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Structure of *Nitrosocystis oceanus* and Comparison with *Nitrosomonas* and *Nitrobacter*[1]

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ABSTRACT

MURRAY, R. G. E. (University of Western Ontario, London, Ont., Canada), AND S. W. WATSON. Structure of *Nitrosocystis oceanus* and comparison with *Nitrosomonas* and *Nitrobacter*. J. Bacteriol. **89**:1594–1609. 1965.—*Nitrosocystis oceanus* has distinctive features: the cell wall (overall thickness, 250 A) has an inner triplet structure and a dense enveloping layer; between these lie the "cell-wall organelles" (two or more per cell; plaques about 0.5 μ in diameter and 0.1 μ thick) of unknown function and genesis. The plasma membrane (ca. 80 A) shows rare intrusions that form irregular peripheral vesicles, which appear to form the component lamellae of the "membranous organelle" and probably detach from the periphery. The membranous organelles consist of about 20 vesicles so flattened that the lumen is only 100 A thick. The outer surfaces are in contact and form a triplet structure with an accentuated center line; these lamellae almost traverse the cell, displace the cytoplasm and the nucleoplasms, and form the prominent, seemingly permanent, feature of the cell. Division is constrictive without trace of a septum, and the act of division divides the membranous organelle. No mesosomes appear to be formed. *Nitrosomonas europaea* shows no sign of a cell-wall organelle or of the outer enveloping layer of wall. The cytoplasm contains intrusive paired lamellae, which might or might not remain connected to the periphery, and they do not fuse or form regular associations. These are thought to be the equivalent of the vesicles in *Nitrosocystis* but remaining almost parallel and close to the plasma membrane. *Nitrobacter agilis* has a unique plasma membrane with a (50 A) dense layer applied to the inside of the usual unit membrane. All of the components are represented in the intrusions, which are arranged over and shape the poles of the cells, with close and regular spacing. Each nitrifier was distinctive; in common they have membrane systems which, it is considered, must relate to the specialized mechanisms for acquiring energy adopted by these organisms.

Nitrosocystis oceanus is a nitrifier ($NH_4^+ \rightarrow NO_2^-$) obtained from the open ocean (Watson, 1962). It has some structural features extraordinary among the bacteria so far described (Murray and Watson, 1963; Murray, 1963), but it is a bacterium, as shown by the kind of flagella and the procaryotic organization (Stanier and van Niel, 1962; Murray, 1962) of the cell. It has been placed in the genus because of features that are strongly reminiscent of *Nitrosocystis* (Winogradsky, 1931), which was obtained from soil samples. *N. oceanus*, however, appears to have the physiological attributes of a marine organism (Watson, 1962). This paper is intended to survey the structure of this organism and to provide a comparison with other and better-known bacteria involved in nitrification.

[1] Contribution no. 1604 from Woods Hole Oceanographic Institution.

MATERIALS AND METHODS

Origin of the organism. The bacterium has been maintained in the Marine Bacteriology Section of the Woods Hole Oceanographic Institution since its isolation in 1960.

Methods of cultivation. Shake flask cultures at 15 C, with daily *p*H adjustment to 7.8, gave populations of 2×10^7 cells per milliliter in 30 to 60 days. The medium was seawater enriched with 0.1 M $(NH_4)_2SO_4$ and other salts. NO_2 was approximately 0.1 M at harvesting. Cells were also produced in fermentors with automatic *p*H adjustment (Watson, Limnol. Oceanog, *in press*).

Light microscopy. Observations in the living state were made by phase and bright-field microscopy. Mounts in 20% gelatin (Mason and Powelson, 1956) in seawater aided internal differentiation in the former.

Electron microscopy. Cells were fixed with osmium tetroxide and embedded in Vestopal follow-

ing a modification of the methods of Ryter and Kellenberger (1958).

For "prefixation," 10% (v/v) of fixative solution was added to the culture. The suspension was then centrifuged at 5 C, and the pellet was taken up in a small volume of melted 2% Noble Agar (Difco) in Michaelis buffer at 50 C. The agar containing the cells was cut into minute blocks and returned to the fixation fluid for overnight (16 to 20 hr). The blocks were then transferred to 0.5% uranyl acetate for 2.5 hr. Dehydration was accomplished by transfer through a graded series of acetone solutions (30, 50, 75, 90, and 100%, two changes of each at 15-min intervals); then the blocks were permeated with increasing concentrations of Vestopal in acetone, with at least an overnight stay in 75% Vestopal. The polymerizing mixture was added to the blocks in gelatin capsules; polymerization proceeded at 60 C for 2 days.

Sectioning was accomplished with glass knives on a Porter-Blum microtome. The sections were stained by floating the grids on 1% uranyl acetate; no advantage was found with other staining processes (lead and other metal salts).

Prefixed cells were placed on grids, washed, and shadowed with tungsten oxide at a 3:1 angle.

Electron micrographs were taken with a modified Philips EM-100 electron microscope, with 1.8-bore pole pieces, 25-μ objective aperture, Ladd anode, mechanical astigmatism compensator, and accessory prestabilization of the input. Recording was on Kodak "fine-grain positive" film at initial magnifications of 5,000 to 15,000 $\times$ on the film.

RESULTS

General morphology of N. oceanus. The cells observed were fairly large (about 2 μ) and slightly ovoid, but this was not a regular form.

The majority of dividing cells in cultures showed a narrow isthmus between individuals (Fig. 1). In some cases, cells were found in tetrad form and gave the appearance of septum formation (Fig. 2). It was presumed that the division habit was dependent upon the growth rate, which was never fast by ordinary standards. (The division time in these experiments was 24 to 48 hr.) Precise correlation of shape, form, and habit with nutritional state was not possible in the flask cultures used for the majority of the observations reported here, and later experience with fermentation vessels has been confused by masses of cells adherent to parts of the apparatus and by indefinable local conditions.

An internal organization of these cells was not obvious; they were quite refractile and seemingly homogeneous in the light microscope in seawater mounts, whether or not phase contrast was used. However, some showed a distinct barlike structure traversing the cell; this was more commonly seen in old flask cultures or in chemostat cultures of low nutrient concentration. In many cases these must have been dead or dying cells, because exposure to any killing agent, "fixative" or not (e.g., osmium tetroxide, Lugols' iodine, and formaldehyde), made visible this sizeable structure in the cell (Fig. 3, 4). Exactly this sort of structure could be seen in virtually all living cells by mounting them for phase microscopy in 20% gelatin or in horse serum (Mason and Powelson, 1956) to reduce the flare and allow the internal detail to be seen (Fig. 5, 6).

The regular demonstration of this strange structure was indicative of something real. Occasionally the "bars" gave the impression of striae along their length. They were usually oriented along the length of a cell preparing to divide (Fig. 6); in other cells, the bar, occasionally double, could be curved or slightly S-shaped (Fig. 5).

The fixed cell was moderately basophilic and the margin of the transverse bar was more intense in staining. The cytoplasm on each side of the bar had areas of lesser staining which corresponded to the areas giving the staining reactions of chromatin bodies. These seemed to reflect the usual cytoplasmic organization of bacteria (Murray, 1960).

As has already been described (Watson, 1962), living cells were motile. They swim by means of a single flagellum of bacterial form or a tuft of a limited number of flagella seen in shadowed preparations. No other regular projections from the cell were observed, except in the early (and less satisfactory) cultures when a proportion of cells seemed to show a slight "beak" or protuberance, making the already slightly asymmetrical ovoid more extreme. Later cultures did not show this.

Fine structure. Embedding of excellent quality was obtained without difficulty. Attempts were not made to find other fixation conditions that would be as good or better. It seemed probable that prefixation in the seawater medium with one-tenth volume of the usual buffered 1% osmium tetroxide employed by Ryter and Kellenberger (1958) prepared the cells for the subsequent steps, enrobing in agar, and fixation with only Michaelis buffer as diluent. As is the case with many marine organisms (MacLeod and Matula, 1962; Brown and Turner, 1963), the living cells disintegrated on dilution of the medium to less than 2% sodium chloride; the conditions of prefixation probably altered permeability and preserved structure to the point that "standard" fixation could be undertaken.

A general view of sections showed (Fig. 7) that the cells were bounded by a well-defined plasma membrane and contained within a multilayered cell wall. Most sections showed a portion

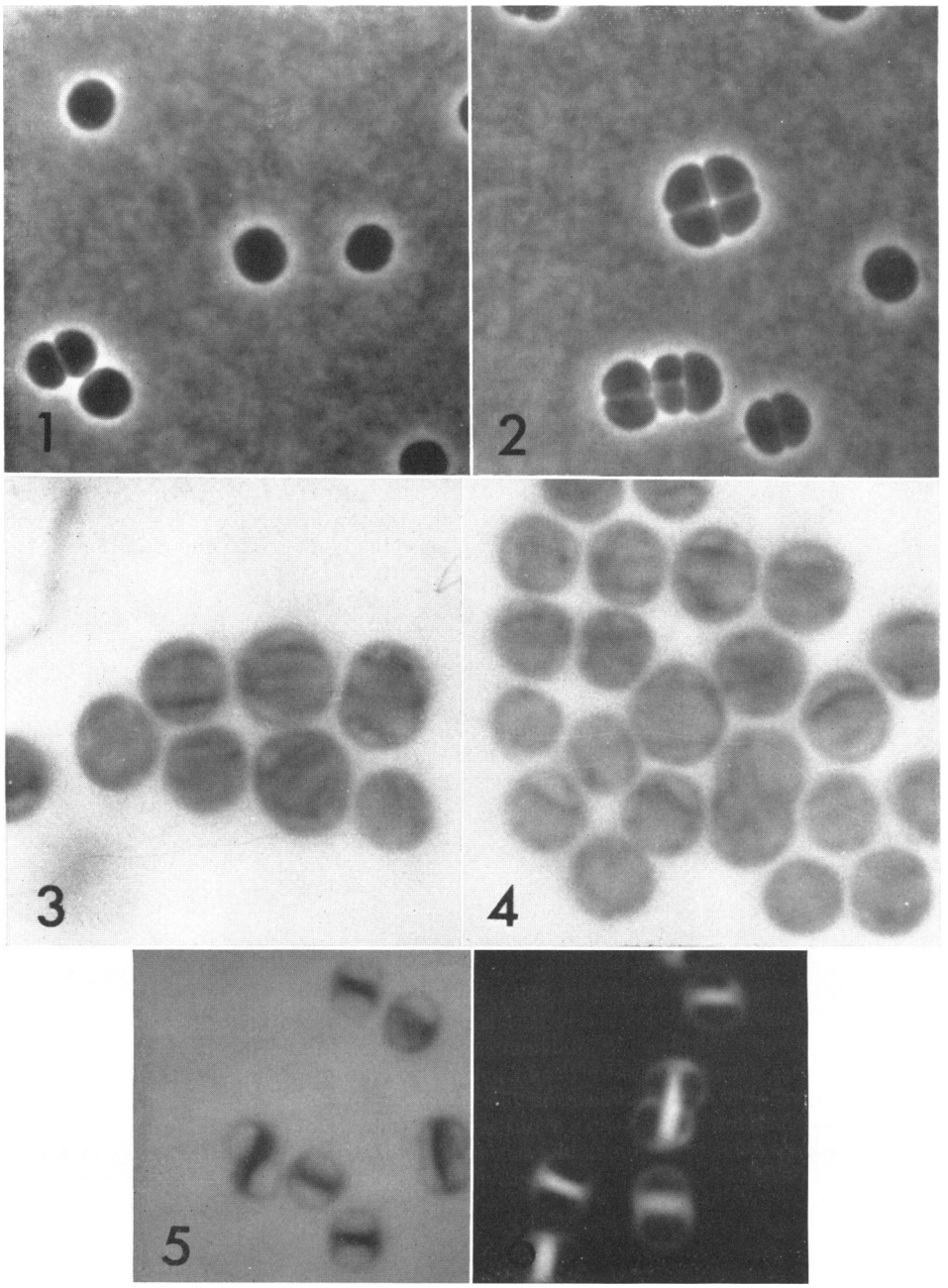
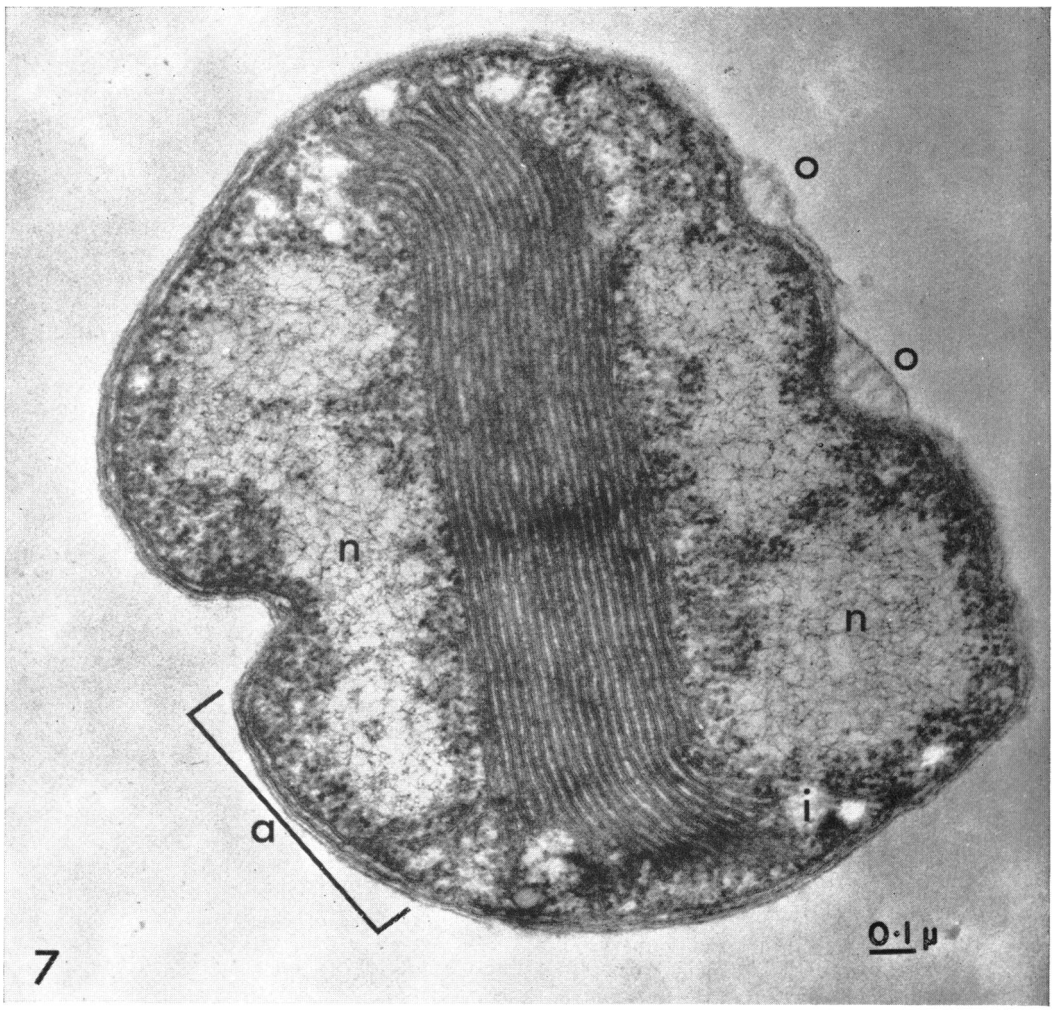


FIG. 1 and 2. *Phase photomicrographs of living cells suspended in seawater, showing single cells, diploids, and tetrads.* × *3,000.*

FIG. 3 and 4. *Photomicrographs of cells fixed with Bouin's fixative and stained with dilute thionine. The basophilia seems to be concentrated in the region of the cell border and, particularly, along the sides of the membranous organelle. The striations may be due to penetration of cytoplasm bearing ribosomes in between some lamellae, as in Fig. 18.* × *4,800.*

FIG. 5 and 6. *Phase photomicrographs of living cells suspended in seawater plus 20% gelatin, showing presence of cytomembranes.* × *4,500.*



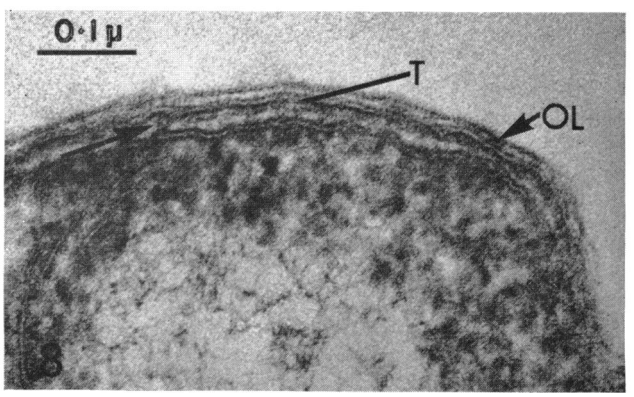


FIG. 7. *General view of a section of Nitrosocystis oceanus, showing the main anatomical features. An area (a) showing the enclosing wall and plasma membrane is enlarged in Fig. 8. Two examples of the wall organelle (o) are visible. Within the boundary are nucleoplasms (n) enclosed by ribosome-packed cytoplasm. Traversing the whole cell are the lamellae of the membranous organelle. The cytoplasm contains some unidentified low-density inclusions (i).*

FIG. 8. *Higher magnification of part of Fig. 7, showing the components of the wall outside the arrow. OL, outer enveloping layer; T, the triplet layer. Inside the gap indicated by the arrow is the plasma (cytoplasmic) membrane. Some traces of intracytoplasmic membrane show at the left.*

of one or more cell-wall organelles (Murray and Watson, 1963). Flagella were visible very rarely; the "polar membrane" (Murray and Birch-Andersen, 1963), characteristic of bacteria with tufts of flagella, was not observed. The most obvious feature of all cells was a stack of closely set membranes (often about 20) traversing the cell. This membranous organelle extended from near, but was not obviously continuous with, the plasma membrane on each side. The cytoplasm contained a fair concentration of ribosomes lying in a matrix of slight density. They did not seem to be of unusual size (120 to 150 A) nor did they show unusual arrangement. It was noted, however, that the ribosomes did not penetrate between the lamellae of the membranous organelle. Within the cytoplasm were prominent areas lacking ribosomes and resembling in all ways the nucleoplasms of bacterial cells, including the lack of a boundary membrane (Kellenberger, Ryter, and Séchaud, 1958). The disperse and disoriented fibrils of the nucleoplasm indicated that either the nucleoplasm of these organisms was particularly dilute or, more likely, the conditions were not well adapted to the best preservation of the nucleoplasm (Kellenberger, 1962). The cytoplasm also contained a few areas of very low density that resembled the lipid (Murray, 1960) or glycogen (Cedergren and Holme, 1959) inclusions of other bacteria; we have not made an attempt to identify them.

A closer view of the cell wall indicated an arrangement slightly unusual among gram-negative bacteria and additional to the extraordinary cell-wall organelle (Murray and Watson, 1963; Murray, 1963). The apparent overall thickness of the cell wall, excluding the plasma membrane and the gap between plasma membrane and the inner element of the cell wall, was of the order of 250 A and consisted of at least four distinct dense layers separated by three layers of lower density (Fig. 8). The layers seemed to be bound together, because they usually retained their spacing over the whole cell surface. In a few places the inner three dense layers were united but thrown into small folds and the outer layer was drawn taut over them, indicating the possibility of separation at this plane. The main discontinuity was provided by the insertion of the cell-wall organelle, which was always found to occupy a space (normally 60 to 100 A) between the outermost layer and the underlying layers (Fig. 9, 15). There were no new observations to add to the existing description (Murray and Watson, 1963).

The three inner dense layers ran parallel and together as a triplet structure (about 160 A, about equally divided but with dense central line) of an appearance resembling the now revised profile of the cell wall of *Escherichia coli* (Murray, Steed, and Elson, Can. J. Microbiol., *in press*) and many other gram-negative bacteria (Claus and Roth, 1964). The innermost layer may then correspond to the "intermediate layer" or mucopeptide layer of other organisms (Murray et al., *in press*). The spacings are shown as approximate measurements in the diagram (Fig. 15).

The plasma membrane, overall thickness about 75 to 85 A, showed the usual (*see* Robinow, 1962) clearly delineated pair of dense layers separated by a zone of low density, but it seemed a bit thicker than in most bacteria. The membrane appeared to be entire and free from cytoplasmic intrusions (Murray, 1963) in virtually all the cells examined. One exceptional area, shown in Fig. 9, could have been an artefact, but it seemed more reasonable to consider that it represented a rare view of the origin of the unit membranes (80 A) found sparsely distributed at the periphery. The latter were often observed to lie close to the inner side of the plasma membrane, and even serial sections usually did not help in tracing the connections due, in large part, to the extreme difficulty of showing these membranes unless they were cut very nearly normal to the surface. On occasion, several individual layers of unit membrane might be found in the first 500 A of cytoplasm. These appeared to be single membranes (Fig. 8), often paired but not highly oriented with respect to each other, and the cytoplasmic components (e.g., ribosomes) were not found between them.

The membranous organelle consisted of a stack of highly oriented triplet membranes. There was strong direct evidence that some of the peripheral unit membranes were continuous with the organelle (Fig. 10), and undeniable evidence (Fig. 11) that the triplet consisted of two unit membranes (each 80 A) so closely joined that the contiguous layers formed the slightly more dense central element. The spacing between triplet membranes, although remarkably regular in many cases, was capable of disruption (Fig. 12). But even in this case of dislocation, the cytoplasmic particles were excluded from the area of the organelle.

A variant type of organization was seen in some cells (Fig. 13) which showed evidence (lack of cytoplasmic density, few or fragmenting ribosomes, and relatively low density of the dense parts of the organelle) of being in a different physiological state to that exemplified in Fig. 7, 10, and 12.

The two types of organization were compatible with a straightforward interpretation based on the observation that the lamellae must be formed of flattened vesicles with contents that may be

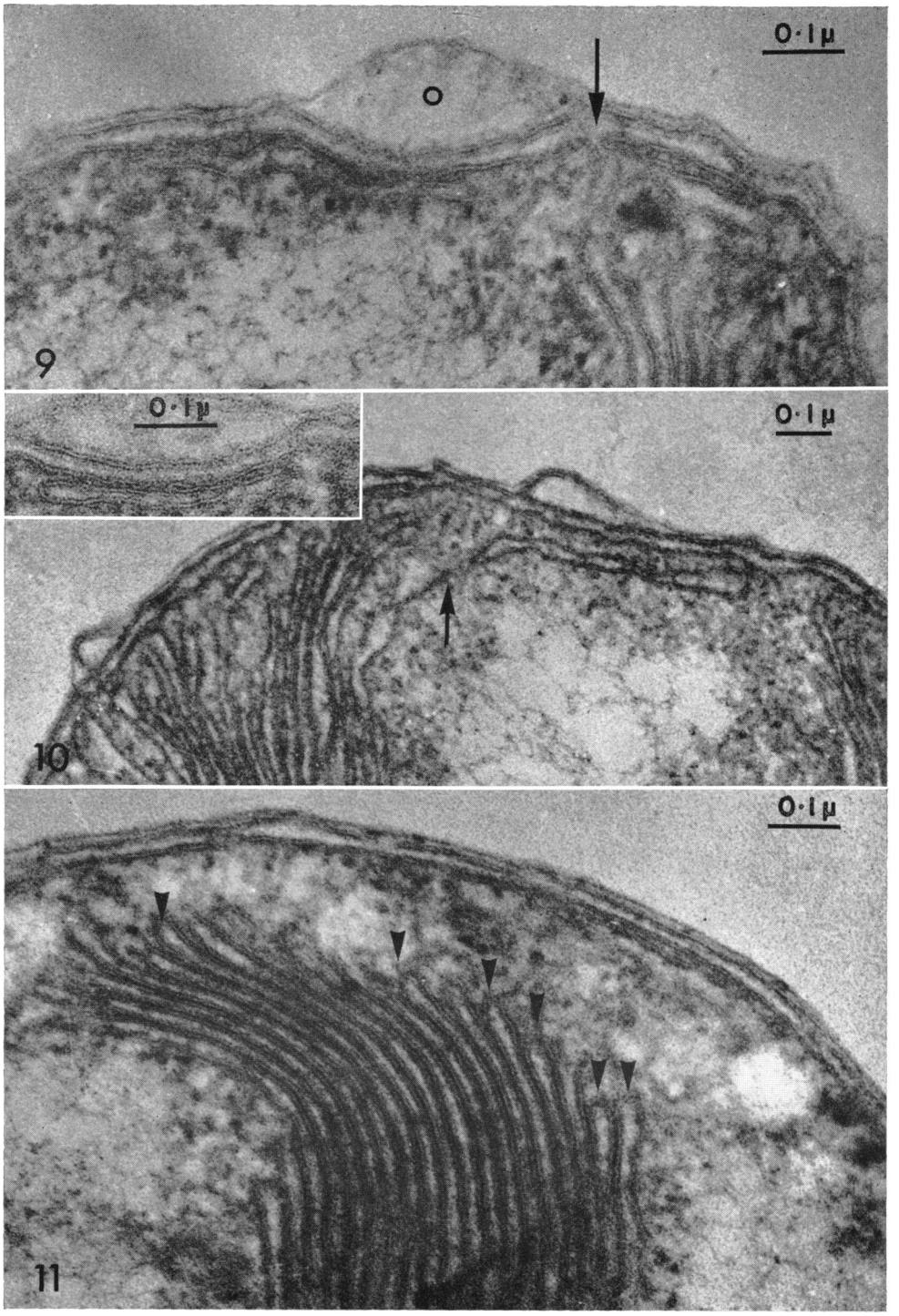


FIG. 9. *Section of Nitrosocystis oceanus, showing a cell-wall organelle (o) between the components of wall. At the arrow is an example of an intrusion of plasma membrane.*

FIG. 10. *Section showing evidence for the peripheral origin of the membranes in the membranous organelle. Neither this nor other profiles of the periphery of the organelle show direct continuity. Such continuity as there is seems to be with less (or not yet) organized bits of membrane, as at arrow and in Fig. 9. This is an enlargement of one end of Fig. 18. × 107,000. Inset shows a flattened vesicle next to the plasma membrane.*

FIG. 11. *Profile of the membranous organelle, showing the continuity of membranes from one lamella to the next. The loops are marked by arrows.*

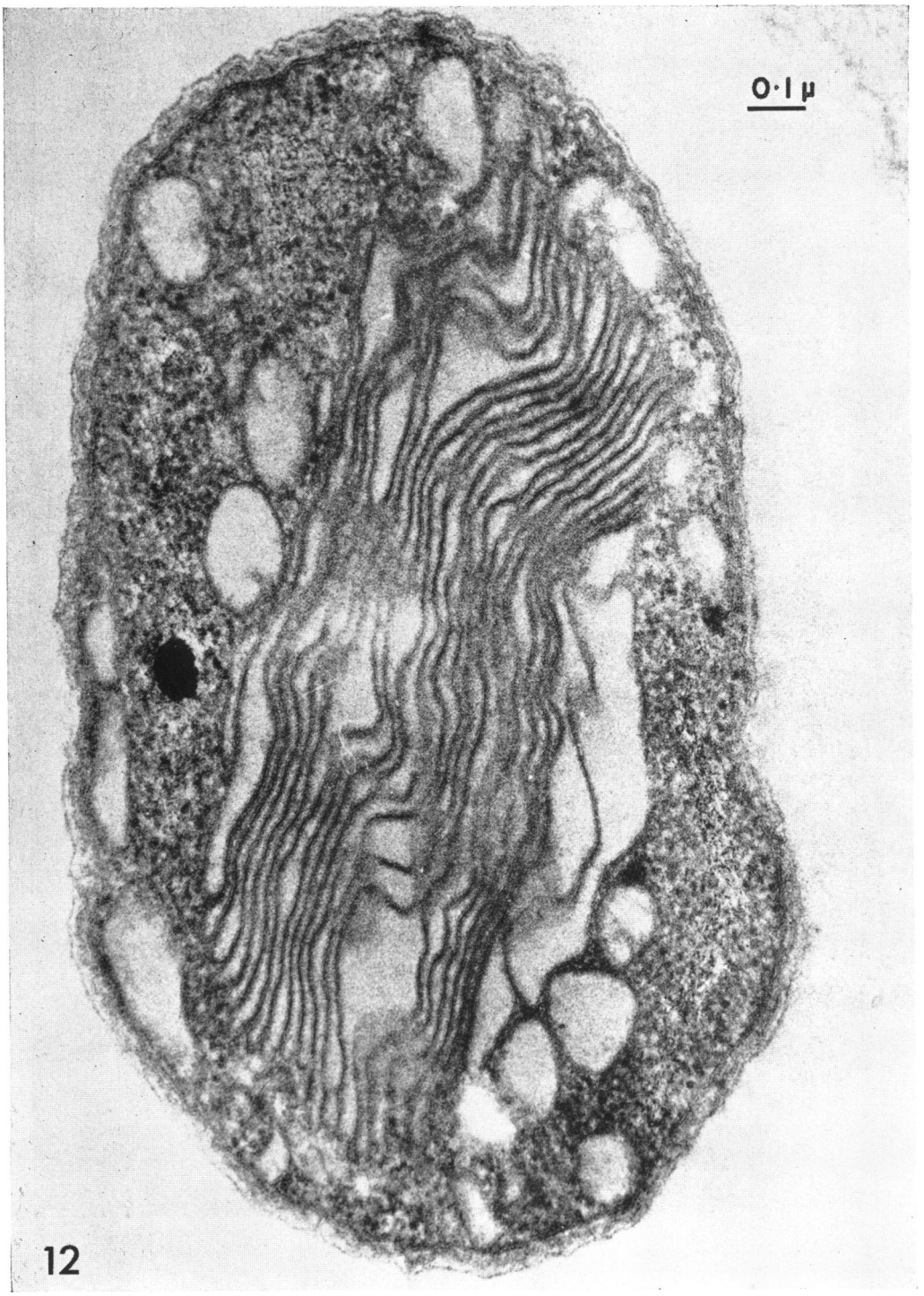


FIG. 12. *Section of Nitrosocystis oceanus in a physiological state that has caused many peripheral vesicles, as well as those in the organelle, to be swollen. The appearance justifies the view that the organelle consists of closely contiguous flattened vesicles and that these are not different in kind from those near the periphery.*

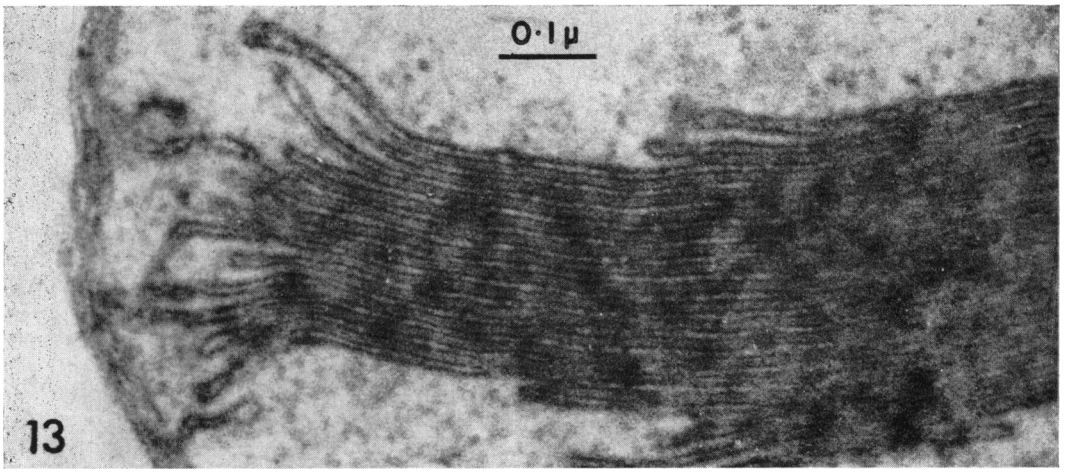


FIG. 13. *Variant appearance of the organelle. One component of the membrane seems to be accentuated. The triplet structure shows where membranes of adjacent vesicles touch, so it is directly comparable with Fig. 11.*

excessive in amount (Fig. 12), or be evenly distributed to separate the elements in a regular fashion (Fig. 7), or be reduced to the point that the membranes of the vesicles made contact (Fig. 13).

The vesicular nature of the lamellae was traced on three different micrographs that fortunately showed clearly enough the full length of the organelle. The outsides of one flattened vesicle were in contact with the outer surface of the next vesicle so closely that a triplet structure formed. It was not surprising then that ribosomes did not appear to penetrate the organelle. The variant forms shown in Fig. 7, 12, and 13 maintained this arrangement, so that the actual difference between the membranous organelles in the various states was considered to be in the volume, distribution, and perhaps the nature of the contents of the vesicle (Fig. 14).

The origin of the vesicles forming the organelle seemed to be the plasma membrane, despite the extreme rarity of traceable intrusions of that structure into the cytoplasm. However, as already pointed out, intrusions were seen (Fig. 9) and considered as the origin of the peripheral membranes. These latter, when seen, were often paralleled at varying distance by another membrane. Serial sections of such a pair showed that some of these were indeed vesicles, probably fully comparable with those of Fig. 12 and already equated with the individual elements of the organelle. So our view (*see* Murray, 1963) of cell division appeared to involve constriction of an enlarged cell into halves (Fig. 7). No septa were seen in electron micrographs. However, appearances such as that in Fig. 2 were a warning that some sort of septum might be expected. In view of recent experience with septum formation in *E. coli* and *Spirillum serpens* (Steed and Murray, *in preparation*), we could not be confident of the observations.

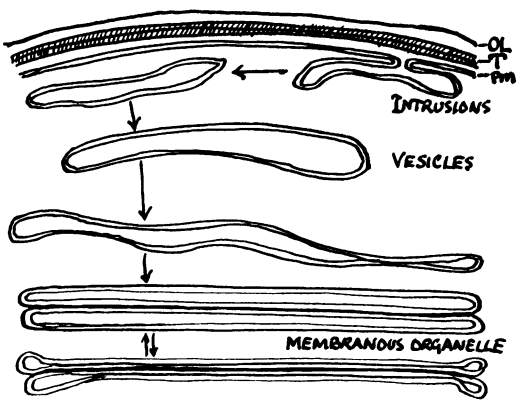


FIG. 14. *Schematic drawing illustrating the relation of the plasma membrane to vesicles and to the structure of the membranous organelle of Nitrosocystis oceanus.*

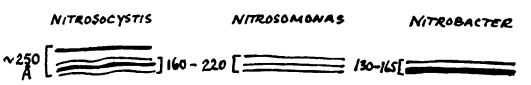


FIG. 15. *Diagram summarizing the structure and dimensions involved in the cell wall of Nitrosocystis oceanus, and comparison with walls of Nitrosomonas and Nitrobacter.*

The interpretation of structure and arrangement of membranes to form the membranous organelle has been summarized diagrammatically (Fig. 14). The same has been done for the cell-wall structure (Fig. 15).

The membranous organelle was so closely knit that we were curious to find out what happened to it during division. Light microscopy showed clearly that the organelle was oriented along the axis of the dividing pair, and there was every indication that the organelle was divided by the fission of the cells, making this the first persistent organelle of bacteria. The direct observations of living cells was supported by the appearance (Fig. 16 and 17) in the electron microscope (Murray, 1963). Rarely, there seemed to be a double bar in a single cell, and a rare electron micrograph showed the same appearance (Fig. 18); in this case, partition of one element to the sister cells seemed to be possible, but light microscopy indicated that this should be a rare event.

More mysterious was the fate of the cell-wall organelle in division and the genesis of that organelle. Nothing adding to our previous description (Murray and Watson, 1963) has been found, and we have not been successful in finding a way of demonstrating these elements in living cells so that their activities and fate could be defined.

Nitrosomonas europaea. This well-known nitrifier showed a different organization despite the physiological correspondence of activities with those of *Nitrosocystis*. The ovoid cell showed (Fig. 19) a simplified cell wall; a cytoplasm containing a few concentric membranes at the periphery of the cell, enclosing the majority of the protoplasm; a scattering of low-density inclusions (reserve substance); and well-defined procaryotic nucleoplasms.

The *Nitrosomonas* wall (inset, Fig. 19) consisted of a tripartite element (160 to 220 A) entirely similar to the inner part of the *Nitrosocystis* cell wall. There was no sign of the outer layer or of the associated cell-wall organelles that were so prominent in the latter.

The membranes in the cytoplasm showed the same spacing as the plasma membrane. There was evidence, again not abundant, that the cytomembranes were formed by intrusion from the surface (Fig. 20). Ribosomes were not prominent in the best of the preparations of this organism, so that decision on which surfaces represented the inside and which the outside of the intrusion was hard to make. In most places there were only one or two pairs of membranes at the periphery. These were not tightly packed; for this reason, no 'triplet' membranes were seen, and the resemblance to *Nitrosocystis* seemed slight. Another embedding did show some regularity and showed that cytoplasmic particles were not in the interstices of the intrusion (Fig. 21). Only rarely were any membranes seen much deeper than 1,000 A inside the plasma membrane, and none was seen penetrating the nucleoplasm or forming any associative structure. They appeared to encircle the whole cell.

Division (Fig. 19) seemed to be constrictive, more elaborately so than any other example that we know. Any membranes present seemed to be sectioned by the fission.

Nitrobacter agilis. Because *Nitrobacter* continues the oxidation performed by *Nitrosomonas* and *Nitrosocystis*, it was felt that a comparison was mandatory to see whether there was any fine structure common to the group. Some aspects of structure were peculiar (Fig. 22–25).

The cell wall was not obviously a triplet structure. The inner layer was very dense and took up the uranyl salt avidly; thus, differentiation was limited to an asymmetrical dense-light-dense profile. Curiously enough, the plasma membrane of this organism had a similar sort of profile: a unit membrane (about 65 to 75 A) with a dense accretion (about 55 A thick) on the inside. This was the more obvious because of extremely regular plasma membrane intrusions (Fig. 22), in which the layers of the membrane seem to be as closely and regularly apposed as possible (Fig. 23 and 24). Each layer of the plasma membrane was represented so that, counting the 40 to 50 A separation between them as a layer of low density from the gap between plasma membrane and wall, there were at least nine layers in the intrusive structure. The intrusions were disposed in two or three thicknesses over the poles of the cell (Fig. 22); this was most obvious in incidental sections of lysing cells (Fig. 24). As a consequence, cross sections near the ends of cells showed concentric rings of membranes within the plasma membrane (Fig. 23). Here, as in *Nitrosocystis*, the membranes were so closely set that there did not seem to be room for other cytoplasmic elements between them.

In other respects the cells were odd in their overall lack of symmetry, being peg-shaped as has been generally observed, probably because of the arrangement and rigid apposition of membrane. They also contained an amorphous inclusion of moderate density. The latter were in the cytoplasm (Fig. 25), and in most cases were in contrast with the nucleoplasm. These nuclei were so extensive that the seeming association could have been fortuitous. They resembled the "chondrioids" seen in *Vitreoscilla* (Costerton, Murray, and Robinow, 1961), and did not have a counterpart in the other nitrifiers. They were

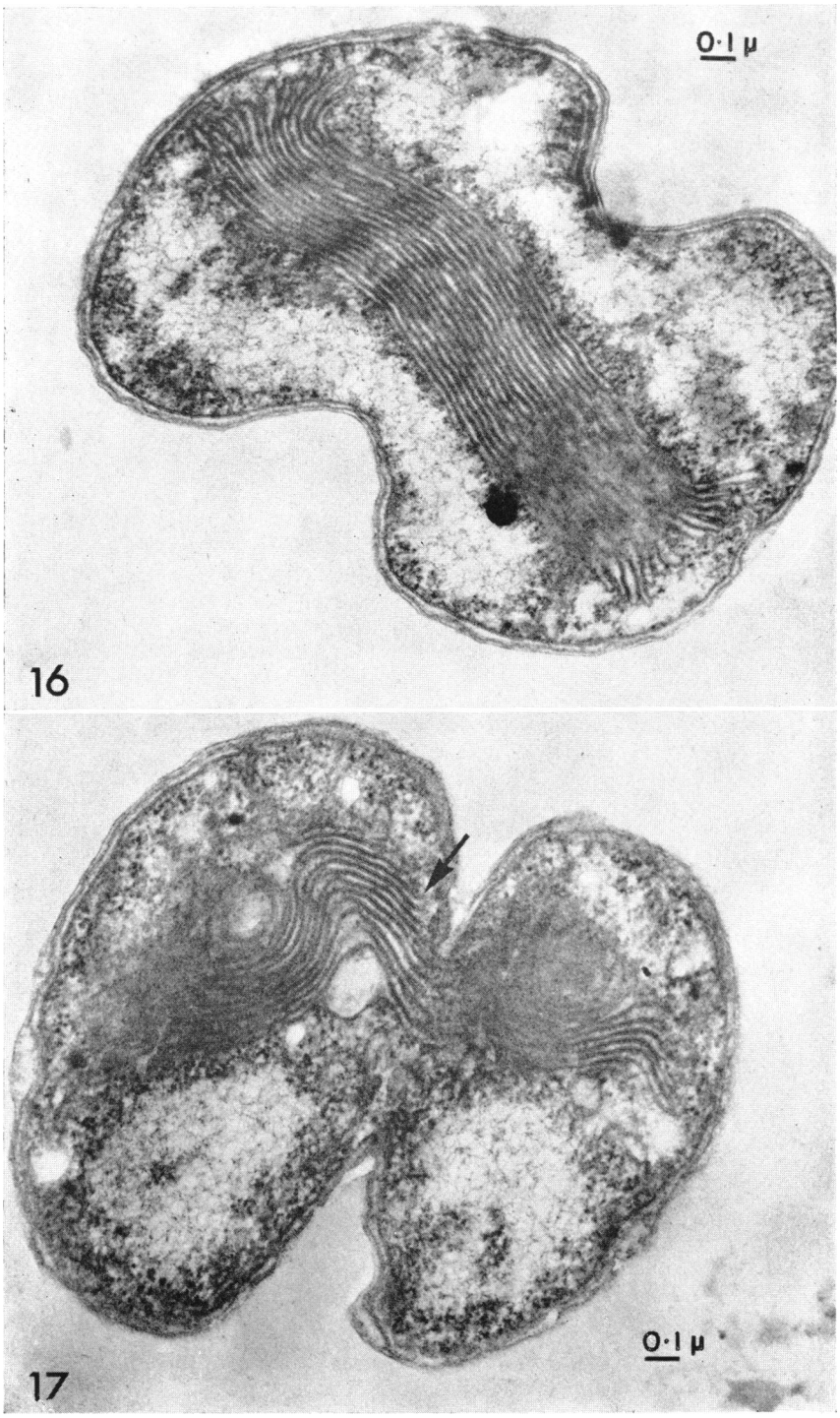
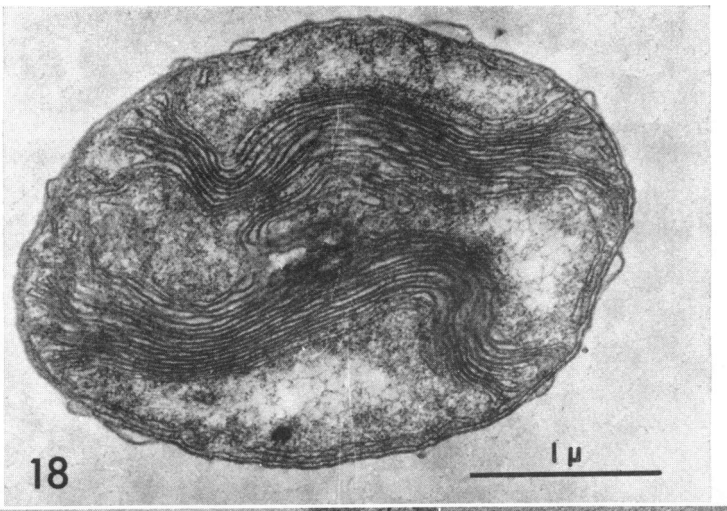


FIG. 16 and 17. *Sections of Nitrosocystis oceanus, showing further steps in constrictive division (Fig. 7 probably shows the earliest stage), in which the membrane organelle is divided as if it were a permanent organelle of the cell. In Fig. 17, it seems clear that the lamellae must be cut by the process. Changes in orientation of lamellae cause the fading of detail.*



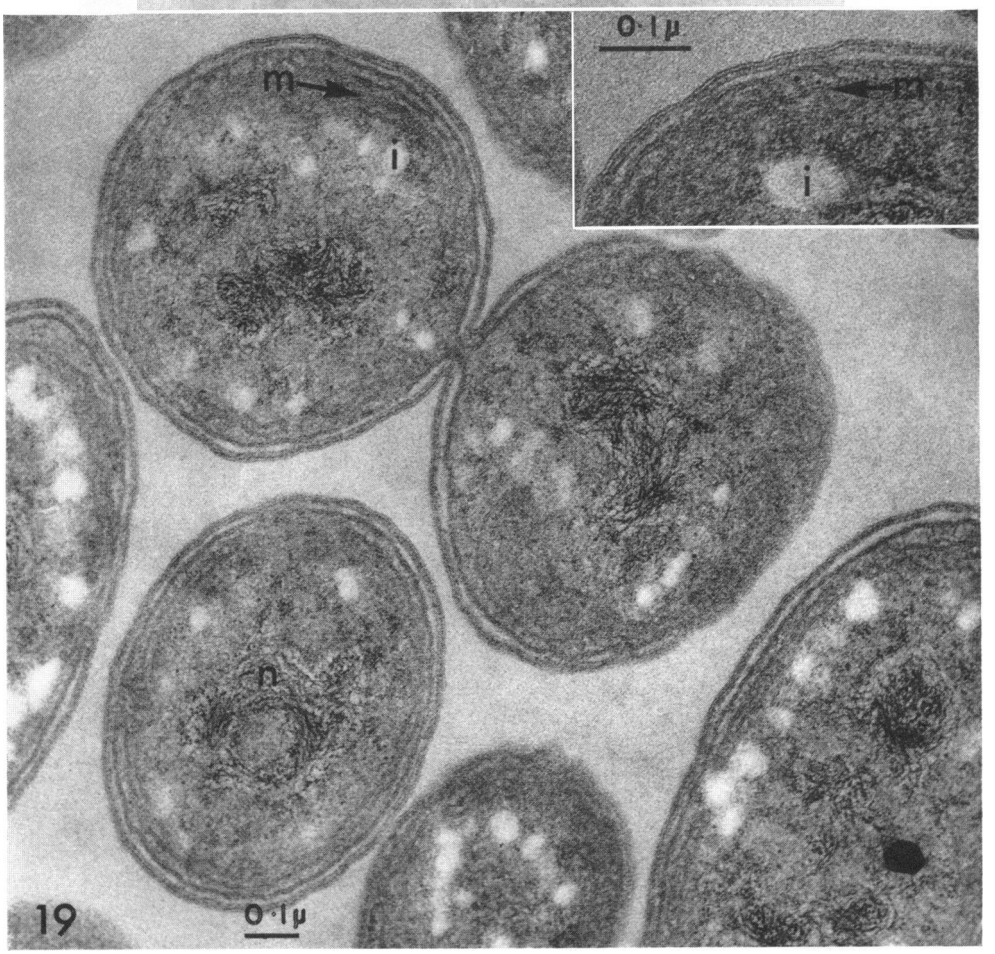


FIG. 18. *Section showing the appearance of two membranous organelles separated by both cytoplasm and part of a nucleoplasm. There are a few lamellae in common that traverse the gap.*

FIG. 19. *Sections of Nitrosomonas europaea, giving a general view of structure and showing a nearly completed division. Nucleoplasms (n) and low-density inclusions (i) are prominent. Less clear because of varying orientation are intracytoplasmic membranes (m). Inset is at higher magnification (same as Fig. 20 and 21), to show the triplet structure of the cell wall.*

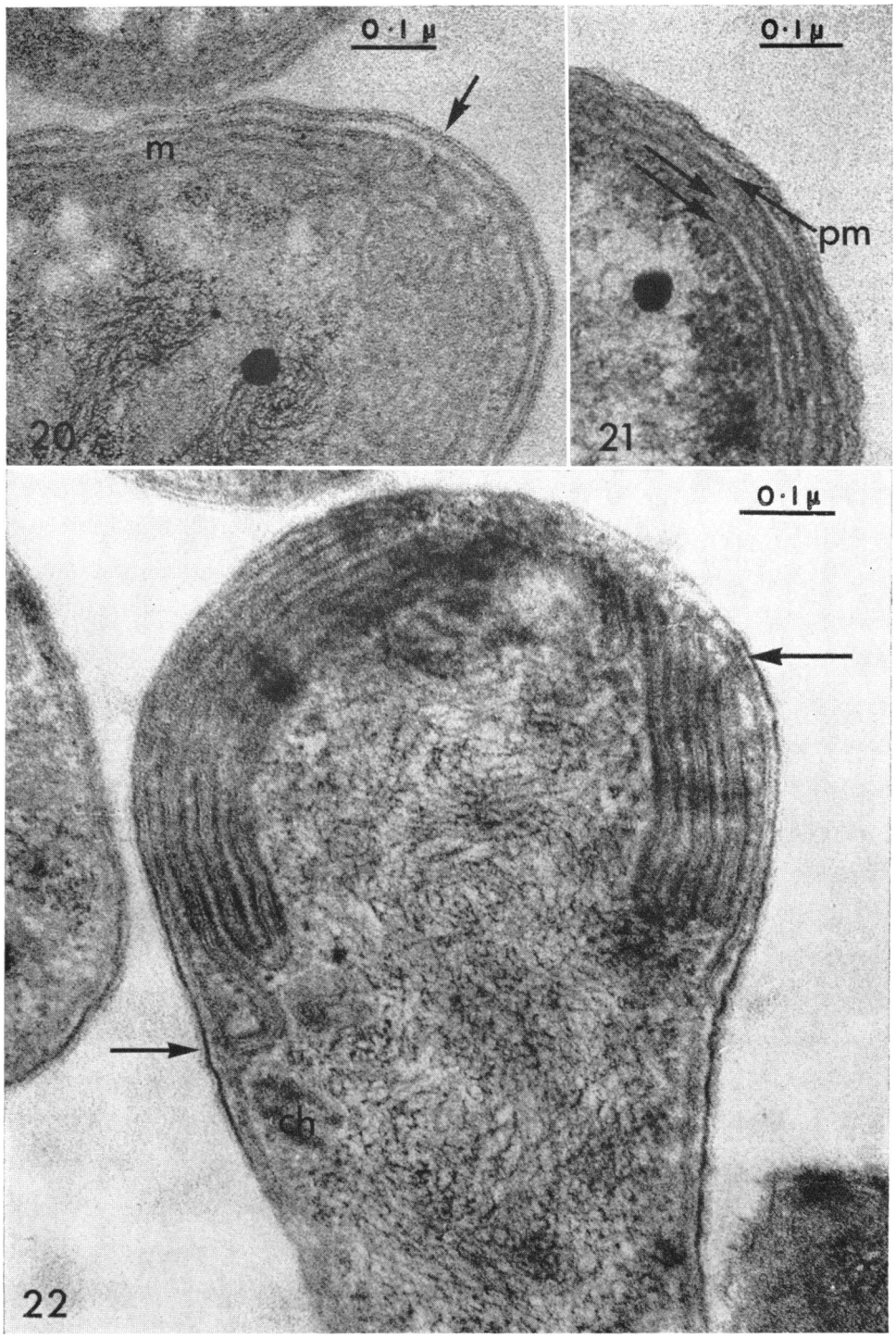


FIG. 20. *Section of Nitrosomonas europaea, showing an invagination (arrow) of plasma membrane, which is considered to form the intracytoplasmic membranes (m); these membranes are thus considered to run in pairs.*

FIG. 21. *Portion of a cell showing that the lumena of two pairs of intracytoplasmic membranes (arrow) are free from cytoplasmic contents. The fifth membrane is the plasma membrane (pm). This section was stained with lead.*

FIG. 22. *Section of Nitrobacter agilis, showing the polar arrangement and regular spacing of closely opposed plasma-membrane intrusions (arrows). The inner border of the plasma membrane and the outer borders of the intrusions carry a thick extra-dense layer that is unusual among bacteria. A portion of a dense inclusion (ch) is shown.*

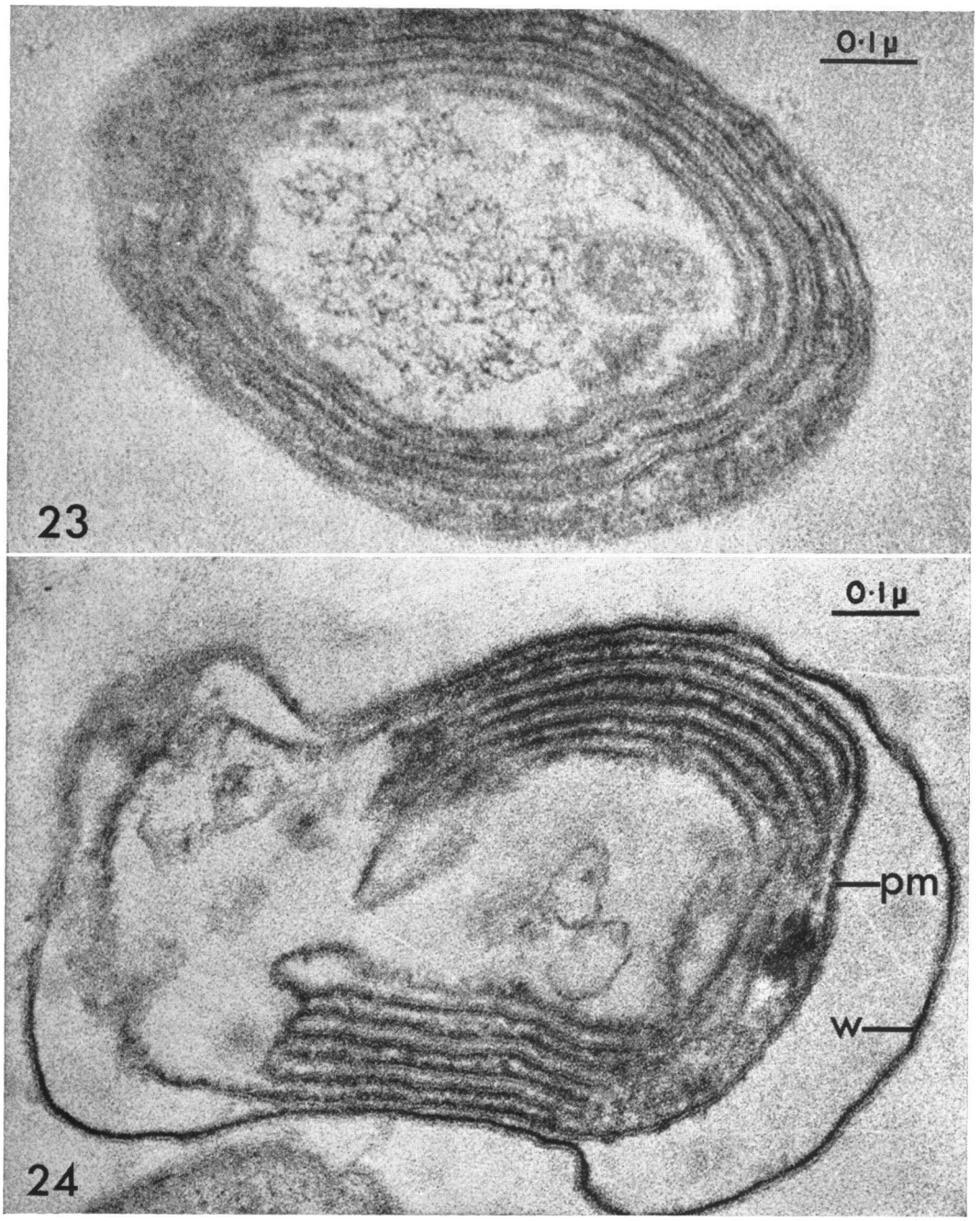


FIG. 23. *Cross section of an end of a Nitrobacter agilis cell, showing that the membranes must expand to enclose the whole hemisphere.*

FIG. 24. *Longitudinal section of a lysing cell of Nitrobacter agilis, showing the form and disposition of membranes inside the plasma membrane (pm) and wall (w). The dense element of the plasma membrane and intrusions is preserved, and must be considered part of the membrane. The wall shows a very dense inner half.*

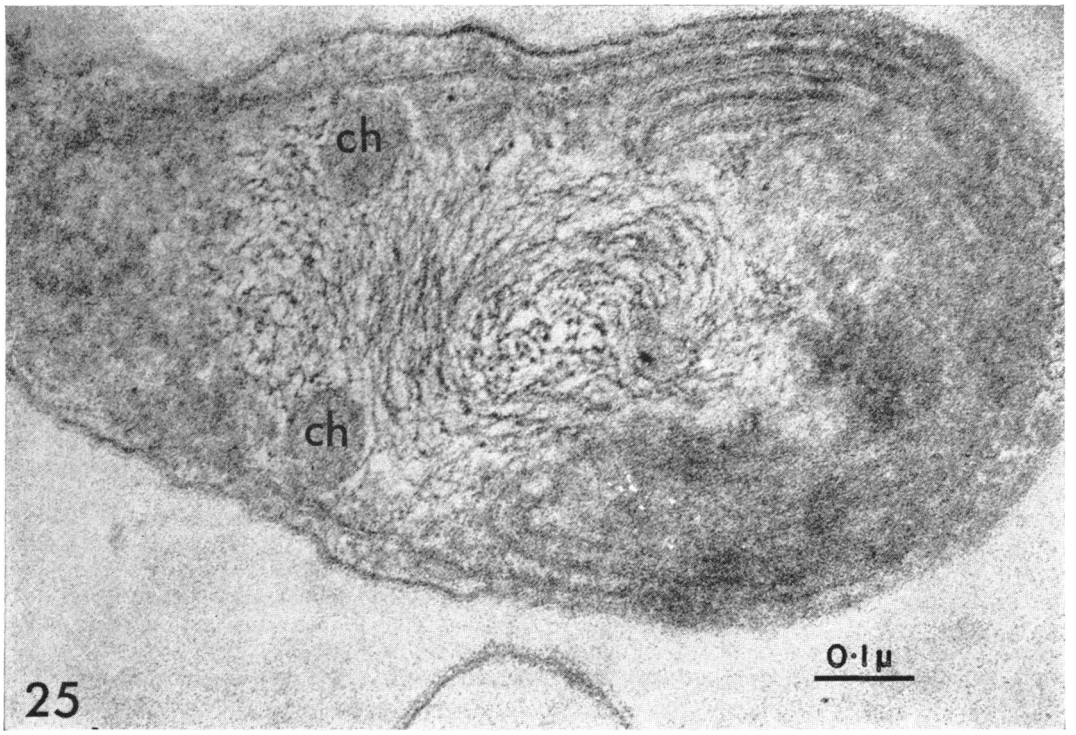


FIG. 25. *Section of Nitrobacter agilis, showing a pair of dense inclusions (ch) in a characteristic site. They are contiguous to the finely whorled fibrils of a nucleoplasm, as in Fig. 22.*

most often seen in the central portion of the cell (i.e., the region not enclosed by the intrusive membranes), but were not exclusively in that region.

Divisions, in this case, did not cut across organized associations of membranes.

DISCUSSION

Nitrosomonas europaea and *Nitrosocystis oceanus* both have to oxidize ammonium ions to nitrite, and this energy-yielding activity has to be undertaken in at least three coupled steps (Lees, 1962). This sort of grouping of energetic reactions is most often membrane-linked, and, consequently, the elaborate membranes and their arrangements in these particular bacteria seem to be quite appropriate (Murray, 1963). The same is true for the metabolically more difficult process of converting nitrite to nitrate, and it is most suggestive that *Nitrobacter agilis* has highly organized membranous intrusions at the poles of the cell. The association of these elegant membranes with function is largely assumptive; it has not yet proved possible to demonstrate in some definitive way that the actual oxidations take place in the membranous organelle.

In *Nitrosocystis* and *Nitrosomonas*, the presence of membranes in the cells seems to be a constant feature. Their distribution in the cell makes it apparent that these massive (in terms of proportion of volume) components must be in the way of cell division. In fact, it would appear that they are divided by the division of the cell. The consequence is that these membranes are essentially permanent organelles of these species; this is a new finding among bacteria. That being so it is perhaps not surprising that it is difficult to trace the formation of this organelle, since it may, following division, be able to add to itself by synthesis *in situ*. However, addition or perhaps replacement in *N. oceanus* of membranes must occur, and we have a strong impression that when this occurs it involves the inward migration of peripherally formed vesicles of the plasma membrane (Fig. 14). In *N. europaea*, the membranes may remain in contact with the peripheral plasma membrane. Nevertheless, if it is a permanent organelle of the cell, the organelle is also peculiar in that it is not membrane-bounded. Thus, there is adherence to the dictum of Stanier and van Niel (1962) that no membrane-bounded organelles of bacteria exist that are smaller than

the cell itself. It is peculiar that so few connections (virtually none) exist between the organelle and the peripheral membrane. However, this connection seems to be quite hard to demonstrate in the case of the photosynthetic lamellae of blue-green algae, and it may be in large part a technical problem if the point of origin of the flattened vesicle forming the lamella is single and relatively small.

It is possible that some or all of the other membranes represent extensions of the plasma membrane for the carrying out of other processes, and they might conceivably be equivalent to mesosomes (Fitz-James, 1960). However, all three of the organisms studied here exhibit peculiar and highly organized membranous intrusions, and neither these nor the small number of other membranous components bear any striking resemblance to the forms and arrangements of mesosomes, plasmalemmasomes, or chondriods studied in other kinds of bacteria (Murray, 1963).

The formation of closely apposed, fusing lamellae in cells has several counterparts in nature, notably the formation of grana or thylakoids in higher-plant chloroplasts (Menke, 1962) and the stacked lamellae in retinal rods (Eakin, 1963). The former may be more comparable in this case, since it has been proposed (Menke, 1962) that the discs are formed by invagination of the enveloping unit membrane of the chloroplast; this is roughly homologous to what occurs in photosynthetic bacteria (Cohen-Bazire, 1963; Fuller, 1963). The closest resemblance to stacked membranes in bacteria has been shown in the photosynthetic *Rhodospirillum molischianum* by Giesbrecht and Drews (1962), and these, too, show connections to the superficial plasma membrane. The circumferential arrangement of membranes seen in *Nitrosomonas* is also rare, but has been described for the photosynthetic *Rhodomicrobium vanniellii* (Boatman and Douglas, 1961) and for the eubacterial species, *Alcaligenes faecalis* (Beer, 1960).

One must assume, because of the slightly less organized membranes exhibited by *Nitrosomonas*, that the highly organized appearance of *Nitrosocystis* is ordained by genetics of the cell and is not essential to the biochemical transformations involved. This must include the extremely close apposition of the membranes of adjacent vesicles to form the triplet structure on contact.

For its part, *Nitrobacter agilis* shows plasma-membrane intrusions that maintain the most regular of spacing, and this may well be functionally important. The packing of the membranes into the polar region of the cells, as well as their close-ordered arrangement, must make for considerable rigidity; they deform the cell and account for its characteristic shape. This seemed to be unique, but there have been recent observations of a similar morphology and cause in the case of *Rhodopseudomonas palustris* (R. Y. Stanier, *personal communication*).

The most striking feature of the membranes in *Nitrobacter* is the very dense layer on the inner side of the plasma membrane that is carried over the outward facing surfaces of the sets of intrusive membrane. This layer is unique in our experience and without explanation. It seems possible on the basis of its extreme density that it might represent a zone of concentration of some metal-enzyme complex such as either cytochromes or perhaps copper enzymes, both of which have been said to be in high concentration in this organism (N. E. R. Campbell and M. I. H. Aleem, *personal communication*). Certainly, the layer is an organized part of the membrane, because it remains intact despite lysis of the cell.

Nitrobacter does show some peculiar inclusions, already mentioned as resembling what have been termed "chondriods" in some other cells (Costerton et al., 1961). It may be only fortuitous that in this case they are near or next to nucleoplasms and commonly close to the midsection of the cells. The midsection is the only part free from the extensive membranes, and the contiguity to nucleoplasms may be dictated by the relatively large volume of the latter.

It seems a pity to have to be quite so silent about the extraordinary cell-wall organelle of *Nitrosocystis*. This has been described separately (Murray and Watson, 1963), and there is nothing new to add to the previous description. Their presence is easily detectable in electron-microscope preparations of sections but not, so far, in the living cell; consequently, we have no real information on their natural history.

The cell walls in general show the basic structure of a triplet, i.e., either three dense thin layers separated by two low-density layers, or one thin and one very thick dense layer separated by a single low-density layer. Both forms can be shown in organisms such as *E. coli* (Murray et al., *in press*), and this seems to depend on metal staining during preparation. This means that the kind of arrangement is similar to that of many other gram-negative bacteria. *Nitrosocystis* does show the outer enveloping layer as an additional feature.

These autotrophic nitrifying bacteria show a most fascinating range of structure that stimulates questions about the functional anatomy of the physiological processes involved. It is obvious that other approaches are needed to resolve

these questions, but the initial successful embeddings have provided us with a point of departure as well as an anatomical description.

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LITERATURE CITED

BEER, M. 1960. Disposition of membranes in *Alcaligenes faecalis*. J. Bacteriol. **80**:659–664.

BOATMAN, E. S., AND H. C. DOUGLAS. 1961. Fine structure of the photosynthetic bacterium *Rhodomicrobium vannielii*. J. Biophys. Biochem. Cytol. **11**:469–483.

BROWN, A. D., AND H. P. TURNER. 1963. Membrane stability and salt tolerance in Gram-negative bacteria. Nature **199**:301–302.

CEDERGREN, B., AND T. HOLME. 1959. On the glycogen in *Escherichia coli B;* electron microscopy of ultrathin sections of cells. J. Ultrastruct. Res. **3**:70–73.

CLAUS, G. W., AND L. E. ROTH. 1964. Fine structure of the gram-negative bacterium *Acetobacter suboxydans*. J. Cell Biol. **20**:217–233.

COHEN-BAZIRE, G. 1963. Some observations on the organization of the photosynthetic apparatus in purple and green bacteria, p. 89–110. *In* H. Gest, A. San Pietro, and L. P. Vernon [ed.], Bacterial photosynthesis. Antioch Press, Yellow Springs, Ohio.

COSTERTON, J. W. F., R. G. E. MURRAY, AND C. F. ROBINOW. 1961. Observations on the motility and the structure of *Vitreoscilla*. Can. J. Microbiol. **7**:329–339.

EAKIN, R. 1963. Lines of evolution of photoreceptors, p. 393–425. *In* D. Mazia and A. Tyler [ed.], General physiology of cell specialization. McGraw-Hill Book Co., New York.

FITZ-JAMES, P. C. 1960. Participation of the cytoplasmic membrane in the growth and spore formation of bacilli. J. Biophys. Biochem. Cytol. **8**:507–528.

FULLER, R. C. 1963. Comparative structure and activities of the microbial photosynthetic apparatus, p. 223–241. *In* D. Mazia and A. Tyler [ed.], General physiology of cell specialization. McGraw-Hill Book Co., New York.

GIESBRECHT, P., AND G. DREWS. 1962. Elektronenmikroskopische Untersuchungen über die Entwicklung der "Chromatophoren" von *Rhodospirillum molischianum* Giesberger. Arch. Mikrobiol. **43**:152–161.

KELLENBERGER, E. 1962. The study of natural and artificial DNA-plasms by thin sections, p. 233–250. *In* R. J. C. Harris [ed.], The interpretation of ultrastructure. Academic Press, Inc., New York.

KELLENBERGER, E., A. RYTER, AND J. SÉCHAUD. 1958. Electron microscope study of DNA-containing plasms. II. Vegetative and mature phage DNA as compared with normal bacterial nucleoids in different physiological states. J. Biophys. Biochem. Cytol. **4**:671–676.

LEES, H. 1962. Some thoughts on the energetics of chemosynthesis. Bacteriol. Rev. **26**:165–167.

MACLEOD, R. A., AND T. I. MATULA. 1962. Nutrition and metabolism of marine bacteria. XI. Some characteristics of the lytic phenomenon. Can. J. Microbiol. **8**:883–896.

MASON, D. J., AND D. M. POWELSON. 1956. Nuclear division as observed in live bacteria by a new technique. J. Bacteriol. **71**:474–479.

MENKE, W. 1962. Structure and chemistry of plastids. Ann. Rev. Plant Physiol. **13**:27-44.

MURRAY, R. G. E. 1960. The internal structure of the cell, p. 35–96. *In* I. C. Gunsalus and R. Y. Stanier [ed.], The bacteria, vol. 1. Academic Press, Inc., New York.

MURRAY, R. G. E. 1962. Fine structure and taxonomy of bacteria. Symp. Soc. Gen. Microbiol. **12**:119–144.

MURRAY, R. G. E. 1963. The organelles of bacteria, p. 28–52. *In* D. Mazia and A. Tyler [ed.], General physiology of cell specialization. McGraw Hill Book Co., New York.

MURRAY, R. G. E., AND A. BIRCH-ANDERSON. 1963. Specialized structure in the region of the flagella tuft in *Spirillum serpens*. Can. J. Microbiol. **9**:393–401.

MURRAY, R. G. E., AND S. W. WATSON. 1963. An organelle confined within the cell wall of *Nitrosocystis oceanus* (Watson). Nature **197**:211–212.

ROBINOW, C. F. 1962. On the plasma membrane of some bacteria and fungi. Circulation **26**:1092–1104.

RYTER, A., AND E. KELLENBERGER. 1958. Étude au microscope électronique de plasmas contenant de l'acide désoxyribonucléique. I. Les nucléoides des bactéries en croissance active. Z. Naturforsch. **13b**:597–605.

STANIER, R. Y., AND C. B. VAN NIEL. 1962. The concept of a bacterium. Arch. Mikrobiol. **42**:17–35.

WATSON, S. W. 1962. *Nitrosocystis oceanus Sp. Nov.* Abstr. Intern. Congr. Microbiol., 8th, Montreal.

WINOGRADSKY, S. 1931. Nouvelles recherches sur les microbes de la nitrification. Compt. Rend. **192**:1000–1004.