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THE DIVISION AND POST-FISSION MOVEMENTS OF BACILLI WHEN GROWN ON SOLID MEDIA.

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(Plates III—VIII and 14 Text-figures.)

The behaviour of living bacteria during artificial cultivation in fluid and semi-solid media has often been observed and described, but very few investigators appear to have studied the changes which take place when bacteria are grown either on the surface or in the depth of solid media. The investigations which are described in this paper were undertaken with the purpose of ascertaining how various bacilli behaved during the early stages of growth on the surface and in the depth of agar, and how far the various types of division and post-fission movement influenced the characters of the colonies subsequently formed.

Hill (1901) devised a method for watching the growth of bacteria on agar and carefully described the mode of growth of *B. diphtheriae* and of *B. typhosus*. His "technique consists briefly in substituting for the ordinary 'hanging drop' of liquid or jelly a cube of solidified agar, on the surface of which the bacteria are distributed. The inoculated surface of this cube is applied to the under surface of a cover-slip, and for convenience is known then as the 'hanging block.' Oxygen probably reaches the bacteria by diffusion through the block or the seal. Certainly aerobic bacteria like *B. diphtheriae, B. typhosus*, etc., grow readily in such preparations."

Hill (1902, p. 204) gives the following directions for preparing hanging block preparations. "Pour melted agar into a Petri dish to the depth of about one-eight to one-quarter inch. Cool this agar and cut from it a block about one-quarter inch to one-third inch square and of

the thickness of the agar layer in the dish. This block has a smooth under and upper surface. Place it, under side down, on a slide and protect it from the dust. Prepare an emulsion in sterile water of the organism to be examined, if it has been grown on a solid medium, or use a broth culture; spread the emulsion or broth upon the upper surface of the block as if making an ordinary cover-slip preparation. Place the slide and block in a 37° C. incubator for five to ten minutes to dry slightly. Then lay a clean sterile cover-slip on the inoculated surface of the block in close contact with it, usually avoiding air bubbles. Remove the slide from the lower surface of the block and invert the cover-slip so that the agar block is uppermost. With a platinum loop run a drop or two of melted agar along each side of the agar block, to fill the angles between the sides of the block and the cover-slip. This seal hardens at once, preventing the slipping of the block. Place the preparation in the incubator again for five to ten minutes to dry the agar seal. Invert this preparation over a moist chamber and seal the cover-slip in place with white wax or paraffin. The preparation may then be examined at leisure." In most of Hill's experiments a warm stage was made use of.

The results obtained by Hill (1901) and by Hill and Rickards (1903), who appear to be the only workers who have investigated the growth of bacteria under such conditions, will be referred to later.

Methods.

1. *Growth on the surface of agar.*

In order to investigate the mode of growth on the surface of agar the following very simple method was employed. A small quantity of melted agar was poured on to the surface of a sterile glass slide and immediately spread with a warm sterile platinum needle, so as to cover at least one square inch of surface. As soon as the agar had solidified a small drop of a very dilute emulsion of the organism to be investigated was placed on the surface of the agar, and a square three-quarter inch cover-glass was then gently lowered over the drop, avoiding as far as possible the formation of bubbles. The best plan of accomplishing this is to place one edge of the cover-glass near the edge of the agar and gently lower the opposite edge with a platinum needle. Next the agar projecting beyond the cover-glass was cut away on all sides[1], and

[1] It was found that if the cover-glass was only sealed down by means of vaseline on to the surrounding agar (without removing the excess of agar) the preparation gradually contracted and the focus of the microscope had to be constantly altered.

the edges of the cover-glass sealed to the slide all round by means of a small quantity of melted vaseline. The specimen was then transferred to the stage of a microscope placed within a Nuttall's microscope thermostat, kept at 37° C. Such a preparation can be continuously studied for many hours or days.

This method is applicable to motile as well as non-motile organisms. The bacteria cannot lie otherwise than horizontally and in the same plane. They are all necessarily in optical contact with the cover-glass and are free to grow (horizontally) without the restrictions imposed by surrounding them with jelly or the freedom to drift allowed if liquid is used.

2. *Growth in the depth.*

In order to study growth in the substance of the agar a small quantity of bacterial emulsion was mixed with melted agar at 40° C. and the latter poured on a slide. When the agar was just beginning to set a cover-glass was placed on it and the specimen finished as in the case of surface preparations.

3. *The bacterial emulsion.*

The emulsion of the bacterium to be investigated was invariably made from a 12 to 18 hour sloped agar culture. In making the emulsion sterile tap water was generally used. Even in the young cultures which were made use of a certain proportion of the organisms were found to be dead, or at any rate incapable of growth under these conditions. This was more frequently the case if a portion of the growth from the surface of the agar culture was used for making the emulsion. The proportion of dead bacteria was much smaller if the growth occurring in the water at the bottom of the tube was used.

4. *The choice of an organism for observation.*

The observations were usually made with a Zeiss 4 mm. objective and an 8 compensating ocular, artificial light being used for illumination, with sub-stage lowered and a narrow diaphragm.

For satisfactory observations a part of the preparation should be sought for where the bacteria are so thinly scattered that only a few are within the field at the same time (see Plate VI, Fig. 13). A well isolated organism, which looks capable of growth and which is lying in a

clean field, should be chosen. After a little experience it is generally easy to decide whether a given organism is going to divide or not. An organism which is likely to divide is usually larger and better defined and more opaque than one which is apparently dead. Moreover in some species the living healthy bacterium, if carefully watched, shows slight changes in outline at intervals, suggesting some plasticity in the bacterial walls.

5. *Method of recording the observations.*

After the preparation had been placed on the stage of the microscope and a suitable organism focussed, the time was recorded and the organism drawn and labelled 1. The specimen was then carefully watched, and any changes noted. Usually a long period of time, up to an hour or more, elapses before any change occurs. In most species the first important change to be seen is an increase in length, accompanied by a slight bending. Later a segmentation interval appears in the middle of the organism, dividing it into two more or less equal portions. These two parts are labelled 11 and 12 respectively in the drawings. After this division, or fission, the behaviour of the organism differs according to the group to which it belongs, but in all cases various movements take place to which the term " post-fission movements " was applied by Hill. The two new organisms (11 and 12) grow and again divide, and ultimately numerous organisms are produced and form a colony. For the purposes of identification the organisms arising from the division of 11 were labelled 111 and 112, and those from 12 were labelled 121 and 122 and so on[1].

The time at which each division or other well marked change occurred was also noted.

6. *Photographic records.*

Photographic records were also obtained, showing the mode of multiplication of a typical member of each group. When a suitable bacterium had been found the microscope was removed from the thermostat and clamped in a horizontal position on a Zeiss camera. Illumination was obtained by means of limelight or a mercury vapour lamp, and the organism focussed on a clear glass screen. The magnification varied

[1] The genealogical relationships of the new cells are indicated by Hill's (1901, p. 84) modification of Rickard's (1901) system for culture record. The modification consists merely in omitting the decimal point and all figures to the left of the point.

between 100 and 500 diameters in the different series, as it was found impossible to obtain satisfactory results with the higher magnifications with some species when growing on the surface. Growths in the depth were necessarily only slightly magnified as the organisms do not remain in the same plane. The photographs reproduced in Plates III, IV, V are shown at the original magnification, but some of those on Plates VI and VII have been somewhat enlarged. In all cases the prints were obtained from untouched negatives. Very fast plates were generally made use of in order to reduce the period of exposure as much as possible. If a long exposure is given a certain amount of movement invariably occurs and spoils the negative. The exposures usually varied between five and ten seconds, though sometimes it was necessary to give up to 30, 60 or even 90 seconds.

As soon as the exposure had been made the microscope was taken back to the thermostat and the preparation watched until sufficient development had occurred to render another photograph desirable. The greatest care had to be exercised in moving and clamping the microscope as the slightest jar was apt to completely spoil the specimen, and many were ruined by such accidents.

Owing to the frequent changes in temperature caused by removing the microscope from the thermostat to the camera development was slow, and in order to obtain a satisfactory series the preparation had to be started very early and watched for several hours.

I wish here to acknowledge my indebtedness to my laboratory assistant, Mr J. Charles, for the great help he rendered in the solution of some of the difficulties met with in obtaining these photographic records, and for the care and attention he bestowed on the negatives.

General observations.

In order to satisfactorily follow the various changes which occur in the formation of a small colony from an isolated bacillus several hours have to be devoted to watching a single preparation. Moreover owing to various causes such as interruptions, accidents and unsuccessful preparations it was almost invariably necessary to spend several days watching a number of preparations of a single species. As only occasional days could be devoted to this work the observations have extended over a period of more than two years. During this time the mode of growth of a considerable number of species of bacilli and vibrios has been investigated.

As has already been stated growth in the long axis of the original organism first occurs and then division, followed by post-fission movements. By repeated divisions and movements colonies are produced. In every species hitherto investigated a decided tendency towards the adoption of a parallel arrangement by the newly formed rods has been noted. This parallel arrangement, which may be of short duration or which may persist indefinitely, is brought about by the post-fission movements. Four different well marked varieties of post-fission movement have been observed, and according to their post-fission movements the organisms investigated have been divided into four groups.

I. The "loop forming" group.
II. The "folding" group.
III. The "snapping" group.
IV. The "slipping" group.

The differences in post-fission movement appear to be explicable on the hypothesis that the variations are mainly due to differences in the strength and behaviour of the capsule.

Up to the present however it has been found impossible to make direct observations on the behaviour of the capsule during division in the living state, either with the ultramicroscope or by any other means. That a capsule or membrane exists may be clearly demonstrated for even in crowded preparations individuals lying side by side are rarely or ever in visible contact, an interval always remaining between them. Very little information has been gained by the study of stained specimens.

It was early clearly recognised that the behaviour of an organism growing in a confined space between agar and glass might be totally different from its behaviour when growing freely on the surface of an agar plate. Parallel observations were therefore frequently made by simultaneously watching a specimen prepared by the method described and the growth occurring on the surface of an agar plate made from the same emulsion. While the former was under almost constant observation the latter was taken out of the incubator at frequent intervals and examined. The growth taking place in the two cultures could thus be easily compared. These experiments showed that bacilli growing under ordinary conditions on the surface of agar plates behave for many hours in exactly the same manner as those growing between agar and glass.

Deep colonies.

In each group the deep colonies differ to a greater or less extent from the superficial colonies, and the deep colonies produced by the organisms belonging to the four groups differ from each other in their appearance and mode of production as greatly as do the superficial colonies. Nevertheless the mode of division, the method of post-fission movement and the function of the capsule remain the same in each group whether growth is occurring in the depth or on the surface, and the differences in the deep colonies can be attributed in each case to the same variations in the strength and behaviour of the capsule, which influence the superficial growths.

Detailed account of observations.

Group I. The "loop forming" group.

The only organism belonging to this group which was studied was *B. anthracis.*

During the early stages of growth the original organism increases in length and divides, producing a chain of closely connected rods, which develops a distinct curve near its centre. This primary curve soon develops into a loop, and secondary loops are rapidly produced at the opposite side of the chain. To accommodate the rapidly increasing length of the chain further loops are produced, and the pressure they exert results in the compression of the central portions of the primary, secondary and later loops to such a degree that for long distances their walls are in apposition. Thus long wavy strands of parallel chains of bacilli are produced. Ultimately therefore the colony is composed of a central portion consisting of numerous wavy strands of parallel chains of bacilli running in various directions, and of a peripheral portion formed by the numerous loops in which the strands terminate. The terminal portions of the original thread can generally also be recognised as delicate wavy processes projecting from the colony, and often showing systems of loops like those seen in the earlier stages of growth of the primary colony.

Diagram 1 is reproduced from a series of drawings made during the development of a superficial colony from a single anthrax bacillus. Over each figure the time in minutes from the commencement of the observation is given.

In Fig. 1 the single bacillus is seen. During the first 100 minutes slow growth occurred and the bacillus divided into two bacilli of equal length (Fig. 2). Subsequently growth was much more rapid. After

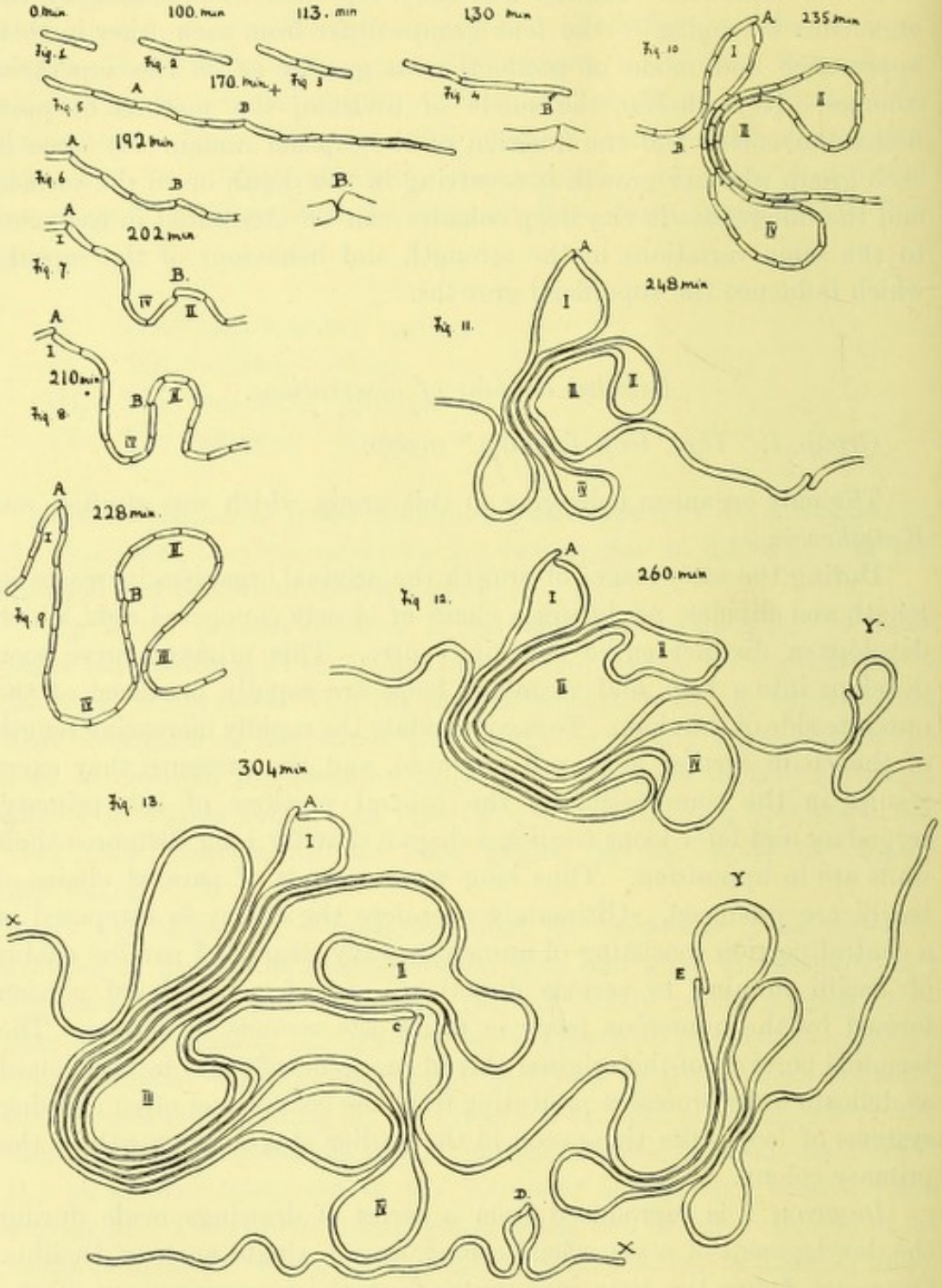

Diagram 1. Illustrating the development of *B. anthracis* on the surface of agar.

130 minutes a chain of four bacilli had been produced (Fig. 4), and after 170 minutes a chain of eleven bacilli (Fig. 5). By this time the chain had developed a decided bend at B, and a less marked one at A. Owing to the fact that the adjacent bacilli slipped slightly out of the line of the chain at these points their positions could be easily recognised during subsequent growth. It is of interest to note that the linear distance separating the points remained approximately the same, as long as the points continued to be recognisable (Figs. 5 to 10). The next four figures (6, 7, 8 and 9) illustrate the formation of a primary (IV) and two secondary (I and II) loops. Fig. 10 shows further development of these loops and the addition of another (III), and also illustrates how the pressure of the developing loops causes the formation of parallel chains of bacilli. The development of further loops and increased parallelism is shown in Figs. 11 and 12, and in the latter figure the commencement of a secondary series of loops in the right free portion of the chain is seen at Y. In Fig. 13 a complete young colony is illustrated. It consists of a single chain of closely approximated bacilli, extending freely as a slightly wavy thread over the surface of the medium at both ends. The centre of the chain is much twisted and has formed numerous loops, and bands of parallel bacilli. On the free portion of the chain extending to the right a secondary system of loops (Y) has developed. The chain is incompletely broken at four points (A, C, D and E). These incomplete breaks are no doubt due to partial ruptures of the capsule, for it seldom happens that the chain becomes completely divided at such places. The appearances seen at one of these points is illustrated on a larger scale at B, B[1].

In *Plate III* a series of photographs (× 240) are reproduced illustrating the formation of a superficial colony from two anthrax bacilli lying close together.

In Fig. 1 a long bacillus is shown with an abrupt angle near its centre. To the left of the angle and above the first bacillus lies the much shorter second bacillus. Fig. 2 (35 minutes) shows an increase in the length of both bacilli, and accentuation of the angle to such a degree that the portions of the bacillus forming it are almost parallel. Fig. 3 (120 minutes) shows a great increase in the length of the chain. That portion which originally formed the angle has grown out into a long curved process, composed of two parallel threads. The

[1] In order to avoid confusion the segmentation is omitted in the later figures, 11 to 13. The continuation of the left free portion of the chain is illustrated in the detached figure below the colony.

early formation of loops in the central portion of the chain is also seen. On the portion of the chain extending to the right a small angle may be noticed. The smaller bacillus is partly following the curves of the larger. Fig. 4 (170 minutes) shows increased growth and increased loop formation. The angle noticed on the right portion of the chain has now developed into a process and a loop is in the process of formation. Fig. 5 (215 minutes) illustrates a further advance in loop formation and the development of parallel strands of bacilli. Both the right and the left ends of the chain are now seen to be producing secondary systems of loops. Fig. 6 (292 minutes) illustrates a more advanced condition of the central portion of the colony. Parallelism is well marked and numerous loops are seen. The rapidly developing secondary system of loops on the left end of the chain is well shown. Fig. 7 (× 100) is a photograph of the complete colony after seven hours' growth. The primary system of loops is well shown in the middle and close to it on each side the secondary systems formed on the two ends of the chain. The two free ends are seen extending downwards. Above each of these is a less curved apparently freely projecting thread. These are in reality processes composed of parallel threads of bacilli produced in the same way as the process in Fig. 3. Fig. 8 illustrates the same colony after 23 hours' cultivation. Growth has mainly occurred in the central portions of the colony resulting in the production of very numerous loops, and large numbers of strands of parallel threads of bacilli. Moreover the whole colony is not now on the same plane as increased growth in the centre has caused some strands to override others.

In this series of photographs the formation of a complete colony is illustrated almost from the commencement of growth to the formation of a typical anthrax colony consisting of a raised centre of wavy strands surrounded by numerous loops and a few projecting threads.

The development of such a colony has been followed on many occasions and complete division of the original chain has very seldom been noticed. Occasionally, however, this does occur after an acute angle has been formed. It may therefore be stated as a general rule that a superficial anthrax colony consists of a single twisted chain of bacilli.

In the depth of the agar as on the surface a chain of closely approximated bacilli is first produced. As the chain grows it becomes irregularly curved; but owing to the resistance of the agar regular loops cannot be produced, and instead distorted loops, which may be

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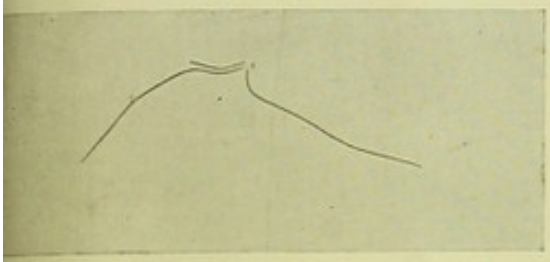

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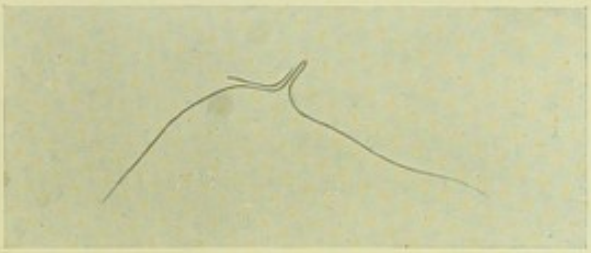

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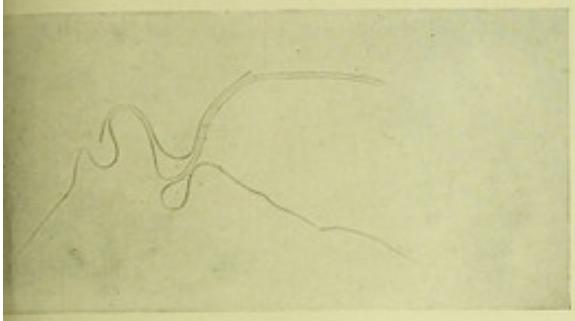

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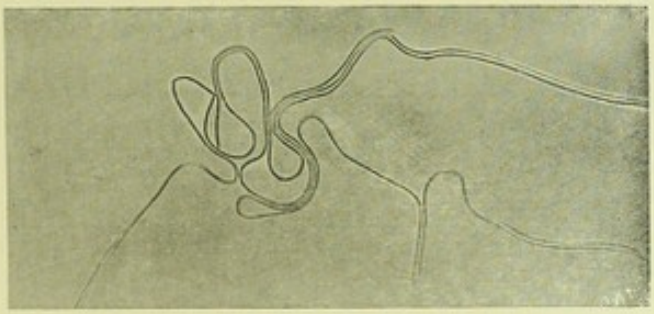

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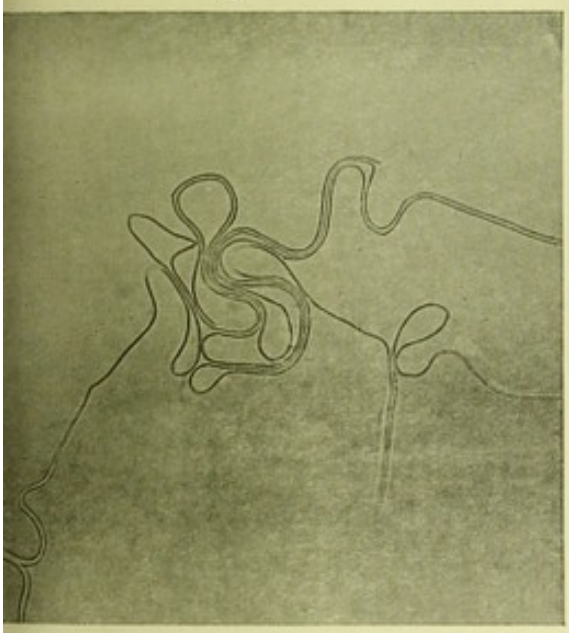

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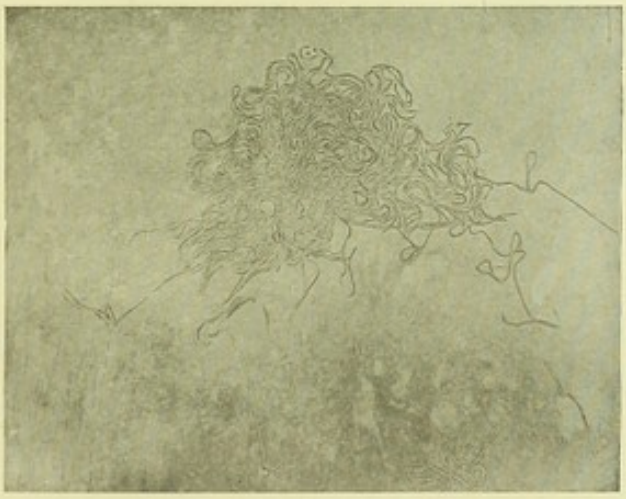

Plate I. The development of *B. anthracis* on the surface of agar.

more accurately described as lateral outgrowths composed of spirally twisted threads, are thrust out into the medium. At other places the attempt at loop formation results in the chain becoming much twisted on itself, and gives rise to the appearance of knots on the chain. Finally the colony consists either of a single knot or a series of knot-like masses, surrounded by wavy outgrowths composed of spirally twisted chains of bacilli.

Parallelism such as is seen in superficial colonies never occurs in deep colonies. In *Plate IV* (Figs. 1 to 10) a series of photographs (× about 80) are reproduced illustrating the formation of a deep colony from a single anthrax bacillus. In Fig. 1 a long wavy chain is seen, whose development from a single bacillus has been followed. Fig. 2 (15 minutes) shows an increase in length and a marked irregularity in the chain some little distance to the left of the centre. In Fig. 3 (45 minutes) the irregular area previously seen has become more distinct. In Fig. 4 (80 minutes) two small processes, just beginning to develop, can be made out one on each side of the irregular area and some distance from it. This figure also shows projecting processes one on each side of the irregular area. In Figs. 5 and 6 (117 and 135 minutes) the development of these processes may be followed. In Fig. 6 the irregular area now resembles a knot. Figs. 7, 8 and 9 (165, 210 and 285 minutes) illustrate further development. Fig. 10 (450 minutes) shows the fully developed colony. The irregular area first noticed has developed into a large knot, and several smaller knotted masses, produced in the same way, are seen along the length of the colony. Many irregular processes project laterally from the colony, some of which have developed knots in their length. At each end the colony terminates in a wavy thread.

In Figs. 6 to 10 another colony can be followed developing above the original colony, and in Figs. 7 to 10 a third colony developing below it.

Owing to their uneven disposition photographs of deep colonies have to be taken at such a low magnification that the details of the development of knots cannot be followed. While the photographs just described were being taken drawings were made at the same time on a larger scale. Diagram 2 reproduces a series of drawings showing the development of the irregular area from the condition seen in Plate IV, Fig. 2 to the condition seen in Plate IV, Fig. 5.

In *Diagram* 2, Fig. 1 (15 minutes) the structure of the irregular area is clearly shown. It is caused by several definite loops in the course of the chain, at two of which (A and B) the chain is sharply

twisted. Figs. 2 and 3 (25 and 35 minutes) show the further development of the loops. In Fig. 4 (45 minutes) continued development has resulted in the formation of a loose tangle. The knot resulting from the further development of the tangle is seen in Fig. 5 (117 minutes). The gradual intertwining of the thread composing the tangle results in the formation of a tight knot at first slightly smaller than the original tangle. The two twisted loops, A and B, seen in Fig. 1 have developed into twisted processes, A_1 and B_1. Two other lateral processes C and D arising directly from the original chain are also seen.

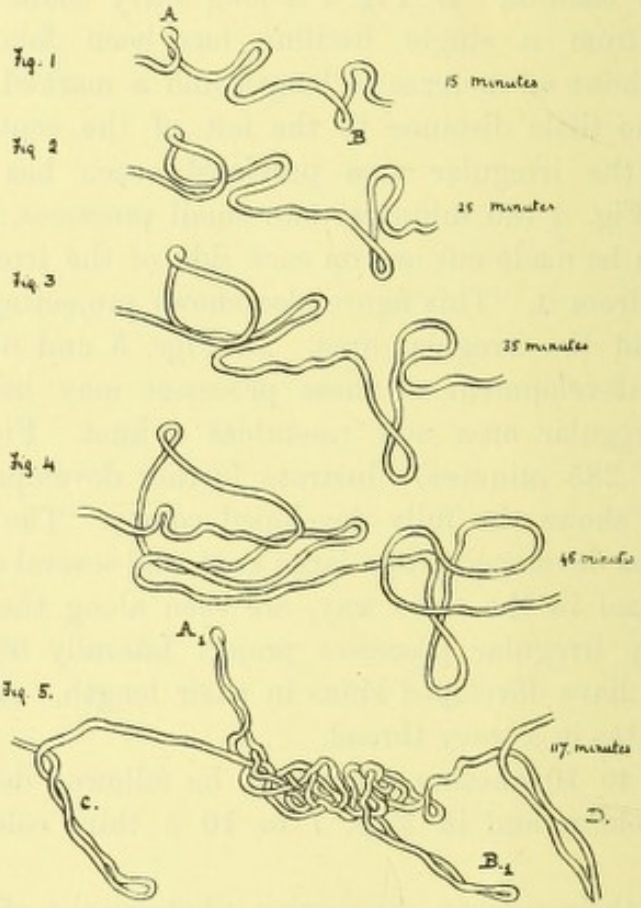

Diagram 2. Illustrating the development of *B. anthracis* in the depth of agar.

The lateral outgrowths of the deep colonies of *B. anthracis* are all due to such spiral or twisted outgrowths from the chain. These processes occasionally develop tangles or knots at various places in their length. In very rare cases only has a complete rupture of the original chain been seen.

Plate IV, Fig. 11 illustrates a typical deep anthrax colony in agar. It consists of a dense central mass with numerous more or less radial

outgrowths, in most of which knots have been formed at various places, and all of which possess secondary lateral processes.

Plate IV, Fig. 12 illustrates some young deep anthrax colonies. These consist of central knots and numerous wavy radiating processes. This preparation was made with very dilute agar which limits the growth to a much smaller degree than does ordinary agar.

Plate IV, Fig. 13 shows deep and superficial growth in the same colony. The colony first developed just below the surface of the agar and grew in the typical manner. This portion is seen in the lower left hand part of the photograph. Later a portion of the colony reached the surface and produced on it a typical superficial wavy growth seen in the upper and right hand portions of the photograph.

In fluid media long chains occur, but owing to their growth being unrestricted they produce neither loops nor tangles.

The conditions seen in stained preparations and in "contact" preparations and the observations on living specimens just described all tend to show that in the "loop forming" group, as illustrated by *B. anthracis*, although the protoplasm becomes segmented and numerous bacilli are produced, the membrane or capsule surrounding the chain apparently remains continuous, and keeps the newly formed bacilli closely connected with one another. This capsule is seldom completely ruptured, and each colony therefore consists of a single uninterrupted chain of bacilli. Under the conditions of observation, or on surface cultures, the bacilli are only free to grow horizontally. As growth is taking place, not only at the ends, but in all parts of the chain at the same time, curves ultimately developing into loops are formed in order to accommodate the rapidly increasing length of the chain.

If the centre of a piece of string is placed between two parallel glass plates, separated from each other by a distance equal to the diameter of the string, and the string is gradually pushed between the plates from fixed points on each side, the changes which occur in a young colony of a loop forming bacillus are to some extent reproduced.

In the depth loop formation is hindered by the consistency of the medium and consequently the chain as it grows tends to develop tangles, which subsequently become knots, and twisted lateral outgrowths.

Group II. The "folding" group.

During the first stages of growth on the surface the original bacillus grows in length and divides into two rods of equal length, separated from each other by a very distinct interval. By further growth a straight

or slightly curved chain composed of three, four or more distinct rods is produced. As growth progresses and more rods are formed the chain no longer remains straight or slightly curved, but develops angles at the points of junction of the various bacilli. These angles rapidly become more and more marked so that the chain exhibits a folded or zigzag appearance. By the further development of the folding process many of the rods come to lie parallel with each other, often with their long axes at right angles to the direction of the original chain. Continued growth then gives rise to the development of strands of parallel chains of bacilli, which ultimately become curved and wavy. Irregularities in the folding process not infrequently produce distorted loops.

The development of a bacillus belonging to this group on the surface of agar is illustrated in *Plate V.* Fig. 1 (× 250) shows a single, slightly curved bacillus. At its lower end a thinner pale prolongation is seen. This either represents an empty portion of the capsule or perhaps a dead bacillus. It is evidently incapable of growth and can be traced in exactly the same condition for 170 minutes (Figs. 1 to 6). Fig. 2 (75 minutes) shows an increase in length. Three bacilli are now seen, the lower two forming a slight angle with each other, and the third joined to the upper of the two at a greater angle by a faint connection, like the prolongation seen in Fig. 1. As in the former case no growth occurs in this region, which can be traced up to Fig. 6. The fact that its diameter greatly diminishes (Figs. 4 to 6) seems to confirm the view that it represents a stretched portion of capsule. In Fig. 3 (95 minutes) further growth has occurred, the two adjacent bacilli having divided into four, which lie at a small angle with each other, and the upper one having divided and formed a chain of two individuals, whose long axis is now disposed nearly at right angles to that of the main chain. Fig. 4 (125 minutes) shows the commencement of the folding process. The main chain has divided into seven individuals, and the angles formed at the junctions of the upper three and the lower three are very marked. In Fig. 5 (150 minutes) the main chain consists of thirteen individuals. The parallel arrangement produced in its upper and lower portions by the continuation of the folding process, resulting in the complete approximation of the bacilli forming the angles, is well seen. In Fig. 6 (170 minutes) the increase in the number of the bacilli forming the chain is well shown, and parallelism is a marked feature. This figure also illustrates the formation of angular loops, which are marked features in the peripheral portions of some colonies of organisms belonging to this group.

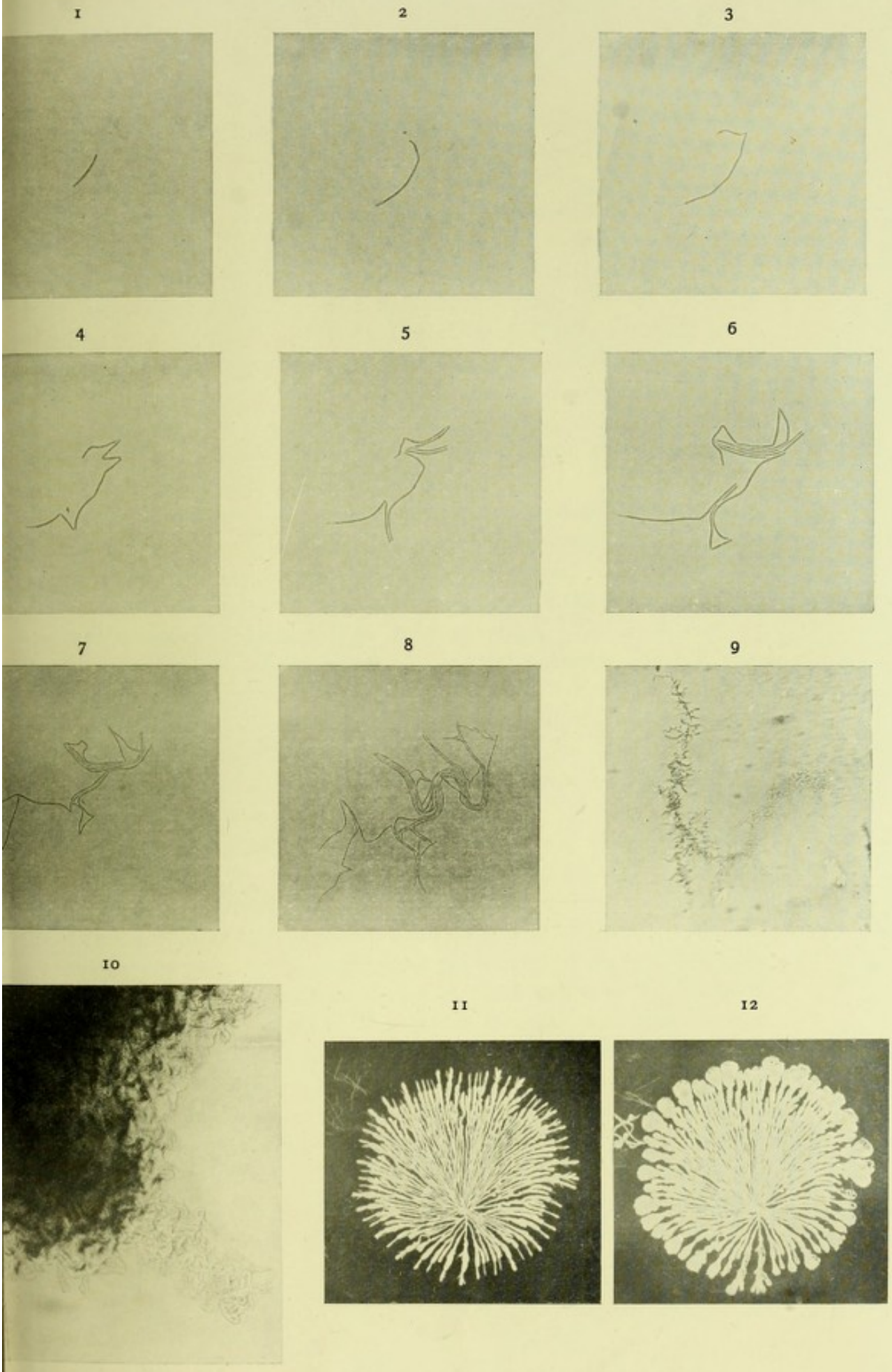

Plate III. The development of a "*folding*" organism on the surface of agar.

Such loops are produced by a modification of the folding arrangement, which is well exemplified in Figs. 4, 5 and 6. As the result of the approximation of the bacilli forming the marked angle at the lower portion of the chain (Fig. 4) a lateral process composed of two parallel bacilli is produced in Fig. 5. In the course of further growth one of the organisms forming this process grows faster than the other and the chain formed from it develops a secondary angle. The result is an irregular loop. Figs. 7 and 8 (× 150; 195 and 243 minutes) show the results of further growth. In the latter it is seen that the parallel chains of bacilli, well shown in Fig. 6, tend to develop considerable curves. In Fig. 7 a chain of bacilli from another colony has grown up and touched a prolongation of the colony under consideration at the left hand lower corner of the photograph.

In spite of the fact that the bacilli appear to have become separated at some of the angles, especially when these have become very acute, no separation really occurs in the early stages of growth. If such a chain as that shown in Fig. 5 is disturbed by a current of water its shape becomes completely altered, but it floats away without losing its continuity. In the later stages of growth complete ruptures are occasionally seen.

Fig. 10 (× about 30) shows the margin of a fully developed colony with a thin expansion composed of numerous angular loops.

Deep colonies show a modification of the process just described. The formation of one is illustrated in *Diagram* 3.

In Fig. 1 a chain with two angles and several curves is shown. Five minutes later (Fig. 2) both the curves and the angles have become better marked. During subsequent growth numerous angles are developed, each of which becomes more acute and ultimately results in a process, composed of two parallel bacilli, united at their distal ends, growing out in a direction more or less at right angles to the direction of the original chain. Most of the curves also sooner or later develop into angles. The lateral processes at a later period produce secondary lateral processes of their own in the same way. Although the parallel bacilli forming the lateral outgrowths are at first united at their distal ends, there is a tendency for the uniting portion of the capsule to rupture after the outgrowth has reached a considerable size, probably due to unequal growth in the chains formed from the two original bacilli producing the outgrowth. This is clearly seen in the outgrowth produced from the well marked angle in the centre of the chain in Fig. 1, and also in that produced from the large curve near the bottom of this figure.

In the figures the formation of various angles and the subsequent outgrowths can be clearly followed up to 135 minutes (Fig. 10). Later development becomes irregular and often results in the production of knot-like masses in the main chain, as may be seen in Figs. 11 and 12, representing the upper portion of the colony after 210 and 310 minutes growth respectively. Fig. 11 represents a later stage of the upper portion of the colony above the point A, and Fig. 12 a later stage in the development of Fig. 11 above the point B.

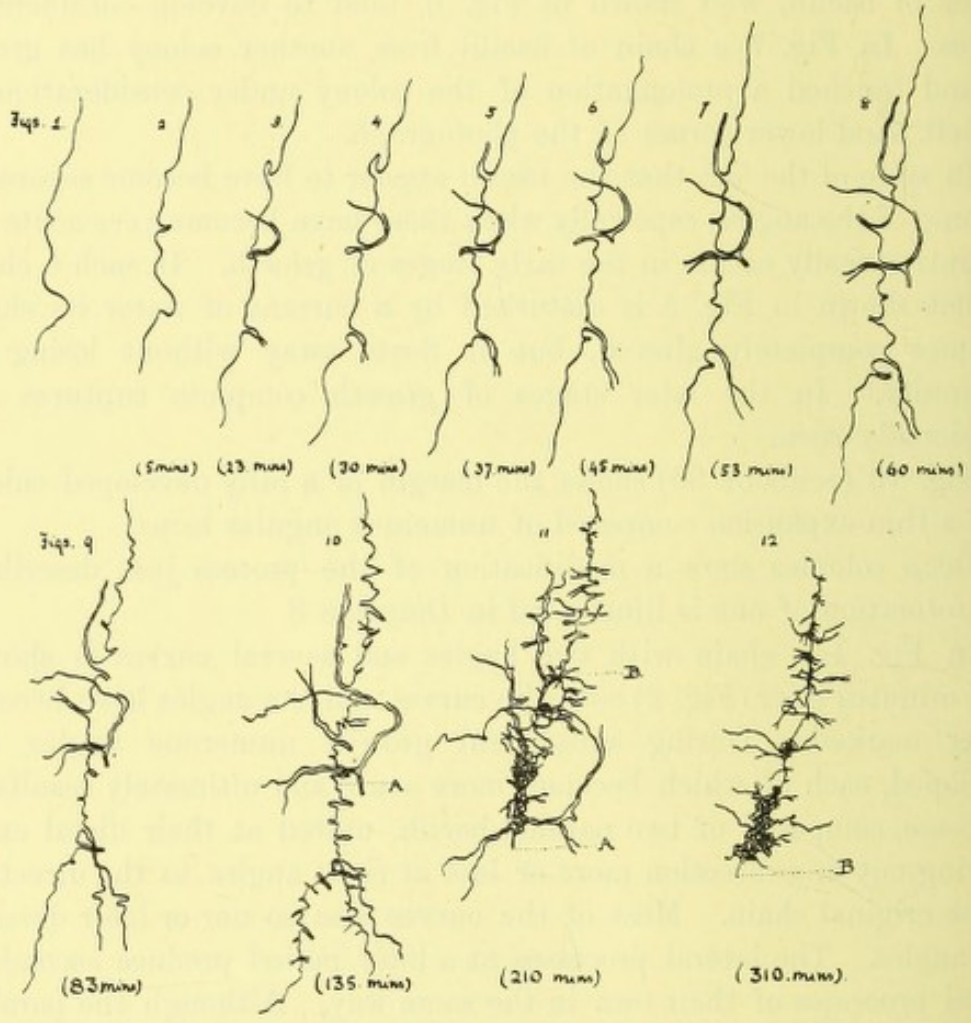

Diagram 3. Illustrating the development of a "folding" organism in the depth of agar.

Plate V, Fig. 9 illustrates the upper portion of this colony at a still later stage (6 hours).

Three organisms, morphologically resembling *B. anthracis*, belonging to this group have been investigated and all of them produce, in spite of the difference in the mode of development, colonies resembling those

of *B. anthracis*. These colonies consist of twisted strands of parallel bacilli occasionally surrounded by fringes of irregular loops (Plate V, Fig. 10). The central portion of the colony has been well described as looking under a low power lens like a mass of cracked ice.

The peculiar folding which is so noticeable during the early development of the colony is probably made possible by the length and flexibility of the parts of the capsule connecting the individual bacilli composing the chain. The length of this connecting portion of the

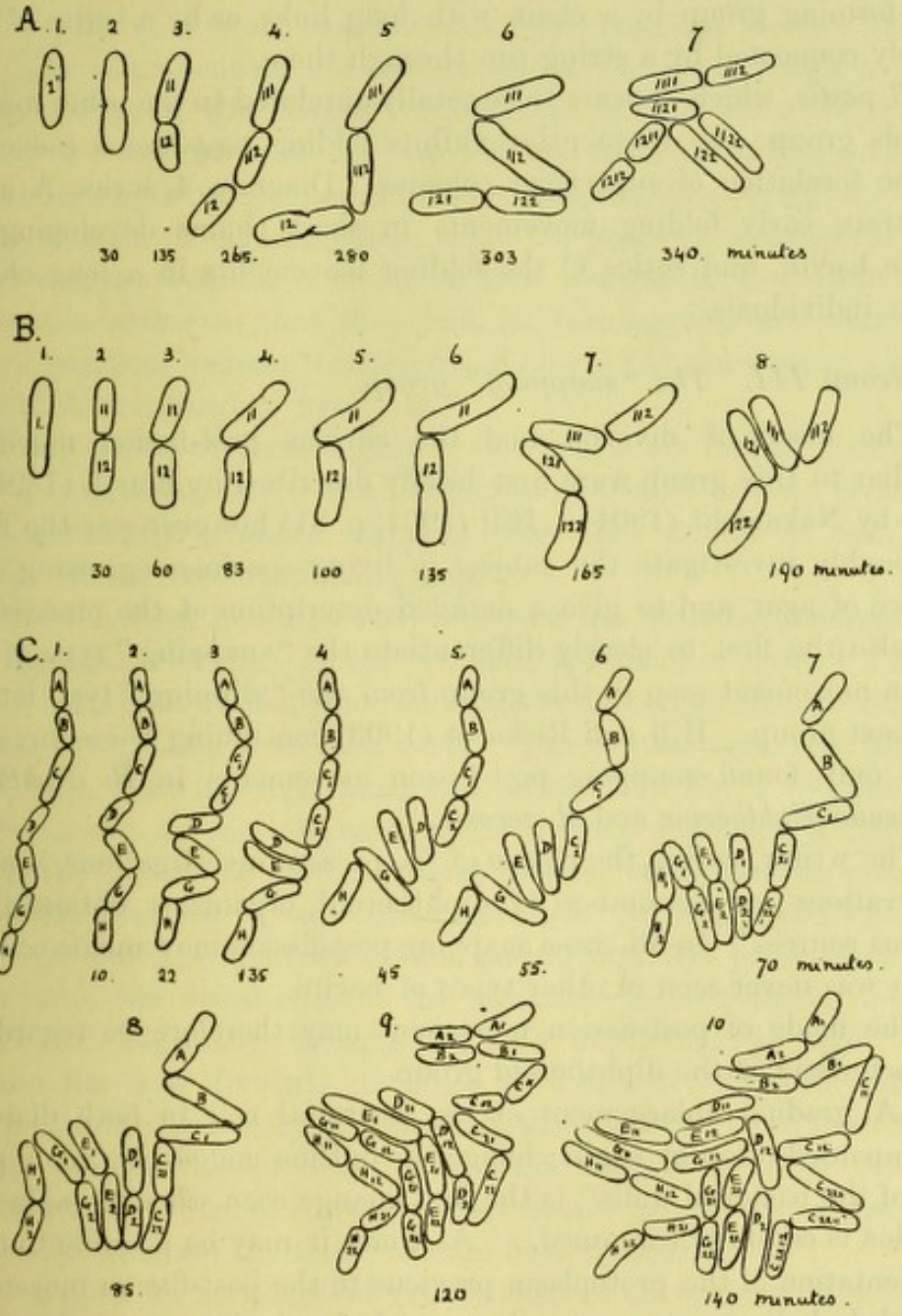

Diagram 4. Illustrating the development of *B. pestis* on the surface of agar.

In the later stages however the disturbance caused by one organism snapping moves a number of others and alters the relative positions of the members of the group.

In Fig. 9 (× about 300) two curved bacilli are seen. The upper one subsequently undergoes no change, but the lower bacillus gives rise to a small colony. Fig. 10 (115 minutes) represents the condition seen shortly after the division of the lower bacillus, and Fig. 11 (130 minutes) illustrates the approximation of the distal ends of the new rods resulting from further growth. Fig. 12 (240 minutes) shows a small colony of seven individuals formed from the lower bacillus. The upper bacillus is straighter than in the earlier photographs, but this change is probably due to pressure on its lower end produced by contact with one of the newly formed organisms.

Fig. 13 illustrates the condition of a suitable field at an early stage of development (55 minutes after the observations commenced). Single bacilli or pairs of bacilli, developed from single individuals, are scattered over the field. Fig. 14 shows the same field after six hours' growth. Here seven large colonies are seen. Four (A, C, E, G) have

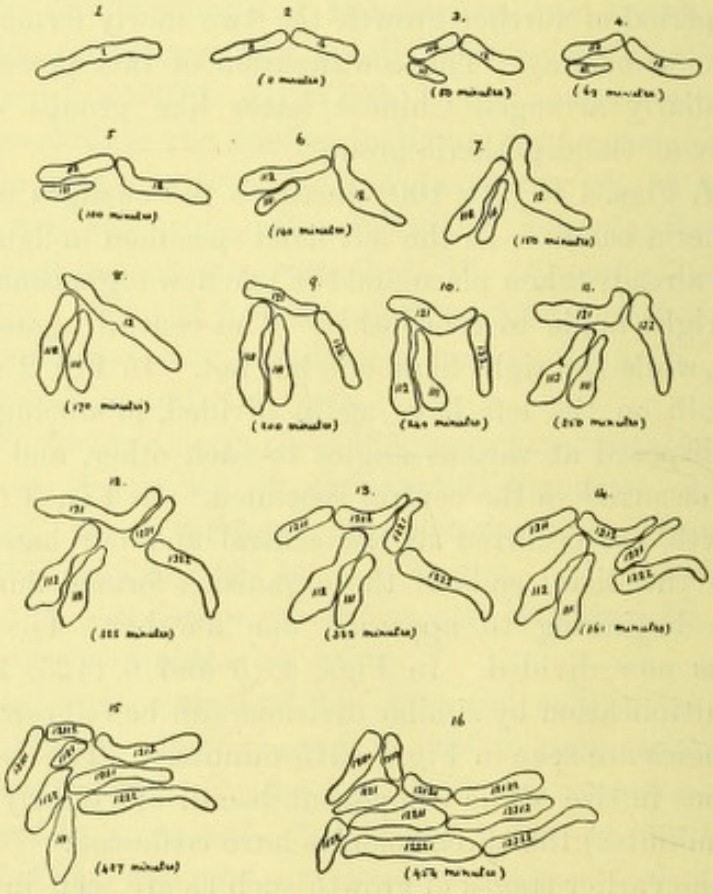

Diagram 5. Illustrating the development of *B. diphtheriae* on the surface of agar.

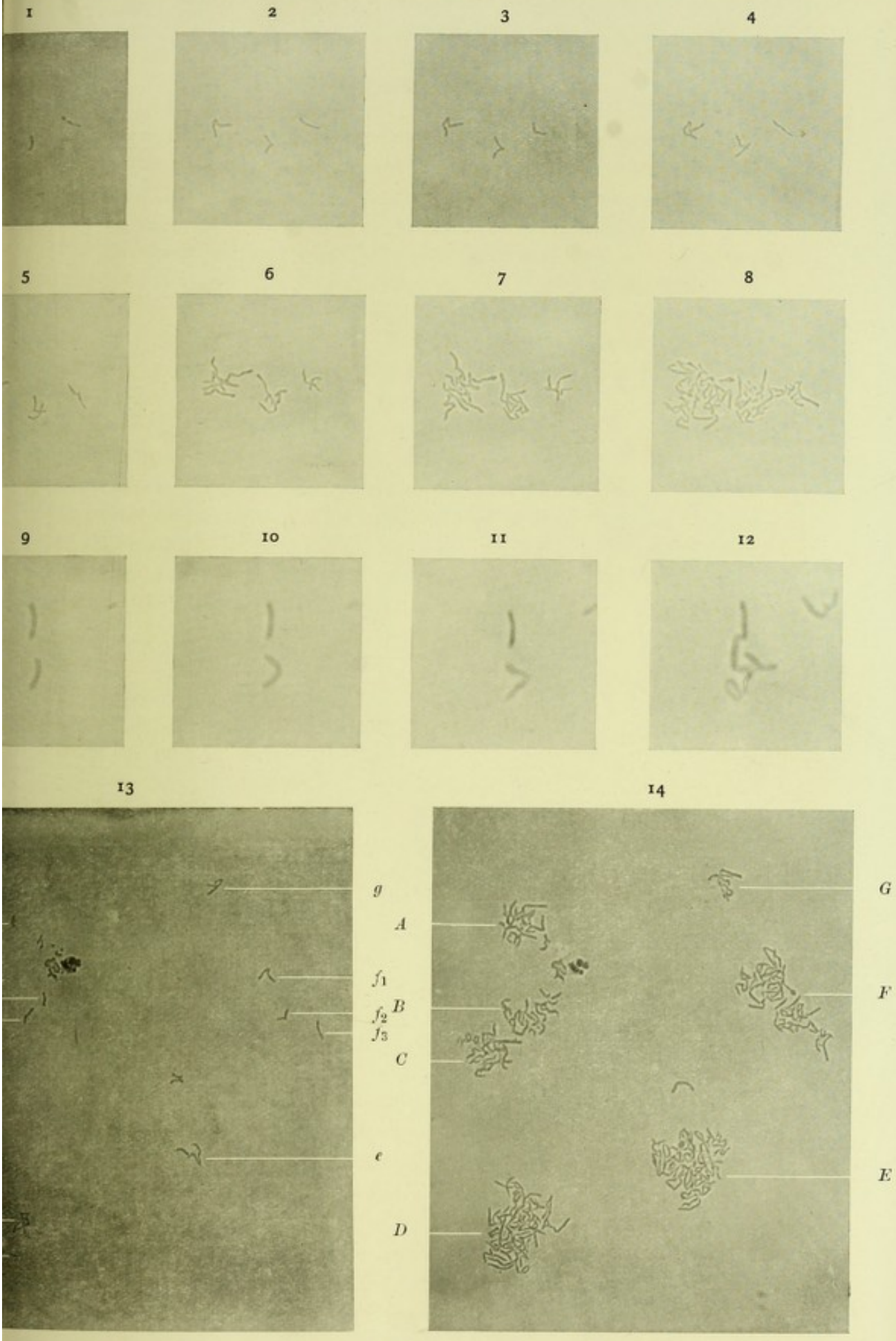

Plate IV. The development of *B. diphtheriae* on the surface of agar.

been developed from single bacilli (a, c, e, g Fig. 13), and three (B, D, F) by the fusion of the products of two or three originally separate bacilli (b_1, b_2; d_1, d_2; and f_1, f_2, f_3 respectively). The right hand colony (F) in this figure is the one whose growth has been followed in Figs. 1 to 8.

Diagram 5 illustrates the development of a colony from a single diphtheria bacillus. The tendency to the production of parallelism is specially marked in this series. During the course of growth one of the newly formed organisms (Fig. 3, No. 111) apparently degenerates. Although it grows large it never divides and later becomes pale and ill defined (Fig. 15) and eventually disappears (Fig. 16).

Diagram 6 also illustrates the development of a diphtheria colony. It differs from the last series in showing a number of giant and irregular forms (Fig. 7, No. 31; Fig. 8, No. 32; Fig. 12, No. 21) most of which subsequently divide. One curious wedge-shaped organism (Fig. 8, No. 313) undergoes no further development. This series also illustrates the division of one bacillus (Fig. 7, No. 31) into three (Fig. 8, Nos. 311, 312 and 313).

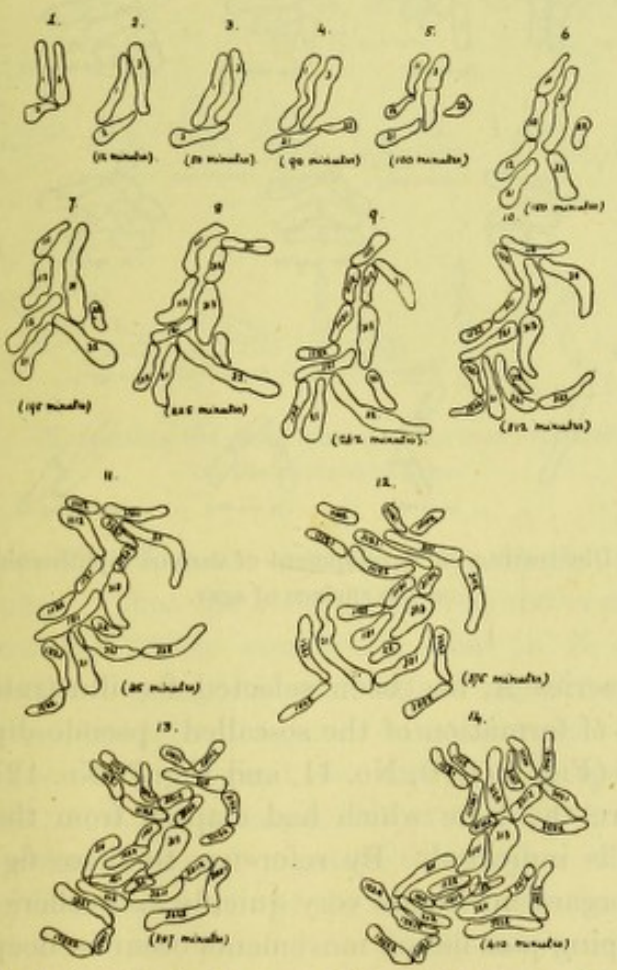

Diagram 6. Illustrating the development of *B. diphtheriae* on the surface of agar.

Diagrams 7 *and* 8 illustrate the same type of division and post-fission movement in eight diphtheroid organisms, (A) *B. Hofmanni*, (B) *B. coryzae segmentosus*, (C) *B. xerosis*, (D) a diphtheria-like bacillus from the eye of a diseased turkey, (E) a bacillus from a plate exposed to the air, (F and G) two different species from the mouth and (H) one from the lung of a grouse.

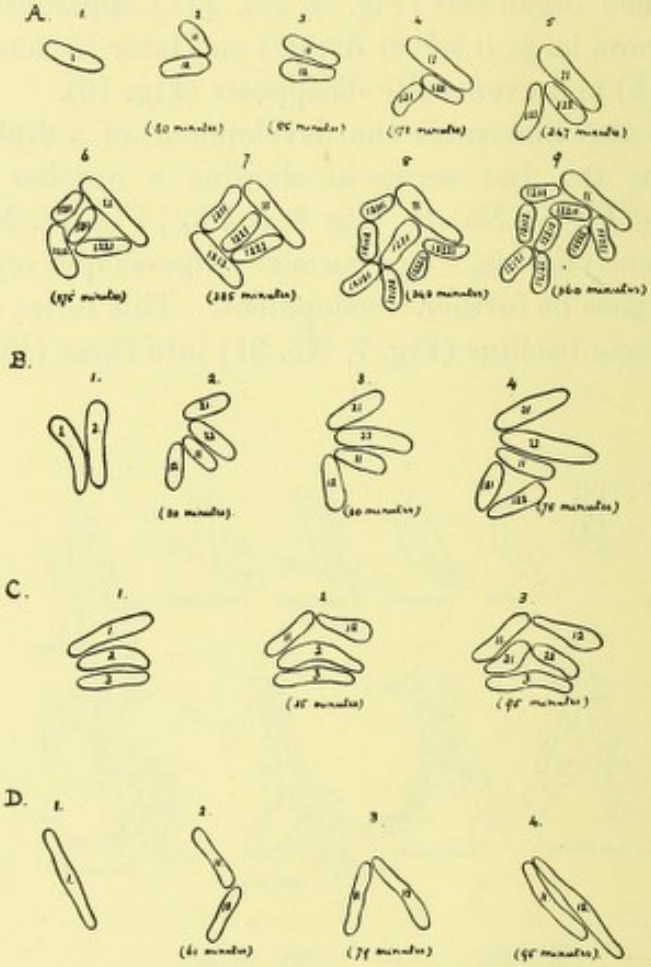

Diagram 7. Illustrating the development of various diphtheroid organisms on the surface of agar.

Diagram 7, series A, has been selected for illustration because it shows the mode of formation of the so-called "pseudo-diphtheria" type of *B. Hofmanni* (Figs. 4 to 9, No. 11, and Fig. 7, No. 1212).

In each figure the time which had elapsed from the beginning of the observation is indicated. By reference to these figures it will be seen that some organisms divide very quickly and others very slowly.

Similar snapping post-fission movements occur in deep growths, but the resulting bacilli remain more nearly in line than they do on surface

growths. As growth continues the distal ends of the newly formed bacilli very gradually approach each other, but the organisms seldom become parallel.

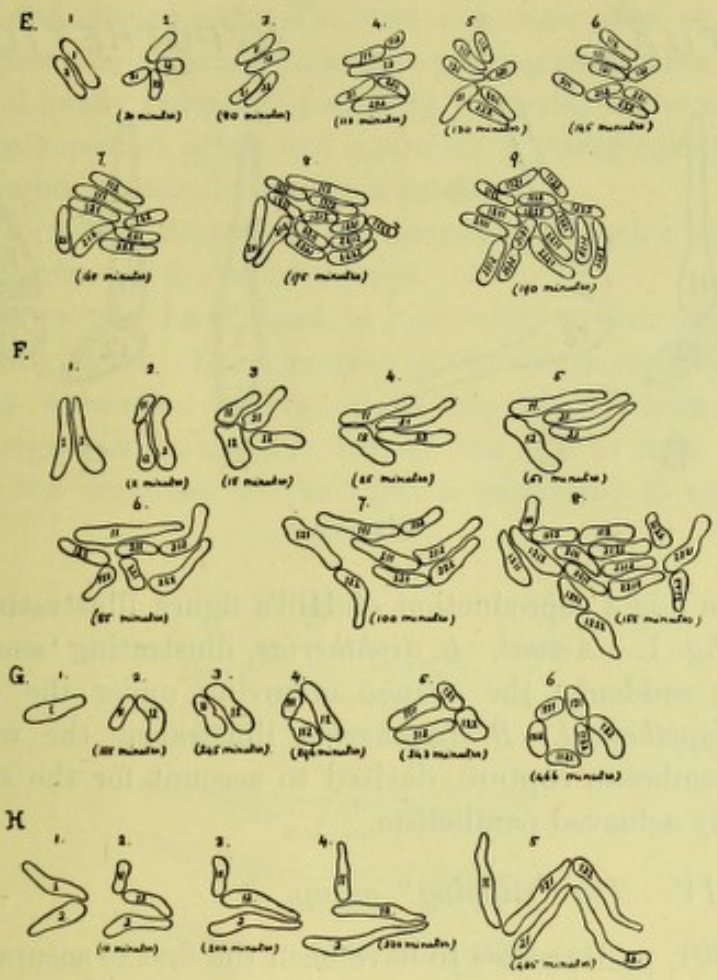

Diagram 8. Illustrating the development of various diphtheroid organisms on the surface of agar.

Hill (1901, p. 81) offers the following hypothesis in explanation of these movements. "That the visible bacterial rod is surrounded by an invisible or scarcely visible membrane; that in *B. diphtheriae* (and other members of this group) only incomplete rupture of this membrane after fission occurs, the line of rupture running round only a portion of the circumference of the original rod, leaving a bridge connecting the new rods formed; that the enlargement of these rods before snapping and the tension thus produced originate the rupture; that the snapping is due to the sudden occurrence of the rupture, and that the preliminary angular position and the parallelism finally achieved are due to the

pressure of the proximal ends of the new rods on each other, in composition with the restraint exercised by the unruptured strap-hinge-like portion of the original membrane."

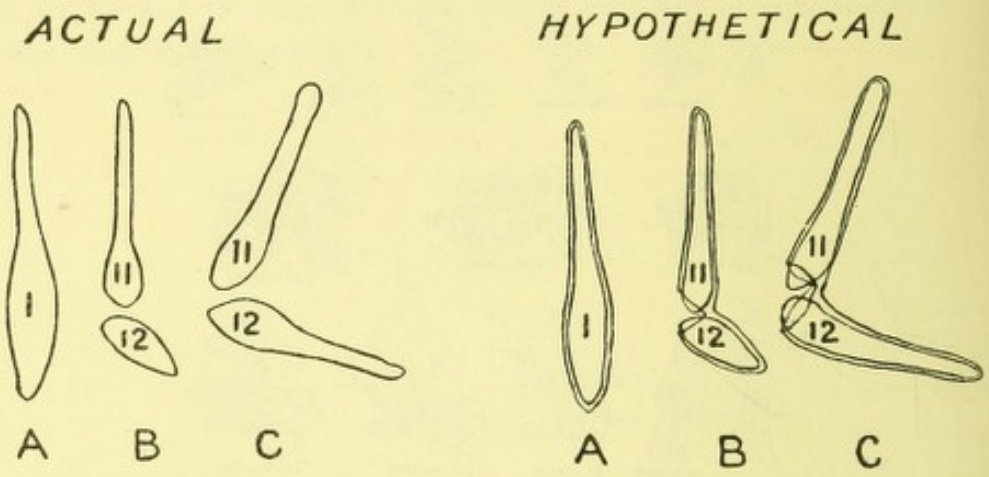

Diagram 9.

Diagram 9 is a reproduction of Hill's figure illustrating his hypothesis. "Fig. 1.—*Actual. B. diphtheriae*, illustrating 'snapping.' (A) became (B) suddenly, the change occurring under the writer's eye; Fig. 1.—*Hypothetical. B. diphtheriae*, illustrating the writer's hypothesis of membrane rupture, devised to account for the snapping and subsequently achieved parallelism."

Group IV. The "slipping" group.

Hill (1901, p. 81) seems to have been the first to accurately describe and illustrate by means of a diagram the post-fission movements of this group, calling them "slipping" movements.

The writer has found that *B. typhosus*, *B. enteritidis* (Gaertner), *B. coli*, *B. pneumoniae* (Friedländer) and allied organisms, *B. pyocyaneus*, the butter bacillus of Rabinowitch, *V. cholerae*, *S. rubrum* and other vibrios, *B. fluorescens*, *B. subtilis* and allied organisms and many other species of non-pathogenic bacilli belong to this group.

The characteristic mode of development on the surface of agar is as follows. The original single rod grows in length and divides, "the fission being always clearly evidenced by a sharp separation of the protoplasm of the original rod into two usually equal portions, by a translucent segmentation interval. Later a slight curve to one side is observed in one of the two new rods still in line with each other" or

both rods become slightly bent, though one is usually more bent than the other. "This curve tends to straighten afterwards, the straightening being achieved by the proximal end of the curved rod slipping slowly out of line with the other rod, towards that side on which was the convexity of the curved rod. The two rods now free at both ends continue to grow, the proximal ends thus passing each other in opposite directions, and finally reaching, if nothing prevents, to the distal ends." This process is repeated again and again till a young colony composed of a large number of parallel bacilli is produced.

Plate VII (× 625) illustrates the formation of such a colony in the case of an organism of the *subtilis* group.

Fig. 1 shows two bacilli end to end, with a clear segmentation interval dividing them. The organism to the left is slightly bent with the convexity upwards. In Fig. 2 (45 minutes) division has taken place in the organism on the left, but all are still in line. In Fig. 3 (55 minutes) the organism on the right is beginning to slip past the

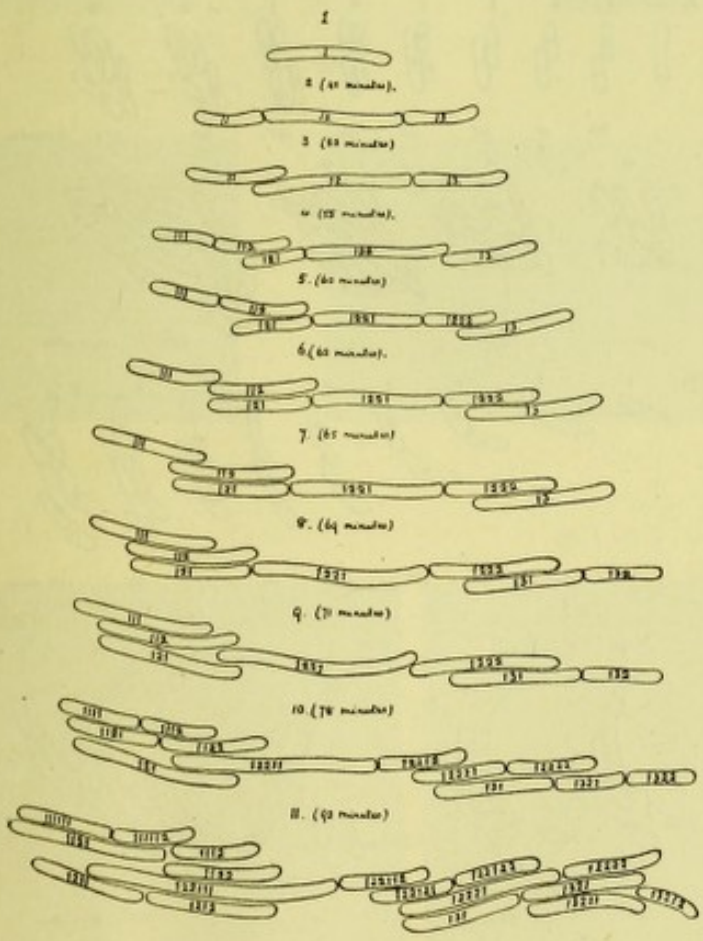

Diagram 10. Illustrating the development of *B. coli* on the surface of agar.

central organism. Figs. 4 and 5 (70 and 90 minutes) show the continuation of this process. In Fig. 6 (105 minutes) the organism to the left is slipping past the central one and in Fig. 7 (120 minutes) the condition is more advanced. At this stage the colony consists of three parallel lines of bacilli. The further development of this colony by the same methods is illustrated in Figs. 8 to 11 (140, 180, 225, 250 minutes respectively). In the latter figure the colony consists of 109 distinctly separated rods and two long unsegmented threads which become parallel with each other at the right hand extremity of the colony. In Fig. 12 (340 minutes) the colony is just beginning to fuse with another colony at the bottom right hand corner of the photograph.

During all this time the bacilli forming the colony remained in one plane.

Diagram 10 illustrates the development of a young colony of *B. coli* from a single cell, and *diagram* 11, series A and B, the development of *V. cholerae.* Series B, Fig. 13 shows some very long forms.

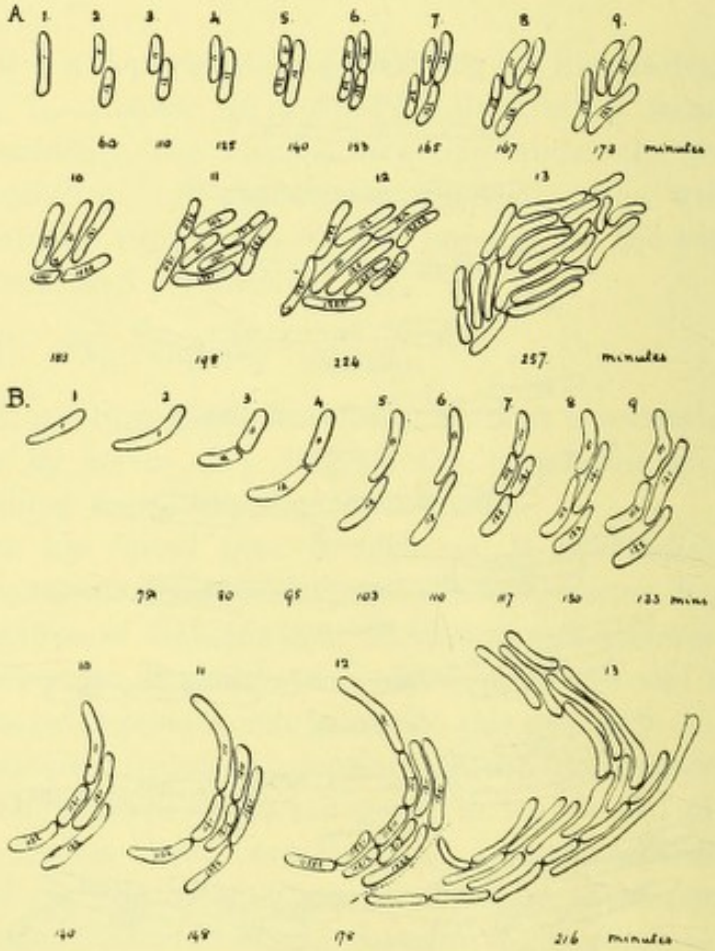

Diagram 11. Illustrating the development of *V. cholerae* on the surface of agar.

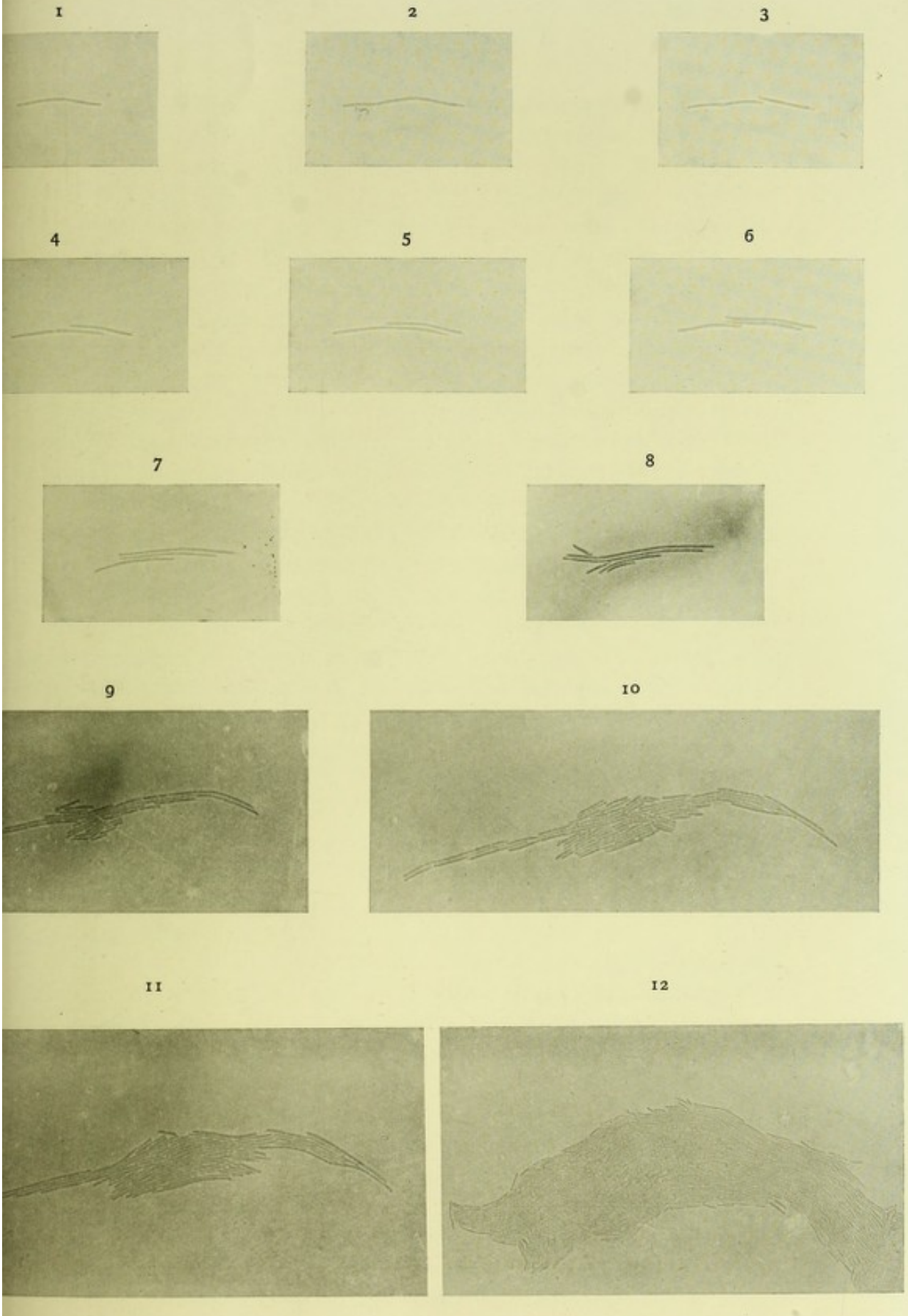

Plate V. The development of *B. subtilis* on the surface of agar.

Irregularities due to extreme rapidity of growth or to the formation of long unsegmented threads may occur, but are seldom met with in cultures of the smaller bacilli belonging to this group. In the former case "the rods may grow so fast after separation that instead of ultimately lying side by side the impinging of one upon the other near the proximal end of the latter carries this latter in front of it, and swings it round so that it lies right across and in front of the axis of the first, forming an irregular T." Irregularities due to this cause are not very common in surface cultures. A more common and more important cause of irregularity of growth, which is more especially apt to be a disturbing factor in the case of large, relatively strong, bacilli of the *subtilis* group, is due to the formation of long unsegmented threads. Very frequently the gradual increase in length in such threads causes them to develop primary and secondary loops, such as occur in surface cultures of *B. anthracis*. Ultimately however segmentation usually occurs and owing to the bacilli composing the loop slipping past each other an irregular network is produced.

Diagram 12, series A, illustrates the formation of long parallel partially segmented threads in a culture of an organism belonging to the *subtilis* group, and series B illustrates the changes which occur in a loop developed from such parallel threads. In series B, Fig. 1 a well formed loop is seen, and in Figs. 2, 3 and 4 the gradual development of secondary loops. In the latter figure however slipping has already commenced at *a*. In Fig. 5 the secondary loop on the left is still better marked, but the primary loop is beginning to break up by segmentation (b, b, b, b) of the threads composing it. Fig. 6 shows the irregular network resulting from the breaking up of the loop by segmentation and slipping.

This series also illustrates another irregular post-fission movement which is not infrequently seen in preparations of some strong film forming organisms. Instead of slipping past each other the ends of the newly divided bacilli press against each other (B, Fig. 1, X), and cause the proximal ends to be directed outwards at a considerable angle from the original chain (B, Fig. 2, X). As growth progresses a process consisting of two parallel bacilli is thus formed (B, Figs. 3, 4, 5, X).

In deep growths of organisms belonging to this group typical slipping post-fission movements follow division, but whether the deep colonies ultimately produced are small and more or less rounded or large and branched and "root-like" seems to depend on the capacity of the organisms producing them to overcome the resistance of the agar.

Strong fast growing organisms, such as *B. subtilis*, produce root-like colonies, while small, relatively weak organisms, such as *B. coli*, rounded colonies.

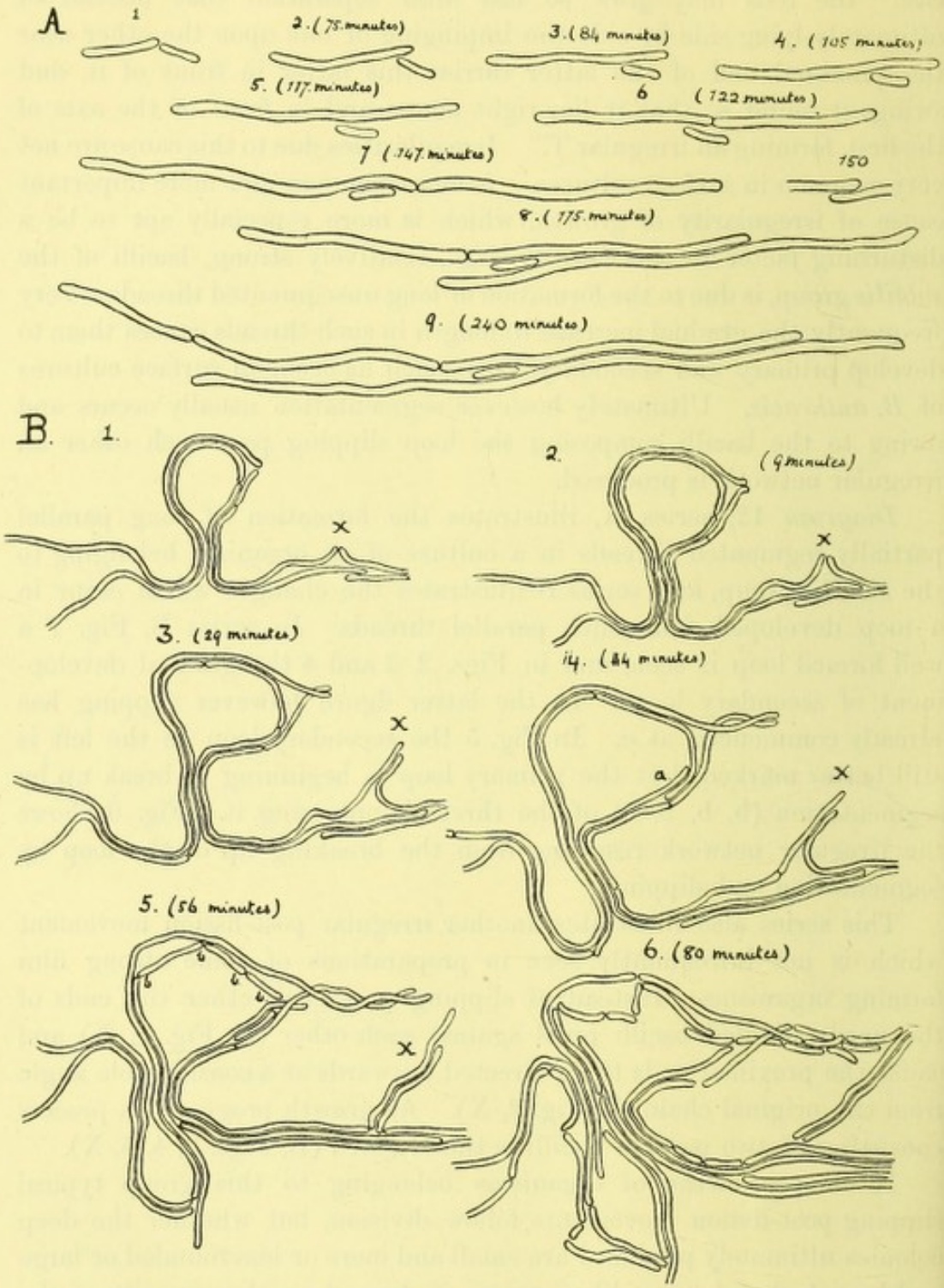

Diagram 12. Illustrating certain irregular modes of development in an organism of the *subtilis* group on the surface of agar.

Many of these small colonies are lens shaped, *i.e.* rounded in one plane and biconvex in the other, and are formed in the following way. By slipping post-fission movements a flat more or less rounded colony, such as that seen in Plate VIII, Fig. 4, is produced. As growth continues large numbers of new bacilli are produced, more especially in the central portions, and begin to override those already formed. The continuation of this process results in the central parts being ultimately composed of many layers and the peripheral parts of fewer layers of bacilli. Hence the biconvex shape on side view.

In the case of the stronger and more rapidly growing bacilli, however, the newly formed organisms in the early stages of growth tend to grow in various planes, and the colony consequently assumes a very irregular branched appearance. During the later stages of growth, when frequent divisions are occurring in the organisms composing the early branches, regular slipping post-fission movements with the formation of parallel strands of bacilli are seen. Possibly the earlier branches produce line of cleavage or areas of less resistance in the agar. The development of a deep colony of this type is illustrated in *Diagram* 13.

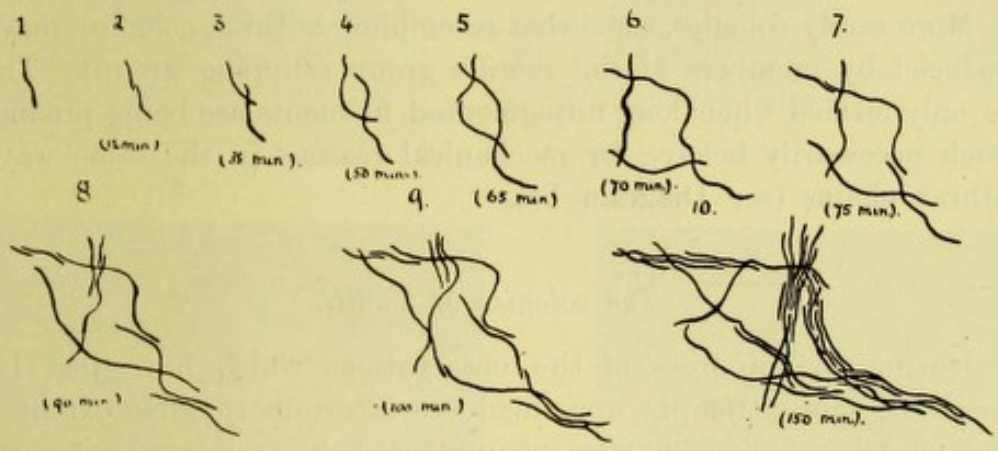

Diagram 13. Illustrating the development of an organism of the *subtilis* group in the depth of agar.

In fluid media cultures of organisms belonging to this group show free, single, usually motile individuals, since there is nothing to retain the rods in their relationship to one another. Pellicles composed of parallel bacilli are however common.

All the facts hitherto observed tend to confirm the hypothesis that in the "loop forming" group the structure of the colonies, superficial and

deep, is due to the strength, close application and non-liability to rupture of the capsule covering the chains; in the "folding" group to its length and flexibility between the bacilli composing the chains; in the "snapping" group to its incomplete rupture during division and the persistence of this condition subsequently; and in the "slipping" group to its complete rupture at the time of division.

The fully formed colonies produced by certain members of the "folding" group may be at times somewhat difficult to distinguish from anthrax colonies. This is due to the fact that in both cases wavy strands composed of numerous parallel chains of bacilli compose the central portions of the colonies. Under a lens both consequently have a "cracked ice" appearance. Usually the margins of the colonies of the folding group are thick and wavy being formed of strands similar in structure to those which compose the centre. More rarely a thin expansion of irregular loops may be seen (Plate V, Fig. 10). On the other hand anthrax colonies almost invariably possess thinner margins, composed of well formed loops, and very frequently exhibit outgrowths of the type shown in Plate IV, Fig. 13. The terminal portions of the chain, with their secondary systems of loops (Plate III, Fig. 7), may also often be found.

More rarely colonies, somewhat resembling anthrax colonies, may be produced by members of the *subtilis* group (slipping group). These are only formed when long unsegmented filaments are being produced, which necessarily behave for mechanical reasons in the same way as anthrax chains (see Diagram 14).

The colonies of bacilli.

During the progress of the observations which have just been described, some attempts were made to ascertain to what extent the mode of "post-fission" movement influenced the appearance of surface colonies, under ordinary conditions of cultivation. For the purposes of diagnosis and investigation considerable importance is necessarily attached to the appearance of surface colonies, owing to the aid it renders in picking out the suspected organisms in mixed cultures. It is of interest therefore to know to what extent the colonies produced by a given organism are capable of variation. Making use of the same batch of agar under constant conditions of incubation the factor which was found to influence to the greatest degree the forms assumed by the colonies was the dryness of the medium.

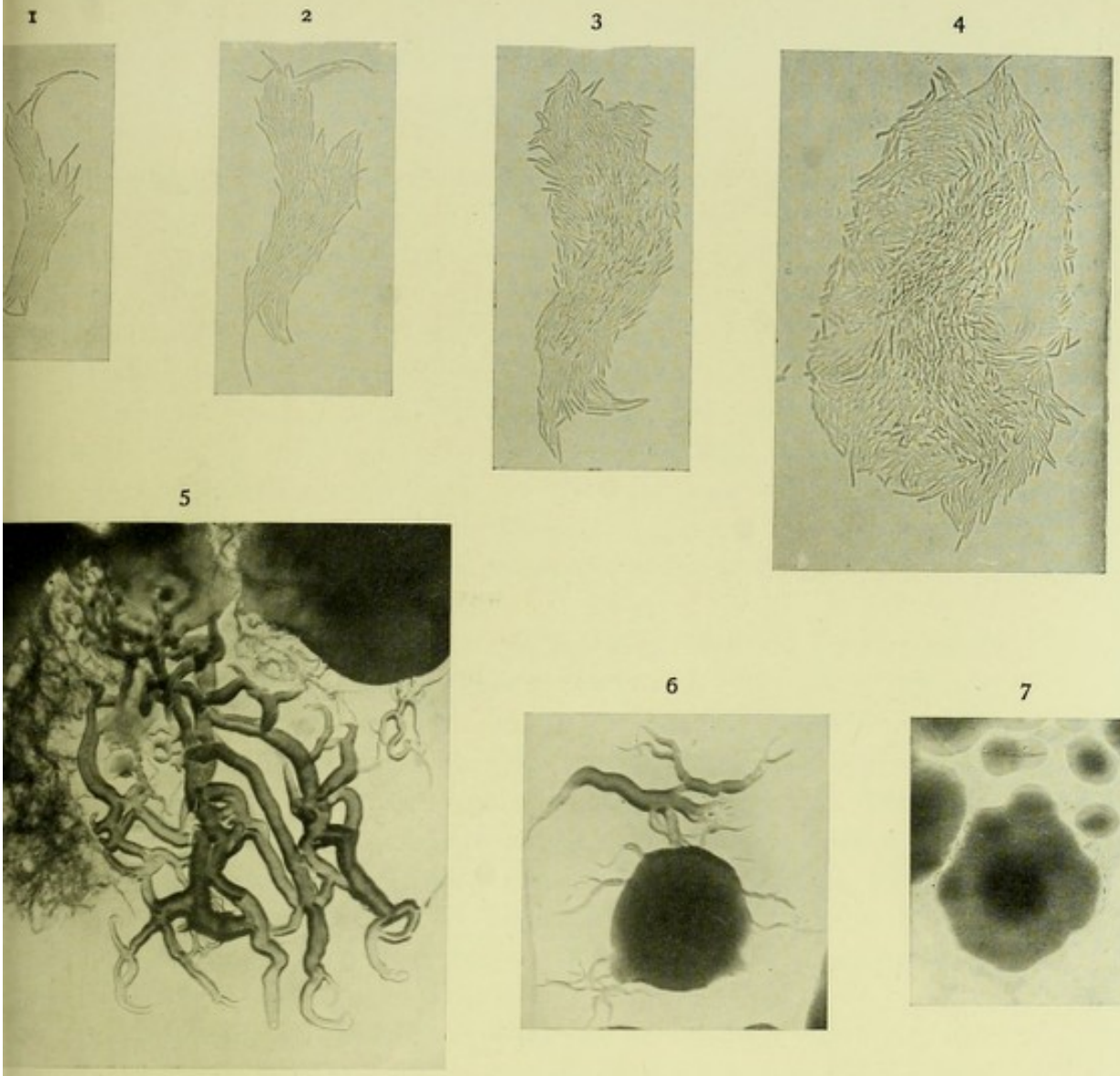

Plate VI. Surface colonies of *B. subtilis*.

The first experiments were made with surface cultures on shallow layers of agar in Petri dishes. In the case of some organisms of the "slipping" group great differences were noticed in the types of the colonies formed on different parts of the dish. On searching for a reason of this phenomenon it was noticed that where the layer of agar was thicker owing to a depression on the bottom of the dish a certain type of colony developed, and where it was thinner another type. The different rates at which layers of various depths dried seemed to afford the most satisfactory explanation of the variations which had been noted. Owing to the uneven construction of these dishes the attempt to follow up these observations in them was abandoned and ultimately the following method was adopted.

Thoroughly clean, sterile glass plates, made from old quarter plate negative glasses, were placed on the bench in a slightly tilted position so that one end was about 0·3 to 0·4 cms. higher than the other. Melted agar was poured on to the plate near the upper end and allowed to run down nearly to the other end. In order to secure an even layer it was distributed with a hot, sterile platinum needle. Several plates were made at a time in this way and allowed to solidify and dry under bell jars for various periods of time. Finally the emulsion of the organism to be investigated was applied usually in three or more parallel longitudinal streaks with a thin platinum loop. The plates, separated by supports, were then arranged horizontally one above the other in a large glass Petri dish. A capsule of water was put into the dish, the lid put on, and the whole incubated at 37° C.

In the "loop forming" group no important differences in the colonies produced on the thick and thin portions of the agar were noticed in thinly sown cultures. Usually the colonies were larger and more inclined to spread at the thicker end, but the general type remained the same. When thickly sown the colonies coalesced to form a continuous film in the thicker portions. Even in continuous growths however indications of the original colonies could be discerned. In the thinner parts the colonies were however separate. In all cases the edges of the growth, whether in the form of a continuous film or as separate colonies, showed the characteristic regularly looped condition, and extensions, such as are illustrated in Plate IV, Fig. 13, were common.

The "folding" group behaved in the same way. The margins of the growths were however usually formed by thick wavy strands composed of parallel bundles of bacilli, and thin expansions of irregular loops, as shown in Plate V, Fig. 10, were uncommon.

In both these groups it is evident from the mode of post-fission movement that widely separated bacilli tend to produce separate, more or less rounded colonies, and that more closely aggregated bacilli tend to produce separate colonies whose peripheral portions fuse with each other, resulting in a slightly uneven continuous film. Such fusion only occurs on moist agar which allows of growth over its surface for a considerable time.

In the "snapping" or diphtheroid group continuous growth was never obtained unless the cultures had been very copiously sown, and in any case numerous elevations in such a growth marked the situations of the originally discrete colonies which had partially coalesced to form this film.

In the "slipping" group, however, the structure and appearance of the colony is very markedly influenced by the condition of the medium. The variations are so great that the colonies in different parts of the culture look as if they had been produced by different species of organisms. To illustrate the range of variation in a single species a member of the *subtilis* group has been chosen.

Plate VIII, Fig. 9 is a photograph of a plate sown shortly after solidification in two parallel streaks with an emulsion of this organism, and incubated for 18 hours. The thicker portion is at the lower end. Over the lower portion a uniform film is seen. (If further incubated folds are often produced on the film, which are due to small areas of the film being thrust away from the surface of the agar by the increasing lateral pressure. Such folds are very commonly present in the lower two-thirds of agar slant cultures.) Towards the middle of the plate the film becomes less dense, and at the upper part discontinuous. Here branched lines of growth, each terminating in a slightly thickened expansion, are seen radiating from the needle tracks. Smaller radiating processes are seen all round the film, being least marked at its thicker portions.

Fig. 8 shows a similar culture on agar which has been dried for a short time after solidification. Here there is practically no film, but in the thicker (lower) parts radiating growths are seen round denser centres. In the thinner (upper) parts the colonies are mostly dense and round.

Though the exact causes of these variations have not been determined, numerous cultivations have shown that they constantly occur under given conditions, and the mode of formation of each variety has been ascertained.

Where the layer of agar is thick and moist a film tends to be formed. In a thickly sown plate each bacillus produces a colony in the manner illustrated in Plate VII, and by the fusion of these colonies a fairly even film results. If the plate is less thickly sown isolated colonies are formed from each bacillus. From these processes run out, and join with those from neighbouring colonies forming a network. The uncovered spaces are filled in by lateral growth from the fused processes. The exact condition ultimately reached depends however on the rate at which the surface of the agar dries, or is in some other way rendered unsuitable for the development of further growth over it. If this occurs before the spaces of the network are filled in a discontinuous film as shown in Plate VIII, Figs. 8 (lower part) and 9 (upper part) results.

Isolated colonies growing on partially dry agar sometimes assume beautifully regular star-shaped forms. Plate V, Fig. 11 (nat. size) shows an extremely fine example of a colony entirely consisting of branched processes, and Fig. 12 illustrates the same colony after a further period of 24 hours' incubation. Here it can be seen that very little lateral extension has taken place, but the processes have enlarged especially at their extreme ends, where large rounded masses of growth have been produced.

In the driest part of the agar the growth is most limited as lateral expansion is early arrested, and rounded or oval heaped up colonies are generally formed. For a certain time the condition of the agar allows of lateral growth, and during this period a flat colony is formed. Subsequent growth results in the bacilli becoming superimposed and a thick colony being produced. Growth under these conditions has been studied and is illustrated in Plate VIII, Figs. 1—4. In Fig. 1 an elongated colony consisting of parallel bacilli all lying in the same plane is seen. Fig. 2 shows further development. In Fig. 3 it is seen that continued multiplication has caused some of the bacilli in the centre of the colony to override others. In Fig. 4 the difference between the central and peripheral portions is still better marked. The central portion consists of at least two layers of bacilli and the parallel arrangement is lost. The peripheral portion still consists of a single layer of bacilli. At this stage a curious phenomenon was noticed, namely very rapid motility at intervals on the part of groups of bacilli lying in the peripheral parts of the colony. These movements often began and ended rather suddenly and the bacilli never passed beyond the original limits of the colony. The blurred appearance

in the right hand peripheral part of the colony is due to active movement during the exposure of the plate and a smaller blurred portion on the other side is due to the same cause. On further incubation the colony only increased slightly in size, but continued multiplication of the bacilli led to the formation of several layers.

Plate VIII, Fig. 7 illustrates a fully formed colony of this type with a thicker centre and thinner margins.

After general surface expansion has been arrested by the drying of the surface of the medium and raised areas of growth of considerable size have been produced further incubation may lead to an attempt at extension in the form of thin twisted, often branched ramifications. Fig. 5 shows on the right a number of such surface ramifications and on the left some thinner twisted threads due to extension in the depth. The two dark masses in the upper right and left corners are rounded masses at the extreme ends of long lateral processes. Fig. 6 shows the rare condition of a small round colony developing branching ramifications.

On somewhat dry agar a great tendency towards the formation of long partially segmented threads, which are probably important factors in the production of colonies with spreading processes, has been frequently noticed. The ramifications just described are also developed from radiating threads which grow rapidly outwards from the colony. Later segmentation and multiplication occur in the threads and consequently thin processes consisting of parallel bacilli are formed along the lines of the original threads.

Another closely allied species, which exhibits a considerable tendency to form long partially segmented threads, behaves somewhat differently. On thin agar the colony after a few hours' growth usually consists of a bundle of parallel threads (Diagram 14, Figs. 1, 1 a) produced by typical slipping movements, as illustrated in Diagram 12, series A. After further growth the bundle has a wavy or sometimes looped appearance, and threads may usually be seen projecting from it in various directions (Diagram 14, Fig. 2). On dry agar further incubation only results in some increase in length and in waviness of the central bundle. On moist agar the central parallel bundle often becomes markedly looped and twisted, and the projecting threads also produce twisted and often looped processes (Diagram 14, Fig. 3). Even though many loops may be produced on the process (Fig. 4) the latter cannot be mistaken for the process of a true loop-forming organism, for in all cases careful observation shows distinct evidence of "slipping," and

single threads projecting from the loops are of common occurrence. Segmentation of the threads forming the processes ultimately occurs (Fig. 5) and indications of the original loops are lost. At this stage the positions of the original loops are marked by bands of parallel bacilli often disposed at right angles to the axis of the process (Fig. 6). If the condition of the agar permits very rapid lateral growth from such processes often occurs at this time.

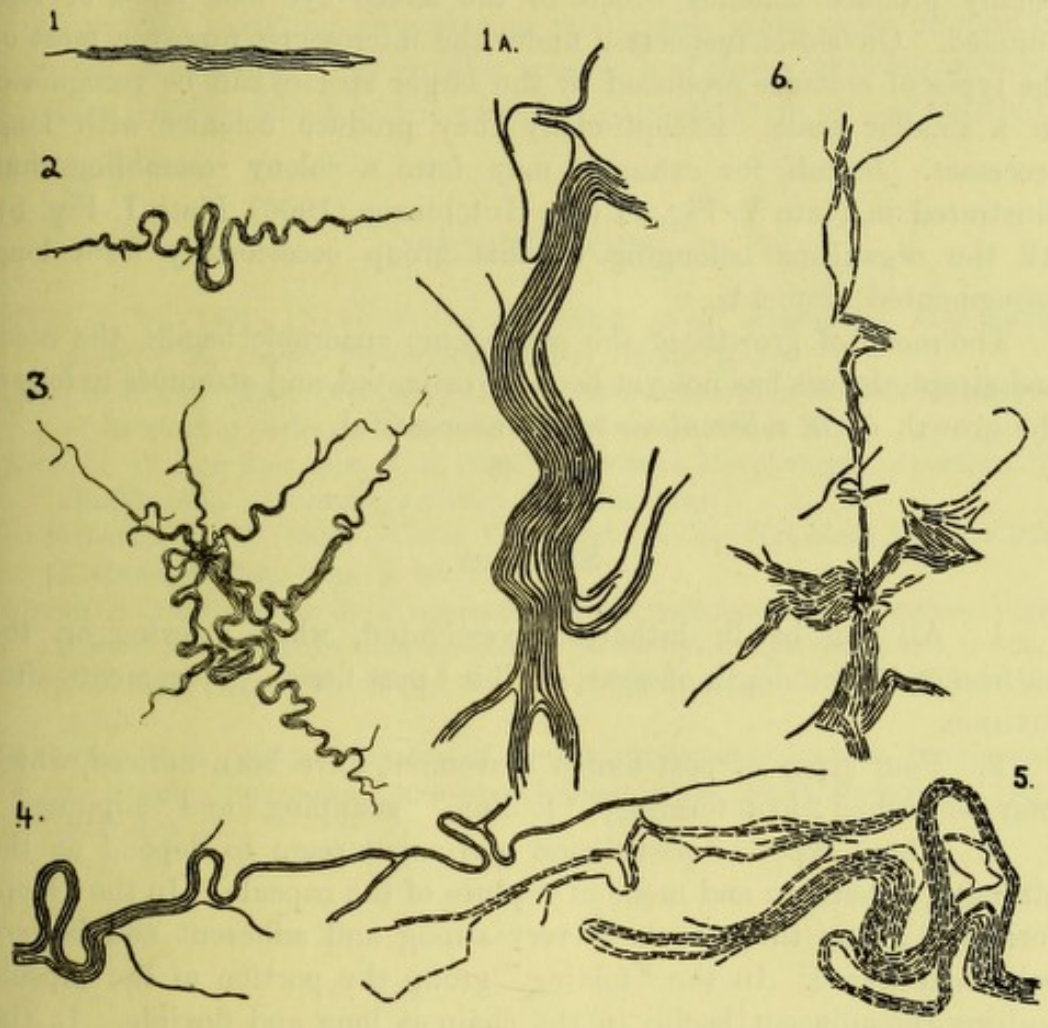

Diagram 14. Illustrating certain irregular modes of development in an organism of the *subtilis* group on the surface of agar.

On thick agar a film is produced by the union of such processes and their lateral projections. During its production many beautiful and fantastic designs may be seen.

Hutchinson (1906, p. 130) illustrates the growth during four hours of a thread-forming organism, *B. lactis albus*, apparently belonging to the

slipping group. The observation ceased before general segmentation had occurred.

All the organisms belonging to the slipping group form films under suitable conditions. Under unsuitable conditions for film formation the larger and stronger members are apt to produce either colonies with radiating processes on moderately moist agar or rounded colonies on thin, dry agar. The smaller, weaker and slower growing members usually produce colonies which to the naked eye look more or less rounded. On closer inspection under the microscope, however, most of the types of colonies produced by the larger species can be recognised on a smaller scale. Exceptionally they produce colonies with long processes. *B. coli*, for example, may form a colony resembling that illustrated in Plate V, Fig. 11 (see Hutchinson (1906), Plate I, Fig. 5). All the organisms belonging to this group occasionally form long unsegmented filaments.

The mode of growth of the pathogenic anaerobic bacilli, the cocci and streptothrices has not yet been investigated, and attempts to follow the growth of *B. tuberculosis* were unsuccessful.

Summary.

1. All the bacilli hitherto investigated, when growing on the surface or in the depth of agar, exhibit "post-fission" movements after division.

2. Four types of post-fission movement have been noticed, which may be termed "loop-forming," "folding," "snapping" and "slipping."

3. These types of post-fission movement seem to depend on the strength, adherence and mode of rupture of the capsule. In the "loop-forming" group the capsule is very strong and adherent and is very seldom ruptured. In the "folding" group the portion of the capsule uniting the adjacent bacilli in the chain is long and flexible. In the "snapping-group" the capsule seems to undergo partial rupture at the time of division, and in the "slipping" group it is completely ruptured.

4. "Loop-forming" post-fission movements are shown by *B. anthracis*, "folding" by *B. pestis* and certain organisms morphologically resembling *B. anthracis*, "snapping" by all diphtheroid organisms and "slipping" by organisms belonging to the typhoid-enteritidis-colon group, vibrios, the butter bacillus (Rabinowitch), *B. pyocyaneus*, *B. fluorescens*, *B. subtilis* and allied organisms and many non-pathogenic species.

5. The characters of the colonies formed by organisms exhibiting loop-forming, folding and snapping post-fission movements are not markedly altered by the condition of medium under ordinary conditions of cultivation. When thickly sown on moist agar continuous growths are formed by all of them, but the edges of these growths resemble the edges of the separate colonies. All organisms exhibiting slipping post-fission movements form films on moist agar. Under unsuitable conditions for film formation the larger and stronger members produce either colonies with radiating processes on moderately moist agar, or rounded colonies on dry agar. The smaller, weaker, and slower growing members produce colonies which to the naked eye appear more or less rounded.

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