SOME FEATURES OF A REMARKABLE ORGANELLE IN *BACILLUS SUBTILIS*

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ABSTRACT

In thin sections of *Bacillus subtilis* certain organelles are observed situated either in the nuclear area from where they can extend into the cytoplasm, or in contact with the cell wall. Inside the nuclear area, the organelle is sometimes composed of concentric layers each seen to consist of two dense borders with a lighter interspace. In other instances, inside as well as outside the nuclear area, the organelles appear as clusters of delicately delimited vesicles. A typical site of occurrence is on the inner rim of the centripetally developing transverse septa, where it appears as a so called peripheral body (2). The micrographs strongly suggest that when the organelles are attached to the walls they have a function in cell wall formation.

INTRODUCTION

In the course of an electron microscopical study of the bacterial nucleus subsidiary observations were made on certain cell inclusions. Pictures were obtained so suggestive of some properties of these organelles that their separate description seemed warranted.

Mudd has presented evidence that some of the cell inclusions encountered in bacteria may be the mitochondria-equivalents of this class of organisms (11 to 13), but there still exists some controversy on this viewpoint (21, 10, 1). It does not follow as a matter of course that all comparatively large bodies of ambiguous nature in bacteria may be reckoned among these mitochondria-equivalents. Therefore, in the absence of any biochemical or cytochemical information on these bodies in *Bacillus subtilis*, we prefer to refer to them here as organelles.

The micrographs to be described are all of *Bacillus subtilis* incubated for 6 hours, starting from spore suspensions. However, comparable observations were also made on much younger cells, even before the first division had taken place.

MATERIAL AND METHODS

In order to synchronize their germination, spores of *Bacillus subtilis*, Marburg strain, were heated for 20 minutes at 80°C. After growth for 6 hours at 38°C. on Difco heart infusion agar, the cells were rinsed off from the plates in a solution of 9 parts 1 per cent Difco casamino acids in distilled water and 1 part Ryter-Kellenberger fixative (19), so that the growth of the cells was stopped in liquid containing 0.1 per cent OsO_4. The Ryter-Kellenberger buffer was prepared with Mg instead of Ca ions, and with Difco casamino acids instead of tryptone; for the rest of the procedure reference is made to the original publication (19). The bacteria were spun down in the prefixation liquid and then fixed overnight with full strength reagent. After embedding in the agar medium the cells were treated in uranyl acetate solution for 1½ hour. After dehydration in graded acetone the embedding was done in vestopal W.

Thin sections were cut on an "LKB ultratome." The electron micrographs were taken with an original Philips electron microscope rebuilt after a design of Le Poole and Kramer at Delft.

OBSERVATIONS

The nucleoplasms in our 6 hour *B. subtilis* cells have a felt-work appearance (Figs. 13, 14) similar to that seen in most of Kellenberger and Ryter's illustrations (6, 7, 19). A difference between these seemingly randomly organized nucleoplasms and those with a higher degree of orderliness in fibrillar organization (5) is that in the former there is more space between the fibers than in the latter. The nucleoplasms with a higher organization in their fiber pattern therefore appear more condensed than those of the more randomly organized type.

The special feature to be described here is that the nuclear area frequently contains a body of particular structure (Fig. 6). In sections this body either looks like a package of more or less concentric rings (Figs. 2 to 4), or as an aggregate of small circular profiles (Figs. 6, 7, 8, 13, 14). That these bodies are situated inside the nuclear area is suggested by those sections of bacteria in which they are seen surrounded by nucleoplasm. But the organelles have also been observed partly or completely surrounded by the cytoplasm (Figs. 8–10, 12, 13).

The organization of the organelles is not clearly distinguishable in all pictures. In Fig. 1, on the left in the nuclear area (arrow), the lack of the usual organization of the dense material in the organelle might be due either to incomplete differentiation or to the obliquity of the section. Here the dense material seems to fuse with the more transparent nucleoplasm. Usually, however, absence of visible differentiation in these organelles is connected with the presence of very dense material, as in the center of the organelle in Fig. 2. Such diffuse dense material, for instance, can also be observed in some areas in Figs. 4, 8, 13, 14.

When the organelles appear as a body of concentric layers, the latter show a substructure of two dense borders, with a width close to 25 A, separated by a more transparent intermediary zone (Fig. 3). In the oblique section of Fig. 4, the layers are less concentric; there is part of a wave pattern bearing some resemblance to the nuclear fiber patterns observed by us in some of the normal or oblique sections through bacilli. The question may be raised whether the new elements follow the course of the original fibers.

A most intriguing organization of the organelles is in vesicle-like elements of about 250 to 300 A diameter (Figs. 6 to 8, 13, 14). Fig. 5 shows a combined pattern of concentric layers and vesicles, and we wonder whether the vesicles originate from reorganization of the layers. The substructure of the layers consisting of two dense borders and lighter intermediary zone disappears in the

FIGURE 1

Organelle material (arrow) appears in close contact with nucleoplasm (*N*). × 100,000.

FIGURE 2

In the center of this organelle there is undifferentiated dense material. × 130,000

FIGURE 3

Organelle composed of concentric layers each consisting of two dense layers with a lighter interzone. × 100,000.

FIGURE 4

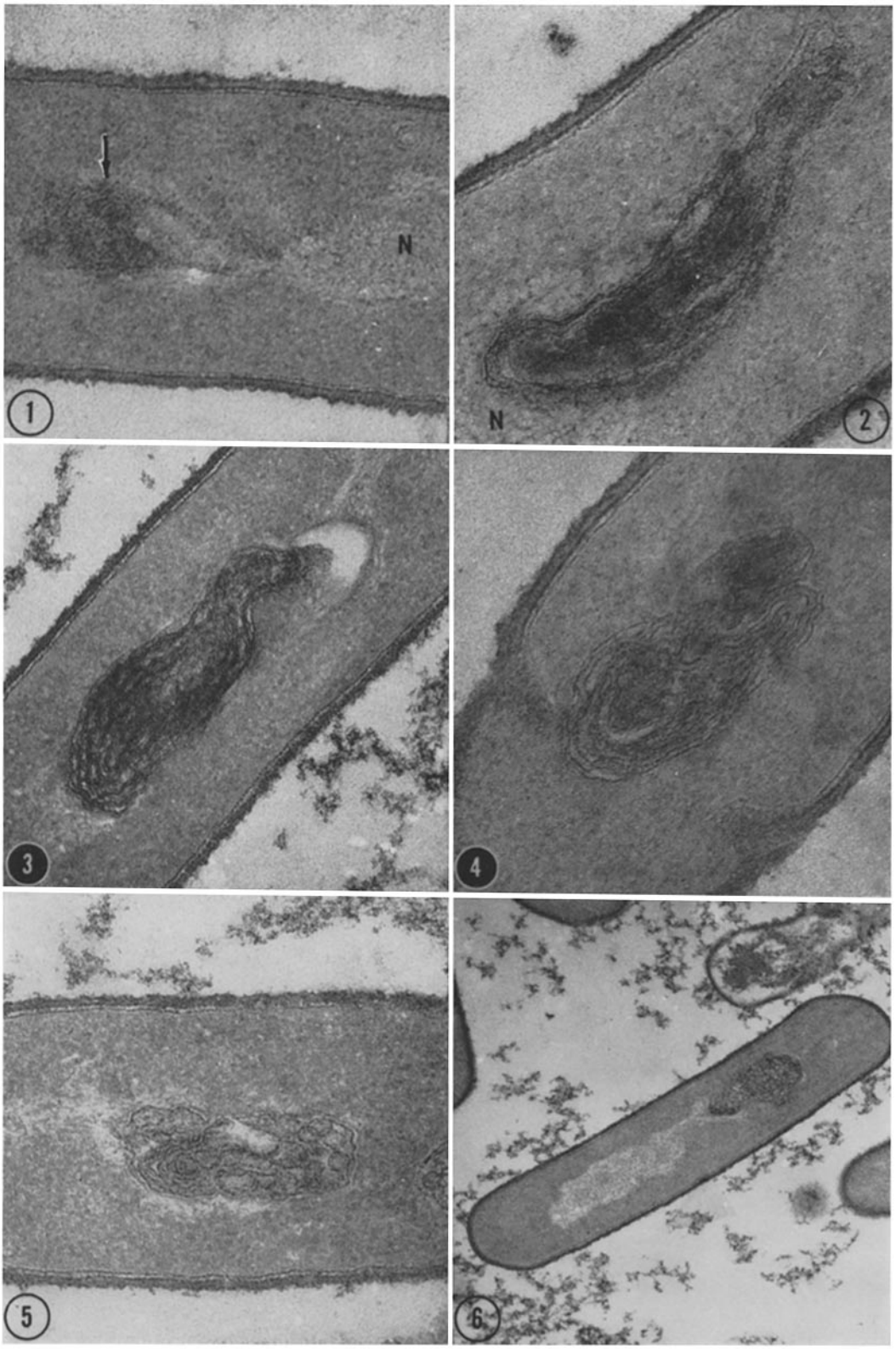
Oblique section. Note the wave pattern of the composing structures in this organelle. × 95,000.

FIGURE 5

In this organelle there appears to be a combined pattern of layers and vesicles. The vesicles (to the right) seem enveloped by a single dense sheet. × 130,000.

FIGURE 6

A low magnification of Fig. 14. Note that the outline of the comparatively transparent nuclear area extends around the organelle to the right. × 40,000.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vesicle texture, each vesicle being bordered by a single narrow dense line only. A similar delicate border seems to enwrap a whole vesicle aggregate (Figs. 5, 7, 8, 10, 12–14).

In cross-section (Fig. 7) the vesicles have essentially the same shape as in longitudinal section (Figs. 8, 13, 14). Although the aggregates of vesicles are most commonly found in the cell center, *i.e.* in the nuclear area, they can extend well beyond this into the cytoplasm. An example of the first situation is represented in Fig. 6, which at low magnification gives a survey of the micrograph of Fig. 14. Around the organelle the outline of the original nuclear area is still visible and its identification is facilitated by the latter's symmetry.

In Fig. 8 an example is given of the extension of an organelle from the nuclear area into the cytoplasm. The dumbbell shape of this organelle is somewhat uncommon. (Reference is made above to the diffuse material which typically obscures part of the organelle in Fig. 8.) In several instances such extensions from organelles in the cell center reached the cell boundary.

Suggestions as to the function of these delicate vesicles are obtained from Figs. 9, 10, 12 and in particular from Fig. 13. In all these pictures a cluster of vesicles can be seen completely outside the nuclear area and in contact with the plasma membrane or apparently even with the cell wall. In Fig. 9 a small aggregate is surrounded by membranes which extend towards, and apparently fuse with, the plasma membrane. The complete plasma membrane[1] (Figs. 7, 13, 14) consists of an about 30-A-thick inner layer which adheres to the cytoplasm and an outer layer which in the pictures is continuous with the cell wall; between these layers is an empty-looking space, while at sufficiently high resolution (Figs. 13, 14) a lighter zone can occasionally be distinguished along the cytoplasmic side of the inner layer. But in the case of plasmolysis the whole plasma membrane is freed from the cell wall and recedes with the cytoplasm. This effect can be seen in Fig. 11, where on one side of the membrane we also observe a number of bulges resembling the vesicles. The plasma membrane contains lipoprotein (9, 24) which probably possesses the property of easily giving rise to vesicles. The layers in Fig. 9 suggest that there is synthesis of membranes,

[1] Or cytoplasmic membrane (*cf.* 9).

FIGURE 7

Cross-section of an organelle composed of vesicles. The inner layer of the plasma membrane (*I*) is adherent to the cytoplasm, the outer layer (*O*) is adjacent to the cell wall (*cf.* with the situation in Fig. 11). × 120,000.

FIGURE 8

The organelle extends from the cell center into the cytoplasm. Note the diffuse dark material in the central part. × 120,000.

FIGURE 9

Adjacent to the cell envelope is a cluster of vesicles surrounded by membranous layers. The loosely layered plasma membrane at this site is different from usual (*cf.* Figs. 7, 11, 13, 14). × 120,000.

FIGURE 10

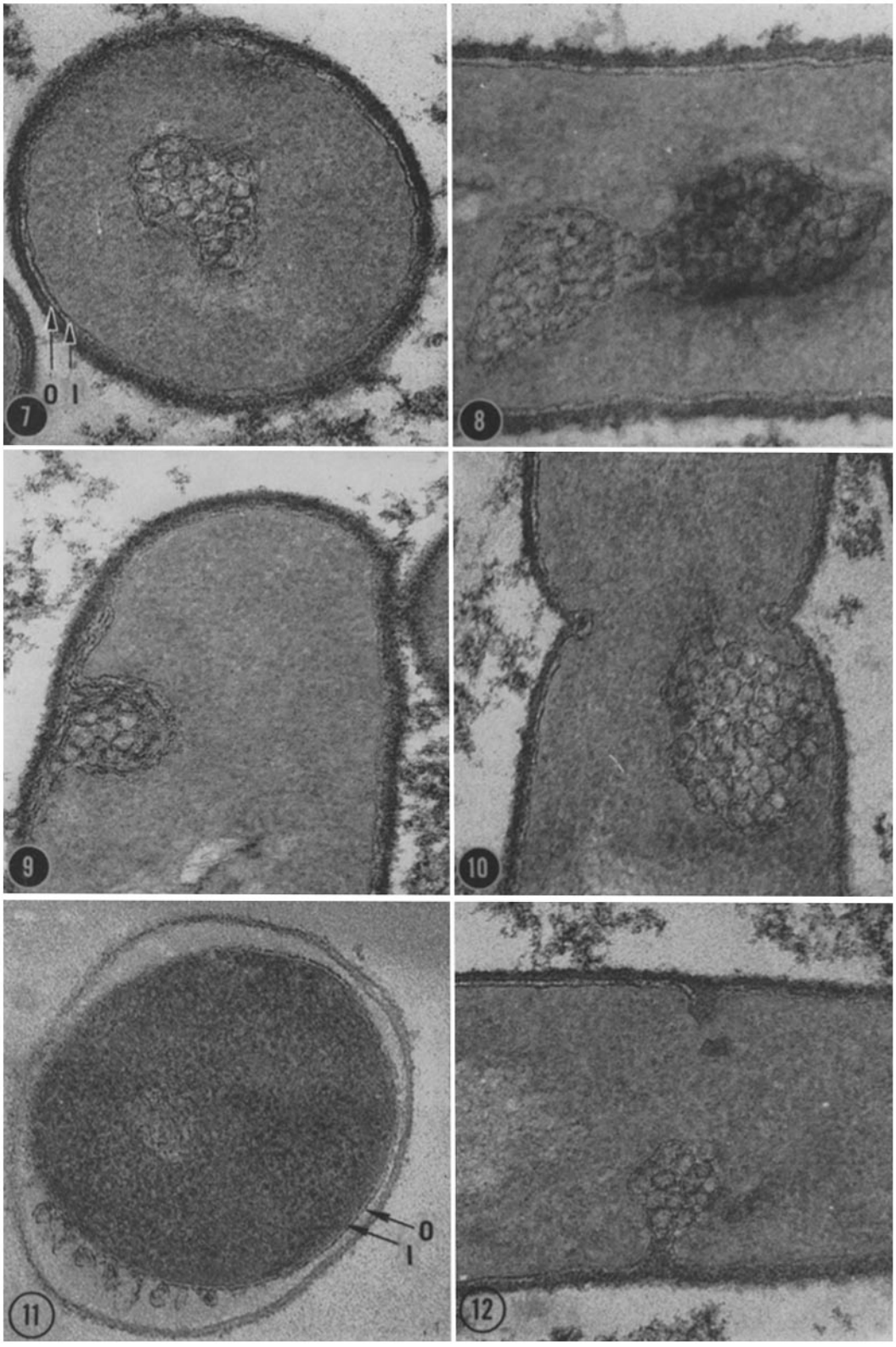
A cluster of vesicles contacts the cell wall apparently without interference of a plasma membrane. × 120,000.

FIGURE 11

On plasmolysis the complete plasma membrane recedes with the cytoplasm. × 120,000.

FIGURE 12

A small cluster of vesicles is situated on the developing cross-wall. × 120,000.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perhaps connected with extension of this presumably still growing cell, or there may perhaps be initiation of a cross-wall. In the original print of Fig. 10, however, the organelle is seen connected to the cell wall without interference of a normally structured plasma membrane.

A striking indication of the functional significance of the vesicle clusters is found in Fig. 12, where one such body is situated on the inner rim of a developing transverse septum. But Fig. 13 is most suggestive. The membrane limiting the vesicle aggregate is continuous with the plasma membrane and the finely granular material of the ingrowing septum abuts directly against the vesicles.

The vesicle cluster on the cross-wall in Fig. 13 seems connected by a vague transition (arrow *A*) with the main aggregate of vesicles in the central area of the cell, and the latter aggregate appears to be in contact with some nucleoplasm (arrow *B*). The fine structure of the vesicles can particularly be studied in Fig. 14, where they show a striation, sometimes resolved as points, the delicacy of which can be appreciated by using the 30-A-wide inner layer of the plasma membrane as reference.

DISCUSSION

The fact that in a number of random sections of bacilli from actively growing cultures[2] a cluster of vesicles is found against the cell envelope would seem to suggest that these organelles function in the process of cell wall growth. This supposition is strongly supported by the finding of vesicular aggregates in close association with developing septa (Figs. 12, 13), and the conclusion that the vesicles may actually build the cell wall material seems hardly escapable when a gradual transition is observed between the fine structure of the new cross-wall and of the adjacent part of the vesicle cluster (Fig. 13). However, if we assume that the organelles are active in cell wall formation, two points remain unexplained: in the first place the bodies have not been found regularly on the cross-wall of other investigated species of bacteria (see below), and secondly, they seem to occupy only part of the rim of the annular septum since they are not seen in the septum area opposite the body (Figs. 12 and 13). But when such an organelle is present on the septum, it is enwrapped with the growing cross-wall in a common boundary, which seems identifiable as the inner layer of the plasma membrane, whereas the dense outer layer was not seen to extend very far into the peripheral body (Fig. 13). The plasma membrane is known to be rich in various enzymes (9, 24). If the body on the annular septum is active in the synthesis of cell wall material it may perhaps move around on the ingrowing septum.

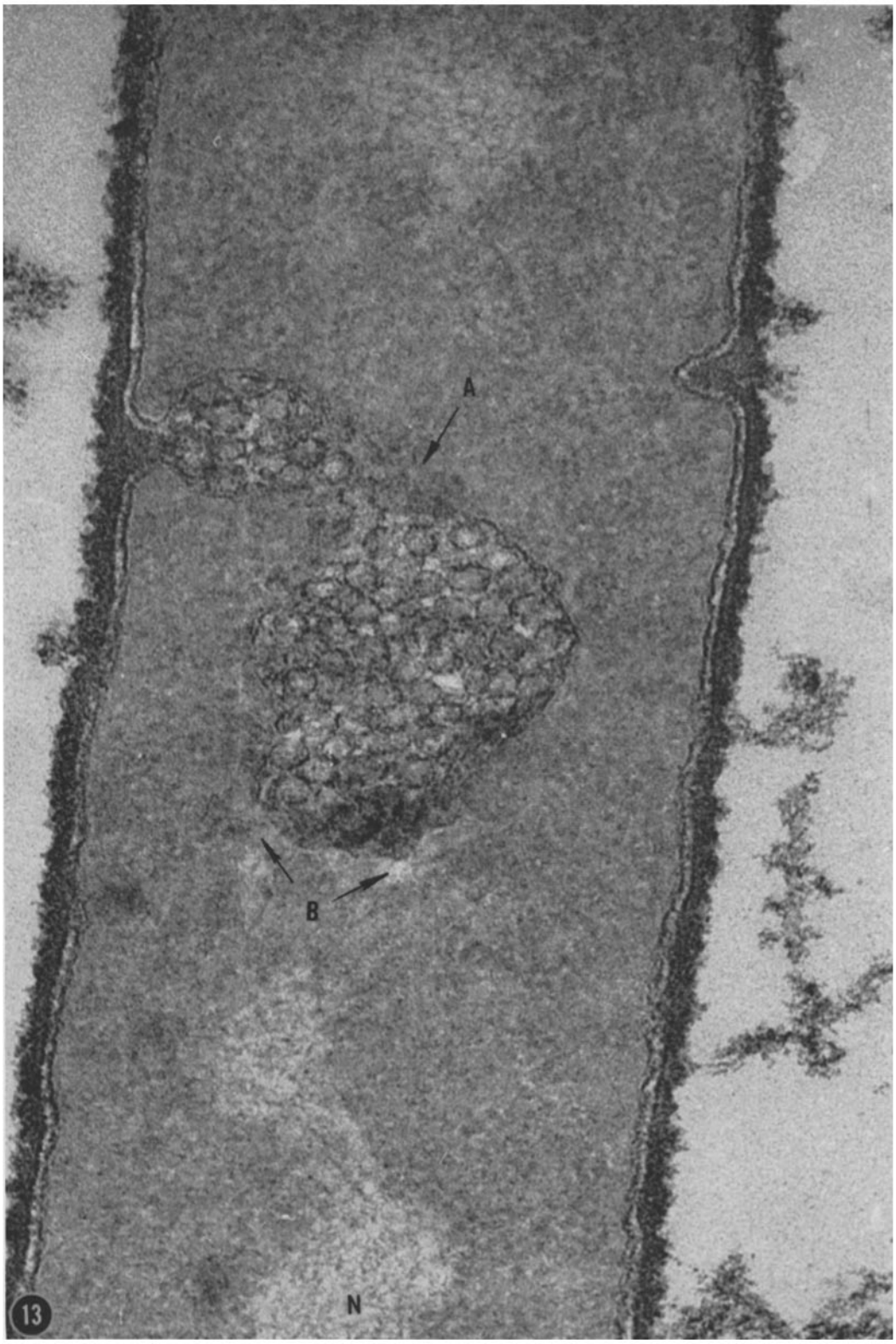
Chapman and Hillier (2) were the first to describe from electron micrographs "peripheral bodies" on the annular discs of transverse walls. These bodies were, however, preserved in their *B. cereus* material as empty vacuoles with a coagulate of dense material. In respect to these peripheral bodies, Salton made some suggestions which agree remarkably well with part of our above interpretation, and in addition he points to the possibility that the bodies may "possess the specific mechanisms for polymerization of low molecular weight components into the insoluble cell wall structure" (20).

The bodies composed of vesicles may originate from the concentrically layered organelles (Fig. 5) which we found in the cell center. Comparisons can possibly be made between the organelles from our study and the mitochondria-equivalents exhaustively reviewed by Mudd (11–13, *cf.* also 8, 16), or with "accessory chromatic granules" (17), but at this stage of research this seems hardly

[2] Studies have been made of spore germination and the resulting vegetative cells up to 6 hours' incubation time at 37°. Starting from the onset of development of vegetative rods the presence of organelles proved a regular feature in this strain of *B. subtilis*.

FIGURE 13

The vesicle aggregate on the new septum seems to be integral with the latter since both are enwrapped in a common boundary. On the side of the wall this boundary is the inner layer of the plasma membrane. The outer layer can be seen (in the original print) to extend into the adjacent area of the organelle. The fine structure of the new wall also continues inside the adjacent part of the organelle. At *A*, transition between the two aggregates of vesicles. At *B*, contact with nucleoplasm. × 200,000.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profitable. An electron micrograph of *B. subtilis*, taken by Ryter and Kellenberger (19, their Fig. 10), shows a "chondrioîde" which somewhat resembles the organelles in Figs. 2, 3, 4. The "two unidentified structures" which Tokuyasu and Yamada (22) observed in *B. subtilis* must also be identical with the organelles described here. The present more general application of the Ryter-Kellenberger technique is bound to reveal the organelles in many more bacteria (*e.g.*, Fitz-James (3), Murray (14, 15)). But it remains an open question why the presence of similar organelles cannot be established for all species of bacteria. We never noticed any structure of this order in two types of spherical bacteria (the so called coccus-C and the *Mycoplasma* cocci) (5), whereas in *Micrococcus albus* concentric membrane systems seemed frequent in the cytoplasm (unpublished).

A point of additional interest is the apparent great versatility in the organization of organelles from various bacteria on application of the same technique. Those observed by us in *B. cereus* (18) seemed somewhat different from the organelles in *B. subtilis*. In the cytoplasm of hyphae of *Streptomyces coelicolor* Glauert and Hopwood not only detected "membranous bodies" but in addition an extensive membrane system continuous with these and with the plasma membrane (4). The authors suggest that the membranes have some features in common with those of the endoplasmic reticulum of mammalian cells.

In higher organisms an intimate relationship is found between the membranous component of the endoplasmic reticulum and the nuclear envelope, which Watson considers to be a specialized element of the cytoplasmic membrane system (23). From the electron micrographs of bacterial nuclei (5) we are confident that in these primitive organisms there is no membranous component to delimit the nucleoplasm from the cytoplasm. The question arises whether the organelles observed in the nuclear area are of cytoplasmic or of nucleoplasmic origin. The organelles might be formed for instance by the plasma membrane and then move into the nuclear area. But when spores germinate organelle systems are sometimes already fairly extensive in the center of the first vegetative cell before the initiation of the first transverse wall. Therefore, even if organelle material should have moved into the nucleus, it may develop further in this area. The possibility that the structures develop between the fibers containing the deoxyribonucleic acid (7, 5) would appear of general interest in view of the present theories on the replication of the genetic code and the latter's expression in the synthetic activities of cells.

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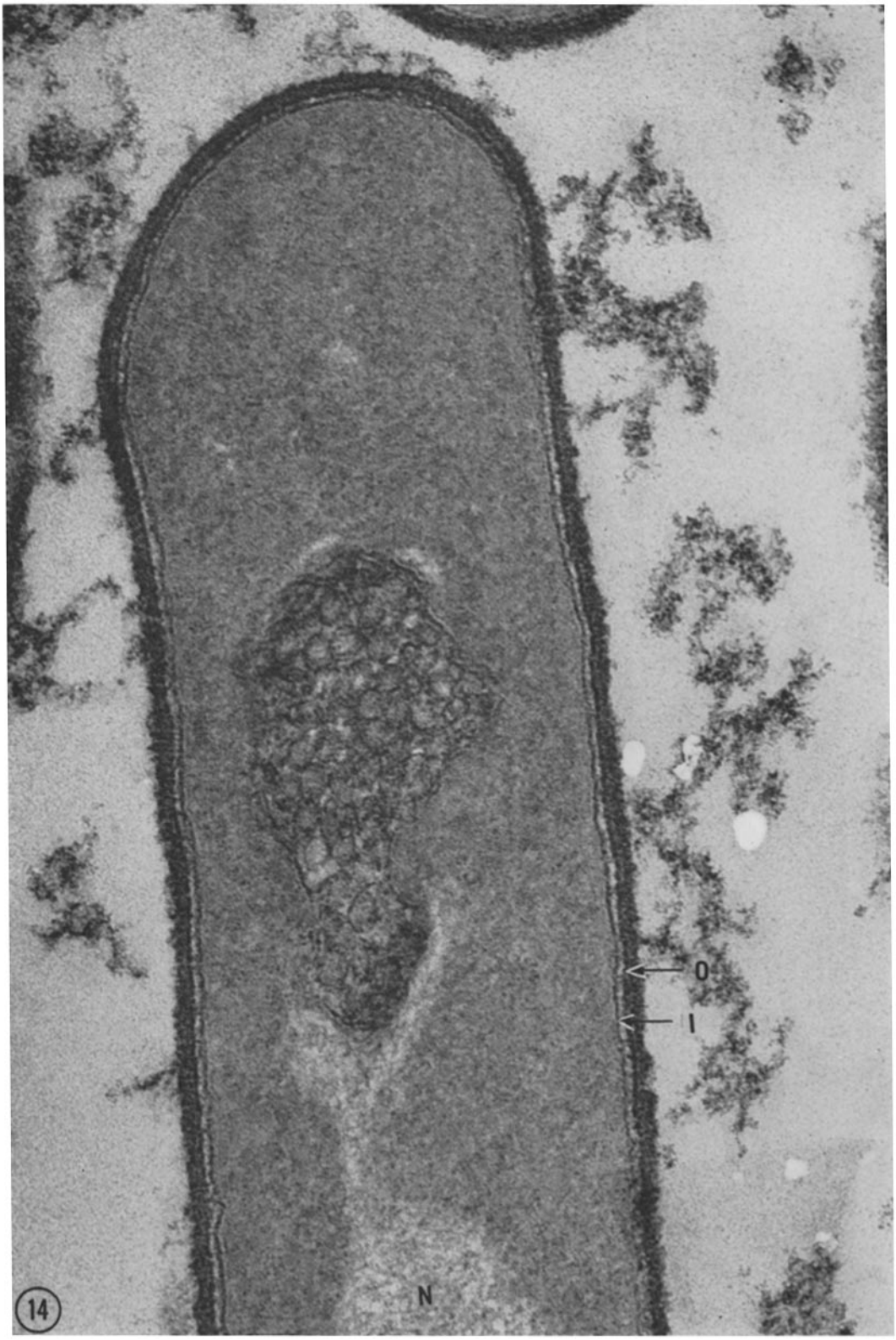
FIGURE 14

Over the vesicles is a striation the delicacy of which can be judged by comparison with the 30 A inner layer of the plasma membrane. The outer plasma layer is adjacent to the cell wall proper. × 200,000.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