A Chlorophyll-Containing Cell Fraction from the Blue-Green Alga, *Anabaena variabilis*.

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Previous studies on the fine structure of blue-green algae including *Anabaena variabilis* (1, 2) revealed the presence of arrays of cytoplasmic membranes similar to those described in the photosynthetic apparatus of higher plants (3). This report is not a systematic study of the morphology of *A. variabilis*, but a description of observations made on the intact cell and one of its fractions. Evidence has thereby been obtained that the cellular chlorophyll and carotenoids are associated with the intracellular membranes.

A. variabilis was cultured according to the method of Kratz and Myers (4). Whole cells and cell-free preparations were kindly provided by Dr. B. Petrack. Harvested cells were washed, broken up by grinding with alumina, and extracted with 40 per cent ethylene glycol in 0.1 M tris buffer, pH 7.8. Two 10-minute periods of centrifugation at 2000 R.P.M. served to remove the abrasive and unbroken cells. The supernatant was then centrifuged at 105,000 *g* for 1 hour to obtain a pellet. All the chlorophyll and carotenoids as well as the photophosphorylating activity of the crude extract was recovered in the pellet. However, the phycocyanin remained in the soluble phase (5). These observations suggest that the pellet contains the photosynthetic apparatus of the whole cell.

Intact cells and the 105,000 *g* pellet were prepared for electron microscopy according to the method previously described (6). As shown in Fig. 1, *A. variabilis* contains evenly spaced, parallel membranes or lamellae arranged concentrically about a lighter region corresponding in location with the central Feulgen-positive area. The lamellae are joined in pairs, forming loops at each end (Figs. 1, inset, and 2). Thus, the parallel membranes are organized into structural units which are closed, flattened sacs or discs approximately 20 mμ thick. In cross-section each sac is represented by two dense parallel lines joined at both ends and separated in between by a lighter space. Dense granules, *g*, are interdispersed among the membranes. Young cells contain fewer granules, suggesting that the latter are storage products.

Cells undergoing rapid division form short chains of individual organisms separated by cell walls and membranes. The general morphology of the dividing cell is similar to that seen in Fig. 1, although young cells contain a reduced number of sacs more widely separated by areas of low density.

In preparations of whole cells, damaged or dying organisms appear among the intact ones. They contain disorganized lamellae and swollen vesicles (Fig. 3). Various stages of cellular disintegration with release of those vesicles also may be observed (Figs. 4, 5).

Thin sections of the chlorophyll-containing 105,000 *g* cell fraction shows that it consists of membranes organized into vesicles (Fig. 6). The resemblance between these vesicles and those resulting from the swelling of discs in damaged cells is used as a criterion for identifying the vesicles in the cell fraction as extensively fragmented discs.

Calvin and Lynch have described a cell fraction isolated from *Synechococcus cedrorum* by a procedure similar to that employed for *A. variabilis* (7). The *S. cedrorum* fraction also contained the chlorophyll and carotenoids of the algae and consisted of "grana-like particles." Notwithstanding the questionable label used to describe them, these "particles" had actually the appearance of dried and flattened vesicles. It appears, therefore, that in *S. cedrorum*, as well as *A. variabilis*, the cellular chlorophyll and carotenoids are associated with vesicles which result from disruption of intracellular membranes (1).

The observations on chlorophyll location in *A. variabilis* are in agreement with those previously reported for the green alga, *Chlamydomonas* (8), and red alga, *Porphyridium* (9). This note lends further support to the hypothesis that in these forms the intracellular membranes are important for the photosynthetic process.

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EXPLANATION OF PLATE 317

FIG. 1. Whole cell of *A. variabilis* containing many lamellae (*l*), nucleus (*N*), and dense granules (*g*). × 58,000.

FIG. 1 *inset*. Section through cytoplasmic membranes showing loop (arrow) formed by pairing of adjacent lamellae. × 70,000.

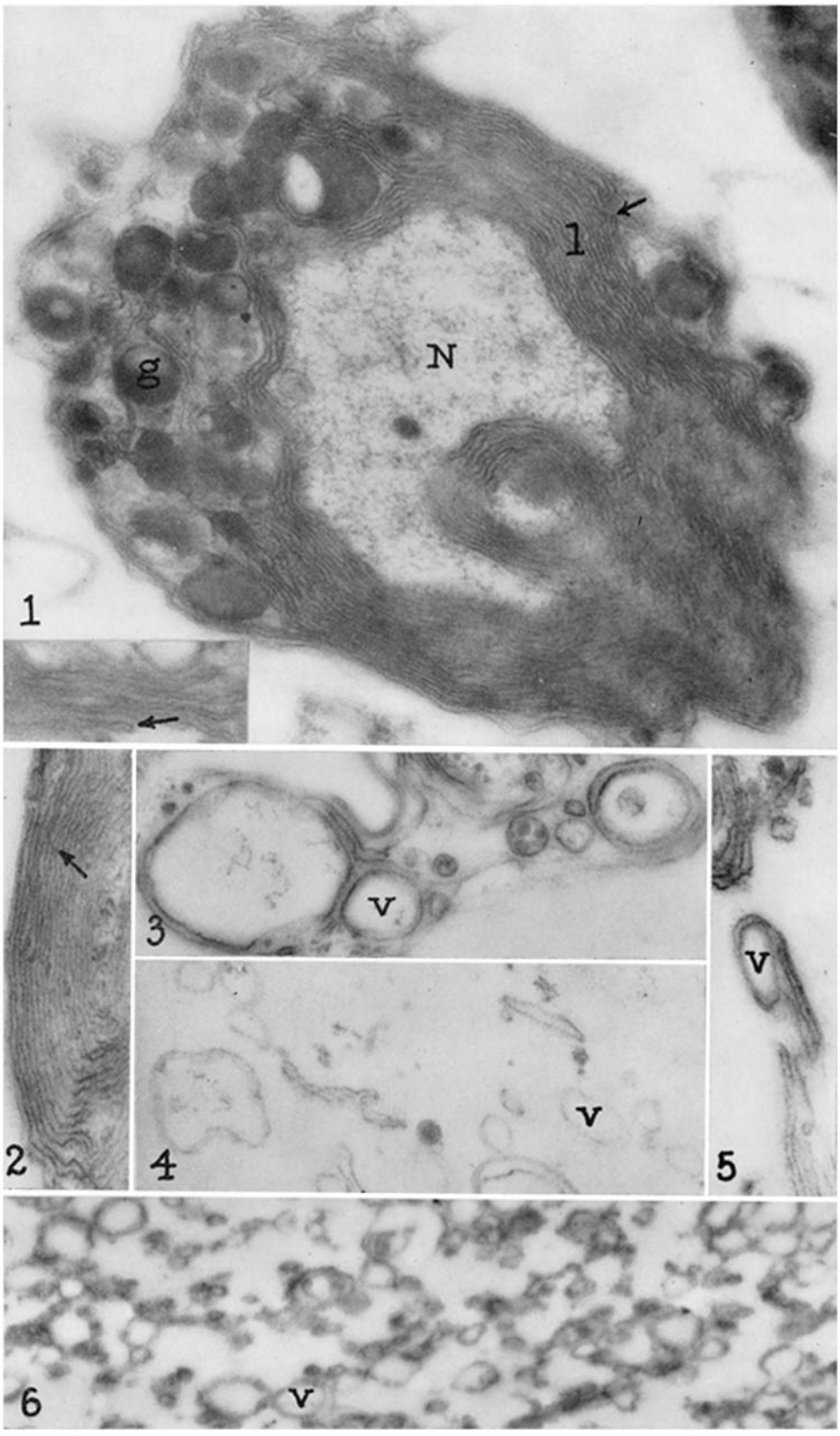
FIG. 2. Same as Fig. 1, inset. × 64,000.

FIG. 3. Part of damaged cell containing several vesicles (*v*). × 34,000.

FIG. 4. Free vesicles (*v*) observed in preparation of whole cells. × 34,000.

FIG. 5. Same as Fig. 4. × 64,000.

FIG. 6. Section of 105,000 *g* pellet which is composed of vesicles (*v*) similar to those in Figs. 3 to 5. × 34,000.



(Shatkin: Chlorophyll-containing cell fraction)