THE PARASPORAL BODY OF BACILLUS LATEROSPORUS LAUBACH

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Plates 306 to 310

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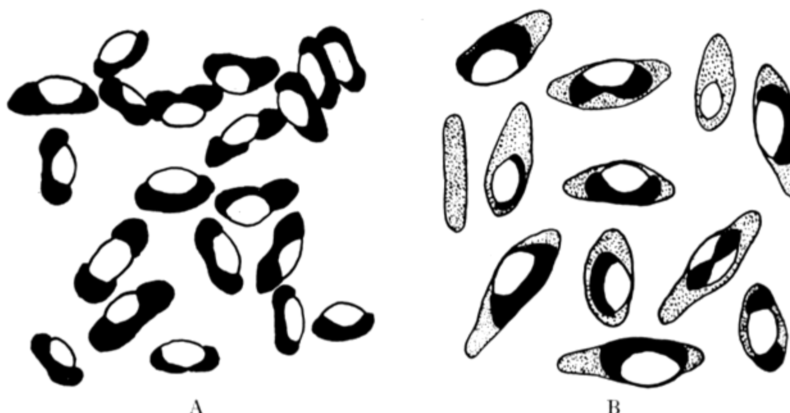
INTRODUCTION

In 1912 White (1) gave the name *Bacillus orpheus* to an interesting sporeformer encountered as a secondary invader of bee larvae infected with European foul brood, but a documented description of this organism was not published until 1917, McCray (2). Attention was drawn by White (3) in 1920 to similarities in the descriptions of two sporeformers isolated from different material, *Bacillus orpheus* from diseased bee larvae and *Bacillus laterosporus* from water. The latter organism had been fully described by Laubach (4) in 1916, a year before McCray published the description of *B. orpheus*. In their careful taxonomic studies of aerobic sporeforming bacilli, Smith, Gordon, and Clark (5) considered that these two organisms were identical and that the name *B. laterosporus* must take precedence.

The interesting feature of *B. laterosporus* is that on completion of sporulation the spores are cradled in canoe-shaped bodies. Laubach (4) described the process of sporulation, "as the organisms swell and assume a fusiform shape the spores appear as slightly staining globules. The protoplasm soon disintegrates but more on one side of the spore than on the other so that the free spores which are almost egg-shaped retain a rim of protoplasm on one side and present a characteristic appearance." White's description (3) agreed with that of Laubach, but he believed that the whole of the vegetative cell cytoplasm adhered to the completed spore. "Soon, as determined from stained preparations, the spore is seen occupying one side of the rod with the protoplasm distributed along the opposite side and the two ends. This relation of spore and rod persists in cultures on solid medium for long periods, especially at room temperature." The drawings by these authors are similar to that in Text-fig. 1 *A* which shows the free spores and their attached canoe-shaped bodies. It is the latter which Laubach regarded as remnants of protoplasm and which White believed to be the vegetive cell rod which persisted so long after sporulation. More recently, when sporulating cultures of *B. laterosporus* were examined before the cytoplasm and the chromatinic bodies had shown signs of disintegration, it was found

that there were already attached to the developing spores canoe-shaped bodies which, with basic stains, were clearly differentiated from the spore and the vegetative cell protoplasm, (Text-fig. 1 *B*). As a consequence of these observations, Hannay (6) listed *B. laterosporus* among those aerobic bacilli which form parasporal bodies. The material adhering to the spore which had previously been thought of as protoplasmic remains he regarded as a particular kind of inclusion, a parasporal body formed as a result of the sporulation processes of the cell.



TEXT-FIG. 1. *A*, drawings of the free spores of *Bacillus laterosporus* lying cradled in heavily stained material which was believed by White (1) and Laubach (4) to be the protoplasm remaining after the completion of sporulation. *B*, drawings showing the transformation of a slender vegetative rod into the typical fusiform-shaped sporulating cell, containing an eccentrically placed spore cradled in a canoe-shaped parasporal body (Hannay (6)). The parasporal body is clearly differentiated from the protoplasm of the cell.

It is the purpose of this paper both to provide the morphological evidence that the spores of *B. laterosporus* are attached to organised bodies as distinct from being attached to protoplasmic remains and to establish the continuity between these observations and those of earlier workers. The free spores with their parasporal bodies have been examined with the light microscope, and the parasporal bodies after germination of the spores with the light and electron microscopes. The intermediate stages of sporulation when the developing spore is refractile but the vegetative cell cytoplasm still appears to be intact has been studied with the light microscope, and ultrathin sections of similar material with the electron microscope. A cytological study of the development of the spore and the parasporal body or a systematic anatomical study of the parasporal body have not been included.

Materials and Methods

The observations reported in this paper were made on a strain of *Bacillus laterosporus* obtained from Dr. A. G. Lochhead.

Preparatory Techniques for Light Microscopy:

(*a*) *Spores*—The bacillus was grown for 4 days at 32° on a potato infusion agar supplemented with 0.05 per cent difco vitamin-free casamino acids. After sporulation was complete, the spores were rinsed off the plates with distilled water and washed three times by alternate centrifugation and suspension in distilled water. The spores after a final centrifugation were suspended in M/30 phosphate buffer at pH 7.2 and stored in the refrigerator.

(*b*) *Sporulation*—The bacillus was grown at 32° on the potato casamino acid medium to the point when sporulation and parasporal body formation was well advanced, but the vegetative cell cytoplasm had not begun to show signs of the lysis associated with the completion of sporulation. After fixation, impression preparations were made, stained, and mounted in water.

(*c*) *Spore Germination*—One ml. of spore suspension was spread over the surface of difco nutrient agar, the surplus removed, and the petri plates incubated at 37°. The germination of the spores was followed by making stained impression preparations. Small blocks of agar were removed, fixed in osmium tetroxide vapour for 1 minute, and the cells transferred to coverslips, stained, and mounted in water.

(*d*) *Staining—Giemsa.* Preparations were stained in a 1:10 dilution of Giemsa in M/30 phosphate buffer pH 7.2 for 30 minutes. After rinsing, the preparations were mounted in water. *Feulgen.* The preparations were hydrolysed in N/HCl at 60° for 8 minutes, rinsed in distilled water, placed in Feulgen's reagent for 1 hour, rinsed twice in sulfurous acid solution, followed by washing in tap water and mounting in water. *Dilute Fuchsin.* Preparations were stained with 0.02 per cent basic fuchsin for 30 seconds, rinsed, and mounted in water. *Air mounted nigrosin films* (*Robinow*). Sporulating cells are mixed in a drop of nigrosin solution and spread over the surface of the coverslip; when dry, the coverslip is mounted in air.

Light Microscopy—The photographs were taken with a Leitz microscope equipped with a first surfaced mirror, achromatic substage condenser of N.A. 1.4, a 2 mm. apochromatic oil immersion objective, of N.A. 1.3, and a $\times 15$ eyepiece. A Bausch and Lomb tungsten ribbon research lamp was used with a green interference filter (5460 A). The lamp and the microscope were adjusted to give Koehler type illumination. The negatives were taken at a magnification of 1,800 on Ilford F.P.3 cut film.

Preparatory Techniques for Electron Microscopy:

Free Spore Coats and their Attached Parasporal Bodies—A spore suspension was incubated at 37° in nutrient broth until the first vegetative cells had erupted from the spore cases. The suspension was centrifuged, washed, and small drops containing empty spore cases with their attached parasporal bodies and the bacterial cells were put on formvar-coated grids and air dried.

Fixation, Embedding, and Section of Sporulating Cells—The bacteria were grown as previously described until sporulation was well advanced. The cells were washed off the solid medium with distilled water containing 1 per cent osmium tetroxide and fixed for 1 hour at room temperature. The cells were then washed twice in distilled water, centrifuged, and the pellet taken up in 0.25 ml. of 20 per cent isopropanol. Isopropanol was used as a dehydrant because in contrast to ethanol it did not remove basophilic material from the parasporal body. A continuous method of dehydration was used; absolute isopropanol was added drop by drop and the suspension was stirred constantly with a fine jet of air from a Pasteur pipette. The addition of isopropanol was interrupted for 30 minutes when the concentration reached 50 per cent, and for 45 minutes when it reached 75 per cent. Isopropanol was then added to give a final volume of 10 ml. The cells were finally dehydrated in three changes of absolute isopropanol. From isopropanol the cells were transferred by alternate-centrifugation and resuspension through a series of isopropanol *n*-butyl methacrylate mixtures containing increasing proportions of methacrylate, and next through three changes of methacrylate, and finally to a mixture

containing fully polymerized methacrylate dispersed in a mixture of methyl and butyl methacrylates and benzoyl peroxide. The composition of the polymerized methacrylate was the same as the mixture of monomers in which it was dispersed. Gelatin capsules (No. 4) containing a mixture of 90 parts *n*-butyl methacrylate, 10 parts methyl methacrylate were polymerized at 70° with 1 per cent recrystallized and dried benzoyl peroxide. After 48 hours the capsules were removed from the oven, the gelatin stripped off, and the methacrylate stored over P_2O_5. One piece of this polymerized methacrylate weighing approximately 0.2 gm. was added to a test tube containing 0.9 ml. of the mixture of monomers and shaken until the solid had completely dispersed (5 to 6 hours); 0.01 gm. of benzoyl peroxide was then added, the bacteria stirred in, and the suspension transferred to No. 4 capsules and polymerized at 70° for 48 hours.

The Porter-Blum microtome provided with a mechanical advance was used to cut sections of the needed thinness.

Electron Microscopy—The sections were examined without removing the plastic. A Philips electron microscope, model 100-A, fitted with a 25 μ objective aperture was used with an accelerating voltage of 60 kv. The original electron micrographs were taken on 35 mm. film at a magnification of 30,000 (screen magnification) and enlarged photograpically.

OBSERVATIONS AND DISCUSSION

The morphological characteristics of *B. laterosporus* which separates it from other bacilli must have given pleasure to those who saw and described them, White, McCray, Laubach. First they observed that as in some other species of bacilli, the slender rod-like form of the vegetative cell changes to a larger spindle or fusiform shape during the onset of sporulation, but that unlike other species in this group the maturing spore of *B. laterosporus* develops, as its name implies, in an eccentric position. Visually, this lateral position of the spores is accentuated when the cells are examined in air-mounted nigrosin films (Fig. 1). Cells in various stages of spore development are illustrated, the mature spores are refractile, the younger spores slightly refractile, but in the earlier stages of spore development, when the cells begin to assume a spindle shape, the spores are non-refractile, and therefore invisible when the cells are negatively stained. While the majority of spores are in a lateral position, a few appear in the centre because of their position in relation to the observer.

Their second observation concerned the appearance of the free spores. Laubach noted that "on completion of sporulation the protoplasm of the cell disintegrates but more on one side than the other so that the free spores which are almost egg-shaped retain a rim of protoplasm and present a characteristic appearance." White wrote that "the spore is seen occupying one side of the rod with the protoplasm distributed along the opposite side and the two ends. This relation of spore and rod persists on a solid medium for a long time." The characteristic appearance of free stained spores is illustrated in Fig. 2. Each oval spore is cradled in a canoe-shaped body and it is this body which was thought to be the protoplasmic remains of the parent cell. However, it can be shown that a similar body is already present in maturing sporulating cells. If cells are stained with a dilute solution of a basic dye (0.02 per cent) when the spores have become refractile but the cell cytoplasm has not yet been

damaged by the lysis which is associated with the completion of sporulation, the spores are seen to be cradled in canoe-shaped bodies which are clearly differentiated from both the lesser stained cytoplasm and the unstained spores (Fig. 6). In contrast, the vegetative cells in this preparation contain no stained inclusion. A comparison of Figs. 6 and 2 would indicate that on completion of sporulation the protoplasm and the cell wall of the parent cell lyse, leaving the free spores with their sidebodies. The explanation of why White mistook the parasporal body for protoplasm may be simple. In many instances the spore with its parasporal body occupies the greater part of the cell (Fig. 6), so that on lysis of the protoplasm and cell wall there is still a close resemblance in the shape and size between the sporulating cells in Fig. 1 and the free spores with their parasporal bodies in Fig. 2.

In general, the adjective "canoe-shaped" is applicable to the appearance of these sidebodies of the spores (Fig. 2). The spores completely fill the canoe and lie cradled so that an undetermined portion of the spore is hidden by the sides and ends. Fig. 3 illustrates the range of variation in the parasporal bodies. In most cases they vary in size and shape, but occasionally spores occur which are only capped at each pole. Usually these caps are connected by a thread lying along the surface of the spore, (Fig. 4), but two exceptions to this observation are shown in Fig. 3. That these odd forms are not caused by the lytic enzymes on completion of sporulation is indicated by the presence of the "capped spores" in sporulating cells (inset to Fig. 6). The variations as seen with the light microscope are interesting but not excessive; for instance, the sidebody has not been seen either as a band encircling the spore or as a coat covering the whole spore surface. The spore without a sidebody is rarely found, but now and again a cell may contain a naked spore at one end and a stainable body at the other (Fig. 5). Such cells are so rare that no attempt has been made to determine whether this object has any properties similar to those of the parasporal body.

Because bacterial cytoplasm is basophilic, it was surprising to find that the parasporal body could be differentiated with fuchsin or with any basic dye. Two factors contribute towards this differentiation, the greater affinity of the parasporal body for basic stains in general and the decrease in the cytoplasmic basophilia from the time of the spores' inception. The parasporal bodies have other staining reactions which are of interest. With an aqueous solution of toluidine blue they stain red, but this metachromasia is not apparent with stains of a low pH. With Ponder's stain, which contains a mixture of toluidine blue, acetic acid, and alcohol and is designed to stain the metachromatic granules and beads of *Corynebacterium diphtheriae*, the bodies stain a bluish purplish colour or occasionally reddish; with Neisser's stain containing methylene blue and acetic acid the bodies do not stain metachromatically. These results would suggest that the basophilic material of the parasporal bodies is not identical with the metaphosphate of the beads and granules of *C. diphtheriae* and *Mycobac-*

terium tuberculosis. The reaction to staining with Giemsa, however, seems to be similar to that obtained with the granules of mycobacteria. It was recently shown by Glauert (7), and by Hartman and Payne (8) that the granules of mycobacteria may stain red with Giemsa's stain, the colour usually associated with the staining of nuclear material. After hydrolysis with N/1 HCl at 60°, the parasporal bodies and the extruded spore chromatin stain strongly with Giemsa (Fig. 7), but with Feulgen's reagent only the spore chromatin stains (Fig. 8). The basophilic material is not removed by the Feulgen procedure, for if the preparations are subsequently treated with dilute basic stains the bodies stain heavily. In hydrolysed preparations the Azure A technique of staining bacterial chromatin stains both the chromatin and the body, but the method is as specific as the Feulgen if it is followed by a sulfurous acid rinse.

The metaphosphate which is said to be responsible for the metachromasia of the granules and beads in mycobacteria can be shifted about the cell by repeated staining and destaining (Porter and Yegian (9)). In contrast, the basophilic material of the *B. laterosporus* sidebodies appears to be contained in an organised fixed structure. If coverslip preparations of the spores are immersed overnight in acetone, there is little or no loss of basophilia, but if they are immersed in chloroform, carbon disulfide, or *N*,*N*-dimethylformamide the basophilic material is removed, leaving behind a body which in positively stained preparations can only be seen in outline, but which in negatively stained preparations appears unaltered.

White's observation that "this relation of spore and rod persists on a solid medium for a long time," has been confirmed. The parasporal bodies in year-old stock cultures appear to be intact and still stain with undiminished vigour. But after removal from the solid medium, the handling and storage of even young spores may present a small problem. On bulk storage of spores in distilled water there is after a few weeks a gradual loss of basophilic material and a deterioration in the structure of the sidebody. Some improvement results from storing the spores in M/30 phosphate buffer at pH 7.0. The temporary storage of coverslip preparations in 70 per cent ethanol is likewise followed by a loss of stainable material; an observation which served as a warning against the use of ethanol for dehydrating spores or sporulating cells prior to embedding them in plastic. In both instances the substitution of isopropanol for ethanol has, in respect to the sidebodies, proved satisfactory.

It has been shown that the parasporal body is formed before the completion of sporulation and persists still attached to the spore after sporulation. In neither instance has it been shown to serve any useful purpose. It might be suggested that the parasporal body is acting as a storage unit against the day when the spore might germinate in deficient media. No tests have been made to determine whether the substance in the sidebody contains phosphorus or even whether it is available to or affects the course of germination of spores on

deficient medium. On a complete medium the spores germinate, swell, and finally erupt, one end of the first vegetative cell is projected through the split spore walls which are rent from the top centre of the oval spore to the thwarts of the canoe (Figs. 10 and 11).

After germination the parasporal body and the spore coat are still attached. If the spores and parasporal bodies had been simply held together by a loose membrane enclosing both, the germination of the spore would have resulted in the disruption of not only the spore walls but also of the enclosing membrane and the two would have separated easily, but even after rough treatment by repeated centrifugation there is no sign of parting. The general impression gained from light microscopy and later confirmed by electron microscopy is that the parasporal body is not a structure which is loosely attached to the spore but is actually part of the spore, a close analogy being that of a porch which is part of a house yet is separate in the sense that it is not lived in. Electron micrographs have not indicated that there is any abrupt line of demarcation between the two (Fig. 9), but have shown that there is a marked difference in the opacity to electrons, the body being more dense than the spore coat.

Whereas with the light microscope the differentiation of the parasporal body depends upon the preferential absorption of a basic dye (Fig. 6), with the electron microscope and ultrathin sections it rest on a more satisfactory structural difference (Figs. 12 to 14). In all the micrographs of sections the surface of the body is wrinkled or folded. Because micrographs of whole freeze-dried bodies, shadowed or unshadowed, are free of wrinkles and of hint of an internal organisation, these wrinkles are believed to be artifacts induced by dehydration and embedding. The vegetative cell protoplasm is slightly granular and no chromatin bodies are in evidence; the parasporal body, on the other hand, comprises, in longitudinal (Fig. 12) and in cross-section (Figs. 13 and 14), closely spaced electron-dense lamellae running parallel with the equally electron-dense spore walls and the relatively less electron-dense material in the interstices of the lamellae. The spore walls consist of alternate layers of differing electron density, concentric and regular, the sidebody of electron-dense lamellae, less regular and less well defined. Whether the dark layers in both represent protein and the lighter regular layers in the spore walls and the amorphous material in the interstices of the parasporal body consist of a lipoidal substance is not known. Regardless of the shape of the spaces between the dark lamellae of the canoe, they are always filled with the lesser electron-dense material, and it is perhaps this material which is responsible for the basophilia. Despite the irregularities in the lamellar structure of the parasporal body, the inner surface of the canoe is contiguous with the spore walls, and it would at present, were it not for the fact that the body does not encircle the spore, be difficult to tell where the one begins and the other ends. Perhaps serious consideration should be given to testing the possibility that the sidebody is a deformation of the spore

wall or of an accessory spore wall. The suggestion of an accessory spore wall is added as a cautionary note. The bacillus species may be divided into two groups according to the staining property of their spores, those which stain lightly and those which stain heavily. If the ability of the spore walls to take up stain is an indication of permeability, it is reasonable to suppose that in those spores which stain heavily there may still be an underlying less permeable coat which confers on the spore some of its resistance to adverse conditions. The spores of *B. laterosporus* stain lightly, whereas the spores of its close relatives *B. brevis* and *B. circulans* stain heavily. Preliminary work with ultrathin sections of the spores of *brevis* and *circulans* has shown that their spore walls are a great deal thicker than those of *laterosporus* and the outer stainable layers are composed of alternately electron-dense and less dense lamellae. The parasporal body may be formed at the expense of this outer or accessory coat. It is not known if such an hypothesis is compatible with the anatomy of the parasporal body; the micrographs of Figs. 12 to 14 are insufficient in number and in detail to allow a reconstruction of the parasporal body and certainly provide no clue as to how membranes which might be intended for covering the spore could be redirected to form a parasporal body.

Attention has only been drawn to similarities between the membranes of the parasporal body and those of the spore coat, but the comparison can be extended to another set of lamellae. Robinow (10) in 1953, while studying ultrathin sections of *B. megatherium* spores, noted a structure which had not been previously described: "In *B. megatherium* spores a layer of poorly stainable and electron—optically very transparent material was found immediately beneath the spore coat, which will be referred to as the 'cortex'." A similar cortex is present in the spores of *B. laterosporus*, and is illustrated in the micrographs of Figs. 13 and 14 as an unresolved layer surrounding the spore core. Unlike the parasporal body and the spore coat which are consistently electron-dense, the resolution of the cortex into its component parts depends upon the addition to the specimen of electron-dense material such as osmium. During the embedding process the methacrylate has a tendency to remove that osmium which is not removed by even prolonged washing following fixation. This loss of osmium is particularly noticeable in areas like the cortex which have initially a low affinity for this metal. With prolonged fixation and lessened washing, this loss in methacrylate is sufficiently offset to show that the cortex of the spores of *B. laterosporus* and of *B. thuringiensis* are composed of closely spaced concentric lamellae (unpublished work). There is here no alternation of electron-dense and less dense layers as in the spore coat of *B. laterosporus*, or alternation of electron-dense lamellae separated by less dense material as in the parasporal body. Of the two lamellar systems of the spore, the spore coat and the cortex, the former seems to be most closely akin to that of the parasporal body.

The lateral position of the spore in the cell has been observed without com-

ment. If in the cross-sections (Figs. 13 and 14) a line is drawn through the centre of the spore and the apex of the triangle formed by the parasporal body the section is bisected, if a second line is drawn through the spore centre at right angles to the first, the section is divided into unequal parts. In these sections the spore is eccentrically placed in one plane only, and it would appear, in the absence of sporulation studies, that this eccentricity could result from the formation of the parasporal body.

SUMMARY

On sporulation the slender vegetative rods swell and form larger spindle-shaped cells in which the spores are formed. When the spores mature they lie in a lateral position cradled in canoe-shaped parasporal bodies which are highly basophilic and can be differentiated from the surrounding vegetative cell cytoplasm with dilute basic dyes. On completion of sporulation the vegetative cell protoplasm and the cell wall lyse, leaving the spore cradled in its parasporal body. This attachment continues indefinitely on the usual culture medium and even persists after the spores have germinated. In thin sections of sporing cells the bodies are differentiated from the cell protoplasm by differences in structure. Whereas the protoplasm has a granular appearance, in both longitudinal and cross-sections the parasporal body comprises electron-dense lamellae running parallel with the membranes of the spore coat and less electron-dense material in the interstices of the lamellae. The inner surface of the body is contiguous with that of the spore coat as if it were part of the spore, rather than a separate body attached to the spore. The staining reactions of the parasporal body are not consistent with those of any substance described in bacteria. With Giemsa the bodies stain like chromatin, but the Feulgen reaction indicates that they do not contain the requisite nucleic acid. With an aqueous solution of toluidine blue they stain metachromatically, but with an acidified solution the results are variable. Neisser's stain for polyphosphate is negative. The basophilic substance is removed from the body with some organic solvents. This basophilic substance has not been specifically identified with any material seen in ultrathin sections, but it is suggested that it might be the less electron-dense material in the interstices of the lamellar structure. In contrast to the spore coat of *B. laterosporus*, those of its two relatives *B. brevis* and *B. circulans* take up basic stain like the parasporal body. Thin spore sections of these species have shown that the walls are thicker than those surrounding the spores of *B. laterosporus*, and it is suggested that the outer stainable layer of *brevis* and *circulans* spores is an accessory coat which in *laterosporus* may have been deformed to give a parasporal body.

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EXPLANATION OF PLATES

Plate 306

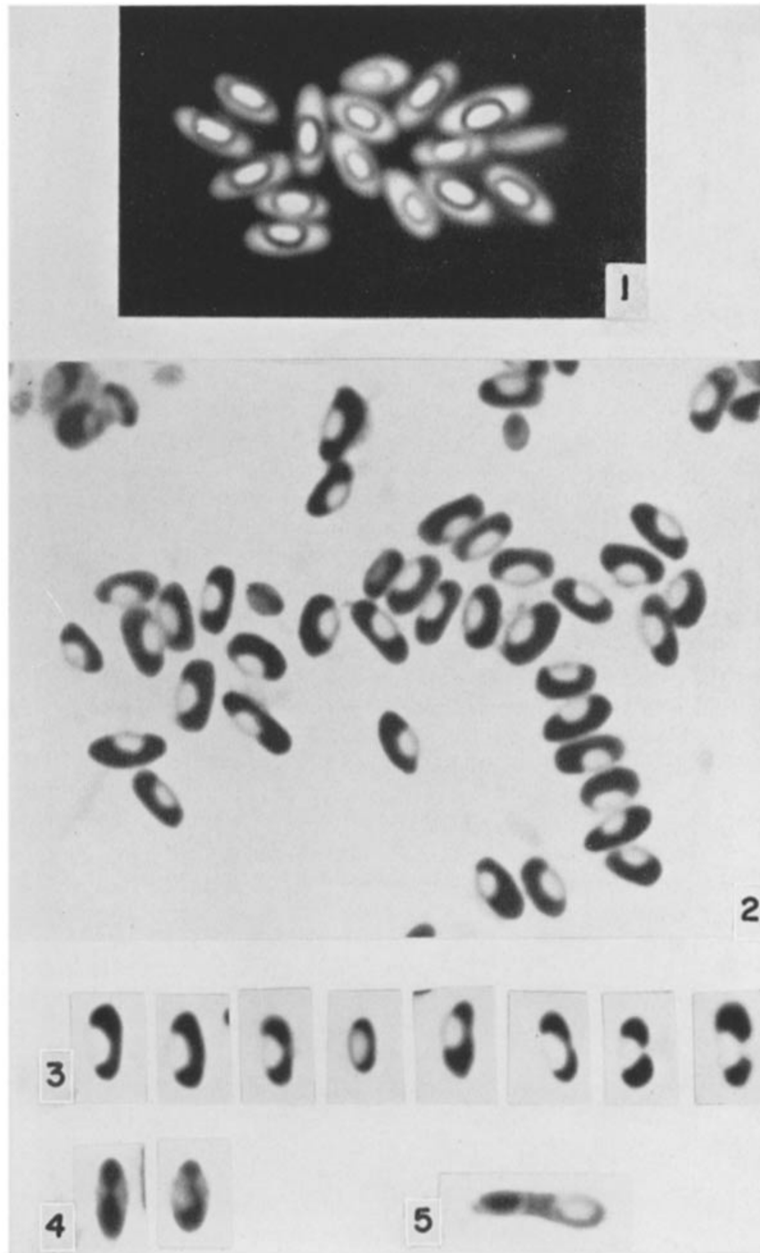
Fig. 1. Photomicrograph of a cluster of fusiform sporulating cells. The emphasis is on the eccentrically placed spores. Immature spores are slightly refractile, and the mature spores are highly refractile. Air-mounted nigrosin film (Robinow's technique). $\times$ 3,600.

Fig. 2. Photomicrograph of free spores and their attached parasporal bodies. The emphasis is on the parasporal bodies. The refractile unstained spores are cradled in stained canoe-shaped parasporal bodies. Dilute fuchsin. $\times$ 3,600.

Fig. 3. Photomicrographs of selected spores illustrating variations in the form of the attached parasporal body. Dilute fuchsin. $\times$ 3,600.

Fig. 4. Photomicrographs of two canoe-shaped parasporal bodies lying keel uppermost. Dilute fuchsin. $\times$ 3,600.

Fig. 5. Photomicrograph of a rare type of cell in which the spore does not lie in a parasporal body. At one end of the cell a refractile spore has developed, and at the other end is a stained irregularly shaped body which may be a parasporal body. Dilute fuchsin. $\times$ 3,600.



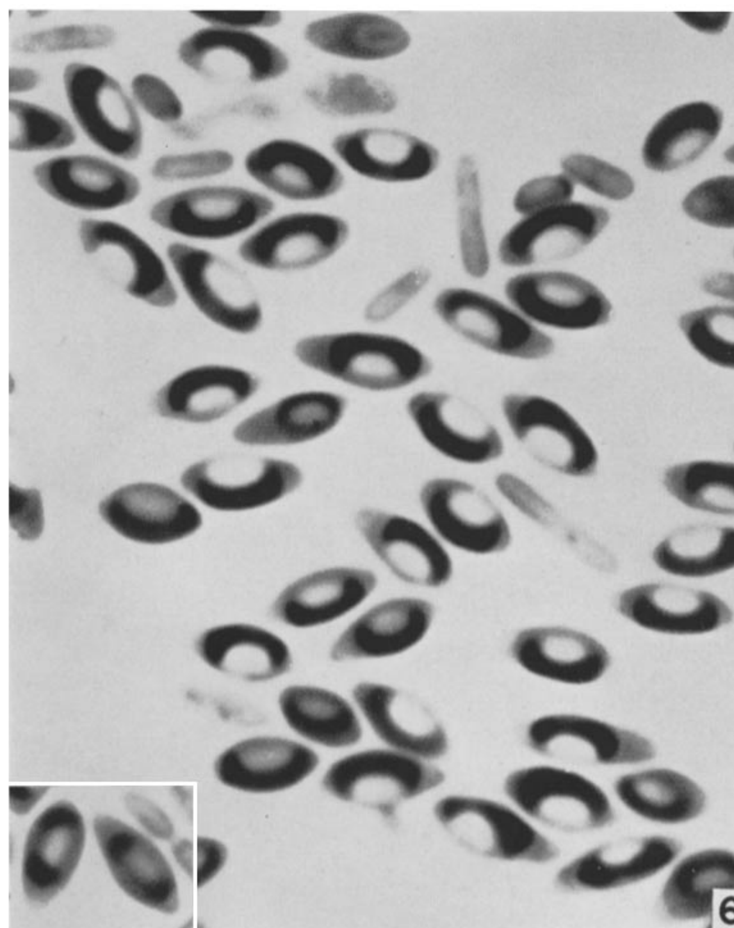
(Hannay: *Bacillus laterosporus* Laubach)

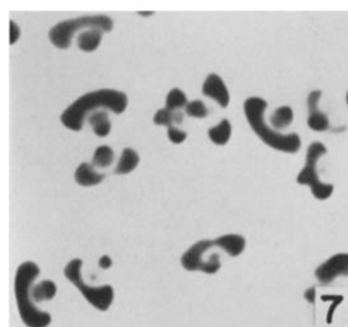
Plate 307

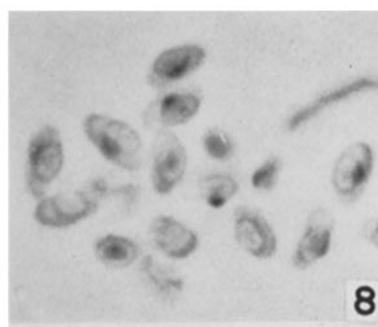
Fig. 6. Photomicrograph of sporulating and vegetative cells. The fusiform sporulating cells contain an unstained spore and a heavily stained canoe-shaped parasporal body, both of which are clearly differentiated from the lightly stained cell cytoplasm. The vegetative cells in contrast to the sporing cells are slender rods and contain no stained inclusions. *Inset.* Two sporulating cells containing parasporal bodies similar to those attached to free spores illustrated in Fig. 3 (the last two spores in the series). Fixed in osmium vapour and stained with dilute fuchsin. × 6,000.

Fig. 7. Photomicrograph of free spores and parasporal bodies stained with Giemsa after acid hydrolysis. The staining of the extruded spore chromatin is normal, but the metachromatically stained parasporal body illustrates one instance of the non-specificity of Giemsa. × 3,600.

Fig. 8. Photomicrograph of free spores and parasporal bodies stained with Feulgen reagents after acid hydrolysis. The extruded spore chromatin gives a positive and the parasporal body a negative Feulgen reaction. Treatment with Feulgen reagents did not alter the affinity of the parasporal bodies for Giemsa or basic dyes. × 3,600.







(Hannay: *Bacillus laterosporus* Laubach)

PLATE 308

FIG. 9. Electron micrograph of a ruptured spore case attached to the relatively electron-dense parasporal body. The specimen was freeze-dried, but not shadowed. $\times$ *ca* 51,000.

FIGS. 10 and 11. Photomicrographs illustrating the persistence of the parasporal bodies and spore coats after germination of the spores and during the subsequent growth of the vegetative cells. Even after germination of the spores, the parasporal bodies are still attached to the spore coats. Preparation fixed in osmium vapour and stained with dilute fuchsin. $\times$ 3,600.

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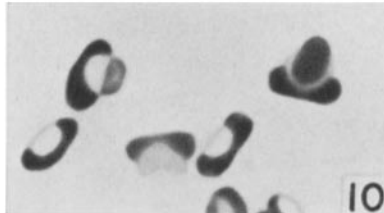

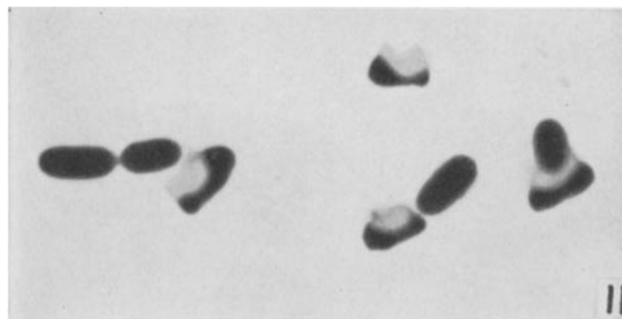

(Hannay: *Bacillus laterosporus* Laubach)

PLATE 309

FIG. 12. Electron micrograph of a longitudinal section through a cell in an advanced state of sporulation. The fusiform shape of the sporulating cell is typical of this species and may be compared with the photograph of sporulating cells in Fig. 9. The spore protoplast (*s.pr*) and its surrounding cortex have been poorly preserved. The ovoid spore is cradled in the parasporal body (*p.b*) which comprises both the electron-dense lamellae running parallel with the equally electron-dense spore walls (*s.w*) and the relatively less electron-dense material in the interstices of the lamellae. No chromatin structures can be recognised in the slightly granular vegetative cell cytoplasm (*v.c.cyt*) which is contained by the vegetative cell wall (*v.c.w*). × *ca* 78,000.

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PLATE 309
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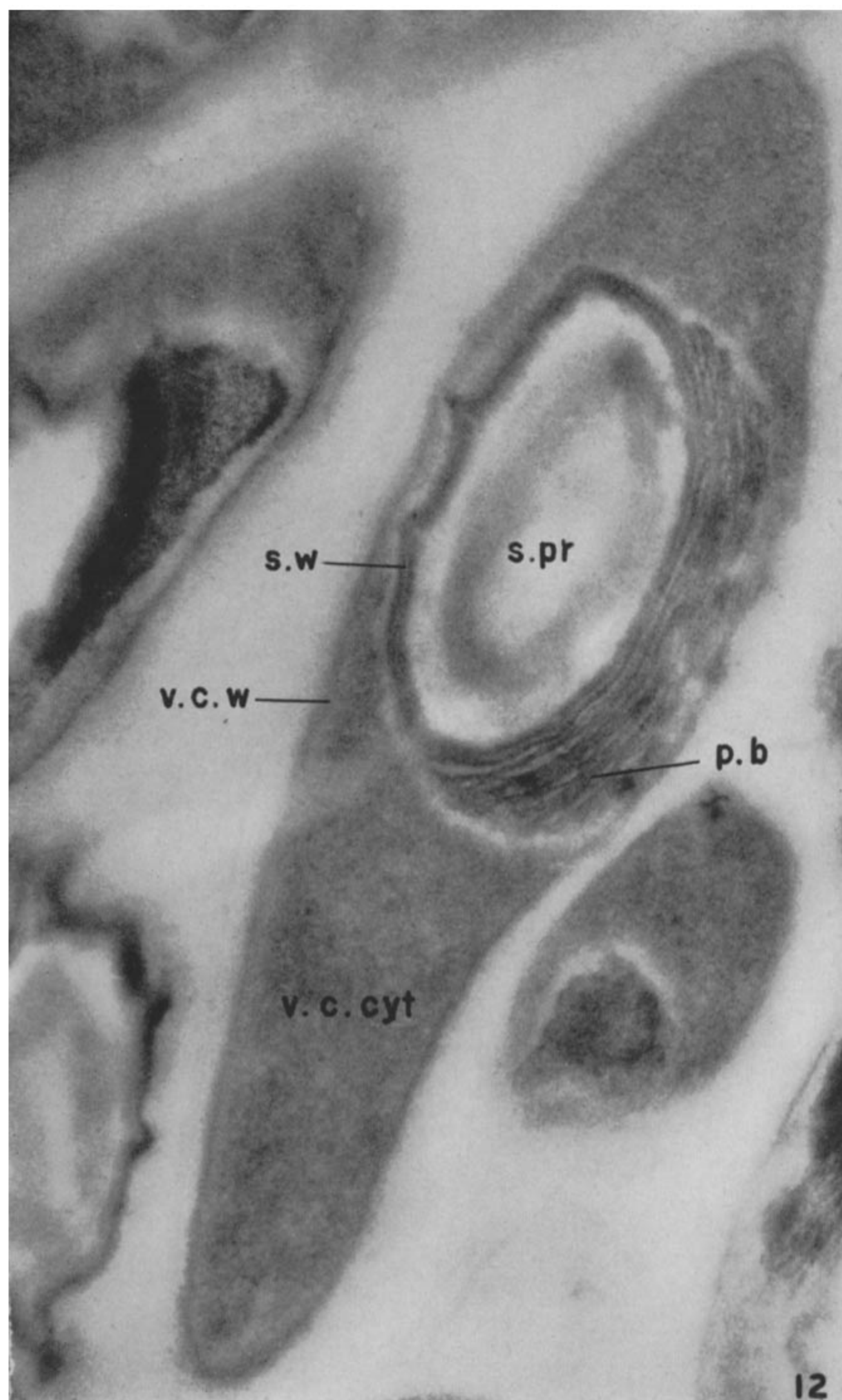
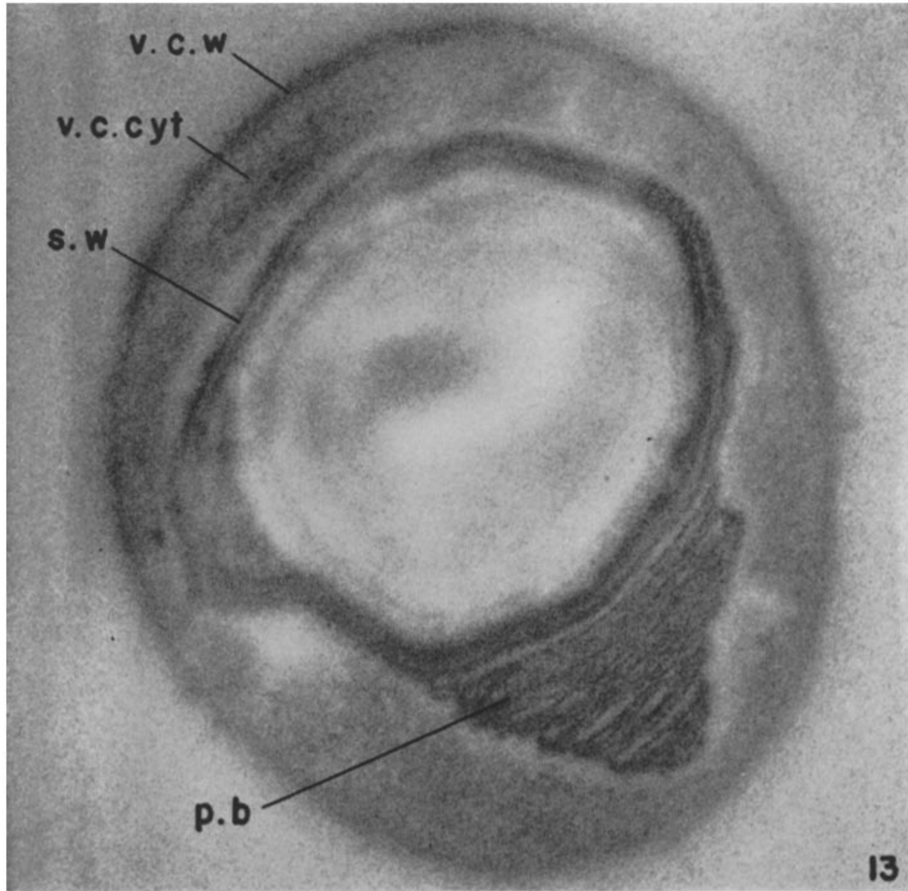


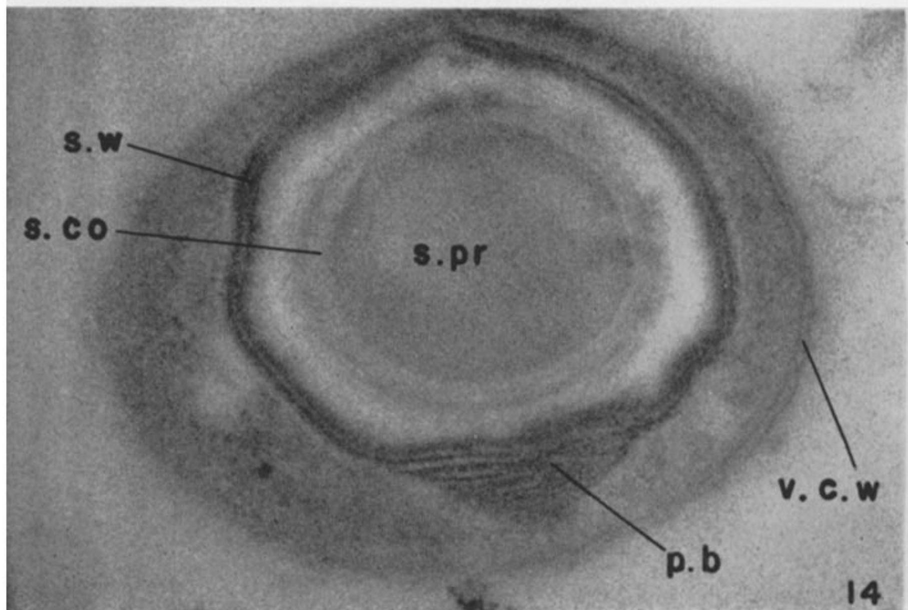
(Hannay: *Bacillus laterosporus* Laubach)

Plate 310

FIGS. 13 and 14. Electron micrographs of cross-sections through two vegetative cells in which sporulation and parasporal body formation are well advanced.

The vegetative cell is covered by a vegetative cell wall (*v.c.w*) which in places is seen as a double membrane system. The details of the vegetative cell cytoplasm (*v.c.cyt*), in which the spore and parasporal body develop, are not resolved in these pictures, and the cytoplasm is of more or less uniform density. The spore protoplast (*s.pr*) is surrounded by a spore cortex (*s.co*), which in turn is covered by the spore walls. The component parts of the protoplast and of the cortex are not resolved; the difference in density of the protoplasts in the two photographs is believed to indicate variations in the changes wrought by fixation and embedding. There is some evidence that the spore walls (*s.w*) are composed of concentric lamellae of high electron opacity separated by layers of less opaque material. The outermost layer appears to be made of the less dense material. In the cross- as in the longitudinal section the parasporal body (*p.b*) is a laminated structure made up of thin electron-dense lamellae running parallel with the membranes of the spore coat. As each layer is shorter than the preceding one, the body is wedge- or keel-shaped. Note the similarity of the lamellae to the membranes of the spore coat. It is not possible from these pictures to ascertain either the anatomy of the parasporal body or the means by which it is attached to the spore coat. $\times$ *ca* 90,000.





(Hannay: *Bacillus laterosporus* Laubach)