noon	25·0	25·0
12.30 P.M.	27·0	27·0
3.30 „	31·0	31·0
9 „	19·0	19·0

At 3.30 P.M. the bichromate culture showed several germinating rodlets, and by 9 P.M. these had grown considerably. That the high temperatures and great range had retarded them, however, even in the orange light, was evident from the much better growth of the control cultures kept constant at 24° C. in the dark incubator. On July 1—*i.e.*, next day—there were plenty of well-grown colonies, but

consisting of much broken filaments and rodlets, instead of forming long tresses.

The culture exposed over *water*, however, never gave any colonies at all: the spores were all killed.

That the light-action on the spores does not depend for its effect on a high temperature, however, follows not only from the previous experiments, but also, and very clearly, from the following one, carried out on July 1, a hot day with a cloudless blue sky, but somewhat hazy, probably owing to London smoke brought up the Thames valley by the light east wind then prevailing.

Spores were sown in 10 per cent. gelatine with traces only of broth and glucose, all the arrangements as before. The sowings were made and exposures started at the open south window at 9 A.M. The following table summarises the results:—

Time.	Temperature.		Remarks.
	Bichromate.	Water.	
	° C.	° C.	
9 A.M.	19	19	
10 ,,	20	20	
10.30 ,,	21	21	
11.0 ,,	22	22	
11.30 ,,	24	24·5	Brought into north laboratory.
12 noon	24	24	
1 P.M.	23·5	23·5	Germination begun over bichromate.
2 ,,	23·5	23·5	
3.30 ,,	24·0	24·0	Germination over water beginning. Returned both to south window.
4 ,,	24·25	24·25	
5 ,,	24	24	
6 ,,	23·5	23·5	
7.30 ,,	22·5	22·5	

At 3.30 several of the filaments under bichromate were measured, and gave from 9 to 10 μ to 20 μ, and even greater lengths (one was nearly 40 μ), whereas the most I could find in the culture over water were a few doubtful cases of commencing germination.

At 5 P.M. the shortest of the filaments over bichromate were about 30 μ, and the longest 130 μ. Twelve measurements gave 30, 36, 30, 45, 54, 45, 90, 99, 76·5, 135, 67·5, and 67 μ respectively, whereas no measurable filaments could be found in the culture over water, a few doubtful rods of 4 or 5 μ alone occurring.

Moreover, it was evident that the bichromate-culture filaments were growing very rapidly, and one was selected for observation, with the following results:—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
5 P.M.	112·5	—	—	—	24·0
5 6 „	121·5	6	9	1·5	23·75
5.11 „	130·5	5	9	1·8	23·6
5.20 „	148·5	9	18	2·0	23·6
5.25 „	157·5	5	9	1·8	23·6
5.32 „	175·5	7	18	2·57	23·6
5.35 „	180·0	3	4·5	1·5	23·6
5.40 „	193·5	5	13·5	2·7	23·6
5.45 „	202·5	5	9·0	1·8	23·5
5.47 „	207·0	2	4·5	2·25	23·5
5.54 „	220·5	7	13·5	1·9	23·5
5.56 „	225·0	2	4·5	2·25	23·25

That is to say, this filament doubled its length in fifty-six minutes at 24—23·25° C.

Further measurements were made during the evening, and showed that the rapid growth continued, for at 6.40 P.M.—the temperature being 23° C.—this filament was approximately 450 μ long, and at 7.30—t = 22·5°—considerably over 1000 μ.

At the same time (7.30 P.M.) and at exactly the same temperature the culture over water showed one or two filaments, very feeble and sharply segmented, about 100 μ long.

At 10 P.M., temp. 22·5° in both cells, the long coils of the bichromate-culture were several thousand μ long, whereas the feebly growing one or two filaments of the water-culture measured from 150 to 250 μ at most, and were much broken up and starved looking.

Even on July 2, when examined at 8.30 A.M., the water-culture had no filament so long as 500 μ, though one or two were over 400 μ long, but by this time the filaments of the other culture measured many thousand μ.

By 9 A.M. on July 3 the bichromate-culture was fully grown, and had developed magnificent spores in every filament; the water-culture showed no trace of spores, though the shortly segmented filaments were very granular.

These facts are of especial interest and importance as disposing effectually of any question as to so weak a food material being unsuitable for the growth, &c., of this bacillus. It is quite clear that very little is needed for normal development.

On July 4, after another day's exposure—but July 3 was dull, hot, and cloudy all day—the culture over water was still devoid of spores. After the whole of the 4th, in dark incubator at 22° C., still no signs of spores.

On July 5 one or two spores were detected at isolated spots here and there in the filaments, but very few.

It was perfectly clear that, exposed at the same temperatures, the culture from which the blue rays were screened flourished well and formed spores, whereas the other was starved and retarded, and could scarcely form spores at all.

Spores sown as before, in weak gelatine, at 8.30 A.M., July 7, were at once exposed to the south window. At 9.30, however, I removed them to the upper room, north window, to avoid high temperatures.

The day opened very bright, with a deep blue sky, about half covered with cumulus clouds, at noon became more overcast, and after 1 P.M. was dull and quite overcast; rain later.

Water.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
					° C.
9.30 A.M.	—	—	—	—	20
11.0 „	—	—	—	—	20·5
12 noon	—	—	—	—	22
1 P.M.	—	Germinating out.		—	22
2.15 „	—	—	—	—	21·75

At 3 P.M. I selected a good rodlet for measurement.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
3.0 P.M.	12·5	—	—	—	20·5
3.30 „	15·75	30	2·25	0·07	21·0
3.50 „	18·0	20	2·25	0·11	20·5
4.17 „	27·0	27	9·0	0·33	20·5
6.0 „	76·5	103	49·5	0·48	19·0
6.30 „	90·0	30	23·0	0·76	19·0
7.40 „	135·0	80	45·0	0·56	18·0

Curve 63, p. 368.

Doubling periods:—

12·5— 25 μ = 3 P.M.—4.12 P.M. = 72 minutes at 20·5—21—20·5° C.
25 — 50 „ = 4.12 „ —5.5 „ = 53 „ 20·5—19·5° C.
50 —100 „ = 5.5 „ —6.46 „ = 101 „ 19·5—19° C.

Reckoned back:—*

135 —67·5 μ = 5.40 P.M.—7.40 P.M. = 120 minutes at 20—18° C.
67·5 —33·75 „ = 4.30 „ —5.40 „ = 70 „ 20·5—20° C.
33·75—16·75 „ = 3.44 „ —4.30 „ = 46 „ 21—20·5° C.

* *I.e.*, the doubling periods calculated back on the curve from the last observation instead of forwards from the first one, and thus giving comparisons of the growth in different sections of the filament.

Bichromate.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
					° C.
9.30 A.M.	—	—	—	—	20·0
11.0 „	—	—	—	—	20·5
12.0 noon	Germinating	out.	—	—	22·0
1.0 P.M.	—	—	—	—	22·0

I now selected a rodlet for measurement:—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
2.15 P.M.	24·75	—	—	—	21·75
3.0 „	42·75	45	18·0	0·4	20·5
3.30 „	56·25	30	13·5	0·45	21·0
3.50 „	72·0	20	15·75	0·79	20·5
4.17 „	99·0	27	27·0	1·0	20·5
6.0 „	270·0	103	171·0	1·6	18·5
6.30 „	324·0	30	54·0	1·8	18·5
7.40 „	585·0	80	261·0	3·26	18·0

Curve 64, p. 368.

Doubling periods:—

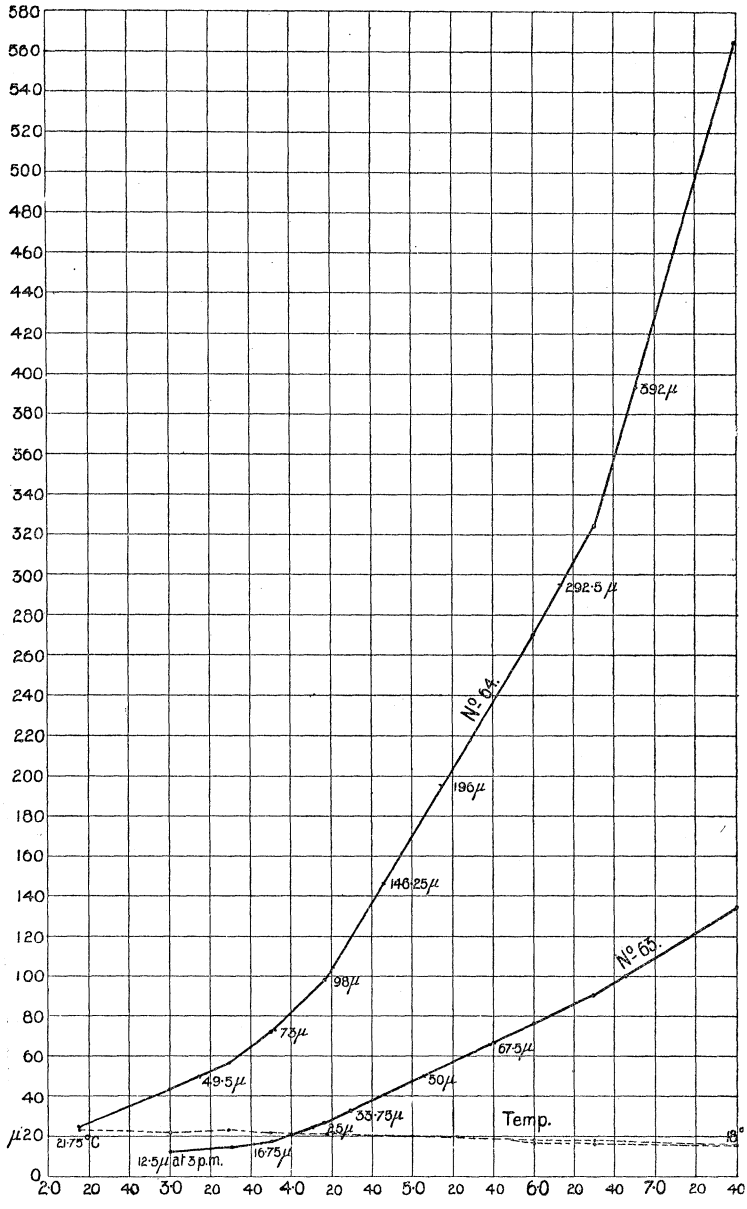
24·75— 49·5 μ = 2.15 P.M.—3.15 P.M. = 60 minutes at 21·75—20·75° C.
49·5 — 98·0 „ = 3.15 „ —4.16 „ = 61 „ 20·75—20·5° C.
98·0 —196·0 „ = 4.16 „ —5.15 „ = 59 „ 20·5 —19·5° C.
196·0 —392·0 „ = 5.15 „ —6.48 „ = 93 „ 19·5 —18·5° C.

Reckoned back:—

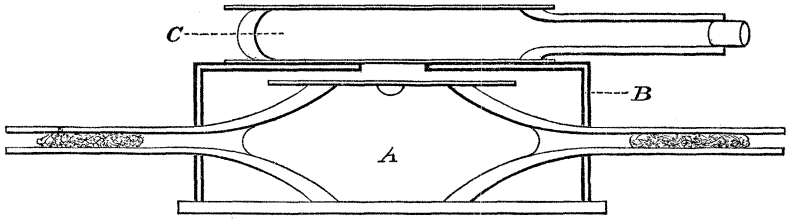
585·0—292·5 μ = 6.14 P.M.—7.40 P.M. = 86 minutes at 18·5—18·0° C.
292·5—146·25 „ = 4.45 „ —6.14 „ = 89 „ 20·0—18·5° C.
73·0— 36·5 „ = 3.51 „ —4.45 „ = 54 „ 20·5—20·0° C.

Here, again, we see that the light effect on *spores* is obvious enough, and that with daylight, which gives no measurable results with *growing* filaments at all. We obtain a more vivid impression of the differences of behaviour of these two cultures if we note the following points:—

The water-culture took 101 minutes to grow from 50 μ long to 100 μ—double its length—at 19·5—19° C., and 120 minutes to grow from 67·5 μ to 135 μ at 20—18° C.; whereas the bichromate-culture grew from 292·5 μ to 585 μ (*i.e.*, doubled its length) in only 86 minutes at 18·5 to 18° C., and only took 89 minutes to grow from 146·25 to 292 μ at 20—18·5°.



On August 3 spores were sown in broth + 1 per cent. gelatine, at 10 A.M., and exposed as follows. Each of two cultures had a blackened matchbox cover pierced with a hole, so as to diminish radiation



A, culture cell (quartz floor, &c.) arranged as usual; B, an inverted matchbox painted black and with a hole over the hanging drop; C, a quartz bottle screen of water or bichromate, placed over the hole, so that no light can reach the hanging drop except through the screen.

and support the (quartz) screen. Each stood side by side, with a dummy cell provided with a thermometer, one pair (culture and dummy) under bichromate, the other under water.

They were on a table outside the north laboratory, exposed to the blue sky, but with no trace of direct sunlight. The amount of white cloud increased from 10.45 to 11.15, and very little open sky was available, except at intervals. The details concerning temperature and weather are given in the following table:—

Time.	Temperature.		Weather.
	Bichromate.	Water.	
	° C.	° C.	
10.10 A.M.	17·5	17·5	Blue sky, with scattered white clouds.
10.15 „	17·75	17·75	
10.34 „	17·9	17·9	
10.55 „	18·75	18·75	Clouding over badly.
11.15 „	20·1	20·1	
11.45 „	21·5	21·1	Threatening rain.
12.5 P.M.	21·7	21·4	A few drops of rain.
12.40 „	23·25	23·0	Blue patches, but much cloud.
12.55 „	23·1	23·0	
1.50 „	21·5	21·2	Very dull.
2.15 „	20·6	20·5	Blue sky, few clouds.
2.40 „	20·5	20·5	Some blue, but many fleecy clouds.
3.20 „	19·3	19·3	More blue.
3.55 „	20·5	20·6	Much cloud and very little blue.
4.40 „	19·0	19·1	
5.0 „	17·8	17·9	Blue sky, but hazy, and a few clouds.
5.38 „	17·5	17·5	
6.5 „	16·6	16·5	Blue, but hazy, and some cloud.
6.30 „	15·5	15·5	
6.55 „	15·4	15 4	

At 7 P.M. all were brought into the laboratory, and the temperature rose to 16·25° at 7.5, 18° at 7.20, 18° at 8.45.

The cultures were examined at 4.40, 7 P.M., and 8.45, in each case with no traces of germinated rodlets in that under water, whereas that under bichromate had germinated out normally and grown well, as the table shows.

Time.	Bichromate.				Water.			
	Average.	Extremes.	Mean.	Temp.	Average.	Extremes.	Mean.	Temp.
	μ.	μ.	μ.	°C.	μ.	μ.	μ.	° C.
4.40 P.M.	70 (15)	31—139	85	19·0	No trace	of germin	ation.	19·0
7.0 „	225 (10)	156—270	216	16·75	„	„	„	16·75
8.45 „	330 (7)	270—460	365	18·0	No rodl spores	ets, but a	few swollen.	18·0
7.0 A.M. Aug. 4	Unmeasu	rably long	—	18·0	No rodl	ets at all.	—	18·0

How the conclusion that the light from a sky only occasionally blue, and generally very cloudy, retarded the spores indefinitely is to be avoided here seems inconceiveable. There is nothing in the minute differences of temperature—the maximum at 11.45 was only 0·4 of a degree—to account for it, and as for the variations (from 17·5 to 23·25), even if they had attained dangerous ranges, they were similar in both cases; but they were in no way dangerous; the range was really very favourable.

On August 4 this experiment was repeated, but with broth instead of 1 per cent. gelatine drops. The cultures were started at 10.15, and kept till 10.55, at a north window in the laboratory, because rain was threatened. The sky then became blue in patches —a very deep blue, but with rolling heavy cumulus clouds—and the cultures were then taken outside and exposed exactly as yesterday, all the cells standing on a glass mirror, the surface of which was covered with black paper except just below the drops and the thermometer bulbs. The variations of sky and temperature are given in the annexed table.

Time.	Temperature.		Weather, &c.
	Bichromate.	Water.	
	° C.	° C.	
10.25 A.M.	19·5	19·6	Very dull and threatens rain, therefore in laboratory.
10.57 „	20·4	20·3	
11.15 „	18·1	18·4	Blue sky and cumulus, therefore put outside.
11.47 „	23·0	23·6	Blue sky and rolling cumulus.
12.30 P.M.	23·6	24·0	Dull and cloudy.
1.6 „	22·8	23·2	Threatens rain.
2.25 „	19·75	20·2	Very thick clouds.
2.57 „	—	—	Brought in to examine. Out at 3.10.
3.10 „	19·1	19·75	Dull, cloudy, threatens rain. Brought in to examine, and kept in.
4.0 „	17·75	18·0	
4.15 „	18·25	18·25	In laboratory at north window.
5.15 „	18·5	18·5	
7.0 „	18·0	18·0	
8 20 „	18·0	18·0	
8 A.M., Aug. 5	17·0	17·0	

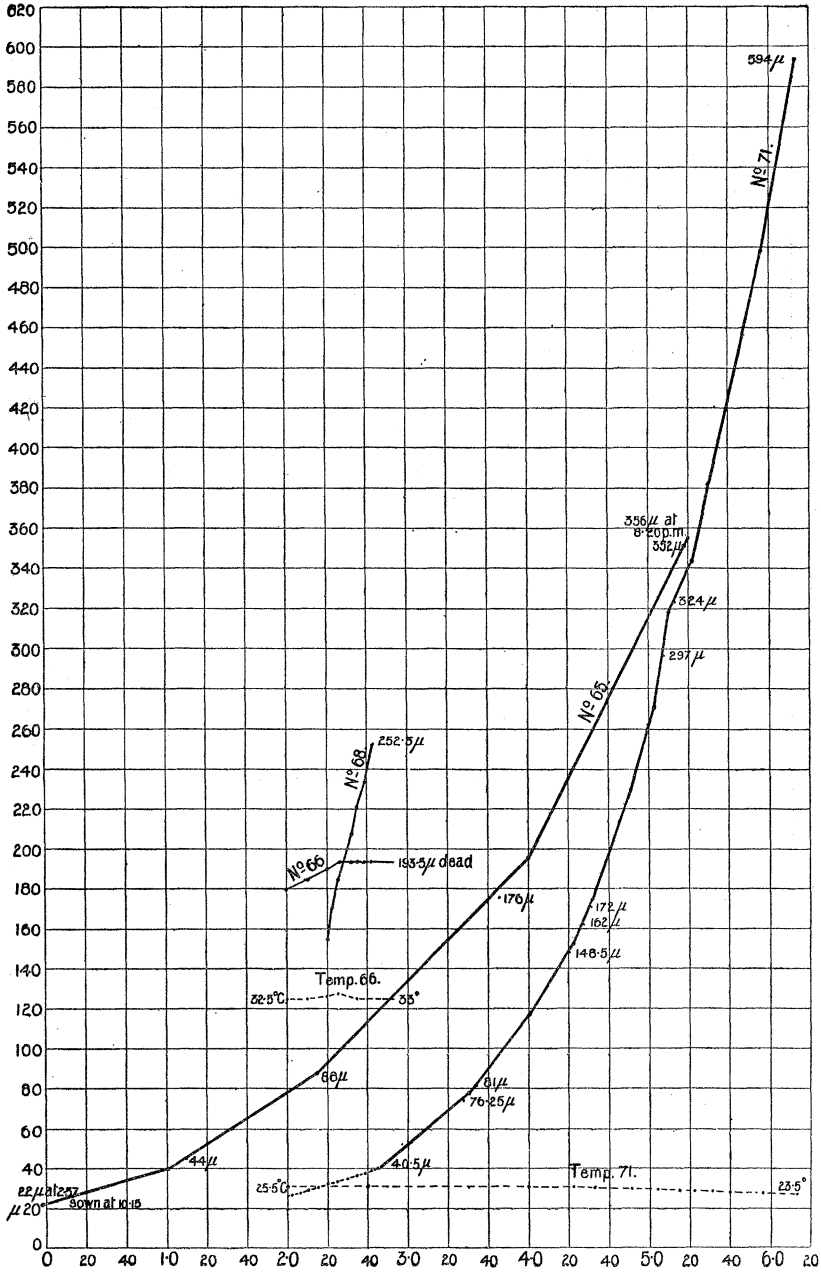
The results of examinations at 2.57 P.M., 4 P.M., &c., are given in the annexed table. They prove conclusively the evident light-action.

Time.	Bichromate.			Water.		
	Average.	Extremes.	Mean.	Average.	Extremes.	Mean.
	μ.	μ.	μ.	μ.	μ.	μ.
2.57 P.M.	22 (10)	18·0— 27·0	22·5	No trace of germination.		
4.0 „	40 (13)	18·0— 67·5	42·75	„	„	
5.15 „	88 (10)	36·0—184·0	110·0	„	„	
7.0 „	175 (10)	40·0—290·0	165·0	„	„	
8.20 „	356 (10)	265·0—450·0	357·5	„	„	
8.0 A.M. Aug. 5	Immeasurably long tresses.			No signs of germination, though plenty of dead spores present.		

Curve 65, p. 372.

If we take the doubling periods of the bichromate-culture, we have (averages)

1. 22— 44 μ = 2.57 P.M.—4.8 P.M., *i.e.*, 71 minutes at about 19·75—17·75° C.
2. 44— 88 „ = 4.8 „ —5.15 „ *i.e.*, 67 „ „ 17·75—18·5° C.
3. 88—176 „ = 5.15 „ —6.45 „ *i.e.*, 90 „ „ 18·5 —18·0° C.
4. 176—352 „ = 6.45 „ —8.19 „ *i.e.*, 94 „ „ 18 0 —17·0° C.


620
600
580
560
540
520
500
480
460
440
420
400
380
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
μ
0 20 40 1·0 20 40 2·0 20 40 3·0 20 40 4·0 20 40 5·0 20 40 6·0 20
No. 71.
594 μ
356 μ at 8·20 p.m.
352 μ
324 μ
297 μ
No. 65.
No. 68.
252·5 μ
No. 66.
193·5 μ dead
176 μ
172 μ
162 μ
148·5 μ
Temp. 66.
32·5°C.
35°
88 μ
61 μ
76·25 μ
44 μ
40·5 μ
22 μ at 2·37
down at 10·15
25·5°C.
Temp. 71.
23·5°

Of course, we must remember these numbers are for averages of lengths, and cannot be compared too closely with the numbers got as averages of detailed observations. Nevertheless, there is a certain and more than merely rough agreement, which suggests that the growth was normal for the rather wide ranges of temperature.

May 22 opened very clear and blue, but with a cold N.E. wind; after 10 A.M. it clouded over, and the afternoon was dull and wet as well as cold.

A culture of spores kept at 12° C. overnight was germinating freely, but slowly. The cell had a quartz floor; the drop was broth-gelatine.

At 10 A.M. this was put over a concave silver mirror and quartz cell of water, and the measurements begun. The exposure was to the open blue sky to the south; no direct sunshine at all was allowed, a cardboard screen being used.

The measurements were as follows, the temperatures being those of a similar cell over a plane silver and bichromate quartz cell; experiments showed this registered the same temperatures.

The experiment was simply a trial to satisfy myself that the apparatus worked satisfactorily. As the following table shows, the growth was very slow, but six hours' exposure to the dull light did not kill the filaments.

The following table gives results :—

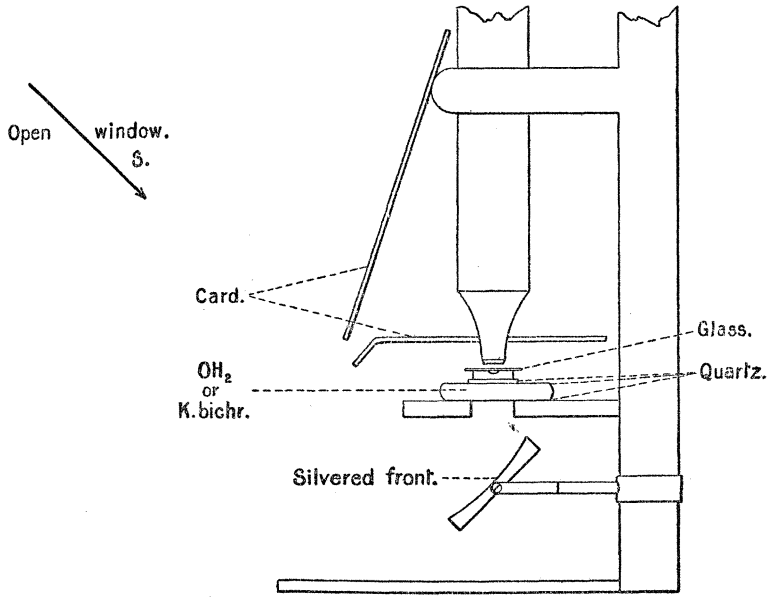
Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
10.10 A.M.	30	—	—	—	12·0
11.50 „	36	100	6	0·06	13·0
12.30 P.M.	44	40	8	0·2	13·0
*2.15 „	48	105	4	0·004	12·0
4.20 „	64	125	16	0·12	12·75

This filament doubled its length in 5 hours and 28 minutes at 12—13—12° C.

On June 21, which turned out a brilliant sunny day, with blue sky, a little hazy, and very hot, the following experiments were made; the apparatus being quartz cells, and screens, and double cardboard shades to microscopes arranged as in the accompanying diagram (p. 110).

The spores, sown in broth-gelatine the night before at 12° C., had germinated evenly to rodlets by 9 A.M., and were exposed at the open south window at 9.45, over screens of water and of K. bichromate, the silver mirrors being turned to the blue sky to the south.

* The window was closed from 2.15 P.M. onwards.



After allowing the light, *from the blue sky only*, to act on the cultures for about $2\frac{1}{2}$ hours, the following measurements were made of two filaments exactly the same length, as near as I could select them :—

Bichromate.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
12.20 P.M.	90·0	—	—	—	26·0
12.45 „	108·0	25	18·0	0·76	26·0
12.55 „	112·5	10	4·5	0·45	27·0
			Water.		
12.25 „	90·0	—	—	—	24·5
12.45 „	99·0	20	9·0	0·44	24·0
12.55 „	108·0	10	9·0	0·9	25·0

Here was evidence that the culture over water was growing less rapidly than that over bichromate, but since the growth of the filaments in the bichromate culture was rapidly approaching the long lengths which I cannot measure, I determined to try the effect of more direct insolation to see if the higher temperature and intensity would bring more rapid action. Consequently I tried the results of

so arranging the mirrors that the solar image was reflected up on to the cultures and thermometers, again using the same cultures, but selecting yet another filament in each of lengths as nearly equal as possible.

The results were somewhat startling and puzzling, as the following tables and curves show:—

Water.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
2.0 P.M.	180·0	—	—	—	32·5
2.10 „	184·5	10	4·5	0·45	32·5
2.20 „	189·0	10	4·5	0·45	33·0
2.26 „	193·5	6	4·5	0·75	34·0
2.36 „	193·5	10	—	—	33·0
2.42 „	193·5	6	—	—	33·0

Curve 66, p. 372.

The growth stopped, and further examination showed the filament was dead.

Bichromate.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
2.20 P.M.	157·5	—	—	—	33·5
2.22 „	175·5	2	18·0	9·0	33·5
2.25 „	184·5	3	9·0	3 0	33·0
2.32 „	207·0	7	22·5	3·2	33·0
2.35 „	221·0	3	13·5	4·5	33·5
2.39 „	234·5	4	13·5	3·4	34·0
2.42 „	252·5	3	18 0	6·0	34·0

Curve 68, p. 372.

Here we see clearly enough that at high temperatures (32—34° C.) the bright light acted as a powerful *stimulus to rapid growth* behind the bichromate, which warded off the blue-violet rays, but brought about rapid *diminution of growth and death* behind the water.

It seems extremely probable therefore that, provided there is plenty of food-material of a highly nutritious nature present, the high temperature is in itself merely conducive to intense constructive metabolism and growth, but if the blue rays gain access at the same time they so interfere with constructive metabolism or so promote *destructive* metabolism (perhaps by promoting respiration ?) that rapid death ensues.

In any case the death cannot here be referred merely to the high temperature, because, on the whole, the bichromate culture was at a higher temperature than the other.

On July 5 spores were sown in weak gelatine at 9 A.M., and cultures kept in dark till 2 P.M. The sky was particularly blue, but unfortunately I had to abandon the measurements at 4.25 P.M., and on my return several hours later they had grown too long to measure.

So far as the measurements show there was no appreciable light-effect up to 4 P.M., but after that the bichromate curve was making a sharp rise out of all proportion to the temperatures; it was unfortunate that an engagement took me away from the observations just at the critical part, because the pursuit of this rise—which ought to have been less than the rise in the other curve, according to the temperatures—would have been interesting.

But these cultures were placed next morning at 22°. C. in the dark incubator, and on the 7th July the one over bichromate had formed excellent and normal chains of spores; whereas the one over water, though some spores were developed, was far behind in that respect. So that, after all, there was an appreciable light effect in the retardation of spore formation.

Spores were sown at 12 midnight on July 5, and kept at 22° in dark incubator till 10 A.M. They were then exposed over a water screen, quartz, as usual. The weak gelatine was used. The day was very hot, and the haze in the early morning soon became thicker, and by noon the sky was overcast; the afternoon was dull and intensely sultry, ending in thunderstorms. From previous experience no light-effect could be expected, and the following table and curve show that no appreciable effect was obtained. The curve, on the other hand, may be regarded as an almost perfect type of the growth record at the temperature used, probably the optimum or a little beyond.

The measurements could only be conducted directly on the whole filament up to 5.3 P.M., but fortunately the filament then segmented so distinctly into two parts, and at 5.30 into three parts, that, since these remained in contact and each quite straight but forming very open angles one with another, I was able to measure each by itself and add their growths. After 6.14, however, this became no longer possible, partly owing to the enormously rapid growth and partly to the lengths being greater than I could measure accurately.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
10.0 A.M.	—	—	—	—	22·0
10.45 „	Germinating.		—	—	23·5
11.30 „	—	—	—	—	26·0
12.0 noon	—	—	—	—	25·5
12.30 P.M.	4·0	—	—	—	26·5
12.45 „	—	—	—	—	26·5
2.0 „	—	—	—	—	25·5
2.45 „	40·5	135	36·5	0·27	26·0
3.30 „	78·75	45	38·25	0·85	26·0
4.0 „	117·0	30	38·25	1·27	26·0
4.22 „	153·0	22	36·0	1·6	25·5
4.32 „	175·5	10	22·5	2·25	25·5
4.51 „	229·5	19	54·4	3·0	25·0
5.3 „	265·5	12	36·0	3·0	25·0
5.15 „	319·5	12	54·0	4·5	24·75
5.21 „	342·0	6	22·5	3·7	24·5
5.30 „	382·5	9	40·5	4·5	24·5
5.40 „	436·5	10	54·0	5·4	24·0
5.56 „	499·5	16	63·0	4·0	24·0
6.14 „	594·0	18	94·5	5·25	23·5

Curve 71, p. 372.

Doubling periods:—

40·5—81·0 μ = 2.45 P.M.—3.33 P.M. = 48 minutes at 26° C.
81·0—162·0 „ = 3.33 „ —4.27 „ = 54 „ 26—25·5° C.
162·0—324·0 „ = 4.27 „ —5.13 „ = 46 „ 25·5—24·75° C.

Reckoned back:—

594·0—297·0 μ = 5.7 P.M.—6.14 P.M. = 67 minutes at 25·0—23·5° C.
297·0—148·5 „ = 4.20 „ —5.7 „ = 47 „ 25·5—25·0° C.
148·5— 76·25 „ = 3.27 „ —4.20 „ = 53 „ 26·0—25·5° C.

On August 6 spores sown in two broth-drop cultures were kept at 23° from 10.15 A.M. to 2 P.M., and germinated out well.

Then placed over bichromate and water respectively during the afternoon to test the light-action, with the following results (pp. 378, 379), a rodlet 6·75 μ long being fixed in the water culture, and one 13·5 μ long in the bichromate culture.

The morning had been very dull, but cleared up between 1 and 2 P.M.; at 2.30 the sky was only just showing blue and hazy between streaky clouds, little or no wind. From 2.37 to 3.10 the sky was overcast with heavy dull clouds, but cleared a bit by 3.30, and from 3.50 to 4.45 again showed some hazy blue between streaky clouds. The clouds get thicker, in fleecy streaks afterwards, and there was some blue showing up to 4.50. Thence onwards was dull and overcast.

The window was open—south laboratory—all the time, up to 6.55, quartz, silver mirrors, card and matchbox screens, &c., as before.

The light from the blue sky was brilliant for ten minutes or a quarter of an hour at the beginning of the exposure.

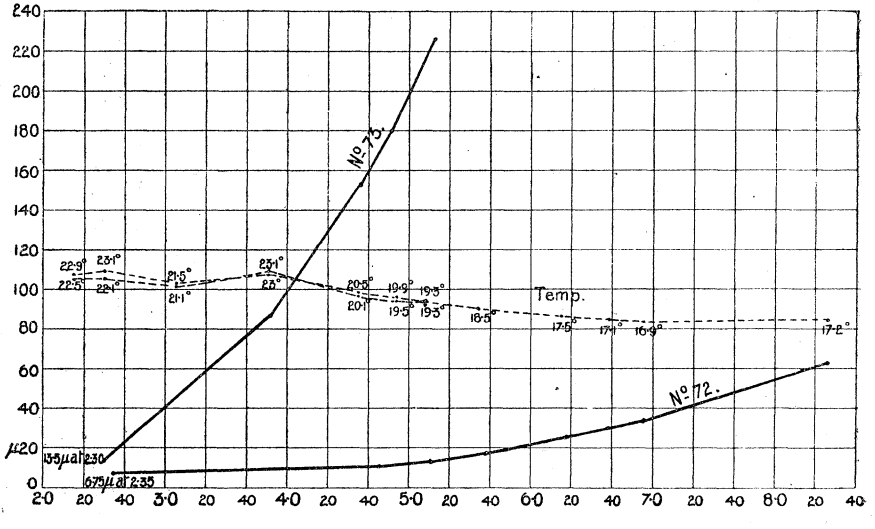
Water.

Time.		Length.	Interval.	Growth.	Rate.	Temp.
		μ.	mins.	μ.	μ.	° C.
2.15 P.M.		—	—	—	—	22·5
2.35	,,	6·75	—	—	—	22·1
2.37	,,	—	—	—	—	22·1
2.52	,,	6·75	17	—	—	22·6
3.4	,,	—	—	—	—	21·1
3.51	,,	6·75	59	—	—	23·1
4.35	,,	11·25	44	4·5	0·1	20·1
4.52	,,	13·5	17	2·25	0·1	19·5
5.11	,,	13·5	19	—	—	19·3
5.39	,,	18	28	4·5	0·16	18·5
6.17	,,	24·75	38	6·75	0·17	17·5
6.28	,,	27	11	2·25	0·2	17·3
6.39	,,	29·25	11	2·25	0·2	17·1
Window closed. 6.55	,,	33·75	16	4·5	0·3	16·9
8.25	,,	63	90	29·25	0·32	17·2

Curve 72.

Doubling periods :—

1. 6·75—13·5 μ = 2.35 P.M.—4.52 P.M. = 137 minutes at 22·1—19·5° C.
2. 13·5 —27 ,, = 4.52 ,, —6.28 ,, = 96 ,, 19·5—17·3° C.
3. 27·0 —54 ,, = 6.28 ,, —7.57 ,, = 89 ,, 17·3—16·9° C.



Bichromate.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
2.15 P.M.	—	—	—	—	22·9
2.30 „	13·5	—	—	—	23·1
2.37 „	—	—	—	—	22·5
3.4 „	42·75	11	2·25	0·2	21·5
3.52 „	85·5	48	42·75	0·89	23·0
4.36 „	153·0	44	67·5	1·5	20·5
4.53 „	180·0	17	27·0	1·6	19·9
5.12 „	225·0	19	45·0	2·4	19·3

Curve 73, p. 378.

Doubling periods :—

1. 13·5— 27 μ = 2.30 P.M.—2.42 P.M. = 12 minutes at 22·9—23·1—22·5° C.
2. 27·0— 54 „ = 2.42 „ —3.17 „ = 35 „ 22·5—21·5° C.
3. 54·0—108 „ = 3.17 „ —4.7 „ = 50 „ 21·5—23·0—21·0° C.
4. 108·0—216 „ = 4.7 „ —5.9 „ = 62 „ 21·0—19·3° C.

It is pretty evident, in spite of the irregular and almost abnormally rapid growth recorded for the bichromate culture at first, that a prolonged retardation occurred in the water culture—a retardation which, for broth cultures, is too long for the temperatures, and can only be referred to the light.

On November 16 I sowed spores in normal 10 per cent. gelatine, and put into 22° C. at 10 A.M. Two cultures were made, and at 12.30 these were exposed as follows, to test the effect of the light from a blue sky on germinating rodlets. The two cultures stood side by side on a mirror, each covered with a matchbox screen; on one lay a blue glass, and on the other a red one. The sky was fairly clear, but clouds passed occasionally.

Two control-cells with thermometers lay beside them, and each of these was arranged exactly like its culture, the glasses used for screens being in each case the half of the same piece of glass as that on the culture.

The temperature was rather low, and ran as follows during the exposure from 12.30 to 4 P.M., on west side of laboratory, and completely sheltered from any direct sunlight.

	Temperatures.	
	Blue cell. °C.	Red cell. °C.
12.40 P.M.	12·75	12·0
12.50 „	10·8	10·75
1.0 „	10·4	10·3
2.0 „	9·9	9·9
2.15 „	9·5	9·5
3.0 „	8·5	8·5
4.0 „	7·0	7·0

At 4 P.M. the cultures were brought into the laboratory, where the temperature rose to 12·5° C., and there is no question as to the temperature being identical in both cells from 2 P.M. onwards, and at no time after the first ten minutes of exposure did the temperature differ by more than $\frac{1}{10}$th of a degree C.

When the cultures were first put out the spores were swollen, and were already beginning to germinate, and if they had remained at 22° C. till 4 P.M. they would have developed filaments from 90 to 150 μ long, as shown by two sister cultures left at 22° C. as controls, and which were examined at 4 P.M.

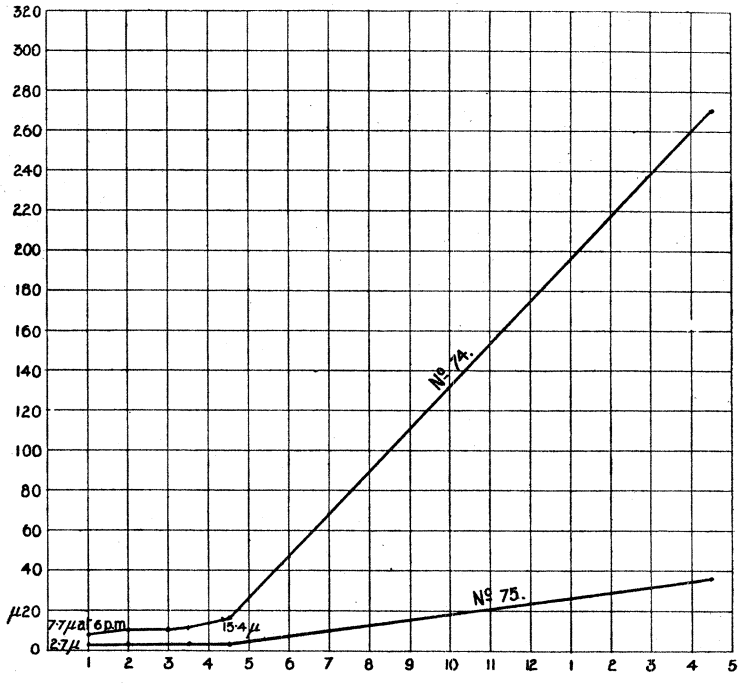
As it was, the exposure to a low temperature—12° to 7° C.—of course checked both cultures.

Nevertheless, at 6 P.M. the red culture showed several rodlets 9 to 10 μ long, one 6 μ, and two or three 4·5 to 5 μ, whereas I could discover plenty of swollen and germinated spores 2—3 μ long, and even one rodlet 4·5 μ long in the blue, but nothing longer. The following table gives the successive measurements.

It will be seen that here we have obvious retardation by $3\frac{1}{2}$ hours of diffuse blue light acting on the young rodlets, and that this retardation was entirely independent of the temperature, which was the same in both cases. (See Curves 74 and 75.)

Time.	Temp.	Blue.			Red.		
		Average.	Extremes.	Mean.	Average.	Extremes.	Mean.
	°C.	μ.	μ.	μ.	μ.	μ.	μ.
6.0 P.M.	12·5	2·7 (10)	4·5— 2·5	3·5	7·7 (10)	4·5— 10·0	7·25
7.0 „	13·0	2·75 (10)	5·0— 2·5	3·7	10·0 (7)	9·0— 12·0	10·5
8.30 „	12·75	2·75 (10)	5·0— 2·5	3·7	11·5 (8)	9·0— 14·0	11·5
9.30 „	12·8	2·75 (10)	5·0— 2·5	3·7	16·0 (7)	11·0— 18·0	14·5
9.30 A.M.	12·5	36·0 (6)	18·0—81·0	49·5*	270·0 (9)	157·5—360·0	258·75

* The mean is raised by a single filament which was (81 μ), much longer than any others.



The curves show that the doubling periods ran approximately thus:—

7·7—15·4 = 6 P.M. to 9.10 P.M. = 190 min. at 12·5—13—12·75 for the red, whereas the blue did not double (2·7—5·4 = 6 P.M. to 10·30 = 270 min. at same temperature) till 80 mins. later.

On November 17 spores which had been sown overnight in normal 10 per cent. gelatine at 13° C. had germinated to filaments 200 to 300 μ long by 10 A.M.; two cultures were examined and exposed at 10.15 A.M., at the south of the laboratory, care being taken that the sun was behind a building, and could not touch the cultures.

One culture was under blue and the other under red glass, each with its control thermometer cell, and side by side on a mirror, all open to the blue sky. The day was cold, and growth very slow. Matchbox screens were used.

The temperature ran as follows:—

Time.	Blue. ° C.	Red. ° C.
10.15 A.M.	12·5	12·25
10.35 „	11·75	11·0
12.15 P.M.	12·25	11·75
1.0 „	12·25	11·75
3.15 „	10·2	10·0

At 3.15—*i.e.*, after five hours' exposure—the red culture had grown considerably more than the blue one, although, so far as there was any difference of temperature at all, it favoured the blue culture. The filaments were too long, and too much curved to measure, but there was no doubt as to the increased growth.

Spores sown in normal gelatine at 10 P.M. on November 17, and kept in the dark at 13° C., had germinated to rodlets averaging 9—12 μ long at 8.30 A.M. on November 18, when two cultures were exposed to the clear blue sky on a mirror to the north and out of the sun. The day was cold and very fine. Each culture was covered by a black match-box screen, and had a thermometer control by its side treated exactly like it. One culture had ruby glass over it, the other blue; the latter was slightly in advance, *i.e.*, had some slightly longer rodlets. In order to be on the safe side I chose the culture which had a slight advance if anything for the blue.

The following table (p. 383) summarises the results of measurements, and the temperatures.

It is not difficult to translate these records. At the very low temperature used growth was extremely slow* in both cases; nevertheless the temperature was above the minimum, and the red glass screen enabled it to go on as usual, because the inhibiting blue-violet rays were cut off, whereas behind the blue glass these rays carried on their destructive work, and the rodlets did not grow at all after the first hour or two.

A word of explanation is necessary, perhaps, regarding the slight discrepancies between the measurements in the blue.

Each drop had about 20 spores in it. In taking the successive observations, two sources of (minute) errors exist: one is, that no doubt the measurements, even of the same rodlet, are not perfectly accurate—it is easy to see how one could go wrong to 0·25, or even 0·5 of a μ—the other is that one does not always find the *same* six or eight rodlets, however carefully one tries. My practice is to seek for the same, but to take care that *every* drop is thoroughly overhauled for the biggest and the smallest rodlets.

On the whole I think it will be admitted that the measurements correspond very well, and that the averages are very good.

At 9.15 P.M. the temperature was falling for the night, and it went down to below 9° C. before morning, rising again slowly after sunrise; this explains the still slow growth of the red culture.

At 8.30 A.M. I put both cultures into the incubator at 22° C., to see if a rise of temperature would make any difference as regards the dormancy of the blue culture.

The result was that while the red culture had filaments from 40 to 300 μ long by 12.30 noon, the blue one showed no further signs of

* The doubling period would seem to be 360—400 minutes, or even longer.

Time.	Blue.				Red.			
	Temp.	Average.	Extremes.	Mean.	Temp.	Average.	Extremes.	Mean.
	° C.	μ.	μ.	μ.	° C.	μ.	μ.	μ.
8.30 A.M.	9·9	10—12	—	—	9·6	9—11	—	—
9.0 „	9·7	—	—	—	9·1	—	—	—
9.40 „	9·1	—	—	—	9·0	—	—	—
10.15 „	10·0	—	—	—	10·25	—	—	—
11.30 „	9·5	—	—	—	9·5	—	—	—
12.30 P.M.	8·5	12·5 (6)	11·25—18	14·6	8·5	10·5 (6)	6·25—13·5	10
2.0 „	8·6	—	—	—	8·5	—	—	—
3.0 „	8·5	—	—	—	8·6	—	—	—
4.0 „	8·5	12·25 (6)	No growth.		8·5	20·25 (6)	18—24·25	21
5.0 „	14·5	—	—	—	14·5	—	—	—
6.15 „	14·5	14·6 (6)	9—18	13·5	14·5	23·75 (5)	18—36	27
7.15 „	14·5	—	—	—	14·5	—	—	—
8.15 „	14·0	13·5 (7)	9—20·25	14·6	14·0	31·5 (8)	18—45	31·5
9.15 „	13·7	—	—	—	13·6	—	—	—
8.30 A.M.	10·0	14·6 (8)	9—22·5	15·75	10·0	48·6 (8)	22·5—76·5	49·5

life than before. Similarly, at 4 P.M., the blue was as before, while the red had filaments several thousands of μ's in length.

On November 18 spores were sown in normal gelatine at 8 A.M., and kept at 22° C. till 12.30, when they had germinated to rodlets 8—9 μ long. Two cultures were then exposed at the south window, the sun being off (behind a building) on microscopes, and over blue and red glass respectively, the light reflected up from ordinary mirrors. Controls as usual. The whole day was clear and cold.

The following are the results :—

The doubling period for the red culture comes out (for the mean) =

$$24 \cdot 75—49 \cdot 5\ \mu = 1.30 \text{ P.M.}—3.47 \text{ P.M.} = 137 \text{ minutes at } 15 \cdot 5—16 \cdot 4—15°.$$

Owing to the fact of my having the previously described cultures, exposed to the open sky behind the same blue and red screens, but at a lower temperature, it is not difficult to see what happened here. The amount of light reflected from the small glass mirrors of the microscopes is not sufficiently intense to kill the rodlets at these temperatures, though it *is* sufficient to inhibit their growth perceptibly.

This explains many of my previous failures. If I arrange the microscopes so as to throw a more intense daylight on the cultures, then the heat rays produce difficulties, because the screens transmit them in different proportions, and I either find the measured filament growing longer than can be measured before the inhibition sets in, or the difference in temperature between the two cultures so great that doubts arise as to how much of the inhibition is due to lower or higher temperature, and how much to the light action.

For, as we now see, the temperature is effective at once, but the light action takes a considerable time to make its effects visible on the growth-curve, and over and over again I have found cultures just beginning to show the retarding, brake-like action of the light injury at, or even after, the conclusion of the short growing period I am able to quantitatively examine and record. Of course, though one may be convinced by inspection that, of two cultures, one has formed a smaller crop than the other, in the absence of measurements the statement wants the definiteness I have been trying to attain.

On December 6, spores in normal gelatine were sown, and exposed at once at 11 A.M. to the hazy, winter sun, at south window of my house.

The "red" stood over bichromate; the "blue" over water tinged with $CuSO_4$, both in quartz cells.

The temperature of controls ran as follows during exposure :—

Time.	Red. ° C.	Blue. ° C.	
11.0 A.M.	13·0	13·0	Window open
11.30 „	11·75	12·5	
12.30 „	11·5	12·25	
12.40 „	12·25	13·0	
1.0 P.M.	13·0	13·5	
1.10 „	15·5	15·0	Window shut
1.20 „	16·5	15·5	
1.50 „	14·5	14·5	
2.0 „	14.0	14·0	
3.10 „	11.0	11·0	

At 3.10 the exposure was stopped, and both cultures put into an incubator at 22° C. in the dark, and they remained under absolutely like conditions to the end of the experiment.

The marked retardation of the blue culture, after the four hours' exposure to a winter sun, never clear of haze, and at temperatures a trifle higher than the red rather than below, is well seen from the following table, in spite of the careful nursing at 22° C., a very favourable temperature, be it noted:—

Time.	Red.			Blue.		
	Average.	Extremes.	Mean.	Average.	Extremes.	Mean.
	μ.	μ.	μ.	μ.	μ.	μ.
4.30 P.M.	4·5 (10)	3·0— 5·0	4	No germination.		
5.0 „	7·0 (5)	5·0— 9·0	7·0	„	„	
6.0 „	15·5 (10)	7·0— 22·5	14·75	„	„	
7.0 „	33·0 (8)	22·5— 49·5	36·0	„	„	
8.30 „	88·0 (10)	54·0—157·5	105·5	9 (8)	4·5—20·0	12·0
	Too long to measure, but about 300 to 500 μ.			45 (8)	9·0—85·5	47·5

Germination had not begun at 3.10, but was commencing at 4.30 in the red, whereas it was delayed till 8.30 in the blue, and would, no doubt, have been still more tardy at a lower temperature.

A review of the foregoing results where no glass was used (except the thin cover slips in some) only confirms the previous results.

1. The spores are distinctly retarded or killed by five or six hours' exposure to daylight, even of low intensity, quite apart from temperature.

2. The *growing* filaments are often not measurably retarded within the period observed, except under conditions such as do not exclude possible temperature effects; but the evidence goes to show that the light slowly retards the growth, acting like a brake on the curve of growth.

3. Further testing of the measurements and curves on growing filaments under like conditions, confirms the confidence in their accuracy, and they may be accepted as very good approximations.

4. Some of these measurements bring out clearly the extreme sensitiveness to changes of temperature of the growing filaments, and emphasise clearly how difficult it is to avoid this source of error.

5. The mode of action of the light may be conceived of in several ways, keeping in view the differences of effect on spores and filaments. First, we might suppose it promotes oxidations in the surrounding food materials, resulting in the formation of poisonous substances which kill the spores but not the filaments; this (taking into account the resistance of the spores to physical agencies, and the evidence previously given) seems unlikely, for unless the *living protoplasm of the actively growing cells* has some extraordinary power of destroying such poisons as fast as they are made, whereas the *dormant protoplasm of the resting spore* is incapable of this, it seems incredible that the spores, otherwise so highly resistant, should succumb more easily than the otherwise so slightly resistant filaments. Secondly, we might suppose that the light action takes effect directly on some easily destroyed reserve material in the spore, which does not exist as such in the actively metabolising growing cell. This would explain the retardation of germination, or the death of the spores, according to the amount of destruction of the spore contents, very well; but it is not easy to accept the assumption that the light is totally without effect on the growing cell. A third possibility seems to be that the light action makes itself effective in promoting some intense metabolic activity in the spores and growing cells alike, and which is connected with enhanced respiration. In this case we might suppose the *spores* to suffer from the too rapid consumption of their unstable reserve materials (as before), while the growing cells do not show the effects *so long as plenty of food material is still available* in the hanging drop, and, therefore, so long as the filaments are still measurable. At a later period, however, the overworked machinery results in the production of much feebler plants, capable of developing a few poor spores only, or even none at all, such as was observed in several cases where the cultures were allowed to go on. Here, then, we can understand why the direct measurements often give no decisive results: the actively growing filaments can only be measured during a short period, but the *cumulative effect of the malnutrition is not evinced by diminished growth until after the measurements have ceased*, or at least till towards the end of the period.*

6. But if the latter hypothesis is accepted, we have to recognise

* This is decidedly against action on the food materials, since one would expect the effect to make itself evident very early in the growth as the injurious bodies reach the sensitive cells.

that the fundamental physiological function affected by the light is *not growth*, but *nutrition*—constructive metabolism; and this would coincide very well with what Elfving found to be the case in macroscopic cultures of fungi.

7. However, the possibilities are not exhausted by the above. There are several points which suggest that the destructive light action may take place *on the enzymes* which the living cell forms and excretes. If Green is right in concluding that the more refrangible rays* are destructive to the action of enzymes outside the cell, it may be that we have here the key to the mystery, and that the cells gradually die of inanition from inability to render their food materials assimilable. Still the experiments throw no light on whether such action takes place outside or inside the cell,† though, perhaps, the results with spores support the latter idea rather than the former one.

8. Whatever the light action consists in, it is evidently exerted by the more refrangible rays, and is the more pronounced the more intense the light, or with feeble lights the greater the proportion of these rays there are in it.

9. The evidence goes to show also, that the rays at the other end of the spectrum, and especially the heat rays in the red and below, co-operate in the light action in question (or, possibly, sometimes antagonise it) in various ways. The probability is that with a given moderate intensity of light, such as occurs in ordinary daylight, the damaging effect of the blue-violet rays on growing cells is dependent on the temperature. If the temperature is about the optimum, the protoplasm, working at its best, seems able to resist—perhaps even undo—the damage; if the temperature is far removed from the optimum, however, the injurious action of the light rays is cumulative, and results in more or less rapid retardation of growth, and eventual death. This is, no doubt, true, whether the protoplasm fails to combat the injury because too *passive*, as at low temperatures, or because over-stimulated and too *active*, as at high temperatures. Taking into account all we know, however, it seems improbable that the organism can resist the bactericidal action of the more refrangible rays *at any temperature*, if those rays are relatively abundant in an intense light.

* Green—"The Influence of Light on Diastase" ('Annals of Botany,' 1894, pp. 370—373).

† It is not inconceivable that when the enzymes begin their work in the food material, the products of their action are easily oxidised. Indeed some experiments point to the probability of this, and suggest that broth with enzymes in it is susceptible to some destruction.

Experiments with the Light of the Electric Arc, and others.

In order to test the validity of previous conclusions as to the essential similarity of action of the electric and solar light, I started the following experiments. In spite of the kindness of my colleagues, Professor Stocker and Mr. Shields, in lending me the apparatus and use of a dark room, and the assistance of Mr. West, who took a lot of trouble in helping me, I had to abandon these experiments, partly because the lamp was not sufficiently powerful, and partly owing to the close attention and long periods of watching they require, and also because of other difficulties. I carried them sufficiently far, however, to show that the matter is worth further attention, and append the results in the hope that someone will take up this line of investigation.

On July 13 I tried the effect of exposing spores, in weak-gelatine, over the water and the bichromate screens, to the electric arc-light, reflected up from silvered mirrors.

The lamp used was a small, old-fashioned Dubosq, and the microscopes, with shades, match-box screens, quartz, and controls, &c., arranged exactly as before, were 3 ft. from the arc. The current used was equal to 8 ampères, and was taken from 32 storage cells. No reflectors or lenses were used, and much light was lost.

The spores were sown at 3 P.M., and the exposures began at 3.15.

The temperatures remained the same, or varied similarly, in both cultures, and during the whole period of exposure—3 hours—only rose from 18° C. to 19·25° C.

On stopping the experiment at 6.15 P.M., the cultures were at once placed in the dark, side by side, at 18° C. The temperature slowly fell to 16° C. at 10.45, when no results were observed.

At 7 A.M., on the 14th July, the bichromate culture showed rodlets and filaments 250 μ long and upwards; but the culture over water did not show a single germinated spore.

I was not entirely satisfied with this result, however, because the gelatine drop had run a little, owing to condensation of water, and therefore regarded the result as negative.

On July 14 this experiment was repeated exactly, the spores being sown at 9 A.M. and exposed at 9.15 A.M. The spectra of the screens were carefully examined, and everything arranged as exactly as possible.

The temperatures were as follows:—

Time.	Bichromate. °C.	Water. °C.
9.15 A.M.	17·5	17·5
9.55 „	18·0	18·0
10.40 „	18·5	18·5
11.30* „	19·0	19·0
11.55 „	19·0	19·0
12.20 P.M.	19·0	19·0

At 12.20 the cultures were put into the dark incubator at 22° C., and examined occasionally during the afternoon, with the following results.

At 3 P.M. there were several germinating rodlets in the bichromate culture, measuring from 4·5 μ to 15·75 μ; but none could be found in the water-culture more than 2 μ long—swelling spores, in fact.

At 4.30 P.M., however, no differences of importance between the two cultures could be detected. I measured 15 rodlets—all that could be found—in the bichromate culture, and these ranged from 5 μ to 64 μ; while fourteen measurements in the water culture gave from 4·5 μ to 54 μ, a difference that could not be insisted upon.

It must be concluded from this that the above exposure (3 hours) has no marked effect on the spores. It is, perhaps, worth mention that a reflection of the western sun, on a hazy day (July 13) thrown from a plane mirror on to the disc of light given by the lamp at about 10 ft. was much brighter than the disc.

After the foregoing, it need hardly be said that 1½ hours' exposure —experiment from 1.35 to 3.5 P.M. on July 14—gave no results.

On July 16, with the same arc, but at 2 ft. distance, I exposed two cultures, arranged exactly as before, for *six* hours—viz., from 9.35 A.M. to 3.45 P.M.

The temperature slowly rose from 16·75 (bichromate) and 17·25 (water) at 9.35 to 18 in both at 9.50; to 20° C. at 12.50, and finally to 21 at 3 P.M., but never passed beyond that.

At 3.50 the cultures were placed in the dark incubator at 22° C. Neither showed any signs of growth up to 10.30 P.M., but at 12 noon, on the 17th July, the culture over water showed one weak tuft of filaments, the longest being 140 to 180 μ long; those in the bichromate culture were 675 to 800 μ long.

This indicates a feeble light action, no doubt; but it is remarkable that the bichromate culture had not made more progress in the time!

On July 26, with exactly similar arrangements, two broth drop-cultures were exposed, from 9.30 A.M. to 3.30 P.M., a full six hours. The temperature rose from 19 at 9.30, to 21·5 at noon, and to 22 at 1 P.M., and so to the end.

* Five minutes were occupied in replacing the carbons.

Before removing to the incubator, at 24° C., I examined both carefully. No trace of a rodlet could be found in the water culture, but 30 or 40 filaments from 200 to 300 and even 450 μ were abounding in that over bichromate.

At 7 P.M., re-examination showed immeasurably long filaments in the bichromate culture, whereas only three filaments 58, 96, and 81 μ long respectively could be found in the water culture.

I think this must be regarded as proof that light-action occurs under the above conditions of exposure; and there can be little doubt that with a more powerful lamp—perhaps aided by quartz condensers, &c.—effects more nearly approaching those with sunlight could be obtained.

Antagonistic Action of a Swarming Bacillus.

The following experiment illustrates very clearly the effect of introducing a rapidly growing, strongly aerobic bacillus to compete for oxygen, &c., with the schizomycete in question.

I selected a well grown culture of *B. ramosus*, and introduced into the broth-drop a few rodlets of perhaps the most rapid growing liquefying bacillus yet isolated from the Thames.

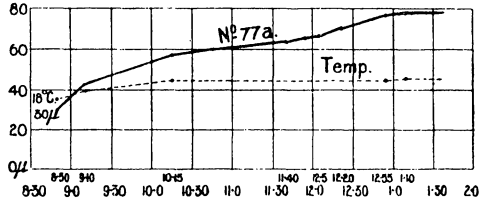
This form belongs to the group commonly known as *B. termo*, and its peculiarities will be described in due course. I call it, for the present, form β. It is a short, stout rodlet, about 1·5 to 2 $\mu \times 1\,\mu$, actively motile, a very rapid grower, and exceedingly greedy of oxygen.

At 8.50 A.M., a portion of the *B. ramosus*, around which the bacillus β was rapidly swarming in considerable numbers, was put into measurements, with the following suggestive results :—

Time.	Length.	Growth.	Interval.	Rate.	Temp. (air).
	μ.	μ.	mins.	μ.	° C.
8.50 A.M.	30	—	—	—	18·0
9.10 „	42	12	20	0·6	20·0
10.15 „	58	16	65	0·25	22·0
11.40 „	64	6	25	0·24	22·0
11.55 „	66	2	15	0·13	22·0
12.5 P.M.	67	1	10	0·10	22·0
12.20 „	70	3	15	0·20	22·0
12.55 „	77	7	25	0·3	22·0
1.10 „	78	1	15	0·07	22·5
1.20 „	78	0	10	0·0	22·5

Curve 77A.

It doubled its length in 115 minutes at (air-temp.) 18—22° C.



The growth had entirely ceased, as I convinced myself by nursing the culture in the incubator for the rest of the day.

Whether this was due entirely to the loss of oxygen brought about by the form β, which showed beautiful chemotactic aggregations about the filaments, by the bye—or to other causes brought about by the action of the intruder, cannot be with certainty determined, but it is obviously a line of enquiry worth further pursuit. In any case the shape of the curve is entirely different from the usual one, and fully supports the conclusion that the intruding bacterium was robbing the filaments of oxygen.

The observation is of considerable interest as touching the questions of antagonism and chemotaxis, and it is probable that the subject would well repay investigation. I had to refrain from pursuing the matter further, however, and merely give this case—not the only one of the kind that has come under my notice—owing to the necessity of following the special line of research I was engaged in.

Effect of probable Poisonous Action.

The following is quoted as probably a case of the poisonous action of some product of combustion of the over-heated cotton-wool in the arms of the cell. It is not *certain* that the action was what I suppose, but it is suggestive that all the filaments in the drops were suffering, whence may be safely inferred that some common action was going on.

On November 11, spores were sown exactly as before, in normal gelatine, at 10.45, at 22° C. : at 4 P.M. the measurements were started at 25° C.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
4.0 P.M.	18·0	—	—	—	25·0
4.15 „	24·75	15	6·75	0·44	25·0
4.30 „	31·5	15	6·75	0·44	25·0
4.45 „	36·0	15	4·5	0·3	25·0
5.0 „	42·75	15	6·75	0·44	25·1
5.11 „	49·5	11	6·75	0·61	25·25
5.22 „	51·75	11	2·25	0·2	25·3
5.30 „	55·0	8	3·25	0·4	25·3
5.40 „	Filament = pathological contraction and abnormal behaviour—see below.				25·4
5.45 „					25·5
5.50 „					25·5
6.5 „	65·25	35	10·25	0·3	25·3
6.16 „	72·0	11	6·75	0·61	25·3

Stopped, because evidently abnormal. Doubling periods :—

18—36 μ = 4.0 P.M.—4.45 P.M. = 45 minutes at 25° C.
36—72 „ = 4.45 „ —6.16 „ = 91 „ 25—25·5—25·3° C.

Normal doubling period* :—

31·5—63 μ = 4.30 P.M.—5.59 P.M. = 89 minutes at 25—25·5—25·3° C.

A very curious phenomenon occurred here. At 5.40 the filament had ceased growing, and apparently measured only 49·5 μ—whereas it was 55 μ ten minutes previously. More careful observation showed a length of *empty sheath* 9 μ long in addition, so that it was really 58·5 μ long, but the solid part had *contracted* itself. The cause of this is not evident, and as the table shows, the temperature, &c., could not have been responsible; but the phenomenon is apparently of the same order as the abnormality described on p. 356 by exposure to intense insolation. At 5.45 the solid part was 54 μ long, and at 5.50 it was 58·5 μ. By 6.5 the empty portion had partly filled up again by a block separated by a piece of empty sheath (about 1 μ long) from the main mass; by 6.15 the abnormality seemed quite repaired.

I have no idea what could have induced this malformation: there is an interesting question involving the maladies of a schizomycete here, but I could only note it in passing.

Its bearing on my present purpose is evident. The disturbance caused the filament to take ninety-one minutes to double its length against forty-five in the previous period; evidently one cause of this would be the loss of cell-divisions and their summation.

* See p. 431 for explanation of the normal doubling period—it is obtained by dating the commencement of the measurements half an hour after starting the growth, to allow the cell time to accommodate itself to the temperature.

There was no reason for concluding that the main mass of the filament grew any slower, but it may have been so.

It scarcely seemed worth while to pursue this, so another filament was taken in the same culture.

The very slow growth, although at and near 25° C., was confirmed by examining another culture, started at the same time at 22° C., and put into Sachs' box at 6.30 P.M.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	°C.
6.43 P.M.	58·5	—	—	—	24·25
6.52 „	65·25	9	6·75	0·7	26·25
7.4 „	69·75	12	4·5	0·4	26·1
7.19 „	74·25	15	4·5	0·3	25·75

Here the curve was actually flattening more and more as time went on.

Another filament was then taken.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	°C.
8.17 A.M.	56·25	—	—	—	24·75
8.37 „	63·0	20	6·75	0·3	25·0
9.11 „	76·5	34	13·5	0·4	25·0
9.24 „	81·0	13	4·5	0·3	25·0

The growth was so slow that I abandoned the measurements. I cannot explain it, unless the following suggestion is to the point. The cotton-wool plugging the arms had been slightly scorched during sterilisation: my practice is not to heat up to browning point, but in this case that had occurred. Can it be that traces of pyridine-like or other bodies get into such a cell, and cause slight antiseptic inhibition? It would probably be worth somebody's while to make a series of experiments with such cultures to this end, with various volatile bodies added in known doses in the arms of the cells.

Experiments on the Effects of Temperature only.

Various results in the foregoing account of my experiments suggested that it would be well to obtain more accurate information as to the effect of temperature alone on the growth curves. Of course a good deal of work has been done on the influence of temperature

generally, on the growth of bacteria, so far as their *macroscopic* characters are concerned, and a few observers—notably Brefeld with *B. subtilis*—have made observations on the rate of cell-division at one or two principal air-temperatures; but, so far as I know, no one has attempted to measure quantitatively and in detail the effect of small differences of temperature over a long range, and that temperature *that of the culture*—which we have seen *may* be different from that of the air—and especially to obtain plotted out curves of growth under such conditions.

It is obvious that my methods admit of this being done, and I thought it might be not only productive of useful information as to the primary point concerned—the definite effect of given temperatures on the growth—but that I might possibly be able to make use of these *normal* temperature-curves indirectly, by comparing them with the foregoing.

I accordingly started what proved to be a very long and laborious series of cultures under a microscope enclosed in an incubator of an improved form modelled on the original type described some years ago by Sachs. The chief difference is that the heating arrangement is a thick, flanged, blackened, iron plate, on which stands a thick sheet of asbestos-board, and on this the microscope. The whole body of the microscope, except the eye-piece and micrometer screw, is enclosed in a wooden box, of which the iron plate is the floor; this box has a glass window in front and two lateral openings (with shutters) for manipulating the culture, and can easily be opened entirely if necessary.

A culture being placed in position, and the microscope focussed on a selected rodlet or filament, the shutters are all closed and the growth goes on in the dark.

The temperature is registered by a thermometer whose bulb is inside and reading column projecting through the roof: the gas passes through a very delicate thermo-regulator, containing mercury and ether, and when once the apparatus (which I shall henceforth term Sachs' box) is heated up, it can easily be kept at a temperature so constant that it does not alter a degree in several hours, while, with a little more care (and provided no large alterations of temperature are going on in the room), it will remain for hours within 0·25° C. of the temperature arranged for.

Of course, the temperature falls or rises quickly when the box is opened—unless it is that of the room—but it returns in five to ten minutes if the opening and shutting are completed in a minute or so; the opening and shutting of the side windows, and the removal and replacement of the shutter during observations, produce very slight slowly acting effects, which, however, cannot be neglected, as we shall see.

The ideal is practically realised in the case of a culture which, once in position, remains without the necessity of any opening at all, and this can frequently be carried out. In order to meet obvious objections, however, I have in all cases made several cultures at each temperature, and to make the results strictly comparable, I adopted the following method of recording.

1. The table of growths was prepared, giving the time of observation, temperature, and length of filament, &c., as in preceding cases.

2. Then the curve of growth was plotted out on sectional paper, care being taken that the squares, &c., were all equal, and that the intervals between the observations were sufficiently short and numerous to give good curves. Since the measurements were all made with the same micrometer-scale and the same microscope, and taking into account the proofs of accurate measurement already given, no more need be said on that head.

3. From these curves and tables I then measured the period occupied by a rodlet or filament of any given length (to start with) in growing to *double its length*, and call this the *doubling period.*

4. The average *doubling period* for any temperature is then obtained in the usual way, by taking the sum of the times and dividing by the number of observations. Before saying more, however, it will be best to examine the actual results, which now follow.

I may add that I had already satisfied myself that the curves obtained at widely different temperatures, are markedly different, and less and less divergent as the temperatures of growth approach one another, facts which are in accordance with experience with other organisms, and which will be evident enough as we proceed.

In what follows I select a number of representative curves from larger series made to familiarise myself with the details; and for the sake of classification—and with reference to some conclusions later—I present them arranged according to the food-materials employed. The media chiefly used were four, viz.: (1) normal beef-broth; (2) the same with 1 per cent. of gelatine added to give a certain degree of stiffness; (3) a stiff 10 per cent. gelatine with mere traces of broth (referred to as weak gelatine); and (4) normal 10 per cent. broth-peptone gelatine.

For various reasons I shall start with the cultures in weak gelatine.

Cultures in Weak Gelatine.

On July 19 spores were sown in the stiff weak gelatine used throughout July hitherto, and put into Sachs' box at 7 P.M. at 17° C.

The temperatures ran as follows:—

Time.	Temp. ° C.
7.0 P.M.	= 17·0
8.30 „	= 16·5
8.30 A.M.	= 16·5 (germination)
11.15 „	= 17·25
11.50 „	= 17·5
12.20 „	= 18·5

And now it was possible to begin the measurements, which ran as follows:—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
12.30 P.M.	9·0	—	—	—	18·5
1.45 „	13·5	85	4·5	0·05	18·5
2.45 „	18·0	60	4·5	0·07	18·0
3.30 „	23·75	45	5·75	0·12	18·0
4.0 „	27·0	30	3·25	0·11	18·0
4.30 „	31·5	30	4·5	0·15	18·0
5.0 „	38·25	30	6·75	0·22	17·9
5.30 „	45·0	30	6·75	0·22	17·75
6.0 „	54·0	30	9·0	0·3	17·8
6.30 „	65·25	30	11·25	0·37	17·8
6.50 „	72·0	20	6·75	0·34	17·9
7.30 „	88·0	40	16·0	0·4	18·0
8.45 „	117·0	85	29·0	0·34	17·25
9.5 „	130·5	20	13·5	0·67	17·25
9.20 „	135·0	15	4·5	0·3	17·4
9.40 „	139·5	20	4·5	0·22	18·0
8.45 A.M.	1100·0	—	—	—	18·0

Reckoned back:—

139·5 —69·75 μ = 6.43 P.M.—9.40 P.M. = 177 mins. at 17·8—18·0—17·25—18·0°C.
69·75—34·75 „ = 4.44 „ —6.43 „ = 119 „ 18·0—17·8° C.
34·75—17·5 „ = 2.40 „ —4.44 „ = 124 „ 18·0—° C.

The doubling periods were as follows:—

1. 9— 18 μ = 135 minutes at 18·5—18° C.
2. 18— 36 „ = 120 „ 18·0—17·9° C.
3. 36— 72 „ = 125 „ 18·0—17·75—17·9° C.
4. 72—144 „ = 170 (approx.) at 17·9—18—17·4—18° C.

partly calculated.

Normal doubling period:—

10—20 μ = 12.50 P.M.—3.4 P.M. = 134 minutes at 18.5—18° C.

From observation next morning, and what was seen during the slowing after 7.30, there can be little doubt the inhibition was partly

due to the increasing stiffness of the gelatine at the low temperatures, and to the difficulty the feeble growth had in peptonising so weak and stiff a medium, and partly to the lack of oxygen consequent on congelation.

On July 17 spores were sown as before, and at once put at 23° in the Sachs' box: this was 8.30 A.M. The temperatures during the morning, &c., ran as follows:—

Time.	Temp. ° C.	
8.30 A.M.	= 23·0	
9.10 „	= 23·75	
9.30 „	= 23·25	
10.5 „	= 23·2	
11.50 „	= 23·25	
12.20 P.M.	= 23·25	
12.40 „	= 23·5	
1.30 „	= 24·0	
1.35 „	= 22·8	(owing to my opening the box)
1.40 „	= 22·75	
1.55 „	= 23·0	
2.10 „	= 24·0	
3.15 „	= 24·0	
3.16 „	= 23·0	(opened box)
4.20 „	= 23·5	
5.5 „	= 23·0	
5.12 „	= 22·0	(opened side door)

I now began the measurements, and continued them till 8.15 P.M. In all these cases where only the doubling periods are given, it must be understood that the table of growths was prepared in detail, and the curve plotted. The tables and curves are too numerous to give in full.

The doubling periods were as follows:—

1. 31·5— 63 μ in 56 minutes at 23·1—22·7° C.
2. 63·0—126 „ „ 53 „ 22·7—24·25° C.

Normal doubling period:—

50·5—101 μ = 6 P.M.—6.51 P.M. = 51 minutes at 23—22·7—23·1° C.

On July 11 I arranged for a culture at 25° C., approximately constant temperature, in Sachs' chamber. Spores were sown at 9.30 in weak gelatine,* and at once put in, though the chamber was not

* Weak gelatine here and throughout = a stiff gelatine (10 per cent.) with only 0·5 per cent. of broth.

as yet low enough in temperature. Germination began about noon, the temperatures meanwhile falling as follows :—

Time.	Temp. ° C.	
9.30 A.M.	= 28·5	
9.45 „	= 29·0	
10.10 „	= 26·25	
10.20 „	= 25·5	
10.30 „	= 25·25	
10.45 „	= 25·0	
11.5 „	= 25·0	(fell to 24·25° on opening to arrange culture)
11.15 „	= 25·0	
11.45 „	= 25·0	
12 noon	= 25·0	
12.15 P.M.	= 25·0	
12.30 „	= 25·0	
1.30 „	= 25·3	
1.35 „	= 25·0	(at 1.55 had to open box, and then fell to 22·5°. At 1.57 = 23°, 2 P.M. = 24°, 2.15 = 24·5°)
2.30 „	= 25·0	

At 2.45 I was able to start the measurements on a vigorous rodlet then selected.

The Sachs' chamber was kept dark by a tinfoil curtain, and the following table shows how constant the temperatures were :—

I had now to abandon this, owing to its breaking up and being too difficult to measure.

Another filament, part of this longer one, was therefore selected, with results given in the following table and curve :—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
2.45 P.M.	18·0	—	—	—	24·5
3.15 „	35·0	30	17·0	0·57	24·5
3.30 „	42·75	15	9·75	0·65	24·75
3.45 „	54·0	15	11·25	0·75	25·0
4.0 „	67·5	15	13·5	0·9	25·5
4.15 „	83·25	15	15·75	1·05	25·5
4.30 „	105·75	15	22·5	1·5	25·0
4.40 „	121·5	10	15·75	1·57	25·0
4.50 „	139·5	10	18·0	1·8	25·0
5.0 „	159·75	10	20·25	2·0	25·0
5.10 „	184·5	10	24·75	2·47	25·25

On analysing the results, the filament doubled its length, as follows :—

1. 18— 36 μ in 32 minutes at 24·5° C.
2. 36— 72 „ „ 47 „ 24·75—25·5° C.
3. 72—144 „ „ 43 „ 25·5—25° C.

Reckoned back :—

184·5 —92·25 μ = 4.21 P.M.—5.10 P.M. = 49 minutes at 25·5—25—25·25° C.
92·25—46·0 „ = 3.35 „ —4.21 „ = 41 „ 24·75—25·5—25° C.
46·0 —23·0 „ = 2.53 „ —3.25 „ = 32 „ 24·5—25° C.

Normal doubling periods :—

35— 70 μ = 3.15 P.M.—4.3 P.M. = 48 minutes at 24·5—25·5° C.
70—140 „ = 4.3 „ —4.50 „ = 47 „ 25·5—25° C.

The following are the results of the second series of measurements; the slight variations in the temperature (not to be overlooked) were due to the necessity of rearranging the culture, and therefore opening the side windows.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	°C.
5.24 P.M.	81·0	—	—	—	25·0
5.30 „	87·75	6	6·75	1·12	24·75
5.40 „	99·0	10	11·25	1·12	24·75
5.50 „	114·75	10	15·75	1·57	25·0
6.0 „	126·0	10	11·25	1·12	25·25
6.10 „	144·0	10	18·0	1·8	25·0
6.20 „	157·5	10	13·5	1·35	25·25
6.30 „	173·25	10	15·75	1·57	24·75
6.40 „	198·0	10	24·75	2·47	25·0
6.50 „	211·5	10	13·5	1·35	25·0
7.0 „	231·75	10	20·25	2·02	25·2
7.10 „	252·0	10	20·25	2·02	25·0

Calculated back :—

252—126 μ = 6 P.M.—7.10 P.M. = 70 minutes at 25·25—24·75—25·2—25° C.

The doubling, from 81 μ to 162 μ, took 59 minutes at temperatures varying between 24·75° and 25·25° C., and, by the shape of the curve, the rate would probably not be far different for the next doubling period.

This specimen, which had grown vigorously during the night of the 11th, was kept at the same temperature, 24·5° to 25·5°, all through the 12th, in the dark. It was necessary to open the chamber at 9.30 A.M. on the 12th, and the temperature fell to 22·5° for a few minutes, but it had recovered before 10 A.M.

At 9 P.M. it had completed the formation of magnificent series of spores, *i.e.*, in less than 36 hours from sowing, the sporification was complete.

On July 15 I repeated the observations in the Sachs' box, the sowing being made—weak gelatine—and put in at 12 noon.

The temperatures of the box ran as follows, before germination :—

Time.	Temp. ° C.
10.0 A.M.	= 23·5
10.30 ,,	= 24·25
11.0 ,,	= 24·75
11.30 ,,	= 25·2
12 noon	= 25·25
12.15 P.M.	= 24·75
12.30 ,,	= 25·25
3.15 ,,	= 26·75
4.0 ,,	= 26·75
5.15 ,,	= 26·75
7.40 ,,	= 27·75

The measurements began at 7.45, and continued till 10.10 P.M., with the following results :—

Doubled its length as follows :—

1. 36— 72 μ in 50 minutes at 28—27·25° C.
2. 72—144 ,, ,, 58 ,, 27·25—25·5° C.

And at its present rate would have again doubled :—

3. 144—288 μ in 54 minutes at 25·5° C.

Normal doubling periods :—

55—110 μ = 8.15 P.M.—9.10 P.M. = 55 minutes at 27·5—26° C.
110—220 ,, = 9.10 ,, —10.6 ,, = 56 ,, 26·0—25·5° C.

At 9 P.M. on the 16th, the culture having been at 25° to 27·5° during that day (and up to 30·5° between 6.20 and 8.30 P.M.), many of the filaments had already formed their spores, *i.e.*, spore-formation had definitely set in in 33 hours. At 8.30 next morning there were fully formed spores in all filaments, large, and very brilliant.

It is clear that at temperatures near 25—27° C. spores may be developed in less than 48 hours.

This experiment was repeated on the 16th July. The spores, exactly as before, were started at 9.45° C., the temperatures running as follows :—

Time.		Temp. ° C.
9.45	A.M.	= 24·75
9.50	,,	= 25.5
10.15	,,	= 25·6
10.55	,,	= 25·9
11.25	,,	= 26·25
11.40	,,	= 26·4
12.15	P.M.	= 26·5
1.30	,,	= 26·75
2.10	,,	= 27·0

Meanwhile, germination had occurred, and the measurements were now begun on a germinal rodlet 9 μ long, and still with its end in the spore.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
2.10 P.M.	9·0	—	—	—	27·0
2.55 ,,	18·0	45	9·0	0·2	26·75
3.20 ,,	27·0	25	9·0	0·36	26·5
3.40 ,,	36·0	20	9·0	0·45	26·6
4.0 ,,	47·25	20	11·25	0·56	26·9
4.20 ,,	65·25	20	18·0	0·9	27·1
4.30 ,,	77·5	10	12·25	1·2	27·2
4.40 ,,	90·0	10	12·5	1·25	27·25
4.50 ,,	105·75	10	15·75	1·57	27·4
5.0 ,,	121·5	10	15·75	1·57	27·5
5.10 ,,	137·25	10	15·75	1·57	27·5
5.20 ,,	157·5	10	20·25	2·0	27·6
*5.30 ,,	186·75	10	29·25	2·9	27·7
5.40 ,,	204·75	10	18·0	1·8	27·5
5.50 ,,	225·0	10	20·25	2·0	27·4
6.0 ,,	254·25	10	29·25	2·9	27·5
6.10 ,,	279·0	10	24·75	2·47	27·6
6.20 ,,	315·0	10	36·0	3·6	27·75
8.30 ,,	Immeasurable		—	—	30·5

Curve 83, p. 402.

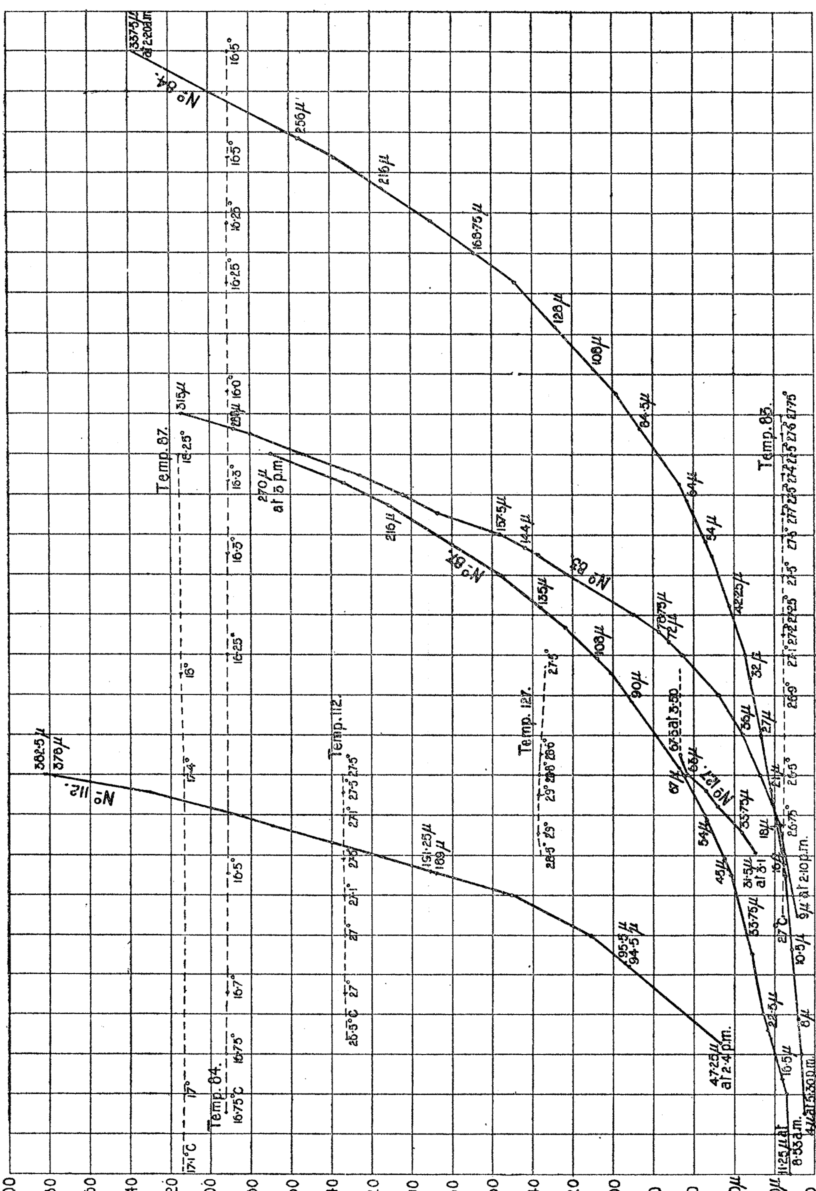
The doubling occurred as follows:—

1. 9— 18 μ = 2.10 P.M.—2.55 P.M. = 45 minutes at 27—26·75° C.
2. 18— 36 ,, = 2.55 ,, —3.40 ,, = 45 ,, 26·75—26·6° C.
3. 36— 72 ,, = 3.40 ,, —4.26 ,, = 46 ,, 26·6—27·1° C.
4. 72—144 ,, = 4.26 ,, —5.14 ,, = 48 ,, 27·1—27·6° C.
5. 144—288 ,, = 5.14 ,, —6.12 ,, = 58 ,, 27·6—27·4—27·75° C.

Reckoned back:—

315—157·5 μ = 5.20 P.M.—6.20 P.M. = 60 minutes at 27·6—27·7—27·4—27·75° C.
157·5—78·75 ,, = 4.31 ,, —5.20 ,, = 49 ,, 27·2—27·6° C.
78·75—39·5 ,, = 3.47 ,, —4.31 ,, = 44 ,, 26·75—27·2° C.
39·5—19·75 ,, = 3.0 ,, —3.47 ,, = 47 ,, 26·75—26·6—26·8° C.
19·75—9·75 ,, = 2.15 ,, —3.0 ,, = 45 ,, 27—26·8° C.

* Had to re-arrange, and open finger windows.



Normal doubling period:—

36—72 μ = 3.40 P.M.—4.26 P.M. = 46 minutes at 26·6—27·1° C.

Doubling Periods.

This is a good opportunity for explaining more in detail what is meant by the above term.

If this long and symmetrical curve 83 is carefully examined, it will be found that, over a great part of its course, *any chosen short length* of the filament *doubles* its length in approximately equal times.

Thus it took forty-five minutes to grow from 9 μ to 18 μ, and the same from 18 μ to 36 μ, and so on. But it is practically the same whatever the length we select, *provided the conditions are constant*, as will be seen by comparing the curves.

I term the period required for doubling the length the "*doubling period*," and the comparison of a large number of curves shows that the nearer we approximate to absolute constancy of the conditions the clearer the truth of the following statement, which amounts to a law, is evident.

Whatever the length of the filament taken, that length is doubled in equal times when the conditions are constant.

For the present it is only necessary to clearly apprehend the meaning of the above term, and the general truth enunciated, so that the reader can understand the figures attached to the tables. Further particulars will come out as we proceed.

The following experiment at 30° was carried out on July 18. The spores, in stiff weak gelatine, were sown and put in the Sachs' box at 9.30 A.M.

The temperatures ran as follows:—

Time.	Temp. °C.	
9.30 A.M.	= 30·5	
10.0 „	= 31·5	
10.20 „	= 30·6	
10.30 „	= 30·2	
11.0 „	= 30·25	
11.20 „	= 30·4	
11.35 „	= 30·5	
12.0 noon	= 31·0	
12.15 P.M.	= 31·1	
12.30 „	= 30·5	
1.30 „	= 30·0	
1.45 „	= 24·75	(due to opening box to arrange)
1.47 „	= 27·0	„ „ „
1·51 „	= 29·0	„ „ „
2.0 „	= 30·0	
2.30 „	= 31·0	
3.15 „	= 31·0	

On now examining I found the germinated bacilli had grown into curiously contorted and stunted colonies, and there stopped. No further growth could be observed, although I slowly lowered the temperature as follows:—

Time.	Temp. ° C.
3.45 P.M.	= 29·5
4.25 „	= 25·0
4.35 „	= 24·75
5.0 „	= 25·4
5.20 „	= 25·75
6.15 „	= 24·75
7.0 „	= 24·0

A rodlet fixed on the scale and measured showed no departure at 7 P.M. from its original length, 9 μ, at 5 P.M. Growth was impossible, apparently, under these conditions.

I presume that what occurred was, germination was effected, but, at the temperature 30—31° C., the delicate young rodlets could not assimilate food-materials from this weak gelatine.

Later considerations, based on further experience, suggests that the variations of temperature may have combined to inhibit the growth also.

On reviewing these curves of growth at various temperatures, kept approximately constant in each case, in the weak gelatine, we find the following facts come out:—

1. As was to be expected, the doubling periods are longer at lower temperatures than at higher ones.

2. There are indications of a temperature near 25° C. being more favourable than any others, *i.e.*, an *optimum* temperature, as contrasted with less favourable lower temperatures nearer a *minimum*, or higher ones nearer a *maximum*.

3. Some facts point to the necessity for distinguishing the germinal phase of growth from the more independent growth which occurs after the rodlet is entirely free of the spore and its reserves.

4. The doubling period is evidently affected not only by the mean temperature employed, but by variations above and below this mean; and this in two ways: (1) according to the *rapidity* of these variations, and (2) according to their *range*.

It is hardly worth while making any more general statements until we have examined other cases, so I now pass to cultures in broth.

B. *Cultures in Broth.*

On July 23 a broth-culture of spores was started in the Sachs' box, darkened, at 1.30. The temperature was 17·2°, and remained steadily

at 17° C. for some time, slowly falling during the afternoon to 16·75° by 4.30. On then opening to search the drop, the temperature rose to 17·4° C., falling to 17° at 5 P.M., and 16·8 at 5.15. Germination had begun at 5 P.M. The measurements began at 5.30 as follows, and were carried through the night.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
5.30 P.M.	4·0	—	—	—	16·75
6.0 „	6·75	30	2·75	0·09	16·75
6.30 „	9·0	30	2·25	0·07	16·7
7.30 „	14.0	60	5·0	0·08	16·5
8.30 „	24·75	60	10·75	0·18	16·5
9.20 „	35·0	50	10·25	0·2	16·25
10.10 „	51·75	50	16·75	0·33	16·3
10.45 „	67·5	35	15·75	0·45	16·3
*11.32 „	99·0	47	31·5	0·67	16·0
12.25 A.M.	148·5	53	47·5	0·9	16·25
†12.57 „	191·25	32	42·75	1·33	16·25
1.27 „	238·5	30	47·25	1·57	16·5
2.20 „	337·5	53	99·0	—	16·5

Curve 84, p. 402.

The doubling periods were as follows :—

1. 4— 8 μ = 48 minutes at 16·75° C.
2. 8— 16 „ = 82 „ 16·75—16·5° C.
3. 16— 32 „ = 86 „ 16·5 —16·25° C.
4. 32— 64 „ = 92 „ 16·25—16·3° C.
5. 64—128 „ = 86 „ 16·3—16—16·25° C.
6. 128—256 „ = 92 „ 16·25—16·5° C.

Calculated back:—

337·5 —168·75 μ = 100 minutes.
168·75— 84·5 „ = 86 „
84·5 — 42·25 „ = 90 „
42·0 — 21·0 „ = 94 „
21·0 — 10·5 „ = 78 „
10·5 — 5·25 „ = 70 „

Normal doubling periods :—

6·75— 13·5 μ = 6.0 P.M.— 7.20 P.M. = 80 mins. at 16·75—16·5° C.
13·5 — 27·0 „ = 7.20 „ — 8.40 „ = 80 „ 16·5° C.
27·0 — 54·0 „ = 8.40 „ —10.17 „ = 97 „ 16·5 —16·25—16·3° C.
54·0 —108·0 „ = 10.17 „ —11.42 „ = 85 „ 16·3 —16·0° C.
108·0 —216·0 „ = 11.42 „ — 1.13 A.M. = 91 „ 16·0 —16·5° C.

* Rose at once to 17·1° C. on opening side windows, falling again to 16·5° C. in five minutes.

† Up to 16·75° C. on opening side window, down to 16·5° C. in five minutes.

As regards the discrepancy between the first doubling period and the rest, I have little doubt it is explicable with reference to the process of germination. It must be noted that the initial length (4 μ) is not that of a free rodlet working up its food-materials from the broth, but of rodlet *plus* spore, and presumably still utilising unexpended stores from the spore. To make the comparisons fair, therefore, we ought to neglect this first period.

On July 21 and 22 the following observations were started with cultures in fresh broth :—

Spores sown 8 P.M. were put at once into Sachs' box at 19·5° C., falling to 18·75° at 9.30 P.M.

On July 22 the growths, followed for 1 hour and 10 minutes, gave the doubling period :—

99—198 μ in 65 minutes at 17—16·75—17° C.

Or, calculating back :—

225—112·5 μ = 8.22 A.M.—9.10 A.M. = 48 minutes at 16·75—17° C.

Normal doubling period :—

112—224 μ = 8.22 A.M.—9.10 A.M. = 48 minutes at 16·75—17° C.

This culture formed spores by 9 A.M., July 25, having been at 16—19° throughout.

On July 22 a culture started in broth at 17° C. at 8 A.M., had germinated out about noon, and the measurements were made from 4.55 P.M. to 8.28 P.M. Sachs' box, &c., as before.

Here the doubling periods were as follows :—

1. 38·25—76·5 μ = 68 minutes at 19—18·2° C.
2. 76·5 —153 „ = 72 „ 18·2—18° C.
3. 153 —306 „ = 73 „ 18—18·2° C.

Normal doubling period :—

56·5—113 μ = 5.30 P.M.—6.41 P.M. = 71 minutes at 18·5—18° C.
113—226 „ = 6.41 „ —7.54 „ = 73 „ 18—° C.

This culture had formed spores at 9 A.M. on the 25th, having been at 16—19° in dark Sachs' box the whole time.

If we calculate at other points in the curve, we get doubling as follows :—

112·5—225 μ = 6.40 P.M.—7.54 P.M. = 74 minutes at 18° C.
50·5—101 „ = 5.20 „ —6.29 „ = 69 „ 18·5—18° C.

On July 24 I sowed spores in broth at 2.30 A.M., and put at once into the Sachs' box at 17° C. The temperature remained constant through the early morning, and measurements were made as follows :—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
8.53 AM.	11·25	—	—	—	17·1
9.40 „	15·75	47	4·50	0·09	17·0
10.20 „	24·75	40	9·0	0·22	16·9
10.50 „	31 5	30	6·75	0·22	17·0
11.30 „	41·5	40	10·0	0·25	17·0
12 noon	55·0	30	13·5	0·45	17·1
12.20 P.M.	65·0	20	10·0	0·5	17·4
1.12 „	101·25	52	36·25	0·7	18·0
1.34 „	123·75	22	22·5	1·0	18·0
2.0 „	156·0	26	32·25	1·24	18·0
2.34 „	211·5	34	55·5	1·6	18·1
2.45 „	234·0	11	22·5	2·0	18·2
3.0 „	270·0	15	36·0	2·4	18·25

Curve 87, p. 402.

The doubling periods are here as follows:—

1. 11·25—22·5 μ = 79 minutes at 17·1—16·9° C.
2. 22·5 —45 „ = 86 „ 16·9—17° C.
3. 45 —90 „ = 79 „ 17 —18° C.
4. 90 —180 „ = 78 „ 18° C.

Calculated back:—

270—135 μ = 1.42 P.M.— 3.0 P.M. = 78 minutes at 18—18·25° C.
135—67·5 „ = 12.22 „ — 1.42 „ = 80 „ 17·4—18° C.
67·5—33·75 „ = 11.0 A.M.—12.22 P.M. = 82 „ 17—17·4° C.
33·75—16·5 „ = 9.46 „ —11.0 A.M. = 74 „ 17—16·9—17° C.

Normal doubling periods:—

13·5— 27 μ = 9.20 A.M.—10.30 A.M. = 70 minutes at 17—16·9° C.
27— 54 „ = 10.30 „ —11.58 „ = 88 „ 16·9—17° C.
54—108 „ = 11.58 „ — 1.19 P.M. = 79 „ 17—18° C.
108—216 „ = 1.19 P.M.— 2.37 „ = 78 „ 18—18·2° C.

In the same culture I selected another filament at 4.45 P.M., July 24, and measured from 4.46 P.M. to 6.24 P.M.

The doubling periods were here as follows:—

85·5—171·0 μ = 77 minutes at 18·75—19° C.

Calculated back:—

220·5—110·25 μ = 5.17 P.M.—6.24 P.M. = 67 minutes at 18·75—19·5° C.

Spores were beginning to form in some filaments at 8.0 P.M. on July 26, but, even on 28th, few, sparse, and small spores, much poorer than those of 25th July.

On July 25 spores in broth were sown at 9.30 A.M., and at once put into Sachs' box, dark, at 19° C. The temperature rose slowly to 19.5° at 10.15, and to 20·25° at 2 P.M.; thence it remained at 20·25—20·75, a rodlet being selected at 3.35 P.M. and kept under observation till 6.20 P.M.

Doubling periods:—

1. 20·25— 40·5 μ in 49 minutes at 20·75—20·25° C.
2. 40·5 — 81 „ „ 56 „ 20·25—20·6° C.
3. 81 —162 „ „ 63 „ 20·6—20·5° C.

Calculated back:—

157 —78·5 μ = 5.18 P.M.—6.20 P.M. = 62 minutes at 20·6—20·5° C.
78·5—39·5 „ = 4.22 „ —5.18 „ = 56 „ 20·25—20·6° C.

Normal doubling periods:—

33·75— 57·5 μ = 4.0 P.M.—5.6 P.M. = 66 minutes at 20.5—20·25—20·6° C.
67·5 —135·0 „ = 5.6 „ —6.6 „ = 60 „ 20·6—20·5° C.

This 25th July culture had formed splendid spores by 10.45 P.M. on the 27th, whence the whole life cycle took about 60 hours at 20—23° C., having been in the dark under a bell-jar since 26th, 9 A.M.

On August 4 a spore culture in broth was started at 7 A.M. at 21° C., rising very slowly, the variations were:—

Time.	Temp. ° C.
7.0 A.M.	= 21·0
9.15 „	= 22·25
9.30 „	= 22·5
9.45 „	= 22·5
9.55 „	= 23·2
10·30 „	= 23·5
10.55 „	= 23·7
11.22 „	= 23·7

Germination began about 9.15, as I saw a spore swollen and elongating then; at 9.45 several were seen from 4 μ to 6 μ long, and at 10.30 one was selected for measurement 10 μ long, as follows:—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
10.30 A.M.	10·0	—	—	—	23·5
10.55 „	20·25	25	10·25	0·4	23·7
11.25 „	31·5	30	11·25	0·4	23·7
11.42 „	40·5	17	9·0	0·5	23·7
12.0 noon	56·25	18	15·75	0·9	23·75
12.20 P.M.	76·5	20	20·25	1·0	24·0
12.40 „	105·75	20	29·25	1·45	24·5
12.50 „	126·0	10	20·25	2·0	24·6
1.0 „	153·0	10	27·0	2·7	24·75
1.25 „	229·5	25	76·5	3·0	25·0
2.25 „	652·5	60	423·0	7·0	25·0

Doubling periods:—

1. 10—20 μ = 10.30 A.M.—10.54 A.M. = 24 minutes at 23·5—23·7° C.
2. 20—40 „ = 10.54 „ —11.41 „ = 47 „ 23·7° C.
3. 40—80 „ = 11.41 „ —12.22 P.M. = 41 „ 23·7—24° C.
4. 80—160 „ = 12.22 P.M.—1.3 „ = 41 „ 24—24·75° C.
5. 160—320 „ = 1.3 „ —1.40 „ = 37 „ 24·75—25° C.
6. 320—640 „ = 1.40 „ —2.23 „ = 43 „ 25° C.

Calculated back:—

459—229·5 μ = 1.25 P.M.—2.5 P.M. = 40 minutes at 25° C.
229·5—114·75 „ = 12.45 „ —1.25 „ = 40 „ 24·5—25° C.
114·75—57·5 „ = 11.59 A.M.—12.45 „ = 46 „ 23·75—24·5° C.
57·5—288·75 „ = 11.19 „ —11.59 A.M. = 40 „ 23·7—23·75° C.
28·75—14·5 „ = 10.40 „ —11.19 „ = 39 „ 23·7° C.

Normal doubling periods:—

22—44 μ = 10.58 A.M.—11.46 A.M. = 48 minutes at 23·7° C.
44—88 „ = 11.46 „ —12.28 P.M. = 42 „ 23·7—24° C.
88—176 „ = 12.28 P.M.—1.8 „ = 40 „ 24—25° C.
176—352 „ = 1.8 „ —1.42 „ = 36 „ 25° C.
*352—704 „ = 1.42 „ —2.32 „ = 50 „ 25° C.

On August 6 a broth-drop of spores was started in the Sachs' box at 10.15 A.M. Temperatures ran as follows:—

Time.	Temp. ° C.
10.15 A.M.	= 22·0
10.50 „	= 24·5
11.5 „	= 25·0
11.30 „	= 24·9
11.45 „	= 24·5
12.0 noon	= 24·5
2.0 P.M.	= 26·0
2.30 „	= 26·0
2.50 „	= 26·0

The measurements were now started on a filament 47·25 μ long, and followed till 4.8 P.M.; the temperature remained at 26—25·75°.

Doubling periods:—

1. 47·25—94·5 μ = 2.50 P.M.—3.20 P.M. = 30 minutes at 26—25·9° C.
2. 94·5 —189 „ = 3.20 „ —3.58 „ = 38 „ 25·9—25·75° C.

Or, calculated back:—

225—112·5 μ = 3.30 P.M.—4.8 P.M. = 38 minutes at 25·9—25·75° C.
112·5—56·25 „ = 2.57 „ —3.30 „ = 33 „ 26—25·9° C.

Normal doubling period:—

47·25—94·5 μ = 2.50 P.M.—3.20 P.M. = 30 minutes at 26—25·9° C.

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* Partly calculated; would probably have been less.

Spores had begun to round off at 7 A.M. on August 7, *i.e.*, life cycle in less than 24 hours.

On August 3 spores were sown in a broth-drop at 11.30 A.M., and put to germinate in the incubator at 23° C.; they did this rapidly and normally about 3.30, and at 3.50 the culture was put into the Sachs' box. During the accommodation period the temperature fell from 28·6° at 3.50 to 27·25° at 4.40, when a germinating rodlet was selected for measurement, as follows:—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
4.40 P.M.	6·0	—	—	—	27·25
5.0 „	8·0	20	2·0	0·1	27·0
5.20 „	10·0	20	2·0	0·1	26·75
5.40 „	14·5	20	4·5	0·2	26·5
6.0 „	22·5	20	8·0	0·4	26·5
6.20 „	33·75	20	11·25	0·5	26·4
6.40 „	47·25	20	13·5	0·67	26·5
7.0 „	68·5	20	21·25	1·0	26·6
7.20 „	103·5	20	35·0	1·7	26·4

Normal doubling period:—

8—16 μ = 5 P.M.—5.44 P.M. = 44 minutes at 27·0—26·5° C.

but as this was the germinating phase, take—

16—32 μ = 5.44 P.M.—6.18 P.M. = 34 minutes at 26·5—26·4° C.

Doubling periods:—

1. 6—12 μ = 4.40 P.M.—5.29 P.M. = 49 minutes at 27·25—26·7° C.
2. 12—24 „ = 5.29 „ —6.3 „ = 34 „ 26·7 —26·5° C.
3. 24—48 „ = 6.3 „ —6.41 „ = 38 „ 26·5 —26·4—26·5° C.
4. 48—96 „ = 6.41 „ —7.16 „ = 35 „ 26·5 —26·6° C.

Calculated back:—

103·5 —51·75 μ = 6.42 P.M.—7.20 P.M. = 38 minutes at 26·5—26·6—26·4° C.
51·75—26·0 „ = 6.6 „ —6.42 „ = 36 „ 26·5—26·4—26·5° C.
26·0 —13·0 „ = 5.33 „ —6.6 „ = 33 „ 26·6—26·4° C.

Another filament, selected at 8.40, gave the following:—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
8.43 P.M.	100·75	—	—	—	28·25
8.56 „	121·5	13	20·75	1·6	28·25
9.15 „	173·25	19	51·75	2·7	27·5
9.32 „	216·0	17	42·75	2·5	27·0
9.45 „	252·0	13	36·0	2·8	27·0

Doubling period :—

100·75—201·5 μ = 8.43 P.M.—9.26 P.M. = 43 minutes at 28·25—27° C.

Or, reckoned back :—

252—126 μ = 8.58 P.M.—9.45 P.M. = 47 minutes at 28·25—27° C.

On August 2 a broth-culture of spores was started at 21·25° in dark at 9.40 A.M. Germination was very rapid at 21·25—21·5°, the temperature being nearly constant, and at 1.45 there were rods from 45 μ to 70 μ and longer.

I then rapidly raised the temperature from 21·6° at 1.45 as follows :—

Time.		Temp. ° C.
2.0	P.M.	= 25·0
2.2	„	= 27·0
2.4	„	= 28·0
2.5	„	= 28·5
2.6	„	= 29·0
2·8	„	= 29·5
2.10	„	= 29·7

and selected a rod, in two segments at an angle of 75° or so, for measurement.

The variations of temperature between 2.10 and 2.40 should be noticed. They were—

Time.		Temp. ° C.	
2.10	P.M.	= 29·7	
2.12	„	= 30·0	Fall due to opening the box
2.13	„	= 29·0	
2.14	„	= 29·3	
2.15	„	= 29·5	
2.16	„	= 29·7	
2.18	„	= 29·9	
2.20	„	= 30·0	
2.25	„	= 29·9	
2.30	„	= 29·9	
2.40	„	= 30·0	

From thence onwards the temperature changed very slowly: equilibrium was attained.

Segment A.

This filament was followed from 2.10 P.M. to 3.10 P.M. and had then

to be abandoned, owing to the pronounced curvatures often setting in at these higher temperatures.

Doubling period :—

18—36 μ = 2.10 P.M.—2.53 P.M. = 43 minutes at 29·7—30—29·9—30° C.

Calculated back :—

51·75—25·75 μ = 2.32 P.M.—3.10 P.M. = 38 minutes at 29·9—30—29·9° C.

Normal doubling period :—

24·75—49·5 μ = 2.30 P.M.—3.9 P.M. = 39 minutes at 29·9—30—29·9° C.

Segment B.

Was followed from 2.10 P.M. to 3.10 P.M., and then abandoned.

Doubling period :—

18—36 μ = 2.10 P.M.—3.0 P.M. = 50 minutes at 29·7—30—29·9—30° C.

Calculated back :—

45—22·5 μ = 2.30 P.M.—3.10 P.M. = 40 minutes at 29·9—30—29·9° C.

Normal doubling period :—

22·5—45 μ = 2.30 P.M.—3.10 P.M. = 40 minutes at 29·9—30—29·9° C.

Total.

Doubling period :—

36—72 μ = 2.10 P.M.—2.57 P.M. = 47 minutes at 29·7—30—29·9—30° C.

Or, calculated back :—

96—48 μ = 2.31 P.M.—3.10 P.M. = 39 minutes at 29·9—30—29·9° C.

Another segment in the same culture was chosen at 3.24 P.M. and followed till 4.10 P.M.

Doubling period :—

118—236 μ = 3.24 P.M.—4.3 P.M. = 39 minutes at 29—29·75—28·75° C.

Or, calculating back :—

260—130 μ = 3.28 P.M.—4.10 P.M. = 42 minutes at 29—29·75—28·75° C.

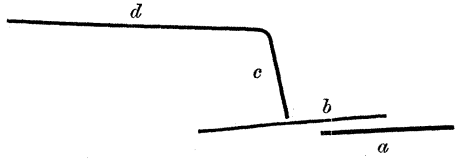
On August 7 a broth-drop of spores was put into the incubator at 23° C. at 7.30 A.M., and left to germinate. At 10.40 there were rods 36 μ to 45 μ long, and the culture was put into Sachs' box at 31° C.; during accommodation the temperatures ran—

Time.	Temp. ° C.
10.49 A.M.	= 29·5
11.0 „	= 31·0
11.15 „	= 31·0

At 11.30 a rod bent thus __ was selected and measured as follows:—The total measurement is given, and the behaviour of the segments into which (as commonly at high temperatures) the filament broke up are referred to later.

This filament was an interesting one in several respects, for its segments broke off so conveniently, or remained joined at such convenient angles, that I was able to measure them much more accurately than sometimes occurs.

When first observed, the filament consisted of two segments joined at an angle of about __. At 12 noon the horizontal leg had broken into two rods, the ends of which were already sliding one over the other, thus __, and by 12.20 the other leg had bent into two segments, which remained joined thus—



Consequently, I was able to measure all four segments separately, and, for convenience, I term them A, B, breaking up into *a*, *b*, and *c*, *d*, as shown in the diagram. The following are their measurements, the intervals occupied in these were too small to record.

First, I take the whole of the leg B, A and the whole of the leg C, D, and give their measurements separately, then their separate segments from the time they became distinct.

Total Length.

(From 11.30 A.M. to 1 P.M.)

Doubling periods:—

1. 42·75— 85·5 μ = 11.30 A.M—12.0 noon = 30 minutes at 31·1 —30·75° C.
2. 85·5 —171·0 „ = 12.0 noon—12.36 P.M. = 36 „ 30·75—30·8—31° C.

Or, calculated back:—

270—135·0 μ = 12.25 P.M.— 1.0 P.M. = 35 minutes at 31—31·25° C.
135— 67·5 „ = 11.52 A.M.—12.25 „ = 33 „ 31—30·75—31° C.

Normal doubling period:—

42·75—85·5 μ = 11.30 A.M.—12.0 noon = 30 minutes at 31·1—30·75° C.

Segment A.

(Comprising, after 12 noon, *b* and *a*.)

Doubling periods:—

1. 22·5—45 μ = 11.30 A.M.—12.0 noon = 30 minutes at 31·1—30·75° C.
2. 45·0—90 „ = 12.0 noon—12.28 P.M. = 38 „ 30·75—31° C.

Or, reckoned back:—

143·75—72 μ = 12.27 P.M.— 1.0 P.M. = 33 minutes at 31—31·25° C.
72·0 —36 „ = 11.52 A.M.—12.27 „ = 35 „ 31—30·75—31° C.

Segment B.

Doubling periods:—

20·25—40·5 μ = 11.30 A.M.—12.0 noon = 30 minutes at 31·1 —30·75° C.
40·5 —81·0 „ = 12.0 noon—12.35 P.M. = 35 „ 30·75—31° C.

Reckoned back:—

128·25—64 μ = 12.23 P.M.— 1.0 P.M. = 37 minutes at 31—31·25° C.
64·0 —32 „ = 11.53 A.M.—12.35 „ = 42 „ 31—30·75—31° C.

Segment a.

(From 12 noon to 1 P.M.)

Doubling period:—

22·5—45 μ = 12.0 noon—12.35 P.M. = 35 minutes at 30·75—31° C.

Or, reckoning back:—

73·25—36·5 μ = 12.27 P.M.—1.0 P.M. = 33 minutes at 31—31·25° C.

Segment b.

(From 12 noon to 1 P.M.)

Doubling period:—

22·5—45 μ = 12 noon—12.41 P.M. = 41 minutes at 30·75—31° C.

Or calculated back:—

67·5—33·75 μ = 12.26 P.M.—1.0 P.M. = 34 minutes at 31—31·25° C.

Segment c.

(From 12.30 to 1 P.M.)

This segment did not reach the doubling point, but, if the curve is prolonged to 1.3 P.M., we get it, which gives—

Part calculated:—

22·5—45 μ = 12.30 P.M.—1.3 P.M. = 33 minutes at 31—31·25° C.

Segment d.

(From 12.30 to 1 P.M.)

Here, again, the doubling period was not reached, but prolongation of the curve to 1.10 P.M. gives—

Part calculated:—

49·5—99 μ = 12.30 P.M.—1.10 P.M. = 40 minutes at 31—31·25°

At 1.40 another filament in this drop was selected and measured as follows, from 1.48 P.M. to 3.10 P.M. :—

Doubling period :—

58·5—117 μ = 1.48 P.M.—2.42 P.M. = 54 minutes at 31·5—31·75° C.

Calculated back :—

170·5—85·25 μ = 2.16 P.M.—3.10 P.M. = 66 minutes at 31·5—32° C.

A review of these broth-cultures bears out the general truth of the previous statements, and justifies the following additional conclusions :—

1. The growth in broth is more rapid than in the weak gelatine, and the doubling periods, especially at lower temperatures, are correspondingly shorter.

2. A curious technical difficulty arises, and often gives great trouble where broth is used (or when, at high temperatures, the gelatine liquefies). On opening the side windows to rearrange the object for measurement a slight draught is produced, and a cooling of the cover-slip ensues; this causes condensation of the vapour in the cell, and, if a drop catches the edge of the hanging drop, currents are produced, and the filament, if short and floating free, may move out of the field. Slight and slow movements may be followed, and all go on as before, but rapid ones are fatal to the purpose.

3. Several of the cultures suggest that a period of exhaustion gradually supervenes as the culture ages; it remains to be seen how far this is due to one or other of the following possible causes: (1) mere using up of the food-materials; (2) fouling of the drop by excreta; (3) or by CO_2 accumulating as the oxygen is consumed in respiration.

C. *Cultures in Broth + 1 per cent. Gelatine.*

On August 1 spores were sown as before, broth + 1 per cent. gelatine, and in at 9.30 A.M., at temperatures as follows :—

Time.	Temp. ° C.
9.30 A.M.	= 22·0
10.25 „	= 22·0
11.00 „	= 22·0
11.30 „	= 22·2
11.57 „	= 22·0
12.10 „	= 22·2
12.40 „	= 21·7
2.00 P.M.	= 20·5
2.20 „	= 20·4
2.28 „	= 21·1

Germination had set in normally, and rodlets up to 50 μ were found. I selected one 37 μ long, divided into two segments of 18 μ and 19 μ respectively, and measured as follows, taking each of its two segments separately; they were joined at an angle of about 165° :—

Segment A.

Doubling periods :—

1. 19—38 μ = 2.28 P.M.—3.24 P.M. = 56 minutes at 21·1—21—21·5° C.
2. 38—76 „ = 3.24 „ —4.35 „ = 71 „ 21·5—22—22·25—21·8° C.

Or, calculated back :—

103·5 —51·25 μ = 3.53 P.M.—5.0 P.M. = 67 mins. at 22—22·25—21·8—22·7° C.
51·25—25·5 „ = 2.55 „ —3.53 „ = 58 „ 21·2—22° C.

Normal doubling period :—

27—54 μ = 60 minutes at 21·5—22·1° C.

Segment B.

Doubling periods :—

1. 18—36 μ = 2.28 P.M.—3.26 P.M. = 58 minutes at 21·1—21—21·5° C.
2. 36—72 „ = 3.26 „ —4.30 „ = 64 „ 21·5—22—22·25° C.

Normal doubling period :—

27—54 μ = 3 P.M.—4 P.M. = 60 minutes at 21·25—22·1° C.

Total.

Doubling periods :—

1. 37— 74 μ = 2.28 P.M.—3.24 P.M. = 56 minutes at 21·1—21—21·5° C.
2. 74—148 „ = 3.24 „ —4.33 „ = 69 „ 21·5—22·25—22° C.

Or, calculating back :—

207·0—103·5 μ = 3.55 P.M.—5.0 P.M. = 65 minutes at 22—22·25—21·7° C.
103·5— 51·25 „ = 2.55 „ —3.55 „ = 60 „ 21·1—22° C.

On July 30 spores were sown in broth + 1 per cent. gelatine, and put in at 8 A.M. Germination was very rapid at 23·75 (8 A.M.), 23·5 (9.20), 24 (10 A.M.), and at 10.45 a rod 9 μ long was selected.

Doubling periods :—

1. 9—18 μ = 10.45 A.M.—11.30 A.M. = 45 minutes at 24·5° C.
2. 18—36 „ = 11.30 „ —12.12 P.M. = 42 „ 24·5° C.
3. 36—72 „ = 12.12 P.M.—12.56 „ = 44 „ 24·5—24·8° C.

Or, calculated back :—

76·5—38·25 μ = 12.17 P.M.— 1.0 P.M. = 43 minutes at 24·5—24·8° C.
38·5—19·0 „ = 11.33 A.M.—12.17 „ = 44 „ 25·0° C.
19·0— 9·5 „ = 10.47 „ —11.33 A.M. = 44 „ 25·0° C.

Normal doubling periods :—

14—28 μ = 11.15 A.M.—11.53 A.M. = 38 minutes at 24·5° C.
28—56 „ = 11.53 „ —12.39 P.M. = 46 „ 24·5—24·8° C.

The rapid germination indicates the neighbourhood of optimum temperature.

This filament was now lost, and another taken as follows :—

Doubling period :—

1. 67·5—135 μ = 2 P.M.—2.50 P.M. = 50 minutes at 24·5—24·75—24·6° C.

Or, calculating back :—

238—119 μ = 2.41 P.M.—3.25 P.M. = 44 minutes at 24·75—24·5° C.

On July 28 spores in broth + 1 per cent. gelatine were sown and put into Sach's box at 23·5° C. at 8 A.M. The temperature was slowly raised to 26° C. as follows :—

Time.	Temp. ° C.
8.00 A.M.	= 23·5
9.00 „	= 24·0
9.30 „	= 24·5
10.10 „	= 25·75
10.45 „	= 26·00
11.00 „	= 25·75
11.15 „	= 25·9
11.35 „	= 26·0
12.30 P.M.	= 26·0 (germination begun)
12.45 „	= 26·0

But fell to 24·75 on opening to arrange.

I now found a rodlet 5 divisions long (= 5 × 4·5) = 22·5 μ, and began measuring.

Doubling periods :—

1. 22·5—45 μ = 12.45 P.M.—1.26 P.M. = 41 minutes at 25·0—26·0° C.
2. 45·0—90 „* = 1.26 „ —2.10 „ = 44 „ 26·0—26·5° C.

This filament had another spore attached to it, and germinating there; possibly disturbances resulted, so I found another filament, and measured it as follows :—

Normal doubling period :—

36—72 μ = 1.10 P.M.—1.58 P.M. = 48 minutes at 25·8—26·4° C.

Or, calculated back :—

74·25—37 μ = 48 minutes at 25·8—26·4° C.

* Calculated from curve.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
2.4 A.M.	47·25	—	—	—	26·5
2.30 ,,	76·5	30	29·25	0·97	27·0
2.45 ,,	90·0	15	13·5	0·9	27·0
3.0 P.M.	112·5	15	22·5	1·5	27·0
3.20 ,,	150·75	20	38·25	1·9	27·1
3.55 ,,	270·0	35	119·25	3·4	27·5

Curve 112, p. 402.

Doubling periods:—

1. 47·25— 94·5 μ = 2.4 P.M.—2.44 P.M. = 40 minutes at 26·5—27·0° C.
2. 94·5 —189·0 ,, = 2.44 ,, —3.32 ,, = 47 ,, 27·0—27·25° C.

This was now too long to measure under this power, so I changed to the Zeiss C:—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
4.0 P.M.	285·0	—	—	—	27·1
4.10 ,,	330·0	10	45·0	4·5	27·5
4.20 ,,	382·5	10	52·5	5·2	27·5

3. 189—378 μ = 3.32 P.M.—4.20 P.M. = 47 minutes at 27·1—27·5° C.

Or, calculated back:—

382·5 —191·25 μ = 48 minutes at 27·25—27·5° C.
191·25— 95·5 ,, = 47 ,, 27·00—27·25° C.
95·5 — 47·75 ,, = 42 ,, 26·5 —27·0° C.

After 4.20, I let the temperature rise as follows:—

Time.	Temp. ° C.
4.30 P.M.	= 28·0
4.45 ,,	= 29·0
8.0 ,,	= 32·0
8.25 ,,	= 32·4

And at 8.25 chose a segment to measure under the C (each division = 7·5 μ) as follows:—

The doubling periods were as follows:—

1. 41·25—82·5 μ = 8.25 A.M.—9.20 A.M. = 55 minutes at 32·4—33·1—32·25° C.

Or, taking 60 μ as the initial length:—

60—120 μ = 8.52 A.M.—10.10 A.M. = 78 minutes at 32·75—32·25—32·75° C.

A somewhat remarkable fact was observed in these July 28 cultures. After standing all night at 32·75°, falling to 26° at 10 A.M. on the 29th July, both the cultures had passed over into spores.

That is to say, at temperatures between 23·5°, rapidly rising to 26°, then up to 33° C., slowly falling to 26° again, two cultures passed through their cycle from spore to spore in twenty-seven hours.

It would be interesting to investigate the properties of these spores thus rapidly formed at high temperatures, and compare them with others.

It is noteworthy that the culture of July 27, though treated exactly similarly from 8 A.M. 28th, to 10 A.M. 29th July, had as yet formed no spores. This latter had grown far more luxuriantly, however (at 27·5 on the 27th, see record), and served to show that the higher temperatures of the 28th culture, though driving the organism to more rapid spore formation, inhibit the growth, as the curve shows. It should also be noted that the spores formed were small, and, in many filaments, sparse, though otherwise normal to all appearance.

On July 29, spores, in broth + 1 per cent. gelatine were started at 10.15 A.M., the temperatures running as follows:—

Time.	Temp. ° C.
10.15 A.M.	= 25·5
10.30 „	= 26·0
10.45 „	= 26·25
11.10 „	= 26·25
11.20 „	= 26·4
11.30 „	= 26·0
11.35 „	= 26·0
11.50 „	= 25·5
12.0 noon	= 25·0
12.15 P.M.	= 24·8
12.25 „	= 25·25
12.40 „	= 25·6
1.0 „	= 25·8
1.5 „	= 26·0
2.25 „	= 26·0

I now sought for a filament, and was surprised to find what rapid progress had been made, for two were found of 67·5 and 70 μ respectively. The shorter was chosen for measurements.

Doubling period:—

1. 67·5—125 μ = 2.30 P.M.—3.7 P.M. = 37 minutes at 25·6—26·2° C
2. 125 —250 „ = 3.7 „ —3.44 „ = 37 „ 26·2—25·75° C.

Doubling periods calculated back:—

276·75—138·5 μ = 3.50 P.M.—3.13 P.M. = 37 minutes at 26·25—25·75° C.
138·5 — 69·25 „ = 3.13 „ —2.40 „ = 33 „ 25·75—26·25° C.

Normal doubling period:—

110·25—221 μ = 3 P.M.—3.37 P.M. = 37 minutes at 26—26·25—26° C.

Evidently the temperatures were near the optimum.

On July 27 spores sown in broth + 1 per cent. gelatine were put in at 7.45, the temperatures running as follows:—

Time.		Temp. ° C.	
7.45	A.M. =	19·5	
8.0	„ =	20·25	
9.0	„ =	23·1	
9.45	„ =	24·5	
10.10	„ =	24·8	
10.30	„ =	25·4	
11.7	„ =	26·0	germination
11.40	„ =	26·75	beginning
	Fell to 26 on opening		
12.30	P.M. =	27·0	
12.45	„ =	27·0	
1.0	„ =	27·2	
1.30	„ =	27·5	
2.0	„ =	27·5	

The measurements were now begun on a stout filament divided into two segments, which were both very straight, and formed an angle of about 150 with each other. It was easy to measure both segments, practically simultaneously, and so get not only the growth curve of each segment but also (as the sum of the two) that of the whole filament, as follows:—

Segment 1.

The doubling period here:—

78·75—157·5 μ in 39 minutes at 27·5—27·7° C.

Or, calculated back:—

234—127 μ = 2.25 P.M.—3.0 P.M. = 35 minutes at 27·6—27·75° C.

Normal doubling period:—

78·75—157·5 μ = 2.0 P.M.—2.39 P.M. = 35 minutes at 27·5—27·9° C.

Segment 2.

The doubling period here :—

72—144 μ in 38 minutes at 27·5—27·7° C.

Or, calculated back :—

225—112·5 μ = 2.24 P.M.—3.0 P.M. = 36 minutes at 27·6—27·8—27·75° C.

Normal doubling period :—

72—144 μ = 2.0 P.M.—2.38 P.M. = 38 minutes at 27·5—27·7° C.

Total.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
2.0 P.M.	150·75	—	—	—	27·5
2.10 „	177·75	10	27	2·7	27·5
2.20 „	213·75	10	36	3·6	27·6
2.30 „	261·0	10	47·25	4·7	27·7
2.40 „	313·0	10	52·0	5·2	27·7
2.50 „	377·75	10	62·75	6·27	27·8
3.0 „	459·0	10	81·25	8·1	27·75

The following measurements were made on another filament in this culture :—

In this case the measurements were made with Zeiss C, each scale division = 7·8 μ, and I regard the numbers as not *quite* so accurate as preceding ones, partly owing to the amplification and partly to the extremely rapid growth.

The doubling period :—

149—298 μ in 35 minutes at 27—27·5° C.

Calculated back :—

343—172·5 μ = 40 minutes at 27·5—27·7° C.

This culture remained in the Sachs' box through all the next day (July 28) at 26—33°, and had not developed spores on the 29th at 10 A.M. It was returned to the box. On the 30th, spores were formed.

On August 3 spores sown in broth + 1 per cent. gelatine were put at 5.15 A.M. into the incubator at 23° C. to germinate. At 10.20 the Sachs' box was ready to receive them. Germination had been rapid and normal, and rods from 40 to 70 μ were present.

The culture was fixed at 10.25 at 24·5° C.

The temperatures during accommodation varied as follows :—

Time.		Temp. ° C.
10.25	A.M.	= 24·5
10.27	,,	= 26·0
10.28	,,	= 26·5
10.29	,,	= 26·8
10.30	,,	= 27·0
10.36	,,	= 27·4
10.45	,,	= 27·4
10.55	,,	= 28·3

At 11.2 the measurements began as follows :—

Normal doubling periods :—

1. 60·75—121·5 μ = 11.2 A.M.—11.37 A.M. = 35 min. at 28·0—28·5 —28·3° C.
2. 121·5 —243·0 ,, = 11.37 ,, —12.15 P.M. = 38 ,, 28·3—28·25—28·75°C.

Or, calculated back :—

252—126 μ = 11.39 A.M.—12.18 P.M. = 39 minutes at 28·3—28·25—28·75° C.
126— 63 ,, = 11.3 ,, —11.39 A.M. = 36 ,, 28·0—28·5 —28·3° C.

On August 2 a broth + 1 per cent. gelatine sowing of spores was put in at 8.30, the temperature as follows :—

Time.		Temp. ° C.	
8.30	A.M.	= 21·3	
9.30	,,	= 21·4	
10.0	,,	= 21·3	
11.0	,,	= 21·3	
11.15	,,	= 21·5	(opened side window)
12.0	noon	= 21·25	
12.10	P.M.	= 21·6	(opened window)
12.25	,,	= 21·5	
		Swollen spore seen.	
12.45	,,	= 21·6	
1.0	,,	= 21·6	Germ. = 3 μ
1.45	,,	= 21·6	,, = 6 μ

The culture was now put on a slate slab at 18° C. until 4 P.M., when (the box temperature having been raised) it was put into culture at 27·5°, and raised to 31·2° by 4.40, and measurements made as follows :—

Doubling period :—

135—270 μ = 4.40 P.M.—5.17 P.M. = 37 minutes at 31·2—31·7° C.

Or, reckoning back :—

283—141·5 μ = 4.42 P.M.—5.20 P.M. = 38 minutes at 31·25—31·75° C.

Normal doubling period :—

135—270 μ = 4.40 P.M.—5.17 P.M. = 37 minutes at 31·2—31·7° C.

In resuming the foregoing series, we note that they fully bear out the previous conclusions, and are closely comparable with the broth-cultures.

Before proceeding to summarise further, it will be instructive to look at a series of experiments made *not with spores*, but with *segments of filaments* transferred bodily to the new media for measurement.

D. *Experiments with Growing Bacilli and Filaments in Broth + 1 per cent. Gelatine, and in Broth alone.*

On July 29 I tried the following experiment. Spores sown in broth + 1 per cent. gelatine in a tube were kept in incubator till 5 P.M. at 25° C. Then I fused the tube end, and thoroughly shook up the filaments to break them into segments. A drop was then used to infect a new tube, and from this a drop put into a cell and used to see if the growth measurements would correspond.

An excellent rod, 24·75 μ long, was easily found in the drop at 5.7 P.M. Giving this a little time to recover the shock of the violent shaking, and to accommodate itself to the temperature, food material, &c., I proceeded to measure it, noting that it began to grow at once, as follows :—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
5.7 P.M.	24·75	—	—	—	25·25
5.15 „	25·87	8	1·12	0·1	25·7
5.20 „	26·9	5	1·12	0·2	25·9
5.25 „	28·0	5	1·12	0·2	26

A very slow, but distinctly perceptible growth.

The regular measurements were then begun at 5.30 P.M.

Normal doubling periods :—

1. 29·25—58·5 μ = 5.30 P.M.—6.15 P.M. = 45 minutes at 26—25·8—26° C.
2. 58·5—117 „ = 6.15 „ —6.55 „ = 40 „ 26·0—26·4° C.
3. 117—234 „ = 6.55 „ —7.34 „ = 39 „ 26·4—26° C.

It is clear that the rate of growth is the same, under the same conditions, as for the filaments normally germinated out from the spores, a point of some importance, as it may perhaps permit an extension of the method in several directions, besides a considerable saving of time.

It seems to me that a good deal of valuable information might be obtained by pursuing this method.

On July 30 I repeated this experiment with a rodlet from a *two days'* culture at 23—25°, shaken in its own liquor and then into fresh (broth + 1 per cent. gelatine in both cases). It was started at 3.25 at 24° C., and remained till 4 P.M. to accommodate itself to the new conditions, the temperature having risen to 24·6°.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
4.8 P.M.	25·5	—	—	—	24·75
4.13 „	„	5	0	0	24·5
4.21 „	„	8	0	0	24·6
4.45 „	„	24	0	0	24·4
4.54 „	„	9	0	0	24·5
5.0 „	„	6	0	0	25·0

I concluded that no growth occurred either (1) because it took a long time for bacilli beginning to pass over into spore formation to accommodate themselves; or (2) because the change of temperature was too sudden; or (3) because something was passed in of the nature of a poison. The shaken tube had remained some hours, and *no* bacilli in the drop were growing at 5 P.M.

On July 31 a broth + 1 per cent. gelatine tube, sown 8 A.M. on July 30, was shaken up after having been till 12.30 at 23—24° C.—*i.e.*, 28½ hours—and a sowing made.

The temperature in Sachs' box was 20·5°, and rose as follows during accommodation period:—

Time.	Temp. ° C.
12.30 P.M.	= 20·5
12.40 „	= 21·0
12.45 „	= 21·75
12.50 „	= 21·6
2.0 „	= 21·0

I now satisfied myself the rodlets were growing, but they were floating about, and I could not fix one till much later.

Time.	Temp. ° C.
2.25 P.M.	= 20·25
2.50 „	= 21·0
3.30 „	= 22·1
4.14 „	= 23·25

One was fixed at 4.18 P.M., and measured as follows:—

Doubling periods (long beyond 1st normal):—

1. 54—108 μ = 4.18 P.M.—5.4 P.M. = 48 minutes at 23·5—23·75° C.
2. 108—216 „ = 5.4 „ —5.46 „ = 42 „ 23·75—24—23·75 C.

Or, calculating back:—

279—130·5 μ = 5.20 P.M.—6·0 P.M. = 40 minutes at 23·9—23·6° C.
139·5—69·75 „ = 4.38 „ —5.20 „ = 42 „ 23·5—24—23·9° C.

Another rodlet was chosen at 6.30 P.M., as follows:—
The doubling period here:—

103·5—207 μ = 6.33 P.M.—7.20 P.M. = 47 minutes at 22·75—22·4—22·5° C.

Or, calculating back:—

230—115 μ = 6.40 P.M.—7.30 P.M. = 50 minutes at 22·65—22·4—22·6° C.

On August 1 a sowing was made of shaken rods from the tube (of rods) made on July 31, and which had stood 21½ hours at 18—20°.

The sowing was made at 9.30, and allowed till 2 P.M. to accommodate itself, at the following temperatures:—

Time.	Temp. ° C.
9.30 A.M.	= 22·0
9.45 „	= 22·0
10.25 „	= 22·0
11.0 „	= 22·0
11.30 „	= 22·2
11.57 „	= 22·0
12.10 P.M.	= 22·2
12.40 „	= 21·7

At 2 P.M. a rod was selected and measured as follows:—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
2.0 P.M.	24·75	—	—	—	20·5
2.10 „	27·0	10	2·25	0·22	20·3
2.20 „	29·25	10	2·25	0·22	20·4

but had to be abandoned. Another was selected at 5.30 as follows [for temperatures between, see spore culture, August 1] in two segments.

Segment A.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
5.30 P.M.	85·5	—	—	—	22·5
5.40 ,,	91·75	10	6·25	6·2	22·25
5.50 ,,	103·0	10	11·25	1·12	22·3
6.0 ,,	112·0	10	9·0	0·9	22·4
6.10 ,,	130·0	10	18·0	1·8	22·5
6.20 ,,	148·0	10	18·0	1·8	22·5

Again I had to abandon the filament, owing to its awkward curvatures. Comparison with another filament enabled me to assure myself of the continued growth, however, at corresponding rates.

Doubling periods :—

85·5—171 μ = 5.30 P.M.—6.31 P.M. = 61 minutes at 22·5—22·25—22·5° C.

Segment B.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
5.30 P.M.	81·0	—	—	—	22·5
5.40 ,,	85·5	10	4·5	0·45	22·25
5.50 ,,	94·5	10	9·0	0·9	22·3
6.0 ,,	103·5	10	9·0	0·9	22·4
6.10 ,,	117·0	10	13·5	1·35	22·5
6.20 ,,	135·0	10	18·0	1·8	22·5

Doubling period :—

81—162 μ = 5·30 P.M.—6.32 P.M. = 62 minutes at 22·5—22·25—22·5° C.

On August 2nd, rodlets, in broth for 9½ hours, at 23—24°, were shaken and sown in broth, and put into dark Sachs' box at 5.30, rising from 27° as follows—half an hour being given for accommodation.

Time.	Temp. ° C.
5.30 P.M.	= 27·0
5.36 ,,	= 29·5
5.45 ,,	= 30·3
5.50 ,,	= 30·7

The rod for measuring was selected at 6 o'clock, the temperature falling a little during the search.

Rodlet in Broth.

Normal doubling periods :—

1. 33·75—67·5 μ = 6.5 P.M.—6.37 P.M. = 32 minutes at 29·6—29·5—30·5° C.
2. 67·5—135 „ = 6.37 „ —7.5 „ = 28 „ 30·5—30° C.

Or, calculated back :—

153—76·5 μ = 6.41 P.M.—7.10 P.M. = 29 minutes at 30·5—30° C.

On August 3, rodlets in broth—from spores sown at 5.15 A.M., at 23° C., and therefore $7\frac{1}{2}$ hours growth—were shaken in broth and a culture put into Sachs' box at 27—28° C., at 12.45 P.M.

During accommodation, the temperatures varied as follows :—

Time.	Temp. ° C.
12.50 P.M.	= 26·75
12.52 „	= 27·25
12.54 „	= 27·5
12.59 „	= 27·75
1.50 „	= 27·5

At 1.54 P.M. the measurements began on a rod 49·5 μ long.

49·5—99 μ = 1.54 P.M.—2.40 P.M. = 46 minutes at 27·2—28° C.

Or calculated back :—

141·75—71 μ = 2.21 P.M —3.0 P.M. = 39 minutes at 27·2—28·5° C.

Another rodlet, in two segments, was selected at 3 o'clock.

Segment A.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
3.1 P.M.	31·5	—	—	—	28·5
3.10 „	37·25	9	6·75	0·75	29·0
3.23 „	49·5	13	12·25	0·9	29·0
3.30 „	54·0	7	4·5	0·6	29·0
3.40 „	63·0	10	9·0	0·9	28·8
3.50 „	67·5	10	4·5	0·45	28·6
4.33 „	67·5	—	—	—	27·5

The growth had ceased entirely, and the filament was the same length as at last observation. Whether this was mere arrest, due to falling temperature (as is probable), or death, could not be determined.

Doubling period:—

31·5—63 μ = 3.1 P.M.—3.40 P.M. = 39 minutes at 28·5—29—28·8° C.

Or calculated back:—

67·5—33·75 μ = 3.5 P.M.—3.50 P.M. = 45 minutes at 28·75—29—28·6° C.

Segment B.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
3.1 P.M.	90·0	—	—	—	28·5
3.10 „	108·0	9	18·0	2·0	29·0
3.23 „	135·0	13	27·0	2·0	29·0
3.30 „	160·75	7	15·75	2·2	29·0
3.40 „	171·0	10	11·25	1·1	28·8
3.50 „	189·0	10	18·0	1·8	28·6
4.33 „	189·0	—	—	—	27·5

The growth had ceased (as said under segment A).

Doubling period:—

90—180 μ = 3.1 P.M.—3.45 P.M. = 44 minutes at 28·5—29—28·7° C.

Or calculated back:—

189—94·5 μ = 3.3 P.M.—3.50 P.M. = 47 minutes at 28·5—29—28·7° C.

If we now summarise all these results, it is seen that there are three points to be considered as regards the growth and behaviour of the organism, (1) The rate of germination: (2) the rapidity of post-germinal growth,—or, what is here the same thing, doubling period, and (3) the time occupied in completing the life-cycle from spore to spore. The labour involved in making out all these points accurately would be enormous, and I have only been able to concentrate my attention on the growth curves, and to go accurately into the doubling periods; though, as we see, some interesting data are given concerning the other phenomena, wherever they could be noted.

Secondly, it will be seen that while paying especial attention to the one point—the influence of temperature on the doubling period—I had occasion to test to some extent the effects of altering the medium in which the growth occurs, and the results are sufficient to show that here, again, there is a large and interesting field for enquiry.

Thirdly, the question arose as to whether any difference is observable between the behaviour of filaments, grown *direct from the spore*, in a given medium at a given temperature, and those grown *from a segment of greater age;* and although here again the enormous

labour involved would be too great to charge myself with at present, sufficient facts came to hand to show that this branch of the enquiry also ought to be taken up and pursued.

Fourthly, the point arose by the way how far the doubling period at a given temperature, and in a given medium, is affected by whether the filament measured has developed all along,—*i.e.*, had germinated—at the temperature of the growth measurements, or whether germination was conducted at some other temperature, and the growing filament then brought into the new temperature.

A fifth point arises by the way also. How far is the matter affected by the age of the food-medium ?

Curves of the Doubling Periods.

If we plot out the results reached so far, some interesting curves can be obtained which help us materially.

Taking any series—say the cultures in weak gelatine—the *averages* of all the doubling periods obtained for the several temperatures may be plotted on ordinates erected on a base line divided into degrees centigrade, and on joining the points thus plotted we get a curve convex to the base line. As a matter of fact, however, the curve so obtained from averages of *all* doubling periods is very irregular, and all that one can say is that, (1) generally speaking, the curve descends from somewhat high numbers below 18° C., to the lowest numbers between 25—30°, and then ascends again.

2. The various jerks on this curve are unequal, and without any evident order, and the re-ascent of the curve beyond 30° C. is not always obtained.

3. On the whole the curve descends most for broth, and least for weak gelatine.

It seems hardly worth while to go more into details, since it was so evident that something had to be explained here, before further steps could be taken, that I made a careful analysis of all the conditions, and found that the irregularities on these curves of all averages were due to a number of more or less controllable causes.

In the first place, it turns out that it was a serious error on my part to start the germination of the spores in the Sachs' box at various convenient temperatures with reference to the temperatures of observation, and many of the irregularities in question are due to the consequent (1) differences in initial vigour of the plantlets started at different temperatures, (2) differences in *phase* of growth of such rodlets, and (3) differences in degree of exhaustion of the food-material in the drops, which had thus been exposed for different lengths of time to different initial temperatures.

It is hardly necessary to go into the details of this very complex

matter, which I had to test in various ways before deciding on a satisfactory plan—for it must be borne in mind that one has to so arrange matters that the necessary phase of growth shall occur at a time convenient for observation and measurement, otherwise it would be imperative on one to be ready to take the measurable phase at any hour of the day or night—it may therefore suffice to say that further reflection on all the circumstances suggests that the following conditions would have to be fulfilled before satisfactory results could be obtained. It will be obvious that some of these conditions can *not* be completely satisfied, so that observers will have to be content to approach them as nearly as possible. I give the ideas as they arise, but perhaps other investigators can suggest improvements.

1. The temperatures employed should be constant, and this condition can be so nearly approached that we may regard practice as according with theory. The difficulties have been sufficiently discussed as they arose.

2. The food-material and medium employed should be constant. This condition can also be approximately carried out, because although (1) the water-contents of the hanging-drop vary at different temperatures, and (2) no food-medium remains exactly the same from day to day, however carefully prepared and kept, considerable accuracy can be attained. This difficulty cannot be got over by using a fresh brew each time, because no two brews can be *exactly* alike. With great care, the nearest approximation to uniformity must be aimed at.

3. The spores used must be from the same culture. This can be realised, and, apart from theoretical differences as the age of spores increases, we may regard this condition as satisfied.

4. The culture-cells used must be prepared in the same way, and be loaded with the same water supply, and have the same air-contents. Practically this condition is also met.

5. The culture-drops should always be of the same size, and supply equal amounts of food-materials. Here we cannot do more than approximate as closely as possible to the requirements, though much can be done by practice, using the same loop, and so on.

6. Strictly speaking, the drop ought always to contain the same number of spores—preferably, no doubt, *one* spore—but it would be impossible to meet this condition exactly, in working with large numbers of cultures, as I have to do, and so approximation only can be hoped for. I regard the matter of importance, however, for it is impossible to overlook the probability—amounting to all but certainty—that each filament is to some extent an antagonist to all its neighbours: competing with them for oxygen, water, and food-materials, and, no doubt, affecting the medium injuriously as its metabolism proceeds. With great care and long practice it is

possible to make the sowings approximately uniform—say 12—25 spores in each drop. More can hardly be hoped for.

7. The spores ought always to be germinated out at the same *constant* temperature, though beyond the confirmation of the fact that some temperatures favour germination better than others, my experiments throw little light or further details on this matter. I select 22° C. as a useful temperature.

8. It seems probable from the foregoing that the germinated culture ought always to be transferred to the temperature, &c., at which the growth is to be examined *at the same age*, so that the filaments to be compared, as to their growth-curves under the new conditions, shall have been exposed to uniform conditions for the same period of time previous to the study of the new reactions, and so that we may always select filaments of *equal length and age* from the spore, for comparison. This is undoubtedly a difficult condition to fulfil, and I have by no means always succeeded in fulfilling it, because it requires that the investigator shall be able to begin his work at any stated times.

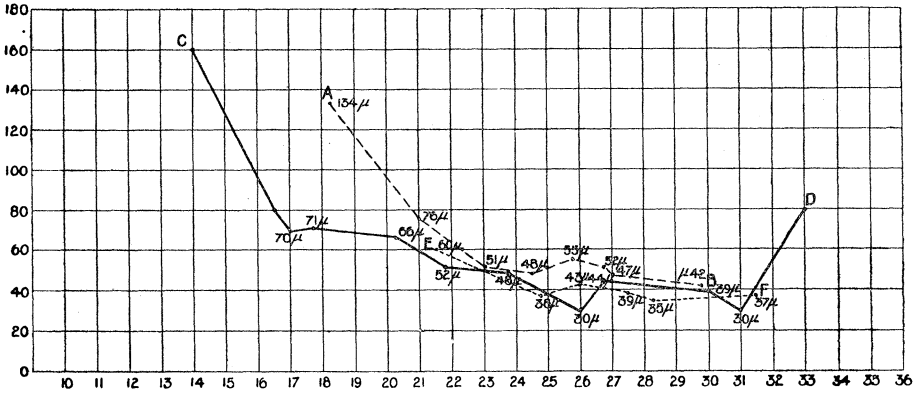
9. Lastly comes the question of what we shall compare on the growth-curves. The outcome of the experiments given suggests that it may not be sufficient to compare *any two* doubling periods, as has been done. Probably it would be better to decide to compare either (1) the doubling of equal lengths—*e.g.*, the elongation from 100 to 200 μ—at the given temperature, &c., or (2) the doubling period starting always at the same age—*e.g.*, whatever the length of the filament after one hour's sojourn at the given temperature, see how long it takes to double that length. Clearly we cannot do *both* with the same series of cultures, though perhaps the ideal to be aimed at is that both be done eventually. Looking at the importance of the subject, it seems worth while to throw out the suggestion at any rate.

The following set of cultures in normal gelatine show very clearly that excellent and clear results are to be obtained by observing the above precautions as closely as possible, and adopting the method of recording now to be described.

1. The culture, when put into the Sachs' box, is allowed half-an-hour for the organism to accommodate itself to the new temperature.

2. The first doubling period selected is that starting from the half-hour accommodation just mentioned, and all previous growth (*i.e.*, growth during the accommodation period) is neglected. I term these doubling periods "normal doubling periods," in contradistinction to the foregoing doubling periods selected anywhere on the curves.

3. If we plot out these *normal* doubling periods, as already described (Curves AB, CD, and EF), we obtain a much more regular curve, which, as will be seen, comes evidently into the category of respiration curves.



On examining these three curves, AB, CD, and EF,* we see that—

1. The general tendency of the weak gelatine—*i.e.*, poor in food materials but stiff in consistency (Curve AB)—is to lengthen the doubling period.

2. That considerable irregularities occur at all the temperatures higher than about 24° C. or 25° C. These are no doubt due to the inequalities in the *phase of growth*, different ages of the culture, and so on, already discussed.

3. It looks as if the curve suddenly shot up at temperatures beyond 31—32° C., as shown by the curve for broth (CD).

4. It might, perhaps, be suggested that although the addition of gelatine delays the doubling period, it also delays the attainment of the maximum temperature, *i.e.*, it protects the food-drop against exhaustion, until a higher temperature than that at which exhaustion sets in when broth alone is used. This may be the case, but I do not regard it as proved, and there are many difficulties with broth-cultures at higher temperatures, as already said.

But (5) it is clear that these three curves, unsatisfactory though they are as yet in detail, show striking general resemblances to a curve of respiratory activity—the steady fall in the doubling period as we pass from lower temperatures, and the sudden rise again beyond 31° C. (seen in the broth curve) being very suggestive—and I believe that is what they really are, for reasons already discussed.

Having so far cleared the way for the understanding of these curves, we shall see that when attention is carefully given to all the points discussed, a much more regular curve is obtained, as the following series show.

* Curve AB summarises the averages for weak gelatine; CD, those for broth + 1 per cent. gelatine; EF, those for broth alone. The ordinates are minutes and the abscissæ temperatures.

Cultures in Normal 10 *per cent. Gelatine.*

Experiments on the effects of Temperature in the Dark, with constant Food-material and conditions of Germination.

In the following series I used throughout the same normal gelatine —10 per cent. gelatine, with broth and peptone—and always incubated the spores at 22° C. until germination was completed and the rodlets in full growth. As far as possible, moreover, I started the growth-measurements on filaments in exactly the same phase of growth, and used drops of as nearly as possible the same size, and containing the same number of spores, though it was found to be quite impracticable to fulfil all these conditions absolutely.

The arrangements—Sachs' box, cells, temperature regulations, and conditions of measurement—were as before.

I select a number of typical curves from a much larger number, and need only say that they are quite representative and fair examples. The labour of recording the growths, working out the tables, and drawing the curves has been very great, as will readily be understood; but it seems worth while to have done it to get the insights here obtained into the marvellous regularity of growth that goes on when the conditions are under control, quite apart from the other information conveyed by the results.

Still, it will be obvious that it has been impossible to carry out more than one series so thoroughly in the time, and it must remain for others to investigate the behaviour of the organism in other media or under other conditions similarly controlled.

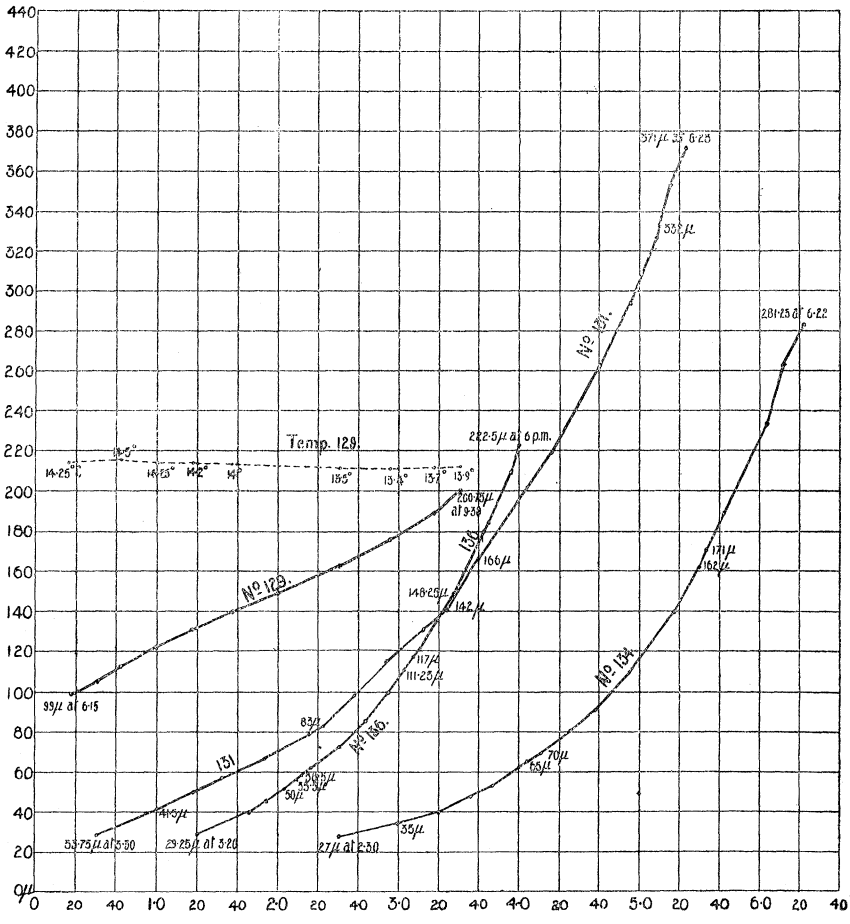
On November 16 spores were sown in normal gelatine at 10 A.M., and put at 22° C. till 6 P.M., and then put into the Sachs' box at 14° C., and measurements made as follows :—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
6.15 P.M.	99·0	—	—	—	14·25
6.42 ,,	112·5	27	13·5	0·50	14·5
7.0 ,,	122·5	18	10·0	0·55	14·25
7.17 ,,	131·0	17	8·5	0·50	14·2
7·35 ,,	140·0	17	9·0	0·53	14·0
8.29 ,,	162·5	54	22·5	0·41	13·5
8.55 ,,	176·0	26	13·5	0·52	13·4
9.18 ,,	191·75	23	15·75	0·68	13·7
9.30 ,,	200·75	12	9·0	0·75	13·9

Curve 129, p. 434.

Doubling period :—

99—198 μ = 6.15 P.M.—9.27 P.M. = 192 minutes at 14·25—14·5—13·4—13·9° C.



On December 11 spores in normal gelatine were put in at 22° at 1.45, and into measurement at 6.15 P.M.

The normal doubling period :—

20·25—41 μ = 6.45 P.M.—9.2 P.M. = 137 minutes at 14·5—15·1° C.

It is a very long and tedious business to work out these slow growths at low temperatures, but several other curves show that about 200 minutes represents the doubling period for 14° C.; the time rises very quickly as the temperature falls, however, and I find it is nearly 400 minutes for 10—12° C., and probably nearly 800 to 1000 minutes for 6—8°. This is, no doubt, partly due to the stiffness of the gelatine.

On December 10 spores were sown in normal gelatine at 22° C. at 10.30, and put into measurement at 3.30.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
3.30 P.M.	33·75	—	—	—	16·8
3.45 „	—	—	—	—	17·0
4·0 „	41·5	30	7·75	0·26	17·0
4.18 „	49·5	18	8·0	0·44	17·0
4.32 „	56·25	14	6·75	0·48	17·0
4.54 „	67·5	22	11·25	0·51	17·0
5.16 „	78·75	20	11·25	0·56	17·0
5·39 „	99·0	23	20·25	0·9	17·0
5·54 „	114·75	15	15·75	1·0	17·0
6.13 „	130·75	19	16·0	0·8	17·0
6.24 „	142·0	11	11·25	1·0	17·0
7.16 „	218·5	52	76·5	1·5	16·5
7.40 „	261·25	24	42·75	1·78	16·25
7.56 „	295·0	16	33·75	2·1	16·7
8.8 „	326·5	12	31·5	2·6	17·5
8.15 „	353·5	7	27·0	3·8	18·0
8.23 „	371·0	8	18·0	2·2	18·25

Curve 131, p. 434.

The doubling periods are (normal) as follows:—

41·5— 83 μ = 4·0 P.M.—5.21 P.M. = 81 minutes at 17° C.
83·0—166 „ = 5.21 „ —6.40 „ = 79 „ 17° C.
166·0—332 „ = 6.40 „ —8.10 „ = 90 „ 17·16·25—17·5° C.

This shows very clearly the steady growth and equal doubling periods at constant temperature, and, at the same time, how slight an alteration in the temperature (causing variation either way) suffices to slow the growth.

Another segment in the same drop was now selected, and measured as follows:—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	C.
8.33 P.M.	56·0	—	—	—	18·0
8.40 „	59·5	7	3·5	0·5	18·0
8.51 „	67·0	11	7·5	0·7	18·1
9.0 „	72·0	9	5·0	0·55	18·0
9.10 „	78·75	10	6·75	0·67	18·0
9.20 „	87·75	10	9·0	0·9	17·8
9.30 „	95·5	10	7·75	0·77	17·6
9.41 „	103·5	19	8·0	0·8	17·5
9.50 „	112·5	9	9·0	1·0	17·5
10.0 „	122·5	10	10·0	1·0	17·5
10.10 „	132·5	10	10·0	1·0	17·5
10 21 „	144·0	11	11·5	1·1	17·6
10.30 „	153·0	9	9·0	1·0	17·6

Here the doubling period was :—

56—112 μ = 8.33 P.M.—9.49 P.M. = 76 minutes at 18—18·1—17·5° C.

On November 17 spores sown as before, remained at 22° C., from 10.30 A.M. till 3.45, and were then put in at 19·5° C.

Doubling period :—

81·0—162 μ = 4.5 P.M.—5.16 P.M. = 71 minutes at 19·8—19·6—19·7—19·3° C.
162·0—324 „ = 5.16 „ —6.26 „ = 70 „ 19·3—18·75—19·5° C.

Calculated back :—

459·0—229·5 μ = 5.49 P.M.—7.11 P.M. = 82 minutes at 19—18·75—19·5° C.
229·5—114·5 „ = 4.37 „ —5.49 „ = 72 „ 19·7—19° C.

Normal doubling period :—

91·0—182·0 μ = 4.15 P.M.—5.26 P.M. = 71 m. at 19·7—19·6—19·7—19·6—19·3°C.
182·0—364·0 „ = 5.26 „ —6.41 „ = 75 „ 19·3—18·75—19·5° C.

On December 8 spores were sown in normal gelatine at 11 A.M., and put into the incubator at 22° C. till 2.30 P.M.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
2.30 P.M.	27 μ	—	—	—	19·5
2.35 „	—	—	—	—	19·0
3.20 „	40·5	50	13·5	0·27	18·5
3.30 „	—	—	—	—	19·0
3.36 „	49·5	16	9·0	0·56	19·0
3.47 „	54·0	11	4·5	0·4	19·0
4.0 „	63·0	13	9·0	0·7	19·0
4.10 „	69·75	10	6·75	0·67	19·0
4.25 „	81·0	15	11·25	0·75	19·0
4.38 „	92·25	13	11·25	0·86	18·9
4.55 „	110·25	17	18·0	1·0	19·0
5.17 „	139·5	22	29·25	1·33	19·4
5.33 „	171·0	16	31·5	2·0	19·6
5.42 „	189·0	9	18·0	2·0	19·5
6.6 „	236·25	24	47·25	2·0	19·25
6.15 „	263·25	11	27·0	2·4	19·25
6.22 „	281·25	7	18·0	2·6	19·3

Curve 134, p. 434.

The normal doubling periods :—

35— 70 μ = 3.0 P.M.—4.10 P.M. = 70 minutes at 19·0—18·5—19·0° C.
70—140 „ = 4.10 „ —5.18 „ = 68 „ 19·0—18·9—19·4° C.
140—280 „ = 5.18 „ —6.21 „ = 63 „ 19·4—19·6—19·25—19·3°C.

On comparing these periods with those of November 17, it will be seen how admirably the agreement comes out, and further shows that

the food-material has not degenerated in quality during the interval of nearly a month, for it had remained in the flask for that interval.

From 6.15 onwards the above filament was segmented into a short and a long segment; in the following table I resume the further growth of the shorter one.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
6.15 P.M.	65·0	—	—	—	19·25
6.22 „	70·0	7	5·0	0·7	19·25
6.35 „	81·0	13	11·0	0·8	19·5
6.45 „	90·0	10	9·0	0·9	19·5
6.56 „	100·0	11	10·0	0·9	19·25
7.6 „	109·0	10	9·0	0·9	19·0
7.17 „	121·5	11	12·5	1·1	19·0
7.25 „	132·75	8	11·25	1·4	19·0

Here the doubling :—

65—130 μ = 6.15 P.M.—7.23 P.M. = 68 min. at 19·25—19·5—19·0° C.

At 8.15 another segment in the same drop was chosen, and measured as follows:—

Some interesting comparisons are possible here. For instance, the original filament was 65 μ long at 4.3 P.M., and it was 109 μ long at 4.54 P.M.; the present segment was 65 μ long at 6.15, and was 109 μ long at 7.6. In both cases, therefore, exactly the same time, *i.e.*, fifty-one minutes, was occupied in growing the 44 μ referred to, in the first case the temperature being 19° to 18·9° C., and in the second 19·25—19·5—19° C.

Then, again, the doubling period is the same as the second one of the original filament, viz., sixty-eight minutes, at nearly the same temperature.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
8.17 A.M.	114·75	—	—	—	19·0
8.35 „	123·75	17	9·0	0·5	19·2
8.57 „	135·0	22	11·25	0·5	19·4
9.10 „	144·0	13	9·0	0·7	19·25
9.27 „	157·5	17	13·5	0·8	19·25
9.57 „	180·0	30	22·5	0·75	19·75
10.10 „	198·0	13	18·0	1·4	19·3

Here the doubling period is becoming extended to more than double the previous ones, indicating the spoiling of the drop either

by exhaustion or by excreta; the latter is more probable, because much growth went on during the next 24 hours, as examination next day showed.

On November 24 spores were sown in normal gelatine at 10 A.M. and kept at 22° C. till 3.15.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
3.20 P.M.	29·25	—	—	—	21·6
3.30 „	—	—	—	—	21·75
3.45 „	40·5	25	11·25	0·45	21·6
3.53 „	45·0	8	4·5	0·5	21·6
4.2 „	51·75	9	6·75	0·75	21·6
4.17 „	63·0	15	11·25	0·75	21·6
4.30 „	74·25	13	11·25	0·86	21·5
4.42 „	85·5	12	11·25	0·95	21·4
4.55 „	100·0	13	14·5	1·1	21·5
5.10 „	121·25	15	21·25	1·4	21·6
5.27 „	148·25	17	27·0	1·6	21·7
5.45 „	184·25	18	36·0	2·0	21·5
5.56 „	211·25	11	27·0	2·4	21·5
6.0 „	222·5	4	11·25	2·8	21·5

Curve 136, p. 434.

Doubling periods :—

29·25— 58·5 μ = 3.20 P.M.—4.12 P.M. = 52 minutes at 21·6—21·75—24·6°C.
58·5 —117·0 „ = 4.12 „ —5.7 „ = 55 „ 21·6—21·4 —21·6°C.

Calculated back :—

222·5 —111·25 μ = 5.2 P.M.—6.0 P.M. = 58 minutes at 21·6—21·7—21·5° C.
111·25— 55·5 „ = 4.8 „ —5.2 „ = 54 „ 21·6—21·4—21·6° C.

Normal doubling periods :—

40·5— 90 μ = 3.45 P.M.—4.46 P.M. = 61 minutes at 21·6—21·4° C.—
90·0—180 „ = 4.46 „ —5.43 „ = 57 „ 21·4—21·7—21·5° C.

Another culture, November 24, was from spores at 22° C. from 1 to 6 P.M.

Doubling periods :—

18—36 μ = 6.0 P.M.—7.0 P.M. = 60 minutes at 21·9 —21·75° C.
36—72 „ = 7.0 „ —8.11 „ = 71 „ 21·75—21·9° C.
72—144 „ = 8.11 „ —9.41 „ = 90 „ 21·90° C.

Normal doubling period :—

27—54 μ = 6.30 P.M.—7.42 P.M. = 82 minutes at 21·75—21·9° C.

This filament was then left until 8.45, and the following further measurements taken :—

Doubling (second normal doubling period) :—

54—108 μ = 7.42 P.M.— 9.4 P.M. = 82 minutes at 21·9° C.
108—216 „ = 9.4 „ —10.31 „ = 87 „ 21·9—21·8—21·9° C.

The foregoing cultures and their growth-curves are extremely interesting and instructive, especially because the temperatures were fortunately so constant.

As we see, the normal doubling period at 21·6°—21·4° was sixty-one minutes in the first case, where the germination period occupied five and a quarter hours, but it was seventy-two minutes (at 21·75—21·9°) in the second case, with a germination period of five hours and a shorter filament. The question arises, Why did the second culture occupy eleven minutes longer in doubling its shorter filament, although it had been exposed a quarter of an hour less time to the 22° C. of germination, and although the temperature was nearer the optimum by 0·3° to 0·25° C. ?

I think the explanation is that the temperatures further from the optimum were brought to bear at an earlier stage on a relatively weaker plant, and the latter therefore coped less ably with the more unfavourable circumstances.

Turning again to the second culture, it is worth noting that its second normal doubling period, *at perfectly constant temperature*, be it noted, is distinctly longer than its own first one—eighty-two minutes against seventy-two—bearing out my previous remarks as to the gradual slowing of growth as the culture ages, and, no doubt, due to the slow exhaustion of the food-materials and fouling of the medium by products of metabolism.

On November 27 spores in normal gelatine were sown at 2.15 and put at 22° C. till 8.5 P.M.

Doubling periods :—

114·5—229·0 μ = 8.5 P.M.—8.55 P.M. = 50 minutes at 22·75—22·25° C.
229·0—458·0 „ = 8.55 „ —9.48 „ = 53 „ 22·25—23·0° C.

Calculated back :—

454·5 —227·25 μ = 8.55 P.M.—9.48 P.M. = 53 minutes at 22·25—23·0° C.
227·25—113·75 „ = 8.4 „ —8.55 „ = 51 „ 22·75—22·25° C.

Normal doubling period :—

176·0—342·0 μ = 8.35 P.M.—9.27 P.M. = 52 minutes at 22·5—23·0° C.

Spores sown November 26 at 12.45 in normal gelatine at 22° C., remained till 7.45 P.M. and were then measured at 23°.

Time.	Length.	Interval.	Growth.	Rate.	Temperature.
	μ.	mins.	μ.	μ.	° C.
7.49 P.M.	218·0	—	—	—	23·0
8.3 „	274·5	14	56·5	4·0	23·0
8.20 „	333·0	17	58·5	3·4	23·5
8.34 „	396·0	14	63·0	4·5	23·5
8.54 „	517·5	20	121·5	6·0	23·5

The rate of growth was now so rapid I could no longer measure.

Doublings:—

218·0—436·0 μ = 7.49 P.M.—8.41 P.M. = 52 minutes at 23·0—23·5° C.

And back:—

517·5—258·75 μ = 7.59 P.M.—8.54 P.M. = 55 minutes at 23·0—23·5° C.

On November 23 spores in normal gelatine were put in at 22° C. at 2 P.M., and observations at 25° started at 6 P.M.

Doubling:—

18·0—36·0 μ = 6.5 P.M.—6.39 P.M. = 34 minutes at 23·5 —25·0—24·75° C.
36·0—72·0 „ = 6.39 „ —7.30 „ = 51 „ 24·75—25·0° C.

Calculated back:—

87·5 —43·75 μ = 6.56 P.M.—7.40 P.M. = 44 minutes at 24·75—25·0° C.
43·75—21·75 „ = 6.16 „ —6.56 „ = 40 „ 24·75—25·0—24·75° C.

Normal doubling period:—

28·0—56·0 μ = 6.30 P.M.—7.16 P.M. = 46 minutes at 25·0—24·75—24·9° C.

Another culture, made at 2 P.M. and hitherto at 22° C., was put in at 8.40 P.M.

Doubling:—

195·75—391·5 μ = 8.45 P.M.—9.31 P.M. = 46 minutes at 23—25° C.

Back:—

490·5—245·25 μ = 9.2 P.M.—9.45 P.M. = 43 minutes at 24·6—25·25—25·1° C.

Normal doubling period:—

245·5—490·5 μ = 9.2 P.M.—9.45 P.M. = 43 minutes at 24·6—25·25—25·1° C.

A curious phenomenon is often observed in these stiff gelatine cultures at temperatures near 23—24° C. This is the neighbourhood of the melting point, and it sometimes happens that *as the drop melts the rate of growth rises quite sharply;* I explain this as due to the sudden access of more oxygen and available food-materials to the rodlets.

On November 12 spores were sown as before at 10.30, kept at 22° C. till 3.30, when into Sachs' box at 24·5° C.

Doubling :—

20·25—40·5 μ = 3.30 P.M.—4.10 P.M. = 40 minutes at 23·0—25·0° C.

Back :—

47·25—23·5 μ = 3.37 P.M.—4.22 P.M. = 45 minutes at 24·75—25·0° C.

Another culture, exactly as before at 22° since 10.30, was put in at 4.30.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
4.39 P.M.	78·75	—	—	—	24·25
4.48 „	90·0	9	11·25	1·2	24·6
5.8 „	135·0	20	45·0	2·2	24·6
5.15 „	155·25	7	20·25	2·9	24·75
5.25 „	177·75	10	22·5	2·2	25·0
5.31 „	202·5	6	24·75	4·1	25·0
5.37 „	220·5	6	18·0	3·0	25·0
5.44 „	245·25	7	24·75	3·5	25·0
5.54 „	292·5	10	47·25	4·7	25·1
6.1 „	328·5	7	36·0	5·0	25·2
6.6 „	360·0	5	31·5	6·3	25·4
6.12 „	396·0	6	36·0	6·0	25·5
6.17 „	423·0	5	27·0	5·4	25·5

Curve 142, p. 442.

I had now to stop, the filament was too long to measure further. The doubling periods here :—

78·75—157·5 μ = 4.39 P.M.—5.17 P.M. = 38 minutes at 24·25—24·75° C.
157·5 —315·0 „ = 5.17 „ —5.58 „ = 41 „ 24·75—25·2° C.

Reckoned back :—

423·0—211·5 μ = 5.34 P.M.—6.17 P.M. = 43 minutes at 25·0—25·5° C.
211·5—105·25 „ = 4.55 „ —5.34 „ = 39 „ 24·6—25·0° C.

Normal doubling periods—

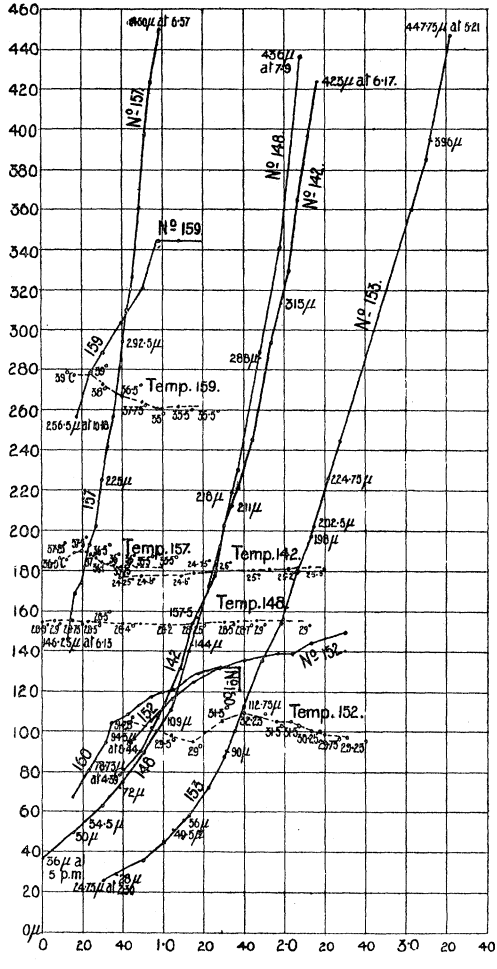
115—230 μ = 5.0 P.M.—5.40 P.M. = 40 minutes at 24·6—25·0° C.
230—460 „ = 5.40 „ —

not completed, but at present rate not far from 6.24 P.M., which = 44 minutes.

On November 13 a sowing, as before, made at 2 P.M., was put in at 9 P.M., and measured as follows :—

Doubling period :—

58·5—117·0 μ = 9.3 P.M.—9.50 P.M. = 47 minutes at 24·5—25·75—24·9° C.



Reckoned back :—

139 ·5—69 ·75 μ = 9.13 P.M.—10.0 P.M. = 47 mins. at 25 ·75—25 ·0—24 ·9—25 ·0° C.

On November 13 spores, as before, were sown, and put at 22° C. at 12.30 and into the Sachs' box at 6 P.M.

Doubling period :—

36—72 μ = 6.0 P.M.—6.45 P.M. = 45 minutes at 26 ·0—26 ·25° C.

Reckoned back :—

92 ·25—46 ·0 μ = 6.15 P.M.—7.0 P.M. = 45 minutes at 26 ·1—26 ·5° C.

Another filament was taken at 8 P.M. and measured as follows:—
Doubling period:—

225—450 μ = 8.13 P.M.—8.55 P.M. = 42 minutes at 25·4—25·9° C.

Reckoned back:—

486—243 μ = 8.17 P.M.—8.59 P.M. = 42 minutes at 25·4—26·0° C.

The following case is a very interesting one. It will be noticed that a drop of KHO was put into the arm of the cell. I was experimenting at the time to see if I could detect any difference by removing what traces of CO_2 diffuse into the cell as fast as they form, but the results were negative in that connection. Incidentally, however, it turned out that the drop of strong KHO often caused a slight dry skin to form on the gelatine drop when first put into the higher temperature, the skin being visible by faint wrinkles in it, and this affects the growth of the organism, possibly by impeding oxygen access. After the drop had melted, all went on quite normally, as we see, and there is no reason to reject the curve as an illustrative one.

On November 10 spores were sown at 10.30 in 10 per cent. gelatine and put into 22° C. A drop of KHO was put in the arm of the cell. At 3.35 the culture was put into Sachs' box at 27·5° C. The opened box fell to 25° C., but recovered to 27° C. at 3.45.

The filament selected did not grow very straight at first, but straightened out later and did well; it was also abnormal in some way, having one joint swollen, but recovered eventually, and after three-quarters of an hour or so behaved quite normally, and the question arose (answered above), had the KHO any effect?

The doubling periods here:—

46—92 μ = 4.0 P.M.—4.52 P.M. = 52 minutes at 27·2—27·0—27·5° C.
92—184 ,, = 4.52 ,, —5.36 ,, = 44 ,, 27·5—27·9—27·75° C.

Reckoned back:—

243·0—121·5 μ = 5.11 P.M.—5.53 P.M. = 42 m. at 27·75—27·9—27·75—28—27·6° C.
121·5— 60·75 ,, = 4.33 ,, —5.11 ,, = 38 ,, 27·0 —27·9° C.

Normal doubling periods:—

48— 96 μ = 4.5 P.M.—4.55 P.M. = 50 minutes at 27·0—27·5° C.
96—192 ,, = 4.55 ,, —5.40 ,, = 45 ,, 27·5—27·9° C.

I regard the first period of growth as unmeasurable and abnormal, due to something wrong with the growth and position of the filament, but after the first hour it seemed quite normal, as said.

A second filament from the same culture was then examined, as follows:—

The doubling periods here were as follows:—

31·5— 63 μ	= 6.5	P.M.	—6.40 P.M.	= 35 minutes	at 29·0—27·2° C.
63·0—126 „	= 6.40	„	—7.20 „	= 40 „	27·2—27·9° C.
126·0—252 „	= 7.20	„	—8.11 „	= 51 „	27·9—27·5° C.

Or, reckoned back:—

319·5 —159·75 μ	= 7.42	P.M.	—8.26 P.M.	= 44 mins.	at 27·75—27·5° C.
159·75— 79·8 „	= 6.53	„	—7.42 „	= 49 „	27·25—27·9—27·75° C.
79·8 — 39·9 „	= 6.16	„	—6.53 „	= 37 „	28·0 —27·2—27·5° C.

A scrutiny of these two tables does not bring out any increase generally in the doubling period during the third to fifth hours of exposure to these temperatures, as compared with the first hour; but the second table suggests that each doubling takes a slightly longer time to effect. It may be that the temperature is not sufficiently far from the optimum to show any great slowing effect in such short periods.

Next morning there was a very good crop in the hanging drop, left at 26·5—27·5° all night, and this supports the above suggestions.

On November 14 spores were sown as before at 11 A.M., and remained at 22° C. till 4 P.M., then into box at 29° C.

The first attempts to fix a filament failed, from floating. Meanwhile the temperature varied as follows:—

Time.	Temp. °C.
4.1 P.M.	= 28·25
4.15 „	= 29·5
4.32 „	= 29·1
4·45 „	= 28·75

and at 5 P.M. I succeeded in fixing a filament, measured as follows:—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	°C.
5.0 P.M.	36·0	—	—	—	28·9
5.6 „	—	—	—	—	29·0
5.15 „	49·5	15	13·5	0·9	28·9
5.24 „	—	—	—	—	28·75
5.29 „	63·0	14	13·5	1·0	28·5
5.40 „	74·25	11	11·25	1·0	28·5
5.48 „	90·0	8	15·75	1·9	28·4
6.2 „	112·5	14	22·5	1·6	28·2
6.12 „	139·5	10	27·0	2·7	28·25
6.26 „	184·5	14	45·0	3·2	28·5
6.37 „	231·75	11	47·25	4·3	28·7
6.48 „	289·0	11	57·25	5·2	29·0
6.58 „	341·75	10	52·75	5·2	29·0
7.9 „	436·25	11	94·5	8·6	29·0

Curve 148, p. 442.

Doubling periods :—

36— 72 μ = 5.0 P.M.—5.37 P.M. = 37 minutes at 28·9 —29·0—28·5° C.
72—144 „ = 5.37 „ —6.13 „ = 36 „ 28·5 —28·25° C.
144—288 „ = 6.13 „ —6.48 „ = 35 „ 28·25—29·0° C.

Reckoned back :—

436·0—218·0 μ = 6.34 P.M.—7.9 P.M. = 35 minutes at 28·6—29·0° C.
218·0—109·0 „ = 5.58 „ —6.34 „ = 36 „ 28·3—28·2—28·7° C.
109·0— 54·5 „ = 5.20 „ —5.58 „ = 38 „ 28·8—28·3° C.

Another partial filament was taken at 8.15.
Doubling periods :—

59·5—119 μ = 8.15 P.M.—8.49 P.M. = 34 minutes at 28—28·25° C.

Reckoned back :—

144—72 μ = 8.25 P.M.—9.25 P.M. = 60 minutes at 28·25—28·5—28° C.

There can be little doubt that here we have a case of slowing of growth after five hours' exposure to the temperatures given; *i.e.*, the measurements are here of a piece running down from exhaustion.

On December 3 spores were put at 22° at 9 A.M., and into measurement at 2.35, as follows :—

Normal doubling periods :—

116—232 μ = 3.5 P.M.—3.35 P.M. = 30 minutes at 28·25—28·6—28·5° C.
232—464 „ = 3.35 „ —4.6 „ = 31 „ 28·5—31° C.

Doubling periods :—

87·75—175·5 μ = 2.54 P.M.—3.24 P.M. = 30 minutes at 28·25—28·6—28·5° C.
175·5 —351·0 „ = 3.24 „ —3.54 „ = 30 „ 28·5—30·5° C.

Calculated back :—

495·0—247·5 μ = 3.37 P.M.—4.10 P.M. = 33 minutes at 28·5—31·1° C.
247·5—123·75 „ = 3.7 „ —3.37 „ = 30 „ 28·25—28·6—28·5° C.

The most interesting feature in this curve was to see how a steady slow rise of temperature did not prevent a second doubling at nearly the maximum speed. I was not able to measure any further, but saw enough to convince me that the organism was breaking down soon after from exhaustion.

On November 7 spores were sown in normal 10 per cent. gelatine at 1 P.M., and put into incubator at 22° C. At 5.30 the germinated rodlets went into the Sachs' box at 30° C., and at 5.54 the measurements began as follows :—

Doubling period (nearly normal) :—

29·25—58·5 μ = 5.54 P.M.—6.23 P.M. = 29 minutes at 29·5—29—32·6° C.

Further examination of this culture brought out very clearly the difficulties of these high temperatures. I started measurements at 6.28, 7.58, and 8.23 P.M. respectively, but each had to be abandoned after less than a quarter of an hour, owing to the excessive dilution, due to the powerful absorption of water by the (melted) gelatine drop, which induced currents and floated the rodlets away.

The general result was that the longer the culture remained at the high temperature the slower the growth. No doubt the dilution was largely responsible for this, but another cause may have been the slowing action of the high temperatures, taking time to act on the filaments.

It is by no means improbable, comparing all one has observed of these cultures at high temperatures, that the bacterium goes through a grand period of growth, *i.e.*, it begins growing slowly, attains a maximum, and then slows down again until fully outgrown. This being so, the total length of bacterium obtained (which, in other words, represents its total crop, or dry weight) will depend on the following factors:—

1. The amount of available food material. This I cannot measure, because it is not necessarily the amount of *broth*, or *gelatine*, &c., but the amount of peptonised materials, &c., it can get from the medium.

2. The temperature. At the optimum it metabolises, and grows, and respires, &c., at its best; but at higher temperatures removed from that it may grow *for a short time more rapidly*, but sooner exhausts itself, and so produces a poorer crop in the end. This certainly seems to be the case with these high temperature cultures, for I never get anything like so large a crop at 30—35° C. as at 22—25° C., other things being equal. Moreover, it seems clear that though the growth may for a short time be as rapid as it is near the optimum, it soon slows down (*e.g.*, compare November 5 experiments). All this suggests that at temperatures above the optimum, the machinery of the cell is being worked at too high a speed, and comes to grief sooner or later. How far this is due simply to over-respiration and rapid exhaustion of the oxygen in the medium, or to inability to peptonise or digest the food-materials, or other causes, it is impossible to say with certainty. Probably the matter is extremely complex, however.

3. The amount of water in the medium. I have repeatedly pointed out my suspicion that variations in the growth result from variations in the precipitation or absorption of water in the culture-cells. It is not easy to trace the effects to mere dilution of the food materials, however, and it is quite possible that in all cases slight, but perceptible, changes of temperature accompany these evaporations and precipitations, &c. In some cases, at any rate, the dilution is traced to precipitations due to slight draughts, causing

cooling of the cover-slips, so that here, at any rate, the effect is at least in part due to temperature.

These reflections seem justified by the following measurements in the culture last referred to :—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
8.44 P.M.	94·5	—	—	—	31·25
8.54 „	105·75	10	15·25	1·5	30·5
9.5 „	117·0	11	11·25	1·1	29·5
9.15 „	126·0	10	9·0	0·9	29·0
9.28 „	132·75	13	6·75	0·5	31·5
9.40 „	135·0	12	2·25	0·19	32·25
9.54 „	139·5	14	4·5	0·3	31·5
10.4 „	139·5	10	0	0	31·5
10.13 „	144·0	9	4·5	0·5	30·25
10.20 „	146·5	7	2·5	0·36	29·75
10.30 „	149·0	10	2·5	0·25	29·25

Curve 152, p. 442.

We see that the growth had become very much slower, and at last had all but ceased. At 8.30 next morning the short crop had begun to try to form a few feeble spores; two control cultures, at 22° C. all the time, had grown more evenly, and developed a crop probably 100 times as big, and had not yet passed over into the spore stage, though they did so during the succeeding forty-eight hours, and then represented a far larger crop, with much more numerous and well-developed spores.

Further criticism of these November 7 experiments confirms the conclusions already arrived at. Thus, during the first hour at 29·5, rising to 32·6° C., and therefore at the large range of 3·6° C. the doubling nevertheless only took twenty-nine minutes; whereas after three hours at similar temperatures, 29·0—32·25° (a range of 3·25) it had not anything like doubled in *two hours*, *i.e.*, the time of exposure to this range tells by slowing the doubling period.

On December 2 spores were put in, at 22°, normal gelatine at 9 A.M., and into measurement at 2.30 P.M., as follows :—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
2.30 P.M.	24·75	—	—	—	30·75
2.35 „	—	—	—	—	29·5
2.40 „	—	—	—	—	29·75
2.45 „	—	—	—	—	30·0
2.50 „	36·0	20	11·25	0·56	30·1
3.0 „	45·0	10	9·0	0·9	30·2
3.13 „	58·5	13	13·5	1·0	30·5
3.22 „	72·0	9	13·5	1·5	30·8
3.30 „	87·75	8	15·75	1·9	31·0
3.40 „	112·5	10	24·75	2·4	31·0
3.48 „	135·0	8	22·5	2·8	31·0
3.58 „	158·0	10	23·0	2·3	31·2
4.6 „	180·0	8	22·0	2·8	31·25
4.15 „	202·5	9	22·5	2·5	31·75

Curve 153, p. 442.

Normal doubling periods:—

45— 90 μ = 3.0 P.M.—3.31 P.M. = 30 minutes at 30·2 —31·0° C.
90—180 „ = 3.31 „ —4.6 „ = 35 „ 31·0 —31·25° C.
180—360 „ = 4.6 „ —5.3 „ = 57 „ 31·25—32·9° C.

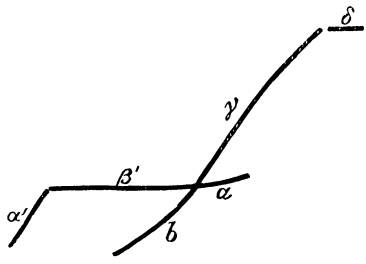
This filament was then left for over half an hour, the temperature continuing the slow but steady rise already seen to have set in, and on resuming the observations it had broken up in a manner very characteristic of such cultures. Since I have not as yet described any of these cases—which occur quite commonly, however—I propose to enter into the details of this one, as an instructive and typical example.

When the filament was measured at 4.6 it showed a slight angular bend near the middle, like this 

, the segment *a* being 85·5 μ long and *b* 94·5 μ long—total length 180, as registered in the table; when left at 4.15 *a* had grown to 95 μ and *b* to 107·5 μ —total 202·5 μ as seen.

On resuming the measurements at 4.50 several interesting changes in length and position were observable. The segments *a* and *b* had separated, and grown across one another as seen in the accompanying diagram.

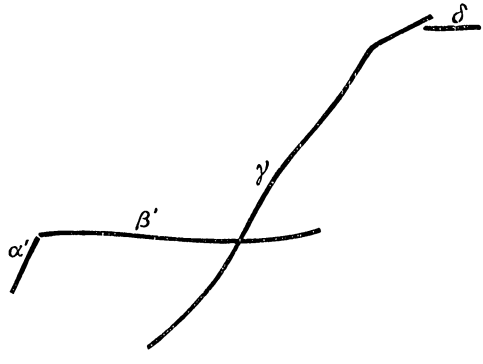
Not only so, but *a* had bent at nearly a right angle into two segments—a short one, α', and a long one, β'—measuring 45 μ and 101·25 μ respectively, while *b* had similarly segmented into a long portion, γ, measuring 153 μ, and a short one, δ, measuring 22·5 μ altogether, but really consisting of a piece of empty sheath—not represented in the diagram—and a piece of solid and normal-looking filament, the latter measured 14 u, and the connecting



piece of empty sheath 8·5 μ. In fact, the disjointing segment δ had *contracted away in its sheath* from the parent filament γ. During the next five minutes the bit of sheath disappeared, and the *rodlet began growing again* as an independent segment.

Now, it is perfectly feasible to measure all these portions of the original filament, only, of course, it takes two or three minutes to accomplish the necessary movements of the culture—to bring the various segments at angles under the scale—and to turn the eye-piece and read off the measurements. Consequently, one has to record the *mean* time of measurement, and a corresponding slight lack of accuracy results in the curves.

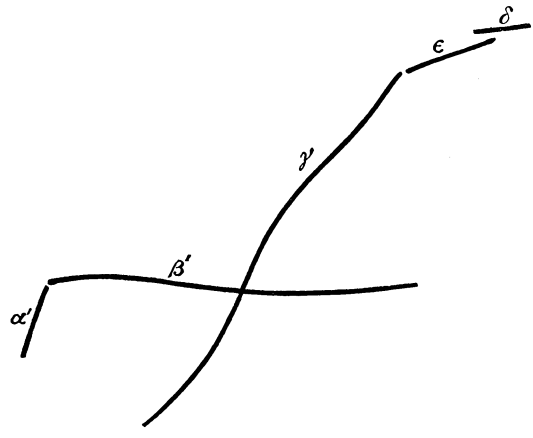
At 5.1, *i.e.*, eleven minutes after the last record, the various segments were grouped as in the following diagram:—



And measured as follows:—

α'	= 48·25 μ	having grown		3·25 μ
β'	= 108·0 μ	„	„	6·75 μ
γ	= 180·0 μ	„	„	27·0 μ
δ	= 18·0 μ	„	„	4·0 μ
Total..	354·25 μ	„	„	41·0 μ

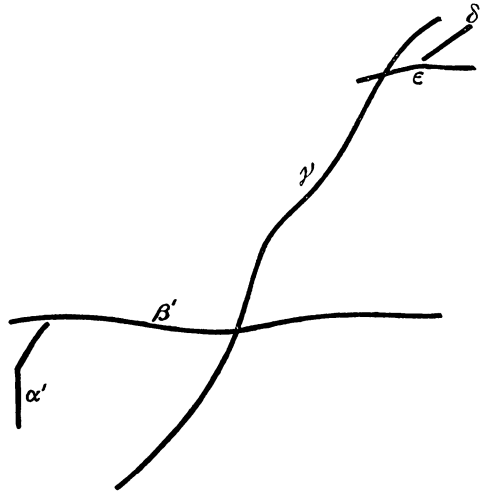
The mean time of the next record was 5·10, and the position was as in the annexed diagram:—



The first change of importance was that a segment, ϵ, already bent at an obtuse angle with γ in the last stage, had now separated from γ as an independent rod, the rest (and it) had grown as follows:—

α'	= 49·5 μ	having grown		1·25 μ
β'	= 112·5 μ	,,	,,	4·5 μ
γ	= 166·5 μ	,,	,,	22·5 μ
ϵ	= 36·0 μ			
δ	= 20·25 μ	,,	,,	2·25 μ
Total..	384·75 μ	,,	,,	30·5 μ

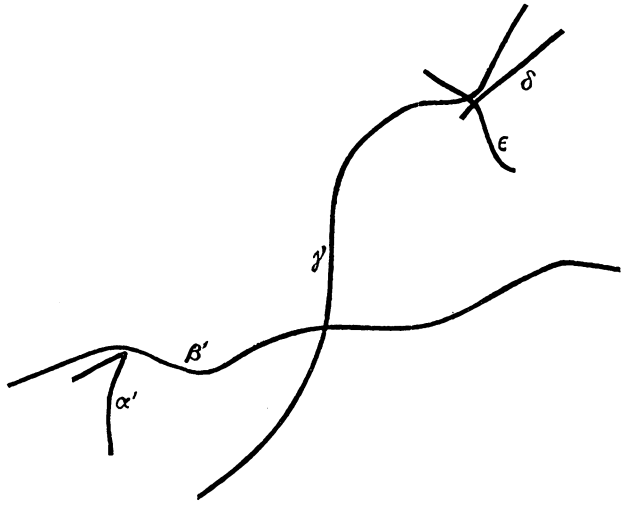
In the next phase the difficulties began to increase. The mean time of measuring was 5·21, and the change of positions and growths are seen from the following diagram and table:—



The changes in position are partly due to slow floating and partly to growth, be it noted.

α'	= 56·25 μ	having grown	6·75 μ
β'	= 121·5 μ	,, ,,	9·0 μ
γ	= 202·5 μ	,, ,,	36·0 μ
ϵ	= 40·5 μ	,, ,,	4·5 μ
δ	= 27·0 μ	,, ,,	6·75 μ
Total..	447·75 μ	,, ,,	63·0 μ

At 5.30, as near as could be judged, the diagram of positions stood thus :—



But it was no longer possible to obtain measurements on which any reliance could be placed, and so I abandoned the culture.

It is interesting at least to put the foregoing totals together as a continuation of the measurements made when the filaments were easily dealt with :—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
4.50 P.M.	313·25	35	110·75	3·16	32·25
5.1 ,,	354·25	11	41·0	3·6	32·5
5.10 ,,	384·75	9	30·5	3·4	32·75
5.21 ,,	447·75	11	63·0	5·7	32·9

Curve 153 (*continued*).

The doubling periods (of entire curve):—

24·75— 49·5 μ	= 2.30 P.M.	—3.4 P.M.	= 34 minutes	at 30·75—29·5—30·25° C.
49·5 — 99·0 „	= 3.4 „	—3.35 „	= 31 „	30·25—31° C.
99·0 —198·0 „	= 3.35 „	—4.13 „	= 38 „	31—31·75° C.
198·0 —396·0 „	= 4.13 „	—5.12 „	= 62 „	31·75—32·75° C.

Calculated back:—

447·75—223·75 μ	= 4.22 P.M.	—5.21 P.M.	= 59 minutes	at 31·75—32·9° C.
112·0 —224·0 „	= 3.40 „	—4.22 „	= 42 „	31—31·75° C.
56·0 —112·0 „	= 3.10 „	—3.40 „	= 30 „	30·25—31° C.
28·0 — 56·0 „	= 2.36 „	—3.10 „	= 34 „	29·5—30·25° C.

It is evident that even if we allow a wide margin for errors, the curve and tables show that the period of breaking up of the filament coincides with a period of slowing of the growth, *i.e.*, the high temperature was beginning to tell on the growth, and the fourth doubling period was nearly twice as long as the preceding ones.

This accords very well with the experiment of November 7, where the first doubling period was twenty-nine minutes, but that of a later rod in the same culture was found to be over 120 minutes, fully bearing out the conclusions there arrived at, namely, that the temperature begins to tell after a longer or shorter time.

This culture had broken up into short separate segments of two to five cells each at 10 P.M. ($t.$ = 31·5), and on December 3 at 9 A.M., had formed a few feeble spores here and there, while many empty bits of sheath were seen. The sister culture kept at 22° all the time was now a fine normal crop of long-coiled tresses, and must have measured several thousand times the lengths of the starved-looking crop above described. Nevertheless, the drop of gelatine in the 22° C. culture was *certainly no bigger* than that at the higher temperature, but, if anything, a little smaller. These cultures alone were sufficient to convince me that it is not a question of quantity of food-materials originally given.

On November 5 spores were sown at 9 A.M. in 10 per cent. normal gelatine, and kept at 22° C. until 3.10, when the germinated filaments were put into the Sachs' box, which stood at 35° C.

A filament was selected measuring 40·5 μ with the following results. It must be remembered that this first series of measurements was made during the period of accommodation to the high temperature, and while the box—necessarily opened for arrangement—was recovering from the consequent lowering of the temperature. Variations of temperature are much more difficult to control as they get higher.

The filaments in this normal gelatine show their septa much more sharply than in broth, and, as we see, grow fairly rapidly at these temperatures.

The curve shows that the doubling period here was:—

Normal doubling period :—

58·5—117·0 μ = 3.40 P.M.—4.9 P.M. = 29 minutes at 35·5—33·75° C.
40·5— 81·0 „ = 3.20 „ —3.55 „ = 35 „ 32—35·5—34·3° C.

Calculated back :—

141·75—70·8 μ = 3.48 P.M.—4.15 P.M. = 27 minutes at 34·5—33·25° C.

I.e., with a range of 3·5° C. it took 35 minutes to double, whereas with a range of 1·25° C. it only took 27 minutes.

But we must scrutinise cautiously these results with varying high temperatures acting on a culture in process of accommodation.

At 4.28 another filament was selected, and this was showing the slight but distinct writhing movements so often seen at high temperatures. The movement was especially noticeable in a segment, about 20 μ long, which was slowly bending backwards and forwards, and which had broken off, when 22·5 μ long, at 4.50 : since it remained close to the parent segment, however, it was easily measurable with it.

This had to be abandoned now, the measurements becoming too difficult. The rate of growth was fairly rapid, and the length was doubled as follows :—

126—252 μ = 4.28 P.M.—5.4 P.M. = 36 minutes at 34·75—35—34—35° C.

Which—taken with the preceding—shows that strong plants, once germinated out, can stand these high temperatures (even varying) very well for a time, though their growth is correspondingly irregular.

This last point is even more evident from the following, however. The culture was left at 35°—varying a little to either side—till 8 P.M., and another rodlet selected and measured, as follows :—

And on analysing the doubling periods we find a considerable retardation, evidently due to the variations in growth at the high temperatures.

Doubling periods :—

67·5—135 μ = 8.19 P.M.—9.13 P.M. = 54 minutes at 35·4—34·25—37·5—35·5° C.

Calculated back :—

166·5—83·25 μ = 8.35 P.M.—9.31 P.M. = 56 m. at 34·25—37·5—35·25—37·75° C.

It should be noted that the drop became copiously diluted after an hour or so, by absorbing water, and the longer doubling period (36 minutes with a range of 1° C.) from 4.28 onwards is possibly referable to this rather than the age of the rodlet ; though I think the latter fact of importance, as we shall see.

On examining these curves of November 5—all from one culture be it remembered—we find that when first put in the doubling took 35 minutes, at 32—35·5° a range of 3·5° ; whereas the part growing at 33·25—34·5° (a range of 1·5°) only took 27 minutes, whence may

be inferred that the slowing was due to either (1) lack of accommodation, or (2) the greater variation of temperature.

Then we find, after 1¼ hours at the temperatures given, the doubling took 36 minutes at 34 to 35°—a range of 1° C. only—which certainly suggests that it is the temperature, and *not the range* which is effective in slowing the growth.

Next—same culture, but having now been 5 hours at these temperatures—we find 54 and 56 minutes respectively necessary to double the length at 34·25 to 37·75° C., a range of 3·5°; and since the same range was experienced by the first filament mentioned, but which was measured during the first hour or so of exposure to these temperatures, we can no longer doubt that the slowing is due to the gradual effect of the temperature, and *not* to the range.

This means that at the temperatures given the rate of growth gradually slows, and the grand curve takes a shorter shape, and, on consulting the previous series of curves, the same fact becomes apparent.

On November 9 spores were sown at 11.30 A.M. in 10 per cent. gelatine, and put in at 22° C.; at 6 P.M. they had germinated to filaments over 100 μ long, and the culture was put into Sachs' box at 36—37° C.

In order to see if any body, such as CO_2, caused or hastened the running down of the culture, I put a little KHO solution in one arm of the cell, next the cotton-wool plug.* This was suggested by the experiments of November 7, and previous observations on the extraordinary irregularities and slowing of the growth at these high temperatures.

The measurements were as follows:—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
6.13 P.M.	146·25	—	—	—	36·5
6.17 ,,	168·75	4	22·5	5·6	37·25
6.20 ,,	173·25	3	4·5	1·5	37·5
6.24 ,,	193·5	4	20·25	5·6	37·5
6.27 ,,	202·5	3	9·0	3·0	37·0
6.30 ,,	225·0	3	22·5	7·5	36·5
6.33 ,,	243·0	3	18·0	6·0	36·25
6.36 ,,	256·5	3	13·5	4·5	36·1
6.39 ,,	281·25	3	24·75	8·2	36·0
6.42 ,,	308·25	3	27·0	9·0	35·75
6.45 ,,	326·25	3	18·0	6·0	36·0
6.48 ,,	360·0	3	33·75	11·2	35·5
6.51 ,,	396·0	3	36·0	12·0	35·5
6.54 ,,	423·0	3	27·0	9·0	35·5
6.57 ,,	450·0	3	27·0	9·0	35·5

Curve 157, p. 442.

* See also experiments on p. 443.

The filament then broke quite suddenly, shooting off a short piece, which I measured by itself (see below).

Doubling periods:—

146·25—292·5 μ = 6.13 P.M.—6.40 P.M. = 27 minutes at 36·5—37·5—36° C.

Reckoned back: —

225—450 μ = 6.30 P.M.—6.57 P.M. = 27 minutes at 36·5—35·75—36—35·5° C.,

and this is also the normal doubling period.

The piece which broke off was measured as follows:—

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
7.0 P.M.	63·0	—	—	—	35·9
7.5 ,,	72·0	5	9·0	1·8	35·0
7.10 ,,	81·0	5	9·0	1·8	35·0
7.15 ,,	90·0	5	9·0	1·8	35·0
7.20 ,,	100·0	5	10·0	2·0	35·0
7.25 ,,	113·5	5	13·5	2·7	35·75
7.30 ,,	122·5	5	9·0	1·8	35·6
7.35 ,,	140·5	5	18·0	3·6	35·5
7.40 ,,	158·5	5	18·0	3·6	35·0

Doubling periods:—

63—126 μ = 7 P.M.—7.31 P.M. = 31 minutes at 35·9—35—35·75—35·6° C.

Reckoned back:—

158·5—79 μ = 7.9 P.M.—7.40 P.M. = 31 minutes at 35—35·75—35° C.

On resuming the measurements at 8.30, I found considerable growth still going on, but the filaments floated about so in the now diluted gelatine, that it was not till after 10 P.M. that I succeeded in fixing one, as follows. Moreover, the temperature had risen considerably (to 39° C.), a fact which makes the following measurements only the more interesting.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
10.18 A.M.	256·5	—	—	—	39·0
10.24 ,,	279·0	6	22·5	3·7	39·0
10.30 ,,	288·0	6	9·0	1·5	38·0
10.40 ,,	303·75	10	15·75	1·5	36·5
10.50 ,,	321·75	10	18·0	1·8	35·75
11.0 P.M.	344·25	10	22·5	2·2	35·0
11.10 ,,	345·0	10	0·75	0·07	35·5
11.20 ,,	345·0	10	0·0	0·0	35·5

Curve 159, p. 442.

The filament had gradually acquired the granulated appearance which comes on when dying.

It will be noted that this curve is more depressed than the preceding, and that it has nothing like doubled itself in the hour; still, it is, perhaps, remarkable that growth occurred at all.

Here, again, we find that during the first hour of exposure to *even higher* temperatures than on November 5 and 7, it only took twenty-seven minutes to double the length at 36—37·5° C., a range of 1·5° C. (*cf.* November 5, at a range of 1·25°), or at 35·5° to 36·5° (a range of 1° C.), whereas during the second hour of exposure it took thirty-one minutes to double, though the range of temperature was only 0·75° to 0·9° C., *i.e.*, from 35—35·9° and from 35—35·75° C.

The matter is rendered the more interesting by what occurred during the fifth hour of exposure (10.18 to 11.20 P.M.), for we find that in sixty-two minutes the filament had not accomplished even half the doubling, but had slowly ceased to grow at all.

It is true, this last case taken alone is open to the objection that the temperature rose so high (35·5—39°), but, taken in conjunction with the experience of November 5 and 7, I think it is important.

Moreover, this experiment of November 9 does not support the suggestion that the gradual slowing is due to the accumulation of CO_2, or any volatile body inhibitive of the growth and absorbable by KHO. Of course it does not disprove any such hypothesis, but it shows that the slowing is not a *mere* matter of CO_2 accumulation, and (considering the capacity of my cells and all the conditions) it can hardly be a mere starvation of oxygen supply.

The results point to intense destructive metabolism, possibly respiratory, as the cause of death, and suggests that the high temperatures over-work the machinery of the cells, and, no doubt, the irregular, jerky growth shown on the curves is an expression of this. At the same time, it is not impossible that the highly stimulated organism is here so extremely sensitive to minute changes in the environment that these sudden variations are, in part, due to alterations not recorded.

On December 3 spores sown at 2 P.M., normal gelatine at 22° C., were ready at 7.50.

Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ.	mins.	μ.	μ.	° C.
8.12 P.M.	153	—	—	—	38·0
8.24 „	171	12	18	1·5	38·0
8.34 „	180	10	9	0·9	37·9
8.49 „	225	15	45	3·0	38·5

At 8.34 the filament had curved badly, and began breaking up, and at 8.49 it was broken into several writhing curved segments, difficult to measure. At 8.51 all growth had ceased, and by 9.5 the broken fragments were becoming granular, the temperature meanwhile having gone up to over 39° C.

On December 3 spores sown at 9 A.M., normal gelatine, at 22° C., were put into measurement at temperatures near 39—40° C. at 9.15 P.M. On opening the Sachs' box the temperature fell from 39·75° to 35°, and had to work up again with some difficulty, as the opening of the side windows, &c., has more effect at these high temperatures than at lower ones.

The results are as follows :—

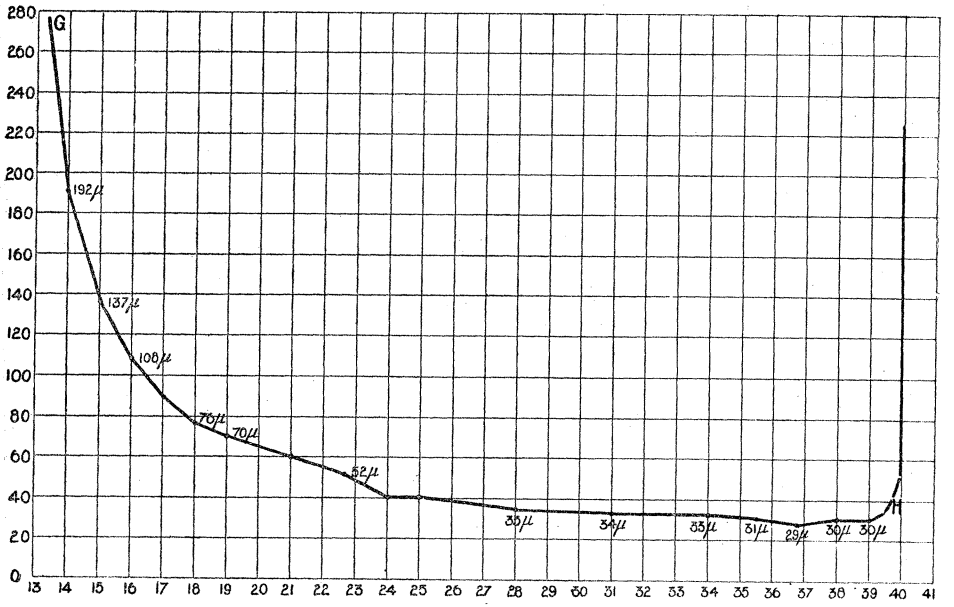
Time.	Length.	Interval.	Growth.	Rate.	Temp.
	μ	mins.	μ.	μ.	° C.
9.15 P.M.	65·5	—	—	—	35·0
9.23 „	81·0	8	13·5	1·7	37·0
9.32 „	94·5	9	13·5	1·5	37·75
9.35 „	103·5	3	9·0	3·0	38·75
9.42 „	108·0	7	4·5	0·6	40·0
9.47 „	112·5	5	4·5	0·9	41·0
9.55 „	117·0	8	4·5	0·6	41·5
10.0 „	119·25	5	2·25	0·4	41·3
10.5 „	121·5	5	2·25	0·4	41·25
10.11 „	126·0	6	4·5	0·7	40·8
10.17 „	128·25	6	2·25	0·3	40·6
10.22 „	130·5	5	2·25	0·4	40·6
10.30 „	132·5	8	2·0	0·25	41·0

Curve 160, p. 442.

At 10.39 the filament, growing no longer, sharply contracted to 117 μ, and passed over into the granular condition which indicates death.

This case is interesting, because it evidently marks the limit of possible growth of this bacillus as nearly as possible. The specimen was a very vigorous one, and there was no reason why it should not have gone on growing vigorously for some time yet at temperatures near the optimum, and, indeed, we see it began growing at a pretty good rate as the temperature rose to 39—40° from 9.15 to 9.35, and thence the curve depressed more and more, and the doubling period became prolonged to infinity.

In the curve G H, I have summarised the results of all averages of my curves of normal doubling periods obtained in this way. The ordinates are minutes, the abscissæ degrees centigrade.



The ordinates are minutes, the abscissæ temperatures.

Summary.

We see from various experiments—*e.g.*, those of November 17 and 18—that growth can and does take place as low as 8·5° C., but so slowly that it is extremely inconvenient to get a curve sufficiently long for my purposes. When we remember that it takes from about 360 to 400 minutes, or even longer, for a filament to double its length at these temperatures, it is obvious that one cannot trace many of them; for even supposing 360 minutes to be the average, it would mean twelve hours' continued attention to get two doubling periods, to say nothing of the six hours or more necessary for germination, so that variations impossible to watch may take place during the long intervals between some of the observations.

Consequently I have but few records of these slow periods at low temperatures near this.

It is interesting to note, however, that 8 5° C. is certainly not the minimum, though it probably approaches it; this is important in connection with the river habitat of the organism.

Some of the experiments show that the growth is still very slow at temperatures near 10—12° C., but it is impossible to say more than that the doubling period is here about 300 to 400 minutes or so. The observations begin to be more definite near 14° C., and we may put the normal doubling period at not far from 200 minutes.

Passing on from this point the doubling periods fall to about 100 minutes near 16° C., and the shape of the curve now begins to be apparent (see Curve G H); about 70 to 80 minutes near 18°, 60 to 70 minutes near 20°, falling to 50 minutes near 22°, and about 40 minutes near 24° C. to 26° C., 35 to 30 minutes near 28—30° C., and this rate of growth may be kept up, for a short time at any rate, at all temperatures thence to 36° or 37° C., but with certain complications to be referred to shortly.

It may be inferred from the facts, that growth occurs, at a *very* slow rate, at some minimum temperature near 5° or 6° C., and that starting just beyond an infinitely long period, the doubling period at the minimum temperature occupies many hundreds of minutes, rapidly shortening for each degree as we raise the temperature to 12—15°, and falling more slowly at each step as the temperature becomes more favourable.

It never seems to fall below about thirty minutes, however (the lowest recorded for a post-germinal filament is twenty-seven minutes), and we may therefore assume that at the quickest rate, cell-division demands thirty minutes or so for its completion—for it will be conceded that the doubling period of a filament is the expression of the time occupied by a complete cell-division and growth, since it is evident from the uniform length of the cells that when the filament is twice as long as at first, it has twice as many cells in it.

Now the experiments show over and over again that at higher temperatures—*i.e.*, temperatures beyond 25—26° C.—this minimum doubling period is only approached during the early stages of the growth at the given temperature, and when we reach temperatures near 30° C. and beyond, the filaments occupy fifty or sixty or even more minutes to complete a second or third doubling, and after a time fail to complete the period if the high temperature is maintained.

In other words, these high temperatures gradually tell on the power of the organism to maintain its best rate of growth, and the doubling period gets longer and longer the further the temperature is beyond the optimum. The optimum temperature thus comes to be that temperature at which the organism can longest maintain its ability to double its length (or, which is the same thing, complete its cell-divisions) in the shortest time, and so turn the maximum amount of food-materials into cell-substance—or, crop. This optimum temperature is 25° C. to 28° C., or very near these, as the curves show.

The maximum temperature, therefore, is not a fixed point, until we approach 39° to 40° C., beyond which no growth seems possible; but it differs according to the length of time the organism has been exposed to the high temperature.

Thus, it frequently occurs that a *first* doubling period is completed

at even 35° or 36° C., in the minimum time—*i.e.*, thirty minutes or so—but the *second* doubling of the same filament will require a longer time, and the *third* may occupy nearly twice as long, and so on, as is well seen from the curves of November 5.

Another way of putting it is the following—the higher the temperature (up to the limit) is above the optimum the *sooner* the total growth of the organism is completed, but as the *rate* of this growth does not increase beyond that of the thirty minutes doubling period, the total length of the filament produced is proportionately less than would have been produced nearer the optimum, *i.e.*, the total crop is a smaller one.

As I understand it, this action of temperature is on the life of the organism, and not a mere exhaustion of the food-medium; and I suppose that in like manner the action of the blue rays of light is similarly on the organism, and not merely on the food-medium.

But it by no means follows that the food medium is totally exempt from oxidation under the action of both temperature and light; and in the case of such extremely oxidisable media as peptone-meat-broth, and similar compounds, *the constitution of which approaches as near to the bodies concerned in metabolism as any media physiologists have been able to prepare*, we must not be surprised to find that they undergo oxidation *outside*, as well as *inside* the living cells, especially when enzymes are present, under the action of light and high temperatures.

Many of the experiments point to this conclusion, and it is particularly to be noticed that the food-medium seems to become more and more subject to such oxidations—promoted by high temperatures or by light—when the action of the peptonising enzymes, which they excrete, is in full swing, as if the whole system—cell *plus* its peptonised medium—of the hanging-drop were respiring, so to speak.

It will, I think, be worth while to institute a careful series of experiments specially directed to secure information on this head; for it opens up a very large and important question.

Elfving's researches* have, it seems to me, already rendered it extremely probable that, in the case of the fungi he examined, the action of the light is to destroy, by promoting oxidation, the constructed metabolites *at the moment they are about to be assimilated*. It looks, in fact, as if the materials to be built up in the protoplasmic structure are in a dangerously unstable—*explosive*, if you like—condition, and no doubt it is at this period that the damage is done, probably in the machinery of the cell itself, though it is not impossible that it occurs outside the actual machinery, in vacuoles, for instance.

Now peptone-broth, *when saturated with enzymes* of the kind referred to, is doubtless an extremely unstable medium, and probably in a

* Elfving, "Studien über die Einwirkung des Lichtes auf die Pilze," 1890.

condition *more like the one in which it enters into the constitution of the living substance than any other organic substance we know*—or at any rate that we can prepare and use outside the living cell.

In a certain sense then, it is possibly not absurd to compare the peptonised broth-drop, in which a bacterium cell is suspended, to a sort of inverted living cell in which the peptonised food medium should normally be *inside* the protoplasmic lining, *i.e.*, in a vacuole. In other words, we might, perhaps, roughly compare it to a vacuolated cell turned inside out, a comparison the more justifiable since the bacterium cell seems to have no obvious vacuole: instead of its assimilable food solution being clothed by the protoplasm, its protoplasm is clothed by the assimilable food-solution (of course I am neglecting the cell-membrane, and do not wish to push the analogy too far) in a highly unstable condition.

The practical aspects of this are also interesting, for it will rarely happen that a bacterium cell, or spore, escapes without organic material clinging to it—a matter of importance even if it were shown that the action of the light was entirely confined to promoting oxidations at the surface of the cells.

Taking all into account, therefore, it might perhaps be worth investigation (if methods can be devised) how far some heliotropic effects and retardations of growth in higher plants, and the retarding action of light on growth generally, are due to *destructive oxidations in the cell-sap of highly combustible food-materials at, or just prior to, the moment they are ready to be assimilated into the living substance of the protoplasm.*

It might possibly remove some of the difficulties connected with the theory of heliotropism of non-cellular cœloblasts, if the light action occurs in vacuoles next the source of illumination—though I confess I see no way out of the difficulties of so-called negative heliotropism, on this or any other hypothesis.

It certainly suggests reasons why so many adaptations occur in nature to protect fluids, which presumably contain such substances as I have referred to, by colour screens of exactly the kind we should expect to be efficient, as I have already referred to elsewhere.

An interesting result follows from the fact that the doubling period is simply the visible expression of the doubling in length and bipartition of all the cells composing the filament.

Suppose a filament to be 50 μ long, and composed of ten cells each 5 μ long, and that that filament doubles its length in thirty minutes at a given temperature: then the filament, now 100 μ long, consists of twenty cells, each of which has taken thirty minutes to divide and double itself; from this we can deduce the number of bacilli formed in a given time from the doubling periods, although the individual bacilli are themselves invisible, and when we find a curve like that of

August 4, where the filament grows from 10 μ to 652 μ in length, at 21—23·7°, with an average doubling period of about thirty-five minutes, it can be translated as meaning that the number of bacilli increased as follows:—

2	bacilli	became		
4	at the	end of the	first	35 minutes.
8	,,	,,	second	,,
16	,,	,,	third	,,
32	,,	,,	fourth	,,
64	,,	,,	fifth	,,
128	,,	,,	sixth	,,

and so on, and we may assume that if the supply of food-material could be kept constant, and no disturbing conditions set in, this would go on. If it went on for only half a day—twelve hours—there would be nearly 4,000,000 of the bacilli produced from the pair started with above, and the filament would be nearly 40,000,000 μ in length—*i.e.*, nearly 40 metres—whence some idea may be obtained of the energy of the growth on the one hand, and of the limits imposed by the culture-drops on the other; for if we take the size of a drop as 1 cubic mm., which is approximately the volume of a hanging drop such as is used in the cultures, and remember that the bacilli in question are about 1·75 μ in diameter, it will be found that the above length of 40 metres, nevertheless, has plenty of room in the drop, for the filaments have a volume of only 96,250,000 cubic μ to pack away in the 1,000,000,000 cubic μ of the drop, so that we see the latter *could* hold ten times the quantity.

We are now in a position to resume the discussion of these growth-curves in detail, and the action of temperature, &c., on this schizomycete, with more hope of success.

It is evident that the normal growth-curve is one which begins to rise slowly, and gradually gets steeper and steeper, and then slowly rises less and less rapidly until the end. This gives a curve like a long drawn out $\int$.

At the optimum temperature the growth is very rapid, and lasts for a long time, and the organism uses the materials to maximum effect and produces from them the maximum amount of its own substance—in other words, the largest "crop."

At temperatures above the optimum, however, the growth, though at first as rapid as at the optimum temperature, lasts for a shorter and shorter time, according as the temperature is further and further removed from the optimum; consequently, the curve, though equally steep in its steepest parts, begins to fall sooner, and growth ceases sooner, and the crop obtained from the same amount of original food-material is smaller and smaller according as the temperature is higher.

At length a temperature is reached where the curve is infinitely short, *i.e.*, no growth occurs at all. This temperature is, however, above 39° C., and indicates the death-point.

Taking temperatures below the optimum. There is a point, somewhere below 8° C., where the curve is indefinitely postponed, *i.e.*, no growth can occur at all. Then comes a temperature, also below 8—10° C., where the curve ascends slowly and never attains the steepness of the curve at optimum temperature. This is the minimum temperature.

At temperatures above the minimum the curve attains more and more nearly, and in shorter and shorter times, to the steepness of the normal curve, the nearer the temperature in question is to the optimum temperature.

This optimum temperature is either 25° C. or some point very near it.

The above case of the normal curve is the hypothetical one where *all* the conditions are constant, a state of affairs never realised.* During the growth, between the period when the germination is completed and the organism no longer obtains any supplies from the spore, but is totally dependent on the food-materials given it, and the period when the curve begins to ascend less rapidly, there is a period of maximum growth, during which the filament doubles its length in equal minimum times. This is the critical period of the curve. The more closely the curve approximates to the normal curve the longer this phase of equal minimum doubling periods lasts; the more external conditions affect the curve the shorter this phase is, and the longer the doubling periods become.

The factors affecting the curve may be regarded as of two kinds, internal and external, though they probably never vary entirely independently.

The internal factors are such as (1) irregularities of cell-divisions: if a single cell fails to divide in due order, the curve is at once affected, because the regularity of the intercalary growth of the filament is destroyed, and this occasionally happens. (2) The separation of the segments: several observations suggest that the growth is slowed at once when the new surfaces of the broken ends come in contact with the food-medium. (3) Nutations and oscillatory movements, though possibly these affect the *measurements* rather than actual growth. (4) Unknown internal factors which affect the rapidity of germination, the ability to assimilate the food-materials, and so forth. In some cases these may be due to pathological conditions, as in the case given on p. 392.

* Theoretically, with absolute uniformity of conditions, including food supply, the curve would go on to infinity, and the doubling periods be equal throughout; the fall above would then be indefinitely postponed.

The external factors are: (1) temperature. Variations in the curve are produced by sudden variations in the temperature, and, apparently, the variations are the more pronounced the *quicker* the temperature changes and the more *extensive* their range; but the amount of variation in the curve, due to any given rise or fall of temperature in constant time, appears to depend on the distance of the temperature (from which the variation is reckoned) from the optimum. In other words, the sensitiveness of the organism to a rise or fall of a degree centigrade varies according to the temperature from which the rise or fall occurs, for, if it has been growing at 30° C., constant temperature, for an hour, it shows a more marked deflection on the curve for a sudden rise or fall of 1° C. than for the same sudden rise or fall from 25° C.

That the variation in rate of growth which has been going on at any hitherto constant temperature is more pronounced when the rise or fall is 2° C. than when it is only 1° C. will be obvious, and similarly for any other range; but, again, it must be noted that the *amount* of deflection of the curve for *any* range of variation depends on the mean temperature, or the hitherto constant temperature, at which the growth has been going on.

The practical importance of all this on experiments on light is very great, and the difficulty, amounting almost to impossibility, of arranging two cultures differently illuminated (if the sunlight is at all intense), so that each shall be growing at exactly the same temperature during the observations, limits the absolute value of the determinations, so far as the curve of growth is concerned.

But there are other points besides (1) the actual temperature when constant, (2) the suddenness of any rise or fall, and (3) the range of temperature, referred to above.

As we have seen, the time during which the organism is exposed to any given (constant) temperature above the optimum affects the growth considerably. The general result of the observations is that the higher the temperature is above the optimum the more rapidly the organism completes its ⌒-shaped curve of growth—*i.e.*, the quicker it passes through the phase of maximum rapidity of growth into that of cessation—and consequently the smaller the crop produced from a given amount of food-materials.

For instance, if the spores germinate out at 22° C., and are made to complete their growth at 30° C., the amount of growth (or, in other words, the crop produced) will be much greater if the exposure to 30° is delayed till the *eighth* hour after sowing than if it is made at the *sixth* hour. In both cases the growth at the higher temperature is at maximum rapidity at first, and then rapidly declines. Suppose two cultures at 22° C., the spores having been sown at 12.0 noon: one is put in at 30° at the sixth hour (6 P.M.) after sowing,

when the filaments are 20 μ long, the other not until the eighth hour (8 P.M.), when 320 μ long. The first will grow at maximum rate, or nearly so, for the first hour, and the filaments double their length twice during that time, therefore = 80 μ long (at 7 P.M.), and then begin to decline; the second will also double twice in the hour (*i.e.*, at 9 P.M.), and therefore be 1280 μ long when growth commences to decline.

Suppose during the second hour at 30° C. the doubling period is twice as long as during the first hour, then the first culture will have its filaments 160 μ long, and the second one 2560 μ long at the finish (8 P.M. and 10 P.M. respectively). If during the third and fourth hours the period is again doubled, the first culture at the end of the fourth hour (10 P.M.) is 320 μ long, while the second culture (at 12 P.M.) is 5120 μ, and so on.

If both had remained at 22° C., and maintained a doubling period of, say, thirty minutes through the whole time regarded above (as experiments show would be more or less the case), then, supposing 20 μ to be the length at starting (6 P.M.), it would be 320 μ at the end of two hours (8 P.M.), and 1280 μ at the end of the next hour (9 P.M.), corresponding to the first hour at 30° above, 5120 μ at the end of the next hour (10 P.M.), and 81,920 μ after two hours more (*i.e.*, 12 P.M.).

That is to say, at the more favourable temperature of 22° C.* the filaments would attain a length of 81,920 μ in the same time as they take to reach 5120 μ, or even 320 μ, if their last four hours or so are spent at the higher unfavourable temperature.

No doubt the ideal case given could not be actually realised, but there can be no question that an approximation to it is what occurs, and the matter resolves itself into this:—At temperatures above the optimum the organism cannot get as much out of the nutrient materials as it can at temperatures near the optimum or below it, evidently because there is some more or less enormous waste of energy expended in doing something which no longer contributes to the nutrition of the protoplasm, and this the more the higher the temperature. To say that the respiration is rendered too intense in proportion to the constructive metabolism is, no doubt, true, so far as it goes; but this does not explain the probably complex matter beyond a certain as yet unsatisfactory point.

There is one aspect of the matter worth noting. I imagine no physiologist would allow that the destructive waste of energy going on here has its seat solely in the food-materials, but would agree that it is in the protoplasm of the cell; this is of importance, because we must conclude that some presumably similar waste of energy goes on

* 22° C. is chosen because it was convenient to work with, as being that used; but 25° C. is nearer the optimum.

at temperatures otherwise suitable for growth when light rays at the blue-violet end of the spectrum act on the growing cells or the spores.

Unless it can be shown that the high temperature kills the organism by acting on its food-medium *outside* the cell, the above is an argument against any such simple explanation of the action of the blue-violet rays, especially since experiments with other plants point to similar destructive actions of such rays in cases where no question of a bathing food-solution can be raised—unless we choose to regard the sap in the vacuole of a living cell as such a bathing medium, as indeed it is, in a sense. It would probably, however, seem a strange proposal in the present condition of plant-physiology to refer the inimical actions of light solely to reactions of the cell sap—though the possibility could perhaps not be denied.

(2.) The second external factor to be considered is light.

The experiments show beyond all cavil that light-rays of higher refrangibility bring about the death of the spores at all temperatures worth consideration;* in this case the curve of growth does not come into account. The evidence also shows that these rays depress the otherwise normal curve; but the difficulties begin here, because we have no means of expressing the intensity of the light used in terms similar to those used in reference to temperature.

It is proved that a light of low intensity, passing through screens which transmit only blue-violet rays, kills the young filaments at low temperatures, which in the absence of these rays does not injure the filaments at all. It is also proved that these light-rays at even higher and more favourable temperatures, seriously retard the growth of more advanced filaments, so that their curve is much more depressed than the curve of similar filaments at the same temperature but protected from the blue-violet rays.

When the temperatures are very favourable to growth, however, it is often difficult to determine quantitatively the effect of the light-rays on the curve of growth, because the latter can only be observed for a period too short for the effect of the light action to be measurable; even in these cases, however, the curve is frequently seen to be commencing its depression towards the end of the observation period, and the cultures exposed to the light are seen to be deficient in crop or in spore-production subsequently.

At temperatures above the optimum it is extremely difficult to judge of the damage due to the light apart from injury due to the temperature, but the general conclusion seems likely that high temperatures act so much more rapidly than the light that most of the disastrous effects are due to the former.

* *I.e.*, it is no use discussing temperatures known to be dangerous on their own account.

From all this it must be inferred that temperatures from the minimum to the optimum are antagonistic to the injurious light-action, and are the more effective in this respect the nearer they are to the optimum; temperatures above the optimum, on the other hand, help in the destructive light-action, or, rather, co-operate with the light in killing, or in retarding the growth.

(3.) The third external factor which helps to complicate these curves is the nature of the food-medium, and here, again, it has been extremely difficult to obtain quantitative results. The following general conclusions seem deducible from the experiments.

Under otherwise similar conditions in the dark the doubling period seems to be lengthened by the addition of gelatine, and the optimum temperature raised somewhat. How far this is due to impeded access of oxygen, owing to its slower diffusion through a more solid medium, or a similar impeding of the action of solvent enzymes, or to mere mechanical opposition to growth, or diminished access of water or food solution to the organism, could not be decided. Experiments with stiff gelatine at lower temperatures suggest the co-operation of all these obstacles; at higher temperatures it seems probable that no mechanical opposition to growth occurs, and that since the warm fluid gelatine absorbs much water, the difficulties regarding that agent disappear also, but it is not improbable that diffusion is still slower than in broth.

Whether the shorter doubling period and lower optimum in broth are due entirely to the absence of the above obstacles, or to the food-materials being really in a more suitable condition for direct assimilation by the cells, must also remain undecided. That the latter factor is not unimportant, however, is borne out by a few experiments made with less suitable food-liquids—*e.g.*, glucose solutions—and is, of course, likely on other grounds.

(4.) That access of oxygen is necessary for the growth follows from direct experiments, and also from the extraordinary depression of the curve when another oxygen-consuming bacterium gained access to the drop. Experiments where KHO was put into the arms of the cells did not support the idea that it is the mere accumulation of CO_2, due to respiration which ultimately causes the depression of the curve at high temperatures, however, and further experiments are needed to show how far, if at all, the organism can endure partial pressures of oxygen.

(5.) Some of the experiments, especially at higher temperatures, indicate the importance of water, the general necessity of which will of course not be disputed.

The curves seem to be affected by sudden dilutions of the drops, owing to condensation of water on the cover-slips of the culture-cells. How far this is due to mere dilution of food-materials, enzymes, &c.,

or to osmotic phenomena, or to minute changes of temperature incident to the precipitation, cannot be determined. I have discussed the collateral effects of these condensations, as difficulties in the observations, in their place, but special experiments are needed in regard to the other points.

(6.) In a few cases there is positive evidence that volatile antiseptic substances—*e.g.*, from scorched cotton wool—inhibit the growth. Here, again, special investigations are needed to determine the effects of these on the curves, a line of experimental inquiry for which the method is admirably suited.

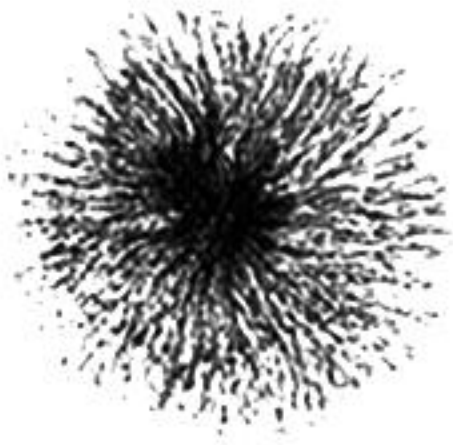
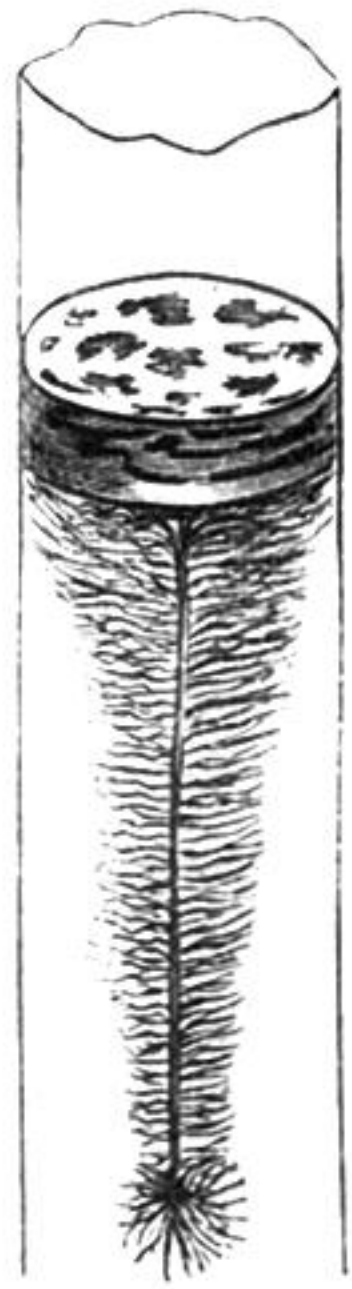


Fig. 1.

Fig. 2.

Fig. 3



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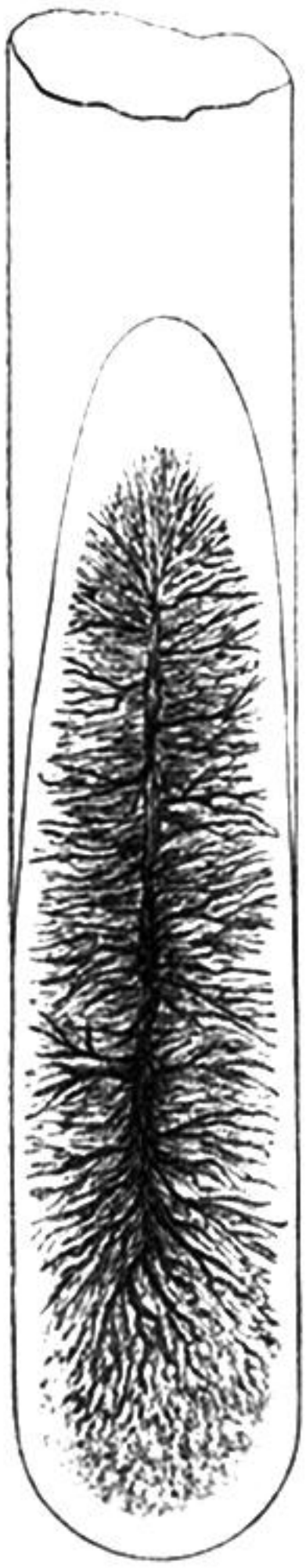

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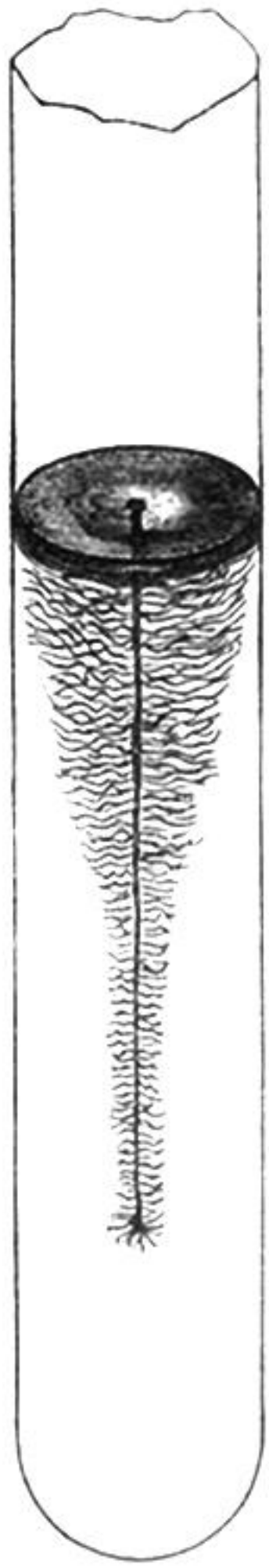

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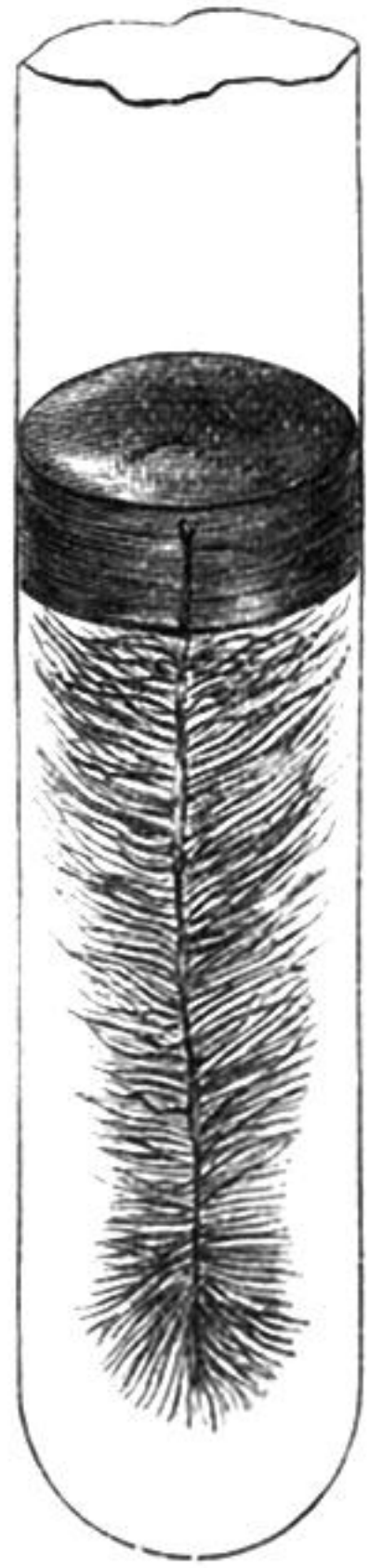

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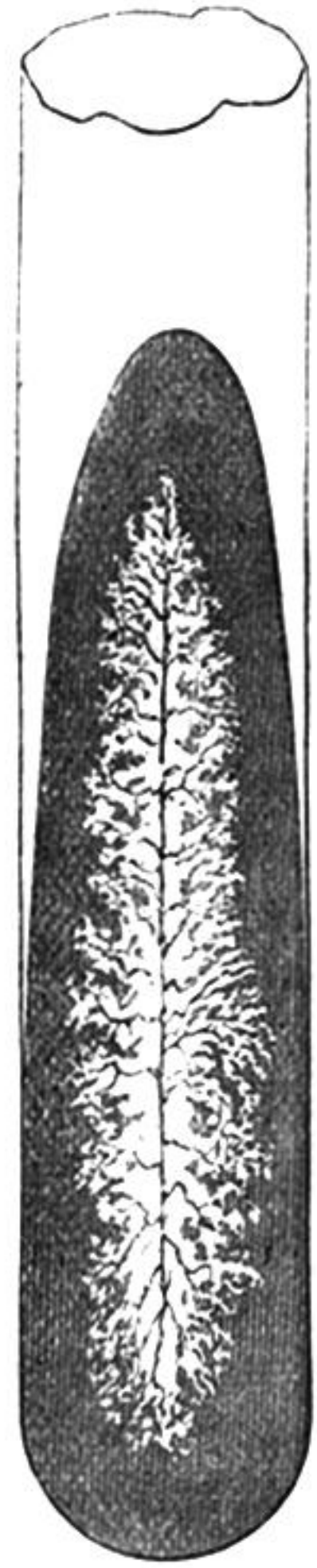

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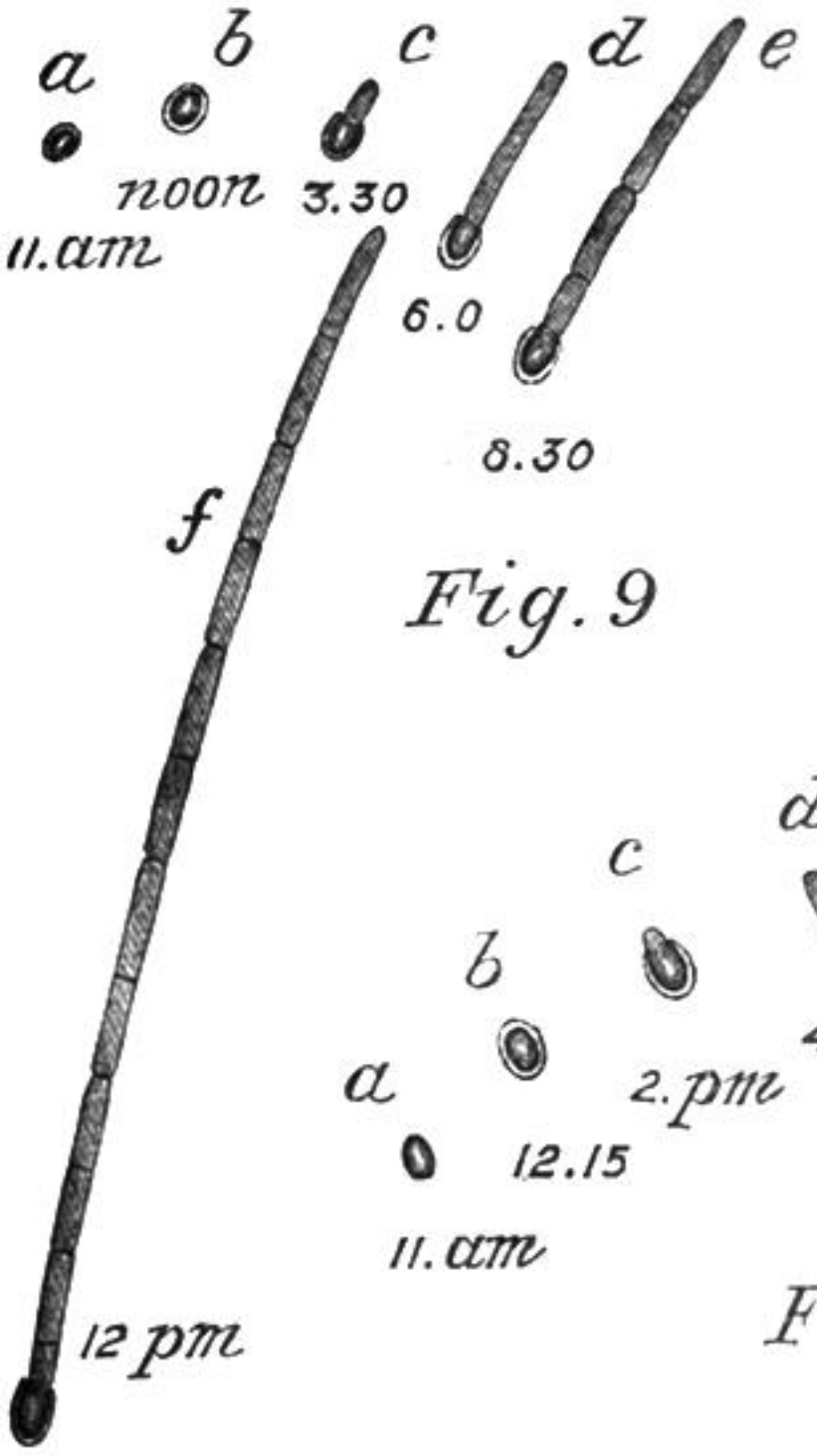

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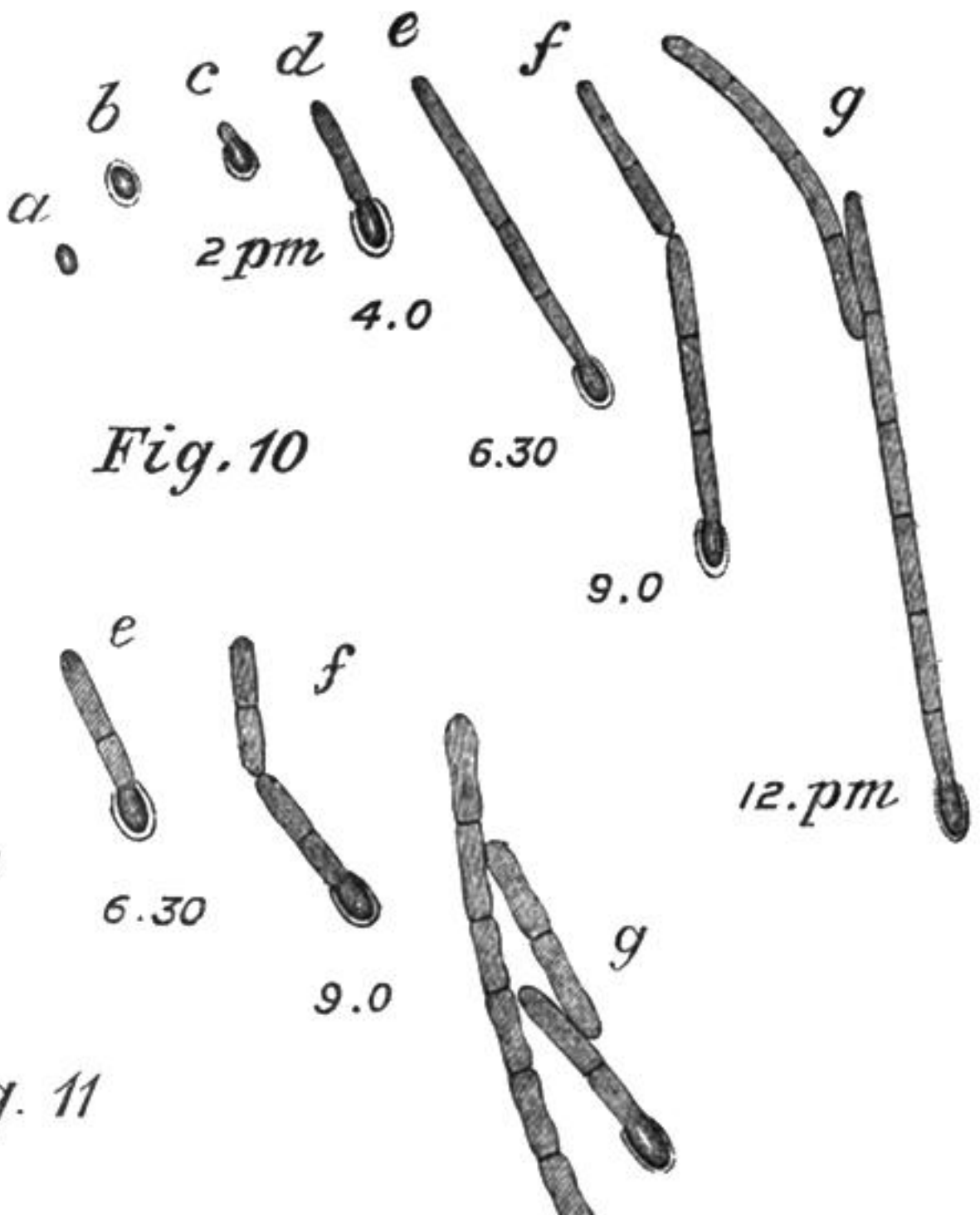


Fig. 10

Fig. 11

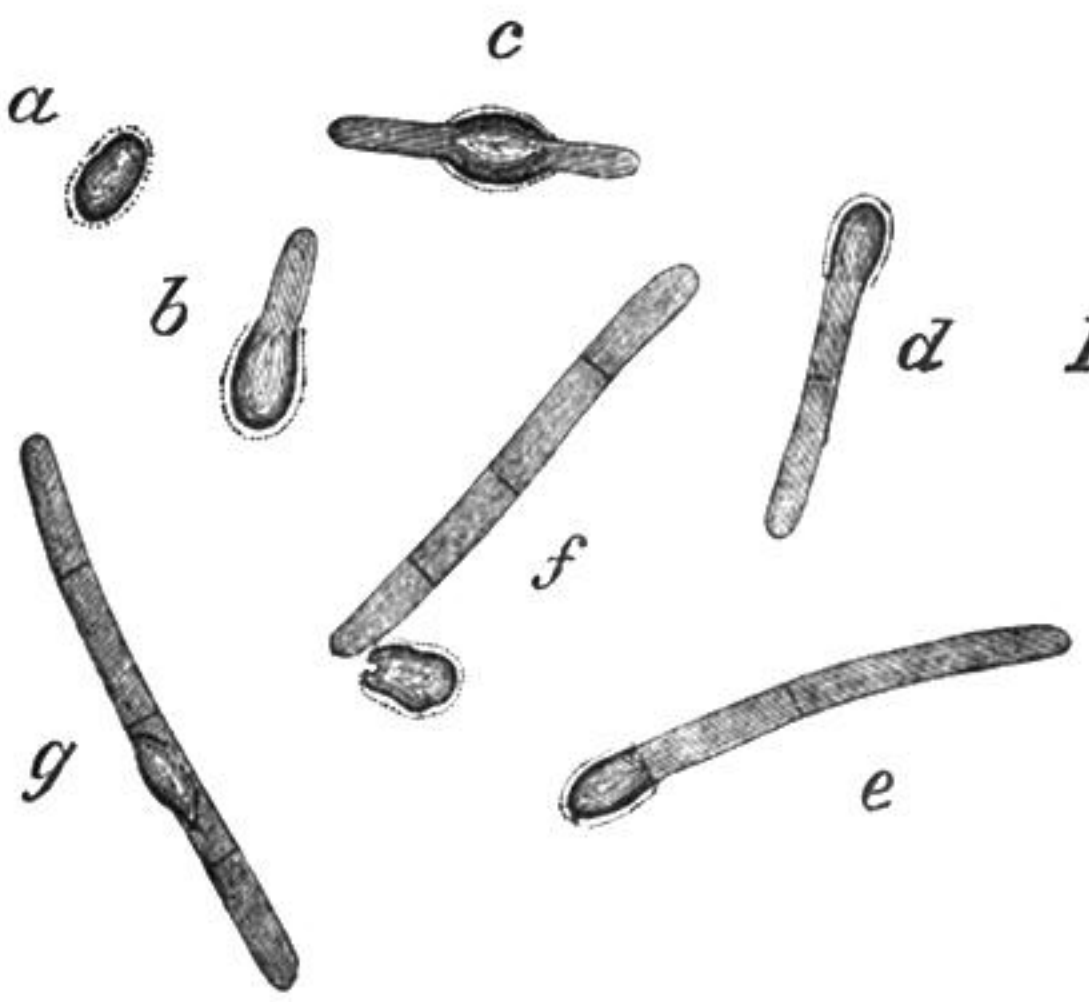
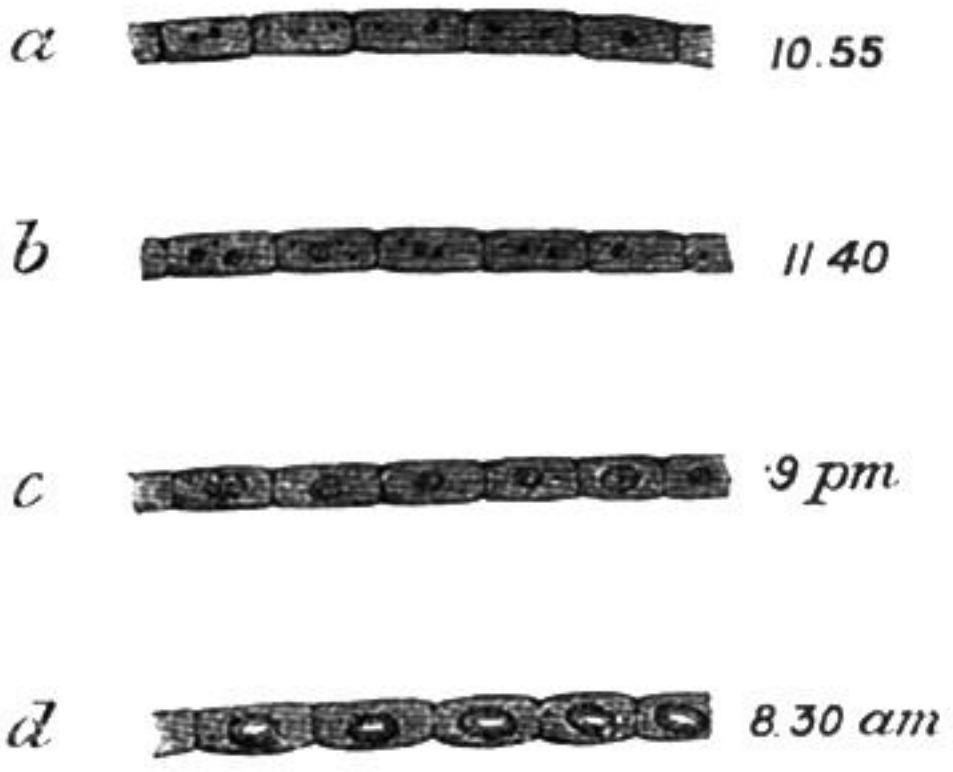


Fig. 12

Fig. 17

Fig. 13



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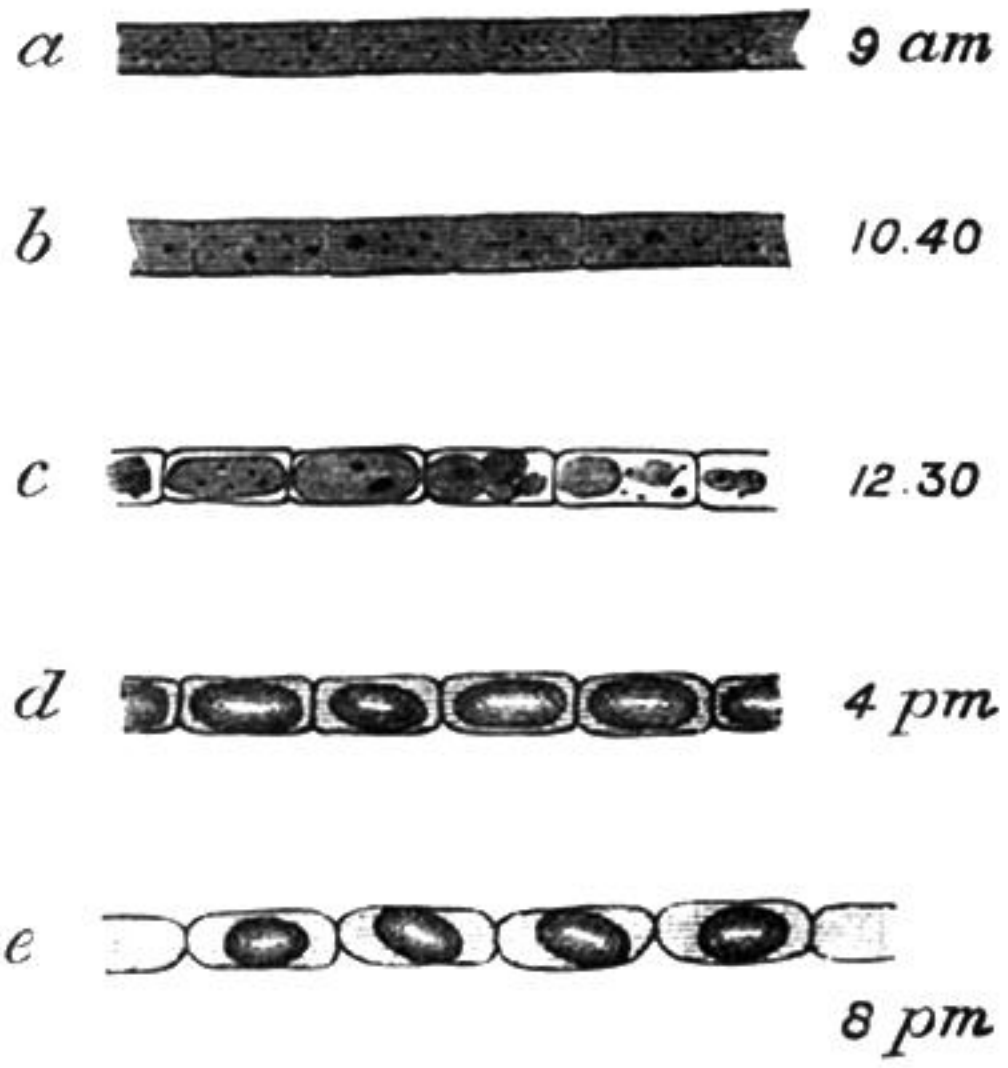

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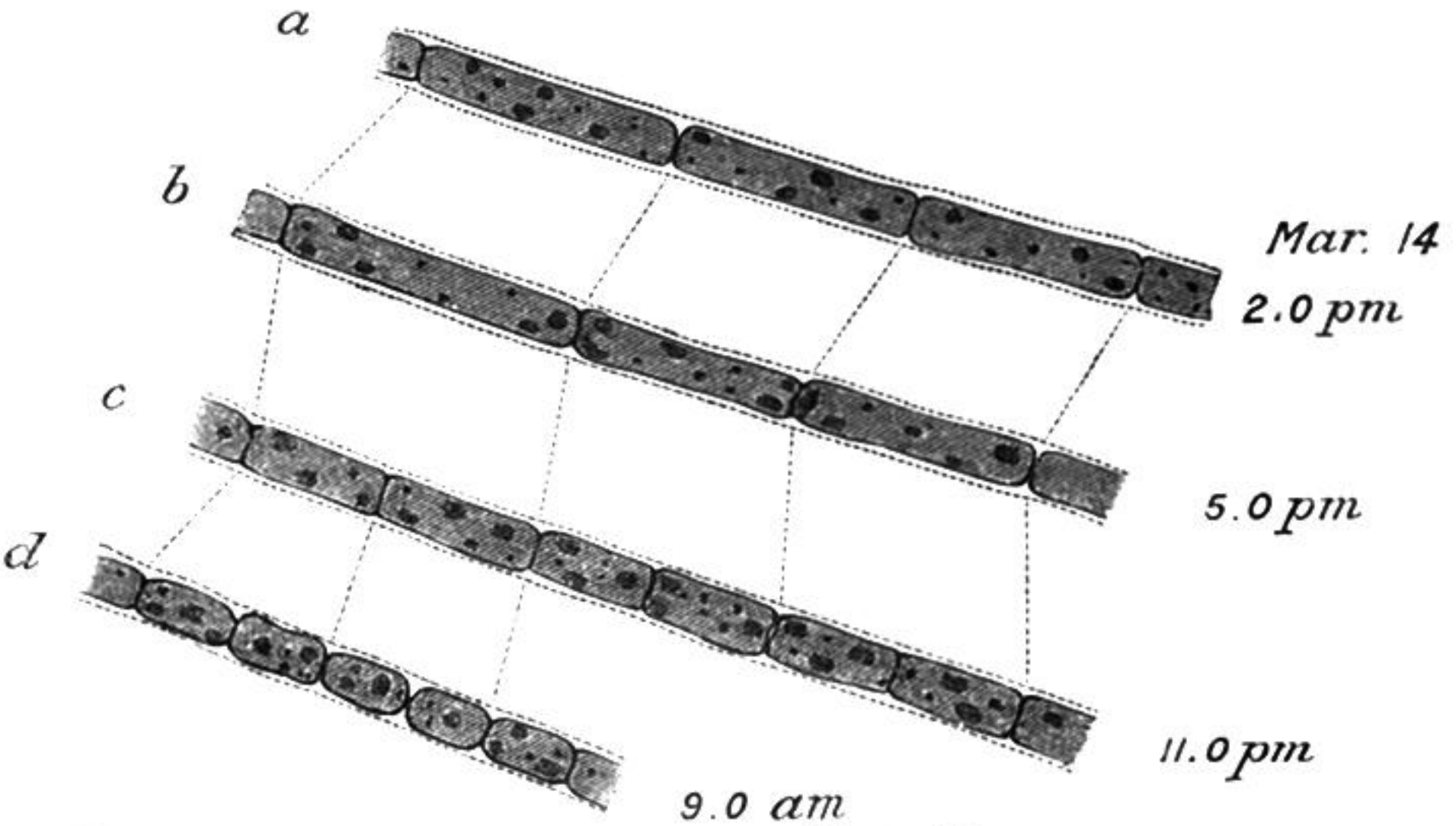

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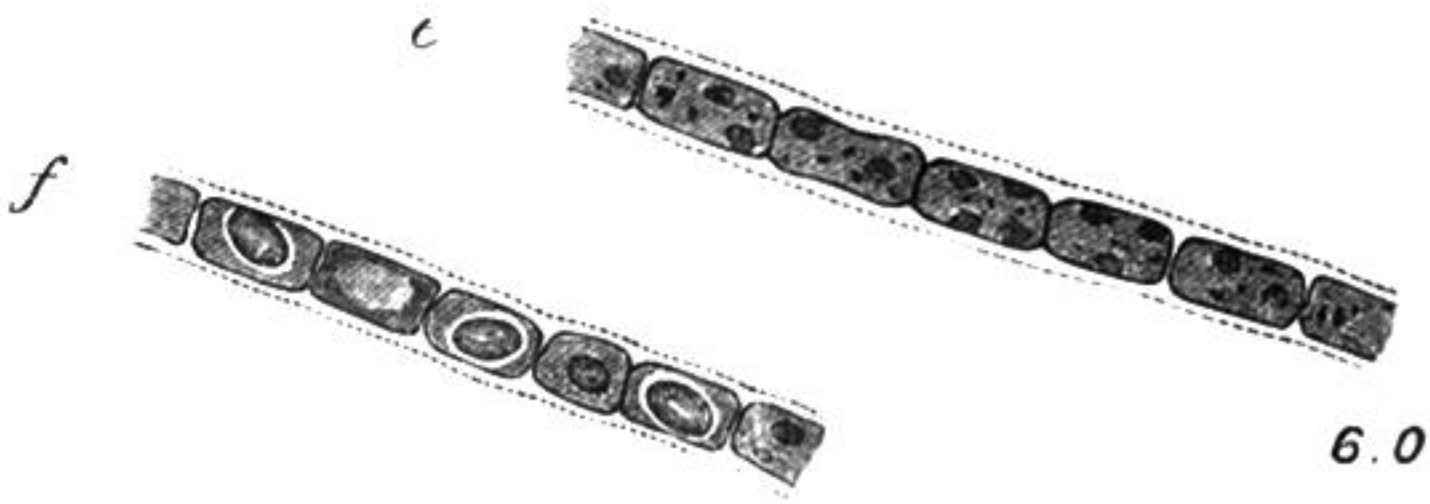

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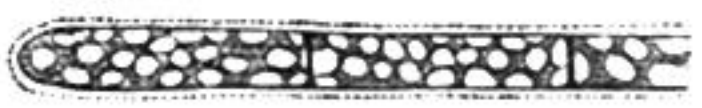

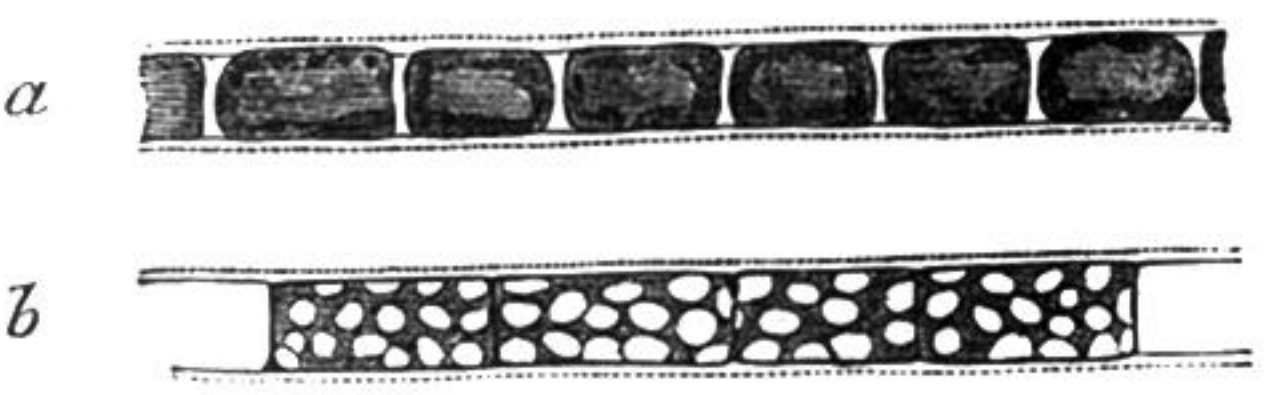

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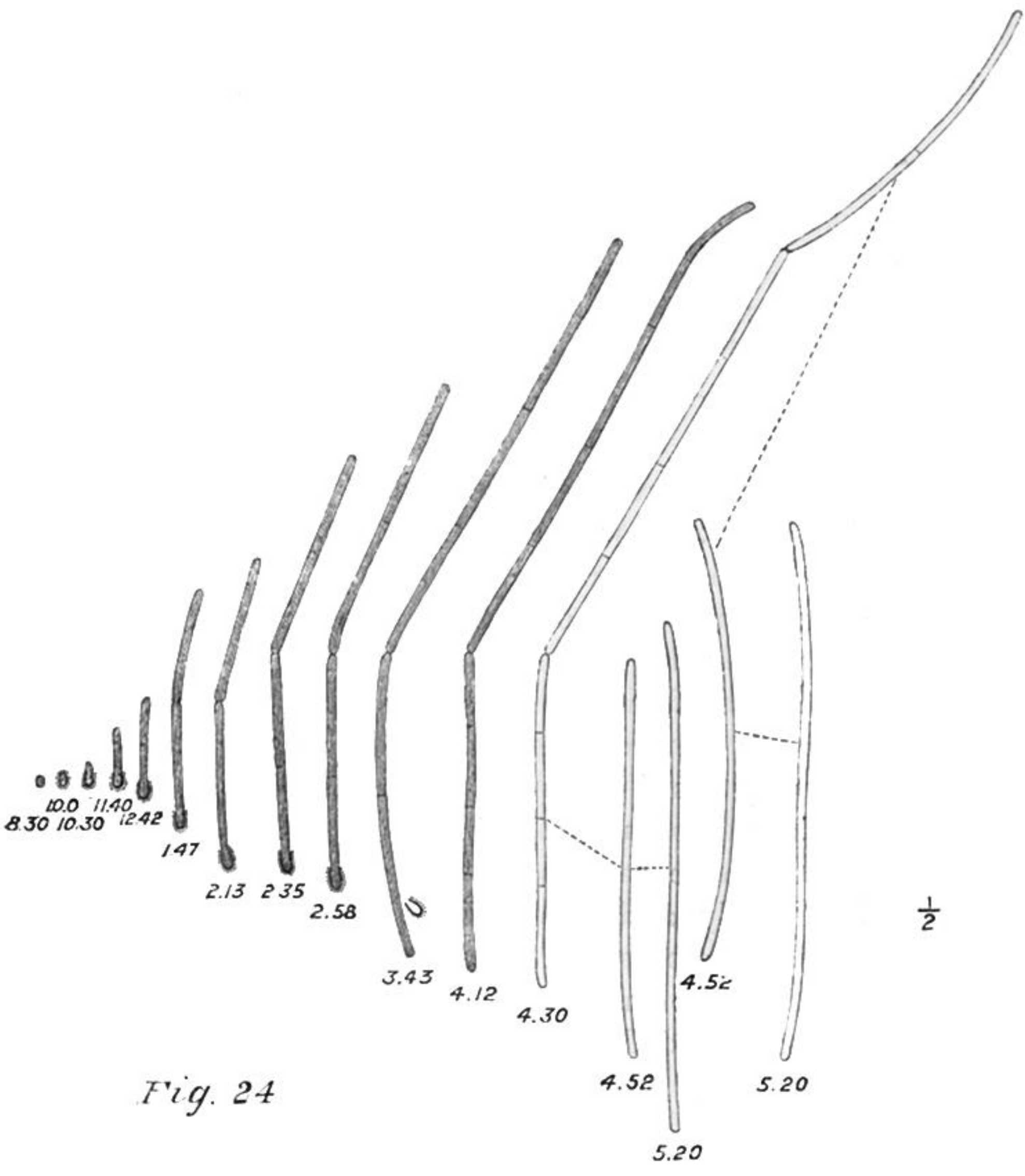
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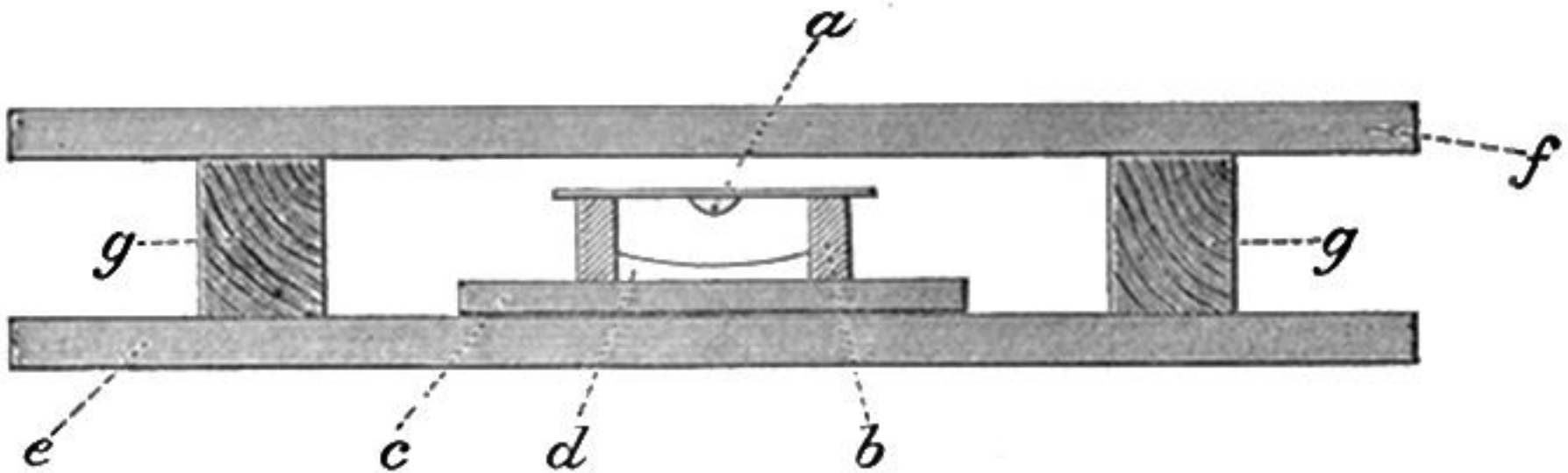
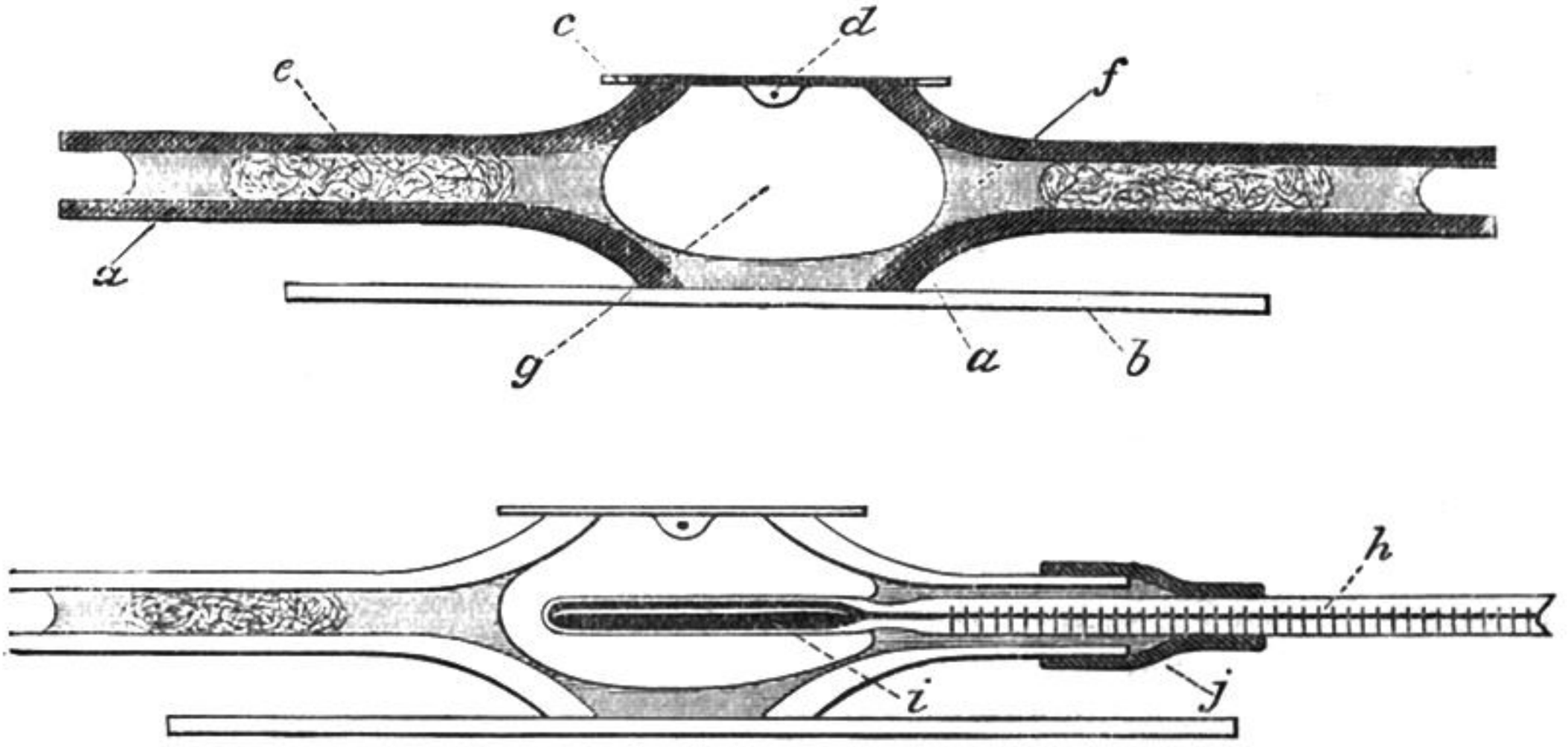
1/2

Fig. 24



a = hanging drop, suspended from cover slip forming roof of moist chamber; *b* = glass cell in section; *c* = glass slide forming floor of moist chamber; *d* = layer of water to prevent hanging drop drying up; *e* = lower coloured or other glass screen; *f* = upper ditto, supported on wooden blocks shown in section at *gg*.



a = cavity of the glass cell (moist chamber) the atmosphere in which is kept saturated by water-vapour which evaporates from the layer of water b and wet cotton-wool plugs c and c'. d the hanging-drop suspended from the cover-slip. The latter is luted to the glass-cell by stiff gelatine; while the floor of the cell is formed by a glass slide (e) cemented by paraffin melting between 55° and 60° C.

At c' the second open arm of the glass cell has been cut short to receive the bulb of the thermometer f (the bulb of which may be blackened). g and g' are the coloured glasses, so placed that all the light reaching the hanging-drop d must pass through them.