Some Thames Bacteria.

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With Plates XX and XXI.

I. A short colourless Bacterium, forming stearine-like colonies [1] : type of *Bacterium ureae* (Jaksch). (Pl. XX.)

THIS form is apparently not uncommon in the river; I have isolated it several times, but have only cultivated it twice through all media.

It occurs on the plates as cocci, about 1 μ diam., not motile, and grouped in pairs, or rows of four, or isolated or in heaps, and evidently developed from the breaking up of short rods 2 × 1 μ found with it (Fig. 1). On old agar-cultures the cocci alone are found; but in actively-growing gelatine-cultures the rodlets prevail and can be seen to be breaking up to cocci in all stages.

No spores have been found in any medium. The rodlets stain easily by ordinary methods—e.g. Loeffler's methylene blue, carbol-fuchsin, &c.—but they are easily decolourized by Gram's method.

[1] This is the type of Group I, referred to in Proc. R. S., Vol. 61, 1879, p. 417.

On plates at 12–15° C. it grows slowly as white, somewhat typhoid-like irregularly circular fronds, beautifully zoned and marked with radial lines. Under the $\frac{1}{3}$ obj. these are contoured, hyaline at the edges, and yellower in the thicker, central portion. There are no pronounced blue or green sheens or iridescence, but after a week or so the fronds appear dull (*matt*), and like thin drops of stearine with irregularly contoured edges (Fig. 2). No liquefaction even after two to three months. Plates at 20° showed in forty-eight hours as white discs, like flattened milk-drops, 1–2 mm. diam. Under the $\frac{1}{3}$ these are yellowish, contoured and opaque, the submerged colonies being very granular.

On the third day the diam. = 2–5 mm., and with the typical opaque, stearine-like appearance.

Fourth day = 9 mm. diam., and similar appearance. On holding up to the light a beautiful shagreen-like appearance, bossed in the centre, edges like ground glass.

Sixth day = 15 mm., opaque. Indented edges to the otherwise circular frond. Traces of zones and radial structure in some.

Fourteenth day = 20 mm., zoned and radiate and with elegantly indented edges. Yellowish.

Microscopic cultures in hanging-drops of gelatine were made, but it was found to be impossible to measure the growth. The short rodlets break up as soon as division is completed, and fall apart to make the colony of cocci. Fig. 3 shows a case where the isolated rodlet (*a*) in gelatine at 20° C., at 3 p.m. had divided into two, and one of these was dividing again at 10 p.m. (*b*). At 11 a.m. next day a colony, oval, pale, and with the normal characters, had formed, measuring 99 × 85 μ in length and breadth, and several rodlets thick (*c*).

If it was ten rodlets thick, such a colony reckoned as a rectangular one would contain about $\frac{99 \times 85 \times 10}{2} = 42{,}075$ rodlets, which indicates fairly rapid rate of growth.

At the same hour (11 a.m.) the following day the colony

measured 300 × 180 μ, which, calculated as before, gives us 540,000 cocci or 270,000 rodlets.

Numerous other cultures only confirmed these results, and neither filaments nor spores could be obtained in any gelatine medium; while the minuteness of the organism rendered futile all my attempts to directly observe its growth in liquid media.

During active growth the rodlets 2 × 1 μ prevail, but as the colony ages these all break into cocci.

Stab-cultures at 12–15° show up in three days, but the development is slow. Yellowish-white dots appear in the tunnel, and a thin, dull, ground-glass-like frond above. This is hard and tough, like stearine, and difficult to scrape off. In fourteen days the surface is nearly covered, the frond with beautifully indented margins and radiate structure. No further growth in the tunnel. The matt appearance is due to a rough shagreen-like surface (Fig. 4).

At 22° the development is equally good on acid or alkaline gelatine, and on the third day the characteristic matt frond appears above. The dots in the tunnel coalesce a little, indicating feeble growth. No trace of liquefaction. In old cultures the gelatine is slightly brownish-tawny above, and the colony has a faint greenish yellow tinge. The stab is sword-shaped. No trace of liquefaction even after three months' culture: the yellowish white, thin, waxy, shagreened growth just covers the top of the gelatine, and has delicate fimbriate margins. Colonies submerged in the gelatine exhibit no growth.

Streak-culture at 20° spreads fairly quickly, as a white, thin, matt film, like stearine or ground-glass, in forty-eight hours. In seven days nearly the whole surface is covered with a beautifully frondescent very thin film, spreading from the thicker streak, flush with the surface, greyish-white like ground-glass (Fig. 5). No sign of liquefaction even after two months. Edges very thin and fern-like.

On agar at 30–35° a faint streak and one or two thin spots appeared in two days: these hardly increased in eight days.

On keeping at lower temperatures a tough paste-like dirty white patch slowly spread in a month.

At 22° white granular discs appeared in twenty-four hours, and coalesced to a thin spreading yellowish white film in two to three days. Slight white deposit in drainage. In a week there was little progress: the individual colonies are thin fronds like those on gelatine plates, but remain small. They are often polygonal where their edges touch, and give a curious mosaic-like or scaly look to the growth (Fig. 6). Under the lens these resemble the scales of a Turbot. In other cases the film is continuous.

Potato at 34° gave no results in a week, but at 22° a dull, white yellowish streak appeared in twenty-four hours, with the peculiar stearine-like look of the gelatine colonies. In forty-eight hours this was thicker, dry, yellowish grey, and in four days had crenate and somewhat mesenteric edges. The colour darkened with age—buff, and like dull wax. In a week this consisted of rodlets 2 × 1 μ, breaking to cocci 1 μ in diameter. Old cultures at 34° showed a slight growth after one month at lower temperatures. After two to three months the growth turns umber-brown.

Broth at 25° formed dense flecks above in twenty-four hours, which easily shook down. In forty-eight hours a dense greasy-looking flocculent veil above, falling at the slightest shake: abundant deposit. These flocculent veils are renewed, the intermediate liquid remaining clear. The very abundant flocculent deposit acquires a slightly buff-white tinge with age.

Milk at 25° showed no change in a month, beyond a distinctly acid reaction. On keeping three months still no change observable.

Glucose at 30° formed white flocks below in twenty-four hours, and this deposit increased in two to three days. But no bubbles or turbidity resulted. At 25° the flocks and deposit were slightly more abundant, and resembled the broth cultures. No turbidity. The deposit slightly yellowish, but not very abundant even in three weeks.

Urine at 25–30° gave very slight traces of turbidity in

twenty-four hours, and subsequently a few bubbles formed, and granular flocks were deposited. No definite turbidity, and no increase in three weeks.

The most likely form I have been able to trace resemblances to is Jaksch's *Bacterium ureae*, which may be distinct, as he believes it is, from Pasteur and Van Tieghem's *Micrococcus ureae*.

It agrees with Jaksch's form in the size of the rodlets, the general characters of the plate cultures, and particularly in the dull (*matt*), ground-glass appearance. The stab-cultures also agree fairly well, though I have never detected the smell of 'Häringslake' noted by Jaksch.

The growth is described as 'ungemein langsam [1].' Of course this is very indefinite: in my form the growth is slow but not uncommonly so. The general behaviour as to temperature agrees, so far as can be gathered from the meagre information to hand.

I prepared tubes of Jaksch's fluid as follows—per 1 litre water :—

$\frac{1}{16}$ gram.	$Mg\,SO_4$.
$\frac{1}{8}$ „	$K\,H_2PO_4$.
5 „	Rochelle Salt.
5 „	Urea.

In this perfectly clear liquid the organism grew very slowly, forming grease-like flecks and films on the surface and a very white deposit. Better at 25° than at 35°. No odour could be detected, and it is pretty evident that if this is Jaksch's form, it grows but feebly in the liquid given.

M. ureae seems to differ from the rodlets chiefly in growing more quickly and at higher temperatures, and in the cocci—which may also be in pairs or tetrads or chains—and in the occasional formation of zoogloea.

The stearine-like plate-cultures are very suggestive, and the stab-cultures agree well, except that I have not noticed the odour described.

[1] Zeitschr. f. Phys. Chemie, Bd. 5, p. 395.

In both cases we are devoid of information as to the behaviour in other media than gelatine and urine or Jaksch's fluid, so that it is impossible to be sure of the identity of these forms.

There are some distinct resemblances also to Zimmermann's *M. concentricus* [1], but he does not note the dull stearine-like appearance which is so striking in all my cultures.

As Tataroff [2] himself remarks, his 'Perlmutter-glänzende Diplococcus' may be *M. ureae*, and the resemblances are noted.

The following tabular summary gives the salient characters of this Bacterium.

Habitat.	Not uncommon in the Thames.
Morphological characters.	Cocci about 1 μ, not motile, single or grouped in pairs, rows of four, or in heaps, and formed by breaking up of rodlets 2×1 μ.
Plates.	At 12–15° C. forms slowly growing white, typhoid-like, irregularly-circular, contoured, zoned, and radially-marked fronds: edges hyaline, centre yellowish. Become dull, matt, and like thin stearine drops.
Stab.	12–15° yellowish white dots in the tunnel, and a thin frond-like ground-glass above. This is hard and tough, and matt, as in plate colonies. Quicker at 20–22° C.
Streak.	At 20° grow fairly rapidly as a white, thin, matt film, nearly covering the whole surface in a week. The streak thicker.
Agar.	At 30–35° grows slowly as a dull white and dryish layer. At 22° the characteristic white waxy matt film forms more rapidly. In some cultures isolated colonies form on the surface and coalesce to form the film, as polygonal Turbot-scale-like mosaic.
Potato.	No growth at 34°. At 22° a dull, yellowish-white, waxy, dry streak-like stearine, darkening to buff, and after some weeks to umber.
Broth.	At 25° forms dense floating flecks and greasy white films, which fall as an abundant deposit, eventually buff-white. The films are renewed and liquid remains nearly clear. The greasy films adhere to sides of tube above.
Milk.	No change at 25° beyond acid reaction.
Glucose.	No turbidity, but white veils and flocks form and fall as in broth, but less abundant. Better at 25° than 30°.
Urine.	At 25–30° a few bubbles and granular flocks only, and only slight traces of turbidity.

[1] Die Bakterien unserer Trink- und Nutzwässer, Chemnitz, 1890, p. 86.

[2] Die Dorpater Wasserbacterien, 1891, p. 71.

Jacksch's liquid. Slow growth and greasy flecks, falling as a very white deposit. Better at 25° than at 35°. No odour.

Pathogenicity. Not pathogenic to guinea pigs, according to Professor Kanthack's report [1].

After remaining from May 28 to June 8 of the following year, i. e. over twelve months, it was found quite easy to revive this form from an agar tube. Good plate-cultures resulted in four days at 20–22° C., and the colonies were quite characteristic. Further cultures in gelatine, agar, potato, broth and milk tubes confirmed this, and the results at 25° and 35° respectively were as above.

This form must therefore be regarded as a very constant and persistent one, in marked contrast to many of the others I have had to deal with.

II. A colourless capsuled Coccus or Bacterium [2]. (Pl. XX.)

An interesting form, isolated and cultivated through all stages at least twice from the river in the autumn, was one which occurred on the plates as small, short, oval, non-motile rodlets over 1 μ long by 0·75 to 1 μ broad, and invested by a tough dense zoogloea or capsule, which occurred round the groups of dividing rodlets—then biscuit-shaped—as well as round individual cocci or rods.

If rapidly stained by Gram's method the capsules are decolourized, and the rodlets coloured: but they are easily decolourized. The stained rods = 1 × 0·6 μ to cocci about 0·6 μ. The capsule = about 6 to 10 μ.

On plates at 12–15° C. the colonies are white, porcellanous, shining discs or fronds, with a central spot and faintly zoned. To the unaided eye the colonies look bluish-white and translucent if held up, the zone or zones appearing yellower: the zones sinuate in agreement with the indented

[1] I have to thank my colleague, Professor Kanthack, for kindly examining a number of these bacteria for me in respect of their pathogenic properties.

[2] Referred to as the type of Group XI in Proc. R. S., Vol. xli, p. 420.

margins. Under the $\frac{1}{3}$ obj. the whole colony looks yellowish, granular, and gradually becomes more opaque in the centre as the frond thickens, the margins thinning out and paler.

The microscopic examination also shows the colonies marked by irregularly and curiously contorted lines and streaks (Fig. 1 *a*), and scattered sets of brighter, rounded, sausage-shaped and vermiform areas. In the older colonies these are less visible in the centre, owing to the opacity as the colony thickens, but the zoning is found to be principally due to these brighter areas nearer the margins.

The submerged colonies are yellow, granular, opaque, and irregularly lobed like a complex glandular acinus or salivary gland (Fig. 1 *b*, *c*).

As the colony ages—three to four weeks—the white becomes tinged with a tawny hue, and a tendency to soften and sink into the gelatine is evident.

Closer examination with a higher power shows that the bright vermiform and rounded areas are dense zoogloea masses embedded in the granular matrix of the colony, and that the glandular submerged colonies and the dark central part of the emerged ones are simply dense and irregularly-lobed zoogloeas containing the cocci and short rodlets, the rest of the colony consisting of irregularly and closely-crowded escaped cocci without any evident capsules (Fig. 1 *d*).

These imbedded zoogloeas are so obviously similar to Cohn's *Ascococcus Bilrothii* that I referred to them throughout my notes as *Ascococci*, but—though unfortunately we do not know the size of Cohn's form—the cells seem to be larger, and they are certainly not permanently cocci, as will be seen later.

At 20° the colonies were visible in twenty-four hours as minute grey points, yellowish and granular under the $\frac{1}{3}$. A higher power (Zeiss D) showed them already lobed and capsuled. On the second day they form white opaque irregular circles 2 mm. diam., and like milk: under the $\frac{1}{3}$ the submerged colonies are lobed and glandular, the emerged

ones form discs, with the Ascococcus-like groups imbedded.

On the third day they are like irregular milky drops, too thick to show structure.

Stab-cultures at 12–15° form a wet, glistening, thin white frond above and yellowish-white, dense, dot-colonies in the tunnel. In a week the frond has nearly covered the surface of the gelatine, and is depressed in the middle, slightly sinking into the gelatine; while the colonies along the tunnel enlarge and tend to radiate into the surrounding gelatine. The sinking goes on until the frond lines the sides of a distinct funnel, devoid of liquid however; and the submerged colonies form cloudy outgrowths and widen the tunnel. The sinking and softening of the gelatine continue, and are very decided in a fortnight to three weeks (Fig. 8).

At 20° C. the phenomena are similar but quicker. In five days the softening of the gelatine is pronounced, the submerged colonies confluent; and a good funnel with signs of liquefaction and running are evident in ten days. The growth is equally good—or even a little better—in slightly acid gelatine, as compared with slightly alkaline.

The growth is easily removed by the needle, but does not lift as a whole membrane, and is firm and waxy or slightly slimy. Even after ten weeks there is no real liquefaction of the gelatine, but the cloudy white growth was penetrated far in.

At 20° in sugar-gelatine, a milk-like spreading drop formed above, and a considerable confluence and growth in the tunnel in three days.

Streak-cultures at 20° show a dull, translucent, white growth, yellowish if held down, bluish by transmitted light, thin at the margins, spreading slowly, and softening the gelatine in eight or nine days, and beginning to sink along the axis (Fig. 9).

In a month a deep spoon-shaped scooping has occurred, in which the cloudy white growth floats in viscid softened gelatine.

Agar. At 20° C. a copious, thick, spreading, glistening, pure white streak with iridescent edges, extending to a frondescent film all over in twenty-four hours. In three days a thick, shining, translucent, waxy, yellowish-white layer. On the fifth day this is a wrinkled membrane, and a white wrinkled veil and precipitate are seen on and in the liquid of drainage.

This glassy-looking membrane is tough and lifts as a whole, and the microscope shows it as a dense zoogloea, with rodlets breaking up to cocci in Ascococcus-like masses. Staining with acetic acid and dahlia-violet shows that the capsules enclose both single rodlets and colonies (Figs. 3 and 10).

At 34° C. the growth is similar, but less rapid. In four days the gum-like, translucent membrane is formed, but even in eight days it had not covered the surface.

In strong growths the Agar is evidently diminished in a few days, serving as food-material. After a week or two at low temperatures the corrugated membrane is renewed on the stripped Agar.

Potato. In forty-eight hours, at 22° C. a thin, wet, spreading, glistening film is formed, white at the thin fimbricated spreading margins, very pale yellow inwards, and with a greyish cast where thickest in the centre. About the fourth day the thin white margin disappears, and the whole patch is wet and slimy (Fig. 11).

The microscope shows that the wet sulphur-white to yellowish-grey slime consists of rods 1·5 to 4 μ × 1 μ, motionless, embedded in a tough slime which draws into long strings on the needle.

At 22° alcaline potato is an equally good medium with normal, the colour of the copious whitish slimy growth being perhaps less grey and more sulphur-yellow in hue.

Artichoke at 25° C. A white irregular patch was formed in two days, and spread all over as a white film on the fourth day, after which no further growth was noted, even in fourteen days.

Carrot at 25° gave good results. In twenty-four hours a rapidly-spreading gum-like layer formed, and extended all over as a wet, thin, watery layer in forty-eight hours. No change on the fourth day, and matters were the same at the end of a week. In fifteen days the tubes were discarded—no further growth.

Turnip at 25° gave no certain results in four days, and even after a week no growth was observable. Kept for three weeks—no further results.

Broth. No growth at 35° in three days. The liquid remains perfectly clear. At 25° C., however, the broth is turbid in twenty-four hours and with a dense precipitate, which is white in three days. Even after a fortnight the liquid is still densely turbid, and a copious flocculent precipitate has fallen.

Glucose at 25° showed no trace of activity in three days. A tube put in at 30°, when the temperature was falling to 25°, gave a slight turbidity in three days, and traces of white precipitate, but no fermentation visible; this remained the same on the ninth day. In other cases no results were obtained in two or three weeks at 25° C.

Milk at 25° C. No change to third day, but in fifteen days a thick custard is formed, and the tube can be upturned. In eighteen days the casein falls. The reaction is acid. No signs of solution in five weeks at 25° C.

Urine at 22° gave a slight turbidity in five days, with traces of a ring, but no signs of further growth.

Pathogenicity. This form was kindly examined for me by Dr. Lazarus Barlow and gave pathogenic results. A guinea-pig inoculated in the peritoneal cavity with about 10 c.c. of a four days' old beef-broth-culture died in twenty-three hours.

On examining Dr. Lazarus Barlow's preparations, I found them exactly to type. That from the peritoneal fluid showed the capsules, faintly but distinctly, but in the others they were almost invisible. It should be noted that no information had been given when the tubes were handed on, and so no

attempt to bring out the capsule had been made (Figs. 4 and 5).

Professor Kanthack also found that this form is pathogenic to guinea-pigs, though as he was working with smaller doses, the results were not so fatal. Inoculation into the thigh produced a large swelling, intra-peritoneal injection made the animal very ill for a day. In both cases, however, the guinea-pigs recovered. The experiments gave the same results on repetition. It is worthy of note that the form sent to Professor Kanthack had been much longer in culture than that sent to Dr. Lazarus Barlow.

On being revived on July 13 from an Agar-culture which had stood since May 13 of the previous year, i. e. fourteen months, the plate-cultures gave normal colonies, showing the characteristic zoogloeas embedded in the mass. On potato also the cultures showed the characteristic yellowish pasty growth with a broad white marginal area on the brownish-grey potato, and the other cultures were normal, and no question could arise as to identity. Even the custard in milk was developed in fourteen days at 30° to 35° C.

All attempts to revive another culture failed.

De Toni and Trevisan[1] have attempted a classification of capsuled micrococci along the following lines:—

They group all the forms under the head of Ascococceae. Then they cut out Winogradsky's *Amoebobacter*, chiefly on account of the amoeboid movements and arrangement in series. The remainder are divided up, first according as the 'capsule' is *general* and around whole colonies, or *special*, i. e. around each individual coccus. Further subdivisions depend on whether the 'cysts' or 'capsules' are lamellated or not, whether the colonies or families consist of few or many individuals, whether the divisions are in one or more planes, and so on.

It seems difficult to accept the details, but no more consistent attempt is to hand, so far as I am aware.

[1] Sylloge Schizomycetum, p. 1035.

There are nine genera, as follows:—

Lamprocystis, which includes only Lankester's *Bacterium rubescens*, with its numerous (real or assumed) synonyms.

Ascococcus, again confined to one form—Cohn's *A. Billrothii*.

Bollingera, comprising two species of *B. equi* (*Micrococcus ascoformans* of Johne, *M. botryogenus* of Rabe) and *B. Vacchetae* (Trev.).

Cenomesia, also with two species—*C. albida* and *C. lilacina*, both from sulphur waters.

Thiocystis, again comprising two forms — Winogradsky's *T. violacea* and *T. rufa*, both from sulphur waters.

Thiothece, including Winogradsky's *T. gelatinosa*, from sulphur springs.

Leucocystis, with only Schroeter's *L. cellaris*, found in caverns, &c.

All the above are regarded as having a general capsule, common to whole colonies or families: the following are devoid of this, but each coccus has its own special investment:—

Chlamydatomus includes the two species: *C. Beigellii*, first described by Beigel as a *Gregarina* found on hair, and *C. cellaris*, found by Hansgirg in cellars.

Gaffkya includes four species: *G. grandis*, the *Microcoque des reins et des ulcères syphilitiques de la peau* of Babes and Cornil.

G. tetragena (Gaffky), *Micrococcus tetragenus*, found in phthisical sputum.

G. Mendozae (Trev.), *M. tetragenus mobilis ventriculi*, a motile form which gives an odour of skatol in cultures.

G. Archeri (Trev.), Archer's *Black Micrococcus*, a deeply pigmented form found on potatoes.

But these are not the only micrococci described as having these 'capsular' investments, as the following list shows:—

Micrococcus of Bovine pneumonia, Poels and Nolen, from the lungs of cattle infected with pleuro-pneumonia, and resembling Friedlander's bacillus in many respects.

Diplococcus of Horse pneumonia (Schütz), a similar but imperfectly described form.

Haematococcus Bovis (Babes); *Pseudodiplococcus pneumoniae* (Bonome), indistinguishable from *M. pneumoniae crouposae* except in its growth at lower temperatures; *M. ureae* (Pasteur); *M. luteus* (Cohn); *M. viticulosus* (Katz), are other species described as capsuled or forming investing zoogloea masses.

I am unable to refer my Thames form to any of the foregoing with certainty, and am inclined to suggest that it should receive a name as a new 'species.'

From a Petri-dish, in which a plate-culture had been made from a drop of water impregnated by shaking up a zoogloea-mass grown on Agar, I removed a little of the gelatine-film with a loop, and transferred it to a culture-cell, suspending it from the cover-slip as for a hanging-drop culture.

The plate-culture had been going twenty-four hours at 20°, and the colonies were just visible—hardly so without a lens—and my idea was to watch the behaviour of a rodlet at the thin margin of a colony.

To do this, however, it was necessary to raise the temperature of the culture chamber just sufficiently to soften the gelatine and make it spread a little, for no matter how carefully one prepares such a culture as the above, the play of lights reflected and refracted at the conchoidal fractures of the solid splinter of gelatine interferes seriously with observations under high powers.

Consequently it was necessary to warm the whole to nearly 25° C., and then let the minute-drop solidify again.

This was done, and several well-isolated rodlets were now found near the margin of the colony and clear of it. I now focussed a pair, lying close together but sufficiently apart for distinct observation: their position was fixed by means of the micrometer, and they were drawn at 10 a.m.; the temperature being 21·5° C.

Their behaviour at subsequent periods of observation is given in Figs. 16 *a–f*. At 10.20 each had divided, though the two halves were still joined: at 10.35 they were free, and now there were four rodlets in place of two (see Fig. 16 *c*). At 11.10 a left-hand rod was dividing, as shown by its biscuit-shape, and at 11.40 there were six rodlets; at 12.20 a rod below, to the right, was dividing, and by 12.45 there were eight rodlets.

Now it was evident that in the successive divisions the sister-halves were not equally capable of dividing. The question arises whether this is due to position, or some other cause. I am strongly inclined to regard it as due to position; in each case the new divisions occurred first in cells *nearest new territory*, i.e. advancing away from the colony into unexplored gelatine.

The above observations had now to be interrupted, and on resuming them at 3.20 p.m. a startling discovery was made—*all the free bacilli were in active swarming movements.* The temperature had slowly risen to 23·5 and remained there, and the gelatine-drop had absorbed a great deal of water: these factors, taken with the liquefying power of the colony, explain why the drop was now liquid.

But the swarming was an unexpected phenomenon. I had got over my surprise at the isolated rodlets, above described, showing no capsules, because earlier examination of the gelatine colonies showed that not all the cocci or rodlets are capsuled. Hitherto, however, they had shown no signs of movements. The obvious suspicion arose that an intruding swarmer had got into my hanging-drop.

That was not the case, however, as the following observations show. As we have seen, the temperature had been slowly rising all the morning, as follows:—

10·0	a.m.	temperature	= 21·5
10·20		„	= 22
10·35		„	= 22·25
11·10		„	= 22·5
11·40		„	= 22·5
12·20		„	= 22·75
12·45		„	= 23·25
3·20		„	= 23·5

And I allowed this rise to go on. The numbers of swarmers increased enormously, and I suspected this was not due merely to the rapid division of those already in motion, but that the increase was partly due to reinforcements from the colony of resting forms.

After some search—principally due to the difficulty of focussing now the drop was enlarging—I got a very typical capsule enclosing six rodlets under observation at 3.40. The temperature was 24·5°, and remained there. But the rodlets inside this cap were no longer quiescent: they were slowly moving, tumbling over one another within the hyaline prison of the capsule.

Numerous free swarming rodlets were now in the neighbourhood, and one saw here and there groups of about six to ten of apparently free ones moving about each other, gliding and tumbling one over the other in the same way as those imprisoned in the capsule referred to.

This capsule was kept under observation from 3.40 to 4.40, and notes made at 3.55, 4.15, and 4.30.

The slow swarming at 3.40 became more and more active as time went on, and at 4.15 was as active as in the apparently free swarming groups around, but the enveloping capsule was now swollen, and so transparent that it could only be known to be there by the limits its presence placed to the swarming movements of the imprisoned bacilli. At

4.30 the diffluent walls had softened and dissolved, and the imprisoned swarmers escaped, and were swimming about as actively as any of the other free rods.

These observations were confirmed several times—as soon as the temperature rises to about 23·5 to 25° active swarming begins.

In some cases a pair of recently divided rodlets behave in a peculiar manner, and this I have seen not once, but several times. Each is capable of movement on its own account, but in some cultures (gelatine) the newly-separated rodlets separate by backing away in a straight line, and then come together again, end on, and remain a few seconds as if they had never separated at all. Thus, in Fig. 13 I have sketched the relative positions of one of these pairs at four stages of their oscillations. At first they were closely applied pole to pole in a straight line (*a*), then they suddenly darted asunder (*b*), till separated by about three times the length of either: after a few seconds they flew together again (*c*), and then again flew apart (*d*); and this went on for at least half an hour.

It is quite a common event to find the rodlets swarming in this way, though the pairs do not invariably approach and recede in the same straight line. It was noticed that in one and the same case the movements in either direction—separation or flying together—might concern both, or only one, or sometimes one and sometimes the other, and by no means equally. If we call the rodlets *A* and *B*, sometimes they darted apart five or six times the length, equally distant from the point where their poles joined, and next time *A* would dart off and leave *B* quiescent, or *A* would move twice or three times as far as *B*: similarly on darting together. I could find no rhythm about this phenomenon, and do not understand its meaning. At one time I thought the darting together might be due to an elastic cilium which they were tugging at, but it seems improbable.

In most cases, however, the swarming is in and out irregularly when several are concerned, and it seems to

depend on the temperature. Fig. 14 gives a case where the single rodlet at 10.25 p.m. had divided at 11 p.m., and the two halves separated at 11.5 p.m.: these two oscillated away from and towards one another as above described, and went on doing this and dividing through the night, and at 7 next morning had developed into a colony about 25 μ in diameter, and containing probably 10,000 to 15,000 of the short rodlets, all swarming actively, in the circumscribed space of their own gelatinous investment.

If the temperature does not rise beyond about 20° the colonies are developed without any swarming. Thus Fig. 15 shows a case where a rodlet was fixed at 4 p.m. in 10% gelatine and remained at 15° C. through the night. Next morning at 8·15 it had formed a small colony about 3 μ in diameter, and consisting of eight to ten rodlets, so far as I could make out—possibly twelve. The temperature was then allowed slowly to rise to 20–25° C., and at 3 p.m. the lobed colony of quiescent short rodlets and 15–16 μ in diameter, shown in Fig. 15 (*c*), had formed. At 8.30 p.m. the whole colony was in active swarming, but next morning was quiescent again.

Numerous attempts to cultivate this form further were made without success.

III. Rose-pink Micrococcus: Type of *M. carneus.* (Zimm.) (Pl. XXI.)

A very pretty rose-pink form was isolated several times and studied during the winter of 1894–95, when it seemed fairly common. It is by no means one of the more frequent forms in the Thames, however. I was for some time puzzled by it, for at one period its alliances seemed doubtful. It occurs as spherical cocci of variable sizes, from 0·5 to 1·0 μ, or even occasionally up to 1·5 μ or nearly so, in diameter, in irregular botryoidal groups, and perfectly quiescent.

It stains easily, and well-stained specimens may show a darker more or less central spot, and a paler halo round

the cells. Specimens in water sometimes seem to be distinctly vacuolated, or even to have granules in them, and some of these characters at first led me to suspect its being an extremely minute yeast-form—for instance, the vacuolations, the paler halo, and the grouping—but I have been able by cultures to determine that this is not so: it is a true Schizomycete. The fact that it does not ferment glucose solutions is, so far as it goes, evidence against the yeast view; but of course it is far from conclusive, since plenty of yeasts do not ferment sugars. In the absence of any proof of budding I considered this form as probably a *Micrococcus*, and the occurrence of diplococci and rows of nearly or quite equal-sized cocci point to the same conclusion.

On cultivating it at 19–20° in broth-drops under the $\frac{1}{12}$th immersion it proved to be a *Sarcina*-like *Micrococcus*[1], which divides in all three directions, but the progeny frequently partially separate later on, and only remain united in zoogloea-masses, and so form irregular botryoidal groups of cocci each 1–2 μ in diameter. The high refrangibility of the gelatinous zoogloea investment makes it impossible directly to see the actual act of division, but enough evidence was obtained (see Figs. 8 and 10) to determine the nature of the organism.

After being sown about twenty-four hours, the cocci are found dividing very regularly in the *Sarcina*-form (Fig. 8), but in the course of another twenty-four hours the cocci partially separate as they rapidly divide, and, rounding off, remain agglomerated in the characteristic grape-like manner shown in Fig. 8 *d* and Fig. 9. As time goes on, the separation is more and more complete, and isolated cocci and diplococci are common in the drop.

The series figured in Fig. 8 (*a* to *d*) will show this. At 11.50 a.m. a group of three *Sarcina*-masses was isolated (*a*) and watched: at 2.30 p.m. the *Sarcina*-divisions had increased as seen in (*b*), though it was impossible to accurately

[1] The type of Group XVII in Proc. R. S., Vol. xli, p. 421.

count the cells. The group had rotated through about 180° in the interval.

At 4 p.m. the further development seen in (*c*) had taken place, and signs of loosening of the individual cells were evident, and at 9.50 p.m. the group was a rapidly increasing botryoidal mass as shown at (*d*). Next morning it was a loose mass of groups like Fig. 9.

The best series, however, is the one in Fig. 10, where I traced the whole course of development under the $\frac{1}{20}$th immersion. The gelatine-drop was prepared at 5 p.m., and after allowing time to solidify, &c., the single coccus drawn in Fig. 10 (*a*) was fixed at 5.55, t=20° C. At 8.5 this had grown to the biscuit-shaped figure shown at (*b*). and at 11.40 p.m., the temperature having fallen to 19° C., there were four cocci in focus (*c*). Whether growth had occurred in the plane at right-angles to the paper I could not with certainty determine, but was of opinion that it had. During the night the temperature fell to 16° C., but was at 18° by 9.10 a.m., when nine cocci were clearly visible (*d*), and certainly some existed in the depth, but I could not focus down to them.

By noon, growth was rapidly advancing, and two groups of four, one of two, and some behind were visible (*e*): the temp.=19° C.

At 2.10 (t.=19°) the group was loosening (*f*), and this went on as the growth and division rapidly advanced (*g*=4 p.m., t.=21°), till at 9 p.m. (t. 22°) there was a mass like a bunch of grapes (*h*).

Plate-cultures at 12–15° C. show slowly-developing, raised, dry, rose-pink points, which even after three weeks are not more than 1–2 mm. in diameter, and do not as a rule liquefy. In a week the submerged colonies, under the $\frac{1}{3}$rd objective, are irregular, roundish, dull-pink and granular; while the emerged ones are prominent, rose-pink, opaque drops, showing a deeper centre, and a paler granular zone around. Even after two months the rose-pink, slightly sunk, projecting points are not bigger than in three-weeks' plates (Figs. 1–3).

Under the $\frac{1}{3}$rd objective the older emerged colonies show

as granular discs with a colourless margin and deeper centre, others are distinctly zoned, pink, and considerable variation in the depth of colour occurs, from pale-brick red to lavender-tinted rose-pink.

Gelatine streak at 20°. In twenty-four hours the growth begins as a dry, pale lavender, tinted rosy streak, much the colour of almond petals. In a fortnight it has a curious appearance of striping, like fresh-cut muscle under a lens. The colour gets more like sealing-wax at the thickened base. The transversely striped appearance—due to ridges—seems a constant character. In the course of a month or more it slowly liquefies, and in six weeks seven-eighths of the gelatine is quite liquid.

Stab-cultures at 12–15° show small dots in the puncture-line in five days, and a protuberant dry pink button above. The colour deepens to plum-pink as the button widens, and in eighteen days no trace of liquefaction occurs.

At 20° the growth is similar (Fig. 4), but traces of sinking are found in five weeks; in six to seven weeks the gelatine is liquefied half-way down, and even more.

It requires three months or more to complete the liquefaction to the bottom.

Agar. In forty-eight hours at 25° a dryish rosy streak of isolated and conjoining raised dots. In three weeks confluent to a shining, pasty, rose-pink, broad streak, with thicker axis, and flattened, radiately striated mesenteric and indented margins (Fig. 5). Consistence pasty. The hue is a lavender-tinted rosy pink, much like almond petals.

After being in culture some time on Agar at 25°, numerous minute dot colonies are formed, hardly showing trace of pink in six days: faint pinkish deposit. The growth at 35° is still more faint, minute pink dots appearing in ten days.

Potato. At 20–22° forms a pink, rather moist, spreading layer in three days, which in five days becomes almost vermilion, thin, and spread all over. The colour is very peculiar; perhaps carmine is the nearest hue (Fig. 6). The growth on alkaline potato is extremely slow, or even *nil.*

Normal potato at 35° shows very slight growth in forty-eight hours. Little progress in four days to a week: merely a few extremely minute red spots in a watery film.

Carrot at 25° gives a thin and very poor pinkish-white growth in fourteen days.

Artichoke. No growth in fourteen days at 25°.

Turnip. No growth in fourteen days at 25°.

Broth. No growth at 35° in a week, nor after a month's subsequent keeping at ordinary temperatures. At 25° a faint pinkish deposit in three days, but no turbidity. In a fortnight the deposit is increased—granular and flesh-pink: no turbidity or other change. The pink slowly deepens in hue.

Milk. At 25° showed no change in fifteen days beyond traces of pink in a small white deposit. This had not increased by the third week, when the liquor was faintly but distinctly alkaline in reaction: no other change, but in the course of two or three months there are traces of peptonization without coagulation.

Glucose at 25°. Showed no growth in fourteen days. Not proved to be pathogenic for guinea-pigs according to Professor Kanthack. The results are doubtful.

The following pink, non-liquefying micrococci and yeasts are on record.

Micrococcus cerasinus siccus (List[1]) is a very minute form, 0·25 to 0·32 μ in diameter, found in water, but growing best at high temperatures—e.g. 37° C.—and not doing well on gelatine. It is interesting to observe that this form is also noted as resembling a Torula in some cases, but it is incapable, according to Adametz, of inducing fermentation. The description to hand is very meagre, but the size, temperature, and other characters seem different from those of the Thames organism.

M. carneus (Zimmermann)[2]. This form, found in the Chemnitz water-supply, presents some striking resemblances to the Thames one. The cocci average about 0·8 μ in

[1] Eisenberg, Bakteriologische Diagnostik, p. 34. [2] Zimmermann, l. c., p. 78.

diameter, and are arranged in irregular botryoidal clumps. It grows best at ordinary temperatures, and poorly at 30–33° C. The growths on Agar and Potato are strikingly similar to my results, but there are minute differences in the description of the plate-colonies, possibly due to differences in the temperature of our cultivations. Lustig [1] describes a red form (*Coccus ruber*) which Maschek found in water, and which he regards as probably identical with Zimmermann's species. The differences in the two descriptions are nearly, if not quite, as great as those between Zimmermann's account and mine, only Lustig gives too few particulars (e.g. as to temperatures, &c.) for a decisive judgement.

Another red Micrococcus is Flugge's *M. cinnabareus* [2], also found in water and air. Excepting that the cocci are described as 'large,' and frequently in pairs or in tetrads, and that the plate-colonies are red-brown under the low power, there is nothing in the short diagnosis to separate this form from the above, and we may well suspect that they are one and the same form, for the naked-eye colours of Flugge's species agree very well. Of course much depends on what 'large' means in his diagnosis [3].

Macé [4] describes under the name *M. roseus* (Flugge) a common air-form, in twos, threes or tetrads, with flat faces, about 1·4 μ along the greatest diameter. It does not liquefy, but the description is too meagre to make much of. Macé also points out how similar these forms appear to be, and remarks that the form termed *M. cinnabarinus* of Zimmermann cannot be distinguished from Flugge's *M. cinnabareus*.

This *M. roseus* of Flugge must however be distinguished from the *M. roseus* of Eisenberg referred to below, as well as from the *M. roseus* described by Maggiora [5], a non-

[1] Diagnostik der Bakterien des Wassers, p. 40.

[2] Flugge, Die Mikroorganismen, 1886, p. 174.

[3] Macé, Traité pratique de Bactériologie, p. 335, gives 0·9 μ, which would strengthen the force of the above.

[4] Macé, p. 334.

[5] Giorn. d. Soc. ital. d'igiene, Anno XI, 1889, p. 356, No. XXII.

liquefying form, 0·6 μ in diameter, associated in irregular glomeruli, and forming a pale rose pigment.

Mention may also be made of *M. agilis* (Ali-Cohen [1]), a motile form, 1 μ in diameter, and which sometimes liquefies slightly after a long time: a pink layer is formed on Agar and potato.

In addition to the foregoing non-liquefying forms, may be mentioned a series which liquefy the gelatine:—Bumm's *Diplococcus roseus* [2], a liquefying air form; *Sarcina rosea* (Schroeter [3]), also a liquefying aerial form; *M. roseus* (Eisenberg [4]), a slowly liquefying form found in sputum.

Sarcina mobilis [5] (Maurea), said to be motile (?), liquefies, and will not develop on potato.

Finally, reference may be made to *Bacillus prodigiosus*, which is often termed *Micrococcus prodigiosus*, owing to the shortness of its rodlets: this seems identical with *M. haematodes* described by Zopf [6] as the form concerned in bloody sweat.

The resemblances to Zimmermann's *M. carneus*, which he regards as probably identical with Maschek's form [7], is so marked that only one point of importance indicates lack of identity. This is as regards the mode of division. Zimmermann says (l. c. p. 78) the divisions occur in one direction only, but I find the divisions occur in all directions, and that in certain stages the groups resemble a *Sarcina*. It is an interesting point that Maschek's form (I quote from Lustig, l. c., p. 40) presents the same similarity to a *Sarcina* that mine does, and we have seen that Zimmermann regards Maschek's

[1] Central-bl. f. Bakt., 1889, VI, p. 36.

[2] D. Mikroorg. d. gonorrhöischen Schleimhauterkrankung, 2. Ausg., Wiesbaden, 1887 (Eisenberg, l. c., p. 12).

[3] Eisenb., p. 16.

[4] Eisenb., p. 408 (quite distinct from Flugge's form: see above).

[5] Sternberg, Manual of Bacteriology, p. 720.

[6] Spaltpilze, p. 60; see also Cornil and Babes, Bactériologie, p. 142, and reference to a form mentioned by Pasteur.

[7] Zimmermann, l. c., p. 79; Maschek, Bakt. Unters. d. Leitmeritzer Trinkw., p. 60; Adametz, Die Bakterien d. Trink- u. Nutzwasser, No. 17.

form and his own as probably identical, and Lustig takes the same view.

It may be worth while to raise the question whether the *Sarcina*-form and the *Staphylococcus*-form of Micrococci are more than growth-forms of one and the same organism. If this turned out to be true, Schroeter's *Sarcina rosea*—and possibly Menge's *Sarcina* of red milk [1] is the same organism—would have to be examined in this connexion.

Several of my micro-cultures in broth-drops showed, as we have seen, that this *Micrococcus* forms evident *Sarcina*-like groups when young and growing slowly, but that the botryoidal (*Staphylococcus*-like) growth prevails later on when development is rapid.

It is perhaps not incorrect to say that the few known forms of *Sarcina* all come from sources (acid media, air, water, &c.) which may be regarded as poor pabula for such organisms. In any case there is nothing absurd in the suggestion, because it is known [2] that *Sarcina*-forms may so alter their habits on certain food-media that the cells become isolated by dissolution of the membranes and only single *Micrococci*, or (when dividing) *Diplococci*, are found, though the 'packet-form' can be obtained by another alteration of the food-medium.

I regard the case as not only interesting, but of some importance, for no one would have been able to infer the existence of the two conditions without actual culture in hanging-drops.

This form was easily revived on July 13 from an Agar culture of the preceding Aug. 14—i. e. eleven months—and soon came up normal.

Its peculiar cherry red (cerise) colour and other characters were as before, and it was interesting to see how the differences between it and certain other red species—e.g. *B. prodigiosus*—were maintained.

[1] Central-bl. f. Bakt., VI, p. 596.
[2] E. g. Macé, l. c., p. 364.

IV. A Pseudo-bacillus [1]. (Plate XXI.)

This occurs as irregular and often curved rods 4 × 1 μ in water, motionless, often with spore-like darker spots in them, and breaking up into cocci. In old gelatine-cultures only the cocci are found, in chains or groups, or as diplococci and single cells, about 1 μ or a little less when stained. They stain by Gram's method.

No true endogenous spores have been found, though easily stained oval bodies occur in the rods as described.

In broth the motionless rods are often slightly curved, and measure 2–3 × 1–1·2 μ, and grow out to short filaments 10–12 μ and segmented. In some cases inflated involution forms occur, nearly 2 μ thick.

Plate-cultures at 12–15° C. show in four days as raised yellowish-white colonies, fairly quickly growing, and already coalescing. The submerged ones are very opaque, yellowish white, not zoned. Liquefaction begins in a week, as a slight sinking, but does not progress (Fig. 1).

After three months in culture, plates at 18–20° showed nothing to the unaided eye until the third day, but in forty-eight hours the $\frac{1}{3}$ detected minute pale discs. On the third day just visible as white points, which under the $\frac{1}{3}$ are greyish, hyaline, coarsely granular.

On the fifth day they look like raised drops of milk, 1 mm. diameter, domed, opaque, glistening yellowish white. Under $\frac{1}{3}$ course, grey-yellowish, and opaque.

On the sixth day they are 1·5–2 mm., on the seventh 2–3 mm., opaque, cream-coloured, flattened domes. On the ninth day 3–4 mm., shining and like drops of cream. No trace of sinking, though some run together when in contact. The peculiar glistening appearance of the colonies is due to their wetness—as if sweating water on the surface.

Stab-cultures at 12–15°. In two days a raised dome-like button, porcellanous white, and slight yellowish dots in tunnel.

[1] Referred to as type of Group XVIII in Proc. R. S., Vol. lxi, p. 421.

In a week the colony above is a pure white, much raised, and shining like wet glazed porcelain. In a month it becomes cream-like and soft.

At 20° it grows equally well on acid and alkaline gelatine. In three days it is a very white raised button, 2–3 mm., with slightly confluent dots in tunnel. On the fourth day it is like porcelain, thick, glistening, raised. After about the sixth day it acquires concentric zones and a cream-colour, and looks as if turned (terraced) out of cream-coloured porcelain. No liquefaction, even in ten weeks.

Streak-culture at 20°. Cream-coloured, raised, glistening streak in forty-eight hours, and this grows fairly rapidly (Fig. 9). In a week it is a thick, glistening, creamy porcelain-like patch, broader below. No liquefaction in two months.

Agar at 30°. Forms a feeble streak, very thin, which makes no progress after forty-eight hours, but fades out as a transparent film. Invisible in eight days. At 35° also no growth in five days, whereas cultures at the same time at 23–25° formed a milk-like, broad, thin, shining, gummy or waxy streak with dense yellowish-white deposit all through the drainage (Fig. 10).

Potato at 22°. In twenty-four hours a wet spot, like dew. In three days this is a diffuse thin streak like milk and water. It thickens on the fourth day to a grey paste, and in a week is a not very extensive patch of cream-like, flesh-coloured paste (Fig. 8). On normal potato the growth is much more raised and distinctly flesh-coloured than on alkaline potato, perhaps because the potato acquires a pale violet hue showing it up. In ten days or so, both cultures are like rich buff or flesh-coloured cream.

At 34° the growth fails. A dew-like patch forms at first, but shows no advance in six days.

But on keeping the tube at lower temperatures, the characteristic flowing cream-like patch forms after some time.

Broth at 25° shows traces of turbidity in forty-eight hours, and a slight deposit in three days. On the fifth day a copious yellowish-white deposit. In a week, still turbid and a white

ring. In three weeks still turbid, white ring, and copious yellowish or buff deposit.

Milk at 25° shows no change in fourteen days. In three weeks it is just acid, but no apparent alteration.

Glucose at 25°. A slight white deposit in three days. In three weeks greasy flecks above and a fairly abundant yellowish-white deposit.

This form is not pathogenic to guinea-pigs, according to Professor Kanthack.

It was easily revived from an Agar-tube which had laid quiescent from May to June in the following year—i. e. thirteen months. It came up very pale and weak at first, but soon recovered all its normal characters as described. From the sum of the characters, including the results of microscopic cultures below, this form presents resemblances to *B. diphtheriae* which cannot be neglected, *but it is not a Bacillus.*

When I came to make micro-cultures of this organism in hanging-drops of gelatine and of broth, some unexpected results were obtained of considerable interest and importance. The following examples will illustrate this:—

A gelatine drop-culture twelve hours old had a rodlet 4 × 1 μ at 8 a.m. (t. = 21° C.), which was fixed and observed under the Zeiss E as shown in Fig. 6 (*a–k*). At 9.30 the much longer and sharply bent rod was behaving very curiously for a Schizomycete, for it appeared to be *putting out a branch at right angles* from its lower segment (Fig. 6 *c*).

At 10.30 the much diluted gelatine was nearly fluid and an end-segment had broken off to the right and floated somewhat to the middle of the parent rod and there divided. The further course of the formation of the colony is visible in the drawings (*d–h*). At 4.40 p.m. a circular colony 24 μ in diameter had been formed (*i*): at 8 p.m. this was 32 μ in diameter (*j*). Next morning at 9 o'clock it measured 75 μ across (*k*)[1], and by noon it was 90 μ in diameter and quite typical.

[1] Sketched under a lower power.

Now it is pretty clear that apart from that curious lateral branch, there is very little to denote that this is not a typical Schizomycete, the segmentation of which is at first into rather long rods (10–12 μ) and then into shorter ones (about 3–4 μ). But there was no doubt that the branch *was* a true branch, and further examination in hanging-drops under the $\frac{1}{12}$ and $\frac{1}{20}$ immersion led to the proof that this organism is *not a true Schizomycete at all*, but an oidium-stage of an extremely minute fungus.

The following series (Fig. 5), traced under the $\frac{1}{12}$ in a broth-drop, will suffice to demonstrate this.

At 6.15 a.m. a rodlet (*a*) 3 × 1 μ was fixed, and at 8.40 a.m. it had grown out to a short curved filament (*b*) about 12 μ long: this was longer and distinctly segmented (*c*) at 9 a.m., and just before 10 o'clock (*d*) the longer segment was forming two branches, which had grown considerably by 11.10, and at 12.30 p.m. had crossed one over the other (*e* and *f*). The long segments now showed several septa, not easy to see but certainly visible with careful focussing. At 2 p.m. (*g*) the segments were breaking apart, after further growth *of the terminal ones*, i. e. the growth was *not intercalary*. At 7 p.m. (*h*) quite a large colony of separated segments, like rods, had formed, only part of which is figured. And next morning the still more broken up rod-like segments—some curved—had spore-like, brilliant oval bodies in them (*j*). These are of the nature of oidia or *chlamydospores*, in fact. These stain easily, with the ordinary alkaline methylene-blue, for instance.

As the figures (Figs. 4, 5 and 7) show, these stainable points appear before the final segmentation of the rods into coccus-like short joints—oidia—and then the membrane appears to thicken round them, converting them into spore-like *chlamydospores*.

Now, the point of special interest is that here we have an organism which, according to all its properties as tested by ordinary bacteriological methods, is a *Bacterium*. Its microscopic appearances, as shown in stained preparations, its behaviour in plate-cultures, and on and in all the usual media

employed by bacteriologists, all suggest its being a Schizomycete. Nothing but cultivation in hanging-drops could have demonstrated the fact that it is not a true Schizomycete at all, but an extremely minute Fungus—at least, I presume no one will dispute that its apical growth, acropetal mode of branching, and other morphological characters constitute more important tests than the bacteriological ones. Such forms are quite common among the Basidiomycetes [1].

In case any one should dispute this, however, it rests with him to construct a new definition of the Schizomycetes. Meanwhile, I emphasize the point—a point which I have insisted on elsewhere—that minuteness, staining reactions, rapid growth and the characters obtained in plate-cultures do not prove that an organism is a Schizomycete, and nothing but micro-cultures, difficult as they may be and are, can ever decide the point.

This point raises another matter of considerable interest, however, viz. that of the multiple origin of the group commonly known as Bacteria, by which I mean not only the Schizomycetes proper, but the totality of micro-organisms usually grouped with them.

Excellent evidence exists for the view that the true filamentous Bacilli (the Eu-bacilli or Endosporous Bacteria of De Bary) and the segmenting Bacteria which form no Endospores (De Bary's Arthrobacteria) must be regarded as having their origin from among the lower Algae, and it is customary to refer the former to groups like Oscillatoriae and the latter to forms like *Nostoc*. Whether the group to which *Cladothrix*, *Leptothrix*, and *Beggiatoa* are commonly referred can be joined to these is a debatable point.

For the various forms of *Sarcina* and *Micrococcus*, again, it is not difficult to find analogous forms among the lower Algae, e. g. Chroococcaceae and Palmellaceae, though it must not be forgotten that Micrococci are often merely the ultimate segments of anthrosporous filaments.

Without entering into the discussion as to alliances between

[1] See Brefeld, Unters. aus d. Gesammtgebiete der Mykologie, Heft 8.

various forms of Bacteria (in the wide sense) with Protozoa and Myxomycetes, and merely admitting that such alliance may well exist among the group, I would simply refer to a possible source of confusion which has become more and more probable since Brefeld has made us acquainted with the frequency of oidium-forms and chlamydospores among the Fungi [1], namely that these forms when very minute may easily be confounded with Schizomycetes. The only test is the acropetal mode of growth.

That minute yeast-forms are also liable to be mistaken for Micrococci is evident. I had recently in my laboratory a minute organism which grows in Canada-balsam, and am as yet unable to say with certainty whether it is a yeast-form or a Schizomycete.

Here then we have a good deal of matter for further research, for it is almost certain that minute organisms which will grow in gelatine and other media, and which stain by ordinary methods, are continually being described as Schizomycetes without the application of the only test which really decides the question.

I am strongly inclined to the opinion that we shall have to revise our views as to the divisions of the accepted Schizomycetes very much before long. For instance, Fischer's recent work on the cilia of Bacteria [2] seems to raise the question whether we must not assume a different origin for the ciliated forms of 'bacilli' and for the non-ciliated ones; and, in view of Ali-Cohen's discovery of a ciliated 'Micrococcus [3]' (*M. agilis*), the same applies to the Micrococci.

In any case it is difficult to avoid the conclusion that the organisms grouped under the common denomination of Bacteria (in the wide sense, but including obvious Fungi) are a heterogeneous collection of organisms with very different alliances, some of which have been indicated [4].

[1] Brefeld., l. c. [2] Unters. üb. d. Bau d. Cyanophyceen und Bakterien.

[3] Centralbl. f. Bakt., Band vi, p. 33.

[4] Migula, System d. Bakterien, 1897, and Fischer, Vorlesungen über Bakterien, 1897, have recently proposed extensive revisions of the classification, and have raised similar questions, but not quite the same points as I have here suggested.

Another point of importance, however, concerns those endosporous bacilli which are never motile, e.g. *B. Anthracis*, and those which have cilia, e. g. *B. subtilis*. I believe no one has suggested that the former may have had a totally different origin from the latter and that both may have been derived from ancestors other than Cyanophyceae; but it seems not impossible that minute reduced forms of Zygnemaceae and allied Conjugatae may have given rise to the non-motile bacilli. In such an event the endospores are probably homologues of azygospores [1], the intercalary growth, division, shapes of cells, and even the tendency to gelatinization of the cell-walls remaining the same.

Indeed we may go further. Many Ulothricaceae would serve as prototypes of ciliated bacilli if they lost their chlorophyll and became reduced. It is not impossible that we may have to abandon the Cyanophyceae as probable ancestors of endosporous forms altogether, for none of the Oscillarieae develop ciliated cells, while many Chlorophyceae have intercalary growth and gelatinous walls.

Even the curious pedicellate bacilli, which form one-sided growths or stalks of gelatinous consistence, such as my *B. vermiforme* [2] and the *B. pediculatum* of Koch and Hosaeus [3], are not without possible parallels among Chlorophyceae, e. g. Naegeli's *Oocardium* [4] and other Tetrasporeae.

Moreover, it would seem probable that some of the Chlamydobacteriaceae have had a totally different origin from any of the other Schizomycetes, as is especially evident when forms like *Phragmidothrix* are compared with *Bangia* and its allies.

The development of endospores has undoubted analogies with the formation of cysts in certain Flagellatae—e. g. *Chromulina* and *Monas*—as Migula has pointed out [5], and there are several other cases.

[1] Klebs, Die Bedingungen d. Fortpflanzung einiger Algen, &c., p. 255.
[2] Phil. Trans., Vol. clxxxiii, 1892, p. 149.
[3] Lafar, Technische Mykologie, p. 247.
[4] Pflanzenfamilien, 1. Th., 2. Abth., p. 51, Fig. 33.
[5] Pflanzenfamilien, 1. Th., 1. Abth. a., p. 11.

The Schizo-saccharomycetes, again, form a group which suggest obvious relationships to the yeasts, while Thaxter's Myxobacteriaceae point to alliances with the Myxomycetes.

All things considered, therefore, I think we should be prepared to accept that the morphological relationships of the minute organisms grouped together as Schizomycetes are neither few nor simple, and that their phylogeny is probably not even comparable with a complex tree-form, but is multiple in origin.

EXPLANATION OF FIGURES IN PLATES XX AND XXI.

Illustrating Professor Marshall Ward's paper on Thames Bacteria.

PLATE XX.

I. Short Colourless Bacterium.

Fig. 1. Rodlets and cocci *a* actively growing on gelatine at ordinary temperature; *b* on gelatine at 20° C.; *c* form from an old Agar-culture.

Fig. 2. Gelatine plate-colonies at 20° C. *a* on the third day, *b* on the sixth day after making plates.

Fig. 3. Culture from single rodlet. At 3 p.m. a rodlet (one of two) $= 2 \times 1\ \mu$ was fixed in gelatine at 20° C. *a*, at 10 p.m. this had divided *b*, and at 11 a.m. next morning it had formed the colony *c*: see p. 288.

Fig. 4. Stab-gelatine, one month at 20° C.

Fig. 5. Streak-gelatine, one week at 20° C.

Fig. 6. Agar-culture, four days at 20° C. The layer in *a* consists of coalescent colonies shown slightly magnified in *b*.

II. Capsuled Coccus or Bacterium.

Fig. 1. Plate-colonies at ordinary temperatures, a week old. *a* an emerged colony under $\frac{1}{3}$ showing the characteristic streakings; *b* a submerged colony natural size, and *c* the same under $\frac{1}{3}$ showing the gland-like appearance; *d* a portion of *a* under E/4, showing the embedded zoogloea-masses.

Fig. 2. Three submerged colonies, two days at 20° C.

Fig. 3. A piece of Agar-culture, showing embedded zoogloeas: *a* under $\frac{1}{3}$; *b* under $\frac{1}{12}$ imm. in water; and *c* the same stained with methylene blue, showing the 'capsule' round the masses embedded in gelatinous matrix. The capsuled masses average 4–6 μ to larger and smaller: the organisms, $2 \times 1\ \mu$ to $1 \times 1\ \mu$.

Fig. 4. Stained specimen after passage through animal; preparation from plastic lymph $\frac{1}{12}$ imm. Capsules hardly visible; cocci $1 \times 0{\cdot}75$ to $1 \times 0{\cdot}9\ \mu$.

Fig. 5. Similar from peritoneal fluid: the 'capsules' visible. Cocci about 0·75 to 1·0 μ.

Fig. 6. Similar preparation from Agar-culture stained by Gram's method. 'Capsuled' masses 6–10 μ; rodlets $1 \times 0{\cdot}6$ to cocci about 0·6 to 0·75 μ.

Fig. 7. Colonies after one year's rest, seven days' plate at 18–20° C. Nat. size.

Fig. 8. A ten days' stab-culture showing commencement of the liquefaction.

Fig. 9. A nine days' streak-gelatine-culture.

Fig. 10. An Agar-culture after revival. Five days at 35° C.

Fig. 11. A potato-culture (revived), three days at 25° C.

Fig. 12. *a*, a rodlet 2·5 × 1 μ sown at 3 p.m. in gelatine-drop at 25° C; *b*, the same, divided into four rodlets at 10 p.m. $\frac{1}{12}$ imm.

Fig. 13. A similar culture showing the oscillating movements in the partially liquefied gelatine. In *a* the pair of rodlets together end to end; *b* they have flown apart; *c* come together again; *d* again apart. This oscillating movement may concern both or only one rodlet at a time.

Fig. 14. Similar culture. *a* rodlet fixed at 10.25 p.m.; *b* the same dividing at 11 p.m.; and *c* oscillating apart at 11.5 p.m. At 7 next morning the colony *d*, 25 μ in average diameter, had been formed.

Fig. 15. *a*, a rodlet fixed at 4 p.m. This remained at 15° C. through the night: at 8.15 a.m. next day it had given rise to 8–10 bacilli *b* forming a minute colony 3 μ diam. The temperature then slowly rose to 25° by 3 p.m., when the colony *c* had resulted, about 15 μ diam. At 8.30 p.m. the rodlets were actively swarming, but came to rest during the night.

Fig. 16. A series showing division &c. of rodlets in gelatine. *a* at 10 a.m.; *b* at 10.20; *c* at 10.35; *d* at 11.40; *e* at 12.20; *f* at 12.45. t. = rising from 21·5° to 23·5° (see p. 301). $\frac{1}{12}$ immersion.

PLATE XXI.

III. Rose-pink Micrococcus.

Fig. 1. Plate-colonies at 15–20° C. for ten days: nat. size.

Fig. 2. The same in a month: nat. size.

Fig. 3. The same, six weeks, under $\frac{1}{3}$.

Fig. 4. Stab-culture, five days at 20° C.

Fig. 5. Agar-culture, three weeks at 25° C.

Fig. 6. Potato-culture, fourteen days at 20° C.

Fig. 7. Groups of cocci under $\frac{1}{12}$: *a* fresh; *b* stained.

Fig. 8. Broth-drop culture under $\frac{1}{12}$, showing Sarcina-form: *a* at 11.50 a.m.; *b* at 2.30 p.m.; *c* at 4 p.m.; *d* at 9.50 p.m.

Fig. 9. Characteristic groups from a strong culture at 20° C. on third day under $\frac{1}{3}$, showing the glandular botryoidal masses.

Fig. 10. Gelatine-drop culture under $\frac{1}{20}$ imm. *a* at 5.55 p.m.; *b* = 8.5 p.m.; *c* = 11.40 p.m.; *d* = 9.10 a.m. next day; *e* = 12 noon; *f* = 2.10 p.m.; *g* = 4 p.m.; *h* = 9 p.m. Temperatures fell from 20° to 16° C., then rose to 22° C.

IV. A Pseudo-Bacillus.

Fig. 1. Plate-colonies: *a* on fourth day at 15–18°, under $\frac{1}{3}$; *b* after fourteen days at 20° C. Nat. size.

Fig. 2. Plate-colonies at 20° after seven days. Nat. size.

Fig. 3. Rodlets from an old gelatine-culture, $\frac{1}{12}$ imm.

Fig. 4. Rods stained with methyl-blue, $\frac{1}{20}$ imm., showing spore-like bodies.

Fig. 5. A rod *a* in broth-drop at 17·5–19° C., under $\frac{1}{12}$ imm., showing

growth. *a* at 6.15 a.m.; *b* at 8.40; *c* at 9.0; *d* at 10; *e* at 11.10; *f* at 12.30 noon; *g* at 2 p.m.; *h* at 7 p.m.; *j* at 11 a.m. next day.

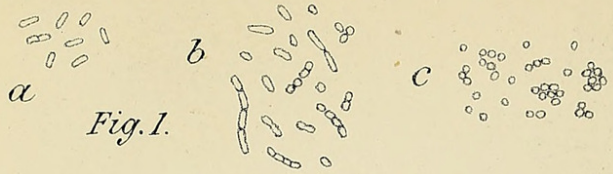
Fig. 6. A series at 21–23° C. in gelatine-drop under E/4. *a* at 8 a.m.; *b* at 8.50; *c* at 9.30; *d* at 10.30; *e* at 11.25; *f* at 12.50 p.m.; *g* at 2.15; *h* at 3.50; *i* at 4.40; *j* at 8 p.m.; *k* at 9 a.m. next day ($\frac{1}{3}$).

Fig. 7. Preparations in broth *a*, *b*, and *c*, and stained with methylene blue *d* under $\frac{1}{20}$ imm.

Fig. 8. A potato-culture, three days at 20° C.

Fig. 9. Gelatine-streak, three days at 20° C.

Fig. 10. Agar-culture, a week at 25° C.



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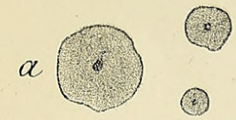

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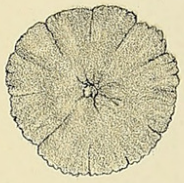

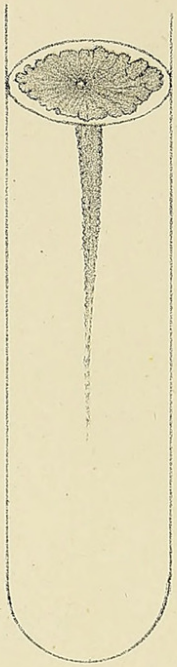

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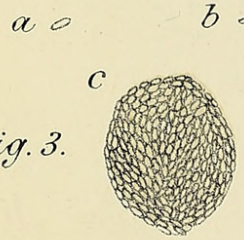

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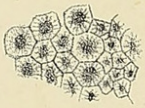

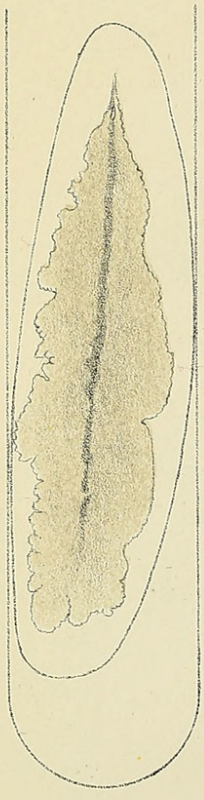

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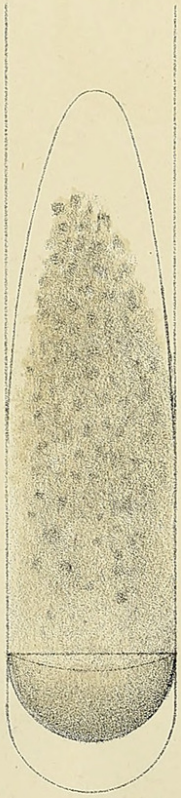

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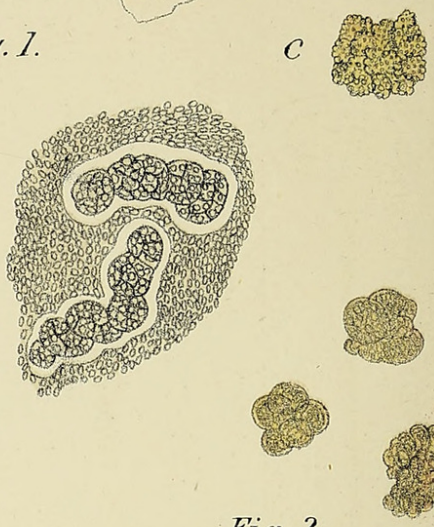

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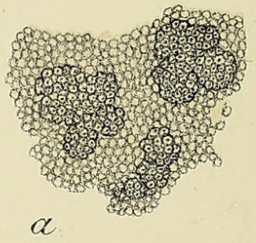

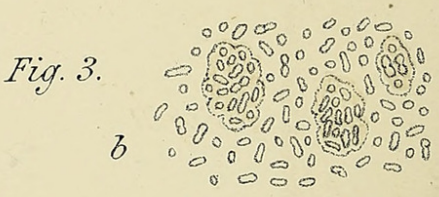

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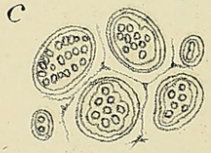

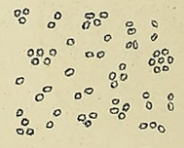

Fig. 4.

I, Short colourless Bacterium.

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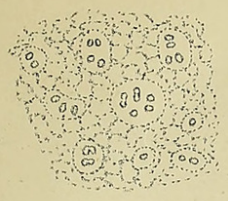

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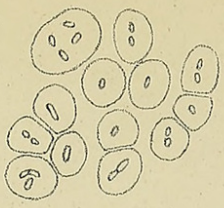

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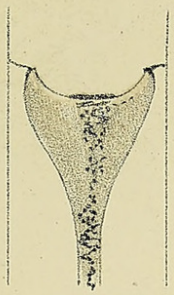

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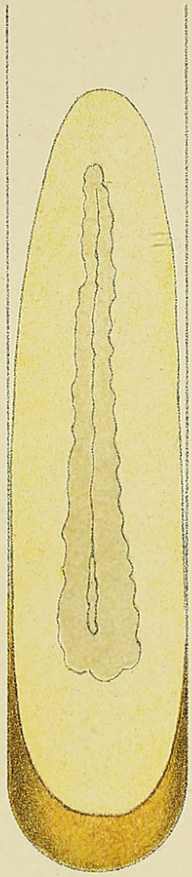

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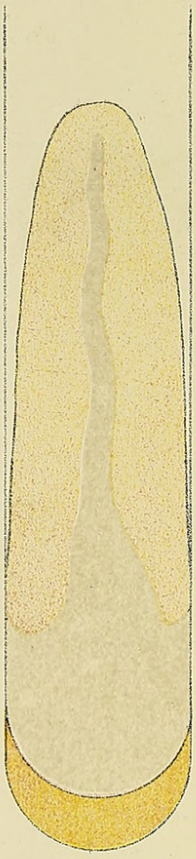

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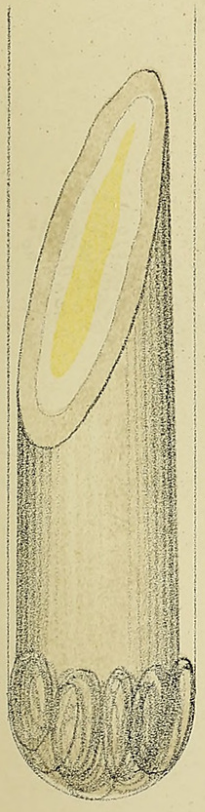

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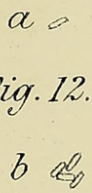

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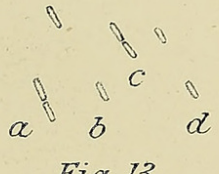

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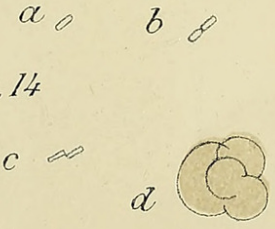

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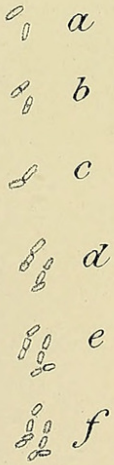

Fig. 16.

Fig. 15.

II, Capsuled Coccus or Bacterium.

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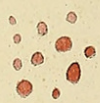

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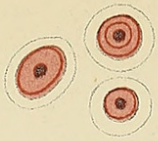

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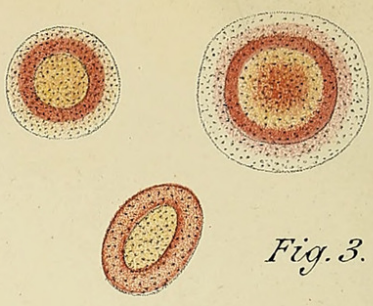

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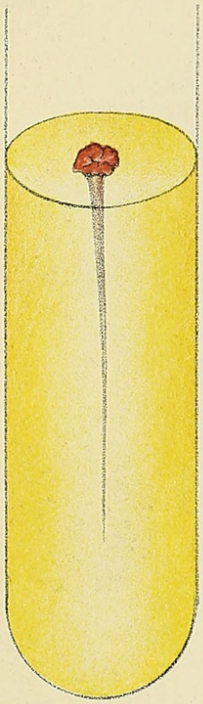

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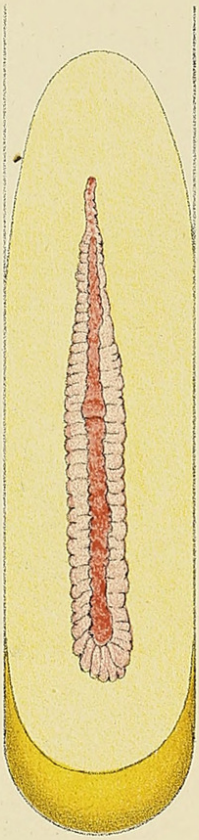

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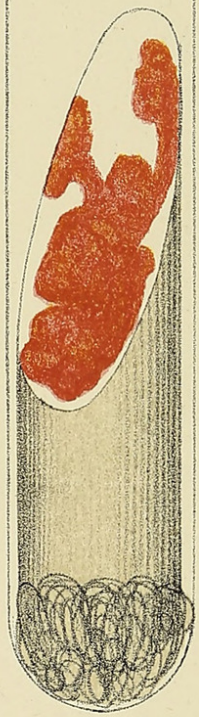


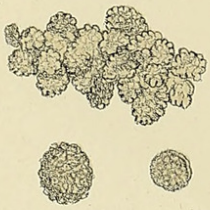
a

Fig. 7.

b

a

Fig. 8.



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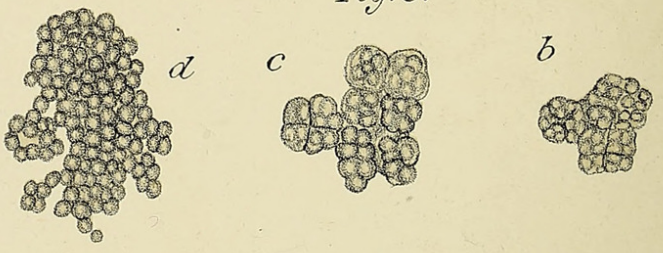
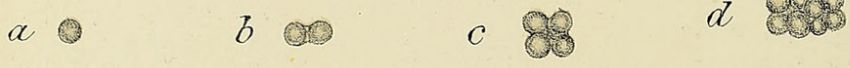


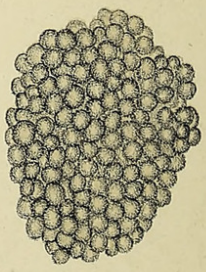
d *c* *b*



a *b* *c* *d*

Fig. 10.

e *f* *g*



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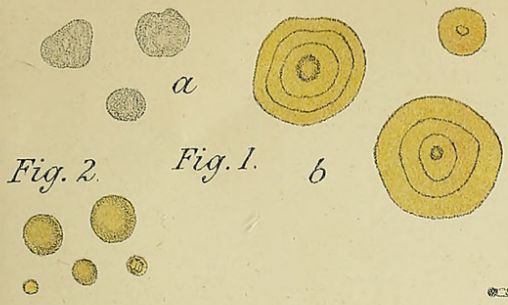
III, Rose-pink Micrococcus.

WARD. – THAMES BACTERIA.



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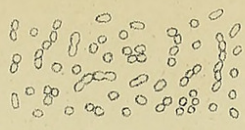

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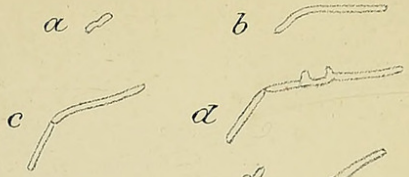

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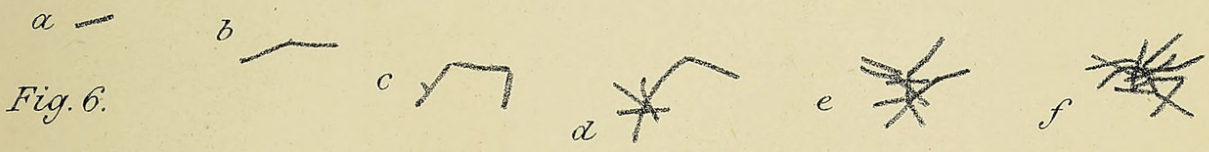

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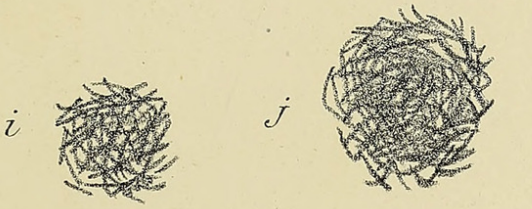

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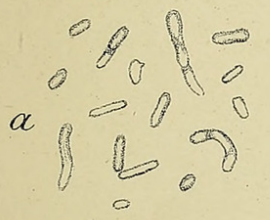
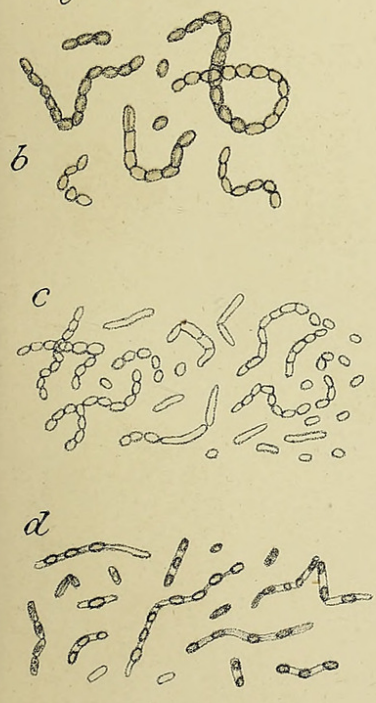
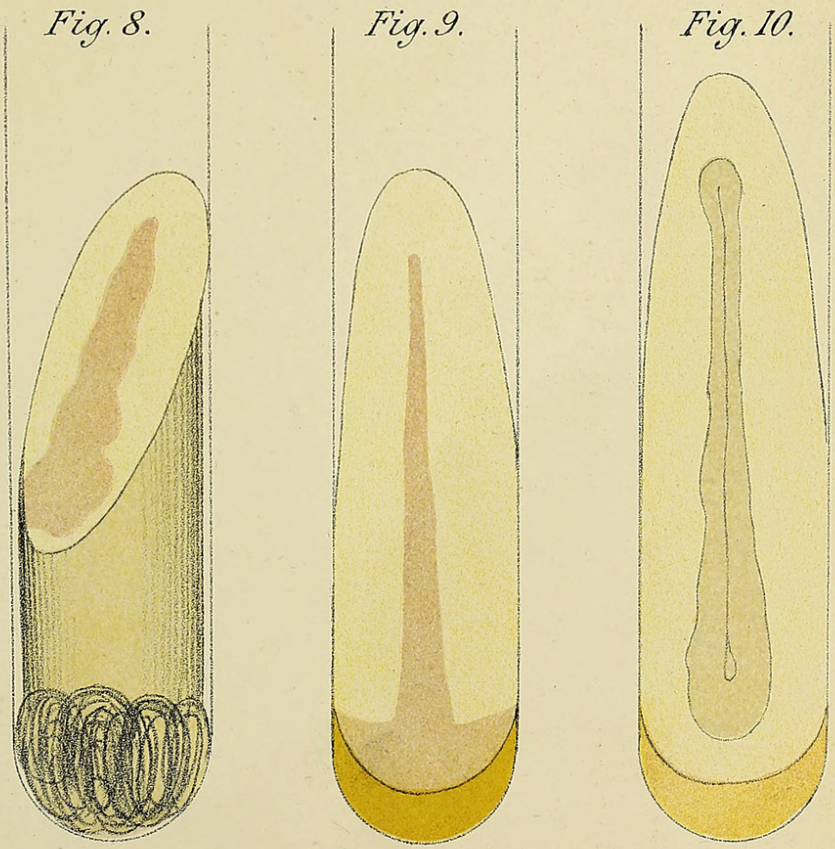


Fig. 7.





IV, A pseudo-Bacillus.

University Press, Oxford.