XI. *Studies on some New Micro-organisms obtained from Air.*

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[Plates 17–20.]

Contents.

In some papers on the micro-organisms present in air, previously communicated to the Royal Society by one of us,* the relative abundance of microbes in the air of different places has been called attention to and the methods of experiment fully described. As these investigations were carried out with the aid of solid nourishing media, we were able to obtain a collection of pure cultivations of a number of micro-organisms derived directly from the air. It appeared to us, therefore, desirable to utilise the opportunity which these experiments furnished for minutely characterising some of the principal forms which are thus obtainable from the atmosphere. There are many reasons which render it of importance that the task in question should be undertaken. Thus, in the methods of cultivation employed by bacteriologists, the experimenter may at any moment be brought face to face with organisms from the air which have accidentally contaminated his cultivations, and it is obvious, therefore, that an intimate acquaintance with the various forms which may thus invade culture-media must be both of interest and importance to all practically engaged in experiments on micro-organisms.

It is not unnatural that the brilliant discoveries in connection with the etiology of infectious diseases should have absorbed the lion's share of the attention of investigators in the field of bacteriology, and that the non-pathogenic organisms should have come to be regarded as comparatively uninteresting by the side of their more formidable brethren. It must, however, be remembered that the functions of the non-pathogenic organisms in the economy of nature are as yet but very imperfectly understood, and that as far as these functions have been investigated they do not yield in point of importance to those of the most virulent pathogenic forms.

Thus the conversion of sugar into alcohol, the decomposition of nitrogenous organic matter with elimination of ammonia, the oxidation of ammonia to nitrous and nitric acids, besides many other natural transformations which are effected through the agency of such micro-organisms, are certainly not second in importance to the results, terrible as they often are, achieved by the pathogenic forms. The organisms producing the above-mentioned changes are known to be present in the air, and there can be little doubt that the numerous other aërial varieties will in the future be found to discharge important duties in the laboratory of nature.

The exactness with which bacteriological research can now be carried out, thanks to the beautiful methods of cultivation which have been developed during the past six years, renders it imperative that all future investigations on the chemical and physiological action of micro-organisms should be made with specific organisms and not with mixtures, as has so often hitherto been the case. On this account the

* "The Distribution of Micro-organisms in Air," 'Roy. Soc. Proc.,' vol. 40, 1886, p. 509.

"A New Method for the Quantitative Estimation of the Micro-organisms present in the Atmosphere," *ibid.*, vol. 41, 1887, p. 443; 'Phil. Trans.,' 1887, B, p. 113.

"Further Experiments on the Distribution of Micro-organisms in Air (by Hesse's Method)," 'Roy. Soc. Proc.,' vol. 42, 1887, p. 267.

first step in investigations of this kind must consist in such careful delineation of the characteristics of specific organisms that their identification may be readily accomplished. In this way it may with confidence be anticipated that the particular chemical and physiological properties of each specific organism will in the future be elaborated, as has been done in a few cases already. It has been especially with this object in view that we have undertaken the task of collecting from the air a number of different varieties of micro-organisms, and, after isolating and obtaining them in a pure state, to carefully delineate the characteristic appearances which they present, both under the microscope and when grown in various cultivating media.

Methods of Study and Examination.

The organisms which we have made the subject of special study were obtained in the examination of the air of various places by means of HESSE's tubes, and by the exposure of dishes filled with gelatine-peptone in the manner already described. In both cases the aërial organisms are deposited on the surface of the solid gelatine, and, by incubation for several days, each organism thus deposited gives rise to a colony frequently possessing a characteristic appearance. If these colonies are not too closely crowded together on the surface of the gelatine, it is easy to transfer a small portion of a single colony to a culture-tube without any admixture from adjacent colonies. This transference is most conveniently effected by means of a sterilised platinum-needle. As the colonies obtained in the HESSE's tubes or on the gelatine dishes are, as already pointed out by one of us, invariably pure, the cultivation which is obtained by inoculating the needle into a sterile culture-tube is also pure if the operation of transference is performed with care and in an atmosphere reasonably free from dust. Thus in the hundreds of cultivations which this investigation has entailed we have scarcely had a single instance of a culture being vitiated through contamination from the air during the process of transference and inoculation.

Gelatine tube-cultivations.—The colonies originally obtained from the air were invariably inoculated, in the first instance, into test tubes, one-third filled with solid sterile gelatine-peptone (for preparation of which see below), and plugged with cotton wool in the ordinary way. The appearances in these gelatine tubes have been carefully watched, described, and in many cases drawn. These appearances are, as is well known, of great importance in serving to characterise specific organisms, and frequently serve to discriminate between organisms of similar and almost identical microscopic appearance.

Composition of Cultivating Media employed:—

Gelatine.—100 grms. gelatine; 10 grms. peptone (dry); common salt, 1 grm.; lean beef, 1 lb.; water 1 litre.

Agar-agar.—10 grms. agar-agar instead of the gelatine; otherwise identical.

Broth.—Similar to the above, only omitting the gelatine or agar-agar.

In making these inoculations, it has been our practice not only to pierce the needle to a distance of an inch or more into the depth, but also to streak it along the surface of the gelatine, as in this manner two characteristic growths are obtained, the one along the track of the needle beneath, and the other upon the surface of the gelatine.

In many instances the growth of the organisms is accomplished by liquefaction of the gelatine, and the manner in which this liquefaction takes place is often very characteristic, both as to the rapidity with which the change occurs and as to the form of the liquefied portion. The differences observed in respect of this phenomenon will be duly noticed in treating of the individual varieties of organisms.

Agar-agar tube-cultivation.—The organisms were in every case also inoculated into similar test-tubes containing agar-agar mixture (for preparation see note, p. 259), and the appearances presented by their growth in this medium have been studied, described, and, where particularly characteristic, drawn.

The agar-agar cultivations frequently serve to establish differences between organisms which, owing to their liquefaction of gelatine, do not furnish characteristic growths in that medium, for the agar-agar is not liquefied by any organisms, and thus surface-growths can be obtained in the case of those which in gelatine produce only liquefaction. On the other hand, there are many cases in which the cultivations on agar-agar are but little characteristic, whilst in gelatine the same organisms present important marks of distinction.

Broth-cultivations.—The organisms were also in every case inoculated into test-tubes containing sterile broth-peptone (for preparation see note, p. 259), and the appearances presented by their growth in this medium have also been carefully observed and described. The appearance of the broth cultivations is generally very much less characteristic than those in gelatine or agar-agar, the principal differences observable being in respect of the formation or non-formation of a pellicle on the surface of the liquid.

There is, however, a special reason for carrying out cultivations in broth, and that is that the form of the individual organisms is generally more uniform and natural in a fluid than in a solid culture-medium, for in the latter the forms are occasionally distorted and rendered abnormal by the pressure to which they may be exposed.

Gelatine plate-cultivations.—Not unfrequently the most striking appearances are presented by micro-organisms when growing in colonies on gelatine-plates. The colonies are often highly characteristic even to the naked eye, and they generally become far more so when examined by transmitted light with a low magnifying power (about 100 diameters) ; these appearances we have in all cases carefully described and drawn.

The plate cultivations are prepared by inoculating with a needle from a tube cultivation into a sterile test-tube containing melted gelatine, thoroughly mixing the latter by agitation, then inoculating with a needle from this into a second test-tube, which is again mixed by agitation. The gelatine in this second test-tube is then

poured out upon a sterile glass-plate and allowed to congeal. Sometimes even a further attenuation is prepared by inoculating from the second test-tube into a third, which is also poured out upon a plate.

In this manner one or other or both of the plates is almost sure to yield colonies sufficiently separated from each other to prevent interference and to enable further inoculations to be made from a single colony.

By means of this method of plate-cultivation we have also controlled the purity of all our other cultivations. For, on submitting the contents of any culture-tube to plate-cultivation in this manner, any impurity in the original culture will become apparent through the production of dissimilar colonies on the plate.

The colonies are also of great importance for the preparation of cover-glasses (see later below) for microscopic examination with a high power (1,000 diameters), as all the forms obtained from a single colony may with certainty be known to belong to a single organism.

We must point out the great necessity of fully describing the appearance of all organisms when growing in colonies, as this forms one of the most important aids in the discrimination and identification of organisms.

Microscopic examination.—In addition to the macroscopic observations already referred to, we have examined, measured, described, and drawn all the various organisms as they appear when viewed under a high power of the microscope. For this purpose we have, in general, employed a $\frac{1}{12}$ oil-immersion (LEITZ) objective, with a No. 3 eye-piece, thus obtaining a magnifying power of nearly 1,000 diameters, whilst for the examination of micrococci we have also employed LEITZ's $\frac{1}{20}$ objective, with the same eye-piece, thus obtaining a magnifying power of about 1,500 diameters.

The organisms have, in general, been prepared for examination according to the KOCH-LÖFFLER* method. Thus a small quantity of a cultivation is transferred, by means of a platinum needle, to a clean cover-glass, a small drop of sterilised distilled water is added, and the mixture is spread as thinly as possible over the glass with the aid of the platinum needle. A second cover-glass is now laid upon the first and then drawn off, each glass being now provided with a thin film of diluted cultivation. The two cover-glasses are laid down with their wet faces uppermost and allowed to become quite dry. One of the cover-glasses is then held by one corner with a pair of forceps and slowly passed three times through the flame of a Bunsen burner or spirit-lamp, the face bearing the film being held upwards. The dried and ignited specimen can then be preserved indefinitely before staining for examination.

The specimen is stained by running a few drops of a diluted alcoholic solution of an aniline dye (gentian-violet, magenta, methylene blue, &c.) with a pipette upon the cover-glass, which is held with forceps by one corner and moved about so as to cause the colouring matter to flow evenly over every part of the film. The dye is allowed to remain on the glass for about one minute, the exact time being varied according to

* 'Die Methoden der Bakterien-Forschung,' HUEPPE, Wiesbaden, 1885, p. 42.

the relative facility with which the organism has by experience been found to take the colouring matter; it is then washed off with distilled water, and, if intended for immediate examination, laid with the film-surface downwards upon a clean glass slip. The excess of water is then carefully removed with blotting-paper, and the preparation is ready for examination. If the preparation is intended for permanent preservation, it is, after washing off the dye, allowed to dry, and then mounted with a drop of Canada balsam.

Drop-cultivations.—We have also studied the motility and progressive growth of the various organisms in "drop-cultivations." The drop-cultivations are prepared as follows:—

A cover-glass is sterilised by holding it with a pair of forceps and passing it several times through a Bunsen-flame or spirit-lamp. A glass slip having a round excavation on one surface, and capable of being completely covered by the cover-glass, is similarly sterilised, and both cover-glass and slip are allowed to cool under a glass shade. When cool, a small drop of sterile broth is placed by means of a sterile looped platinum needle in the centre of one surface of the cover-glass, and immediately after a very minute quantity (the smaller, the better) of a cultivation of the organism under examination is introduced by means of a sterile platinum needle into the drop of broth, and the cover-glass bearing the latter is then carefully placed so that the drop upon its under-surface projects into the centre of the excavation. The periphery of the excavation has been previously coated with a thin layer of vaseline, so that when the cover-glass is placed in position as described the vaseline produces an air-tight seal to the small circular cell which is thus formed.

If the above manipulations are performed with due care, a perfectly pure cultivation is obtained, and its progress can be observed under the highest powers of the microscope.

By the aid of the above-described methods we have endeavoured to accurately characterise a number of micro-organisms which we have obtained from air. A few of these, such as the *Micrococcus prodigiosus*, the *Bacillus subtilis*, the yellow and orange *Sarcina*, have been more or less closely described by other observers, but the remainder are entirely new, or, if previously observed, they have not to our knowledge, after careful reference to the most recent literature, been so described as to render them capable of identification. Under these circumstances we have felt it necessary to provisionally give names to all those forms which have not been hitherto described. The names which we have selected for this purpose are generally of such a nature as to indicate some striking peculiarity which the organisms present either in their cultivations or when viewed under the microscope. By adopting this plan, we believe that the description of, and reference to, the organisms which we have had under observation will be facilitated more than if we had only designated them by numbers or other symbols.

In our terminology we have, following the practice of many recent writers, abandoned the term *bacterium*, distinguishing those forms which are distinctly elongated as *bacilli*, and those which are spherical, or approximately so, as *micrococci*.

I.—MICROCOCCI.

The following different varieties of micrococci have been found and examined by us : —

1. Micrococcus carnicolor.
2. M. albus.
3. Streptococcus liquefaciens.
4. Sarcina lutea.
5. S. aurantiaca.
6. S. liquefaciens.
7. M. gigas.
8. M. chryseus.
9. M. rosaceus.
10. M. candicans.

1. Micrococcus Carnicolor.

Occurrence.—This micrococcus was obtained as a pinkish surface-expansion on a gelatine-dish exposed to the air of the Close at the base of Norwich Cathedral, 26th April, 1886.

Microscopic appearance.—Under the high power (× 1,000) it is seen to consist of almost round cocci varying in size from ·5μ to about 1·5μ. The larger forms almost invariably exhibit a division (see Plate 17, fig. 2, No. 2*c*). Otherwise the cocci present no definite arrangement.

When viewed in drop-cultivations, they exhibit the usual vibratory motion of micrococci.

Appearance in Cultivations.

Gelatine.—In tube after four days (August 6—10, 1886) the needle-track in the depth exhibits but very slight growth ; over the surface, however, there is a pink expansion extending laterally on either side of the streak of the needle. At a later period the needle-track beneath the surface becomes beaded, and the colour of the surface-growth is seen to be of a lighter tint in the centre than round the periphery. (See Plate 17, No. 2*b*.)

In very old cultivations the gelatine becomes slightly liquefied.

Agar-agar.—Grows rapidly, producing a smooth flesh-coloured surface-expansion having a glazed appearance. (See Plate 17, No. 2*a*.)

Broth.—After nine days (August 7—16, 1886) the liquid is clear, free from a pellicle, and has a pinkish deposit at the bottom.

Appearance on plate-cultivation.—The colonies are seen to the naked eye to be of a faint pink colour. Under a low power (×100) they appear as almost perfectly circular smooth-edged colonies, the interior of which is exceedingly finely granular in its nature. They are brown in colour, the shade becoming deeper as the colonies develop further. (See Plate 17, No. 2*d*.) When the colonies reach the surface of the gelatine they form a very thin and round—almost colourless—expansion, which subsequently acquires the characteristic pink tint. Under a low power this expansion is seen to be finely granular, with an almost perfectly smooth edge. (See Plate 17, No. 2*e*.) Tubes were inoculated both from the surface-colonies and from those in the depth of the gelatine, and from each the same characteristic growth was obtained.

This organism has many points of similarity with the *Micrococcus rosaceus*, from which it is chiefly distinguishable (1) by its more rapid growth, (2) by the fainter colour of the pigment, and (3) by the different appearance of its colonies. (See page 269.)

2. Micrococcus Albus.

Occurrence.—We have obtained this as a white surface-expansion on a gelatine-dish from the same place as the *Micrococcus carnicolor* (p. 263).

Microscopic appearance.—Viewed under a high power (× 1,000), this is seen to consist of cocci varying in size from ·8μ to 1·5μ, the larger ones presenting a division (see Plate 17, fig. 5, No. 5*b*); they have no characteristic arrangement.

Appearance in Cultivations.

Gelatine.—In tube after four days (August 6—10, 1886) the needle-track below the surface shows a faint saw-like growth, whilst on the surface there is a narrow, white, shining expansion spreading on either side of the needle-streak. The edge is lobular (see Plate 17, No. 5*a*) and smooth, not serrated. No liquefaction of the gelatine takes place.

Agar-agar.—It appears as a faintly white, almost colourless surface-expansion, with a smooth, but lobular, edge.

Broth.—After nine days (August 7—16, 1886) the liquid is very slightly turbid, free from pellicle, with a yellowish-white deposit at the bottom.

Appearance on plate-cultivation.—The colonies are visible to the naked eye as small milk-white discs. Under a low power (× 100) they are seen to be circular, sharp-edged, and finely granular in nature. In colour they exhibit varying shades of brown, according to the degree of their development. (See Plate 17, No. 5*c*.)

3. Streptococcus Liquefaciens.

Occurrence.—This was obtained from air as a yellow surface-expansion on a gelatine-dish.

Microscopic appearance.—Under a high power (× 1,000) this is seen to be a small micrococcus, varying in size from ·5μ to ·8μ, the dimensions being thus fairly uniform. The cocci are arranged in short chains, as seen in Plate 18, fig. 3, No. 3*a*.

Appearance in Cultivations.

Gelatine.—After four days (August 6—10, 1886) the needle-track below the surface is very faint; at the top there is a slight depression, with a light lemon-yellow deposit, slight liquefaction of the gelatine having there taken place. As the cultivation becomes older, the liquefaction slowly proceeds, the needle-track in the still solid gelatine remaining very faint.

Agar-agar.—Forms an almost colourless shining growth extending even after a month, but slightly to left and right of needle-streak.

Broth.—After nine days (August 7—16, 1886) the liquid is clear, free from pellicle, and has a dirty yellowish-white deposit.

Appearance on plate-cultivation.—To the naked eye the colonies appear as yellowish pin-heads on the surface, each being surrounded by a slight depression. Under a low power (× 100) the smaller colonies appear irregularly circular (see Plate 18, No. 3*b*), the edge is smooth, and the interior is more or less granular.

In gelatine the growth of this organism presents points of similarity to that of *Sarcina lutea*, from which, however, it is most sharply distinguishable, not only by its appearance under the microscope, but also by its growth in agar-agar.

4. Sarcina Lutea.

Occurrence.—We have not ourselves found this organism in the air, but a cultivation of the same was brought by one of us from Dr. Koch's laboratory at the Hygienic Institute of Berlin.

It has already been partially described by Klein ('Micro-organisms and Disease,' 1885, p. 43), Eisenberg ('Bakteriologische Diagnostik,' 1886), Crookshank ('Introduction to Practical Bacteriology,' 1886, p. 120), Flügge ('Die Mikro-Organismen,' 1886, p. 179).

Microscopic appearance.—Under a high power (× 1,000) there are seen large cocci, mostly grouped together in cubical packets of four or more. The individual cells vary in diameter from 1·5μ to 2·5μ, and are best seen when lightly stained with methylene-blue. The staining is very liable to be too intense and so prevent the grouping being recognisable, owing to their lying in heaps, the division of the cells taking place both vertically and horizontally. (See Plate 18, fig. 6, No. 6*b*.)

The appearance in drop-cultures is particularly characteristic, the arrangement in cubical packets being most beautifully shown. It is, of course, non-motile.

Appearance in Cultivations.

Gelatine.—In the tube it grows slowly, forming numerous minute yellow centres in the track of the needle, whilst on the surface it produces a shining lemon-yellow

expansion consisting of small hump-like protuberances. In nine days (August 6—15, 1886) the surface-growth was still very restricted, but had formed a depression filled with lemon-yellow semi-liquid matter. Even after eighteen days (August 6—24) there was but little change in the needle-track, but the surface-depression, which was considerable (see Plate 18, No. 6*a*), was filled with liquid, at the bottom of which was a lemon-yellow deposit.

This organism thus causes a very slow liquefaction of the gelatine, and produces a very decided lemon-yellow pigment.

Agar-agar.—Forms a thick chrome-yellow moist mass extending over the surface.

Broth.—After nine days (August 7—16, 1886) the liquid is clear and free from pellicle ; there is a lemon-yellow deposit at the bottom.

Appearance on plate-cultivation.—The colonies are visible to the naked eye as small yellow centres which, under a low power (× 100), appear of irregular shape, finely granular near the periphery, the edge being nearly smooth (see Plate 18, No. 6*c*). The centre of the colony has a dark greyish-green colour. No liquefaction was observed during the first six days.

5. Sarcina Aurantiaca.

Origin.—This organism is also occasionally found in the air, although we have not ourselves met with it there. A cultivation was brought by one of us fron Dr. Koch's laboratory in Berlin. The only references to this organism which we have been able to find are in Eisenberg's 'Bakteriologische Diagnostik,' which contains a very brief description of its appearance, and its existence is mentioned by Flügge ('Die Mikro-Organismen') without, however, any description being appended.

Microscopic appearance.—Under the high power (× 1,000 or × 1,500) there are seen packets of cocci which are much smaller than those of *Sarcina lutea*. The complete packet of four cocci measures only about 1·7μ across. (See Plate 18, fig. 4, No. 4*b*.)

Appearance in Cultivations.

Gelatine.—In the tube, after four days (August 6—10, 1886), liquefaction has taken place along the path of the needle, producing a funnel-shaped canal which is filled with clear liquid, at the bottom of which is a flocculent orange deposit.

The Plate 18 (No. 4*a*) exhibits the condition of the cultivation after seven days' growth (August 6—13) ; the liquefaction even then has not extended across the tube, and the lower extremity of the needle-path is still comparatively undeveloped.

Agar-agar.—Forms an abundant and moist surface-growth of a strong orange colour. The growth is for the most part continuous, but numerous little heaps are distributed over the remainder of the surface.

Broth.—After nine days (August 7—16, 1886) the liquid is turbid at the surface,

but clear below, with a dirty white deposit at the bottom. After eighteen days the deposit has become of an orange colour.

Appearance on plate-cultivation.—To the naked eye the colonies are visible on the fifth day (October 13—18, 1886) as small, round, yellow colonies, each of which exhibits a circular surface-depression of varying size. On examination with a low power (×100), the colonies are seen to be circular and granular, with a slightly denticulated edge, which in the less developed colonies is not so marked. (See Plate 18, No. 4*c*.)

6. Sarcina Liquefaciens.

Occurrence.—We have found this organism in the air collected on the roof of the Science Schools, South Kensington Museum. It was particularly abundant on the 8th July, 1886, when it was found producing small granular liquefying colonies on the surface of gelatine-dishes which had been exposed there.

Microscopic appearance.—Under a high power (× 1,000 or 1,500) it much resembles *Sarcina lutea*, the cocci, which are about 1·5μ in diameter, being arranged in packets of four and upwards, a very large number sometimes remaining aggregated together. (See Plate 18, fig. 5, No. 5*b*.)

Appearance in Cultivations.

Gelatine.—After four days (August 6—10, 1886) the needle-track below is composed of small isolated whitish centres, whilst above there is a large depression with an air-space and cloudy liquid contents, at the bottom of which there is a greyish-white deposit. The liquefaction has not extended across the tube. On the ninth day (August 6—15) the liquefaction has extended across the tube to depth of about half-an-inch (see Plate 18, No. 5*a*), the liquid portion being very turbid, with a yellowish-white deposit resting upon the surface of the still solid gelatine below. The lower portion of the needle-track exhibits no material alteration. Subsequently the liquid portion becomes quite clear.

Agar-agar.—The growth is very rapid, producing an almost colourless (very faintly green) expansion, very much resembling that of *Sarcina aurantiaca*, excepting as regards the colour.

Broth.—After nine days (August 7—16, 1886) the liquid is clear, free from pellicle, and with a dirty-white deposit at the bottom, which subsequently becomes of an orange colour.

Appearance on plate-cultivation.—To the naked eye the colonies appear almost colourless (very faintly green). They had not caused liquefaction of the gelatine on the fifth day (October 13—18, 1886); a day later (October 19) they formed a surface-depression like *Sarcina aurantiaca*. Under a low power (× 100) the colonies appear (see Plate 18, No. 5*c*) as highly irregular in contour, with a denticulated and lobular edge and granular contents.

Distinctive Differences existing between the three Forms of Sarcina described.

Under the microscope the *Sarcina aurantiaca* is sharply distinguished from the other two by the smaller size of its cells, whilst it presents a still more striking contrast to the other two in the colour of the pigment which it produces when cultivated in gelatine or on agar-agar. From *Sarcina lutea* the other two are also distinguished by the far more rapid liquefaction of the gelatine which they produce. The property which the almost colourless Sarcina has of liquefying right across the tube presents a marked point of distinction from *Sarcina aurantiaca,* in which the liquefaction takes place in the form of a bag.

7. Micrococcus Gigas.

Occurrence.—This was found by us in the air of a cow-shed, forming a large white expansion on a gelatine dish exposed there.

Microscopic appearance.—Under a high power (× 1,000 or 1,500) this is seen to be a large micrococcus, sometimes as much as 1·7μ in diameter; the cocci are frequently adherent in pairs. (See Plate 17, fig. 3, No. 3*a*.)

Appearance in Cultivations.

Gelatine.—It liquefies the gelatine slowly, rendering it turbid.

Agar-agar.—It forms a yellowish-white smooth surface-growth, extending in lobules to right and left of the needle-streak. Later on, the colour becomes cream-yellow, and the lobules, which are numerous, remain small. There is a considerable granular growth in the track of the needle beneath the surface.

Broth.—After six days (September 7—13, 1886) the liquid is clear, free from pellicle, and has a whitish deposit at the bottom.

Appearance on plate-cultivation.—After four days the colonies appear on the surface as pin-heads of a faint cream colour, each causing a depression in the gelatine. Under a low power (× 100) the colonies are seen to be circular in shape, with a slightly irregular edge, and the contents, which are cloudy at the centre, become distinctly granular towards the edge. (See Plate 17, No. 3*b*.)

8. Micrococcus Chryseus.

Occurrence.—This was found by us in the air collected on the roof of the Science Schools, South Kensington Museum.

Microscopic appearance.— Under the high power (× 1,000 or 1,500) it appears as a micrococcus of variable size, up to 1μ in diameter; the largest cells exhibit a division. The cocci are not arranged in any definite manner. (See Plate 19, fig. 3, No. 3*a*.)

Appearance in Cultivations.

Gelatine. — After four days (August 6—10, 1886) there is a slight surface depression filled with semi-liquid cream-coloured matter; even after sixteen days the semi-liquefaction has but very slightly increased.

Agar-agar. It forms a surface shining growth of light-orange colour.

Broth.—After nine days (August 7—16, 1886) the liquid is clear, free from pellicle, and has a dirty-white deposit at the bottom.

Appearance on plate-cultivation.—After four days the surface-colonies are visible to the naked eye as pin-heads of yellowish colour. Under a low power (× 100) they are seen to be generally round (see Plate 19, No. 3*b*), the more developed colonies showing a finely granular edge, whilst the less developed have a smooth edge.

NOTE.—The dark semi-circular edge seen in the figure indicates the depression produced by the colony on the surface of the gelatine.

9. MICROCOCCUS ROSACEUS.

Occurrence.—We have frequently met with this organism in the course of our experiments on air; we have also compared it with a cultivation which was brought by one of us from Dr. KOCH's laboratory in Berlin. On gelatine plates or dishes which have been exposed to the air, it produces small, smooth, shining, bright pink expansions.

Microscopic appearance.—Under a high power (× 1,000 or 1,500) the cocci are seen to be very variable in size, the largest being as much as 2·5μ in diameter; the larger forms exhibit a well-marked division. (See Plate 17, fig. 4, No. 4*a*.)

Appearance in Cultivations.

Gelatine.—It forms a shining, smooth, pink expansion on the surface, whilst the needle-track below remains almost undeveloped. As the cultivation becomes older, the margin assumes a radiated appearance. Still older cultivations frequently exhibit slight liquefaction.

Agar-agar.—Forms a smooth, bright-pink surface-expansion devoid of any further characteristics.

Broth.—After nine days (Aug. 7—16, 1886) the liquid is clear, free from pellicle, and exhibits a pink deposit.

Appearance on plate-cultivation.—To the naked eye the more developed colonies appear as pin-heads on the surface, and are bright-pink in colour. Under a low power (× 100) they are seen to be of a distinctly reddish tint, the edge being irregular, but smooth; but as the colonies approach the surface the irregularity diminishes. (See Plate 17, No. 4*b*.)

10. Micrococcus Candicans.

Occurrence.—This was found in the air collected on the roof of the Science Schools, South Kensington Museum. We believe this organism to be identical with that described by Flügge, *loc. cit.*, p. 173, but he does not mention that it liquefies gelatine.

Microscopic appearance.—Under a high power (× 1,000 or 1,500) the cocci are seen to be variable in size, the larger ones exhibiting a division and reaching 1μ in diameter; they are devoid of any definite arrangement. Plate 17, fig. 1, No. 1*c*, represents the appearance of the cocci taken from an agar-cultivation and viewed with a magnifying power of 700; in No. 1*d* they are taken from a gelatine-cultivation and are magnified 1,000 times.

Appearance in Cultivations.

Gelatine.—After four days there is a surface-depression containing an intensely white and opaque mass. As the cultivation becomes older, liquefaction slowly proceeds downwards, the liquid formed being highly glutinous and turbid. (See Plate 17, No. 1*b*.) The mode of liquefaction in the case of this organism is very dependent upon temperature; thus in warm weather, or if the temperature is maintained at about 22° C., the liquefaction takes place in a long funnel, as seen in the Plate, whilst at a low temperature the liquefaction is mostly confined to the surface.

Agar-agar.—Already in the course of three days there is a strong growth forming a smooth and dazzling white mass upon the surface. The brilliancy of the white mass, which resembles a moist patch of Chinese white, is especially characteristic. (See Plate 17, No. 1*a*.)

Broth.—After nine days (August 7—16, 1886) the liquid is pervaded with a fine turbidity; there is no pellicle, but a white deposit is found on the bottom.

Appearance on plate-cultivation.—The colonies are milk-white and, under a low power (× 100), they are seen to have a smooth edge, the interior being granular; and, whilst the older colonies are somewhat irregular in shape, the less developed ones are nearly circular. (See Plate 17, No. 1*e*.)

II.—BACILLI.

1. Bacillus aurescens.
2. ,, aureus.
3. ,, citreus.
4. ,, plicatus.
5. ,, chlorinus.
6. ,, polymorphus.
7. ,, profusus.
8. ,, pestifer.

9. Bacillus lævis.
10. ,, cereus.
11. ,, subtilis.
12. ,, (Micrococcus) prodigiosus.

The above is a list of the various forms of bacilli which have been found by us in air, and which, with the exception of the two last, have not, as far as we are aware, been previously described. We have again ventured to designate these new forms by names which are indicative of some striking characteristic which they possess. Thus in the case of Nos. 1, 2, 3, and 5, the pigments which are produced on cultivation being very marked, the names have been selected with regard to this property. In the case of No. 4, again, the peculiar appearance of the cultivations is suggested in the name; whilst in No. 7 the microscopic appearance, and in No. 8 the strong and highly disagreeable smell possessed by its cultivations, are indicated by the names assigned to them.

1. Bacillus Aurescens.

Occurrence.—This was met with by us as a yellow growth on a gelatine-dish which had been exposed to the air of a railway-carriage.

Microscopic appearance.—Under a high power (× 1,000 or 1,500) this is seen to be a short bacillus occurring singly, in pairs, and in threads of three and four. The individual bacilli are from three to five times as long as broad, with rounded ends. Their length varies from 1·5μ to 3·5μ. In the threads the divisions are not always distinctly visible, and it has then the appearance of a long slender bacillus. In Plate 19, fig. 4, No. 4*b*, the appearance of the bacilli when grown in broth is represented, the magnifying power being 1,000. In No. 4*c* the bacilli are taken from a gelatine-cultivation, and are only magnified about 600 times.

Viewed in drop-cultivations, they exhibit vigorous vibratory and rotatory motion, but no movement of translation was observed.

Appearance in Cultivations.

Gelatine.—The growth is very faint in the track of the needle below, but on the surface it forms a light orange-coloured, *dry*, and much crumpled expansion, which does not cause liquefaction of the gelatine even in very old cultivations. The appearance is very characteristic.

Agar-agar.— Forms a *dry* light-orange surface-growth, much crumpled, with an irregular edge, which is of lighter colour than the central portion. (See Plate 19, No. 4*a*.)

Broth.—After six days (August 7—13, 1886) the liquid is clear, but there is a

plentiful deposit of cream-yellow matter, and the surface is covered with a delicate dirty-white pellicle, which subsequently becomes faintly cream in colour.

Appearance on plate-cultivation.—To the naked eye the colonies are visible as small pin-heads of a faint orange colour. Under a low power (× 100) they are seen to be not perfectly circular, finely granular inside, and with a very slightly jagged edge. (See Plate 19, No. 4*d*.)

2. Bacillus Aureus.

Occurrence.—This was also found forming an orange-coloured pin-head on a gelatine-plate, which had been exposed in the same place as the last.

Microscopic appearance.—With a high power (× 1,000 or 1,500) this is seen as a bacillus forming fine graceful threads (see Plate 19, fig. 5, No. 5*b*), which are considerably longer than those formed by *Bacillus aurescens*. In drop-cultivations they exhibit vibratory motion only.

Appearance in Cultivations.

Gelatine.—There is but little growth in the path of the needle below, but on the surface it forms a dry crumpled expansion, which is of a much deeper orange colour than *B. aurescens*. In old cultivations it causes slight liquefaction of the gelatine.

Agar-agar.—Forms an orange growth, which is less crumpled and less dry in appearance, but deeper in colour than that of *B. aurescens*. (See Plate 19, No. 5*a*.) The cultivations, from which the drawings of these two bacilli were made, were started on the same day, and, although all the conditions were precisely similar, the difference between the two growths was very marked.

Broth.—After six days (Aug. 7—13, 1886) it resembles *B. aurescens*, but the deposit and pellicle were deeper in colour.

Appearance on plate-cultivation.—The colonies differ but little from those of *B. aurescens*, forming pin-heads on the surface, which are, however, of a deeper orange colour, and are more rapid in their growth. See Plate 19, No. 5*c*.

3. Bacillus Citreus.

Occurrence.—This was found producing a yellow pigment on the surface of a gelatine-dish which had been exposed to the air in Hyde Park.

Microscopic appearance.—Under a high power this is seen to be a short fat bacillus about one-and-a-half to twice as long as broad. It frequently exhibits a median transverse division, which can, however, be only well seen with a very high magnifying power ($\frac{1}{20}$ oil-immersion), 1,500 times. Sometimes the bacilli hang together in chains of three and four. The average length of a pair is about 3·4μ; the ends are rounded and sometimes pointed, especially in those cases where division has taken place. Not

unfrequently there are found forms of very peculiar shape. Some are bent and often club-shaped, and present other irregularities in thickness. That these forms are only modifications of the same organism is distinctly proved by the fact that they are found along with the ordinary forms in one and the same colony when the organism is submitted to plate-cultivation. These forms do not appear to be due to involution, as they occur in fresh cultivations and stain well. Neither were spores observed in these nor in any of the other forms. A very large number of microscopic preparations were made from different cultivations of this organism in order to confirm these observations. (See Plate 20, fig. 2, No. 2*b*.)

In drop-cultivations the bacillus is seen to be non-motile.

Appearance in Cultivations.

Gelatine.—After four days (August 6—10, 1886) the needle-track below presents a slight saw-like growth, whilst on the surface there is a small leaf-like expansion extending on either side of the needle-streak. (See Plate 20, No. 2*a*.) This expansion is of a distinct lemon-yellow colour, with a smooth shining surface. The growth does not extend much on keeping the cultivation longer, and no liquefaction of the gelatine takes place.

Agar-agar.—Forms a moist shining surface-expansion of sulphur-yellow colour, and with a lobular edge. The growth, even in old cultivations, was not found to extend over the whole surface.

Broth.—After nine days (August 7—16, 1886) the liquid is clear, free from pellicle, and has a very slight yellowish deposit at the bottom.

Appearance on plate-cultivation.—The colonies are visible to the naked eye as small white discs after four days, which, on keeping longer, become of a strong yellow colour.

Under a low power ($\times$ 100) the colonies are seen to be highly granular, more or less regularly circular in shape, and with an almost smooth edge. (See Plate 20, No. 2*c*.)

4. Bacillus Plicatus.

Occurrence.—This was found forming a white irregular protuberance on the surface of a gelatine dish which had been exposed to the air in one of the wards of the Brompton Hospital for Consumption.

Microscopic appearance.—Under a high power this is seen to be a very minute bacillus, about $1\frac{1}{2}$ times as long as broad. Usually several bacilli are adherent together, thus forming short threads (see Plate 18, fig. 7, No. 7*b*), the length of which varies from 1·7μ to 5μ.

Seen in drop-cultivations, it was found to be very motile. No spore formation was observed.

Appearance in Cultivations.

Gelatine.—The growth to which this organism gives rise in gelatine is exceedingly characteristic. On the surface there appears along the needle-streak a much crumpled and folded greyish expansion, the peripheral corrugation of which causes the surface to become abundantly pitted and excavated. The growth in the needle-track *below* is much less vigorous than *on* the surface, although in course of time it becomes developed to a considerable extent and has a beaded appearance.

It causes no liquefaction of the gelatine, even in old cultivations. (See Plate 18, No. 7*a*.)

Agar-agar.—The appearance is very similar to that of the cultivation in gelatine; the surface is, however, of a somewhat more moist texture, and the edge extends in thin fern-shaped expansions over the surface of the agar-agar.

Broth.—After nine days (August 7—16, 1886) the liquid is very slightly turbid, has a dirty-white deposit, and there is a small amount of flocculent matter on the surface; and, adhering to the sides of the tube, this develops later on into a tough irregular pellicle.

Appearance on plate-cultivation.—After four days the colonies appear to the naked eye as small white discs, the larger ones, which have reached the surface, exhibiting an indentation in the centre. As growth proceeds, the centre of the colony remains depressed, whilst the circumference becomes irregularly folded and raised, so that the colony is only attached to the surface of the gelatine by a comparatively narrow pellicle. The substance of the colony is very tough in character, so that the whole growth can be easily removed in its entirety by means of a needle.

Under a low power (× 100) the small colonies have a rough irregular edge varying in shape and degree of roundness. The larger colonies are dark-brown near the edge, but of a lighter shade near the centre; they are very irregular in shape; the contents are finely granular. The different stages of development are exhibited in Plate 18, No. 7*c*.

5. Bacillus Chlorinus.

Occurrence.—This was found as a yellow slowly-liquefying expansion on the surface of a gelatine dish exposed to the air on the spire of Norwich Cathedral. We have found it on numerous occasions to be very prevalent in air.

Microscopic appearance.—Under a high power this is seen to be a very short bacillus, varying from ·5μ to 1·5μ in length and about half as broad as long; the extremities are rounded. It occurs singly and in short chains. (See Plate 17, fig. 7, No. 7*b*.) In drop-cultivations only vibratory motion was observed.

Appearance in Cultivations.

Gelatine.—After four days (August 6—10, 1886) the needle-track below exhibits only a very faint growth, whilst on the surface there is a liquefied depression with a lemon-yellow deposit. Liquefaction proceeds slowly, the track of the needle below the surface remaining very faint.

Agar-agar.—Produces a strong, almost uniform, shining surface-growth of a greenish-yellow colour.

Broth.—After nine days (August 7—16, 1886) the liquid exhibits a fine turbidity; there is no pellicle on the surface, but a dirty-yellow deposit on the bottom.

Appearance on plate-cultivation.—On the third day (October 29—November 1, 1886) the colonies appear as greenish shining expansions, rapidly extending on the surface, but remaining small in the depth of the gelatine.

Under a low power (× 100) the larger surface-colonies exhibit very fine granulation, with a thin smooth edge. The smaller colonies have also a smooth sharp edge, with a cloudy interior. (See Plate 17, No. 7*a*.)

6. Bacillus Polymorphus.

Occurrence.—This was obtained as a small colourless pin-head with radiated rim on the surface of a gelatine dish which had been exposed to the air on the roof of the Science Schools, South Kensington Museum.

Microscopic appearance.—This organism exhibits a great variety of forms, even in cultivations only one day old. In the first place there are seen small fat bacilli, almost like micrococci; then there are longer or more oval individuals, frequently occurring in pairs, and also forming strings of irregular thickness. In these strings there is frequently no division visible, and such an irregular band sometimes reaches 17μ in length. The isolated bacilli are ·8μ in length and nearly as wide, whilst when united in chains they appear several times this size.

At first sight this variety of form has the appearance of an impure cultivation. We have, however, found the same variety in examining the contents of single colonies from plate-cultivations of this organism (see Plate 17, fig. 6, Nos. 6*b*, 6*c*), and there can, therefore, be no doubt that all these forms belong to one and the same organism. No. 6*b* was taken from a colony obtained on plate-cultivation, and No. 6*c* from an agar-tube cultivation. Viewed in drop-cultivations, they appear almost like micrococci, singly and in chains of varying length. Vibratory motion only was observable.

yellow. (See Plate 17, No. 6*a*.) The needle-track below the surface exhibits a fine saw-like growth, which is more considerable than that of many of the organisms described above.

Agar-agar.—The growth again exhibits a highly serrated edge, but the rate of extension over the surface is more rapid than in the case of the gelatine.

Broth.—After nine days (August 7—16, 1886) the liquid is turbid above, but clear below, and is clothed with a thin cloudy-white pellicle on the surface. There is also a white deposit at the bottom.

Appearance on plate-cultivation.—To the naked eye the colonies are circular and bluish-white, with a small yellow spot in the centre. On the surface of the gelatine they form distinct pin-heads. Under a low power (× 100) the larger surface-colonies are seen to be circular, with an irregular corrugated edge, enclosing coarse granular matter. The central portion is cloudy and surrounded by a distinct ring. (See Plate 17, No. 6*d*.)

The smaller colonies in the depth of the gelatine are very irregular in shape and resemble the corolla of a flower. The contents of the colony is more finely granular than those of the larger surface-colonies, the centre being also clouded. (See Plate 17, No. 6*e*.)

As in other cases, the cultivations were made by inoculating from both kinds of colonies, and the identity of the two proved.

7. Bacillus Profusus.

Occurrence.—This was found in the air collected on the roof of the Science Schools, South Kensington Museum, producing a beautiful iridescent growth on the surface of gelatine.

Microscopic appearance.—Under a high power it is seen to be a short fat bacillus with rounded extremities. The length reaches about 1·7μ and the width about ·5μ. As seen in Plate 18, fig. 2, No. 2*a*, the dimensions of the bacilli are very variable even in one and the same colony (the drawing was made from a preparation taken from a colony). These larger forms are comparatively rare; their length is more than 1·7μ, the width even sometimes reaching that figure.

Viewed in drop-cultivations, they were found to exhibit vibratory motion only, and were seen isolated as well as hanging together in short chains of two, three, and four.

Appearance in Cultivations.

Gelatine.—There is but little growth in the path of the needle *below*, but *on* the surface it frequently extends in a very thin layer which has a beautiful opalescent appearance when viewed by transmitted light.

Agar-agar.—On this medium it forms a much thicker growth, giving rise to a smooth, whitish, lobular expansion, the thinner foliated margin of which exhibits beautiful iridescence by transmitted light.

Broth.—After seven days (August 17—24, 1886) the liquid is clear, excepting the surface, on which there is some thin, granular, floating matter, and at the bottom there is a small amount of whitish deposit.

Appearance on plate-cultivation.—The surface-colonies are seen with the naked eye to form an opalescent expansion of increasing size, with a very irregular contour. (See Plate 18, No. 2*d*.) In the depth of the gelatine, on the other hand, the colonies appear as grey dots. Under a low power (× 100) the surface-colonies exhibit a dense centre, surrounded by a very thin and granular expansion having a highly irregular contour. (See Plate 18, No. 2*b*.) The drawing represents a colony in which this surface excrescence is commencing. No. 2*c* represents a colony in the depth of the gelatine. Viewed against the light, these surface-colonies are of a beautiful azure-blue colour.

8. Bacillus Pestifer.

Occurrence.—This was found forming a small white expansion on the surface of a gelatine-dish which had been exposed to the air in a garden near Hughenden, Bucks.

Microscopic appearance.—Under a high power this is seen to be a large thick bacillus about 3·4μ in length and from ·8μ to 1·7μ in thickness; the length is difficult to determine, owing to the formation of threads, which are frequently of great length, extending far beyond the field of the microscope, and giving rise to winding vermiform figures. (See Plate 19, fig. 7, No. 7*b*.)

Viewed in drop-cultivations, the bacilli are seen to exist singly, in pairs, threes and fours, &c., up to exceedingly long threads. Their movement is slow and undulating, the single bacilli exhibiting most motility. It also forms non-motile tangled masses, but in no case was spore-formation observed. In Plate 19, No. 7*c*, which is drawn from a drop-cultivation, the arrangement of the bacilli in smaller groups is shown. Although we have examined a very large number of preparations of this organism, both in young and old cultivations, in gelatine, agar-agar, and broth, we have never observed any spore-formation.

Appearance in Cultivations.

Gelatine.—On the surface it produces an almost colourless feathery expansion, which causes slow liquefaction of the gelatine.

Agar-agar.—Commences by forming a grey-white smooth surface-growth, which rapidly extends over the agar; the surface-growth sometimes becomes very much wrinkled, like that of the *Bacillus subtilis* (see below), but it has a more moist and shining appearance than the latter, and is of a grey, transparent, almost colourless hue. The wrinkles are very highly convoluted and twisted. (See Plate 19, No. 7*a*.)

Broth.—After four days (August 31—September 4, 1886) the liquid is slightly

turbid, free from pellicle, and has a small quantity of white deposit at the bottom. Even after thirteen days there is only a thin film on the surface, which falls to the bottom on shaking, and there is very little deposit.

Appearance on plate-cultivation.—After two days the colonies appear to the naked eye only as white specks, but seen with a low power (× 100) those on the surface exhibit a very irregular contour, consisting of branchings into the surrounding gelatine of threads; the interior of the colony has the appearance of being composed of threads closely packed together; as they develop further, the centre becomes very dark and cloudy, but the edge remains very light, and thus much resembles a crystal branching out in feathers into the surrounding gelatine; after five days the feathery contours can be seen with an ordinary magnifying glass. In the depth the colonies appear compact and almost circular. (See Plate 19, No. 7*d*.)

In all cultivations this organism gives rise to a most disagreeable odour, somewhat resembling that of putrid blood.

9. Bacillus Lævis.

Occurrence.—This was found forming a yellowish-white liquefying growth on the surface of a gelatine-dish which had been exposed to the air in one of the wards of the Brompton Hospital for Consumption.

Microscopic appearance.—Under a high power this is seen to be a bacillus the average length of which is 1·7 to 2·5μ, and it is about 5 times as long as broad; the ends are distinctly rounded. It occurs singly, often in pairs, and occasionally in threads. It gives rise to spores which are nearly as long as the bacillus itself, but more oval in shape, and which exhibit the characteristic highly refractive appearance of spores in general. All the well-known forms of *Bacillus subtilis* were observed, including the thickened form, only on a much smaller scale, and the threads being considerably shorter.

In preparations made from the surface of agar-agar cultivations frequently nothing but spores were visible. Whilst the bacillus is readily stained with any of the ordinary aniline colours (gentian-violet, &c.), the spores prove refractory as usual.

In Plate 19, fig. 6, No 6*c*, the preparation was made from a gelatine-cultivation of ten days' age. In No. 6*b* the appearance is shown when a preparation is made from a colony after three days' growth. Tube-cultivations started from such colonies yielded in course of time all the various forms represented in No. 6*c*.

In drop-cultivations the bacilli are seen to be exceedingly motile, occurring singly, in pairs, and occasionally in threads; subsequently stationary masses of bacilli make their appearance, and shining spores are visible.

Appearance in Cultivations.

Gelatine.—After four days liquefaction has commenced at the top of the needle-track, forming a round depression, the bottom of which is filled with a white cloudy liquid. After nine days the liquefaction has extended across the whole tube to a depth of half-an-inch. The liquid is turbid and has a tough, greyish, wrinkled pellicle upon its surface and a flocculent deposit at the bottom. The lower part of the needle-track does not exhibit much alteration even at this stage. Ultimately the whole contents of the tube becomes liquid. (See Plate 19, No. 6*a*.)

Agar-agar.—The growth is but little characteristic. It forms a moist, shining, greyish-white surface expansion, which rapidly extends over the whole agar-agar.

Broth.—After nine days (August 7—16, 1886) the liquid is turbid near the surface and clear below; there is a dirty-white flocculent deposit at the bottom, and a thin granular pellicle on the surface. Subsequently the liquid becomes clear, the pellicle remaining on the surface.

Appearance on plate-cultivation.—In three days the colonies are visible to the naked eye as small white dots, the surface-colonies exhibiting a slight flocculence, which indicates the commencement of liquefaction; as the colonies increase in size, liquefaction of the gelatine slowly proceeds.

Under a low power (× 100) the colonies in the depth of the gelatine are seen to have a smooth edge, which is irregular in shape and encloses granular contents.

The surface-colonies exhibit a very fine thin film of irregular shape, extending from a small centre, indicating the spot where the colony first reached the surface and began to liquefy. (See Plate 19, No. 6*d*.)

The characteristic differences between this organism and *Bacillus subtilis* will be pointed out after the latter has been fully described.

10. Bacillus Cereus.

Occurrence.—This was found producing a large liquefying colony on a gelatine-dish which had been exposed to the air in a cow-shed.

Microscopic appearance.—The bacilli are from 3·4 to 12μ in length. There are also seen thickened forms about 3·4μ long and 1·7μ wide. The ends of the bacilli are generally slightly rounded, whilst some are almost quite square. The bacilli form threads which are very variable in length, some being composed of ten segments or more.

Spore-formation was also observed as seen in the Plate. (See Plate 20, fig. 3, No. 3*a*.)

In a drop-cultivation the following changes were observed to take place:—

When examined directly after inoculation from an agar-agar cultivation, there were visible isolated bacilli, many of which contained a single spore, and free spores were also present; there was practically no movement taking place. Within 12 hours

there were numerous very motile bacilli, generally isolated, but occasionally forming longer threads.

After 24 hours the bacilli were perfectly motionless, generally in pairs or in threads of three and four. After 48 hours the bacilli were still stationary, and there was abundant spore-formation, each segment exhibiting a shining oval spore in its interior. As the cultivation increased in age the threads were gradually broken up and the spores liberated. The free spores exhibit vibratory movement.

Appearance in Cultivations.

Gelatine.—The mode of growth essentially resembles that of the *Bacillus subtilis* in this medium, the only difference being that it causes more rapid liquefaction of the gelatine than the latter.

Broth.—Growth practically identical with that of *Bacillus subtilis* in this medium.

Agar-agar.—The growth in this medium presents a marked difference to that of *Bacillus subtilis.* It forms a moist, grey-white, *smooth,* wax-like expansion, which rapidly extends over the surface of the agar-agar. Even in very old cultivations no wrinkling, but only slight granulation of the surface, takes place.

Appearance on plate-cultivation.—Owing to the exceedingly rapid liquefaction of the gelatine which this organism causes, it is necessary to examine the plates within 24 hours of their being poured, in order to observe the first appearances presented by the colonies.

We have examined a number of plate-cultivations of this organism, but the following description will serve to illustrate the progressive development of the colonies.

After keeping the plate at 18–20° C. for 18 hours, the colonies were just visible to the naked eye as small white dots, no apparent liquefaction having yet set in. Under a low power (× 100) the colonies appear as round or oval woolly masses having a finely spinose edge, from which, in many cases, long whip-like and spirally-coiled threads extended into the surrounding gelatine. Some of the colonies, on reaching the surface, gave rise to highly-irregular filamentous growths consisting of bands of fine threads, as subsequently described and drawn in the case of the colonies of *Bacillus subtilis.* (See Plate 20, fig. 5, Nos. 5*e*, 5*f*.) These filamentous surface-growths sometimes appear as though they were not derived from any colony of the usual kind, but had arisen quite independently ; this appears to be due to the colony from which they proceed having been situated very near the surface, and having only attained very insignificant dimensions before reaching it ; and, having once arrived there, the growth on the surface is enormously more rapid than in the depth, and soon produces liquefaction. Other colonies, again, situated in the depth of the gelatine, exhibit a more uniformly spinose contour, as seen in Plate 20, fig. 3, No. 3*b*.

After 24 hours the colonies had considerably increased in size, being very apparent to the naked eye, although active liquefaction had not yet set in. Under a low power

(× 100) the whip-like extensions noticed above had enormously increased, the greater number of the colonies having the appearance presented in Plate 20, fig. 3, No. 3*c*; others, again, like that shown in Plate 20, fig. 3, No. 3*b*; and others partaking of the character of both these, as shown in Plate 20, fig. 5, No. 5*f*. After 36 hours the colonies had further increased in size, and in many cases the whip-like extensions had become much thickened; in some colonies these gave rise to a star-fish appearance, which is easily visible to the naked eye.

We have established beyond doubt that all the above forms of colony are derived from one and the same organism, inasmuch as we have repeatedly prepared plates by inoculation from single colonies and again obtained colonies of the same diversity in appearance.

11. Bacillus Subtilis. (Hay Bacillus.)

Although this micro-organism has become classical through the great care with which it has been described by numerous authorities, including Cohn, Koch, Klein, Flügge, and many others, it is only recently that the appearances to which it gives rise on plate-cultivation have been recorded (Eisenberg, *loc. cit.*; Flügge, *loc. cit.*). We have had occasion to carefully examine the appearances produced by this organism in order to compare them with those resulting from some of the organisms described above. For the purposes of this comparison, we have employed a cultivation which was obtained by one of us from Dr. Koch's laboratory in Berlin.

Microscopic Appearance.

The single bacilli vary in length from 1·7μ to 6·8μ, and are about 1·7μ in width; the ends are slightly rounded, but sometimes nearly rectangular. Prior to spore-formation the bacilli become thicker and more square (see Plate 20, fig. 5, No. 5*c*), and, as described in the case of *B. cereus*, these thicker forms present a very different appearance to the ordinary bacilli. The bacilli also grow into threads, which are frequently of great length. The spores, which are to be seen in all but the newest cultivations, have a length of about 2·5μ, and are about 1μ in width; they are oval, and present, as usual, a bright and shining appearance, which, together with their property of not staining with aniline colours, renders them easily distinguishable from the bacilli. In Plate 20, fig. 5, Nos. 5*c* and 5*g*, these various forms are represented; thus in No. 5*g* are the ordinary bacilli, also the thickened bacilli, also bacilli containing spores; whilst in No. 5*c* a thread is shown composed of numerous segments, also a similar thread which has become thickened and exhibits a spore in each segment.

Viewed in drop-cultivations, the isolated bacilli are seen to be very motile, but there are also stationary masses of bacilli which are non-motile. Subsequently threads and spore-formation are observable, as previously described in the case of *B. cereus*.

Appearance in Cultivations.

Gelatine.—The growth gives rise in the course of a few days to liquefaction of the gelatine in the form of a long funnel, the lower part of which throws out feathery lateral extensions into the adjacent gelatine. (See Plate 20, fig. 5, No. 5*a*.) Soon the liquefaction extends across the tube at the surface, and ultimately involves its whole contents, a tough white pellicle forming on the surface, the liquid below becoming clear, and a large quantity of flocculent matter becoming deposited at the bottom.

Agar-agar.—The growth rapidly extends over the surface as a white opaque expansion, which soon assumes a dry appearance and becomes copiously wrinkled and puckered. (See Plate 20, fig. 5, No. 5*b*.)

Broth.—Grows rapidly, rendering the liquid turbid and giving rise to a white deposit at the bottom, and forming a pellicle on the surface which gradually increases in thickness and tenacity.

Appearance on plate-cultivation.—The colonies become visible to the naked eye in about two days' time as small white dots when beneath, whilst *on* the surface they exhibit a very small liquefied circle of a greyish hue.

Under a low power (×100) the colonies in the depth of the gelatine are seen to have an irregular contour, with short spinose extensions in parts of the circumference, and the interior of each colony has a wavy structure, as if composed of coiled threads. (See Plate 20, fig. 5, No. 5*d*.) As the colonies increase in size the internal structure becomes less defined, whilst the circumference becomes uniformly spinose (See Plate 20, fig. 3, No. 3*b*.)

In the early stages (about after two days' growth) the surface of the gelatine presents in places small cloudy expansions, which, when viewed under a low power (×106) exhibit a most characteristic appearance, which seems to have hitherto escaped observation, consisting of a highly-irregular figure (see Plate 20, fig. 5, No. 5*e*), composed of parallel bands of fine threads arranged in a much-contorted pattern. This appears to be the form assumed by the colonies *on first reaching the surface of the gelatine*, for, on further preserving a plate exhibiting a number of such "thread" colonies, in the course of a day or two their appearance will be found to have entirely changed, their place being taken by a liquefied surface, the margin of which exhibits the usual spinose character first described. In Plate 20, fig. 5, No. 5*f*, an ordinary spinose colony in the depth is seen to be breaking out into a thread-expansion where it has reached the surface.

Tubes both of gelatine and agar-agar were inoculated from both varieties of colony, each giving rise to the same characteristic appearances already described. Plates were again prepared from these separate cultivations, and both varieties of colony obtained from each cultivation.

It thus appears that *Bacillus subtilis* as well as *B. cereus*, described above, both form colonies of several different types, the form of which depends upon the position in the gelatine-film of the bacilli from which they are derived.

In the first place there are compact colonies in the depth of the gelatine, which soon show small spinose or hair-like extensions from the periphery. These extensions increase in thickness, but remain fairly uniform in length, and ultimately the colony produces a liquid circle in the gelatine, and the periphery of this circle has also a finely spinose appearance. This appears to be the only form of colony which has been described by other observers. The appearance of these colonies may be compared to that of a "*crown of thorns.*"

In a modification of the first class of colony, which appears to arise when the plates are incubated at a somewhat higher temperature, the hair-like extensions from the compact colony in the depth are much longer and irregular, often spiral and twisted, or resembling the lash of a whip. The formation of these longer extensions is probably accounted for by the smaller resistance offered by the gelatine at the higher temperature. These likewise ultimately produce liquefied colonies which do not differ in appearance from the liquefied colonies of the first class. This second class may be designated *whip-colonies.* These whip-like colonies appear to be especially characteristic of *Bacillus cereus.* (See Plate 20, fig. 3, No. 3*c*.)

Thirdly, there are found surface-growths of very remarkable appearance, and consisting of parallel bands of threads meandering over the surface of the gelatine in the most capricious manner, and frequently expanding into coils. These surface-growths are often quite independent of any compact colony, their production being, as far as we can see, due to the original individual organism or group of organisms from which they have sprung being situated so near the surface of the gelatine that the first out-growth, having promptly reached the surface, has there grown out in free contact with the air with enormous rapidity. Thus in very young plates these surface-growths are already found of enormous dimensions compared with the compact colonies in the lower strata of the gelatine. This third class may be fitly named *meander-colonies.* They also give rise to liquefaction, the edge of the liquefied portion being similar to that of the liquefied colonies of the other two classes. We have found these meander-colonies in the case of the *Bacillus cereus* as well as in that of *Bacillus subtilis.*

In Plate 20, fig. 4, we have represented a colony of the *Bacillus anthracis* which presents many points of resemblance to those of the *Bacillus subtilis,* more especially to the "whip" and "meander" modifications.

Points of Distinction between B. lævis, B. cereus, *and* B. subtilis.

From the descriptions which we have given above it will be seen that these three micro-organisms resemble each other very closely in many points; they can, however, be sharply distinguished on the following grounds:—

Liquefaction of gelatine.—B. cereus causes by far the most rapid liquefaction of the gelatine; *B. subtilis* stands second in this respect, the liquefaction being very

decidedly less rapid; whilst *B. lævis* possesses the power of liquefying gelatine to a much less extent than either of the other two.

Agar-agar cultivations.—*B. subtilis* is very sharply distinguishable from the other two by the property which it possesses of imparting a characteristic wrinkled appearance to the surface of the agar-agar.

Colonies on gelatine plates.—The colonies produced by *B. lævis* are, as has been shown above, very different from those of the other two, whilst we have not been able to establish any substantial difference between the colonies of *subtilis* and *cereus* beyond the difference in the rate of liquefaction, already referred to, and the longer and more spiral form of the whip-like extensions which we have constantly observed to be characteristic of *B. cereus.*

Microscopic appearance.—In this respect, again, *B. lævis* exhibits a marked difference from the other two, its dimensions being very decidedly smaller.

12. Bacillus (Micrococcus) Prodigiosus.

This organism was first described by Cohn as a micrococcus, but is now generally regarded as a bacillus.

We have met with this organism both in air and water, but we have especially studied it from a cultivation obtained by one of us from Dr. Koch's laboratory in Berlin.

Microscopic appearance.—The cells are rather longer than broad, the largest forms being about $1{\cdot}7\mu$ in length and about 1μ in width; they are frequently found hanging together in pairs. More distinctly bacillar forms have been described by Cornil and Babès ('Les Bactéries,' 1886, p. 141) as occurring in broth-cultivations. We can fully confirm these observations, having ourselves seen them in drop-cultivations, and, what is more convincing, in single colonies. (See Plate 20, fig. 1, No. 1*c*.)

Appearance in Cultivations.

Gelatine.—Grows very rapidly, liquefying the gelatine in the form of a conical sack (see Plate 20, No. 1*b*) which soon extends across the tube at the top and, gradually passing downwards, involves the whole tube. The liquid formed is very turbid, with an abundant flocculent deposit of an intensely crimson colour. Near the surface there is generally seen adhering to the glass a thin layer of still darker red colouring matter which has the peculiar fluorescence of an aniline colour when in a concentrated state.

Agar-agar.—Grows very rapidly over the surface, producing a deep, blood-red, smooth and shining expansion, the colour being only developed on the surface. (See Plate 20, No. 1*a*.)

Broth.—Grows rapidly, rendering the broth turbid, and producing in the first

instance a white deposit, which later on becomes of a pinkish colour, whilst at the surface the colour is visible much sooner.

Appearance on plate-cultivation.—After two days the colonies are seen to the naked eye as circular depressions, each having a red centre. Under a low power (× 100) the less-developed colonies in the depth of the gelatine are devoid of red colour; they are finely granular, with a very irregular contour. (See Plate 20, No. 1*d*.) The surface-colonies, on the other hand, have a distinctly red nucleus surrounded by a very thin and finely granular brownish growth having a very irregular contour. (See Plate 20, No. 1*d*.) No. 1*e* represents the appearance of the colonies to the naked eye.

III.—SACCHAROMYCES.

We have found two varieties of Saccharomyces in the air, the one colourless, and the other producing a red pigment. They are both of very frequent occurrence.

1. Saccharomyces Liquefaciens.

This organism produces very characteristic star-shaped liquefying colonies on gelatine-plates which have been exposed to the air.

Microscopic appearance.—The individual cells are oval and 7 and even 9μ in length, and from 3 to 5μ in width. They appear only to bud from the apex, and frequently there are twin buds from the same apex of the parent cell; occasionally they are seen hanging together in long strings. (See Plate 19, fig. 1, No. 1*d*.)

Appearance in Cultivations.

Gelatine.—After a few days the needle-track below the surface exhibits small feathery centres in its lower portion, whilst in its upper portion the track is continuous and throws out hair-like lateral extensions of increasing length into the adjacent gelatine. On the surface there is a depression filled with white, cloudy, liquefied matter. Later on, the liquefaction extends across the tube, and ultimately involves the whole of the gelatine. (See Plate 19, No. 1*a*.)

Agar-agar.—Forms a shining surface-growth exhibiting radial marking from the central puncture. The surface is *very* faintly pink in colour. The track of the needle beneath the surface exhibits beautiful feathery lateral extensions. (See Plate 19, No. 1*b*.)

The growth both in gelatine and agar-agar is exceedingly characteristic.

Broth.—After nine days (August 7—16, 1886) the liquid is clear, with a dirty-white deposit at the bottom.

Appearance on plate-cultivation.—The colonies when young appear as small cloudy centres to the naked eye. As they increase in size they assume a star-shaped appearance, the rays of which gradually extend and liquefy the gelatine in a highly characteristic manner.

Under a low power (× 100) the branches of the star are seen to be composed of the individual cells closely compressed together in packets. The appearance is shown in Plate 19, 1*c*. No 1*e* represents the naked-eye appearance of a colony.

In its microscopic appearance this saccharomyces appears to correspond with the description of *S. apiculatus*, but the behaviour of the latter in cultivations has not been described.

2. Saccharomyces Rosaceus.

We have found this on gelatine-plates which have been exposed to the air. It forms small pink pin-head colonies on the surface of the gelatine.

Microscopic appearance.—The cells are long, oval, about 8·5μ long and 3·5μ in width. They appear to bud from the extremities only, and there are frequently two twin buds adhering to the same extremity of the parent cell. They are sometimes seen hanging together in chains of four. (See Plate 19, 2*b*.)

Appearance in Cultivations.

Gelatine.—It forms a shining pink expansion on the surface, there being but little growth in the track of the needle below.

Agar-agar.—On the surface it forms a smooth shining growth of a beautiful rose colour. The growth exhibits lines of darker tint radiating from a lighter centre (see Plate 19, 2*a*). There is no colour and but little growth in the needle-track below the surface.

Broth.—After six days the liquid is clear, with a dirty-pink deposit at the bottom, which later on becomes of a bright-pink colour, the liquid remaining clear.

Appearance on plate-cultivation.—The colonies are seen to the naked eye as shining pink pin-heads, which do not cause liquefaction of the gelatine. Under a lower power (× 100) the individual cells composing the closely packed masses of which the colonies consist can be distinctly seen, especially in the case of the surface colonies. (See Plate 19, 2*c*.)

IV.—MOULD PRODUCING A BROWN PIGMENT.

This was found by us producing a dark-brown pigment on a gelatine-plate which had been exposed to the air on Norwich Cathedral.

Microscopic appearance.—It forms a tangled network of branching filaments without visible division; in thickness it goes up to ·8μ. In Plate 18, fig. 1, No. 1*d*, the filaments are shown magnified about 600 times. No spore-formation has been observed.

Appearance in Cultivations.

Gelatine.—After a few days the needle-track below the surface exhibits small, isolated, flocculent centres. The surface is depressed and exhibits greyish flocculent

plaques fringed with brown, which shades off into the surrounding gelatine.* As the age of the cultivation increases, the brown colour becomes more intense and gradually extends throughout the tube. The gelatine becomes slowly liquefied, the fluid being of the same dark-brown colour. (See Plate 18, No. 1*b*.)

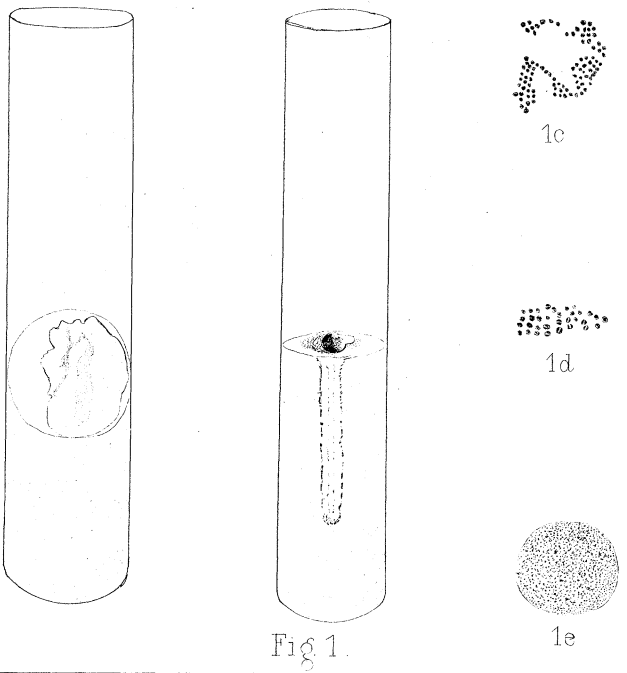
Agar-agar.—The surface of the agar becomes covered with flocculent greyish plaques around which the brown colour is formed, the latter extending gradually into the depth of the tube. (See Plate 18, No. 1*a*.)

Broth.—The appearance is very characteristic. Numerous small, isolated, spherical tufts make their appearance throughout the liquid, adhering to the sides and bottom of the tube. (See Plate 18, No. 1*c*.) The liquid, which is quite clear, becomes of a deep sherry colour.

Appearance on plate-cultivation.—The colonies appear as cream-white disks, each of which is surrounded by a cloud of brown colouring matter extending over a distance many times the diameter of the colony.

Under a low power ($\times 100$) the colony is seen to consist of fine branching threads radiating from a central tangled mass.

* This surface-growth is of such a tough nature that it is with some difficulty that a portion can be removed with a platinum-needle.



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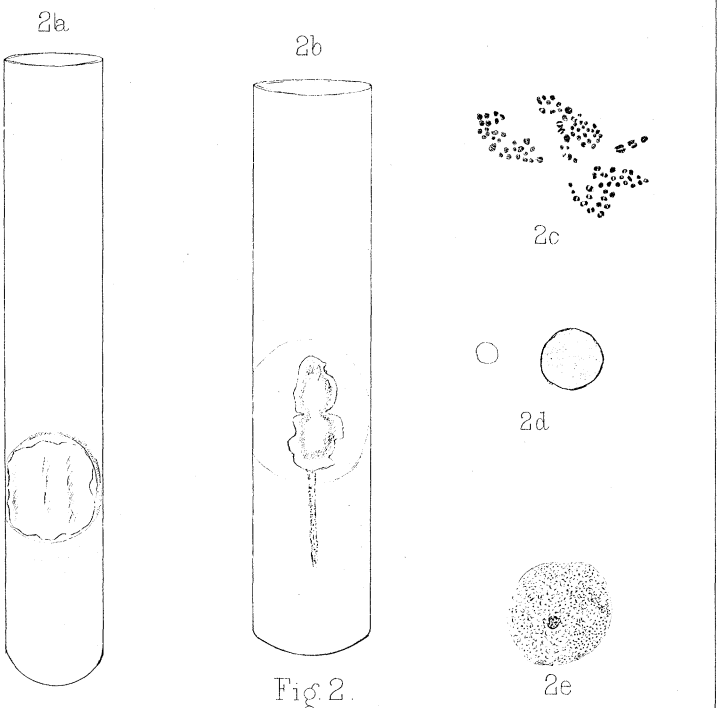

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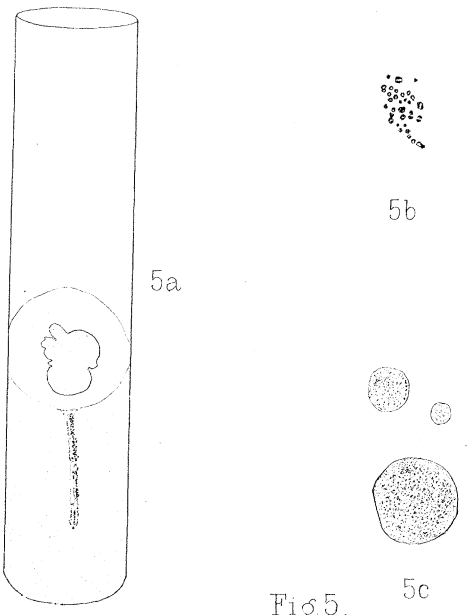

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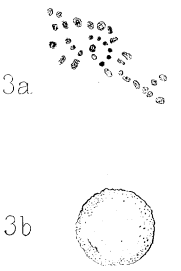

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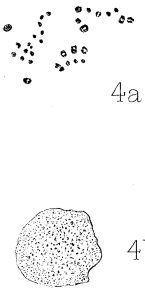

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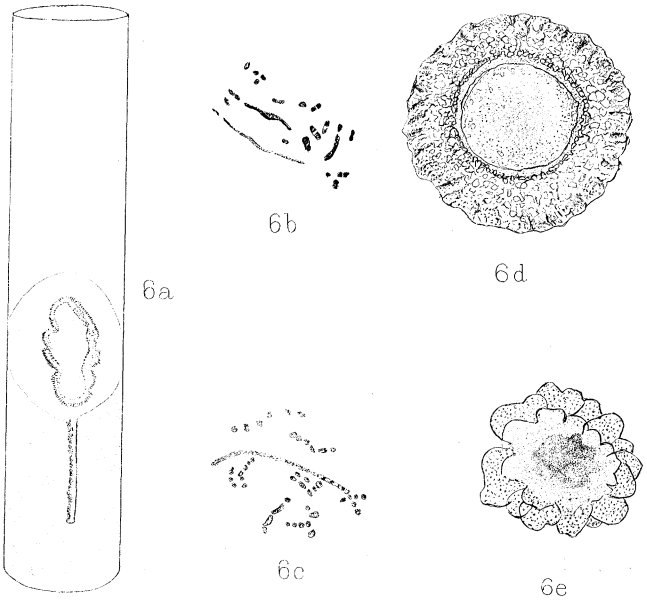


Fig 6

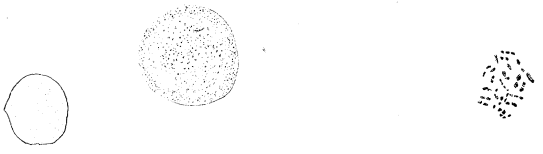
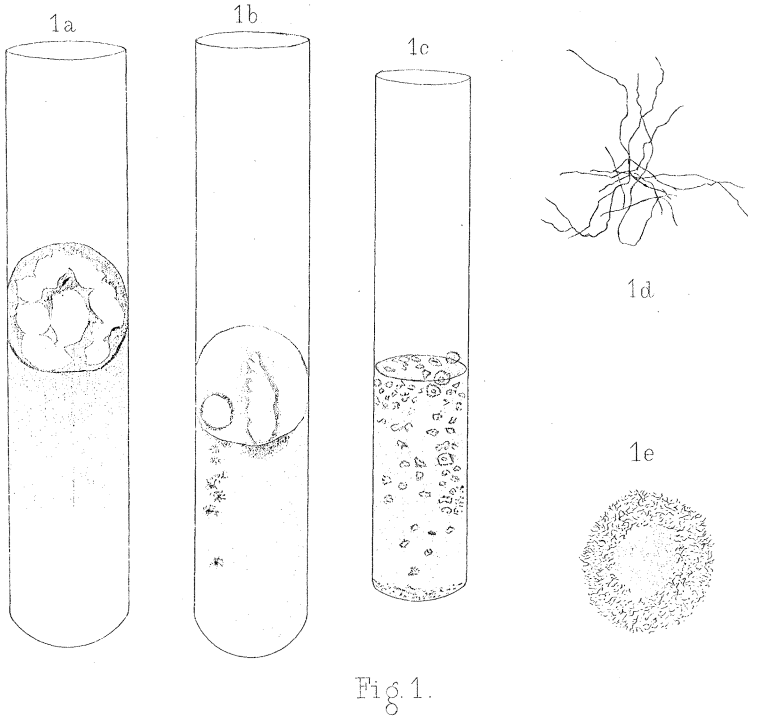


Fig 7



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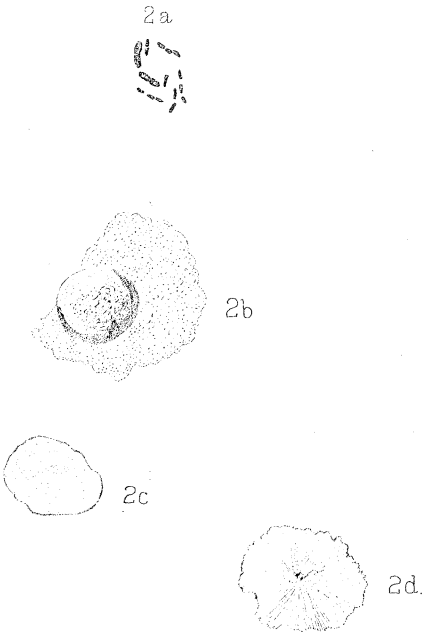

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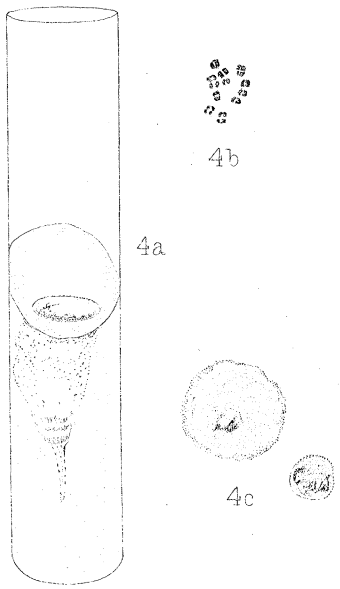

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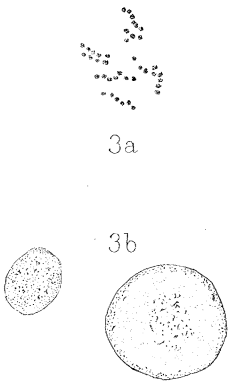

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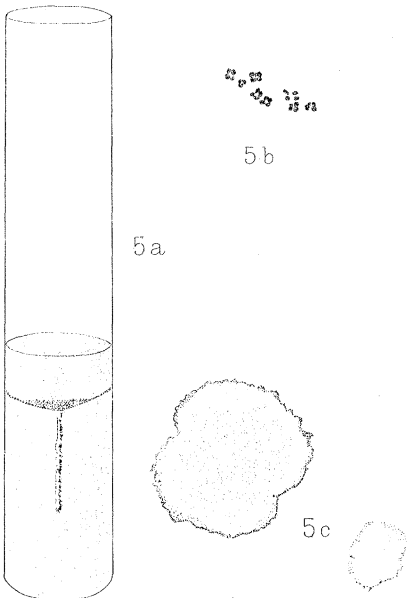

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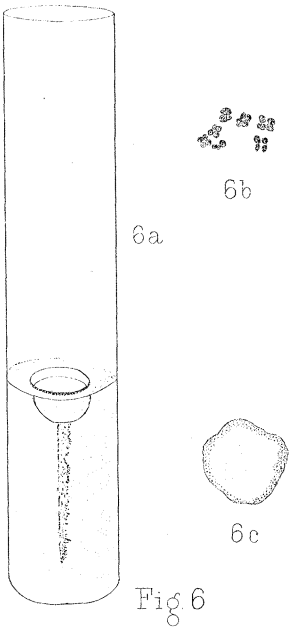

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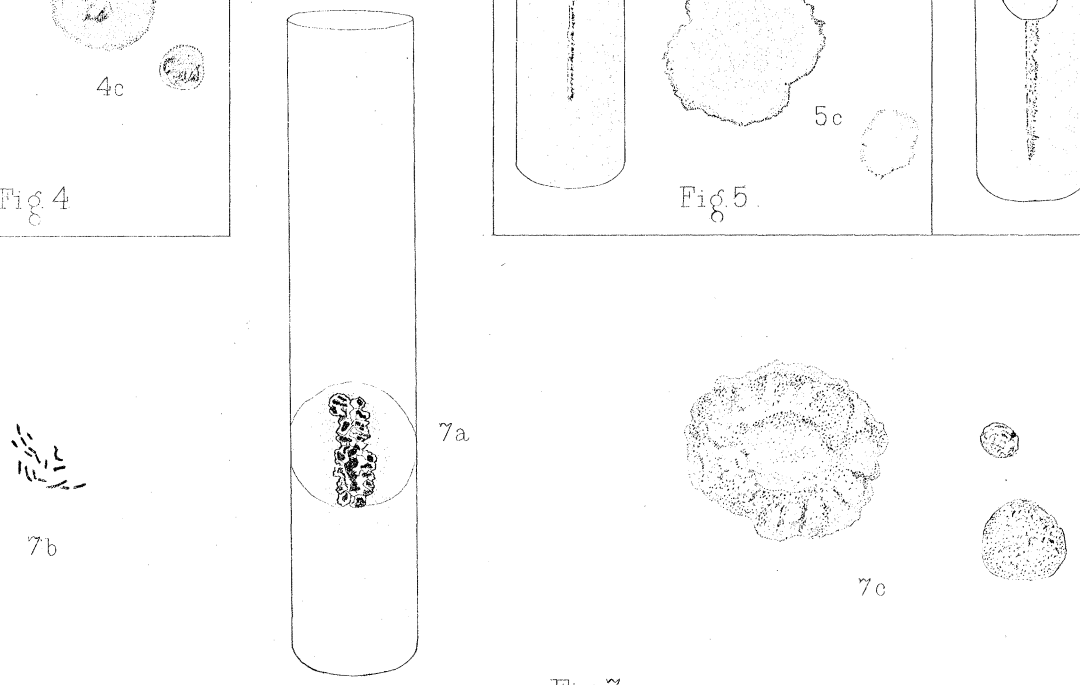

Fig. 7

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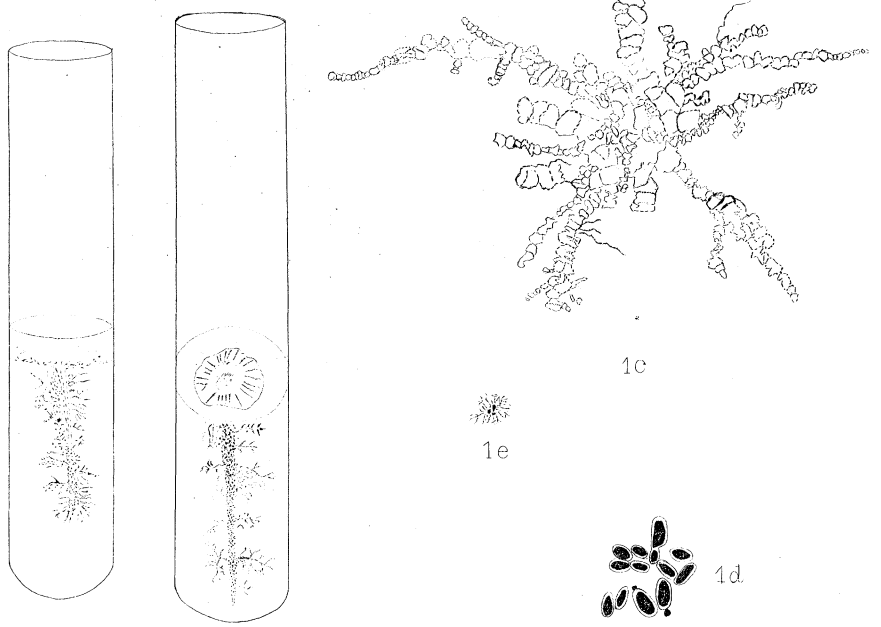

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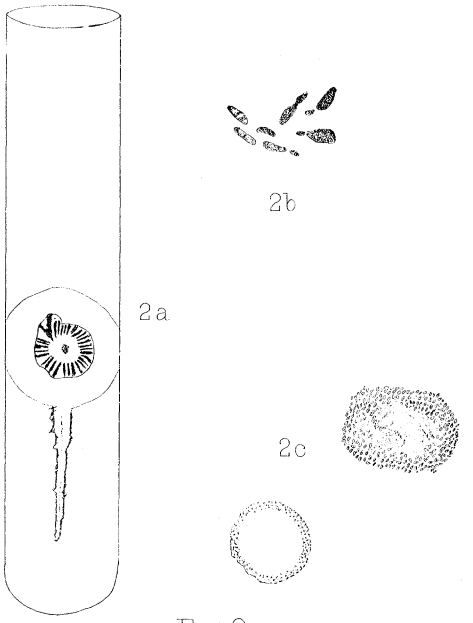

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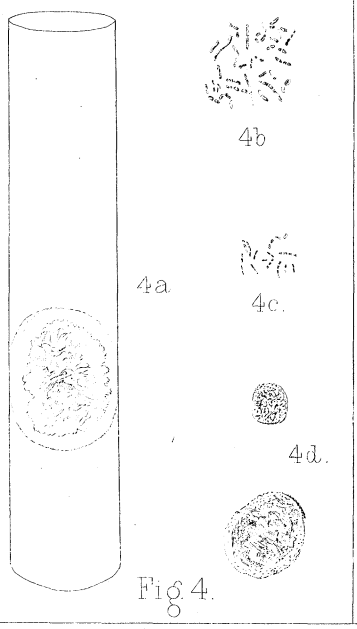

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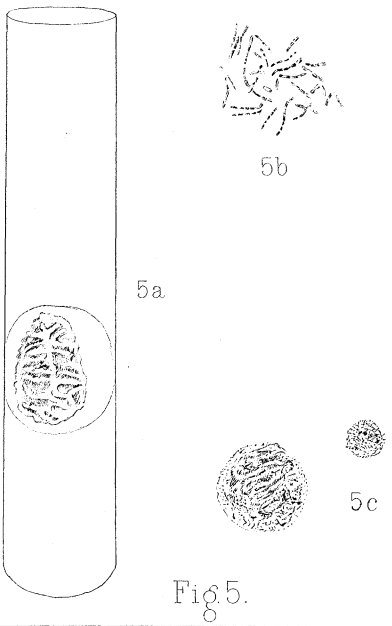

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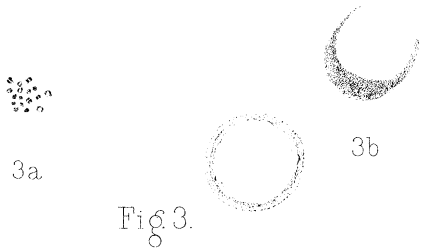

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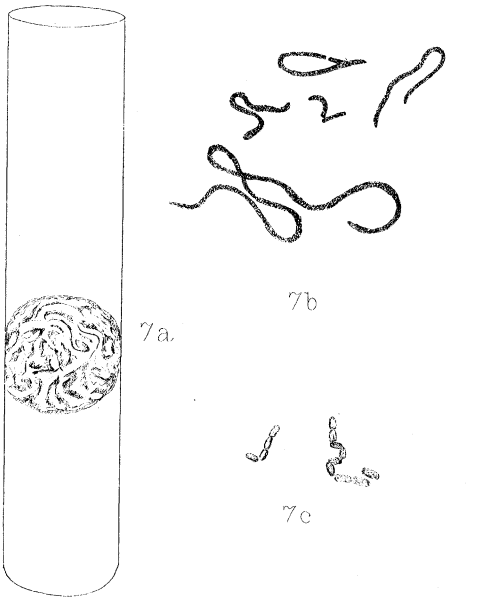

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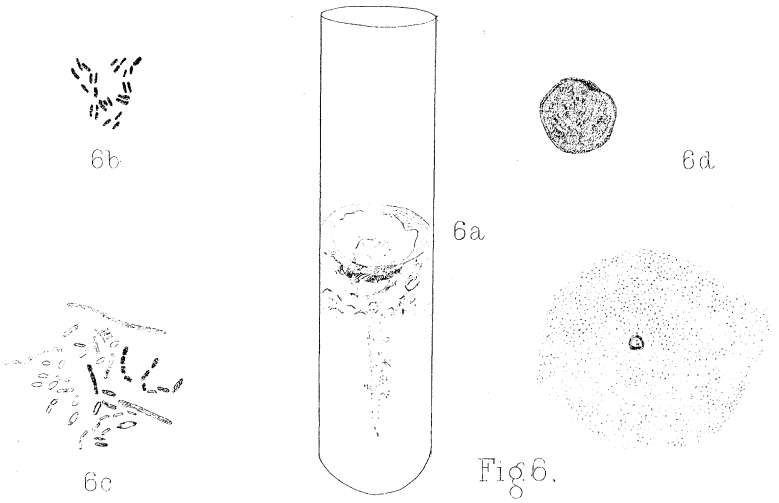

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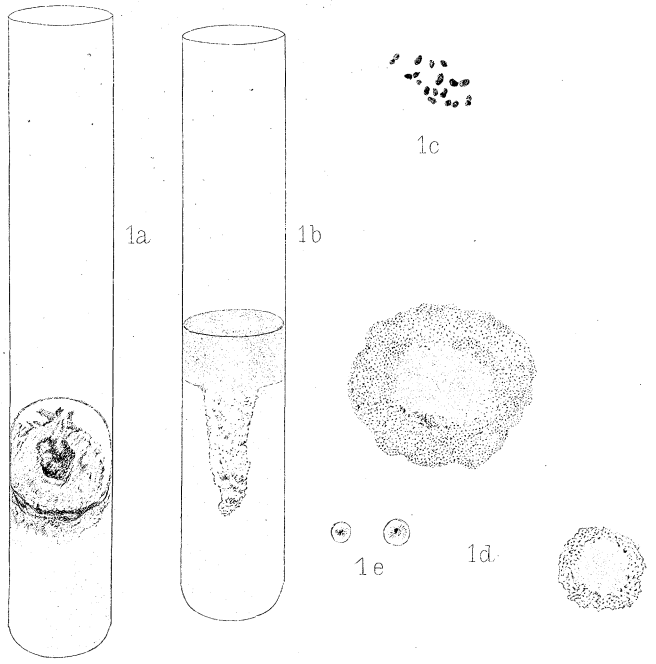

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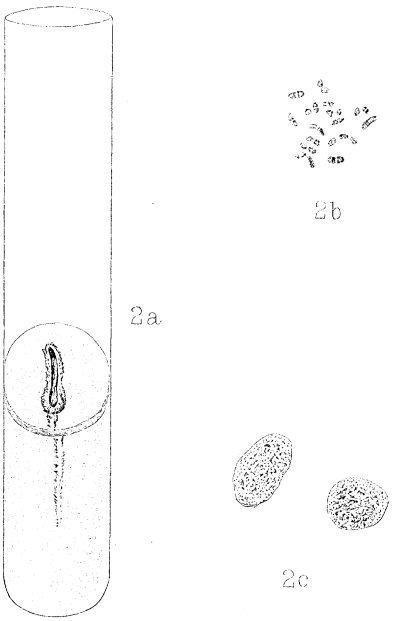
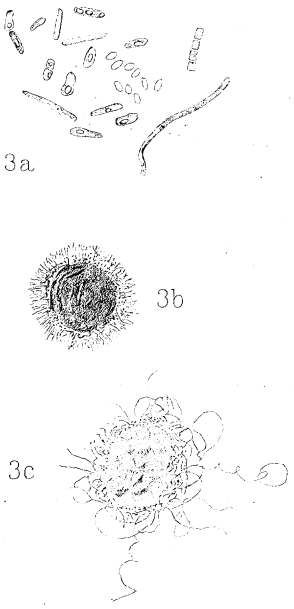


Fig. 2



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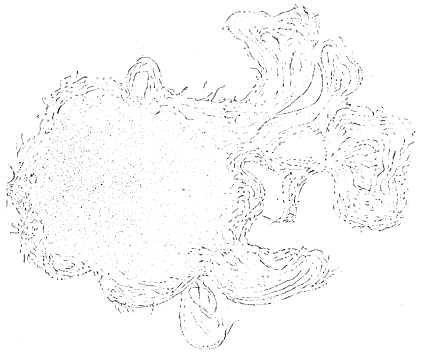

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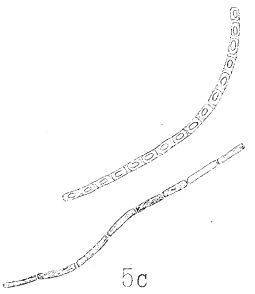

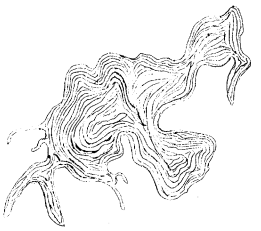

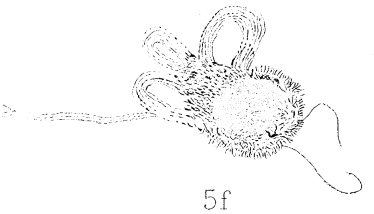

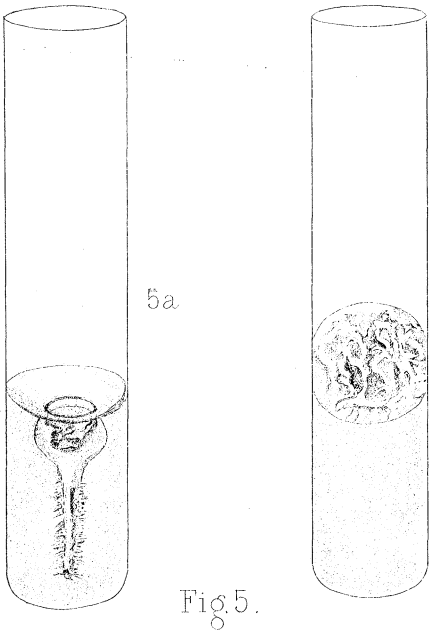

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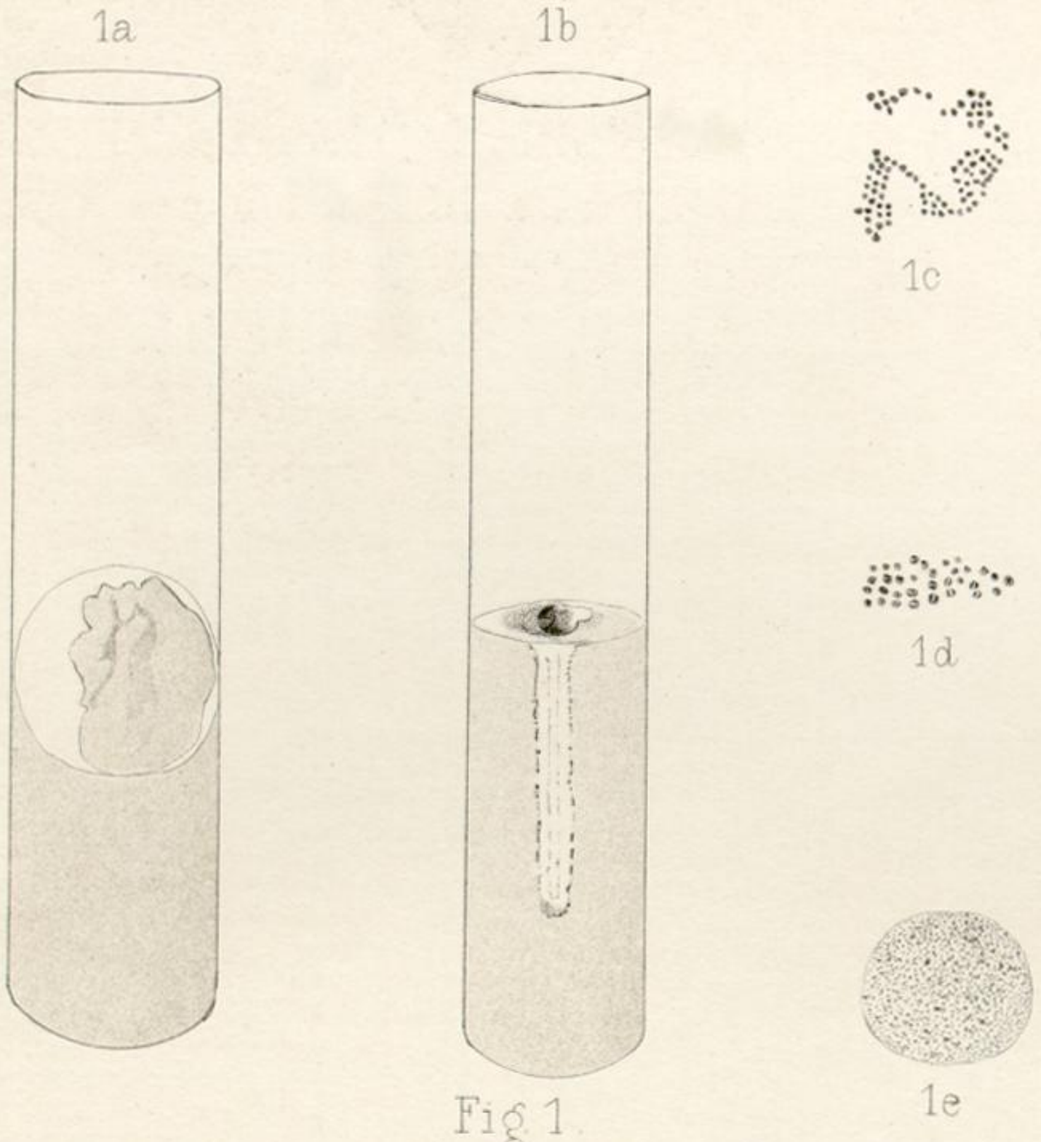

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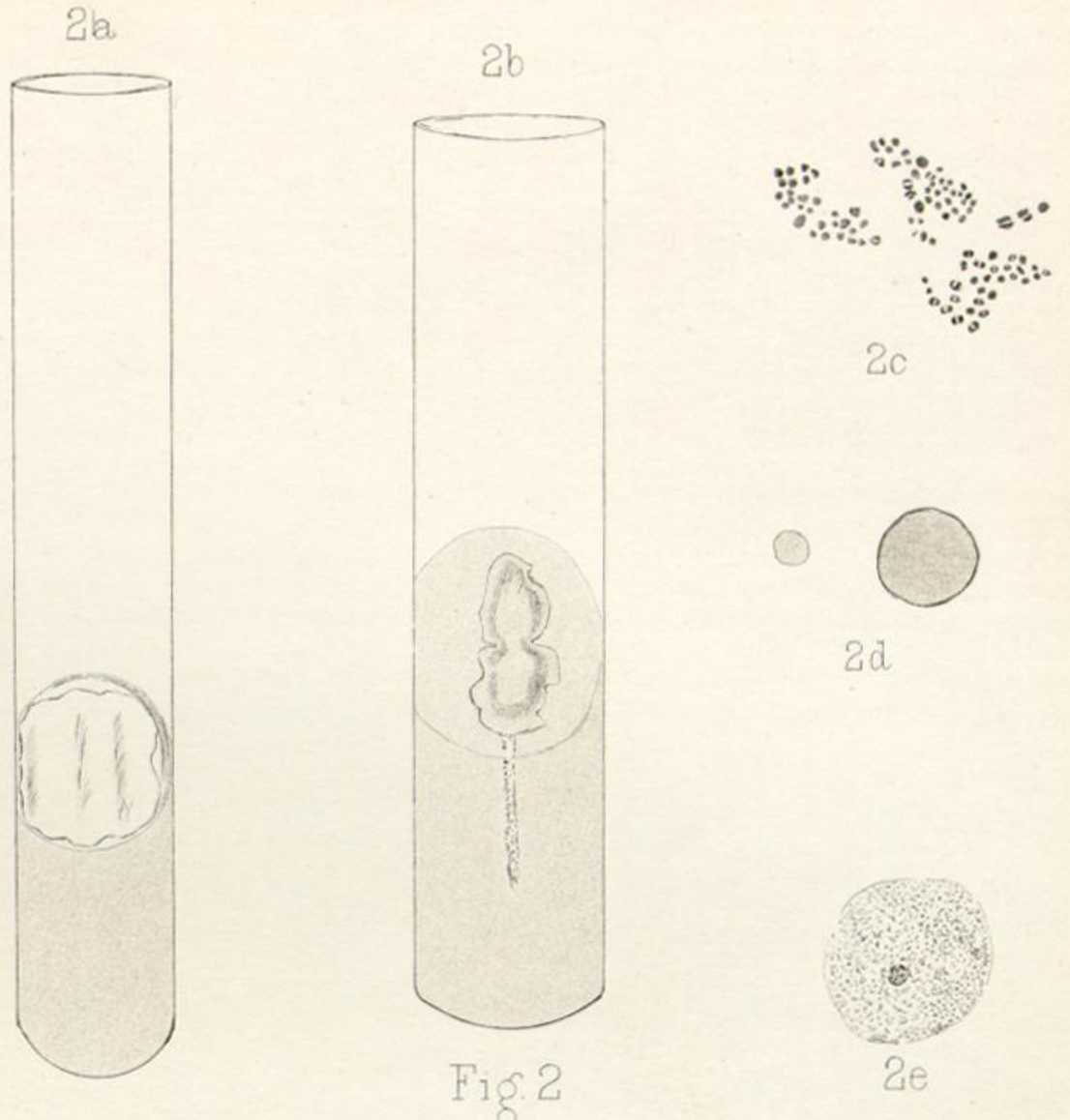

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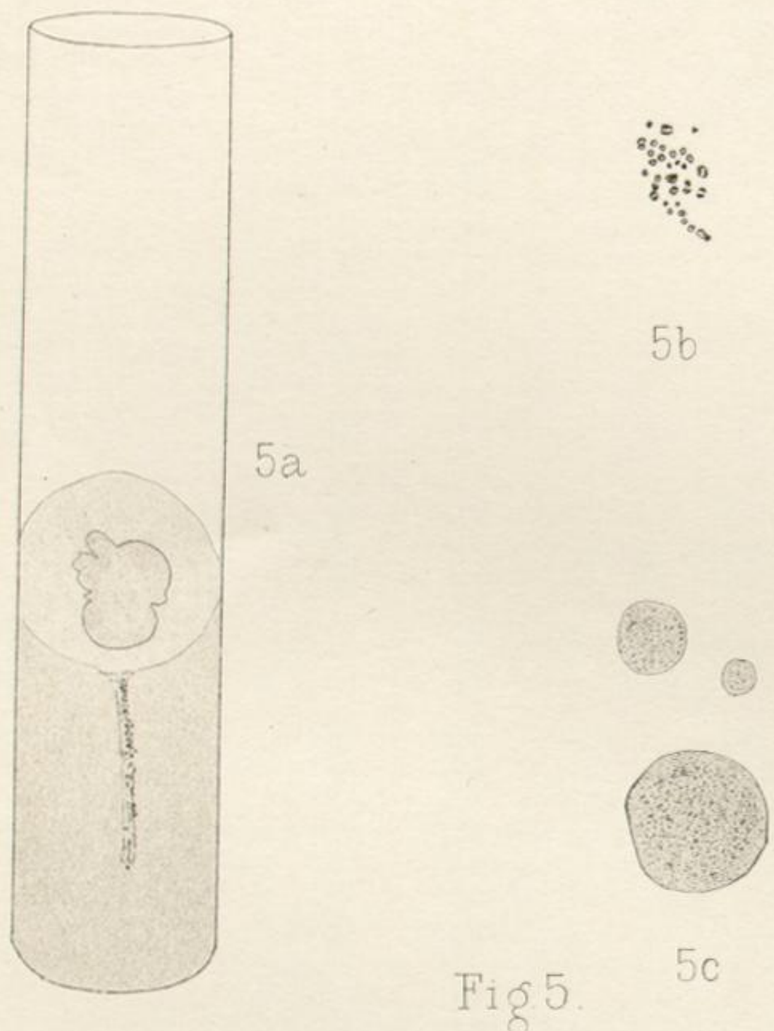

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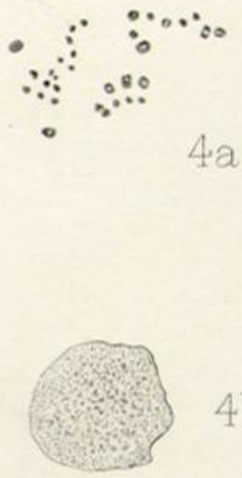

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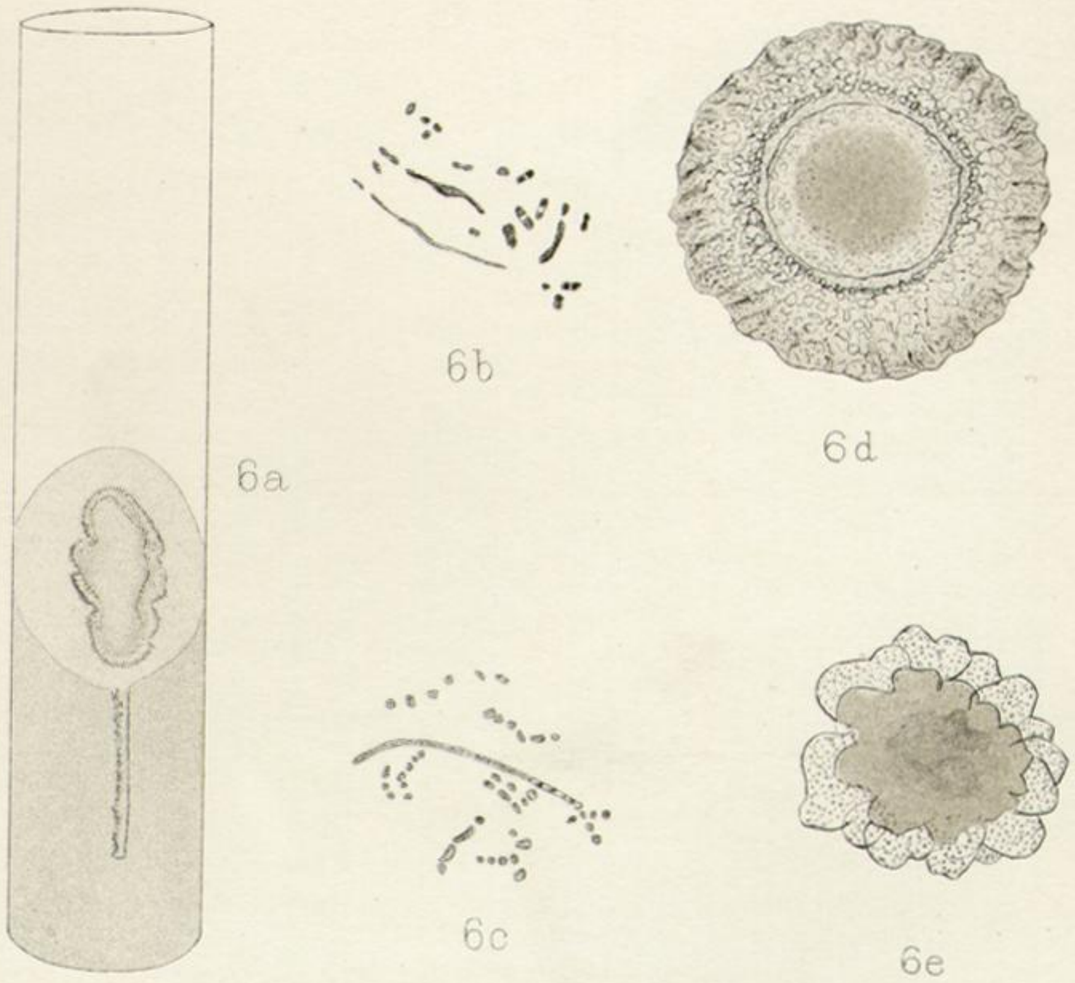


Fig 6

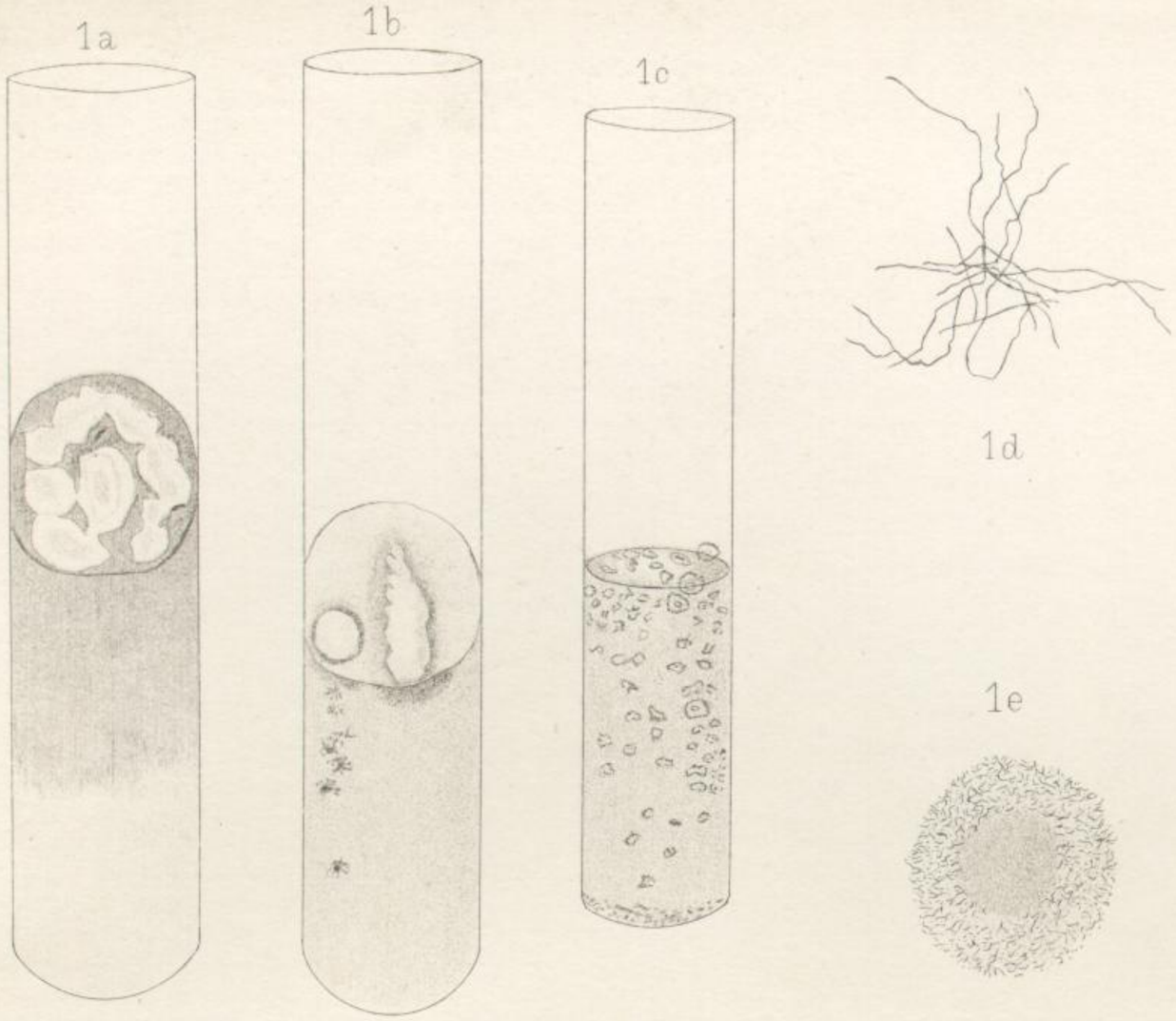
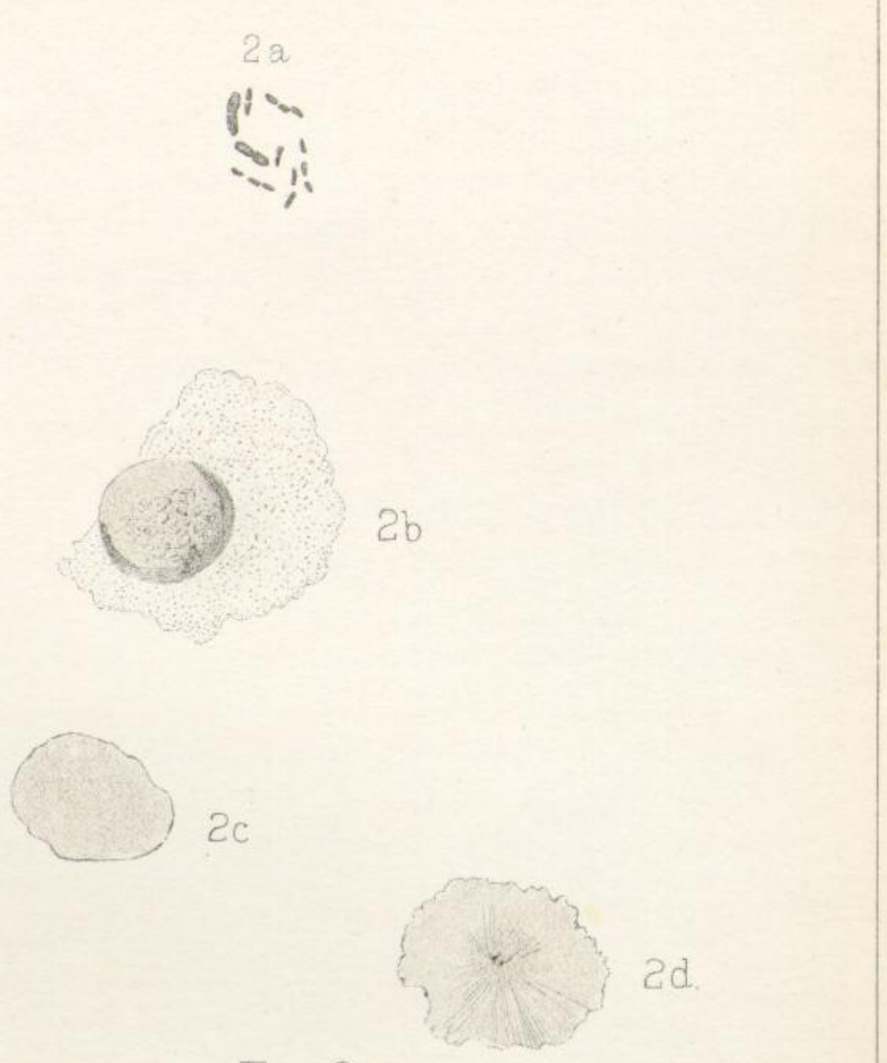


Fig. 1.



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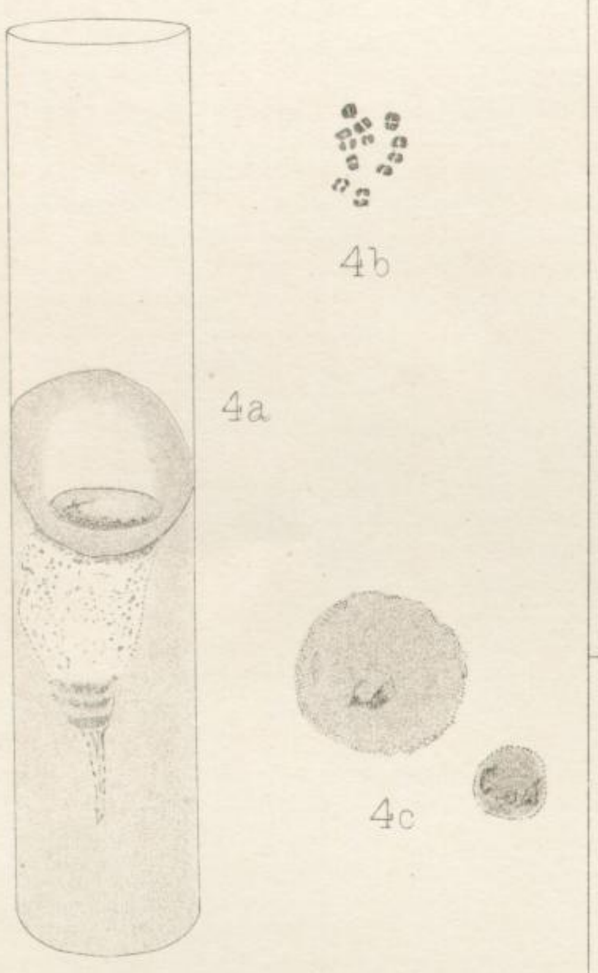
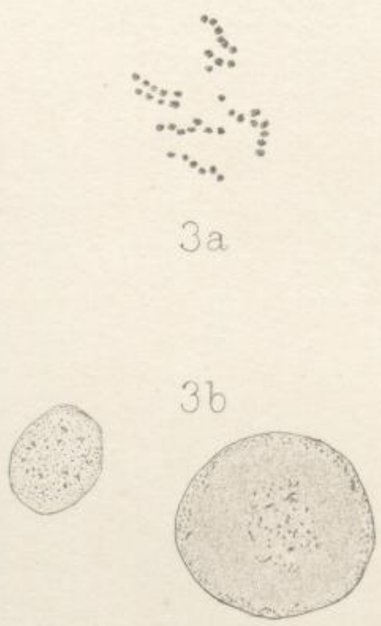


Fig. 4



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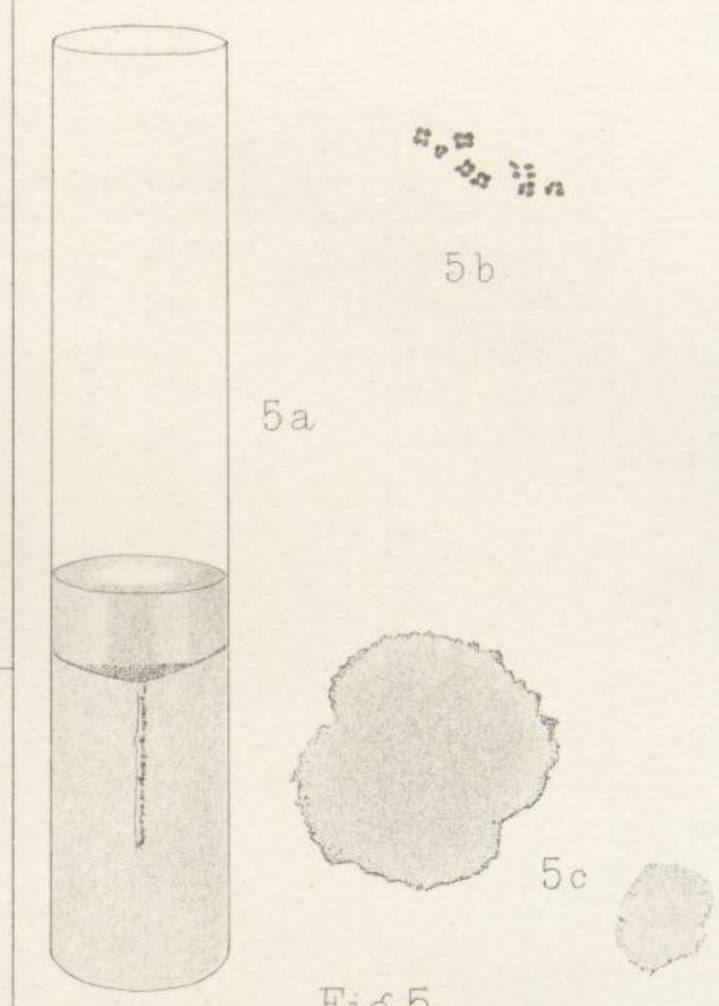
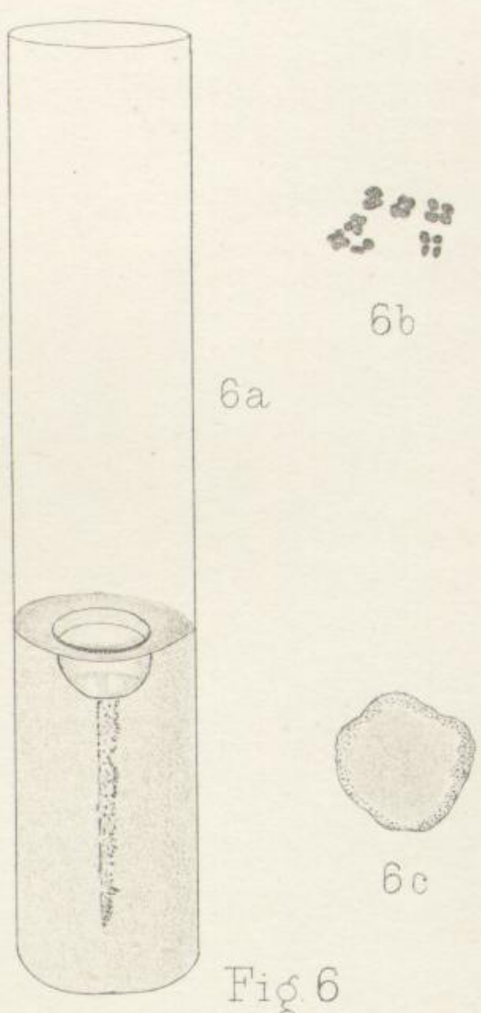


Fig. 5



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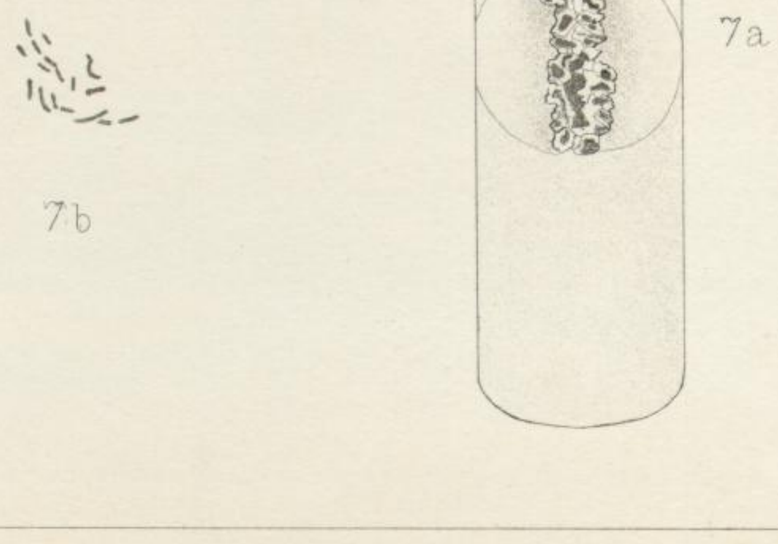


Fig. 7

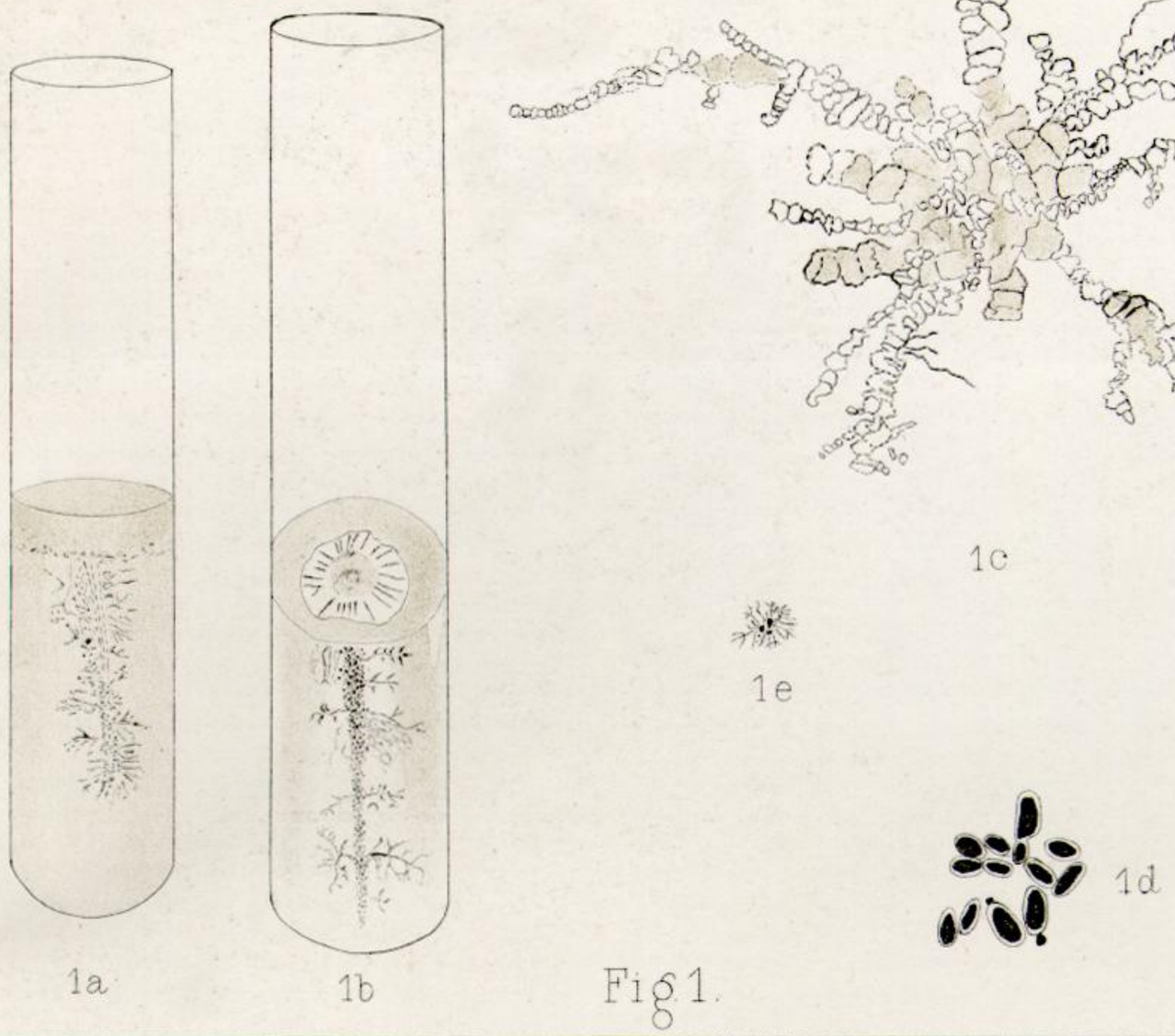


Fig 1.

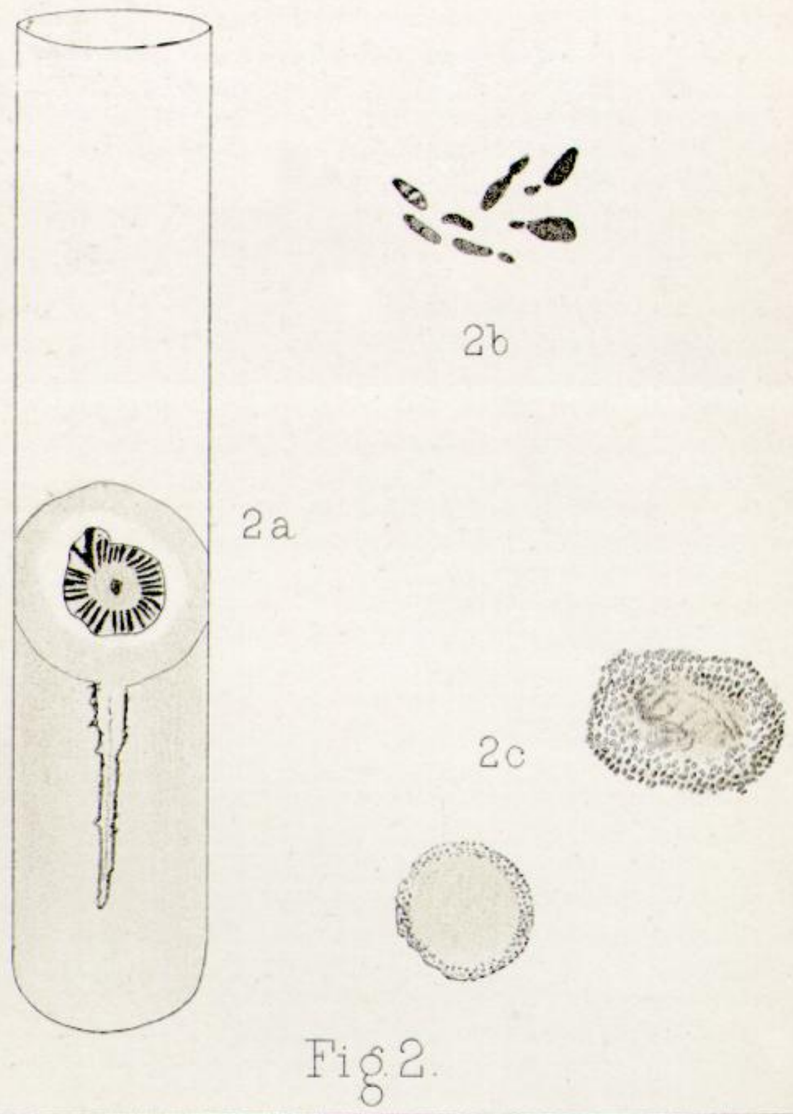


Fig 2.

Fig 3.

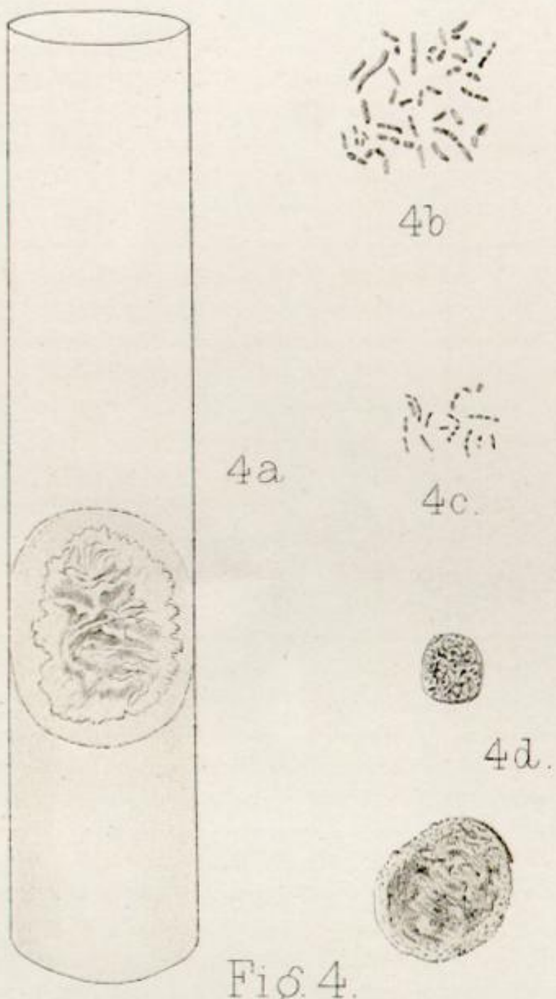
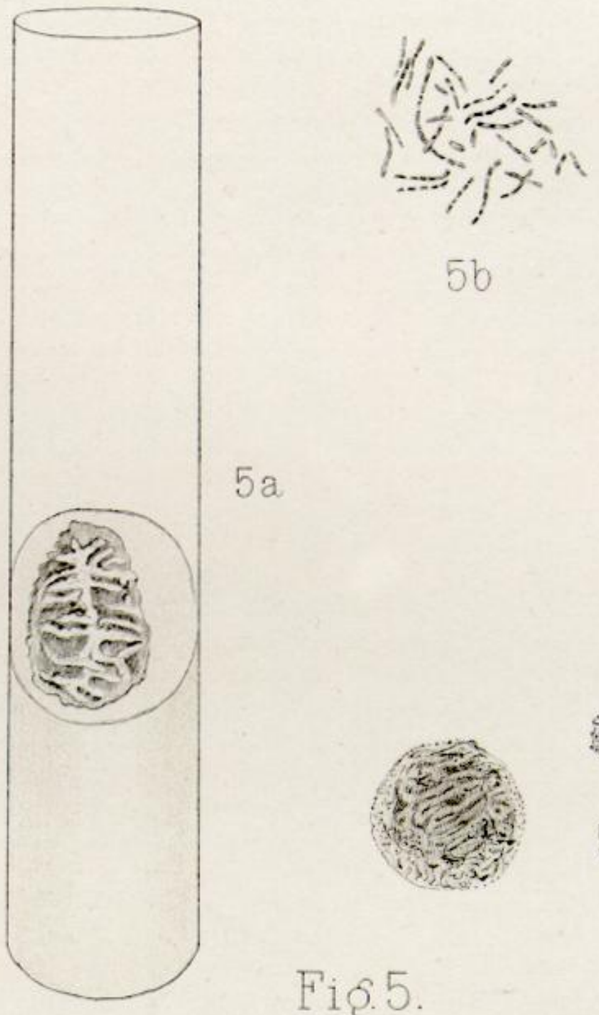


Fig 4.



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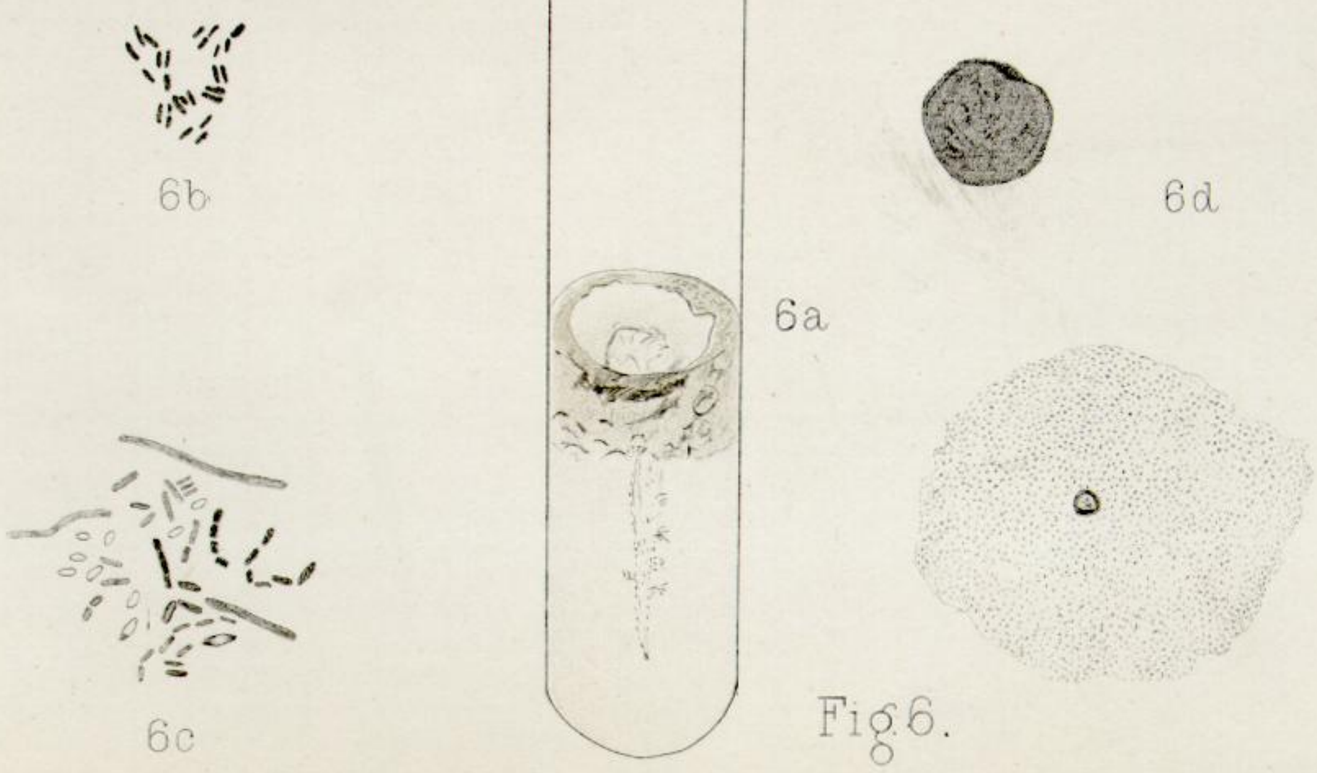


Fig 6.

Fig 7.

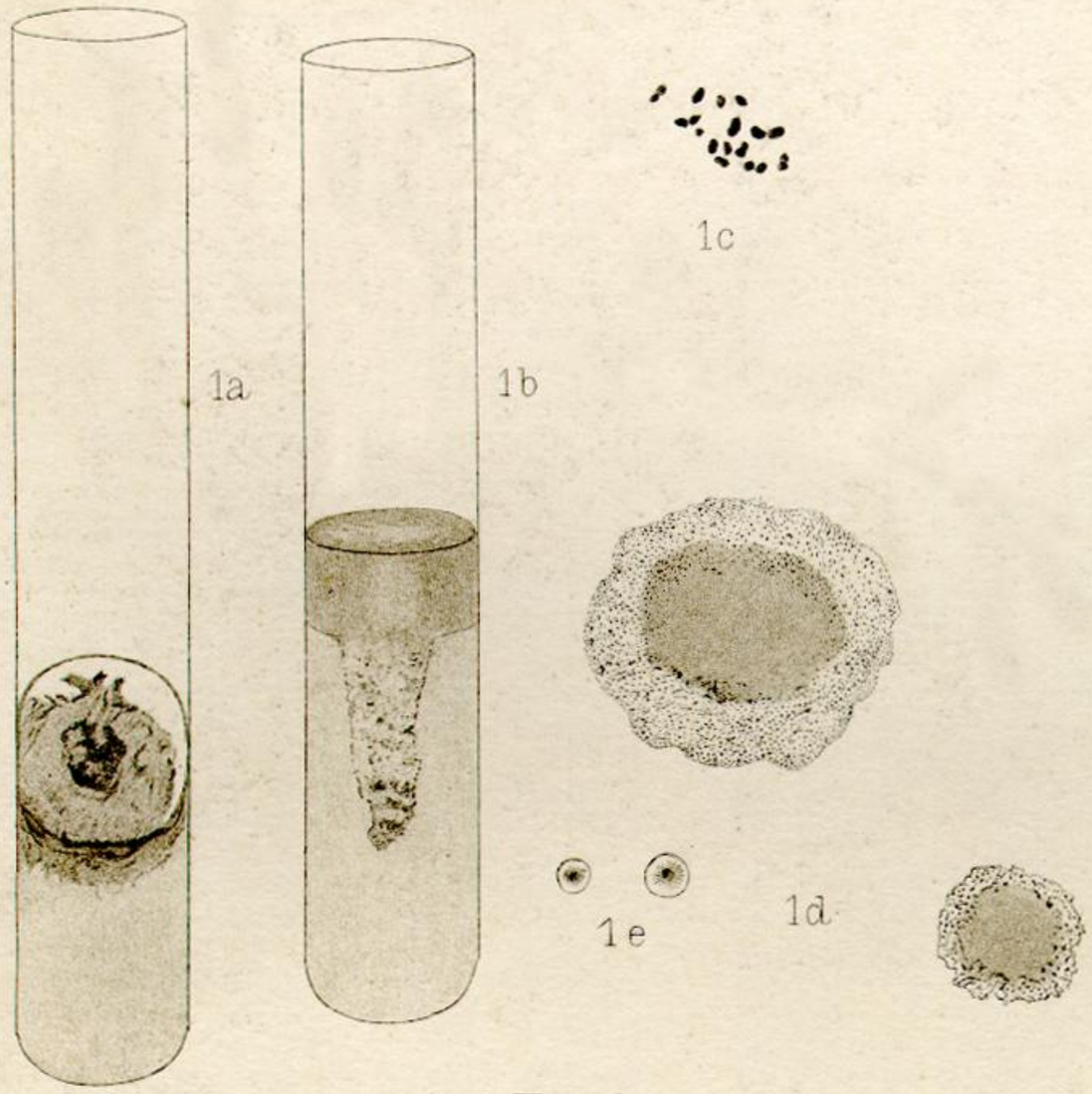


Fig. 1

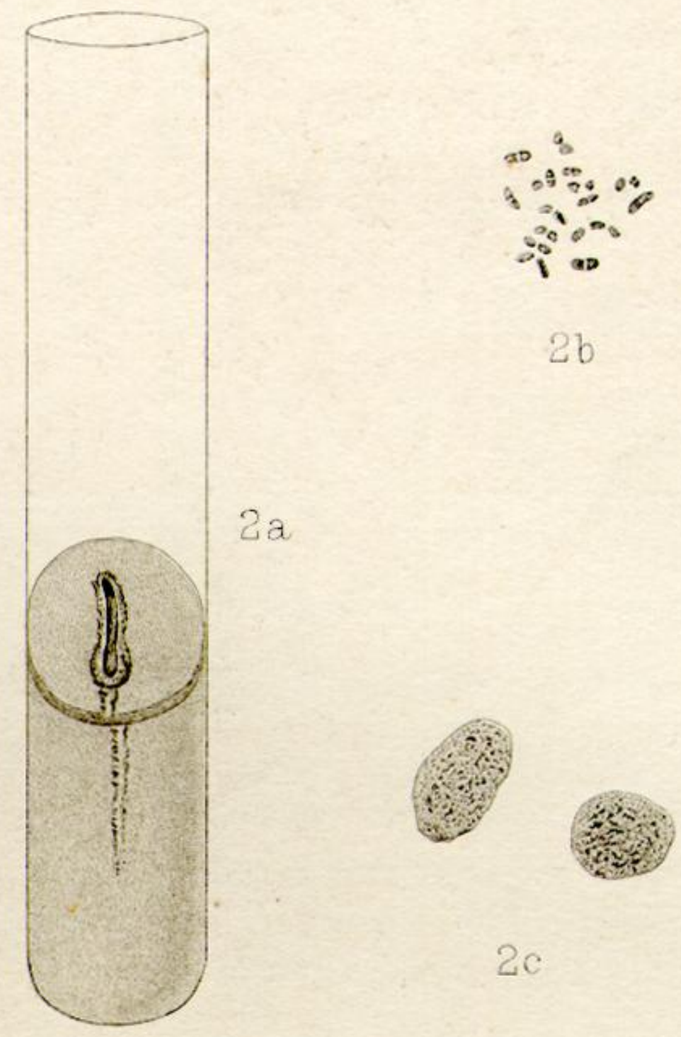


Fig. 2

Fig. 3.

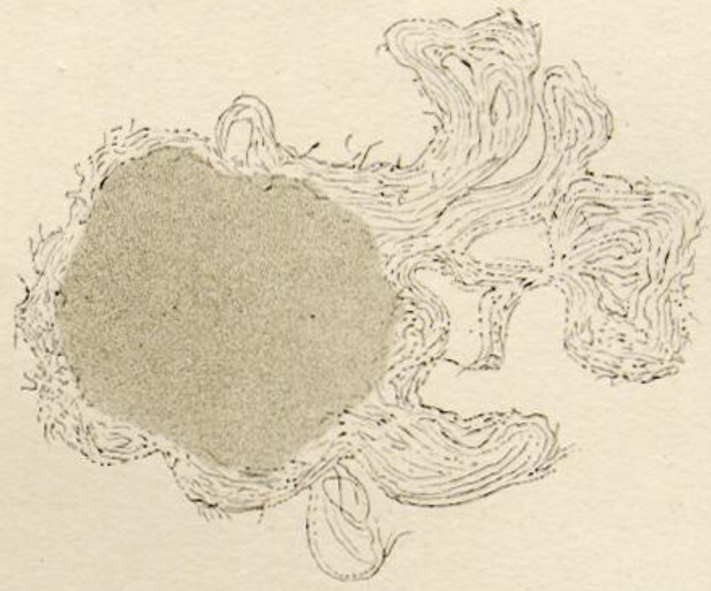
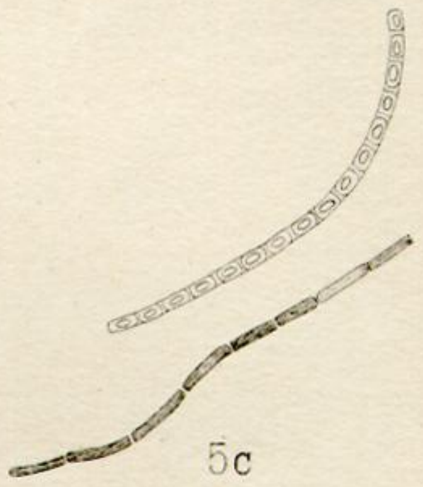
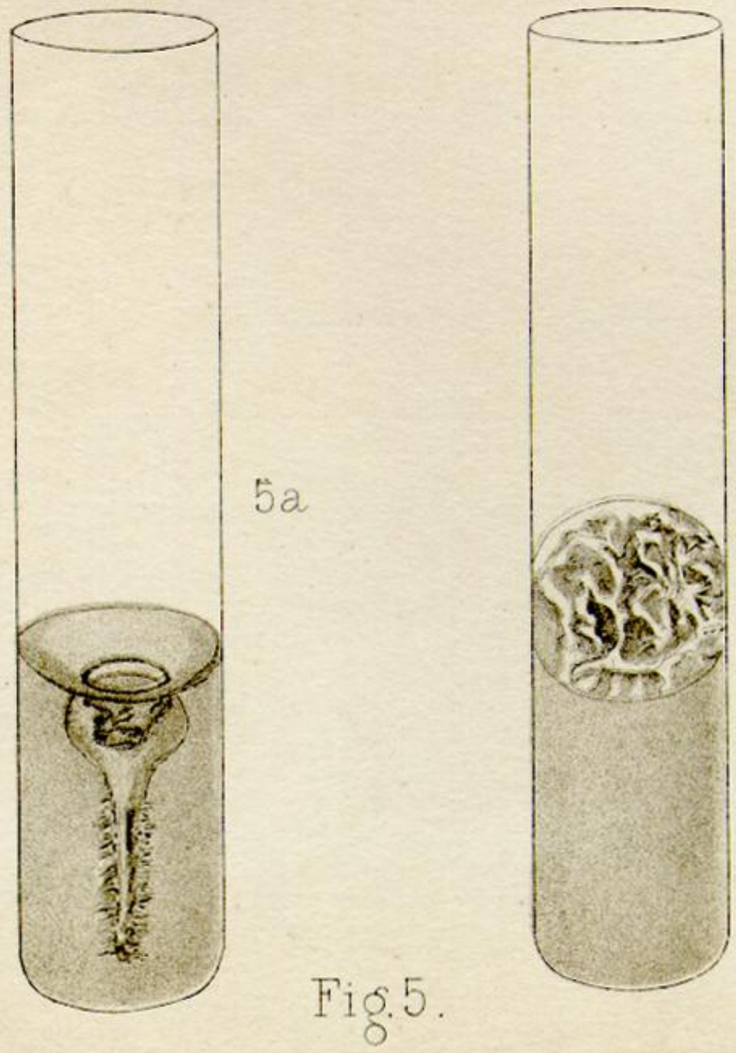


Fig. 4





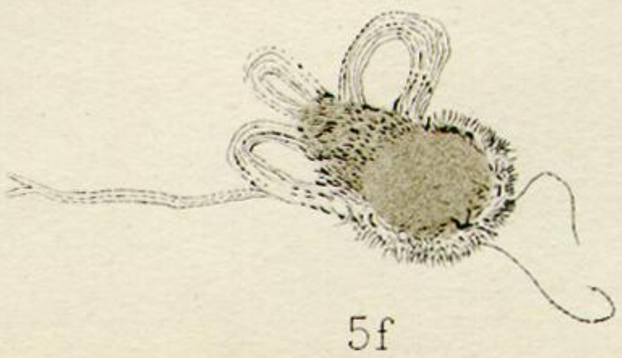


Fig. 5.