On a Red Pigment-forming Organism, B. corallinus (?).

By

Charles Slater, M.B.(Cantab.).

With Plate XXXI.

THE number of micro-organisms known which form red pigments is already considerable, and include the M. prodigiosus, B. indicus, B. ruber, and B. rouge de Kiel. The organism described below differs from any of these in morphology, cultural characteristics, and tint of pigment produced. It occurred as a coral-red, slow-growing, circular, non-liquefactive colony on a gelatine plate which had been used in an examination of the tap water of the laboratory. As the plate had been withdrawn from the moist chambers and examined at least once before the colony appeared, it is impossible to say whether it was derived from the water or was an air contamination. It was most probably the latter.

The colony was found to consist of short, thick bacilli with very rounded ends. Their breadth, which is very constant, is about 1 μ, and the average length of the individual cells from 2 to 3 μ. Very frequently two cells are joined end to end, but it is unusual for more than two fully formed cells to remain in apposition. The organism is motile, the movements being of a rolling recurving character, with but slow motion of translation, except in the case of certain apparently young short cells. The character of the motion recalls that of B. megaterium, but is somewhat more active. The apparent curving and

straightening movements seem to be due to the rolling motion of a slightly curved organism round its longitudinal axis.

By far the most noticeable characteristic is the highly refringent nature of the poles of the cells. This refringence is noticed in a very large proportion of the cells of a culture examined at any stage of growth, and will be again referred to.

On gelatine peptone growth takes place easily, though not very rapidly, and does not produce liquefaction of the medium. The growth forms a regular, raised, moist-looking, somewhat glistening streak on the surface of the gelatine, and is frequently surrounded by a white opalescence due to a deposit of oxalate of calcium. The colour is pinkish or coral red, and though this tint deepens during the first week the coloured colonies are not preceded by any definite uncoloured stage, as is the case in the Prodigiosus, &c.

In a stab culture the growth takes place very scantily along the needle track, and remains colourless. On the upper surface the growth spreads from the point of inoculation, and colours well. The organism is distinctly aërobic.

On agar the growth is a little slower, and is rather more vermilion in colour. The addition of carbohydrates to the agar seems to make no difference in either the rapidity or colour of the growth. In the case of the B. rouge de Kiel, M. Laurent (Ann. Inst. Past., iv, 465) has shown that the colour is not produced in the presence of carbohydrates. The addition of glycerine to the medium is, however, inhibitory to the growth of the bacillus now described. At the most there is a slight pink growth at the margin of the fluid which always collects at the bottom of these tubes.

In liquid media the growth is never copious, and the colour extremely ill-developed. The media tried were ordinary bouillon, bouillon with carbohydrates, and Pasteur's fluid with and without sugar. The addition of sugar both to the bouillon and the Pasteur's fluid certainly increases the growth and assists the colour formation. The growth in these media tends to collect at the edge of the fluid and to attach itself to

any extraneous body, and forms delicate, slightly resistant, gelatinous films. The organism in these films does not appear to have any distinct morphological peculiarities. There is a decided tendency to the formation of a gelatinous material round the cells in the cultures on solid matter, especially on potato.

On potato the growth is copious and the colour well developed. The organism forms a raised irregular waxy-looking coating with a bright reflecting surface. The growth is gelatinous, adherent to the surface of the potato, and the colour is much the strongest in the superficial layers. A greyish blue discoloration of the potato occurs round the growth, appearing early but subsequent to the development of the red pigment, and obviously connected with the growth of the organism. The potato acquires within the zone of grey pigment a slight alkaline reaction, but no distinct odour is developed, as in the case of M. prodigiosus. In old potato tube cultures the pigment becomes a pale chocolate-brown.

Temperature.—The organism grows well at the ordinary temperature, and has its optimum between 20° and 23°. Above this the growth is slower, and at 37° ceases, but the cultures grow rapidly again on removal to a lower temperature. Exposure for one hour to a temperature of 60° kills the organism. Simple drying at 37° on a cover-glass does not impair the vitality.

Pigment.—The pigment in this organism is largely contained in the cells, and is not an excretion. It cannot be extracted by simple shaking with water, ether, alcohol, or chloroform, but requires to be first liberated by disruption of the cells by boiling. It is set free by boiling in water, but is not dissolved. Alcohol and chloroform dissolve it easily, but ether does not. It has not been obtained in a crystalline form. When acted on by alkalies it is turned a yellowish brown, and this is probably the reason of the change of colour in old potato cultures. Acids restore the colour affected by alkalies, but do not themselves cause any change in the original pigment. With the spectroscope no absorption bands could be detected,

the reddish solution simply cutting off the blue end of the spectrum.

The production of the pigment is unaffected by light, taking place as well in the dark as in diffused light.

The pigment-producing power is remarkably constant, and in no case have any colourless colonies been obtained when free access of air is possible. In stab cultures, and in the depths of liquid media, the colour is absent.

If recent cultures of the organism on gelatine or potato are examined, the growth is found to consist chiefly of cells scarcely twice as long as broad, and so rounded at the ends as to appear oval. These small cells are actively motile, and the protoplasm appears to be collected as a refringent mass at each pole. These cells are not unfrequently united in pairs, connected by a thin filament. The bicellular organisms are actively motile, and present the appearance of a flagellated organism. They are especially noticeable in bouillon cultures. Besides those two forms which seem to be characteristic of young and active growth, there occur cells which are three to four times as long as broad, some showing signs of division into the bicellular stage, and others no trace of this separation.

The organism increases by fission, which is somewhat slow, a cell dividing at the temperature of 15° C. in about twelve hours. The most noticeable feature in this organism at whatever stage examined is, as mentioned above, the irregular refractive power of its protoplasm, and its tendency to collect in masses, having various positions in the organism, but very frequently to be found at the poles of the cells. Stained specimens present, also, a very great variety of appearances, owing to their extremely irregular staining. A stained preparation of this bacillus recalls, very strongly, a culture of the Bacillus typhosus when in the condition which shows the "clear space" and the so-called spores, and the appearances presented by this pigment-forming organism are not without interest when compared with those of the pathogenic microbe. Certain cells show, both when stained and unstained, caps of

protoplasm, with an intermediate hyaline scarcely stained cell body. These resemble the clear space cells of the B. typhosus. In other cells the refractive mass is gathered into a distinct rounded spore-like mass at each pole, and in stained preparations a very common appearance is that represented in fig. 3, where a strongly stained central mass is flanked by two oval unstained bodies, apparently corresponding to the refringent masses, and having the appearance of terminal spores.

Büchner has shown that the refringent terminal mass, described as a spore in the case of B. typhosus, was really a collection of protoplasm, and stained deeply, and did not correspond to the unstained oval space in stained specimens. Similarly, by direct staining on the slide, it may be seen that the rounded refractive polar masses are protoplasmic and stain deeply, and that the two forms *a* and *b* in figs. 1 and 2 are not corresponding stained and unstained appearances, but the forms *a* and *c*. That the oval unstained spaces seen in fig. 3 are not spores is indicated by their rather irregular shapes and somewhat faint outline, which suggest rather the space left by the withdrawal of the central protoplasm from that part of the cell. Further, no attempts at differential staining or any of the usual methods of spore staining have been successful, and no free spores can be demonstrated in the cultures. The cultures containing these forms are sterilised by one hour's heating to 60° C.

Regarding the irregularity of staining as due to irregularities in the distribution of the cell contents, the question arises as to whether the forms noticed are produced artificially in preparation, or are the expression of normal changes in the cells, or are the results of degenerative processes. That the various forms are not produced artificially is shown by the fact that similar results are obtained whether the specimens are fixed by heat, alcohol, or simply drying, and that a parallel irregularity is seen in fresh specimens, and in preparations made by staining directly on the slide without fixation.

Bütschli has advanced the theory that the cell contents of bacteria represent the nuclei of other cells, while the protoplasm is reduced to an extremely thin layer, in many cases only represented by the cell wall. He bases his views ('Bütschli ueber den Bau der Bacterien, etc.,' Leipzig, 1890) on the study of the large flagellated organisms found in sulphur waters, which he shows to possess an internal cell substance, having the structure and staining properties of nuclei, and containing the granules of Ehrlich. The refractive portions of the organism described in this paper are strongly stained by the ordinary nuclear stains, such as alkaline aniline dyes, and especially logwood. On this view the forms which have been described as occurring in young freely growing cultures, viz. the short oval cells with refractive poles occurring either singly or in pairs, would apparently be the forms resulting from direct division, and the polar collections of protoplasm would represent the direct division of the nucleus. The successive stages of this division would be represented (fig. 4, *a*) by—1, the cell, with refringent poles ; 2, a form resembling a large diplococcus, stained throughout, and formed by division of stage 1—this form occurs but rarely; 3, a bicellular organism found by the growth of stage 2. In this third stage the cell stains at first equally throughout, but soon, either before the cells separate or soon afterwards, the protoplasm collects at the poles, i. e. the nucleus divides directly, and stage 1 is reproduced. The division of the cell in stage 1, and the formation of stage 3, have been directly observed.

Dr. Delépine, who kindly examined some of the specimens, suggested that some of the other appearances were due to karyokinesis, and, having regard to the columnar form of the organism and its small size, various forms may be distinguished which would represent the formation of a central plate and its subsequent division and gradual separation. These forms are represented in fig. 4, *b*. Starting with a cell, whose contents stain equally and moderately, these contents representing a nucleus, there is a gradual gathering of the chromatin until an intensely staining central plate is formed with unstained

poles. The whole cell is at the same time larger. The central chromatin then shows signs of division, and the two halves gradually separate and travel away from one another towards the poles. Division of the cell takes place between the two halves, and the chromatin is once more equally distributed over the cell. The appearance of constriction in fixed and stained specimens at the central plate is partly artificial, as in organisms examined in a drop culture containing methylene blue the outer border of the cell may be made out, preserving its original breadth until the division is about to take place. It has already been mentioned that the B. typhosus presents similar appearances to the above, and Babes ('Soc. Anatomique,' December, 1884) figures and describes very similar forms occurring in the comma bacillus of Koch. He states that at first the poles of the cells are deeply stained, but as the organism grows the deeply staining portions pass to the centre and become fused; and that then a clear space forms, dividing the central mass into two parts, which indicates the commencing cell division.

This organism, when grown under somewhat unfavourable conditions, readily shows involution forms. When grown on gelatine with scanty access of air the cells become three to four times as long as broad without, in many instances, dividing, and after some days become vacuolated. These vacuoles are generally three in number, and symmetrically arranged. This vacuolation explains many of the appearances seen in the stained specimens. The cultures in fluid media, especially in the depths and in sugar-containing fluids, show long, almost leptothrix, forms, and there is a great tendency to the formation of bud-like projections, which in some instances are so prolonged as to resemble branchings. The protoplasm in these forms also presents irregularities of distribution.

The following is a short list of the principal red pigment-forming bacilli, with their differential characteristics:

Name.	*Character of Cells.*	*Culture on Gelatine.*
M. prodigiosus .	Oval and round cells ·5 to 1 μ, also has a bacillus form. Motile.	Liquefies gelatine quickly.
B. ruber (Frank) .	Bacilli from 6 to 8 μ long, by 1 μ broad. Very motile.	Liquefies gelatine.
B. rouge de Kiel (Breunig)	Bacilli 2·5 to 5 μ long, by 7 to 8 μ broad. Motile.	Liquefies gelatine, which is rapidly coloured throughout.
B. indicus (Koch)	Short cells with rounded ends.	Liquefies gelatine.
B. granulatus roseus (Babes)	Straight rods 3 to 4 μ broad, 5 to 6 times as long.	Does not liquefy gelatine.
B. corallinus . .	Oval cells and rods 1 μ broad, 2 to 3 μ long.	Does not liquefy gelatine.

DESCRIPTION OF PLATE XXXI,

Illustrating Mr. Charles Slater's paper "On a Red Pigment-forming Organism, B. corallinus."

FIG. 1.—Organism unstained.

FIG. 2.—Organism stained alkaline methylene blue.

FIG. 3.—Organism stained logwood, showing apparent spore-like bodies.

FIG. 4.—(*a*) Stages indicating direct division. (*b*) Forms possibly due to karyokinesis.

FIG. 5.—Budding and branched forms occurring in liquid media.

FIG. 6.—Vacuolated forms unstained.

FIG. 7.—Involution forms—torula-like forms.

FIG. 8.—Organism from fluid, sugar-containing medium; stained.

FIG. 9.—Organism growing in gelatine medium.

FIG. 10.—The same colonies twenty-four hours later, showing growth and cell division.

Figs. 9 and 10 are not so highly magnified as the others.

FIG. 11.—Potato culture.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 11.

Fig. 9.

Fig. 10.