Thames Bacteria, III.

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With Plates XII—XIV.
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IN previous papers I have given some types of Bacteria from the Thames, selected as showing very definite characters, some of which were studied in detail. During my investigations of the Thames Flora, however, it was constantly found that certain well-known types of Bacteria are represented by varietal forms of considerable interest because the close and continued study of them might be expected to throw light on the vexed questions of species and fixity of type in Schizomycetes. Some of these groups of varieties were studied for long periods, and the results of such work on one well-marked group are here offered. I regard the forms described below as all belonging to one type; but since this breaks up very naturally into a series of yellow forms and one devoid of colouring-matter, it was judged convenient to regard them as forming two groups (VI and VII) in the survey given in the Fifth Report to the Water Research Committee of the Royal Society¹.

¹ Proc. Roy. Soc., Vol. lxi, No. 376, 1897.

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As will be shown below, some very remarkable biological characteristics have been determined for this type, which would doubtless repay more prolonged and closer study than I have accorded them.

That this type is related to a whole series of yellow pigmented Bacteria on the one hand, and of powerfully liquefying non-pigmented forms on the other, is rendered probable from the following results.

GROUP VI.

The *Proteus* Type.

Nos. 103, 34, 51, 61, 9, 9a, ι , ϕ , ζ , μ and θ .

In the present section I bring together a series of forms which include some 'species' already well known to water-bacteriologists, and best typified by *Proteus vulgaris* and its allies. Several authorities have already disagreed as to how far these do or do not liquefy gelatine—my studies lead to the conviction that the power of liquefaction varies with the conditions, both external and internal.

In his remarkable book entitled 'Ueber Fäulnisbakterien'¹ Hauser published in 1885 the results of his investigations into the bacteriology of three forms or species of *Proteus*, as he named them: these are *P. vulgaris*, *P. mirabilis*, and *P. Zenkeri*. Various criticisms have been directed against Hauser's naming of this new genus, and many observers have refused to take these forms out of the genus *Bacillus* on the evidence adduced: I do not propose to discuss this, merely remarking that the genus *Bacillus*, as at present defined, should depend on the formation of endogenous spores in the rodlets, whereas the literature teems with so-called species of *Bacillus*, in which no such spores have been observed. In any case Hauser seems to have hit on a good name, for the forms are evidently eminently protean in character, so far as peculiarities of colonies go.

¹ Leipzig, 1885, F. C. W. Vogel.

A more important point seems to be that no one has contradicted Hauser's main facts, which are the following:—these three 'species' occur together in putrefying flesh-infusions, and form rodlets in long filaments: the rodlets are about $3-4\mu$ long and 0.6μ broad, or somewhat longer or shorter, *P. Zenkeri* being smaller and only about 0.4μ broad. All motile: no spores known, but all endure drying up.

The principal characteristics as given by Hauser are the remarkable yellowish-brown and grey plate-colonies which consist of radiating mycelium-like, tendril- and hair-like filaments thinning out from a central mass of twisted tresses which in all but *P. Zenkeri* form zoogloea-masses. On the surface of the gelatine these spread out as 'wandering islets' of closely serried rodlets arranged in the most remarkable helicoid, tendril-like and irregular figures, which continually change their shapes. The first two forms liquefy the gelatine, but *P. Zenkeri* does not.

In stab-cultures the gelatine is liquefied rapidly by *P. vulgaris*, slowly by *P. mirabilis*, and not at all by *P. Zenkeri*. On agar, *P. vulgaris* rapidly spreads as a thin greyish layer, and on potato it forms a dirty slimy mass. Hauser gives no particulars as to the behaviour of the other two on these media. All these forms do best at $20-24^{\circ}\text{C}$., and grow quickly. They are also facultative anaërobian, and their products are toxic.

Zimmermann¹ found *P. vulgaris* and *P. mirabilis* in the river, and confirms Hauser's descriptions², adding particulars as to their behaviour in broth and on potatoes. The broth becomes turbid, and greyish flocks are deposited. Lustig³ gives all three species, and quotes several observers as having found them in water. He also adds that *P. Zenkeri* does slowly and partially liquefy the gelatine after some time.

¹ l. c. pp. 66 and 68.

² Zimmermann says, however, that *B. mirabilis* does not liquefy 10% gelatine, and does best at $24-30^{\circ}\text{C}$.: this suggests either great variability or that he did not keep his gelatine cultures long enough.

³ l. c. pp. 89-91.

The first 'form' or 'variety' of this type which I have isolated has the following characters :—

No. 103 : *Proteus mirabilis* (Hauser).

This form is not common, but occurs in the summer. On the plates it consists of short rodlets 1.5 to 1.25μ long by 1μ broad, breaking down to cocci about 1μ diameter (Fig. 4). It is quiescent, and no spores have been found.

In broth, however, it forms rods $3-6\mu$ long by 1μ broad, breaking to shorter rodlets or joined into short filaments $10-12\mu$ long : all active, the filaments with gliding serpentine movements, the rodlets darting hither and thither. On potato the cocci are soon formed.

On plates at $18-20^{\circ}\text{C}$., the colonies are visible in twenty-four hours as zoogloeoas spreading into very characteristic white spider-like and moss-like forms (Fig. 1); and under the $\frac{1}{3}$ rd they are seen to consist of moniliform irregularly branched submerged zoogloeoas, sending floating islands off on the surface, exactly as in No. 34 in principle, but more attenuated and divided (Figs. 2 and 3). To the unaided eye the figured colonies are quite white, or slightly yellowish in a few days: under the lens the zoogloeoas are dull whitish ochre, and the floating islands transparent and difficult to see at first. As the culture ages, these islands become yellower and denser and are then easily seen. One curious feature is the rapid course of the growth: after the second day the colonies did not enlarge at all, apparently, but more careful examination showed that the tenuous films of floating islands were spreading over the surface. There was no sign of liquefaction in fourteen days.

In other plates the first signs are visible in 12-20 hours under the $\frac{1}{3}$ as isolated coils and filaments, tresses, &c., which imitate tendrils of varying degrees of fineness according to the number of filaments and the closeness of the coiling. These lie chiefly on the surface. The zoogloeoas result from the same closely coiled up into skeins and balls.

Stab-cultures at 20°. In forty-eight hours the axis has developed outgrowths like root-hairs, and a tenuous film with a bluish sheen forms above (Fig. 6). In time the film spreads as a bluish-white layer above, but the very delicate root-hairs grow no further. No liquefaction in a month. The surface growth soon forms a continuous opalescent white layer, with a peculiar bluish cast on the surface.

Submerged cultures in gelatine show that it is very slightly anaërobic, but grows far better at the surface than in the depth.

Streak at 20°. A thin delicately fimbriated layer in 24–48 hours, spreading all over the surface in a few days, with the margins delicately cut like moss, and the whole film presenting an astonishing resemblance to a frosted window-pane. The layer is yellowish-white with iridescent edges and surface. No trace of liquefaction in a fortnight. There is, however, a slight growth in the gelatine just below the surface. The growth itself is matt, like ground glass, and soon acquires metallic iridescence on the surface like glass which has been buried (Fig. 5).

Agar at 35°. A yellowish-white streak with slightly spreading thin margins, cut and fimbriated, rapidly develops in 24–48 hours and then grows no further. It then remains unaltered for a month.

At 25° the growth is much better, as a delicate white film spreading all over in 24–48 hours, with radiating filaments from the streak forming moss-like outgrowths as on gelatine (Fig. 7). This also remains stationary after about 4–5 days, and appears unaltered in five weeks.

Potato. At 35° hardly any growth perceptible. At 25° a faint whitish film in 24–48 hours, but it does not spread. The potato acquires a bluish-grey hue in a week, and the thin dryish film then shows up a little clearer, but even in the best cases it is an indistinct grey-white film. It consists entirely of cocci, isolated for the most part.

Broth. At 35° rapidly turbid all through, and remains so for many days. No ring or veil, but a white deposit accumu-

lates. In a week this is fairly abundant and the liquid clears. At 25° the course of events is the same, but with more vigour and rapidity. An abundant white deposit, easily shaken up into the clear supernatant broth, which undergoes no change in colour. Even in 3-4 weeks there is no trace of ring or veil.

Milk. At 35° shows no change in a week, but in 2-3 weeks it is quietly peptonizing without any coagulation. At 25° no change in a week, but in three weeks shows signs of peptonizing without coagulum. The peptonization is very slow and in some cases does not begin—so far as the clearing of the milk shows—until the third week.

There can be little doubt that No. 103 is the form called *Proteus mirabilis* by Hauser¹ and *Bacillus mirabilis* by Zimmermann², and which has been found in water by a number of observers.

Tataroff³ met with a variety which formed a dirty yellow colouring, but regards it as identical.

No. 34: Type of *Proteus* (Hauser).

This form has an interesting history. It was isolated in March, 1894, and kept in culture throughout 1895.

It showed at first no signs of liquefaction, but during culture a tendency to sink into, and slightly liquefy was manifested, and alterations of various kinds made themselves apparent in the characters of the colonies. The principal features when first isolated were as follows:—

Habitat. Thames, in March, 1894.

Morph. In gelatine it forms short active rodlets $3 \times 1 \mu$.

Plates. Nothing visible even under the $\frac{1}{3}$ in forty hours, but visible to the unaided eye in ninety hours as thin, irregular, film-like plates with a bluish sheen when held up, contoured fimbriated edges, and a darker central spot (Fig. 8). The submerged colonies are yellowish,

¹ Hauser, l. c.

² Zimmermann, l. c. p. 68.

³ Tataroff, l. c. p. 19.

irregular and granular. No liquefaction, but in sixteen days the colonies are sunk in the gelatine, and open out with radial striations from the darker central mass, in some cases the radii appearing fimbriated (Fig. 9).

Streak. Begins to appear in twenty-four hours at 15° , and in forty-eight hours is very thin, white, slightly lumpy and dull. In seven days slowly sinking as a dull opaque white mass.

Slab at $15-18^{\circ}$ C. Begins by forming a delicate arachnoid film on the surface, and very slender outgrowths like root-hairs with tufts and granules along the axis. In a week or two there is a striated membrane above (Fig. 10). In a month one-eighth of the gelatine is liquefied—not reaching the bottom of the tunnel—the liquid faintly turbid. A very copious white deposit on the flat base. The tunnel shows very faint root-hairs here and there.

In a year there is a clear yellow liquid, and cloudy white (yellowish) deposit.

Agar. Growth apparent in fifteen hours, but slow, as 2-4 days show. In a month a thin, glistening, slightly spread yellowish-white layer, with somewhat fimbriated margins.

Potato. Doubtful in three days, or else forms a thin film, the colour of the potato and almost invisible except as a wet patch. All grey. Same in a week.

Broth. Turbidity just perceptible in eighteen hours, but only slight on the fifth day; seventh day hardly turbid, but with a white deposit. Other cultures gave slight turbidity in forty-four hours, and in four days a considerable flocculent deposit, and nearly clear liquor: by the eleventh day the deposit was a little yellower.

When again brought into continued culture, in 1895, No. 34 gave the following characters, and clearly showed its nature as a feebly liquefying member of the *Proteus* type, allied to Nos. 51 and 103 respectively—in fact, midway between them.

It occurs as rods up to 10μ long, breaking up to rodlets $1.5-2\mu \times$ about 1μ , or in couples or fours. In water it is more than 1μ thick, and the rods about $2-3\mu$ long, and motile. The ends are apt to stain more deeply. Old broth-cultures give rods up to $10-12\mu$, and longer, and about 1μ

thick. Decolorized by Gram's method. Plate-cultures at 12–15° give typical colourless, contoured, typhoid-like colonies, growing very slowly.

Plates at 20° showed white flecks $\frac{1}{2}$ mm. in diameter in two days. These have a spider-like appearance under the lens, and are composed of moss-like tufts of filaments and tresses from zoogloea-centres (Fig. 11 *a*). On the third day the corkscrew-like zoogloea-tresses radiate to the surface and form queer spider-like groups, and thin contoured fronds, up to 2 mm. in diameter (Fig. 11 *b*). Many colonies present dendritic or Moss-agate figures, but as the filaments reach the surface they flow out into flat, flame-like and delicately contoured lobes. No signs of softening on the fifth day (Fig. 11).

Stab-cultures at 12–15° give white dots in the tunnel, and an extremely thin hyaline frond-like film above in two days. In five days the film is still extremely tenuous, and the growth very slow.

In two days at 22° a beautiful and very delicate transparent frond, and irregular fine root-hair-like radiations from the tunnel. It grows in slightly acid gelatine also, but best in alkaline. On the sixth day the film is still exquisitely thin and transparent.

After six weeks the centre has sunk a little, but no liquid results: it is a dry hole 3–4 mm. deep and same diameter.

Streak at 20°. An extremely delicate thin and transparent film in two days, with whiter dots along the streak. In a week the film has spread nearly all over, but is scarcely visible at the transparent jagged edges, where it is flush with the gelatine and broad.

Agar at 34°. White spots along the streak in twenty-four hours. In forty-eight hours these have extended rapidly and are coalescing, like very thin scales, to a broad, rather waxy patchy streak. In four days it forms a very thin, flat, shining, yellowish film, with blue iridescent margins, and radiate zones. The streak thickens later to a shining, dirty-white, gum-like layer.

In some cases the growth at 34° is exactly like form 24 side by side, but the margins are a little thinner.

Potato. No visible growth, though possibly a transparent thin layer exists.

Broth at 35° . Shimmering turbid in twenty-four hours, and in forty-eight hours a slight deposit falls. In a week it is still slightly turbid, with flocculent, floating, serpentine masses, and traces of deposit. In a fortnight the liquid is clear and yellow, the flocculent deposit yellowish-white, and consists of rods $10-12\mu$ and longer, and about 1μ thick. At 25° the progress is very similar. The turbidity increases in forty-eight hours, flocks and deposit as before. In a week it is still shimmering: in a month the liquid is clear, and an abundant yellowish-white deposit has formed.

Milk at 25° . No change in fifteen days, but in a month nearly all is peptonized and the turbid buff liquor is strongly alkaline.

Glucose solution at 25° gave no results.

Pathogenicity. According to Dr. Kanthack¹, a guinea-pig, after intra-peritoneal injection, suffered no effects whatever.

No. 51 : Type of *Proteus vulgaris*.

Much less common than No. 50, though among the common types in the Thames; is a powerfully liquefying, non-pigmented form with the following characters.

It occurs on the plates as short rodlets about $3 \times 0.75\mu$ or a little thicker, and is motile. In broth the rods are chiefly 3×0.8 or $4 \times 0.8\mu$ or a little thinner, but filaments up to 30 or even 40μ long occur, and have a serpentine motion. The shorter rods are often paired. The movement consists in a wagging about the centre of the rod, as well as forward motion, but not the rapid darting movements of No. 50.

¹ My late colleague, Dr. Kanthack, Professor of Pathology in the University of Cambridge, kindly examined many of these forms for me as regards their pathogenic properties.

Cultures in gelatine-drops gave positive results, and helped materially in further distinguishing this form. In a gelatine-drop made at 10.30 a.m., temp. = 21° , a short rod $3 \times 0.75 \mu$ was fixed at 11.15, and had (Fig. 16 *b*) divided at 11.50 into two rodlets each $1.5 \times 0.75 \mu$: by 1 p.m. each had grown to $2 \times 0.75 \mu$, and by 2.40 one was distinctly constricted and the other faintly so—each $4 \times 0.75 \mu$, and by 3.30 each had again divided and four short rodlets now lay side by side. (See Fig. 16 *f*.)

By 6.15 p.m. there were about sixteen rods crowded into an oval colony measuring $10 \times 6 \mu$, temp. = 20.5° ; and by 10.30 next morning the colony was perfectly spherical, and 44μ in diameter: the temperature had fallen to 18° during the night and was now 19° C. At 3 p.m. the colony was of the same shape, and with no sign of liquefaction or movement, and measured 52μ across; temp. = 21° . At 9 p.m. the diameter was 56μ , temp. = 19° , and no further progress was observed, the gelatine-drop having dried up somewhat.

In another case, in a broth-drop made at 10.30 a.m., the rodlets measured $3 \times 1 \mu$ or somewhat thinner, and moved with the characteristic waggling movement. Rapid division occurred, and in a few hours most of the rods measured $4 \times 1 \mu$, and some had grown to filaments of 10–12 up to 30–40 μ , and with serpentine movements. On the second day most of the rodlets measured 1.5 to $2 \times 1 \mu$, and on the third day most of them were so short as to be nearly cocci.

Plate-cultures at 18 – 20° C. showed colonies under the $\frac{1}{3}$ in twenty-four hours, the submerged ones being smooth in outline but irregularly oval or circular, yellowish, granular; and the emerged colonies coming out as thin, hyaline, faintly granular more or less circular films.

In forty-eight hours the colonies are visible to the eye as pale white circles with a central spot, and exhibit a curious crystalline appearance and bluish sheen when held up. Under the $\frac{1}{3}$ rd each emerged colony is found to have a dense liquefying core of dark granules, whence radiate coils and tresses,

which straighten to rays as they approach the periphery and give the whole colony a curious resemblance to an *Acineta*. (See Fig. 12.)

But more careful observation—and easily overlooked, as it was by myself at first—shows that beyond the radiating tresses is an extremely tenuous and hyaline irregularly lobed coronal film on the surface of the gelatine, and formed by the radiating tresses as they emerge. Liquefaction rapidly sets in towards the end of the second day, and the plate is usually destroyed by the third day.

The liquefaction differs in character from that of most common liquefying Bacteria, and does not consist in a scooping out of the gelatine in circles and throughout its depth, but is universal over the *surface* of the plate only; even on the fourth day the lower parts of the film may be firm. Unlike the ordinary cases also, the watery liquid has a very slight odour, not decidedly unpleasant.

One of the most remarkable features about these plate-cultures at 20° and near that temperature, was the extremely rapid liquefaction that sets in towards the end of the second day, and for some time I was excessively puzzled by this phenomenon. Over and over again I left a plate looking quite 'safe' at 8 or 9 p.m., to find it liquefied all over next morning. Several times I have visited the plates at various hours between midnight and 4 a.m. with the same result; the plates looked quite 'safe,' with colonies only 4 or 5 mm. in diameter, and yet by 8 or 9 a.m. they had liquefied.

One point struck me, and eventually gave the clue to the explanation. When the liquefaction was observed carefully next morning, one saw that it was at first only superficial; the whole top-layer of gelatine was a watery liquid, but the gelatine below was still firm and had its colonies firmly imbedded.

Then—by an accident in the first case—I found that just before the sudden liquefaction, if the plates were held obliquely to the light of a lamp, a faint iridescent shimmer is to be discovered around each of the emerged colonies,

in the form of an irregular zone with jagged and indented edges (Fig. 13). More careful microscopic examination showed that this almost invisible zone is an extremely tenuous film of Bacteria arranged in coils and tresses and lying on the surface of the gelatine in curiously lobed, fimbriated, flame-like and other figures, extending round the more visible colony. In fact, what had hitherto appeared to be the periphery of the colony was only the periphery of the denser central parts, and this extended radially for long distances around as the tenuous lobed film, only *one* Bacterium deep in most of its parts. But this was not the most wonderful part of the phenomenon. When I had succeeded in seeing this film by careful focussing under the $\frac{1}{3}$ rd it was possible to make out that its lobes are composed of the most curiously convoluted and doubled-up tresses of filamentous series of Bacteria. (See Figs. 19 and 22.) A very fair idea of the convolutions is got by saying that they are a good imitation of those obtained by Mr. Galton in his well-known finger-prints.

While looking at this convoluted coronal film by lamp-light one night, I found that the lobes at the extreme periphery perceptibly moved, and careful focussing soon showed that the whole system of this coronal film is in a state of creeping movement, and gradually extending itself radially over the surface of the gelatine.

If a lobe is carefully watched it is seen to put out processes like an Amoeba; these processes thicken up, or spread out, or thin out into fimbriae sometimes so fine as to be nearly invisible, and the Bacteria of the convolutions are also seen sliding one over the other. Or a piece of a lobe becomes constricted off, and moves freely away, over the surface of the gelatine; or two neighbouring pseudopodial processes curve over and fuse, and so on—the movements are so exactly like those of an Amoeba that no other term is applicable.

In Figs. 17–20 I have reproduced outline-drawings, and in Fig. 22 more detailed drawings, of some of these movements,

and they will give better ideas of their character than a lengthy description in words would do. In Fig. 17 two of the pseudopodia, as I shall term them for the sake of brevity, were sketched in outline (*a*) and their changes recorded for each succeeding minute (*b* to *e*). First, the lower thin tongue curved up towards and fused with the flame-like pseudopodium above; then it broke away from its basal connexion with the corona (to the right below), thickened up, and gradually fused laterally with the small blunt lobe near the $\times$ —a nearly fixed point throughout, and so converted the bay in which the $\times$ lies into a closed space; the changes during the next two consecutive minutes are seen in *d* and *e*.

Even more astounding are the changes seen in Fig. 18, where the changes of outlines (*a* to *n*) were drawn at intervals of half to one minute—practically as fast as I could record them, in fact.

The curved outline in which *a* is placed was the peripheral end of a blunt lobe of the coronal film, and just outside to its left was a serpentine free mass of the Bacteria. This latter was writhing like a worm, and in less than a minute broke in two (*b*), its distal part continuing to writhe freely, while the proximal one first joined the blunt lobe and then stretched out and again joined the free part (*c*). Again it broke and a free shorter part wriggled off (*d-f*), and this alternate breaking off (two pieces in *g*) and joining up again went on for a minute or two (*d-h*). At last the free curved end of the worm-like part curled over and made a loop (*i, k*), and it remained a constituent part of the larger pseudopodium, its further changes being sufficiently evident from *m* and *n*.

The series in Fig. 19 shows the somewhat slower changes observed in one of the broad and blunt lobes of the coronal film, the most marked alterations being observable in the outlines of the enclosed area in which the $\times$ is placed. *b* was drawn four minutes after *a*, *c* a minute after *b*, and *d* a minute later.

One of the most interesting cases is shown in the series

Fig. 20. At 8.20 p.m. the ring *a* was lying some distance away—to the left—from a peripheral lobe, not shown in *a* to *k*, and the outline sketches *a* to *h* were made at intervals of a minute.

It was evident that this ring was made up of a series of parallel, or nearly parallel, coiled series of Bacteria, and from 8.20 to 8.28 (under Zeiss B. occ. 4) these coils were revolving from left to right as one faces the ring, sliding one over the other more or less regularly and smoothly—as was quite clear by watching the notch-like free ends depicted in the inner and outer outlines. At the same time the flat mass of these coils was surging from side to side, sometimes widening, sometimes narrowing the ring. The movements of the ring as a whole had a curious resemblance to those of a smoke-ring—a vortex-ring. Between 8.28 and 8.31, however, the direction of revolution of the coils was *reversed*, as seen by the arrows in *i* to *l*.

I was curious to see how stable this ring was [for although it often thinned out dangerously at some part (*d* and *g*) it never broke] as compared with other free groups (e.g. those in Fig. 18), and should have watched it as long as possible, but at 8.39 the large lobe (to the right of *b*) referred to above had crept nearer and nearer, and between 8.41 and 8.42 it came into contact with and soon (*o*, *p*, *q*) fused with the ring, which now became merely a part of the lobe.

These illustrations will suffice to show how the progress of the coronal film is effected radially over the surface of the gelatine. The pseudopodia go on creeping outwards, and occasionally long tongue-like processes shoot out, and bits of these separate off as outlying free colonies, which may again join one to another or to the main corona as they wander over the surface, and *it is these outliers and the all but invisible coronal film which so mysteriously liquefy the surface of the gelatine.* In fact, at the very time I thought the visible colonies were extending so slowly that the gelatine could not liquefy before morning, the surface of the

latter was already covered with these wandering colonies and outliers, and with a tenuous all but invisible reticulate film of Bacteria.

The next question that arose was as to the mechanism of the movements of the pseudopodial-lobes, &c.; and to solve this I tried to cultivate the colonies in gelatine-drops under the $\frac{1}{12}$ th immersion.

The series in Fig. 21 is a good one. The gelatine-drop was made at 11 a.m. and a rod *a* fixed: temp. = 20°C . At 4 p.m. there were about eight rods in a clump *b*, and at 8 p.m. an oval colony $8 \times 4 \mu$. At 9.30, the temperature having fallen to 19° , the colony measured $10 \times 8 \mu$, and at 11.10 p.m. $12 \times 10 \mu$: temp. = 18.5° .

The temperature fell to 16° during the night, and at 8 a.m. the colony was circular and consisted of short rodlets closely packed and perfectly quiescent. Diameters $34 \times 32 \mu$. At 9.30 a.m. it was a circle 36μ diameter, temp. = 17.5° , and through the day it slowly grew as follows:—

10.55 a.m.	44μ	temp. = 19.5°
12 noon	59μ	20.5°
3.30 p.m.	64μ	21.0°
8.40 p.m.	72μ	19.5°

At 3.30 next day the hitherto granular circle was showing signs of differentiation (*e*). It was very opaque—really a sphere—but one could make out several curved filaments among the rest. At 6 p.m. the sphere was much clearer, and had evidently become looser, and the presence of numerous long curved filaments was observed (*f*), though no movement was discernible. I am strongly of opinion that the clarifying of these spheres is due to a slow increase of a slime between the cells, no doubt the swollen cell-walls. At 8.40 the clarification was more marked, especially in the middle, and slow writhing movements of the filaments and rods could be detected, the nature of which suggested the existence of a sort of zoogloea-slime as the medium in which they were occurring.

Just before 9 p.m. the softening gelatine gave way and the sphere began spreading itself out as a film, but I was unable to do more than satisfy myself that the rods and filaments *themselves* move.

Repeated attempts to bring the colony in the hanging-drop to the creeping stage observed in such perfection on the plates failed in some cases, evidently because the small drop of gelatine becomes exhausted before the colony is ready to spread, in other cases because the drop got diluted and too thick for focussing even with the E/4.

Eventually I abandoned this method in favour of another, the extraordinary success of which suggests its wider application in such researches as these.

I took a clean cover-slip, and sterilized it by holding it over the flame between talc plates, and spread a thin film of sterile gelatine on it and allowed it to set. I then lifted it by sterile forceps so arranged that one point held the middle of one edge and the other point the middle of the opposite edge; hence the cover-slip was so jammed between the forceps that when the latter were held vertical, the former was horizontal; and I then brought the sterile cover-slip, gelatine-side down, on to one of the creeping films on a two 'days' plate-culture. In fact, I made a 'Klatsch-präparat' of the creeping film, and after a few attempts obtained a very perfect specimen. The cover-slip was then fixed as the roof of one of my sterile culture-cells, and treated exactly as in the case of a hanging-drop.

After a few minutes at 20° C., I then saw under the $\frac{1}{12}$ immersion one of the most magnificent sights in the way of bacterial movements it has ever been my lot to witness.

The lobes and flame-like protuberances of the coronal film, as well as some of the looser bacterial aggregates from the centre of the colony, had been taken up and fixed by the gelatine to which they adhered in their relative positions: the impress of the film was particularly perfect, and after recovering from the shock, the movements of the lobes and wandering outliers went on in the now swelling film of

gelatine—taking up water-vapour from the cell—exactly as if no disturbance had happened.

The movement consists in the sliding of the rods and filaments one over the other, and under the $\frac{1}{12}$ is far too rapid to draw in detail, whence I had to make line-sketches from which the figures were copied later.

The filaments are of various lengths, some being segmented very clearly into separate rods, others less clearly into longer segments. They are evidently growing and dividing as the active gliding movement continues, though of course it is impossible to trace that in detail. It is to be inferred, however, from the action of the film or lobe as a whole, for the filaments are laid flat in tresses, coils, &c., in one plane on the gelatine, and they evidently grow by intercalary growth and segment into shorter and shorter rods.

The worm-like segments glide over one another, as said, and by no means always in the same direction.

Of three parallel segments, for instance, any one may go for a time in one direction while the others are slipping past it in the opposite one. In the same sense, there is no order as to stoppage, reversal of movement, &c.

It is very interesting to see how fresh arrangements of the moving series are instituted. This may occur simply by an outside segment starting off on its own account, as it were, as *a* in the annexed diagram ;

or *a* may meet *b*, and form a new series, and in this case *c* will curve out into the increased space as indicated by the arrows.

Similarly when two series meet, the free ends may slip on e between the other, as in the annexed diagram, or collisions

may occur. Sometimes such collision results in a bending on itself of one of the filaments, and it may snap, or a vortex may result, and the filament go round and round in one direction for a time as in the diagrams annexed, and so on with infinite varieties in the details of the movements.

Stab-cultures at 18–20° (see Figs. 14 and 15). Good growth in the tunnel in twenty-four hours, and a thin film has spread over the surface and liquefied the uppermost layer to a slime.

There is considerable variation as to the rate of liquefaction, however, some cultures forming a big 'pouch' in three days, while others only form a 'thistle-head' funnel in that time.

In gelatine, submerged, and the tubes put into pyrogalllic acid and KHO in a closed bottle at 20° C., slow but perceptible growth occurred in the first twenty-four hours, and increased during the second day.

Agar at 20°. In forty-eight hours a broad layer, perfectly smooth and glistening, chalk-white, and with a bluish cast in oblique light. Slight white deposit.

Potato at 20°. A grey moist layer spreads all over in three days. It is much thinner than the flesh-coloured, raised, lumpy, and matt patch formed by No. 50, and totally different from it. In a week it is a little thicker, and spread all over as a wet grey-white layer.

Broth at 18-20°. Dense turbidity in 24-48 hours, and a white deposit falls. No ring or veil in four days: still very turbid.

At 33-35° a dense satiny turbidity in twenty-four hours, increasing during the second day. Still more turbid later, and with white flocks and deposit.

Milk at 35°. Separation of clot on third day. Distinctly but not strongly acid.

This form is pathogenic to guinea-pigs according to Dr. Kanthack.

No. 61.

No. 61, an imperfectly studied form, was evidently the same or very closely allied. There are doubts as to the pathogenicity, as the cultures were not successful. It seems, therefore, unnecessary to give details.

No. 9: Proteus type.

A not uncommon liquefying non-chromogenic *Bacillus* (old cultures of which, however, are orange-coloured) occurred as very short rodlets 1.5-2 μ long by 0.75-1 μ broad, hardly if at all motile, and often oval 1.5 $\times$ 1 μ or so, which developed plate-

colonies as follows. Gram's method decolorizes it, but it is easily stained by ordinary methods.

Plates at 12–15° C. Yellowish-grey, granular, circular discs, often with irregular edges, appeared in two to three days. On the fourth day liquefaction began, each colony being merely a zoned brownish circular disc about 1–2 mm. diameter, the edges of each colony being fimbriated or even with irregular tresses. Liquefaction very rapid on the fourth day, the small colonies—white to the unaided eye—retaining their circular shape as they float. Under the $\frac{1}{3}$ each was a grey disc, hollowing out in the centre, and with fimbriated edges (Fig. 24).

After-plates gave the same dimensions, i. e. ordinary round-ended rodlets $1.5\text{--}4\mu \times 1\mu$. The thickness not uniform, but in some cases $0.75\text{--}1\mu$.

After three months in culture, plates at 15–18° showed white-grey spots in three days, each sunk in a shallow lenticular depression. Under the $\frac{1}{3}$ the submerged colonies are moruloid, tawny, granular: as they emerge they thin out with anthrax-like tresses and fimbriated edges. On the fourth day they are about 1–4 mm. diameter across the lenticular depression, the ochre-coloured central eye being solid, or 1-zoned, and under the $\frac{1}{3}$ the zone is found to be due to clouds around the more solid centre. Others are masses of tresses, with fimbriated edges. They lift as a whole from the depression.

On the fifth day the lenticular depressions are coalescing: they measure 5–8 mm. in diameter. The central ochre-yellow eye resembles a sieve, and is composed of dense and less dense clouds. The fluid in the depression is turbid. In a week the plate is nearly liquid.

Stab-cultures at 12–15° C. Rapidly forms a thistle-head funnel, and liquefaction occurs and spreads to the sides. Repeated tough whitish veils form on the surface, and fall successively, and the resulting deposit is very shiny and tough. The top of the liquid may be clearer, or it may be more turbid above (Fig. 25). In a fortnight nearly all the gelatine is liquefied with a white ring and veil above: dense clouds fall

into the nearly clear liquid on shaking, and go to swell the dense yellowish-white deposit. In a month all is liquid, with a white ring and fallen veils.

Agar at 22° C. Shows a spreading, white, granulated layer in twenty-four hours, and in three days this is very white, and extends as a veil over the clear drainage. In six days the marked whiteness and granular appearance persist (resembling the growth on potato), and new veils, wrinkled, replace those deposited in the clear liquid.

At 34–35° the growth is also rapid, as yellowish-white spots confluent in twenty-four hours, and a veil and deposit already occur in the drainage. In two days it is an abundant white shining layer, with outlying dot-colonies. It consists almost entirely of paired rodlets 1.2×0.5 or 0.6μ when stained, thicker before staining. It is more tawny than form No. 16. After two or three months the agar-growths are deep ochre-yellow.

Potato at 22° C. In forty-eight hours a dense chalky-white, granulated, spreading growth appears, becoming mamillated, and of a peculiar milk-white appearance later. No further extension after four or five days, but in three weeks the colour is dull, and a slight metallic iridescence is observable.

Broth. At 35° becomes rapidly turbid in twenty-four hours, and densely so in two to three days, when a white ring and deposit form. In a week an abundant white deposit has fallen and the liquid is still extremely turbid, but there are no distinct veils.

At 25°, in broth infected from agar-culture, the course of events is much the same, but definite veils form. In twenty-four hours a slight white greasy-looking ring and veil occurs, and from the third day onwards the thin veils fall successively. The dense turbidity and copious deposits are as before, the latter being very white, and even in a fortnight the turbidity is dense.

Milk, infected from an agar-culture, gave definite separation of the casein in forty-eight hours at 25° C., and by the sixth day much of the precipitated coagulum was dissolved,

the reaction being distinctly acid. In eleven days solution was rapid, and a chalky-white deposit had fallen.

Glucose solution. At 25° C. an infection from agar gave no results, except very minute floating flecks and deposit in eleven days, the liquid remaining otherwise quite clear.

No. 9 seems to be pathogenic to guinea-pigs according to Dr. Kanthack's report.

Attempts to revive No. 9 after a year failed.

Since this form presents several differences from the typical form, and suggests alliances with Groups VII and XIV, I set forth its characters in tabular form.

Number 9 (Proteus type), Figs. 24 and 25.

Habitat. The Thames.

Sizes, &c. Short rodlets $1.5-2 \times 0.75$ to 1μ and hardly if at all motile. No spores observed.

Plates. At 12–15° yellowish-grey circular discs, edges irregular or fimbriated. Liquefaction rapid on the fourth day, the colonies floating and retaining their shape. Under $\frac{1}{3}$ each colony zoned, brownish, with tresses at edges.

Stab. At 12–15° rapid thistle-head funnel, and liquefaction, and repeated tough white veils form and fall. All liquid in three weeks or so, and a white ring and veil above, turbid liquid and slimy deposit of successively precipitated veils.

Agar. At 22° and at 35°: rapid growth as a very white layer of confluent granulated or circular colonies becomes markedly white and shining.

Potato. At 22° a peculiarly chalky- or milk-white granulated or mamillated spreading layer in forty-eight hours onwards. Becomes dull, and with slight metallic iridescence later.

Broth. At 35° rapidly and densely turbid in twenty-four hours, and in 2–3 days an abundant white deposit, and trace of white ring. At 25° the same, but more definite veils form and fall successively. Even after a fortnight the turbidity persists. The deposit is very dense and white.

Milk. At 25° separates in forty-eight hours, and in six days was rapidly dissolving the coagulum. Reaction distinctly acid. Chalky-white deposit in eleven days as solution proceeds rapidly.

Glucose. Merest traces of flecks and deposit in eleven days at 25° C. The liquid remained clear.

I am strongly inclined to the belief that No. 9 is a variety connecting the *B. Termo* type with the *Proteus* type, and if so it bears out a widespread suspicion among bacteriologists. The same applies to the following forms, the tabular summaries of which give the principal characters. Several of them appear to me to be weakened forms, but some had not been sufficiently studied before passing out of cultivation.

Number 9 a (Variety of Proteus type), Fig. 26.

Habitat. The Thames.

Morph. In six weeks, gelatine, practically cocci 1 μ in diameter, or short rodlets 1 $\times$ 1.5 μ fresh.

Plates. Nothing in forty hours even under $\frac{1}{3}$, but quite visible to the unaided eye in ninety hours, and slowly liquefying in 115 hours. By the sixth day the colonies float in a yellowish watery liquor, as balls of a grey-white colour and 1–2 mm. diameter.

Streak. In twenty-four hours at 15° an extremely thin white streak, and in forty-eight hours this is nearly flush with the gelatine-surface, very thin, matt, white. In seven days much slower and thinner than form A, but similarly white, flush and granulated. Rapid liquefaction in eleven days.

Stab. In a month the gelatine is half liquefied, with a very turbid liquor over a white deposit on the floor. Grows in acid gelatine. After some months a viscous clear yellow liquid and yellow-white very flaky deposit.

Agar. In five days at 20° a very thin dull white layer confined to the streak. In a week, a mere extremely thin film where the loop touched the agar.

Potato. In five days at 20° a very thin, wet, creamy-white film. In eight days extensive, thin, with matt edge, yellowish with metallic iridescence on the surface in the middle.

Broth. Faintest turbidity in eighteen hours, but white turbid in forty hours and with slight deposit. Very turbid in three days and with white deposit, but on the fifth day clearer above and only slightly

turbid. On the seventh day a thin veil, which breaks to flecks on shaking. In a month turbid above, and a slight ring and veil and yellow-white precipitate. It shows alliances with several other forms, and may be a weakened form of No. 9.

ι (*Variety of Proteus type*), Figs. 27 and 28.

Habitat. The Thames.

Morph. Extremely small and active rods $2-3 \times 0.75$ to 0.5μ .

Plate. Yellow-grey, slightly sunk, zoned and radiate circles, just visible as points under the $\frac{1}{8}$ in forty hours, visible to the unaided eye in ninety hours. Gelatine completely liquefied in 115 hours.

Streak. Slowly scoops and liquefies the gelatine with a white deposit.

Stab. Very slow growth as white, leafy, frondose colonies, and dots in upper part only. In a month it has liquefied $\frac{1}{3}$ of gelatine, and forms a white deposit on the flat base: liquid perfectly clear.

Agar. Merest film or nothing visible on the fifth day, and hardly visible in three weeks. After being some time in culture, a rapid white streak extending in fifteen hours; a milk-white broad streak in two days; spreading, yellow-white, and thicker in four days. Thin, yellowish, and extended in fourteen days. In a month a thin white layer not spread much. Recalls forms ϕ and ζ .

Potato. Yellow-buff wet film, not unlike form π but more waxy. At 20° nothing appears, or a mere wet film in three to seven days, and even in eleven days a mere trace is seen as a faint white film.

Broth. At 35° no trace in a month, and another attempt gave nothing in a week. At 25° the broth becomes turbid and has a whitish deposit.

Milk. Precipitates casein in five days. Alkaline reaction. Solution later; nearly all gone in three weeks, liquor yellow. All clear in a month.

Glucose. No results.

Several points (e. g. its behaviour on potato) suggest its resemblance to π , which may be a stronger form.

Agar suggests resemblances to forms ϕ and ζ , and the negative results on broth suggest ϕ and γ .

On the whole, colony *ι* seems to be one of the *Proteus* group, probably a very weak form.

Variety φ, Figs. 29, 30.

Habitat. The Thames.

Morph. Short swinging paired rodlets $2 \times 0.5 \mu$.

Plates. Trembling liquefying area with grey-white centre and dots around, but in further cultures the one or two colonies visible under $\frac{1}{3}$ in 115 hours are still invisible in six days. In ten days the gelatine is liquefying and the feeble colonies running together and white.

Streak. Slow white colonies sinking and scooping the gelatine. White flecks.

Stab. $15-18^{\circ}$ C. It begins by forming a faint film on the surface and faint clouds in the tunnel. In fourteen days an eighth of the gelatine is slowly liquefied and a dense white-yellow deposit forms: liquid turbid. Tiny dots in tunnel. In a month three-fourths of the gelatine is liquefied and clear, white deposit resting on the solid base. In a year a very slight deposit, brownish liquid.

Agar. Thin white film. Hardly visible in fifteen hours. White wet patches in two days. Shining milk-white layer in five. On the second day a yellow-white streak; spreading on the fourth, as a thin white layer. On the fourteenth day still extremely thin and slight: little growth in a month—white flecks in drainage.

Potato. Nothing in three weeks. Other cultures have a thin white streak first seen on the seventh day.

Broth. Failed in all cases.

Milk. Precipitate in twenty days, alkaline. In three weeks it shows peptonization, but slow. Yellow liquor. Liquor nearly clear in a month. Nearly clear yellowish-brown liquor and clots.

Glucose. Nothing.

I cannot understand the negative results in broth. The whole behaviour suggests a very weak form.

The behaviour on agar joins ϕ , ζ , and ι perhaps. It seems more like ζ in some respects, but the milk- and broth-cultures are sharply different.

On the whole I think it is an extremely feeble form of *Proteus*.

Variety ζ, Figs. 31-33.

Habitat. The Thames.

Morph. Non-motile rods to nearly cocci. Rodlets often paired. Fairly large, $1 \times 0.5 \mu$.

Plates. Grey, granular, dense and structureless colonies, just visible in forty hours. Slow and non-liquefying—at least at first, but (see streak) they slowly liquefy later.

Streak. Grey-white, spreading, and eventually scoops and sinks, slowly liquefying—white.

Stab. White points in tunnel. In fourteen days a thistle-head and yellowish-white precipitate, and dots in tunnel. In a month half-liquefied, turbid, copious white deposit. Grows also in acid gelatine. In a year a brownish-yellow liquid, clear, and very glairy deposit, pale brown.

Agar. White along the streak, or greyish, often hardly visible in three weeks. Growth visible in fifteen hours, and forms a white mesentery in two days: waxy and very white in four; but even in fourteen hardly spreads beyond the streak. In a month it is little spread. White flocks in drainage.

Potato. Nothing in four days: in seven a very thin wet white film. In three days at 20° are formed white flecks, wet, and exactly the colour of the potato. In seven days a thin dew-like colourless wet film.

Broth. Soon clouds, and deposits a white precipitate. Very turbid in eighteen hours and same to third day. On fifth day not very turbid, slight deposit, no veil. In a month no veil, turbid throughout, and yellow-white deposit.

At 20° C., distinctly turbid in forty-four hours, and in four days turbid but no veil: white deposit. Similar in seven days, and on the eleventh day there is still no veil, only yellowish-white flocculent deposit, &c., as before.

Milk. No visible change in seven days. Acid reaction.

Glucose. No change in a week.

Perhaps μ and ϕ are connected, but they are slower in broth.

Agar suggests the union of ϕ , ζ , and ι , and broth connects ζ , μ , and θ more or less.

I am inclined to regard ζ as a form of *Proteus*; while the potato-cultures strongly support this, the microscopic characters do not help us. On the whole it is probably a weakened form.

Variety μ , Fig. 34.

Habitat. The Thames.

Morph. Extremely short and small rods: nearly cocci: no motion: 1 to 1.5 $\times$ 0.75 μ .

Plates. Crenate circular colonies, orange-yellow and sinking. Nothing visible even under $\frac{1}{3}$ till ninety hours, and very slow at 115 hours. In 140 hours small colonies, yellowish and granular, are visible, the largest being $\frac{1}{20}$ mm. In seven days the largest is 1 mm. finely granular greyish-yellow circles. Slowly liquefy.

Streak. Soon sinks and forms a yellow eye. Liquefies slowly: pale and dull yellow.

Stab. Rapid dots in tunnel in forty-eight hours. In three days a slight depression (thistle-head) with a dark eye and cloudy liquid: deposit yellowish and dense. In fourteen days one-eighth gelatine liquefied, and a funnel forms with rusty yellow-white deposit, and dots in tunnel. Resembles ζ but is more orange. In a month one-fourth liquefied, but not to the bottom of the funnel; very turbid, a yellow clot rests on the solid base. In a year the liquid is brownish with a slight dirty-white deposit.

Agar. White layer in twenty-four hours. Yellowish-white broad wet streak in five days. Cloudy-white and slightly mesenteric.

Grows in fifteen hours. In two days a thick, wet, white mesentery, and in four days copious yellowish-white wet mesentery. In fourteen somewhat buff, thick and wet.

In a month it is white with a buff tinge¹, thick, wet, not much spread, and forms a somewhat buff deposit in the drainage.

Potato. Nothing, or an invisible film in three days at 20° C.; a thin slightly buff streak in six days. Nothing in three weeks at 15° C.

Broth. Traces first visible on the third day; on the fifth slightly turbid with traces of white deposit. Same seventh day. More on tenth. In a month densely turbid, and yellowish-white deposit: no veil.

Milk. Very faintly alkaline in twenty days, but no visible change in a month.

In a year a brown liquid with white clots, like cream clots.

Glucose. Doubtful traces of deposit.

This form seems, in some respects, to connect this typical *Proteus* group with the yellow species considered under the next type.

Colony θ , Fig. 35.

Habitat. The Thames.

Morph. Very small rodlets $1 \times 0.5 \mu$, or nearly cocci on old gelatine.

¹ Yellower than β .

Plates. Mycelium-like very thin, circular, radiating colonies, with very transparent wedge- or chisel-shaped fimbriae at margins, and liquefying in centre. Two days' growth at 15° shows a dark eye and faint zone. All liquefied in seventy-two hours.

Streak. Rapidly scoops and liquefies at 15° C., and forms a very white turbid mass. All liquid in ten days, with white turbidity and deposit.

Stab. At 15° yellow-white dots in tunnel. Rapidly spreads above as a yellow-white, radiating mycelium-like layer with an eye. In five days an eighth of the gelatine is liquefied, turbid. In three weeks half is liquid, very turbid, white deposit. In a year a red-brown liquid and dirty deposit.

Agar. In twenty-four hours at 20°, yellowish-white, wet, thin, confined to the streak, and even in seventeen days there is only the thin yellow-white streak.

Potato. In three days at 20°, wet, yellowish-grey, spreading. In six days spread all over and very wet. In seven days very like form *v* but rather yellower.

Broth. Turbid in twenty hours at 20°, very turbid in forty-four hours, and more so in sixty-eight hours. On the fourth day a white deposit, and still very turbid in a week: no veil, but deposit very white on eleventh day.

All the characters seem to point to this being a weakened form of *Proteus*.

GROUP VII.

Colonies 7, 17, 23, 31, (B), K, and *B. arborescens*.

The Yellow Proteus Type.

Among the commonest Bacilli, or at any rate rod-like Schizomycetes, to be found in the Thames at all seasons, are a series of forms of which the following are representatives isolated and cultivated by me.

While their differences in detail are not to be overlooked, they present a number of peculiar characters in common so strongly marked that there can be little doubt they must

be brought together as a group of allied varieties, or if you will, 'species.'

They all agree in forming the peculiar tenuous cloudy colonies on plates, composed of coarse and fine radiating and much interlaced tresses and filaments, like an extremely fine mycelium sunk in the gelatine. The coarser of these masses and tresses are in the condition of zoogloaeas, i. e. the rods and filaments are held together by their swollen cell-walls. They also agree in liquefying the gelatine more or less rapidly.

The filaments of which the tresses are composed are always very thin—about $0.5-0.75\mu$ —and break into rodlets about $4-6\mu$ long, which may again break up into shorter ones $1-2\mu$ long. These are either quiescent or only slightly motile, and no spores could be obtained in any cultures.

Another point of general agreement is the gradual assumption of a more or less pronounced ochre-yellow to chrome-yellow colour in the older plate-cultures, and other gelatine cultures; though, as we shall see, there is great variation as to the extent and depth of this.

The growths on agar differ considerably in small details, but they agree in being more or less yellow, and in failing at temperatures above 25°C .

On potato also, while great differences were observed as to the depth of colour and vigour of growth, all agree in forming some shade of yellow, and in refusing for the most part to grow above 25°C .

In broth again, with differences in detail they agree in not growing above 25° , and in forming a shimmering turbidity and more or less yellow deposit.

Similar resemblances in milk. No peptonization or, as a rule, marked coagulation occurs, but a faint acid reaction occurs in all.

It is for these reasons that I propose to unite all these forms under a common type, which for further reasons which will come out as we proceed, I term the *Yellow Proteus* type.

In order to make clear the details to follow, I begin with

the description of No. 23, a form studied very carefully and for long periods—I have kept it under continuous observation for some months—with interesting results.

No. 23: B. radiatus (Zimm.). Figs. 36-42.

This form is very common in the river at all seasons apparently.

It occurs on the separation-plates as slightly motile rods $2-6\mu \times$ about 0.6μ . After three months in culture the plate-colonies gave similar measurements. No success was obtained with Gram's method, though it stains readily by ordinary methods, and impression preparations are remarkably beautiful.

Plates at $12-15^{\circ}$ gave ochre-yellow colonies, more or less regularly circular, and often surrounded by a sort of yellow halo (Fig. 36). The central part opens out like a star, and the clouds around are found to consist of floating or 'wandering islets' of much coiled and curved filaments or series of rodlets in a zone of liquefaction.

Plates at 20° : in two days there were circular bluish-grey clouds 10-15 mm. diameter, but so tenuous as to be nearly invisible. Under $\frac{1}{3}$ these give the appearance of central tresses breaking up into flocculent granular masses. Liquefies. (Fig. 37.)

Plates at $18-21^{\circ}$ in forty hours show the characteristic bluish-grey clouds, circular and very indistinct, which under the $\frac{1}{3}$ exhibit coils and tresses radiating like mycelial strands from a dumb-bell-like or spider-like centre, with serpentine tresses running about: the whole hardly yellowish. On the third day the circles are about 5 mm. diameter, and pale ochre with bluish margin; granular, opaque and zoned, and beginning to run and sink. The centre opens out into reticulated or sieve-like clouds of granules; the margin has radial tresses like striae. All is composed of tresses of filaments falling into cloudy aggregates of rodlets (Fig. 37 *d* and *e*). On the fourth day liquefaction is commencing, and most of the

colonies are irregular cloudy aggregates; others still preserve the zoned and sieve-like appearance to some extent. Colour more yellow and colonies sinking or running. In a week the circular colony (15 mm. diameter) is composed of bright ochre cloudy masses floating in the lens of liquefaction, and this is surrounded by a nearly colourless zone of radiating tresses in the still solid gelatine, and with floating 'islands' on the surface around.

The most extraordinary feature in all these colonies, however, is the formation of the primary tresses—the serpentine central tresses referred to above. These are in reality compact zoogloea-masses composed of numerous twisted tresses, the walls of which swell and hold the whole together, forming the curious dumb-bell-shaped or jointed or serpentine masses referred to. The thinner and thinner tresses which radiate out from these become more and more tenuous as the filaments remain isolated and break up into rows of segments or free Bacilli.

Later on one finds that many of these at first almost free filaments can also become intertwined to serpentine zoogloea-masses, and the Figs. [36–38] give examples of the various ways in which this can come about: it appears to depend on the submergence in the gelatine.

The explanation of this phenomenon in detail is difficult, but there can be no doubt that these zoogloea-tresses are the same as those figured by Hauser in his monograph on 'Fäulnisbakterien,' as seen in the various forms of *Proteus*, and as those described on pp. 207–214 above.

The following observations throw more light on this subject.

A rodlet isolated in 5 per cent. gelatine was fixed at 20° under the $\frac{1}{12}$ immersion at 9.20 a.m. (Fig. 39 *a*). At 11 o'clock it had grown to nearly three times its original length, and divided once (Fig. 39 *b*). At 11.50 each of the two rodlets had slipped a little past the other, and one of them had divided again (*c*). At 12.30 there were four rodlets, two still in connexion, the other two free (*d*). At 5 p.m. a small

elongated colony of about 20–25 rodlets (perhaps more behind; it was impossible to count accurately) had formed, and some were slowly wandering off (Fig. 39 *e*). At 10 p.m. the initiation of a colony as a beautiful double-fan or constricted bundle of parallel or intertwining filaments breaking up into rodlets was established (*f*), and next morning this appeared as an extremely tenuous cloud of slowly wandering rodlets, filaments and tresses of the types Figs. 36 and 37 *b*. Some of these filaments intertwine to curious serpentine zoogloea-tresses, like those shown in Fig. 36 *b*, the frayed ends of which go on emitting rodlets (Fig. 37 *e*).

Tracing the colony of No. 23 further in gelatine it begins as coloured mycelium-like tresses, radiating offshoots in all directions from the one or more denser coiled and serpentine central zoogloea-tresses, and in forty-eight hours the whole of the circular and extremely tenuous cloud-like colony is built up of the radiating strands branching from the central zoogloea-mass (Fig. 37 *b*).

On the third day in 10 per cent. gelatine the marginal strands become closely serried, and radiate like flames (see Fig. 37 *d*); or if the surface of the gelatine is suitable, these marginal filaments grow out on the moist surface as coiled islets, or compact contoured films.

In this way the different marginal zones shown in Figs. 36–38 are produced, the last-mentioned thin filmy borders (e. g. Fig. 37 *d*) being very iridescent.

The central portion of the colony breaks up into cloudy masses arranged more or less like a network, and very often this more or less membranous part looks just like a sieve, fine or coarse, as the case may be (Fig. 38). The differences in colour, iridescence, zoning, &c., are entirely due to the rate of liquefaction and attendant pigmentation, the amount of flowing of the contoured margin, and the mode of breaking up of the tresses into radiating spokes, networks, sieves, or mosaics, according to the distribution of the clouds of segments into which they disintegrate.

The behaviour of the colonies of No. 23 in 5 per cent.

gelatine is suggestive. Plates kept at 15–18° C., for forty hours, showed the characteristic clouds consisting of the fimbriated mycelia with snake-like zoogloea-tresses as centres. On putting the plate under the Zeiss C, in Sachs' box at 20° C., the characteristic swarming islets described above (p. 209) were seen on the surface and their movements traced, an example being given in Fig. 40, where the changes are recorded at intervals of one minute. These islets, which are composed of the slender rods or coiled filaments, closely arranged side by side, are seen to change shape, advance and retreat, form new connexions or break away from old ones, in a manner so suggestive of the movements of an *Amoeba* that one might almost take them for something of the kind. They are exactly as Hauser describes them. The whole plate is liquid in five to six days. One point of difference between Hauser's *Proteus* and this one is that the movements *do* occur on the moist surface of 10 per cent. gelatine, though very much more slowly than on 5 per cent. gelatine.

Stab-cultures at 12–15° C. begin with the development of yellow dots in the tunnel and a film above.

In five days the latter is sinking, and is a circular yellow colony, spreading like a yellow star from a central eye; the dots in the tunnel are larger. The colour is brighter, more decidedly yellow than No. 7, but they are much alike. In three weeks about one-tenth of the gelatine is liquid, turbid, and with yellow floating flocks, and a yellow deposit on the flat floor: the tunnel is somewhat wider. In two months half the gelatine is liquefied, and an ochre deposit lies on the flat floor below the turbid liquor. Almost the only distinction between this and No. 7, is the floating yellowish flocks, forming an imperfect veil.

At 18–21° a bluish-white circular film and cloudiness all round the axis in forty-eight hours, and sinking just beginning at the yellow eye (Fig. 41). In three days the faint cloudiness pervades the upper part of the gelatine, and a cloudy yellowish film above covers all; liquefaction supervenes by the fifth day, no cloudiness is left, and the clear liquid in

a thin layer extends to the sides, with a pale-yellow cloudy mass above; the axis being hardly visible—faded away.

At 24° C. it rapidly liquefies above in 48–60 hours, with yellow-white clouds.

Streak at 15° C. In forty-eight hours a scooped grove, along which the mass slides as a viscid yellow and whitish mass. Fimbriated thin edges to the groove, like No. 7. On the third day the colour is ochre to orange, very like No. 7. In fourteen days the resemblances to Nos. 7 and 17 are pronounced: turbid liquid (half liquefied), slight veil, flecks, and abundant orange-yellow deposit—rather paler than in No. 7. In twenty-two days, four-fifths is liquid, turbid, and with an ochre deposit. This deposit consists of cocci and rods up to 6 μ long. Floating flecks. Submerged in gelatine it will not grow, indicating that it is aërobic.

Agar at 34° C. No signs of growth.

At 25° C. slow growth. In five days an abundant dirty-white deposit, and a smooth, thin, nearly white film on the agar. This becomes yellowish-white, and the colour and thicker growth alone distinguish it from No. 7.

At 22° the growth is somewhat better. A pale yellowish, cloudy and iridescent film spreads in three days, and a yellowish-white deposit forms. The filmy edges of the growth are diffuse and very iridescent, greenish: the drainage becomes densely buff-white, turbid. When scraped with a needle the growth is dirty-white.

At 12–15° on agar a wet yellowish or dirty-white streak forms in two days, with the edges fimbriated. It seems to grow more rapidly than at 25° C. This becomes a dirty-white shining gum-like layer all over. No growth under anaërobic conditions in fourteen days at 22–25° C.

The growth on agar is decidedly thicker and whiter than either No. 7 or No. 17, but otherwise similar, and the curious faint iridescence with slight tinges of green to pink in various lights is marked. The abundant deposit is flocculent, nearly white. The broad streak-layer has thin jagged edges: colour yellowish, but far paler than No. 7 in eight days.

Potato. At 34° no growth. At 25° a yellow, thin, diffuse watery patch is formed in two or three days; this remains pale—hardly visible as more than wetness in places—and in a week has scarcely spread.

At 22° the growth was thicker and eventually more orange and wax-like: in a week it resembled the corresponding growth of No. 7, but more ochre-yellow in hue, spread, and bright like wet gum.

Broth at 25° C. Slightly turbid in forty-eight hours. In a week hardly turbid, but an abundant flocculent yellowish-white deposit has fallen. On the ninth day there is a flocculent incomplete veil, and falling flocks. In a month the floating veil is easily shaken down, and the greyer colour of the deposit distinguishes it from No. 7.

No growth at 35° , but at 18° it is as good as at 25° .

The old cultures turn the broth sherry-colour, but the deposit remains greyish-ochre, not orange as in No. 7.

Milk at 25° C. No change observed for three weeks, when the reaction was acid. In a month there are doubtful traces of clotting, but no true separation occurs.

Glucose at 25° C. No results.

Not pathogenic to guinea-pigs according to Dr. Kanthack's report.

No. 23 was revived in June from an agar-culture thirteen months old, which had remained quiescent from May 23 to June 24 of the following year. It came up on the plates very white and rapidly liquefied on the fourth day at 20° . The spider-like zoogloea-masses, characteristic fringed edges, and flame-like films on the surface of the gelatine suggested the type, and further cultures confirmed its identity, including the yellow colour.

The *Stab-cultures* gave a thistle-head funnel, very slowly liquefying at 20° , but in all other respects to type.

Streak-cultures, side by side with the stab, gave rapidly scooping streaks, and in five days an abundant ochre growth and deposit formed in the liquefying gelatine.

On *agar* and *potato* the cultures were normal; except that

on potato the very deep orange waxy appearance was developed forthwith at 25° C. In broth the growth stops below 35° C.

In spite of minor variations there could be no doubt of the identity, and the characters obtained undoubtedly strengthen the resemblances between this form and No. 7.

If Zimmermann's *B. radiatus*¹ is compared with my No. 23, it will be found that the coincidences are so numerous that they point to identity. He gives the thickness as about 0.65 μ , the rods being slightly motile. He notes the whitish-bluish-grey colour, and a 'root-like' central mass; the branching to finer and finer twigs of this root-like central mass (my central zoogloea-tresses) and the general resemblance to a mycelium. Most of his details, including liquefaction on the fourth day, the yellow colour, and radiate bundles, come into accord with my description, the slight discrepancies being easily referable to differences in growth.

The stab-cultures will also bear close comparison, though we have each employed different modes of expressing the similar events.

The same is true for the agar-cultures, and we have both noticed the greenish hue. On potato also, Zimmermann's 'ochre-yellow, often tending to red-brown' seems not inconsistent with my 'eventually more orange.'

Similarly with the broth-cultures, he also notes the falling flecks and yellowish-white deposit. The temperature and other conditions, and even the failures with Gram's method, agree, and the whole impression I get from Zimmermann's description leads to the conviction that we are both working with a *Proteus* form, either identical or closely allied and belonging to the same type.

No. 7: *B. radiatus* (Zimm.). Figs. 43-45.

A peculiarly indistinct colony frequently made its appearance on the isolation plates, like a faint mycelium sunk in the gelatine. This was found to consist of radiating rows

¹ Zimmermann, l. c., p. 58.

of rods, each about $3-4\ \mu$ long by 0.5 or $0.6\ \mu$ thick, in series. Each rod $4\ \mu$ long seemed to consist of four segments, so that we may take the unit-rodlet as about $1 \times 0.5-0.6\ \mu$. Old gelatine-cultures give cocci.

Plate-cultures at $12-15^{\circ}$ yielded variable colonies of the mycelioid and branched zoogloea- and arborescent forms (Figs. 43 and 44) so common in *B. arborescens* and its allies, and comparable with those of No. 23. After being in culture some months, plates at $18-21^{\circ}$ C. showed the tenuous bluish, hardly visible, circular clouds (like No. 23) in forty-eight hours, and these on the third day are about 5 mm. diameter. On the fourth day 8-10 mm., very tenuous and cloudy, and with offsets like nebulae around (Fig. 43 *a*).

Under the $\frac{1}{3}$ any group of these offsets is seen to be composed of extremely fine, curved, reticulated and branching colourless tresses, radiating from serpentine, yellowish, central, stouter, curved and branched zoogloea-tresses (Fig. 43 *b*). On the fifth day the colonies are 15-20 mm. in diameter, very grey and cloudy, and spreading into the gelatine, which softens. On the sixth day the ochre-yellow colony opens into sieve-like mottled nets, with thin contoured iridescent margins (like No. 23).

After passage through broth, the cultures at 20° (see Figs.) go through the serpentine zoogloea stage, to dendritic and mycelium-like forms, exactly as in *B. arborescens*, and in six days liquefy and become more and more ochre-yellow. These colonies fall to pieces and float before the sieve-like stage is reached.

Stab-cultures at $12-15^{\circ}$ showed in two or three days as a yellowish or ochre film, spreading like a spider's web, or like irregular stars over the surface, while ochre dots appeared in the tunnel (Fig. 45). The gelatine liquefies, and in a week the yellow film has sunk, one-tenth of the gelatine being liquefied and somewhat turbid, and an ochre-yellow precipitate lying on the flat floor. Growth is very slow in the tunnel, cloudy masses occupying its narrow calibre. In two months half the tube is liquefied, and a bright orange deposit lies on

the flat floor, under a nearly clear yellow liquid. The liquefaction does not quite reach the bottom of the still slender tunnel, but it gradually extends to the bottom of the tube in the course of three or four months.

At 18–20° the thin film above is rapidly developed in forty-eight hours, with a yellow liquefying centre, and the axis is surrounded with a pale cloudiness as in No. 23 under the same conditions. On the third day yellow cloudy liquefaction sets in above, and cloudiness throughout the solid gelatine, exactly as in No. 23; and so also on the fifth day, i.e. the axis fades, and a watery layer with yellowish cloudy masses occurs all over the top.

At 24° rapid liquefaction as a cloudy yellowish mass above, and clouds in the gelatine.

Streak at 15° C. Rather rapid ochre-yellow streak, scooping in forty-eight hours and with a delicate fringed filmy border to the scooped channel. The orange mass slides down into the viscid liquid at base. In fourteen days nearly all is liquid, bright orange deposit and a turbid liquid exactly like No. 17, but with no veil. All liquid in twenty-two days: deep orange deposit.

At 23° the behaviour is almost exactly the same—rapid scooping of a channel off which the mass runs, leaving a thin fringe of radially arranged film attached to the gelatine. On the third day the action is somewhat slower than at 15°, but in longer cultures (fourteen days) it is a little more rapid: otherwise exactly the same. All liquid in twenty-two days, and deep orange deposit, consisting of cocci only (examined in water).

Agar at 34° C., showed no growth.

At 25° the growth is also slow and thin. In seven days a scarcely visible film, smooth, glistening and transparent, and only visible owing to its faint iridescence, and yellow colour when viewed edge on: a yellowish deposit in the drainage. Even in fourteen days the layer does not thicken, but on passing the loop over one collects a yellow mass.

At 22° a similar thin orange film grew, and orange deposit.

In about a week the glassy layer can be made out to be orange-yellow, and the deposit is deep orange. Older cultures, kept a month or more at 12–15°, show a more distinct growth—still thin, but more opaque and evident, and scraping off as a thin orange paste.

After being three or four months in culture, this form became so weak that it hardly grew at all on agar at 15°, 25°, or 30° if infected from agar; if, on the other hand, infected from old gelatine-cultures it grew very well at 15° as a glassy film to type.

The failures are not due to aëration; no growth was got in anaërobic conditions in fourteen days at 22–25° C.

After cultures through broth and again isolated on gelatine, however, the agar-growths at 18–21° were as good as ever: yellow gummy layers in forty-eight hours with pale lilac iridescence and spreading all over. This lilac iridescence is sometimes very marked about the fourth day. In eight days the lilac tinge has gone, the thickish layer is ochre-yellow, gum-like with jagged thin edges, and deposit yellow. Much more yellow than No. 23.

On *Potato* at 22° C. it often refuses to grow, but in some cases a slight golden-yellow patch forms, and in a few cases this became a dull orange fairly thick patch in seven days. It does not spread, and becomes dull, dry and wax-like. At 25° the best growths were very slight, pale golden-yellow, hardly extending beyond the streak. No growth could be got on potato at 34° C.

Broth at 25° C. Slightly turbid in forty-eight hours. In a week a slight turbidity and faint ochre deposit. In a month the same shimmering turbidity and orange deposit. No growth at 35°, but at 18° it is as good as at 25°. The colour of the deposit deepens to red-orange, and the broth becomes sherry-colour.

Milk at 25° showed no signs in fourteen days, but traces of separation were observed on the fifteenth, and in three weeks a stiff clot had formed; the reaction faintly acid. In a month all the casein was down as a coagulum below a straw-coloured

liquid; but no signs of peptonization could be detected in six weeks.

Glucose at 25°. No results beyond a very faint shimmer about the ninth day, and an extremely slight, hardly white deposit.

No. 7 was found by Dr. Kanthack to be non-pathogenic to guinea-pigs.

No. 7 was revived from an agar-tube thirteen months old (May 28 to June 14 of following year), but it was so weak¹ that I could not get the plate-colonies beyond the stage of golden-yellow circular colonies of the λ type—minute points not more than 0.5 mm. diameter just sinking into the non-liquefied gelatine in fourteen days, at 20°, and then growing no larger; liquefaction never took place.

It certainly appears that here we have a weakened liquefying form becoming non-liquefying. If we compare No. 7 with No. 23 the following points of difference come out.

No. 7 breaks up to shorter rodlets. In the plate-colonies there are slight differences of colour and rate of liquefaction and pigmentation only.

In stab-cultures also the differences are chiefly as to depth of colour, and No. 23 forms a veil.

Agar-cultures of No. 7 differ little in colour and thickness, and it appeared somewhat weaker, and this feebleness appeared on potato also.

But revival in broth was easy at first. In broth No. 23 differs by its veil and greyer deposit.

In milk No. 7 slowly coagulated but did not peptonize, and in this is distinct from No. 23.

These differences can hardly be insisted on as distinctive, and I therefore regard No. 7 as the same as No. 23, and both as identical with Zimmermann's *B. radiatus*.

I have already referred to the connexion between these forms and the *Proteus* of Hauser, and this is strengthened by the evident preference for peptones and broth as against

¹ i.e. I infer it is weakened, by its behaviour in gelatine, and because no attempts to invigorate it by broth-cultures succeeded.

carbo-hydrates. As we shall see, also, several other varieties exist which behave similarly.

No. 31 : Yellow Proteus Type. Figs. 46–48.

This form was often met with in the Thames. It occurs on the plates as rods $2-4\mu \times 0.5$ or 0.6μ , or as coiled filaments of rodlets $2-3\mu$ long and about 0.7μ (in water) thick. Other measurements give $2-6 \times 0.5$ to 0.6μ .

Plates at $12-15^{\circ}$ give white circles with star-like central portion, opening out to arborescent or sheaf-like branched forms, with 'wandering islets' and rapidly liquefying.

At $18-21^{\circ}\text{C}$., the colonies appear on the fourth or fifth day as translucent, circular, contoured, colourless or bluish fronds with a yellowish central boss; the edges extremely tenuous, and the central parts varying to spidery and contoured figures. In a week the colour becomes pale-ochre, and liquefaction begins; the colonies 10 mm. or so in diameter forming flocculent mottled and flame-like masses in the liquefied depression. All the stages from the contoured type (Fig. 47 *a*) to the forms with mosaic-mottled clouds are to be traced (Figs. 46, 47, and 48).

Plate-cultures after passage through broth show in twenty-four hours as spider-like colonies. In forty-eight hours all stages of the type are seen: the submerged yellowish ovals and the spidery-like zoogloes with tails. They emerge as thin contoured plates, colourless, and with wandering ringlets (see Figs. 47 *b* and *c*). On the fourth day they open out as pale ochre sieves, like No. 23, and liquefy (Fig. 48).

Stab-cultures at $12-15^{\circ}$. A sieve-like flecked yellowish-white membrane grows above, and floats in a week on the softening gelatine as a clouded patch: yellowish dots in the tunnel. About the ninth day the dirty-white to yellow-ochre cloudy growth is sinking, and in a fortnight it resembles No. 23. In two months half the tube is liquefied, rather turbid, and has a floating flecked veil.

At $18-21^{\circ}$, in forty-eight hours a yellowish-white small

sinking spot above, and dots in tunnel. No cloudiness. On the third day a shallow funnel of liquefaction above: no change in tunnel. Thistle-head in five days, white flocculent mass; axis with yellowish-white dots, sword-shaped.

At 24° also much slower than Nos. 7 and 23.

Streak at 15°—very slow, merely shows bluish-white in forty-eight hours.

At 23° also slow: mere streak in forty-eight hours.

Agar at 34°. No growth. At 25° a thin, smooth, yellowish-white layer, pinkish in some lights, covers the agar in five days, and an abundant yellowish-white deposit falls. It is like No. 23.

At 22° rapidly forms a thin, glassy, yellowish-white film with a greenish cast. In three days the fimbriated edges are remarkably iridescent: yellowish precipitate in the slightly turbid drainage. In four days, a greenish-white glistening layer—the green sheen only in some lights.

The layer, when scraped off with a loop, is dirty-white.

Exactly the same difficulties as with No. 7 occur as regards old agar-growths losing power. No growth under anaërobic conditions in fourteen days at 22–25°C.

At 18–21° a greenish-white flecked layer with pale lilac iridescence in some lights, rapidly spreads in forty-eight hours. The curious faintly marbled or flecked appearance was sometimes marked about the fourth day; it has a slight iridescence of greenish and lilac. In eight days, much like No. 23, but edges less jagged, and green to lilac iridescence still marked. Yellow-white deposit.

Potato at 30°C. No growth. At 25° a greyish film in twenty-four hours becoming drier and not spreading. The growths on potato at 22° varied considerably according as the medium was alkaline or not. On ordinary potato a pale ochre patch formed in forty-eight hours, and this spread but little, as a dry small ochre streak with a whitish rim: on alkaline potato a thin gummy ochre film formed in forty-eight hours, and in three days this was flowing all over, with lilac margins. This pink-lilac hue spreads as the

yellow, wet, flowing mass runs over the potato, and in a week the general tone is more and more golden-yellow, displacing the ochre and the pink. All wet and gummy-looking.

Broth at 25° C. Slightly turbid in twenty-four hours. In a week a faint white ring, slight turbidity, and abundant yellowish-white deposit. In nine days a dense white free veil, no ring and the liquid turbid. Resembles No. 23. In old cultures the liquor is sherry-colour and the deposit pale ochre.

Milk at 25° C. No visible alteration in three weeks, but the liquid is acid. In a month the casein is partially separated.

Glucose at 25° C. No result.

Dr. Kanthack found it was not pathogenic for guinea-pigs. I found it impossible to revive No. 31 from agar-tubes a year old.

On comparing No. 31 with No. 23, the following are the only differences to be noted.

The sieve-like film on the surface of stab-cultures—not essentially different in type from the stellate film of No. 23.

The very slow streak-cultures at both 15° and 23° C.

The pink hue on potato-cultures is a difference, though it did not persist. Here again we cannot insist on the differences, though they appear in parallel cultures, and I therefore place No. 31, with Nos. 23 and 7, as mere varieties of the same form—*B. radiatus* (Zimm.) and belonging to the *Proteus* group.

No. 17: B. ochraceus (Zimm.). Figs. 49–53.

Morphology &c. Composed of very short rods $1 \times 0.5 \mu$ in rows as stained. Fresh preparations show pairs of rodlets 2×0.6 to 0.7μ , or long series, i.e. filaments, segmented into rods about $1 \times 0.6 \mu$. Old gelatine-cultures give cocci about 0.6×0.5 to rods $2-1 \times 0.6 \mu$, all quiescent: old agar-cultures give all cocci or very short rodlets about $1 \times 0.7 \mu$.

On plates at 12–15° very small yellow colonies, which in three to four days float in a viscous liquid, as circular, yellow,

granular firm dots, pale and very transparent under the $\frac{1}{3}$. These are apt to stick to the glass (Fig. 49).

On re-sowing and obtaining less crowded plates, the colonies form yellow-eyed, sinking circular colonies, with spider-like or dumb-bell-shaped zoogloea-mass in centre, and floating islets around by the end of a week. Most of the surrounding clouds or islets are spider-like zoogloea (Fig. 50).

In three weeks the gelatine is softened, and the colonies pale yellow. Sour smell. Plates of No. 17 after being some months in culture give, at 18–21°, colonies just visible in forty hours, under $\frac{1}{3}$ these are moruloid, or spiky, or spider-like zoogloea-forms, yellowish.

On the third day (see Figs. 50 and 51), while most of the colonies are as above, but larger, others emerge as plates 1 mm. diameter with spider-like zoogloea in the centre. On the fourth and fifth days the small yellow dots present similar features, with much variation in details, and the yellow colour is more pronounced; some are still moruloid or gland-like, and submerged; others are cloudy centres with outlying nebulae, and consist of much branched, dendritic, zoogloea-tresses; others are contoured plates as before, with zoogloea in the centre. In the course of two or three days more liquefaction sets in, and the floating cloud-like colonies are yellow with a deep chrome-yellow central eye, reminding one of a weak form of No. 35.

The film is more frondose at the edges, however, and sinking begins about the fourteenth day, and a yellow frondose floating mass remained above, like egg-yolk.

Stab-cultures at 12–15°. Begins by forming an extremely thin yellow film above, and pale yellow dots in the tunnel in three days. In six days the yellow centre is surrounded by a bluish or pale purple iridescent border: slimy and viscous to the needle.

At 18–21° a slight sinking yellowish spot is formed above in forty-eight hours, and short root-hairs along the axis (Fig. 52 *a*). Small funnel in three days. In five days rings around the axis may replace the root-hairs (Fig. 52 *b*).

At 24° rapid spread above and root-hairs along the axis, producing a cloudy appearance all the way down the latter.

In two months the liquefaction has proceeded one-fourth down the tube, the viscid liquid being slightly cloudy. All else as in the type, except the floating yolk-like scum.

Streak at 23°. A very slight yellow streak shows in forty-eight hours. On the third day it is sending pale-yellow clouds into the gelatine, i. e. sinking, but is still only a narrow wet streak. In a fortnight nearly all is liquid, and evenly turbid. Thin yellow floating veil, and orange deposit. In nineteen days all the liquid is nearly clear. Deep orange deposit, of quiescent cocci, and floating flecks composed of rods up to 6–10 μ long.

Agar. No growth at 34–35° C. But at 22° there slowly forms an extremely thin pale-yellow, shining, gum-like film, all over the surface in three to five days, with a slight deposit in the nearly clear drainage, and with bluish iridescence at the margins. The growth stops sharp at the clear drainage. In five weeks the ochre layer is little thicker, but the liquid is turbid and has a copious deposit.

At 25° the faint, filmy, nearly white streak formed in twenty-four hours, becomes a thin canary-yellow smooth layer, and floating flecks and a yellow-white deposit form in the turbid liquor in a week. The growth was better from old gelatine (Fig. 53).

At 18° C., the agar-cultures were exactly like No. 23, except that the amount of deposit in the drainage (in eight days) was much smaller.

Potato at 22°. Extremely slow; in a fortnight only a small ochre patch, duller than No. 18 and far slower.

Broth at 25°, infected from agar, was shimmering in twenty-four hours. And in fourteen days was exactly like No. 18, with a yellow deposit at the base of the turbid liquor. No growth at 35°, but equally good at 18° and 25°. The deposit turns more orange-yellow and the liquor is of a clear sherry-colour: the orange hue is far less deep than in No. 7, however.

Milk at 25°, infected from agar, gave no results in fourteen days beyond acid reaction, and a *trace* of perceptibly yellow deposit.

Glucose at 25°, infected from agar, gave doubtful shimmering in twenty-four hours. In fourteen days no further results, except a very pale ochre deposit as in the type.

No. 17 was found by Dr. Kanthack to be non-pathogenic for guinea-pigs.

All attempts to revive No. 17, from cultures a year old, failed.

If we compare Zimmermann's *B. ochraceus* with No. 17, the resemblances come out strongly. In sizes and measurements they agree very well, and the capsule-like envelope he found might well be due to the zoogloea investment.

Zimmermann observed movements, 'slow and pendulous,' however, but could detect no cilia. His comparison of the warted young colonies to *Viel-beinigen Gliederthiere* when the prominences become marked, is very suggestive, and taken with the other characters points to strong resemblance, if not identity.

The only essential difference in the stab-cultures is that a floating yolk-like mass not mentioned by him is formed in my No. 17.

On agar the general resemblances are obvious, but in my cultures the growth was much stronger, while on potato they seem alike, and the same may be said of the broth-cultures. As regards temperature and air-requirements, the slow growth and slow liquefaction, failure to obtain spores, and general characters of the yellow pigment, the two descriptions agree.

I think it extremely likely therefore that my No. 17 is identical with Zimmermann's *B. ochraceus*.

If No. 17 is compared with No. 23, the following differences are noteworthy.

The rodlets are much shorter, and even break up to cocci. But we must not overlook the fact that equally short rodlets and cocci occur in No. 7. On crowded plates liquefaction was rapid, and old cultures have a sour smell. On the whole

the type of plate-colony is the same, but the zoogloeoas are more condensed, and the colour more golden or chrome-yellow than No. 23.

The chief differences in stab-cultures were the root-hairs in early stages, and the deep chrome-yellow yolk-like veil; the streak-cultures also liquefied more rapidly.

The agar-cultures were also much brighter yellow in hue, but the growth on potato was feebler than the type.

In broth, the brighter colour and absence of veil distinguish it.

It seems likely, in view of all we know, that the more condensed growth on the plates and the deeper colour go hand in hand. If this is so, there may be no sufficient reason for removing No. 17 from the *Proteus* type. I have already shown that No. 17 and Zimmermann's *B. ochraceus* are closely allied, and they must go together.

That these forms are closely allied to Zimmermann's *B. radiatus*—see Nos. 23 and 7 above—will scarcely be denied; and this being so, the position of all these forms in the *Proteus* type seems justified.

***B. arborescens* (Frankl.). Figs. 54–62.**

This form is by no means rare in the Thames, and was described by Frankland in 1889¹, but it is evidently merely a form-variety of the group we are considering, as the following study of it shows.

It exhibits very neatly the connexion between the liquefying and non-liquefying conditions, and I have no doubt it can be modified much more than I have succeeded in modifying it. There can be little hesitation in connecting it with Zimmermann's *B. radiatus* and *B. ochraceus* on the one hand, and with the *Proteus* type and allies on the other.

Habitat. Fairly common in the Thames, especially in the winter.

Morph. Non-motile rods, about 5 or 6 μ long by 0.5–0.6 μ broad, often in pairs, or associated in irregular chains. No traces of spore-formation observed.

¹ Zeitschr. f. Hygiene, B. VI, p. 379.

Plate. Extremely variable according to the conditions. The colonies begin as yellowish granular disks or ellipses. These grow out radially or at the ends into branched zoogloea and fimbriated tufts, or may elongate into snake-like zoogloea-forms branching at the two ends: thus, according to conditions, there are formed in from two to four days, circular, dumb-bell-shaped, irregularly branched arachnoid or serpentine zoogloea-colonies, ochre-yellow in colour, and running into delicate filaments at the margins or ends, which ramify copiously (Figs. 54-57). To the unaided eye these colonies appear as delicate, mould-like, faintly iridescent growths, or as more obvious, ochre-yellow, somewhat zoned, oval or round colonies. At low temperatures, especially when the plates are exposed to light, the emerging colonies grow very slowly as thin, frondose, typhoid-like, irregular plates, marked with striae, and almost colourless, except at the thicker centre, where there is an irregular yellowish mass (Fig. 62): the submerged colonies under such conditions are dull yellow, very irregular in outline, and marked with constrictions. The mycelium-like, more delicate and branched form (Fig. 56) rapidly liquefies the gelatine completely in 24-48 hours, the dumb-bell-shaped and zoned circular colonies (Fig. 55) only liquefy slowly and incompletely after four or five days. The frondose plates (Fig. 62) show no signs of liquefaction even after a week.

Streak. At 15° C., an ochre-yellow, streaky and flecked growth is formed, which slowly liquefies the gelatine to a glairy fluid. In ten days half the tube is liquefied to a viscid fluid, in which a yellow deposit forms.

Stab. In two days at 15° C., a thistle-head funnel of liquefaction is formed, with greyish dots in the tunnel, and an ochre-yellow somewhat stellate mass floating on the liquid above. As the funnel approaches the sides of the tube and deepens (four to five days) the colonies in the tunnel show slightly yellow, and ochraceous flecks are deposited at the bottom of the liquefied gelatine above. In two to three weeks the gelatine is liquefied one-eighth down, a thick yellow deposit forming below the liquefied portion. (Fig. 58; cf. also Fig. 45.)

Agar. In twenty-four hours at 20° C., the streak is yellow, with thin, glistening, frondose spreading margins, which are whiter and slightly iridescent. In three days (Fig. 59) the broad and more frondose expansion is a brighter ochre-yellow, thicker, glistening, with delicately fimbriated, thin, irregular, iridescent margins.

Potato. In twenty-four hours, at 20° C., forms a deep golden-chrome spot. On the third day the colour is still richer, the surface waxy, but rather dull, growing slowly. In seven days a thick waxy mass, red-orange, especially in the centre, surface rather dull, margins striated (Fig. 60).

Broth. Slight turbidity in twenty hours, at 20° C., and this increases during the second and third days. On the fourth day a yellow deposit is formed, but no veil above. In ten days or so the turbidity diminishes and a copious yellow deposit has formed below.

Milk. Slowly peptonized, without previous coagulation.

Requirements as to air. Aërobic.

Temperature. Grows slowly at 10° C., fairly rapidly at 15° C., more so at 20° C.

Liquefaction. Sometimes hardly, or even not at all observable. In other cases fairly rapid.

B. arborescens shows obvious resemblances both to No. 23 and to No. 17, and the forms allied to them, and there can be little doubt as to the propriety of placing it here. But the principal interest attaching to *B. arborescens* is the experimental proof of the extreme variability of the colonies according to conditions.

When the temperature is very low, or the *Bacillus* has been exposed to unfavourable conditions for some time, e.g. exposed to light at 10–12°, or in very old gelatine-tubes, its feeble growth is expressed by slowly emerging as transparent, contoured, thin plates, like a feeble colony of *B. coli-commune*, only that one can still recognize the dense spider-like or star-fish-like zoogloea in the depth (Fig. 62). The fine contours are due to the much curved parallel filaments on the surface of the gelatine (cf. Fig. 11): these lie closely side by side and remain so because no liquefaction occurs, and the segments are therefore held in place flat on the surface of the gelatine.

When the plates are exposed to sunlight for three or four hours, at ordinary temperatures, taking care that the gelatine does not melt; or if the sowings are made from tubes of water exposed to the sun; the type of colony produced is more like

that of No. 23, i. e. slowly growing circular colonies with an ochre-yellow or leather-yellow central spot, and sometimes a zone of the same colour, and with very evident radial structure. On magnifying this, one finds densely branching and interwoven filaments radiating and crossing one another from a central zoogloea of the arborescent or serpentine type. The extreme tips of the irregularly radiating strands are very thin, and give the fimbriated appearance shown in Fig. 57 (cf. Fig. 37). These colonies slowly liquefy the gelatine, and each lies for some time in its own lenticular circle of liquefaction.

When, on the other hand, the colonies are developed from Bacilli in full vigour, e. g. after passing through broth and not exposed to light, they appear in forty-eight hours or so as serpentine zoogloea with thin fimbriated branches from the ends, and at once liquefy the gelatine throughout, in the manner typical for a *Proteus*, without developing into formed visible colonies. This is because the Bacilli separate off and wander all over, and liquefaction is so vigorous that no condensed growths are formed after the first (Fig. 56, and cf. Fig. 44).

There is no doubt that in these experiments the form of colony is dependent on the conditions to which the Bacilli have been exposed, because the latter have their powers of liquefaction, and therefore of nutrition and growth, affected. Unfortunately it is impossible to so arrange matters that only one factor at a time is varied in the environment, and the experimental proof of the action of each individual factor is therefore wanting. We have here, however, ample proof that it is important to keep the conditions as constant as possible in plate-cultures.

Sanfelice¹ had already suggested that *B. arborescens* is a variety of Hauser's *Proteus*, as is also *B. aquatilis* of the same authors. My studies of the yellow liquefying forms which I have classed as Group IX in my fifth report to the Royal Society², suggest that Zimmermann's *B. fulvus*,

¹ Lustig, l. c., p. 85.

² Roy. Soc. Proceedings, Vol. lxi, p. 419.

B. subflavus and *B. ochraceus* are also connected by this form to the true *Proteus* type.

Having regard now to all the facts, there can be little doubt that my Nos. 7, 23, and 31 are allied to the *Proteus* type; and if we admit this, and bear in mind that they all become yellow as the cultures progress, the same is true of Nos. 17, K, and *B. arborescens*, in spite of their assumption of the yellow pigments at so early a date in their development. My own conviction is that we have in Hauser's three 'species' of *Proteus*, and in my six forms 7, 17, 23, 31, K, and *B. arborescens* a set of varieties of the same species, the definition of which rests on their agreement in size and form, mode of growth, formation of zoogloeoas and tresses, method of liquefaction, requirements as to temperature and so on, but that they vary in detail as to rapidity of growth and liquefaction, and consequent differences in the extent and appearance of the colonies, and as to the intensity of pigmentation, which may well be assumed to vary with the above.

But if this is so, we shall have to go further, and to regard Frankland's *B. aquatilis* as belonging to the same series—it may well be a merely weaker variety of his *B. arborescens*—and Zimmermann's *B. radiatus* also, for this is almost certainly identical with my No. 23.

The most serious difficulty here is as regards No. 17, for it shows unquestionable resemblances to Zimmermann's *B. ochraceus*. If these are also identical, then we shall have to consider the question of a possible (indeed probable) alliance between this *Proteus* group and the whole of the golden-yellow liquefying forms which I have brought together as Group IX.

The following varietal forms of this type may be sufficiently characterized by diagnosis as below.

Varieties K and (B). Fig. 63.

Habitat. In the Thames in 1893-4.

Morph. Slender inactive rods 3 to 4 μ $\times$ 0.75 μ .

Plate. Circular, radiated and zoned colonies with a yellow eye : arachnoid. Soon fail.

Streak. Soon sinks and forms yellow-chrome flocks. In a month half the gelatine liquefied with an ochre-yellow deposit.

Stab. Rapid in twenty-four hours, cloudy along the axis. In fourteen days no growth visible on the surface, but 'root-hairs,' cloudy and mycelium-like processes round the axis. In a month the same, but with slender cloudy outshoots into the gelatine.

Agar. Merest film or nothing appears up to the fifth day, and hardly any certain growth in three weeks. In a month a very slight buff-whitish deposit forms in the drainage : no streak visible.

Potato. Nothing visible for certain in three days at 20° : in a week a thin, yellowish, filmy streak, nothing further.

Broth. Faintly turbid on the third day, but others show nothing till the seventh day, when a faint turbidity and white deposit are seen. In a month slightly turbid, and a brittle slight veil and ring, and faint yellowish deposit. Others show a doubtful ring.

Milk. No change in a month visible, though perhaps slightly acid in twenty days.

Glucose. No results.

This appears to be an enfeebled form of this type, and it soon failed in the cultures.

EXPLANATION OF FIGURES IN PLATES XII, XIII, AND XIV.

Illustrating Professor Ward's paper on Thames Bacteria.

PLATE XII.

Group VI. The *Proteus* Type.

Number 103.

Fig. 1. Plate-colonies twenty four-hours at 20° C. Natural size.

Fig. 2. A small colony under the $\frac{1}{8}$, showing zoogloeas and floating islands.

Fig. 3. Young colonies from a plate-culture twenty hours at 20° , showing the origin of the coils, tresses, zoogloeas and islands.

Fig. 4. Cocci from a gelatine-culture three weeks old, $\frac{1}{12}$ imm.

Fig. 5. Streak-gelatine-culture at 20° : *a* after two days ; *b* three weeks.

Fig. 6. A two-days' stab-culture at 20° C., showing 'root-hairs,' and moss-like film above.

Fig. 7. Agar-culture twenty-four hours old at 25° C.

Number 34.

Fig. 8. Plate-colonies at the ordinary temperatures ($15-18^{\circ}\text{C.}$): *a* in ninety hours, *b* in 115 hours, under the $\frac{1}{3}$. In both cases the smaller colonies are submerged.

Fig. 9. The same in sixteen days, at beginning of liquefaction.

Fig. 10. Stab-cultures at $15-18^{\circ}\text{C.}$, fourteen days.

Fig. 11. Plate-colonies of strong cultures at 20°C. : *a* on the second day; *b* on the third day; both under $\frac{1}{3}$, natural size. *c* and *d* two of the colonies on the fifth day, under the $\frac{1}{3}$.

Number 51.

Fig. 12. Colonies of No. 51 in forty-eight hours: *a* natural size, showing the almost invisible tenuous film *b* and *c* under the $\frac{1}{3}$; in *b* the surrounding film was too tenuous to see; in *c* only part of the margin is shown.

Fig. 13. A plate-colony of the largest size on the second day at 20°C. Natural size; the tenuous film just visible.

Fig. 14. A stab-culture after a week at 18°C.

Fig. 15. A stab-culture, more vigorous, after three days at 18°C.

Fig. 16. Successive divisions of a rodlet isolated in gelatine: *a* at 11.5 a.m.; *b* 11.50; *c* 1.0 p.m.; *d* 1.50; *e* 2.40; *f* 3.30; *g* 6.15. (See text, p. 206.)

Fig. 17. Outlines of the pseudopodial films from a two-days' plate at 19°C. : *a* to *e* drawn at intervals of one minute. Under $\frac{1}{3}$.

Fig. 18. Similar series at intervals of $\frac{1}{2}$ to one minute, $\frac{1}{3}$.

Fig. 19. Similar series: *a* at 8.4; *b* at 8.8; *c* at 8.9; *d* at 8.10, $\frac{1}{3}$.

PLATE XIII.

Fig. 20. Similar series: *a* at 8.20; *a* to *h* at intervals of one minute; *i* at 8.39; the rest at intervals of a minute. The arrows mark the direction of rotation, $\frac{1}{3}$.

Fig. 21. Culture under E/4 in a hanging drop of gelatine, at $16-21^{\circ}\text{C.}$: *a* the isolated rodlet at 11 a.m.; *b* progeny at 4 p.m.; *c* at 8 p.m.; *d*. at 9.30. The resulting colony measured $10 \times 8 \mu$. At 11.10 it measured $12 \times 10 \mu$; at 8 a.m. next day $34 \times 32 \mu$; at 9.30 it was a sphere 36μ diameter; at 10.55 = 44μ , and so on. See text, p. 211. At 3.30 it was a sphere too large to measure, and *e, f, g* show the further changes under the $\frac{1}{3}$; *e* 3.30; *f* 6 p.m.; *g* 8.40 p.m.

Fig. 22. Details of movement of the filaments: *a-c* under $\frac{1}{12}$ imm.; *d* under E/4.

Fig. 23. Rods, filaments and zoogloea-clumps from advanced liquefying colony, $\frac{1}{12}$ imm.

Number 9.

Fig. 24. Plate-colonies of No. 9 at $15-18^{\circ}\text{C.}$: *a* on the third day under $\frac{1}{3}$; *b* on fourth day, natural size; *c* the same under $\frac{1}{3}$; *d* on the fifth day, natural size.

Fig. 25. Stab-cultures at $15-18^{\circ}\text{C.}$: *a* in forty-eight hours; *b* on the fifth day; *c* on eleventh day.

Fig. 26. No. 9 *a*. Plate-colonies as isolated: *a* in ninety hours, submerged under $\frac{1}{3}$; *b* in 115 hours, natural size; *c* the same magnified.

Variety ι.

Fig. 27. A colony under the $\frac{1}{3}$ as seen on isolation plate.

Fig. 28. Plate-colonies under the $\frac{1}{3}$: *a* in ninety hours; *b* in 115 hours.

Variety φ.

Fig. 29. A large colony as first isolated. Natural size.

Fig. 30. Plate-colonies: *a* in 140 hours; *b* in ten days.

Variety ζ.

Fig. 31. Plate-colonies at 12–18° C.: *a* in forty-two hours; *b* in ninety hours; *c* in 115 hours; *d* on sixth day. In *a*, *b* and *c* the small colonies are submerged. In *d* the small colony is natural size.

Fig. 32. From a ten-days' plate at ordinary temperature: *a* submerged under $\frac{1}{3}$; *b* emerging (natural size); *c* the same under $\frac{1}{3}$.

Fig. 33. From a six-days' plate: *a* submerged under $\frac{1}{3}$; *b* emerged, natural size.

Variety μ.

Fig. 34. A plate-colony as isolated.

Variety θ.

Fig. 35. A two-days' plate-colony. Natural size.

Group VII. The Yellow Proteus Type.

Number 23.

Fig. 36. Plate-colonies of No. 23 on a five-days' culture at 12–15° C., showing the great variety in type: *a*, *c*, *d* natural size; *b* part of the tenuous margin of *a* under the $\frac{1}{3}$.

Fig. 37. Plate-colonies at 20° C.: *a* two of the very tenuous colonies after two days; *b* one of them under the $\frac{1}{3}$; *c* colonies on the third day, natural size; *d* part of the margin of a colony; *e* one of the strands in *d* more highly magnified.

Fig. 38. Plate-colonies on third day under a lens to show structure, at 20° C.

Fig. 39. Culture in five per cent. gelatine-drop, $\frac{1}{12}$ imm.: *a* at 9.20; *b* at 11.0; *c* at 11.50; *d* at 12.30; *e* at 5.0; *f* at 10 p.m. The rods feebly motile. See p. 227.

PLATE XIV.

Fig. 40. Swarming islets drawn at intervals of one minute, in five per cent. gelatine; order = *a-g*.

Fig. 41. Stab-cultures of No. 23 at 18–21° C.: *a* in forty-eight hours; *b* in a week.

Fig. 42. Plate-colonies from a nine-days' culture after lying dormant a year: *a* natural size; *b* under $\frac{1}{3}$.

Number 7.

Fig. 43. Plate-colonies of No. 7 on the fourth day at 18–20° C.: *a* natural size; *b* one of the outlying groups under the $\frac{1}{3}$.

Fig. 44. Colonies from a forty-eight-hours' culture-plate under the $\frac{1}{3}$.

Fig. 45. Stab-culture four days at ordinary temperature.

Number 31.

- Fig. 46. Colonies from a six-days' plate at 18–21° C., natural size.
 Fig. 47. Similar colonies under $\frac{1}{3}$ from a forty-eight hours' plate : *a* emerged ; *b* submerged ; *c* 'floating islands' more highly magnified.
 Fig. 48. A colony from an eight-days' plate, natural size.

Number 17.

- Fig. 49. Plate-colonies of No. 17 at 18–21° C., for three days : *a* natural size ; *b* an emerged colony under $\frac{1}{3}$; *c* submerged colony under $\frac{1}{3}$.
 Fig. 50. The same on the fifth day : *a* an emerged colony, natural size ; *b* a part of one of its marginal outlyers ; *c* a submerged colony under $\frac{1}{3}$.
 Fig. 51. A colony twelve days at 20° C., natural size, liquefying.
 Fig. 52. Stab-cultures at 18–21° C. : *a* on third day ; *b* on fifth day.
 Fig. 53. Agar-culture one month at 25° C.

B. arborescens.

- Fig. 54. A typical plate-colony of *B. arborescens* isolated from the river, two days at ordinary temperatures (15–17° C.) : *a* natural size ; *b* under the $\frac{1}{3}$.
 Fig. 55. *a* A slowly grown plate-colony on sixth day under $\frac{1}{3}$; *b* another colony on sixth day, and *c* the same on eighth day, both under $\frac{1}{3}$.
 Fig. 56. Quickly grown colony in dark at 20° C., forty-eight hours, under $\frac{1}{3}$. In this condition they rapidly liquefy the plate.
 Fig. 57. Colonies retarded by light, four days old : *a* natural size ; *b* under $\frac{1}{3}$.
 Fig. 58. Stab-cultures at 15° C. : *a* and *b* after two days ; *c* fifth day ; *d* three weeks.
 Fig. 59. Agar-culture three days at 20° C.
 Fig. 60. Potato-culture seven days at 20° C.
 Fig. 61. Rodlets from gelatine-cultures.
 Fig. 62. Colonies from a seven-days' gelatine-plate in the dark at low temperatures (6–12° C.), showing retardation and complete change of form.
 Fig. 63. Plate-colonies of variety (B) as isolated : *a* natural size ; *b* the same under $\frac{1}{3}$; *c* natural size ; *d* the same under $\frac{1}{3}$.

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