PLATE 22.

FIGS. 8, 9, 10, 11, 12, 13.—Spermatocytes from same material as fig. 7, showing abortive acrosome formation.

FIG. 14.—Same material, part of nucleus, showing acrosome formation.

FIGS. 15, 16.—Abortive acroblasts, drawn free-hand.

PLATE 23.

Microphotographs of cells given 4½ times erythema dose and killed some hours afterwards.   For description see p. 461.

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576 . 851 . 4 B . *malvacearum* . 094 + 633 . 51—2 . 3

The Morphology and Cytology of Bacterium malvacearum, E.F.S.

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[PLATES 24–26.]

Introductory.

During the course of studies on the angular leaf-spot disease of cotton, caused by the organism *Bacterium* (*Pseudomonas*) *malvacearum*, E. F. Smith, the production was constantly observed of bacterial forms differing from the normal structureless cell as seen in stained films from 24-hour old cultures of the organism.   Of these the most conspicuous feature was the presence of deeply staining structures within the body, especially in preparations from cultures more than 4 days old.   For some time these unusual forms were considered as artefacts produced by the staining technique, or else as contaminations.   Observations of living bacteria from cultures derived from single cells, and especially evidence obtained from the examination of unfixed wet films by a technique described below, proved conclusively that neither of these explanations was correct, and that the organism possessed hitherto unrecorded internal structure and variations in morphology.

An enormous mass of literature on the subject of bacterial structure and variation has now accumulated. Reference need only be made to the review by Löhnis (8) of the literature on the subject up to 1918, to the analysis of the problem of microbic dissociation by Hadley (5), to the relevant papers in the symposium edited by Jordan and Falk (6), and to the work of Enderlein, most of which has been brought together in his book " Bakterien-Cyclogenie " (3).

The purpose of this paper is to present evidence for adding another bacterial species to those for which variation from the so-called " normal " type of reproduction has been shown to occur. It is of interest that *Bacterium malvacearum* belongs to the rather small group of plant pathogens, since with the exception of the investigations by Levine (7) and Rosen (10) on *B. tumefaciens* little work has been done on this group.

So far as can be ascertained from the literature, *Bact. malvacearum*, first described in 1901 under the name of *Pseudomonas malvacearum* by Erwin F. Smith (11), has always been reported as an invariably structureless slender rod multiplying solely by transverse fission. Faulwetter (4) states specifically that " no capsules, granules, or endospores have been demonstrated." That the phenomena to be described are not peculiar to the particular strain of the organism used has been proved by examination of a number of other strains obtained from sources as widely apart as America, China and the Sudan. Although there is some evidence that the strains differ in detail, the general principles of development have been found to be the same.

Material and Methods.

In these studies a single strain of *Bact. malvacearum* has been used, originally isolated by the writer from infected seed supplied by the courtesy of Mr. R. E. Massey, Botanist to the Sudan Government. The purity of the strain has been ensured, not only by repeated platings, but in addition the culture has been twice restarted from a single cell isolated by means of the Dickinson " Micro-Isolator " (1). The strain is a strongly virulent one and has been used for infection experiments concurrently with these investigations.

In order to obtain uniformity of results and to eliminate the possibility of changes produced by varying external conditions, the greater part of this work was carried out on agar slope cultures on standard media incubated at a constant temperature of 25° C., which is near the optimum for the organism. The media used were a standard potato-extract containing 1 per cent. of saccharose, and a synthetic medium. The potato-extract is prepared by boiling 200 gms. of sliced potato to a pulp and straining through muslin. The extract is made

up to 1 litre, 1·5 per cent. of agar added, the whole autoclaved at 15 lbs. pressure, filtered through paper-pulp under a vacuum, 1 per cent. of saccharose added and the medium tubed and sterilised at 115° C. for 15 minutes.   Some hydrolysis of the sugar doubtless occurs, but as it has been found that the substitution of dextrose or levulose for saccharose has no effect on the organism the latter sugar is used on account of the ease with which it may be obtained in a pure condition.   The synthetic medium was devised originally for the purpose of studying nutritional phenomena.   The composition is as follows :—

	Per cent.
Di-potassium phosphate (K$_2$HPO$_4$)	0·1
Potassium nitrate (KNO$_3$)	0·2
Magnesium sulphate (MgSO$_4$)	0·1
Sodium chloride (NaCl)	0·1
Dextrose	1·0
Agar	1·5

The phosphate, chloride and nitrate are dissolved together in a little water, the magnesium sulphate dissolved separately, the two solutions mixed and made up to the correct volume with water.   The agar is then added, melted in the autoclave at 115° C., and the medium filtered through paper pulp under pressure.   The sugar is then added and the medium brought to a reaction of 7·0 P$_H$.   It is then tubed and sterilised at 115° C. for 15 minutes.

The organism has been found to grow freely on this medium and to produce the same forms as on the potato-extract.   The medium has the advantage of being constant in composition, reasonably well buffered, and permitting of alterations in the nutritional factors without difficulty.   Except where otherwise stated these two media have been used throughout the investigation.   It is, of course, well known that with bacteria as with fungi certain stages of development are produced only in response to altered conditions of the medium or of the external physical factors, but it was thought better in this study of the primary morphology of *Bacterium malvacearum* to keep all factors constant and to leave the investigation of the effect of altered conditions to a later date.

In the earlier part of the enquiry the work was carried out on dried films prepared in the usual way, fixed and stained by a variety of methods.   When it became apparent, however, that the visible appearances bore some real relation to an internal structure in the organism, it was recognised that some technique must be employed which would avoid the drastic processes involved

in the preparation of a dried film. It is obvious that in the process of drying alone, even if the bacteria have been previously fixed by a chemical reagent, some degree of distortion is produced. Further, in the staining of a dried film there is always the risk of deposition of precipitates, staining of particles adhering to the slide, distortion due to the concentration of the stain, and so on. In fact, with dried films, even with the most careful technique it is never possible to be certain that appearances seen represent real structures present in the living organism and not artefacts produced by the method of preparation. Some control of the observations, it is true, can be obtained from examination of the living cells, but their extremely small size precludes the possibility of exact observations in this manner. This point will be dealt with later.

Dobell (2) in his studies of nuclear behaviour in bacteria found no essential difference in appearance between bacteria stained in wet films and the same organisms in stained dry preparations. With *Bact. malvacearum* this is not the case ; even after preliminary fixing dried films invariably show some degree of shrinkage of the internal structure, associated with a flattening and consequent broadening of the cell-body. The photomicrographs on Plate 24 (Nos. 5 and 6) illustrate this point. It is true that in the dried films the central structures to be described are much more sharply defined, but the fact that shrinkage has obviously occurred makes it impossible to interpret the appearance as representing the true forms of the structures, although the clarification of outline is of value in confirming the observations on the stained wet films.

The method finally adopted was a modification of that used by Nakanishi (9) in his studies on nuclear structures in bacteria. Chemically cleaned slides which have been kept in ammonia-alcohol, are further cleansed by flaming. A drop of the stain to be used (Ziehl's carbol-fuchsin diluted with an equal quantity of water has been most employed) is placed at one end of the slide, and a thin film of the stain made by drawing the edge of a strip of typewriting paper over the drop and along the slide. This film should dry rapidly and evenly and be barely perceptible when held to the light. A small drop of sterile water is then placed in the middle of a flamed thin cover-glass, touched with a wire carrying the organisms from the culture, and the cover-slip inverted and dropped on the stained slide. The mount is then sealed with vaseline by means of a camel-hair brush, or, if more permanence is required, with gold size.

The organisms take up the stain quite slowly if the film is correctly prepared, and the process of gradual intensification can be watched under the microscope.

The structures appear successively in the order of their affinity for the stain, and this makes their visual differentiation less difficult.   This phenomenon of progressive staining, coupled with the fact that the organisms are not fixed by drying or subjected to any other distortional process, makes it reasonable to assume that the appearances seen represent real structures and not artefacts. This is further confirmed by the fact that the appearances are practically the same with all the usual basic aniline dyes.   Preparations have also been made by the same method with bacteria previously fixed by corrosive sublimate or picric acid ; no difference in the appearances presented can be observed.

Structural Changes in Young Cultures of Bact. malvacearum.

General Appearances.—Examination of a culture 24 hours old on either of the standard media, stained by the method described, shows a preponderance of slender rods, which stain more or less evenly and deeply.   These conform in every respect to the typical descriptions of the organism given by previous workers.   In size they vary from about $1 \cdot 5$ to $4 \cdot 0$ μ by $0 \cdot 5$ to $0 \cdot 9$ μ, and in shape they are cylindrical with rounded ends.   If the stain is not too intense, however, or if the preparation is examined immediately it is made, before the staining process is complete, a certain number of the cells are seen to contain a deeply staining spherical granule (rarely two or more) usually situated towards one end of the cell and conspicuous by reason of its greater affinity for the stain.   This structure is soon obscured by the staining of the rest of the body.

The same general appearance is presented in films from cultures up to about 3 days old, with the exception that the cells containing granules become more numerous and the latter more conspicuous, by reason of the tendency of the cell-body to stain less deeply.   After the lapse of 3 to 4 days the cells in the culture lose their power to stain evenly and another structure becomes visible. This is centrally placed and presents a different appearance in different cells as described on p. 474.   The change in staining reaction begins with the bacteria at the top of a slope culture and progresses downward, so that in a culture about 72 hours old films prepared from the top of the slope will show the differentiated staining, while those from the water of condensation will still resemble preparations from 24-hour cultures.   In a time varying from 5 to 8 days the whole culture shows the differentiated reaction.   The significance of this point will be discussed in the consideration of the nature of the structures.

Concurrently with the appearance of the central body the granules previously

referred to become very conspicuous, by reason of their intense stain in contrast with the light staining of the ends of the cells. These granules and the changes they undergo are described on p. 479.

The Central Structure.—This body becomes most clearly and sharply defined in cultures about a week to 10 days old, while the cells are of the normal size and shape and before the changes which occur in old cultures have begun to appear. It is readily observable that the central structure bears some relation to the condition of the cell with regard to the division process. In immature cells that have clearly been recently formed by the division of a mother cell the structure appears usually as a homogeneous, more or less spherical, centrally placed body, staining with basic dyes, but less intensely than the granules referred to previously (Plate 24, figs. 1, 3 and 4). In some cells apparently in the same stage, however, the body presents the appearance of a four-lobed structure, or alternatively of four very small bodies all close together in a " tetrad " formation.

It is necessary here to emphasise the optical difficulties encountered in the examination of these internal structures. The extreme limit of resolution for a well-corrected lens-system is given approximately by the quotient of half the wave-length of the light used divided by the effective numerical aperture of the lens. That is, the expression

$$\frac{\lambda}{2 \times \text{N.A.}}$$

gives the least distance between particles that can be observed by the given lens working under the best conditions. It follows that if two particles of any size are separated by a distance less than this, it will be impossible to resolve them by that lens and they will appear as a single particle.

Working with an apochromatic objective of N.A. 1·40 in monochromatic green light (λ = approximately 5000 Ångstrom units = 0·5 μ) the limit of resolution would, theoretically, be about 0·17 to 0·18. In practice this degree of resolution is rarely attained, since it is not usually possible to utilise the full theoretical numerical aperture of the lens, and for most purposes the limit may be put at about 0·2 μ to 0·25 μ.

Now the diameter of a cell of *Bact. malvacearum* is usually about 0·6 μ, so that the central structure will be somewhat less than this, say 0·5 μ, and the diameter of the separate components of the " tetrad " (if a real appearance) must be of the order of 0·2 μ, or perhaps a little more, while their distance apart will be less than this. It will be seen therefore that the resolution of

this structure under usual conditions presents an almost insuperable problem, and it must be admitted that, although it is *seen* as a "tetrad," its existence as a *real* "tetrad" formation is conjectural.

In cells which are obviously in the process of division the central structure presents a different picture.   Normally in cells of this stage it appears as an elongated "dumb-bell," comprising two rounded bodies, one in each half-cell with a connecting strand between (Plate 24, figs. 1 and 2).   Again in some cells in this condition each end of the "dumb-bell" appears more or less bi-lobed or double.

These two (or possibly four) conditions of the cell give fixed points of reference in an attempt to construct a consecutive story from the other appearances. In some cells the structure appears elongated and with only slight indications of a "dumb-bell" shape ; this being commonly associated with an elongation of the whole cell such as occurs before the usual transverse fission takes place. Again in other cells the central structure appears to have divided completely into two before the elongation of the cell has progressed far.

These last two appearances are apparently associated with two distinct methods of fission of the cell.   In the first case where the central structure elongates with the cell the latter seems to divide by a pinching through, much as if it has stretched and given way in the middle.   In the second case, which seems to be derived from the previously described "dumb-bell" by separation of the two halves, the division of the cell appears to be accomplished by the laying down of a transverse wall across the cell between the two halves of the central body.

Reference to the photomicrographs (Plate 24) makes clear the various changes through which this central structure may pass.   It seems reasonable to conclude that these different appearances represent phases in a division-cycle (possibly entailing two alternative modes of division) of the originally single central structure, and that this cycle (or cycles) is intimately correlated with the normal fission-processes of the cell.   In some cases the central structure apparently divides more or less completely before the somatic division has progressed far, but in every case, so far as has been ascertained, the two processes of division are either simultaneous or immediately consecutive.

From these observations alone it seems justifiable to construct a coherent story for the division process, with a possible alternative.   An attempt has been made on these lines to connect the various appearances into two such division-cycles ; these are represented diagrammatically in text-fig. 1.

Before passing to a consideration of the probable nature of these structures
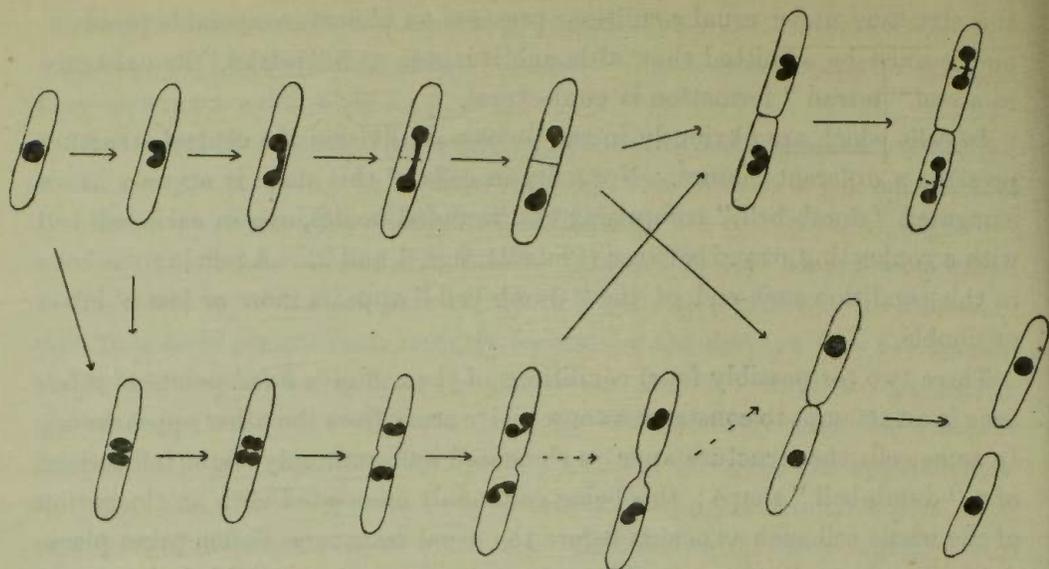
Text-fig. 1.—Diagrammatic representation of hypothetical alternative division-cycles in vegetative cell of *Bacterium malvacearum*.

reference should be made to the phenomena observed in examinations of the living dividing cells.

Observations on Living Cells.

The examination of living bacteria for evidence of their internal structures and division-processes is attended by many very great practical difficulties. The difficulties of optical resolution already referred to apply with even greater force in this case, since in the examination of the living unstained organism it is usually impossible to utilise more than at the most some two-thirds of the total aperture of the objective. This is in part due to the fact that the refractive index of the bacterial body and of its internal structures, being not very different from the surrounding medium, makes it necessary to restrict the aperture of the illuminating cone from the sub-stage condenser. In addition to this purely optical difficulty the problem is complicated by the necessity for the avoidance of contamination, combined with the provision of sufficient oxygen for growth, and at the same time the prevention of desiccation of the mount during the period of examination.

Probably the ideal would be an examination of the dividing bacteria under dark-ground illumination, for under these conditions it is theoretically possible with some types of reflecting condenser to utilise the full aperture of an oil-immersion lens, resulting in a great improvement in resolution. At the same time this method of visual examination renders it possible to make out the

structures much more clearly than by transmitted light. For high-power dark-field illumination, however, it is necessary to have a continuous system of approximately the same (or at least a high) refractive index between the front lens of the condenser and the objective. This condition is sufficiently nearly satisfied if the organisms are in a continuous film of liquid between the slide and the cover-glass. Despite numerous attempts, however, it has not yet been found possible to obtain a continuance of growth under this condition. Division has only been seen to occur where the bacteria had access to free oxygen, as when lying on the surface of a film of agar medium.

Recourse has therefore been made to transmitted light, and although it has not yet been possible to define the structures exactly under these conditions, sufficient evidence has been obtained to confirm the correlation between the division of the central structure and cell-fission. The method found most suitable for these observations was as follows :—An ordinary thin glass slide is perforated with a hole half-an-inch in diameter and on the underside is cemented a thin cover-slip, so as to make a shallow chamber. A drop of filtered sterile melted medium is allowed to run down an extra thin cover-glass, and when this has set the excess of the agar film is cut away with a sterile scalpel, leaving a small square of very thin film in the middle of the slip. This is touched with a wire carrying the organisms and inverted over the shallow chamber. The mount is then sealed with vaseline and examination made for an area where only a few organisms are present in the field. Most modern 1/12-inch objectives will work through this thickness, provided that only extra thin (No. 0) cover-slips are used.

Using this method it was possible to make out the central body as an ill-defined refractive structure. Continuous observation of cells during the process of division shows that this structure at first elongates, then becomes dumb-bell shaped, and finally divides into two, each half passing into one of the daughter-cells. For the reasons given, resolution of the central structure into its details has been impossible, but the evidence is conclusive for the presence and division of the central structure coincidently with the division of the cell.

Significance of the Central Structure.

The evidence of correlation between the division of the central body and that of the cell immediately raises the analogy of nuclear division. The affinity for basic dyes, the position in the cell, and the shape and behaviour of the central structure, all lend support to the hypothesis that these central structures are of the nature of nuclei which undergo a process of division.

In this connection the work of Enderlein (3) referred to in the introductory part of this paper is of much interest. He concludes that the nuclear unit (" Mych ") is the caryological constituent of the primitive cell (" Mychit "). The nucleus is spherical or oval in form and occupies a position close to the wall of the cell. In the coccus there is but one nuclear body, while in all other forms there are two or more. The diameter is $0\cdot1$ μ to $0\cdot25$ μ. It contains no chromatin and stains hardly any more strongly than the cytoplasm of the cell. The nuclear body is observable only when the cell holds but little reserve food-substance. The latter is distributed throughout the young cell in ultra-microscopic granules, the " *trophoconia*," and constitutes the " chromatic " material of the cell, staining strongly because of its high content of nucleic acid and nucleo-proteins. When this food reserve substance is abundant, as in young cultures, the material forms a dense aggregation about the nuclear body, forming a large strongly staining spherical body, the " *trophosome*." Even when nearly all the reserve has been used up the last remnant clings tenaciously around the nucleus, forming the element known as the " *trophosomelle*." Large amounts of food reserve substance, which may conceal not only the nucleus but also the trophosome or the trophosomelle, may be removed by alcohol. Under these conditions, when properly stained, it is observed that, in coccus forms, only a single point takes the stain. In other forms two or several such bodies become evident. These are often at the poles of the cell and represent the true bacterial nucleus or nuclei. After cell-division the heavily staining reserve substance soon appears in the daughter-cells.

It will be seen how closely the phenomena described for *Bact. malvacearum* fit this general hypothesis. Using the technique described by Enderlein it has been found that the central structures gain in clearness by treatment with alcohol, and the " tetrad " appearance referred to becomes even more evident. Whether it will ultimately be possible to resolve the apparently single body into two in all cases remains to be seen. In that case the two alternative cycles of changes suggested in text-fig. 1 would be resolved into one only. At present it appears as though both were possible.

On all points one is led to the conclusion that these central structures represent either true nuclei or possibly (following Enderlein's theory) nuclei imbedded in a mass of " chromatic " food reserve substance. Further these " nuclei " undergo a division process, either immediately prior to, or coincidently with, the division of the cell-body, the latter division being related to the nuclear process.

Gonidia.

Reference has already been made to other bodies, distinct from the central ("nuclear") structures which have been described, found commonly in the cells of young cultures of *Bact. malvacearum*. These are spherical granules, up to $0\cdot3$ μ or so in diameter, characterised by their intense affinity for basic dyes. In a wet film prepared by the technique described these granules are the first to take up the stain and are usually intensely stained before the other cell-structures are appreciably coloured. This fact allows of their differentiation from the "nuclear" structures, with which they might otherwise be confused when produced near the middle of the cell. They may be formed in any position, but most commonly occur rather towards one end of the cell, occasionally even occupying a polar position. Usually they are found singly, but two or more may occur in one cell.

The granules appear to be formed either closely adpressed to, or actually in, the cell-wall, and as they grow in size protrude slightly when seen in profile (Plate 25, fig. 1). Liberation appears to be accomplished in one of two ways, either by simple extrusion through the wall, or else by growth on an elongated stalk, which may reach a length of 1 or 2 μ (Plate 25, figs. 3 and 4). It has not been possible to ascertain whether this stalk is formed by an evagination of the cell-wall or by actual growth of a new organ *sui generis*. The former hypothesis is lent support by the fact that the granule appears to be invested by a membrane or sac which is continuous with the stalk. This sac is not clearly visible surrounding the granule, but after liberation of the latter the stalk, which remains attached to the parent cell, commonly carries at its end the collapsed remains of the membrane, appearing as a faintly staining irregular fragment.

The photomicrographs (Plate 25) show these various stages of the stalked granules and old granules bearing fragmented membranes. The liberated granules can be seen free in the culture under dark-ground illumination. Owing to their extremely small size their Brownian movement is very great and it has not been possible to prove conclusively whether or not they are truly motile. Flagellæ have not been demonstrated attached to the granules.

Bodies of this nature have been observed by other workers in all types of bacteria, including an immense number of species. An exhaustive review of observations and conclusions of a great number of workers, and a critical analysis of the data, are given by Löhnis in his "Studies upon the Life-cycles of the Bacteria" (8). He concludes that the Babes-Ernst "metachromatic

granules," the "microsomes" of Klebs, the "Eier" of Ehrenberg, the "Blastia" of Perty and the "infective granules," "swarm-spores," "conidia," and many of the "buds" and "umbels" of other writers, are all of the same type, which he classes as "gonidia." This term was originally applied to reproductive organs of the lower algæ, and "gonidia" were defined by Sachs as organs of asexual reproduction, formed by contraction of the plasmatic cell content, which leave the parent cell either by breaking the cell-wall or become liberated when the cell dissolves.

That the granules observed in *Bact. malvacearum* are of this type there can be little doubt, and it seems reasonable to regard them as true gonidia, despite the fact that they have not as yet been observed to grow out into normal rods. This point, together with the developmental history of the organism within the plant, will be dealt with in a later paper. It may, however, be pointed out here that both the "nuclear" structures and the gonidia have been demonstrated in the bacteria occurring within plant lesions.

Changes Observed in Old Cultures.

The general appearances described above persist in cultures for some weeks, with the exception, however, that a greater variability in size and shape of the individual organisms becomes more and more apparent. There is a general tendency towards the production of smaller forms, averaging about 2 μ in length, but a certain number of larger and more oval forms are also produced. After the lapse of about 6 weeks the variability becomes very marked. While the majority of the cells are either of the normal type or smaller, a small percentage (5–15 per cent.) of "giant cells" are found. These are of various sizes and shapes, up to as much as $5 \mu \times 10 \mu$ (Plate 26, figs. 1 and 2). Many of them show no internal structure and stain only lightly, while others may contain numerous staining granules ; they resemble the so-called "involution" or "degeneration forms" so commonly described for many species of bacteria and appear to be produced in response to the unfavourable conditions for growth. This is borne out by an experiment on the growth of the organism on the synthetic medium described earlier in this paper, using a variety of sugars in place of dextrose. The most striking effect was produced by the use of maltose. On this medium growth is very scanty and soon ceases. Examination of the culture microscopically shows the presence of large numbers of giant "balloon-like" cells of a great variety of shapes and internal structures. The significance of these "giant-cells" has not yet been worked out, but it

seems unlikely that they represent any real stage in the normal life-cycle of the organism.   They have not as yet been observed in the plant lesions.

A common appearance in these old cultures is the occurrence of rather large, swollen, oval cells, occurring in pairs attached at their ends, and remarkable for the fact that they appear usually at a constant angle to one another. Whether they represent daughter-cells of an incomplete but normal division, or are produced by some other process, is not yet clear.   Several of these " angled " forms may be seen in Plate 26, fig. 1.

Associated with these diverse types produced in old cultures is yet another form.   These are spherical or slightly pear-shaped cells 2–3 μ in diameter and containing a single deeply staining point.   In cultures which have been sealed with paraffin wax immediately after inoculation and left for several months these coccus-like forms are produced in great numbers.   In such a culture the cells are all of two forms, very short rods of the typical structure and these spherical cells.   The mode of production of the " cocci " has been followed in stained films from these old cultures prepared by the technique described.

Method of Formation of the Cocci.

The spherical coccus-like forms are produced by a process of budding of the normal or somewhat swollen rods and oval forms in old cultures.   The bud appears first as a small protuberance from the wall of the parent-cell, which enlarges and swells into a spherical form.   The bud is usually formed at the point in the cell where the " nucleus " lies.   During its growth it remains attached to the parent cell by a very short and narrow neck, which provides continuity between the plasma of the cell and that of the bud.   As the bud increases in size a portion of the " nucleus " of the parent-cell passes through the narrow neck and ultimately becomes abstricted as the " nucleus " of the free coccus.   In this respect the " nucleation " of the coccus appears to differ from the process observed in ordinary vegetative division.   In the latter a symmetrical division of the " nucleus " takes place about the transverse axis of the cell, while in the former a portion of the parent " nucleus " seems to squeeze through the narrow channel of attachment and then to become cut off by the separation of the bud.   This passage of a part of the " nucleus " into the bud is shown in Plate 26, figs. 3 and 4, while text-fig. 2 shows drawings of cocci in various stages of development.

The biological significance of these coccus-like reproductive forms is not yet clear.   The fact that they are normally produced only under adverse conditions for vegetative development of the culture suggests that they may represent
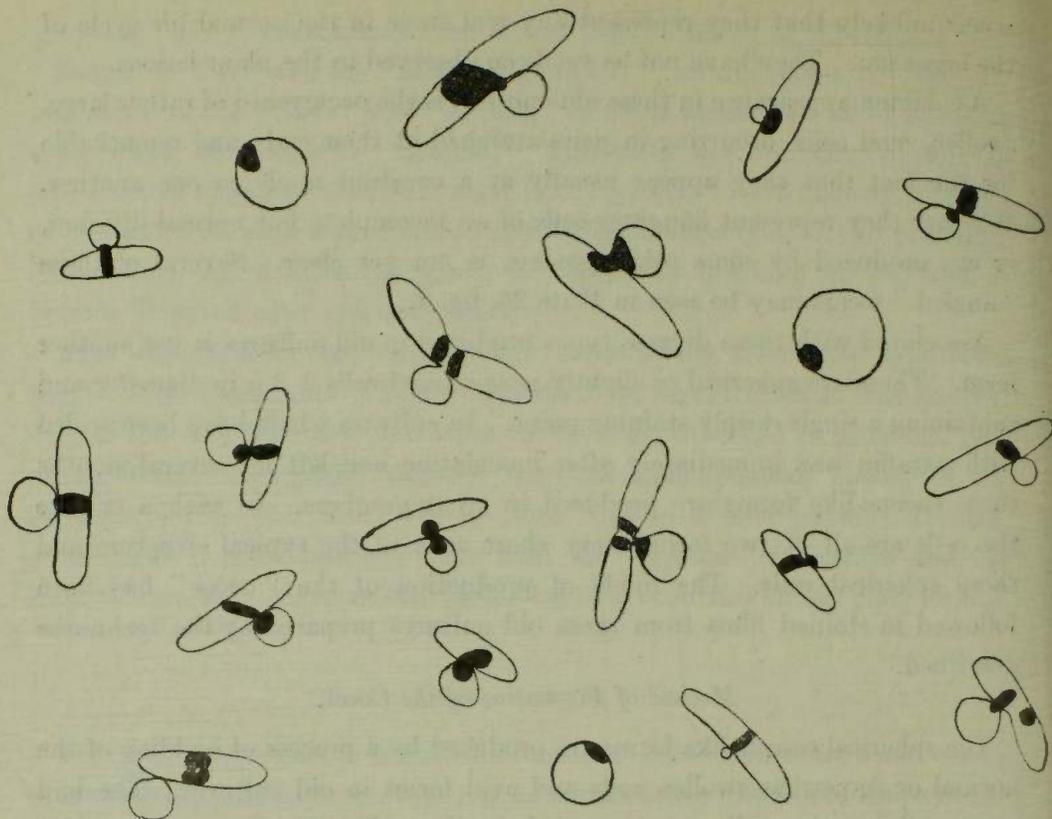
TEXT-FIG. 2.—Drawings of " cocci " in various stages of formation.   Magnification about
17,000 diams.

some more resistant form of the organism than the normal vegetative rod.
If this is so it would help to explain the many inconsistencies observed by
different workers on the resistance of the organism to various lethal agents,
such as temperature, desiccation, and disinfectants, and they (or the gonidia)
might prove to be a factor in the persistence and spread of the disease in the
field.   The fact that no " normal " resistant spores are formed by organisms
of this group has always been a stumbling-block in attempted explanations of
the method of persistence of the various diseases caused by them from season
to season.

Summary.

1. *Bacterium* (*Pseudomonas*) *malvacearum*, E.F.S., has hitherto been described
as possessing no internal structure or reproductive bodies, and as multiplying
solely by transverse fission.

2. A technique is described for staining the bacteria without previous drying
or fixing.

3. Using this technique several structures and a variety of different morphological forms have been observed in *Bacterium malvacearum*.

4. An internal central structure is described, which passes through a division cycle, which is correlated with the division of the cell-body, and is suggestive of nuclear division.

5. Small granules with a strong affinity for basic dyes are described. These are formed in the wall of the cell and are liberated by simple extrusion, or grow out on a stalk from the end of which they are freed. These bodies resemble the " gonidia " of other writers.

6. The occurrence and mode of formation of spherical coccus-like bodies in old cultures is described.

7. " Giant-cells " and other atypical forms have been found to occur in old cultures.

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EXPLANATION OF PLATES.
PLATE 24.

FIGS. 1–4.—2 to 4-week culture of *Bacterium malvacearum*, showing stages in the division of the central structure. Preparations made by the wet-film method. Stained carbol-fuchsin. × 2000 approx.

FIGS. 5 and 6.—Dried films from 2-week cultures, showing shrinkage of internal structures and distortion of cells. Stained Bleu de Roux. Fig. 5, × 1500 ; fig. 6, × 2200 approx.

PLATE 25.

FIGS. 1 and 2.—2-week culture, showing gonidia in the cells and in process of liberation.
   Fig. 1, × 2150 ; fig. 2, × 1500.   Wet-film preparation.
FIGS. 3, 4 and 5.—Ditto.   Stalked gonidia and remains of stalk and membrane.   × 2200,
   approx.   Wet-film preparation.
FIG. 6.—Living cells under dark-ground illumination, the lowermost cell showing a
   gonidium in the wall.

PLATE 26.

FIGS. 1 and 2.—4-week cultures, showing production of " giant-cells," " angled " pairs
   and atypical forms.   Wet-film preparation.
FIGS. 3–6.—4 to 8-week cultures, showing production and method of " nucleation " of
   " cocci."   Fig. 4, × 3000 ; others, × 2000, approx.   Wet-film preparation.

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The Vascular Filaments on the Pelvic Limbs of Lepidosiren, their Function and Evolutionary Significance.

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In 1900 Prof. J. Graham Kerr published the results of his investigations
into the habits and reproduction of Lepidosiren in the swamps of the Gran
Chaco, in Paraguay (' Phil. Trans.,' Series B, vol. 292).   He found that just
before the breeding season papillæ which occur on the pelvic limbs of the male
rapidly develop into long, bright-red, vascular filaments.   These persist
throughout the breeding period, during which the male fish remains in the
nesting burrow with the eggs and larvæ.   After this period the filaments
disappear, by atrophy of the tissues and disintegration, not by absorp-
tion.   Neither filaments nor papillæ usually occur on the pelvic limbs of the
female, but papillæ in a very rudimentary condition occur occasionally in
female specimens, and, judging from the analogy of sex-limited characters in
other vertebrates, these specimens are possibly the oldest, and perhaps no
longer fertile.

Prof. Graham Kerr discusses the question of the function of these vascular
filaments in the male.   Sir Ray Lankester had suggested that they were