(5) The active principle lessens the hyperglycæmia induced by oral administration of glucose in partially depancreatised dogs.

(6) The probability that the substance is an insular hormone is discussed.

REFERENCES.

Bainbridge and Beddard (1906). 'Biochem. J.,' vol. 1, p. 429.
Bayliss and Starling (1902). 'J. Physiol.,' vol. 28, p. 225.
Dixon and Wadia (1926). 'Brit. Med. J.,' vol. 1, p. 820.
Heller (1929). 'Arch. exp. Path. Pharmak.,' vol. 145, p. 343.
Heller (1931). 'Wien. klin. Wschr.,' vol. 44, p. 476.
Ivy and Fisher (1924). 'Amer. J. Physiol.,' vol. 67, p. 445.
Laughton and Macallum (1930). 'Can. Med. Assoc. J.,' vol. 23, p. 348.
Laughton and Macallum *et al* (1931). 'J. Biol. Chem.,' vol. 92, Proceedings 20.
Macallum, A. B., Sr. (1929). 'Can. Med. Ass. J.,' vol. 20, p. 445.
Mellanby, J. (1928). 'J. Physiol.,' vol. 66, p. 1.
Moore, Eadie and Abram (1906). 'Biochem. J.,' vol. 1, p. 28.
Monroe (1906). "Manual of Medicine," London, p. 241.
Pickardt and Pierce (1930). 'J. Amer. Med. Assoc.,' vol. 94, p. 1134.
Still and Shpiner (1929). 'Amer. J. Physiol.,' vol. 91, p. 496.

---

576 . 851 . 4B . malvacearum : 576 . 809 . 56

The Morphology and Cytology of Bacterium malvacearum, *E.F.S. Part* II.—*Reproduction and Cell-Fusion.*

By R. H. Stoughton, B.Sc., A.R.C.S., Department of Mycology, Rothamsted Experimental Station, Harpenden.

(Communicated by Sir John Russell, F.R.S.—Received February 2, 1932.)

[Plates 1 and 2.]

In an earlier paper (Stoughton, 1929) an account was given of certain observations on the morphological and cytological changes undergone by *Bacterium* (*Pseudomonas*) *malvacearum*, E.F.S., the causal organism of the angular leaf-spot disease of cotton plants.

A central, deeply-staining body was demonstrated in the bacterium, and by means of a special staining technique the changes through which it passes were traced. The structure divides simultaneously with the division of the cell-body and the method of this division was described. Further evidence was

Downloaded from http://royalsocietypublishing.org/rspb/article-pdf/111/769/46/132115/rspb.1932.0043.pdf
by Rothamsted Research Limited user
on 19 February 2026

adduced tending to show that this body is of the nature of a true bacterial nucleus or, alternatively, a nucleus embedded in a matrix of "chromatic" material. The formation and liberation of very small deeply-staining bodies, which appear to be identical with the "gonidia" of other workers, were described.

An account was also given of the method of production of spherical coccus-like bodies, formed by a process analogous to the budding of yeasts, which appear to constitute a method of vegetative reproduction not previously described for the organism. At the time the previous paper was written the subsequent development of these "cocci" had not been traced, but further work has thrown light on this point.

Throughout the work a single strain of *B. malvacearum* of proved virulence has been used. The strain has been kept in a state of assured purity by constant re-plating and occasional re-starting from a single cell isolated by means of the Dickinson micro-isolator (1926). The cultures have been grown throughout on potato-extract agar containing 1 per cent. of sucrose and their virulence maintained by frequent passage through cotton plants. All cultures were kept for at least 1 week in an incubator at 25° C. and then transferred to an unheated storage chamber.

The technique employed in preparing the slides was given in the previous paper, but further work with the method has shown certain points worthy of note. A common source of failure has been the use of too much dye solution in preparing the film of stain. Experience alone will show how much stain is necessary for a good result with any particular organism or culture, but the amount will always be small. The film should be barely perceptible when held to the light, and the final preparation should show the nuclear-like bodies strongly stained and the outer membrane distinct, but the remainder of the cell almost unstained. A second source of failure is the use of too thick a water-film. Using cover-glasses of 2 cm. diameter a loopful of water from a 2 mm. platinum loop just fills the space between the cover and the slide without excess, forming a film approximately 5–10 microns thick. One other point is that slides prepared by this method are in no sense really permanent, but remain good for about 2 days only. After this period granulation of the contents of the cells occurs and entirely misleading appearances may be seen.

For routine examination of the slides a high-power water-immersion objective is a convenience as the covers are not soiled and, if necessary, the slides may be examined repeatedly during the day or two that they remain good. Such an objective is also more suited to the examination of objects mounted in a watery

Downloaded from http://royalsocietypublishing.org/rspb/article-pdf/111/769/46/132115/rspb.1932.0043.pdf
by Rothamsted Research Limited user
on 19 February 2026

fluid than an oil-immersion lens. For photography, or for critical examination of the finer details an objective with higher aperture and consequent greater resolving power is needed. In this case better results are obtained with the organisms which are in contact with the cover-glass than with those lying on the slide and viewed through the water-film. The latter interferes with the corrections of the objective, though some improvement may be effected by suitable extension of the tube-length. If, however, the film exceeds about 10 μ in thickness, a satisfactory image of the organisms on the surface of the slide cannot be obtained with an oil-immersion lens. The presence of the water-film reduces the working aperture in any case to a maximum of about 1·3, but this is sufficient for a clear resolution of the main details.

Life-cycle of the Cocci.

Certain stages in the production of the coccoid bodies were described in the previous paper, but fuller details of the earlier stages and of the subsequent development have now been obtained. Figs. 1–3, Plate 1, show the first stages in the formation of the bud, before the division of the nucleus in the parent cell. Fig. 4 on the same plate shows the beginning of this division, the later stages of which were given in figs. 3 and 4, Plate 26, of the previous paper. Owing to the optical limitations discussed in the earlier paper and the considerations referred to above, the minute details of the division cannot be finally determined. The process appears to be a " pinching in two " of the " chromatin " material more or less coincidently with the abscission of the neck joining the coccus to the parent rod. In fig. 5, Plate 1, the coccoid body has attained its full size and is on the point of liberation. The coccus now becomes free in the medium, and in a suitable culture large numbers of the free cocci can be seen, each with its single deeply-stained nucleus-like body. These coccus forms are extremely thin-walled, especially while still growing attached to the parent cell, and in consequence are very easily distorted or destroyed. This fact explains the failure to see the structures in dried-film preparations. Even in a well-stained wet-film preparation critical optical conditions are essential for a clear picture of the formation.

After an interval, as yet undetermined, the cocci germinate. A small papilla appears at one point and this grows out into a rod, apparently identical with the normal vegetative cell, figs. 6, 7 and 8, Plate 1. So far, the cytological changes associated with the germination have not been determined. In this stage the cell appears to contain a large amount of food-material which stains rapidly and deeply, the dense stain rendering it difficult to make out the

Downloaded from http://royalsocietypublishing.org/rspb/article-pdf/111/769/46/132115/rspb.1932.0043.pdf
by Rothamsted Research Limited user
on 19 February 2026

structure. The behaviour is similar in this respect to that of normal vegetative cells from a very young culture, where, as noted in the previous paper, the dense staining rapidly obscures the internal structures.

The development of these bodies seems, therefore, to follow a closed cycle comparable with the vegetative spore-cycle of the lower fungi.

Cell-fusion and "Zygospore" Formation.

In the previous paper reference was made to the occurrence in old cultures (3–6 weeks or more) of characteristic "angled" forms, consisting of two cells apparently united at one end and forming an obtuse angle to one another, fig. 9, Plate 1. It was suggested at that time that this formation might possibly represent an incomplete but more or less normal vegetative division, but further observations have failed to confirm this, and indicate rather that the appearance represents the first stage in the fusion of two independent cells uniting by their extreme ends. A number of forms have been repeatedly observed which fit into a series interpretable as stages in the production of a fusion-cell or "zygospore," and which are difficult to explain on any other basis.

At times the pairs are united by an unmistakable bridge or neck of variable length, fig. 9, Plate 1, but in most cases an obvious tube is not present, and the connection appears to be formed by a breaking-down of the wall of each cell at the point of contact. Here a small swelling appears, very similar in its early stages to the coccoid bodies previously described, fig. 10, Plate 2. This protuberance is at first very thin-walled, and stains only lightly, but at once begins to thicken its walls; the contents become denser and more deeply staining, while, usually, the parent cells become distinctly less dense. The newly-formed body attains a diameter equal to, or rather greater than, the width of the parent cells, figs. 12, 13, 14 and 15, Plate 2. The whole structure stains deeply at this stage, having, as a rule, considerably more affinity for the stain than the vegetative rods. Here again the cytological processes are difficult to determine. In the early stages, where the cells are joined at the tip, the two "nuclear" bodies of the joined cells lie near the point of junction, fig. 10, Plate 2, but in the later stages it is often possible to see the "nuclei" in the middle of each of the subtending cells, fig. 12, Plate 2.

The subsequent history of these spherical spore-like bodies has not yet been determined with certainty. The frequent appearance of two unequal "arms" in the fully-formed structure, fig. 16, Plate 2, where one "arm" seems to be

Downloaded from http://royalsocietypublishing.org/rspb/article-pdf/111/769/46/132115/rspb.1932.0043.pdf
by Rothamsted Research Limited user
on 19 February 2026

undergoing a process of degeneration, suggests that after the structure has attained its full size the parent cells shrivel and fall off. This inequality of the " arms " was at first thought to be due to the production of the spore-like body from the fusion of two cells of originally different sizes, but in all cases so far observed, when the " fusion-cell " is not full-grown, the subtending cells are equal in size, indicating that the difference is due to subsequent unequal shrivelling of the parent cells. In fig. 15, *b*, Plate 2, the parent cells are shrivelling simultaneously. The " zygospores " when free are similar in size and shape to the vegetatively-produced " cocci," but may in some preparations be distinguished by their affinity for the stain. The fusion-bodies during the course of their development acquire a power of staining strongly and appear as dense spherical cells in which no structure is visible, while the cocci usually stain much more lightly, and this distinction is maintained after liberation. When the coccoid bodies germinate, however, they also become easily and deeply stained. There appears therefore to be no way of determining by the observation of stained preparations alone whether the fusion-cells or " zygospores " subsequently germinate, since no culture containing " zygospores " but free from cocci has yet been obtained, although the latter may occur in the absence of the former.

Bodies similar to these zygospore-like forms have been observed in other plant-pathogenic bacteria, but their development in these cases has not at present been worked out in any detail. A striking example of such formation is given in fig. 18, Plate 2, which is taken from a culture sent as *B. stewarti* to the writer by Dr. A. J. Riker, of the University of Wisconsin. Several of the zygospore-like bodies are shown in one field. In these the subtending cells are nearly empty of stainable material although, in each, one very small granule, which may represent the nucleus, is discernible.

Many of the appearances observed in *B. malvacearum* closely resemble Mellon's figures of " zygospores " in *B. coli* (Mellon, 1925, 1926, 1927). More recently Stapp and Zycha (1931) have observed similar appearances in dried-film preparations of *B. mycoides*, but have interpreted them as artefacts produced by gross overstaining. That the bodies described for *B. malvacearum* cannot be so considered, has been demonstrated by their recognition in preparations of the living, unstained organisms. Three photomicrographs of such unstained cells are shown in figs. 11 and 17, Plate 2, under dark-ground illumination. Fig. 11 shows the early stage of " zygospore " formation immediately after fusion, while in fig. 17 the body is fully formed. The latter photograph shows the highly refractive nature of the mature " spore," which

Downloaded from http://royalsocietypublishing.org/rspb/article-pdf/111/769/46/132115/rspb.1932.0043.pdf
by Rothamsted Research Limited user
on 19 February 2026

is associated with its strong affinity for the stain in the wet-film preparations. Immature bodies are less refractive than the parent-cells, fig. 11, *b*, Plate 2, a fact which again agrees with the staining capacity.

Further evidence that these zygospore-like bodies are not artefacts is afforded by preparations made by the standard protozoological methods. Fig. 13, Plate 2, is taken from a cover-glass preparation fixed while still wet in hot Schaudinn's fluid, stained by Heidenhain's iron-hæmatoxylin process and mounted in balsam. The appearance is essentially the same as with the wet-film method.

Numerous attempts have been made to watch the various processes occurring under continuous microscopic observation. The difficulties inherent in such work were discussed in the previous paper, and they are reinforced in this case by the time factor involved in the germination of the cocci or "zygospores." Different methods have been tried, including the preparation of minute hanging drops by the use of the Chambers micro-manipulator, the observation of thin liquid films under dark-ground illumination, the hanging-block method, and the agar-film method (Stoughton, 1929). Partial success only has attended these experiments, growth of the rod from germinating cocci having been observed during a few hours. In nearly all cases where the conditions are such that growth can take place, the multiplication of the ordinary rods is so rapid that they soon overgrow the single cell under observation. Further, any interpretations placed on even apparently successful observations should be accepted with great caution, since the optical conditions under which such observations must be made, involving very considerable reduction in the numerical aperture of the illuminating cone, favour the production of false images. The exception would be observation under dark-ground illumination of high obliquity but, as pointed out in the previous paper, this method seems impossible of application in the case of *B. malvacearum*, which requires free access of air for growth.

Summary.

(1) Using a technique described in a previous paper, new morphological forms have been observed in *Bacterium malvacearum*.

(2) The production of coccoid bodies, their liberation, and subsequent germination to form apparently normal rods, are described.

(3) The formation of densely-staining spherical bodies, apparently arising from the point of fusion of two cells, is described. These bodies are apparently liberated by the degeneration of the parent cell.

Downloaded from http://royalsocietypublishing.org/rspb/article-pdf/111/769/46/132115/rspb.1932.0043.pdf
by Rothamsted Research Limited user
on 19 February 2026

EXPLANATION OF PLATES.

Plate 1.

Figs. 1–5.—4-12 week cultures of *Bacterium malvacearum*, showing stages in the formation of the coccoid reproductive body. Wet-film preparations. Photographed on panchromatic plates with green filter. Leitz 2 mm. fluorite objective N.A. 1·32. × 10 "periplanatic" ocular, Leitz aplanatic condenser, N.A. 1·40. × 2000.

Figs. 6–8.—Stages in the germination of the "cocci" to normal rods. Figs. 6 and 7 photographed with Reichert 1/12-inch achromatic objective, other details as figs. 1–5. × 1600. Fig. 8 as figs. 1–5. × 2000.

Fig. 9.—4-week culture showing early stage of fusion. Photographic details as figs. 1–5. × 2000.

Plate 2.

Fig. 10.—4-week culture showing early stage of formation of zygospore. Photographic details as Plate 1, figs. 1–5. × 2000.

Fig. 11.—5-week culture mounted in sterile water. Photographed on panchromatic plate with green filter. Watson 1/12-inch achromatic objective with funnel stop (N.A. 0·95 approx.), × 10 "periplanatic" ocular, Leitz dark-ground illuminator. (*a*) × 1400, (*b*) × 2200.

Fig. 12.—4-8 week cultures showing formation of zygospores. Photographic details as Plate 1, figs. 1–5. × 2000.

Fig. 13.—4-week culture fixed in Schaudinn's fluid, stained iron-hæmatoxylin. Photographic details as Plate 1, figs. 1–5, *a* and *b*. × 2000.

Fig. 14.—8-week culture showing nearly mature zygospore. Photographic details as Plate 1, figs. 1–5. × 2000.

Fig. 15.—7-week culture showing (*a*) mature zygospore, (*b*) mature zygospore with parent cells beginning to degenerate. Photographic details as Plate 1, figs. 1–5. × 2000.

Fig. 16.—4-week culture showing mature zygospore and degeneration of parent cells. Photographic details as Plate 1, figs. 1–5. × 2000.

Fig. 17.—5-week culture in sterile water. Mature zygospores. Unstained, living forms, dark-ground illumination. Photographic details as fig. 2. × 2200.

Fig. 18.—2-week culture of *B. stewarti* with mature zygospores. × 1500.

N.B.—All preparations are made by the wet-film process except fig. 13, Plate 2, which is a fixed preparation mounted in balsam, and the dark-ground figures, which are water-film preparations unstained.

REFERENCES.

Dickinson, S. (1926). 'Ann. Bot.,' vol. **40**, p. 273.

Mellon, R. R. (1925). 'J. Bact.,' vol. **10**, p. 481.

—— (1925). *Ibid.*, vol. **10**, p. 579.

—— (1926). *Ibid.*, vol. **11**, p. 203.

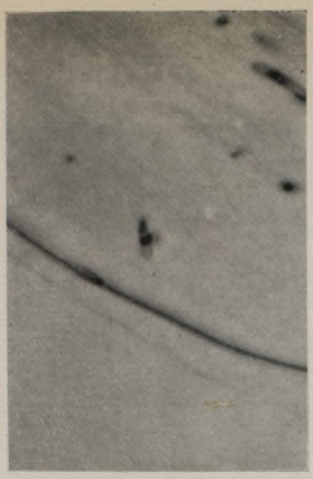
—— (1926). *Ibid.*, vol. **12**, p. 355.

—— (1927). *Ibid.*, vol. **13**, p. 79.

Stapp, C., and Zycha, H. (1931). 'Arch. Mikrobiol.,' vol. **2**, p. 493.

Stoughton, R. H. (1929). 'Proc. Roy. Soc.,' B, vol. **105**, p. 469.

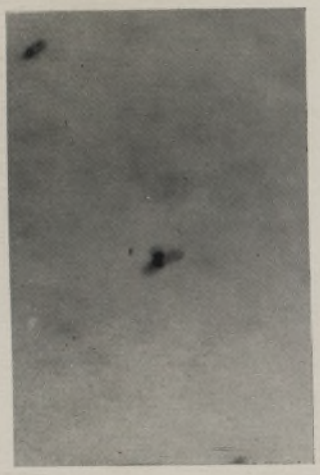
Downloaded from http://royalsocietypublishing.org/rspb/article-pdf/111/769/46/132115/rspb.1932.0043.pdf
by Rothamsted Research Limited user
on 19 February 2026



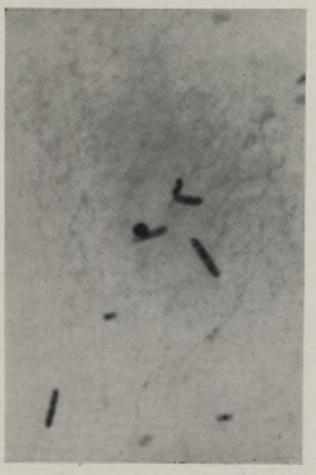
1

2

3



4

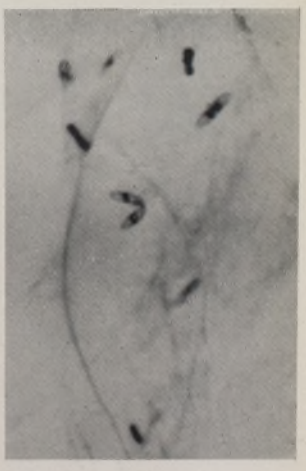


5

6

7

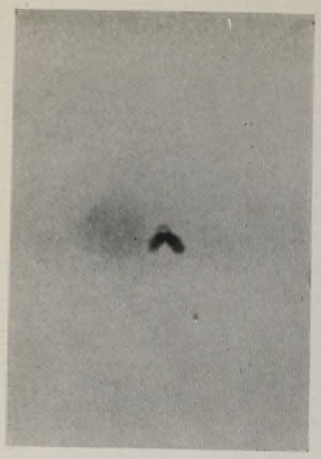
8



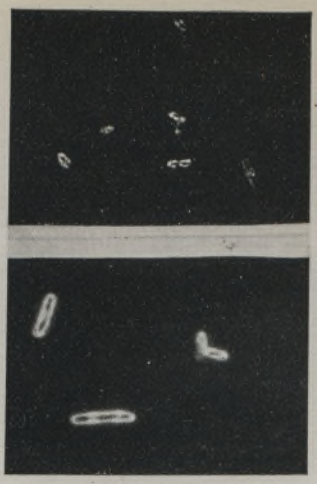
9

Downloaded from http://royalsocietypublishing.org/rspb/article-pdf/111/769/46/132115/rspb.1932.0043.pdf
by Rothamsted Research Limited user
on 19 February 2026

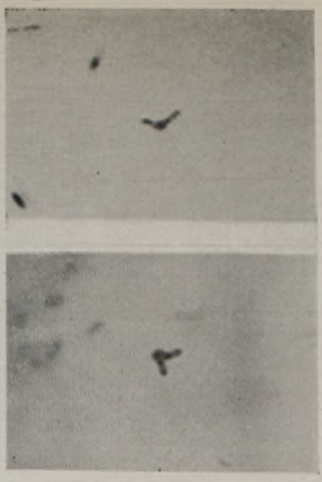
(*Facing p.* 52.)



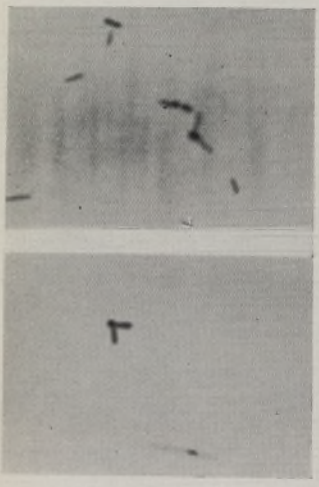
10



11

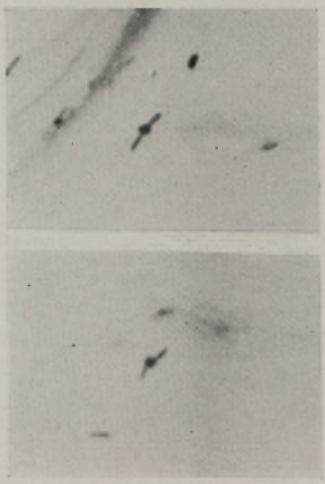


12



13

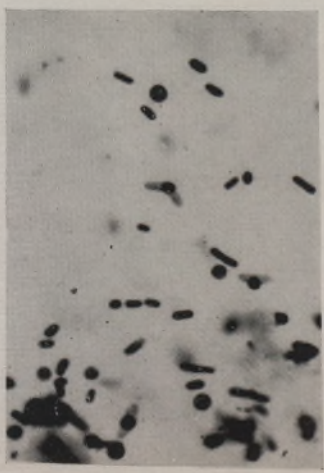
14



15

16

17



18

Downloaded from http://royalsocietypublishing.org/rspb/article-pdf/111/769/46/132115/rspb.1932.0043.pdf
by Rothamsted Research Limited user
on 19 February 2026