The Fine Structure of Gas-vacuoles Released from Cells of the Blue-green Alga *Anabaena flos-aquae*

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Summary. Cells of the blue-green alga *Anabaena flos-aquae* were ruptured and the gas-cylinders, which comprise the gas-vacuoles, were released. The gas-cylinders released retained their same shape and structure: 70 m\(\mu\) or more wide, 0.2—0.9 \(\mu\) long, with conical ends. Staining with phosphotungstic acid showed the membrane to be made up of particles arranged in rows, about 5 m\(\mu\) apart, running round the cylinder at right angles to the long axis. Some of the cylinders became flattened during the preparation. Rounding-off of the gas-cylinders on isolation, described by others, was not encountered. However, some of the isolated gas-cylinders were considerably wider than 70 m\(\mu\) and it is possible that they tend to shorten and widen on isolation from the cell.

Recent electron microscope studies on the fine structure of the blue-green algae have shown that the gas-vacuoles, which appear under the light microscope as regions of low refractive index, are made up of cylinder shaped sub-units stacked very closely together in the cell. The sub-units have been called variously "Hohlspindeln" (Jost, 1965; Jost and Matile, 1966); "gas vesicles" (Bowen and Jensen, 1965) and "gas-cylinders" (Smith and Peat, 1967); the last term will be used here. In broad agreement with the other authors, Bowen and Jensen (1965), found that the cylinders average 75 m\(\mu\) in diameter, range from 0.2 to 1.0 \(\mu\) in length, have conical ends and are bound by a single membrane measuring 2 m\(\mu\) wide after fixation in osmic acid. Smith and Peat (1967) found that the membrane appeared thicker, 3 m\(\mu\), after permanganate fixation. Jost (1965) did not get good preservation of the membrane structure with permanganate and obtained the best results with the freeze-etching technique. Jost showed that the membrane was constructed of particles arranged in ribs which ran round the cylinder at right angles to the long axis of the structure.

In all of the above studies, the gas-vacuoles were examined in sections of the intact cells, where the structure of the cylinders would be supported by the fixation and embedding medium, or by ice crystals in the case of freeze-etching. Jost and Matile (1966) have described the isolation of structures said to be identical with the cylinders seen in freeze-etched and sectioned cells. The cells were ruptured by agitation.
with glass beads in sucrose solution and the cylinders released rounded off to form small bubbles about 0.1 μ diameter. When the bubbles were separated from other cell components by centrifugation in a sucrose gradient, they formed a yellow-orange layer from which a lipid and two carotenoids were isolated and characterized. The carotenoids may account for the reddish colour of gas-vacuoles seen under the light microscope.

Using rather different methods of preparation we have found that the cylinders comprising the gas-vacuoles retain their appearance and similar fine structure when released from the cell.

During the preparation of surface replicas of *Anabaena flos-aquae* it was noticed that when whole cells were dried down on formvar coated grids and viewed under the electron microscope at low magnification, groups of well defined ‘light’ (electron transparent) areas could be seen against the dark background of the cell (Fig. 1). The light areas were of cylindrical shape, about 70 μ wide, and obviously corresponded to the gas-cylinders described above. The cylinders could be seen in unstained and unfixed preparations because their contents, presumably gas (Fogg, 1941), has much less electron absorption than the surrounding cytoplasm; although the surrounding organic material is but weakly electron absorbing, in such a thickness it does offer considerable contrast to the gas-cylinder spaces it encloses. The gas-cylinders which show up are an unusual example of fine structure revealed without prior fixation or staining with electron dense material, and should be free of the associated artefacts.

Some of the cells broke open on drying on to the grid, and their contents were released (Fig. 2). Although the gas-cylinders released were not free of other cellular material they were plainly intact and apparently unchanged. This provided an indication that the cylinders might not depend on the surrounding cytoplasm for support. Conditions were further investigated which would allow the rupture of the cells and the release of the gas-cylinders without distortion of their structure.

Iodine solution, originally investigated to see if it would preserve filaments containing gas-vacuoles, did not cause deflation or distortion of the gas-cylinders. Cells exposed to it showed a tendency to break when subject to very slight pressure, such as from the weight of a coverslip during light microscope examination. Under the light microscope minute bright objects not fully resolved, possibly individual gas-cylinders or stacks of cylinders, were seen to be released on breakage of the cells. Preparations were made for electron microscope examination.

**Material and Methods**

1. *Anabaena flos-aquae* obtained from the Freshwater Biological Association, Windermere, was the same strain used in the comparative study of **Smith** and **Peat** (1967). It was grown in unialgal but not bacteria-free culture in the medium of