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Comparative Study of the Structure of Gas Vacuoles

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The fine structure of gas vacuoles was examined in two blue-green algae, two green bacteria, three purple sulfur bacteria, and two halobacteria. The gas vacuole is a compound organelle, composed of a variable number of gas vesicles. These are closed, cylindrical, gas-containing structures with conical ends, about 80 to 100 nm in width and of variable length, ranging from 0.2 to over 1.0 μm. The wall of the gas vesicle is a non-unit membrane 2 to 3 nm in thickness, bearing very regular striations with a periodicity of 4 nm, oriented more or less at right angles to the long axis of the cylinder. This fine structure could be clearly resolved in isolated gas vesicles prepared from a blue-green alga and from *Halobacterium halobium*, and its presence in the gas vesicles of the green bacterium *Pelodictyon clathratiforme* was inferred from thin sections. The gas vacuole thus appears to be a homologous organelle in all of these procaryotic groups. Minor differences with respect to the length and arrangement of the gas vesicles were observed. In blue-green algae and green bacteria, the vesicles are relatively long and tend to be arrayed in parallel bundles; in purple sulfur bacteria and *Halobacterium*, they are shorter and more irregularly distributed in the cell.

Some blue-green algae and bacteria contain gas vacuoles, highly refractile cytoplasmic inclusions of irregular contour, which occur singly or in small numbers in each cell. Strodtman (24) and Klebahn (10) were the first to suggest that these structures are gas-filled, a hypothesis subsequently confirmed by the observations and experiments of Klebahn (11, 12) on blue-green algae. Walsby recently showed that the gas vacuoles of blue-green algae are freely permeable to gases: hence the nature of the gases contained in these organelles is determined by the ambient atmosphere (26). Cells that contain inflated gas vacuoles usually have a density lower than that of water and hence float to the surface of liquid media. Sudden, sharp pressure on a suspension of cells deflates the gas vacuoles which then become invisible with the light microscope. At the same time, the cells lose their buoyancy and refractility (4).

Gas vacuoles have been found only in procaryotic organisms, among which they have a wide but sporadic distribution (Table 1). They occur in some representatives of the blue-green algae, the purple sulfur bacteria, and the green bacteria, as well as in a few nonphotosynthetic bacteria. The known gas vacuole-containing procaryotic organisms are remarkably diverse with respect to other structural and functional properties. In fact, the only common denominator that can be perceived is an ecological one: they are all aquatic.

The blue-green algae that contain gas vacuoles occur most commonly in the surface layers of oceans and fresh water lakes, where they often produce water blooms. Extremely halophilic nonphotosynthetic bacteria with gas vacuoles (members of the genus *Halobacterium*) are confined to the surface layers of brine ponds and salt lakes. However, the gas vacuole-containing photosynthetic bacteria, all strict anaerobes, are not surface inhabitants; as shown by Pfennig (17), they occur abundantly in a narrow horizontal band just within the deeper, anaerobic layer of meromictic lakes, many meters below the air-water interface. There is no information concerning the distribution of the recently discovered prosthecate freshwater bacteria that contain gas vacuoles (22), but these organisms are probably inhabitants of the surface layers of ponds and lakes. The gas vacuole can therefore be interpreted as a cellular device which enables its possessors to regulate their vertical position in a water gradient. It should be noted that many (though not all) procaryotic organisms that produce gas vacuoles are permanently immotile

TABLE 1. *Distribution of gas vacuoles among procaryotic microorganisms*

Major group	Representatives with gas vacuoles	Reference
Blue-green algae		
Unicellular	Several species of *Coelospherium* and *Microcystis*	5
Filamentous	Species of *Oscillatoria, Lyngbya, Nostoc, Anabaena, Anabaenopsis, Aphanizomenon, Gleotrichia, Calothrix, Spirulina*	4, 5
Eubacteria		
Purple bacteria	*Lamprocystis roseopersiciana, Rhodothece* species, *Amoebobacter bacillosus, Thiodictyon elegans*	17
Green bacteria	*Pelodictyon clathratiforme, Pelodictyon aggregatum*	17
Nonphotosynthetic	*Halobacterium halobium* (some strains), *Ancalomicrobium adetum, Prosthecomicrobium pneumaticum*	15, 22

and hence have no other means of maintaining their position in a water gradient.

Although the first electron microscopic observations on gas vacuoles were made on *Halobacterium*, the fine structure of these organelles has been principally studied in blue-green algae (2, 7–9, 21, 25). Electron microscopy of thin sections and freeze-etched cells of blue-green algae reveals that the gas vacuole is a complex organelle, composed of an array of gas vesicles. Each gas vesicle is a tubular structure with conical ends, some 75 nm in width and ranging in length from 0.2 to as large as 1.5 μm. The wall of the vesicle is a non-unit membrane, 2 to 3 nm in thickness, with a regular striated fine structure. In many blue-green algae, the vesicles are arrayed in parallel bundles which resemble a honeycomb when sectioned transversely (21).

The gas vacuoles of *Halobacterium* have a similar organization and fine structure (13, 23). Gas vesicles have been recently demonstrated in the cells of two other nonphotosynthetic bacteria which possess gas vacuoles, *Prosthecomicrobium* and *Ancalomicrobium* (22).

We shall describe the results of a comparative study of the structure of the gas vacuole in blue-green algae and bacteria, including several representatives of the photosynthetic bacteria.

MATERIAL AND METHODS

Blue-green algae. A pure culture of *Oscillatoria agardhii* var. *suspensa* (19) was kindly provided by E. G. Pringsheim. A unialgal culture of *Aphanizomenon flos-aquae* containing gas vacuoles was isolated from a water sample taken from Lake Washington (Washington) in the fall of 1966 and sent to us by W. T. Edmondson.

Cultures were maintained and grown in liquid medium (1) at 25 C under constant fairly low illumination (1,000 lux), provided by fluorescent, cool white lights.

Photosynthetic bacteria. All strains examined were isolated and characterized by Pfennig (17). *Pelodictyon clathratiforme* strains 1831 and 2730 were cultivated as already described (18). *Rhodothece conspicua* strain 6611, *Thiodictyon elegans* strain 3011, and *Amoebobacter bacillosus* strain 1814 were cultivated in screw-cap bottles completely filled with the medium described by Pfennig (16). The concentration of sulfide was kept low (0.03% $Na_2S \cdot 9H_2O$) and maintained by repeated additions. The cultures were grown at low temperature (10 to 20 C) and at a low light intensity (50 to 200 lux), provided by a 25-w tungsten lamp.

Halobacterium. Two strains of *Halobacterium* containing gas vacuoles were kindly provided by H. Larsen. Strain 5 had originally been isolated from sea salt in his laboratory. Strain Delft is probably identical with the strain originally isolated by Petter (15) and subsequently studied by Houwink (6). Cultures were grown at 30 C in the liquid medium devised by Larsen et al. (13). For growth on solid medium, 2% (w/v) agar (Difco) was added to the liquid medium.

Preparation of specimens for electron microscopy: blue-green algae and photosynthetic bacteria. Since gas vacuoles can be readily collapsed by pressure, cells from liquid cultures were collected by careful centrifugation at a low gravitational field or by filtration through membrane filters (Millipore Corp. Bedford, Mass.). When the suspension was adequately concentrated, the cells were fixed, stained, and embedded by the method of Ryter and Kellenberger (20), except that the period of main fixation at room temperature in 1% osmium tetroxide was reduced to 2 hr for bacteria and 4 hr for blue-green algae. When glutaraldehyde [50% (w/v) aqueous solution; Fisher Scientific Co., biological grade] was used as a prefixative, its final concentration was 3 to 4% (v/v) in phosphate buffer (0.1 M, *p*H 6.8). After 45 min to 1 hr of prefixation, the cells were washed several times with phosphate buffer and then once with water, after which they were fixed with osmic acid.

Halobacterium. For fixation and electron microscopic examination, *Halobacterium* was grown on 47-mm sterile filter pads (Millipore Corp.) deposited on nutrient agar plates. Four filter pads were placed on each plate and inoculated with drops of liquid culture. The Delft strain grew very well under these

conditions; strain 5 did not grow as abundantly, but growth was adequate for our purpose. After growth had occurred, the filter pads were removed, and the cells were fixed in situ.

Without prefixation with glutaraldehyde, osmic acid provokes an immediate lysis of *Halobacterium* cells, even when it is dissolved in Larsen's medium. The cells were accordingly prefixed for 45 min at room temperature by immersing the filter pads in 2.5% (v/v) glutaraldehyde in 0.1 M sodium cacodylate-hydrochloride buffer (*p*H 7) containing 4.3 M NaCl and 0.01 M $CaCl_2$. The filters were then rinsed several times with cacodylate-NaCl-$CaCl_2$ buffer. The cells were gently scraped off into *Halobacterium* medium and fixed with 1% osmic acid dissolved in Veronal-acetate buffer (20) containing 4.3 M NaCl for 2.5 to 3 hr at room temperature. The fixed cells were then washed by centrifugation at a low gravitational field (1,500 × *g*) with Veronal-acetate buffer containing 4.3 M NaCl and further processed by the method of Ryter and Kellenberger (20).

After dehydration with acetone, all specimens were embedded in Maraglas 655 or Vestopal. Sections were cut with a diamond knife with a Porter-Blum microtome MT2, mounted on uncoated 300-mesh copper grids, poststained with lead hydroxide (14), and examined in a Siemens Elmiskop 1A electron microscope operating at 80 kv.

Negative stains were performed with a solution of uranyl acetate [1% (w/v) in distilled water]. The preparations, made on Formvar-coated, carbon-stabilized, 300-mesh grids, were examined immediately.

RESULTS

Gas vacuoles of blue-green algae. Our observations on the organization of gas vacuoles in two species of blue-green algae, *O. agardhii* and *A. flos-aquae*, fully confirm published reports (2, 19, 21). Figures 1 to 3 show the appearance in the light microscope (phase-contrast illumination) of filaments of *O. agardhii* containing gas vacuoles in the expanded and collapsed states, respectively. The form and intracellular arrangement of the gas vesicles, characteristic of the blue-green algae that have been examined in thin sections, are shown in Fig. 8 for *O. agardhii* and in Fig. 9 for *A. flos-aquae*. The width of the gas vesicles varies between 70 and 80 nm for *O. agardhii* and between 65 and 70 nm for *A. flos-aquae;* their length varies between 200 and 500 nm.

In old liquid cultures of *O. agardhii* which have undergone extensive autolysis, the liberated gas vesicles accumulate as a milky layer at the surface of the medium. This material can be collected with a Pasteur pipette and washed free from contaminating materials by repeated centrifugations at relatively low gravitational fields (1,500 × *g*) for several hours: the gas vesicles remain inflated and form a milky layer at the surface of the liquid. Preparations of such gas vesicles negatively stained with uranyl acetate are shown in Fig. 10 and 11. Their overall shape corresponds to that observed in thin sections. They consist of cylinders with an apparent diameter of about 90 to 100 nm, narrowing at both extremities to a sharp point. The walls of the vesicles show characteristic fine, beaded striations, oriented perpendicularly (or nearly so) to the long axis and having a periodicity of 4 nm. The conical extremities show the same fine structure as the body of the vesicles. Most of the gas vesicles shown in Fig. 10 and 11 are still inflated; one partly collapsed vesicle is indicated by an arrow. Isolated gas vesicles of *O. agardhii* kept for more than 2 years in distilled water at 4 C show no tendency to collapse or disintegrate.

Gas vacuoles of green and purple sulfur bacteria. The characteristic microscopic appearance of gas vacuoles under phase-contrast illumination is shown in Fig. 5 for a green bacterium, *P. clathratiforme*, and in Fig. 6 and 7 for two purple sulfur bacteria, *R. conspicua* and *T. elegans*. As a rule, each cell contains a single gas vacuole of irregular contour and variable size.

Electron micrographs of thin sections of the green bacterium *P. clathratiforme* (Fig. 12 to 16) reveal that the shape and dimensions of the individual gas vesicles are closely similar to those of blue-green algae; the width of individual vesicles, measured on thin sections, is approximately 80 nm and their length varies from 300 to 600 nm. However, the cells of *P. clathratiforme* are considerably smaller than those of such filamentous blue-green algae as *Oscillatoria* and *Aphanizomenon*, and the number of gas vesicles comprising a gas vacuole is correspondingly much smaller; generally, each cell of *P. clathratiforme* contains only four to seven gas vesicles. Occasional tangential sections through a gas vacuole show a configuration of the adjacent gas vesicles which suggests that they may be interconnected (Fig. 15). However, this is probably an electron optical illusion, caused by the extreme thinness of the vesicle membranes, which cannot be made visible when cut tangentially.

It is extremely difficult to avoid completely a deflation of gas vacuoles when cells are harvested by centrifugation. Consequently, thin sections of *P. clathratiforme* almost always contain occasional cells in which the vesicles are collapsed or partly collapsed. The apposition of the two non-unit membranes which constitute the walls of such collapsed vesicles produces a structure with a profile similar to that of a typical unit membrane but slightly thinner (Fig. 16). Figure 16 shows a longitudinal section which has passed through a partly collapsed gas vesicle and a mesosome (M); the mesosomal membrane is a true unit membrane, 7.0 to 7.5 nm in thickness,

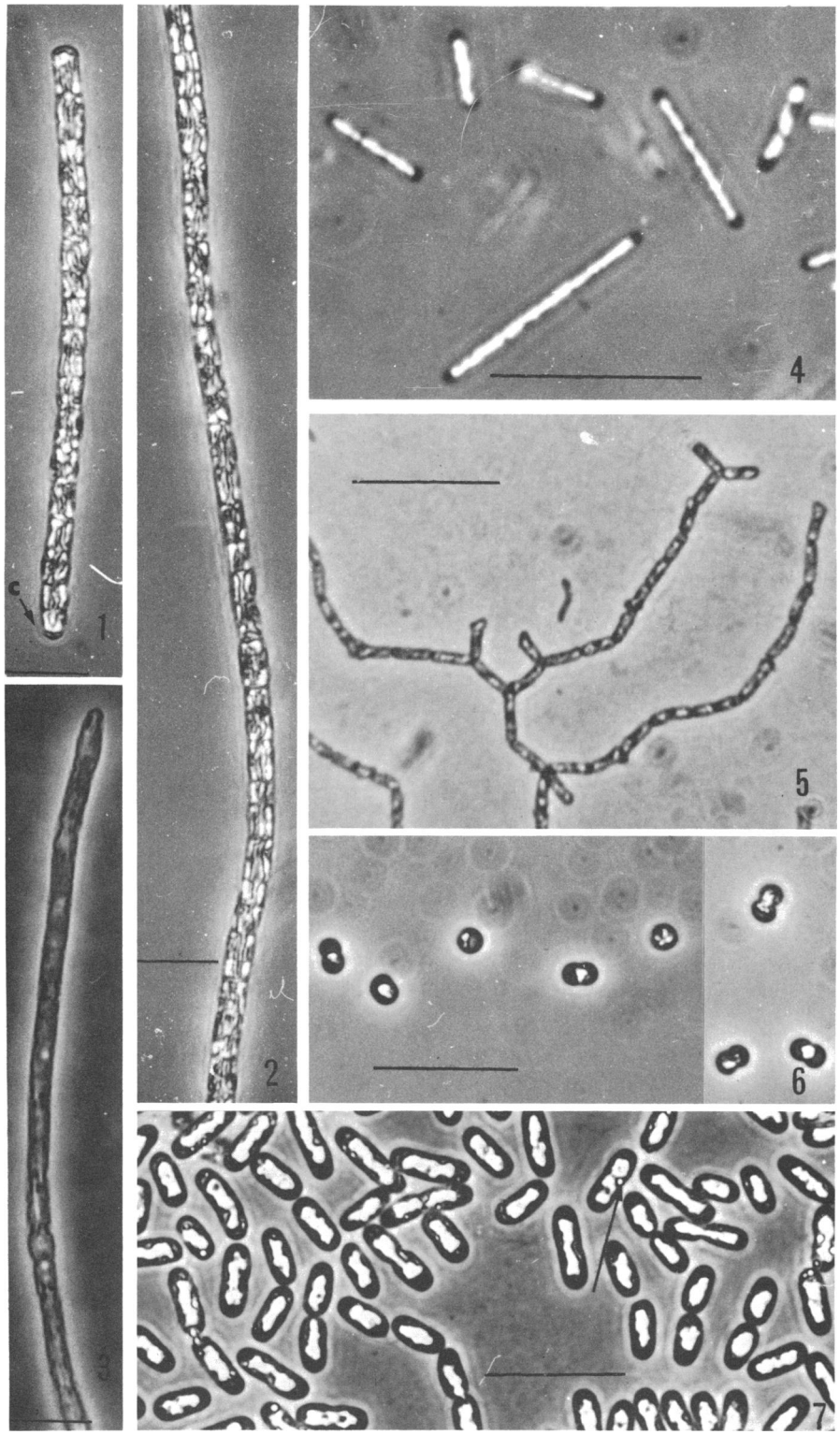


FIG. 1 to 7. *Appearance of gas vacuoles in living cells of blue-green algae and bacteria examined with phase-contrast illumination. The markers indicate 10 μm. (Fig. 1 and 2) Two trichomes of Oscillatoria agardhii var suspensa containing inflated gas vacuoles (bright areas of irregular shape, mostly elongated in the direction of the long axis of the trichome). Each cell in the trichome contains several gas vacuoles. In Fig. 1, the apical cap (c) described by Pringsheim (19) is visible. (Fig. 3) Trichome of O. agardhii var. suspensa in which the gas vacuoles are almost completely collapsed; its width is less than that of the trichomes containing expended gas vacuoles shown*

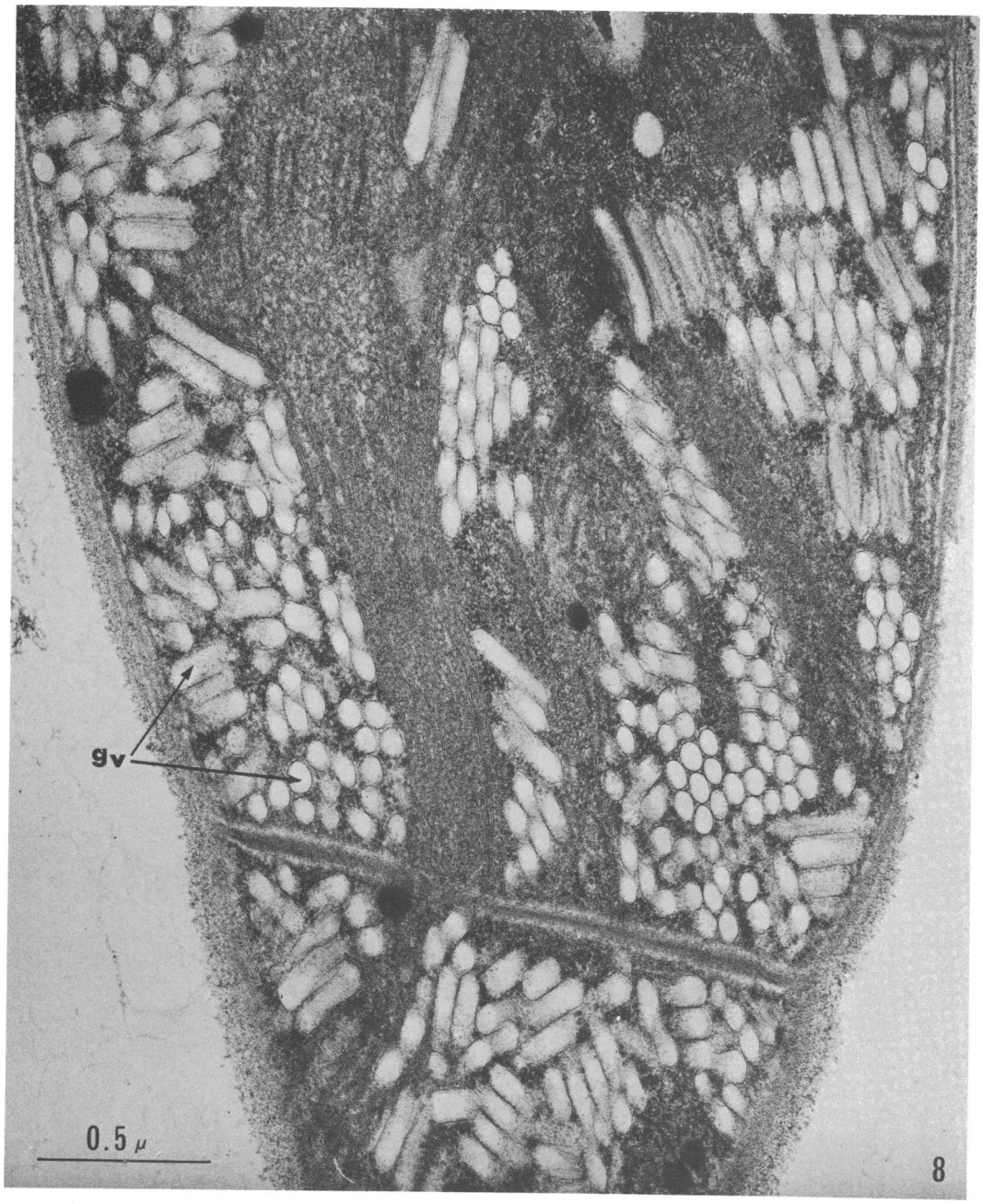


FIG. 8. *Oscillatoria agardhii var. suspensa. Tangential section through part of a trichome. The gas vesicles (gv) are somewhat irregularly oriented in this blue-green alga. Cells were fixed with glutaraldehyde and osmium and embedded in Maraglas.*

in Fig. 1 and 2. (Fig. 4) Halobacterium strain Delft. Each rod-shaped cell contains large gas vacuoles (light areas) which appear to fill almost the entire cell with the exception of the polar regions. (Fig. 5) Pelodictyon clathratiforme strain 1831. The branched chains of cells typical of this genus (18) contain numerous gas vacuoles (light areas of irregular contour). (Fig. 6) Rhodothece conspicua strain 6611. Each of the relatively small cells contains a single, highly irregular, small gas vacuole. (Fig. 7) Thiodictyon elegans strain 3011. Each cell contains a large centrally located gas vacuole (bright area). Many cells also contain small, round, refractile sulfur inclusions (arrow).

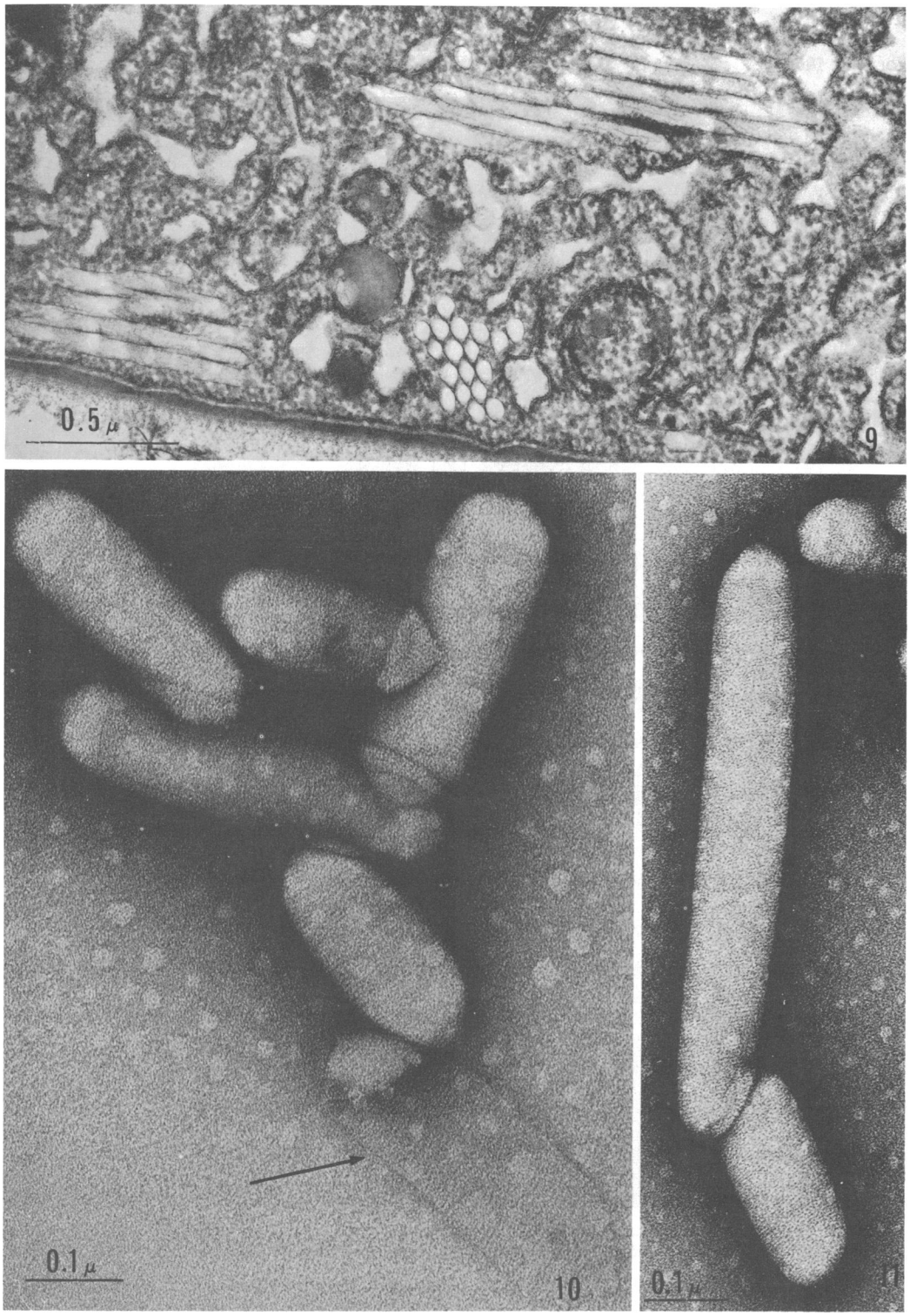


FIG. 9. *Aphanizomenon flos-aquae. In this blue-green alga, the gas vesicles are grouped in bundles. The vesicles of each bundle are oriented parallel to one another. Shown is part of a cell containing three small bundles of gas vesicles, two in longitudinal section and one in transverse section. Cells were fixed with glutaraldehyde and osmium and embedded in Maraglas.*

FIG. 10 and 11. *Isolated gas vesicles from Oscillatoria agardhii var. suspensa, negatively stained with uranyl acetate. In Fig. 10, one vesicle (arrow) is partly collapsed. Figure 11 shows a particularly long gas vesicle and a short one; they appear to be interconnected. Note the fine striations, which extend through the entire length of the vesicles.*

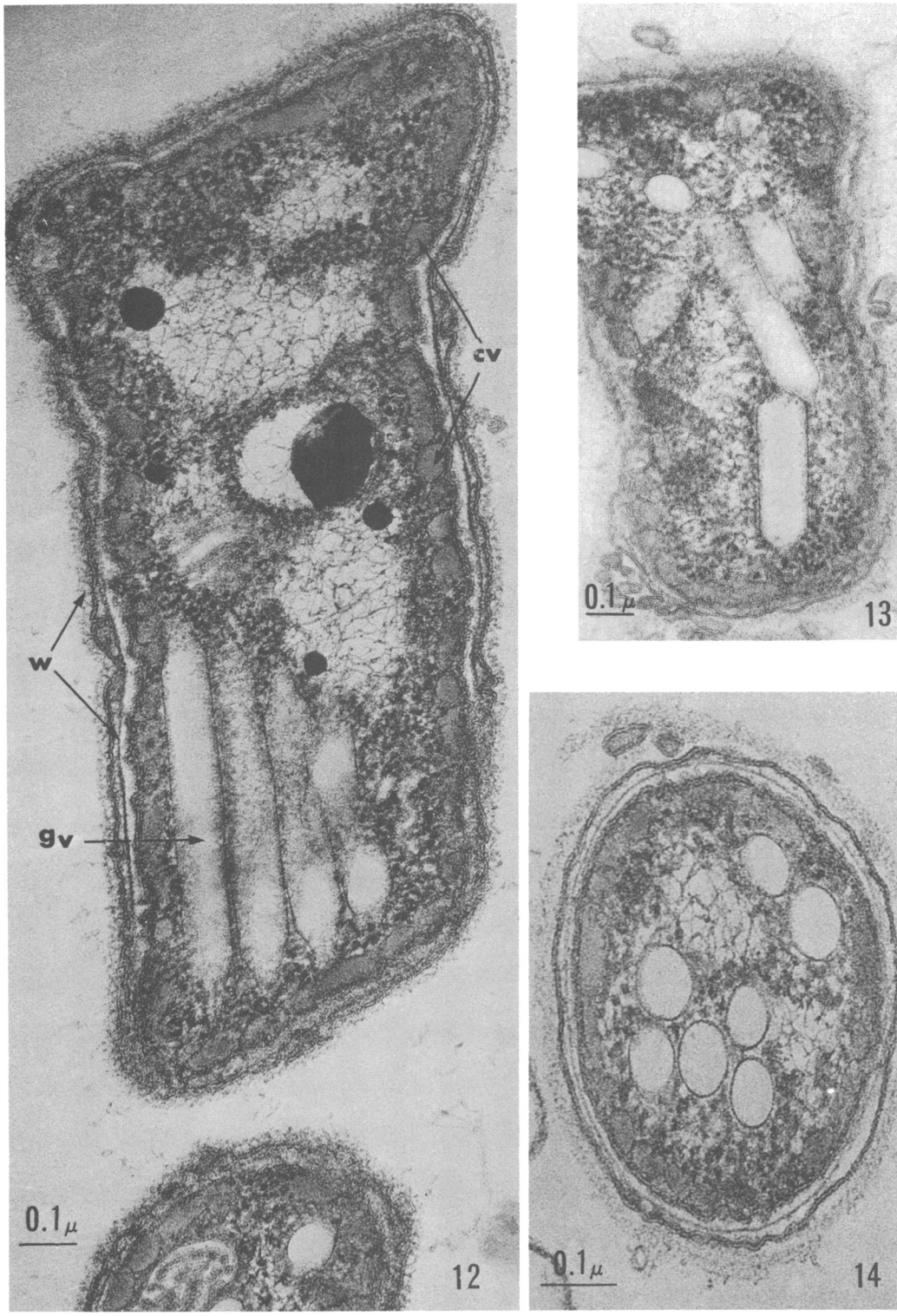
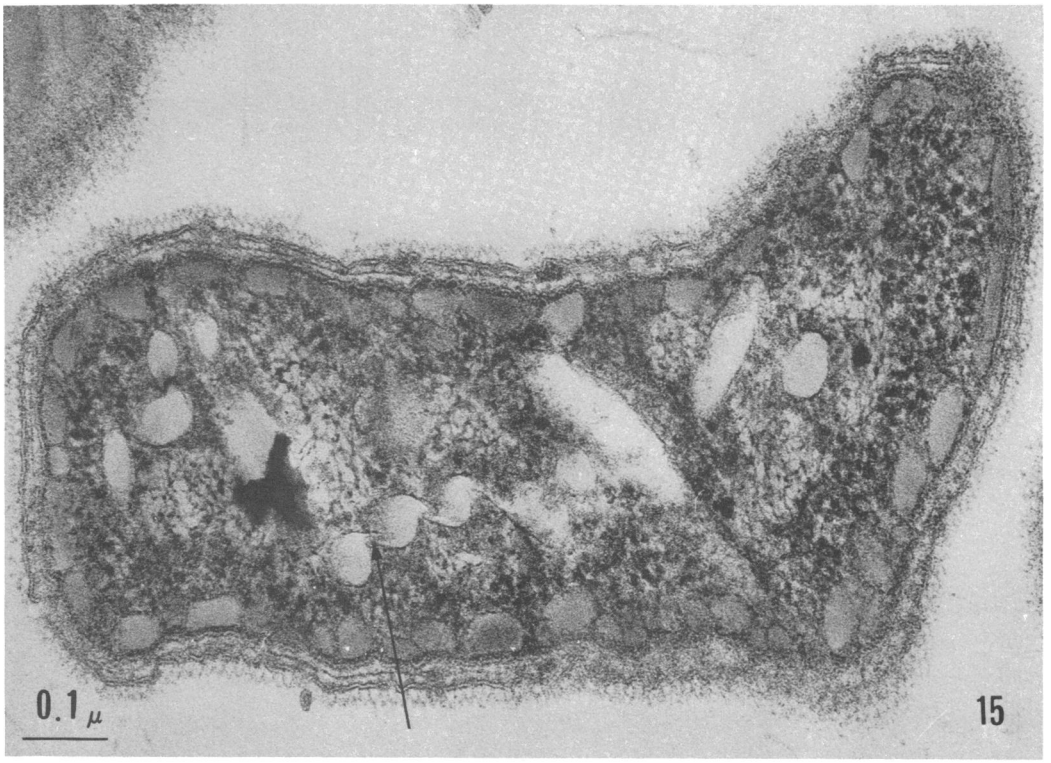


FIG. 12 to 14. *Sections of strains 1831 and 2730 of the green bacterium Pelodictyon clathratiforme. Cells were fixed with osmium and embedded in Maraglas. (Fig. 12) Strain 1831. A longitudinal section through a branching cell. This cell contains a single gas vacuole composed of four closely appressed gas vesicles (gv) in parallel array, which have been sectioned longitudinally. Note the elaborate cell wall (w) characteristic of this species and the cortical array of the photopigment-bearing chlorobium vesicles (cv) which are a distinctive structural feature of all green bacteria. (Fig. 13) Strain 2730. Longitudinal section of a cell showing gas vesicles sectioned both in the longitudinal and in the transverse plane. (Fig. 14) Strain 2730. Transverse section of a cell showing seven gas vesicles sectioned in the transverse plane.*



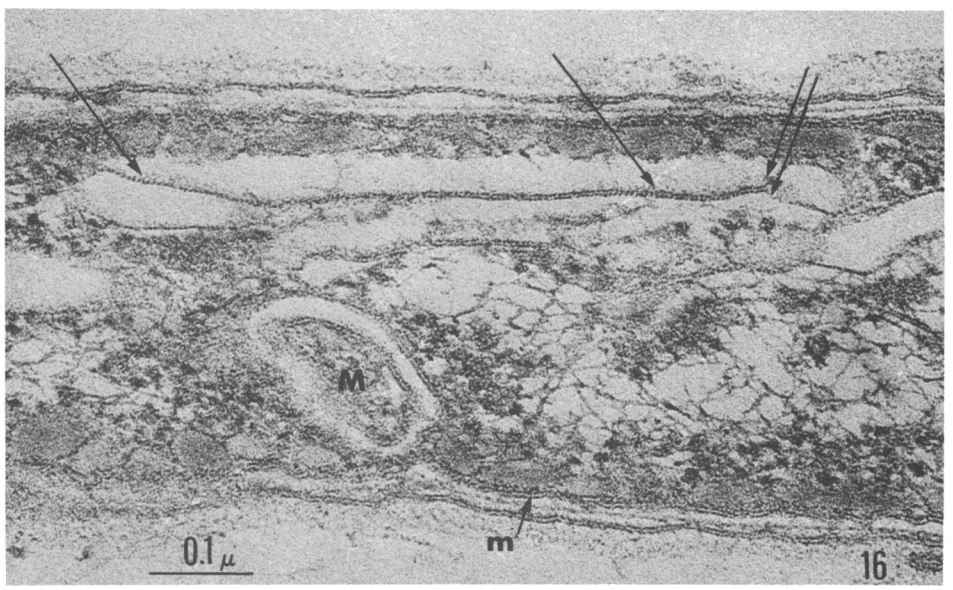
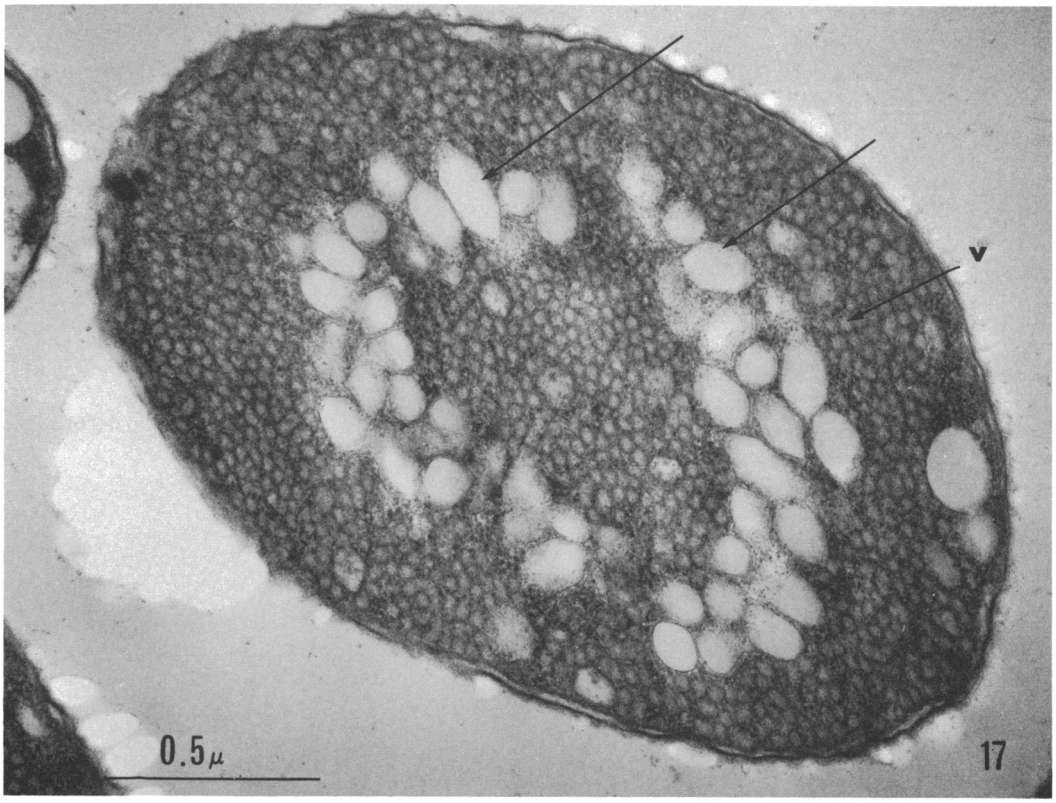


FIG. 15. *Section of Pelodictylon clathratiforme strain 1831 in which a group of gas vesicles has been sectioned tangentially (arrow) in a manner which suggests that they are interconnected. This is probably an optical illusion resulting from the extreme thinness of the vesicle membranes which makes it impossible to observe their individual contours in tangential sections.*

FIG. 16. *Section of Pelodictylon clathratiforme strain 2730 showing the appearance and structure of a collapsed gas vesicle. The double arrow indicates the point of apposition of the vesicle walls of a partly collapsed vesicle. Also present in the section is a typical mesosome (M). Note the slight difference in thickness between the mesosomal membrane, which is a typical unit membrane, similar to the cytoplasmic membrane (m), and the apparent unit membrane formed by the walls of the collapsed gas vesicle. The vesicle membranes show a regular substructure in the regions indicated by a single arrow.*



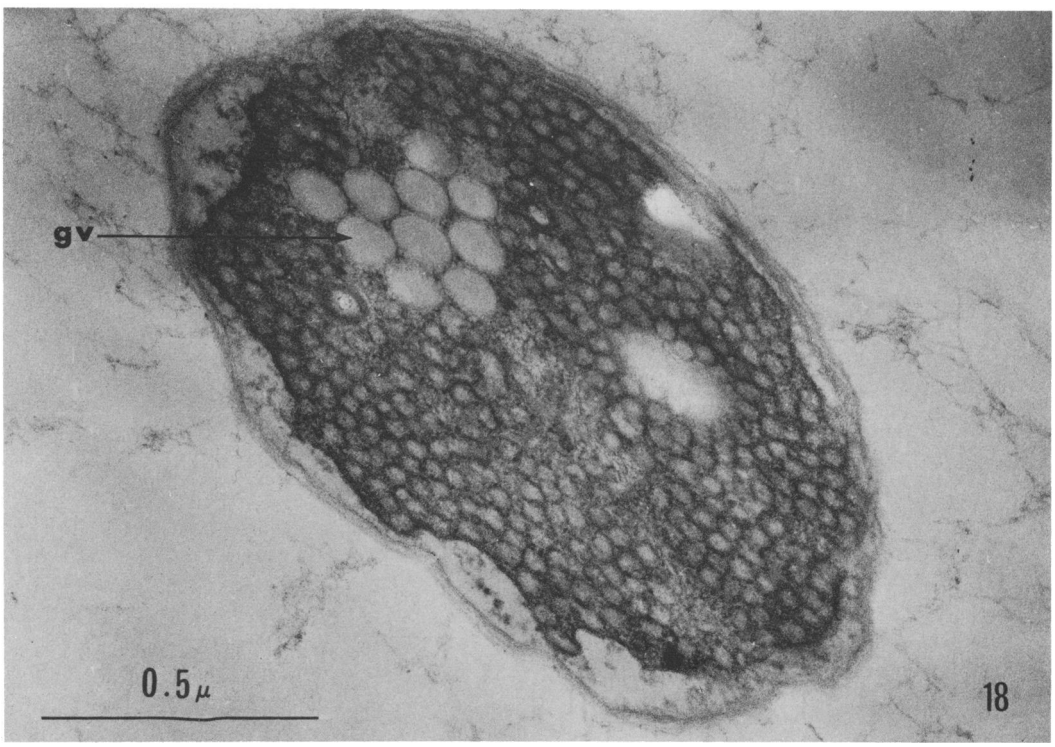


FIG. 17. *Longitudinal section of the purple sulfur bacterium Thiodictyon elegans strain 3011, fixed with osmium and embedded in Maraglas. In this species, the gas vesicles grouped in the nucleoplasmic region of the cell are oriented more or less randomly relative to one another. The surrounding cytoplasm is densely filled with photosynthetic vesicles (v) bounded by unit membranes. As shown by the longitudinal profiles (arrows), the gas vesicles of this species (and of other purple bacteria) are much shorter than those of blue-green algae and green bacteria.*

FIG. 18. *Section of the purple sulfur bacterium Rhodothece conspicua strain 6611, fixed with osmium and embedded in Maraglas. The cell contains a cluster of gas vesicles (gv), all oriented parallel to one another and sectioned transversely.*

resembling the cytoplasmic membrane (m). The walls of the partly collapsed vesicle come together at the point indicated by a double arrow to form a paired structure, 5.0 to 5.5 nm in width, containing globular subunits with a 4.0- to 4.5-nm spacing. Similar subunits can be seen in part of the wall of the vesicle which has been sectioned slightly tangentially (single arrow). These configurations suggest that the walls of the gas vesicles of *P. clathratiforme* have a regularly striated structure, like that in the walls of the gas vesicles of blue-green algae.

In the three species of purple sulfur bacteria examined in thin sections, the gas vesicles are much shorter than those of blue-green algae and *P. clathratiforme* and do not seem to occur in regular parallel arrays, except in the case of *R. conspicua*. However, they have the same general shape (cylinders with conical ends) and a width very similar to that of the gas vesicles in *P. clathratiforme* and blue-green algae. The thickness of the enclosing non-unit membrane is also similar (~3 nm).

In *T. elegans* (Fig. 17), the numerous gas

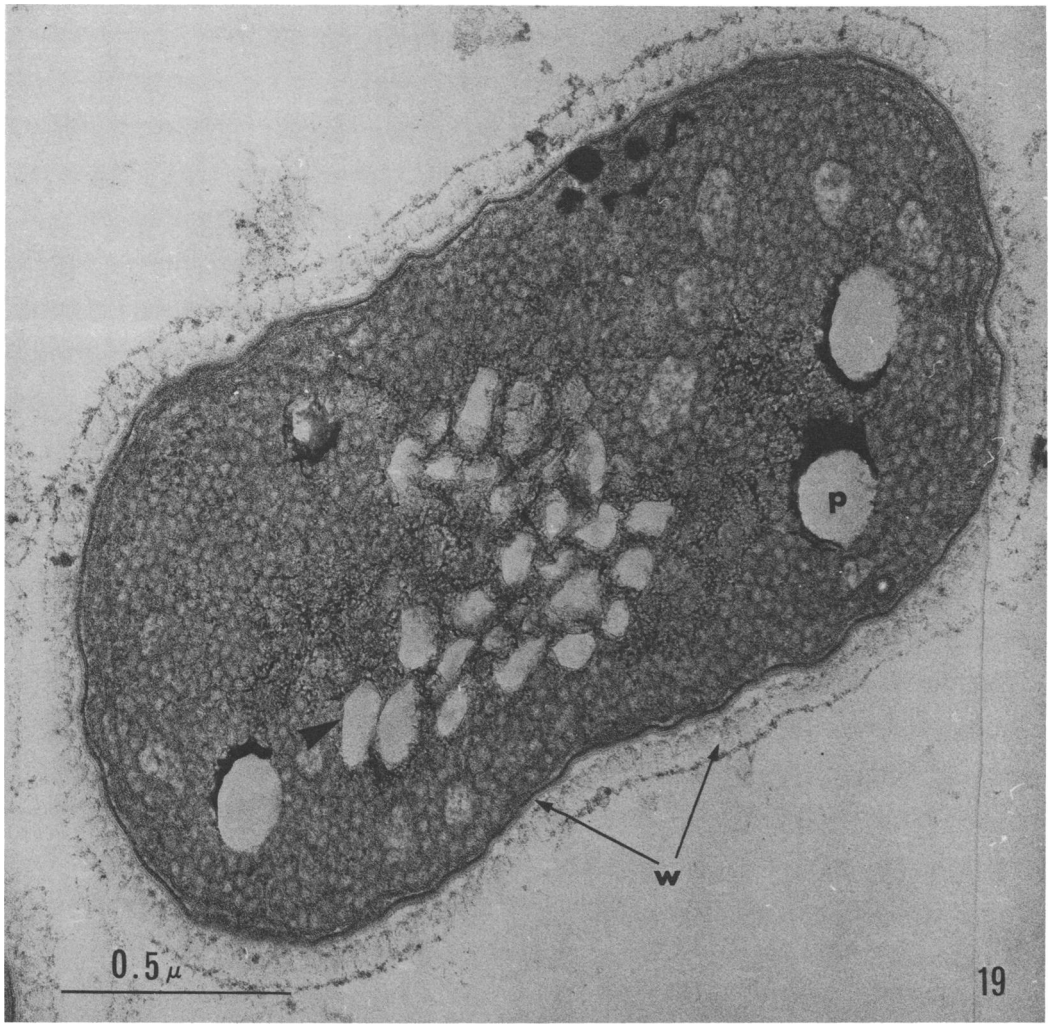
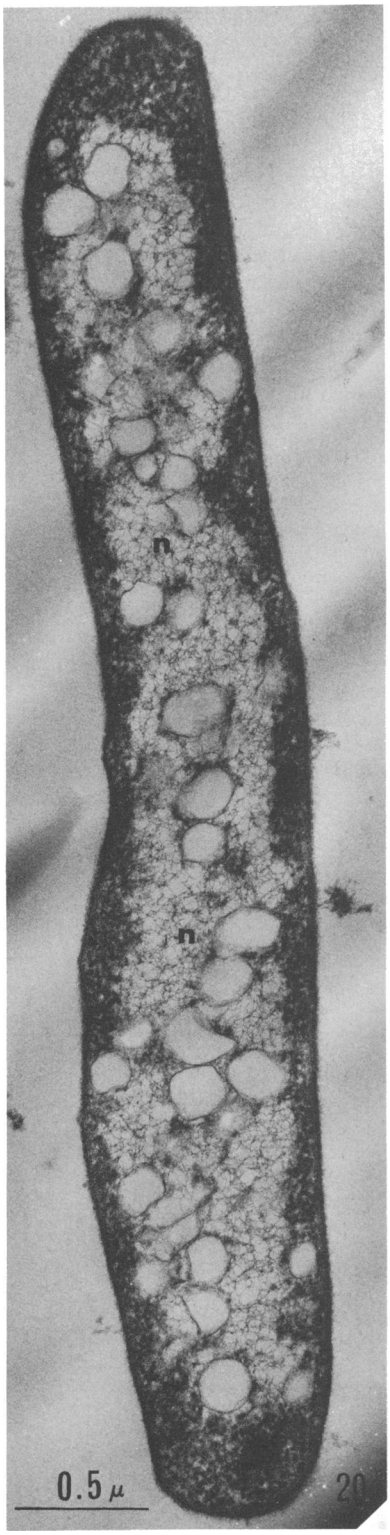


FIG. 19. *Longitudinal section of the purple sulfur bacterium Amoebobacter bacillosus strain 1814, fixed with osmium and embedded in Maraglas. The gas vesicles are not well preserved and show distorted profiles; the arrow indicates a gas vesicle of relatively normal form, which closely resembles those of other purple bacteria. The photosynthetic vesicles are similar to those of Rhodothece conspicua and Thiodictyon elegans. The large electron-transparent areas (p) partly surrounded by a very electron-opaque region are sites of partly vaporized polyphosphate deposits. Note the complex cell wall (w) and its elaborate outer layers.*



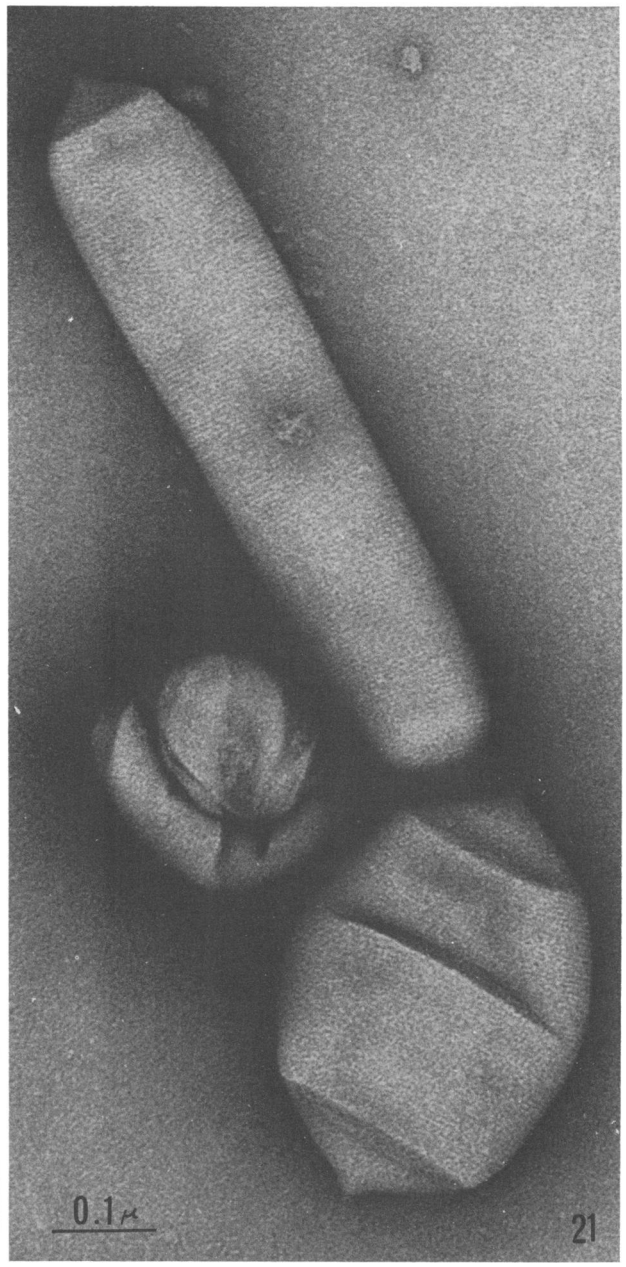


FIG. 20. *Longitudinal section of Halobacterium strain Delft, fixed with glutaraldehyde and osmium and embedded in Vestopal. The short gas vesicles are scattered irregularly throughout the cell and occur for the most part in the nucleoplasm (n).*

FIG. 21. *Isolated gas vesicles prepared from Halobacterium strain Delft and negatively stained with uranyl acetate. The striations characteristic of the walls of gas vesicles are particularly clear in this preparation. Long cylindrical vesicles are relatively rare in this strain but are seen occasionally. Short spindle-shaped gas vesicles are predominant.*

vesicles appear arranged in the form of a hollow sphere, more or less centrally located in the cell in the region occupied by the nucleoplasm. In *R. conspicua* (Fig. 18), the gas vacuole is much smaller, containing only about 10 individual vesicles. It proved difficult to preserve inflated gas vacuoles in cells of *A. bacillosus*. Figure 19 shows one of the best preparations obtained; the individual vesicles, though distorted, do not appear to differ from those of the other two purple bacteria examined. *A. bacillosus* has an extremely elaborate multilayered cell wall, clearly evident in Fig. 19.

Gas vacuoles of Halobacterium species. In strains of *Halobacterium* that contain gas vacuoles, the appearance of cells in the light microscope (Fig. 4) suggests that the gas vacuole occupies most of the cell; frequently, only the poles of the cells are nonrefractile. However, thin sections (Fig. 20) show that this appearance is deceptive: the gas vesicles are scattered irregularly throughout the cytoplasm and nucleoplasm. Their intense light-scattering ability creates the illusion of a continuous, refractile area.

As Larsen et al. (13) and Stoeckenius and Kunau (23) have already shown, *Halobacterium* provides an exceptionally favorable biological material for the isolation of gas vesicles, since the cells can be rapidly and completely lysed by simple dilution with water. We have used a method somewhat different from the methods described by these authors to prepare purified inflated gas vesicles in relatively large quantities.

Cells of *Halobacterium* strain Delft were grown on the surface of large agar plates containing the solid medium of Larsen et al. (13) and lysed by flooding the plates with distilled water. The highly viscous pink lysate was first treated at room temperature (~25 C) with deoxyribonuclease (10 μg/ml of lysate) and then for 12 hr with Pronase (100 μg/ml of lysate). The treated lysate was diluted with an equal volume of a saturated solution of NaCl and poured into a separatory funnel. The gas vesicles were collected after several hours at the surface of the liquid and were washed by drawing off the vesicle-free lower layer and replacing it with a 14% (w/v) solution of NaCl. Five successive washings yielded a milky suspension of intact vesicles which could be stored in the cold for at least 1 year with no apparent change. Figure 21 shows an electron micrograph of this material, negatively stained with uranyl acetate. Long cylindrical gas vesicles are rarely seen in these preparations; most of the gas vesicles are short and are probably flattened somewhat during the drying of the preparation, since their apparent diameter is considerably larger than that measured in sections of fixed cells. The fine structure of the vesicle wall is similar to that in blue-green algae; the striations, oriented perpendicularly to the long axis of the vesicles, have a periodicity of approximately 4 nm and appear to be composed of linearly arranged globular elements.

DISCUSSION

The gas vacuole has essentially the same organization and fine structure in all procaryotic groups in which it occurs. It is composed of a variable number of gas vesicles (hollow cylinders terminated by conical ends). The diameter of the inflated gas vesicle is approximately 80 nm and varies little from group to group (Table 2). The length and intracellular arrangement of these structures are more variable. The gas vesicles of blue-green algae and green bacteria are relatively long and are usually arranged in parallel bundles; those of purple bacteria and *Halobacterium* are shorter and are distributed more irregularly through the cell.

Observed in thin sections after osmium fixation, the wall of the gas vesicle is a non-unit membrane 2 to 3 nm in thickness, appearing as an electron opaque line. Its regularly striated surface is best seen in negatively stained or freeze-etched material.

Since suspensions of gas vesicles which are

TABLE 2. *Width of gas vesicles observed in various microorganisms*

Major group	Width (nm)
Blue-green algae	
Nostoc muscorum (25)[a]	70
Oscillatoria agardhii (21)	70
O. agardhii var. *suspensa* (sections)	70–80
O. agardhii var. *suspensa* (negatively stained)	100
Aphanizomenon flos-aquae (2)	75
A. flos-aquae (21)	70
A. flos-aquae	65–70
Green sulfur bacteria	
Pelodictyon clathratiforme (two strains)	75–80
Purple sulfur bacteria	
Rhodothece conspicua strain 6611	80–90
Thiodictyon elegans strain 3011	90–100
Amoebobacter bacillosus strain 1814	70–80
Halobacterium strain 5 (sections)	100–130
Halobacterium Delft strain (sections)	80–100
Halobacterium Delft strain (negatively stained)	
Spindle shaped	up to 250
Cylindrical	137

[a] Numbers in parentheses represent references.

still inflated can be prepared from lysates of *Oscillatoria* and *Halobacterium*, it follows that each vesicle is an independent unit of structure, completely enclosed by its non-unit membrane. The gas vacuole is thus a compound organelle, its apparent size and shape being determined by the number, arrangement, and intracellular location of the gas vesicles.

Only one other procaryotic organelle that shares some of the properties of the gas vesicle is known. This is the chlorobium vesicle, which is the site of the photosynthetic pigment system of green bacteria (3). Like gas vesicles, chlorobium vesicles are completely enclosed by a thin non-unit membrane, neither connected with nor derived from the cytoplasmic membrane. The special nature of the enclosing membrane of gas vesicles and chlorobium vesicles readily distinguishes both these structures from intracellular eucaryotic organelles (chloroplasts, mitochondria, Golgi vesicles, and vacuoles in the strict sense) which are enclosed by a typical triple-layered unit membrane.

ACKNOWLEDGMENTS

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