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The Ultrastructure and Development of the Colonial Sheath of *Microcystis marginata*

MARTIN KESSEL and JACOBUS N. ELOFF*

Department of Microbiological Chemistry, The Hebrew University–Hadassah Medical School, Jerusalem

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Abstract. The colonial sheath of *Microcystis marginata* has a definite structure as seen by light and electron microscopy, consisting of a relatively smooth inner surface and densely packed, long fibrils on the outer surface. The sheath initially forms around the single cell and expands by continual deposition of sheath material to accomodate the synchronously dividing cells of the colony.

Key words: Cyanobacteria – Colonial sheath – Ultrastructure.

The genus *Microcystis* comprises cyanobacteria found in all temperate and tropical zones, and includes rod and coccoid forms with and without gas vacuoles (Geitler, 1932). One of the most important characteristics used for the classification of *Microcystis* has been the colonial structure. Most descriptions state that the individual cells of *Microcystis* are embedded in a structureless slimy layer (Stanier *et al.*, 1971; Fogg *et al.*, 1973).

Fogg *et al.* (1973) suggested the term "mucilage" in the case of *Microcystis* and *Nostoc* for the matrix holding the individual cells together, but stress that a distinction between the terms "sheath" and "mucilage" has not been made by any author. The current view is that structures, fibrillar in appearance and composed of pectic acids and mucopolysaccharides, should be referred to as sheaths (Dunn and Wolk, 1970). Such sheaths are found surrounding individual cells and have been described for filamentous and unicellular forms of cyanobacteria (Leak, 1967; Tuffery, 1969; Lamont, 1969). Colonial forms have also been described as being enveloped by such sheaths.

In older literature the colonial sheath was used as a taxonomic criterion. *Microcystis viridis* and *M. marginata* were described as having a colonial envelope with a definite refractive margin, sometimes lamellate (Geitler, 1932; Desikachary, 1959). Various authors, cited by Desikachary, discussed the importance of the shape of the colony and limits of the colonial sheath. Though colonial sheaths are well documented in the taxonomical literature, no study has been made of their ultrastructure and mode of formation. During the study of physiological properties of cyanobacteria, we sampled from fishponds in the Upper Galilee in Israel a cyanobacterium organized in distinct colonies that fitted the description of *Microcystis marginata* given by Crow (1923) and was resistant to photooxidation (Eloff *et al.*, in press). The formation, structure and properties of the colonial sheath of this species are described in this communication.

* Permanent address: Botany Department, University of the Orange Free State, Bloemfontein, South Africa.

Materials and Methods

Collection and Treatment of Cells. Samples were collected from a natural bloom of *Microcystis* in a fishpond in the Upper Galilee and filtered through a 225 μm mesh, plankton filter (Zürich Bolting Cloth Mfg. Ltd., Ruschlikon, Switzerland) to remove suspended matter. The filtrate was centrifuged (2000 g, 10 min) and the floating *Microcystis* colonies collected. The colonies were maintained in a modified Chu 11 medium (Safferman and Morris, 1967) under continuous fluorescent light (incident intensity of 1000 ergs/cm^2/sec) at 26° C.

Colonial sheaths were ruptured by shaking without glass beads in a Nossal cell disintegrator (McDonald Instrument Co., Bay Village, Ohio) for 30 sec, causing rupture of some of the colonies. Single cells were separated from the intact colonies and the empty sheaths by filtering through a 25 μm pore size membrane filter. Gas vacuoles in cells were collapsed by applying a pressure of 1500 lb/inch^2 to their suspension.

Chemical Tests. Partially purified sheath preparations, relatively free of individual cells, were obtained after shaking and filtering. Sheath preparations were treated with 100 μg lysozyme/ml dissolved in 0.15 M NaCl in 0.1 M EDTA at pH 8

and 37° C; tests with 2% (v/v) sodium dodecyl sulfate (SDS) were conducted at 60° C. Tests with cellulase (10 mg/ml) were conducted at pH 4.5 and 45° C.

Light Microscopy. Cells were photographed in either phase contrast or Nomarski interference contrast using a Zeiss Universal microscope.

Electron Microscopy. Fixation: Cells were fixed overnight at room temperature in 2% glutaraldehyde in either 0.05 M Na cacodylate (pH 7.2) or 0.05 M phosphate buffer (pH 7.2) to which 0.5% sucrose was added. Buffers and fixatives were prepared in filtered pond water. Cells were washed in buffer and postfixed overnight in 1% OsO_4 in the respective buffers. Postfixed cells were then washed three times in distilled water, embedded in agar and stained in 2% uranyl acetate for 1 hr; the material was dehydrated in a graded alcohol series and embedded in Spurrs Low Viscosity Medium (Spurr, 1969). Sections were cut on a LKB ultratome III with a diamond knife and stained with lead and uranyl acetate. In preparations using ruthenium red, the stain (0.5%) was added at both the glutaraldehyde and osmium fixation steps (Leak, 1967).

Shadow casting: A drop of empty sheath preparation (after mechanical rupture) was placed on a carbon-coated collodion grid, and the liquid was withdrawn after a few minutes. The grid was shadow-cast with palladium at an angle of 10°.

Freeze etching: After being washed free of medium, living and fixed colonies were frozen in liquid Freon 22 and freeze etched in the Balzers BAF 301 apparatus. Cells were fixed in 2% glutaraldehyde in 0.05 M Na cacodylate buffer, washed in buffer and maintained overnight in a 25% glycerol solution.

All the above preparations were viewed in a Philips EM 300 operating at either 60 or 80 kV.

Scanning electron microscopy: Colony suspensions were either washed with 0.1 M Na cacodylate buffer or fixed in 2% glutaraldehyde in 0.05 Na cacodylate for 30 min at room temperature, then washed with buffer and freeze dried for 1 hr. Dried specimens were coated with gold and viewed in a Cambridge Stereoscan Mk II operating at 20 kV.

Results

Fig. 1 compares a mature *Microcystis* colony enveloped in an intact sheath with a colonial sheath ruptured mechanically and emptied of cells. Within the intact sheath the cells appear very tightly packed. Tests with the partially purified sheath preparations showed that the sheaths were insoluble in boiling water even after 2.5 hrs of treatment. The sheath stains with methylene blue and ruthenium red. Although the colonial sheath was not attacked by lysozyme or SDS, walls of individual cells were lysed when treated with SDS, as observed in the strain 7005 (Stanier *et al.*, 1971). There was no evidence of any breakage of the sheath on exposure to cellulase.

Examination of fresh samples of the pond *Microcystis* revealed sheaths enclosing various numbers of cells. In most cases examined, the number of cells was 2^n, up to $n = 5$, indicating synchronous division in the colony (Fig. 2). Cells (shown in Fig. 2a–f) were subjected to pressure in order to collapse the gas

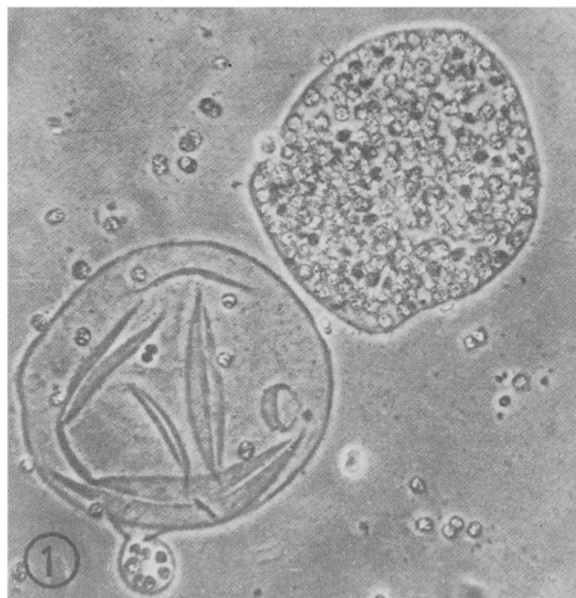

Fig. 1. Intact and dispersed pond *Microcystis* colonies, showing empty and full colonial sheaths. Phase contrast micrograph. ×280

vacuoles and reduce refractility. Under these conditions it was possible to distinguish fibers arranged irregularly around the cells, suggesting a mucilagenous excretion of some kind. In some cases the fibrous material seems to fill the entire intrasheath space, whereas in other cases this extracellular material is absent and cells within the colonial sheath appear smooth. Loose mucilage surrounding both the colonial sheath and most of the cells liberated from colonies was clearly observed in intact and ruptured colonies mounted in India ink. When mature pond *Microcystis* colonies were examined 4 days after mechanical rupture of the sheaths, unsheathed cells as well as cells at late stages of sheath development were observed within the original ruptured sheath (Fig. 3).

The sheath structure was observed in greater detail by staining glutaraldehyde osmium fixed cells with ruthenium red, a stain specific for acid mucopolysaccharide and pectate (Leak, 1967). Examination of the stained cells by Nomarski interference contrast revealed a sheath composed of a thickened, doubled inner layer approximately 0.7 µm wide with an external flattened layer, 1.5–2 µm wide.

Electron microscope examination of thin sections (Fig. 4) of the sheath stained by ruthenium red revealed two amorphous electron-dense layers of 0.15 µm width each, separated by an 0.3 µm thick, more transparent amorphous layer. The total width of 0.6 µm of these three layers corresponded to the doubled inner layer observed by light microscopy. Numerous fibrils, 60 nm in diameter, projecting from the external

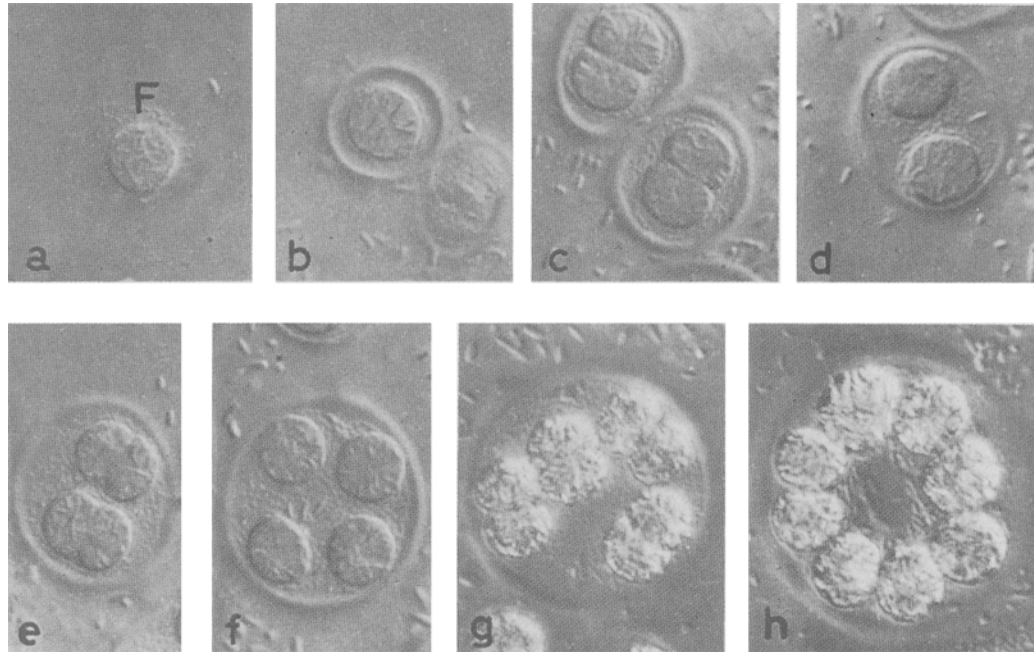

Fig. 2a–h. Stages in the development of pond *Microcystis* colonial sheath. (a) Single cell without sheath, showing extracellular fibers (*F*). (b) Single cell surrounded by sheath. (c)–(h) Colonies in different stages of synchronous division, surrounded by colonial sheaths. Colonies shown in micrographs (g) and (h) were not subjected to pressure and the highly refractile gas vacuoles remained intact, obscuring extracellular details. Nomarski interference contrast micrographs. ×1380

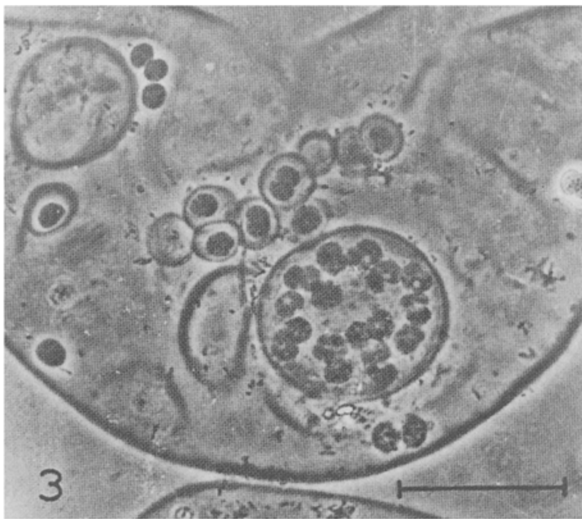

Fig. 3. Various sheathed colonies and single unsheathed cells within sheaths of ruptured pond *Microcystis* colony. Phase contrast micrograph. ×440; scale equals 50 μm

dense layer, had variable lengths of 2–4 μm. The internal dense layer of the sheath appeared to be covered from within by fibrils forming a flattened fibrillar layer, 0.15 μm thick.

Some insight into the surface texture of the sheath was obtained by shadow casting a partially purified preparation of sheaths (Fig. 5). The sheath surface appeared as a very fine network of 50-nm thick fibrils, lacking any particular orientation; their density seemed to depend on the degree of spreading of the sheath on the grid. The fibrils were concentrated at the sheath edges and extruded outwards in varying length and thickness, possibly due to drying and heat produced during shadow casting. Since fixed and sectioned sheaths show a more regular distribution of sheath fibrils, the shadow-cast preparation seems artifactual, but nevertheless reveals the sheath surface structure.

Confirmation of the fibrillar network was obtained when cells were freeze etched (Fig. 6). Although we did not succeed in obtaining fracture planes revealing either the surface or subsurface of the sheath, cross fractures of the sheath revealed a thickened rib with a broad network of fibrils spread on either side. At high magnification, the rib network appeared to consist of fine, 40 nm thick fibrils.

A further aspect of the sheath structure could be demonstrated by scanning electron microscopy (Fig. 7) in which the sheath appeared as a dense structure enclosing the cells. In these preparations, the sheaths were seen as sacs with a relatively smooth surface. The drying process in preparation for the scanning electron microscopy prevented visualization of the fibrillar surface detected by shadow casting and thin sectioning. The juxtaposition of the cells within the

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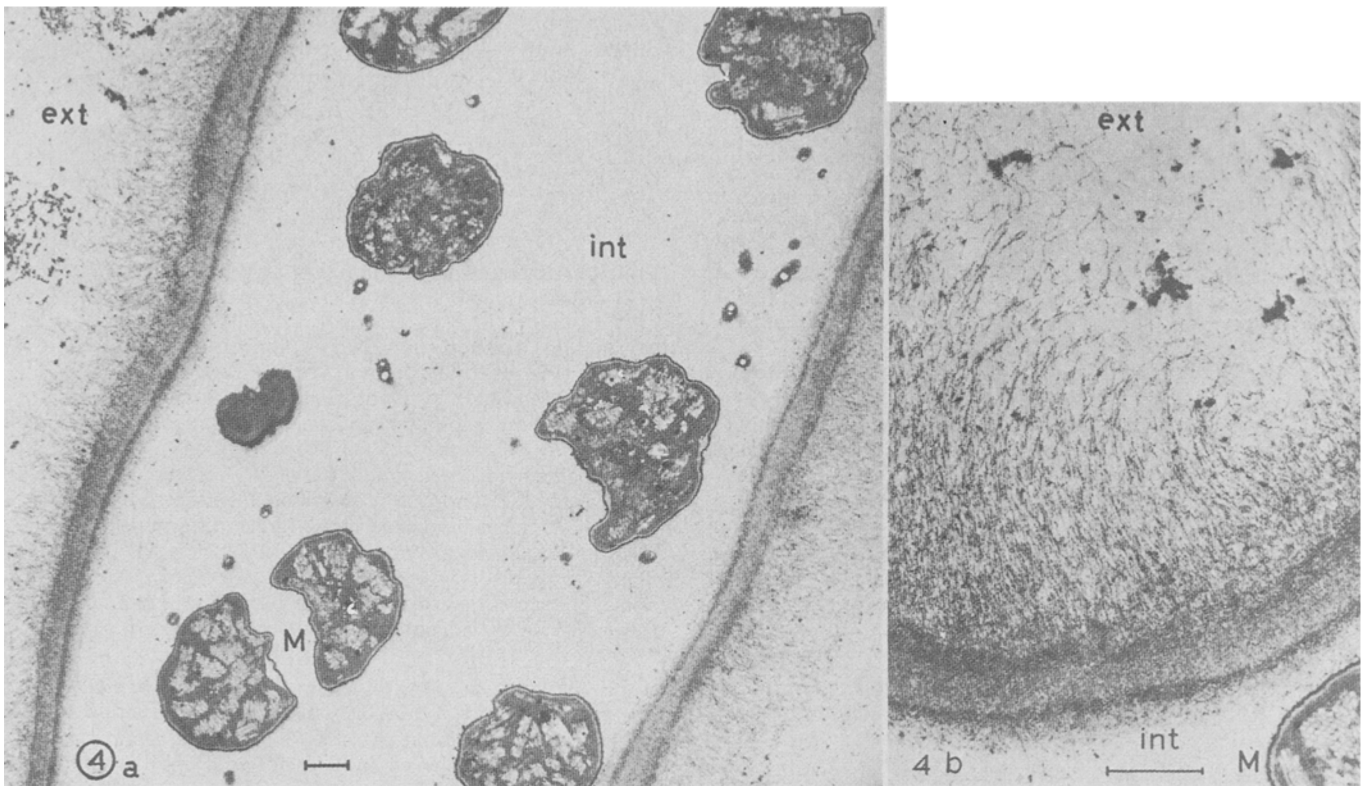

Fig. 4a and b. Thin section of ruthenium red-stained colonial sheath. *int* intrasheath; *ext* extrasheath; *M Microcystis* cells. (a) ×7980; scale equals 1 µm; (b) ×17100; scale equals 1 µm

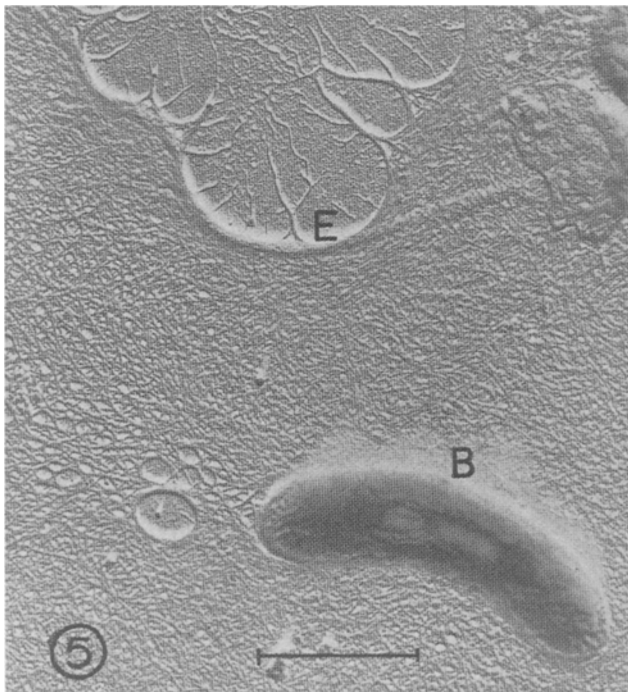

Fig. 5. Shadow-cast partially purified sheath preparation. *E* sheath edge; *B* bacteria lying on sheath surface. ×20235; scale equals 1 µm

sheath could be seen through fortuitous cracks caused by the drying process. A weblike matrix of the extracellular mucilage remained in locations where cells were detached during preparation. By end-on orientation of the cracked edges, the sheath thickness and cellular topography could be evaluated. The relatively thick white flecks on cell surfaces correspond to extracellular mucilage. The outlines of the cells, clearly seen projecting through the sheath layer, demonstrate the flexibility of the sheath in these preparations.

Discussion

The colonial sheath of *Microcystis marginata* has a distinct morphological structure and serves to demarcate the colonial entity in this species. This defined colonial form contrasts with the organization of other *Microcystis* species whose cells are held together in an amorphous hyaline slime.

The *M. marginata* colony is formed by successive synchronous divisions of cells within a common sheath. After initial solidification around the single cell, sheath material produced by the daughter cells most likely becomes progressively intercalated and deposited on the surface of the sheath as the latter

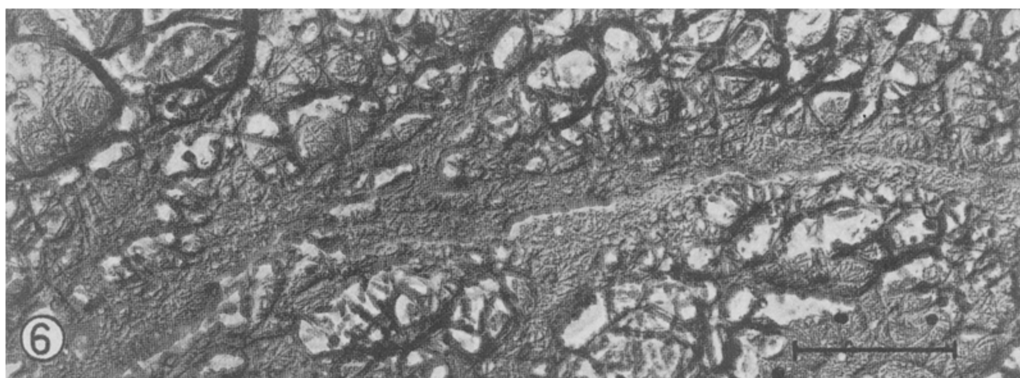

Fig. 6. Freeze fractured preparation showing the fibrillar structure in a cross fracture of the sheath. × 51 300; scale equals 0.5 μm

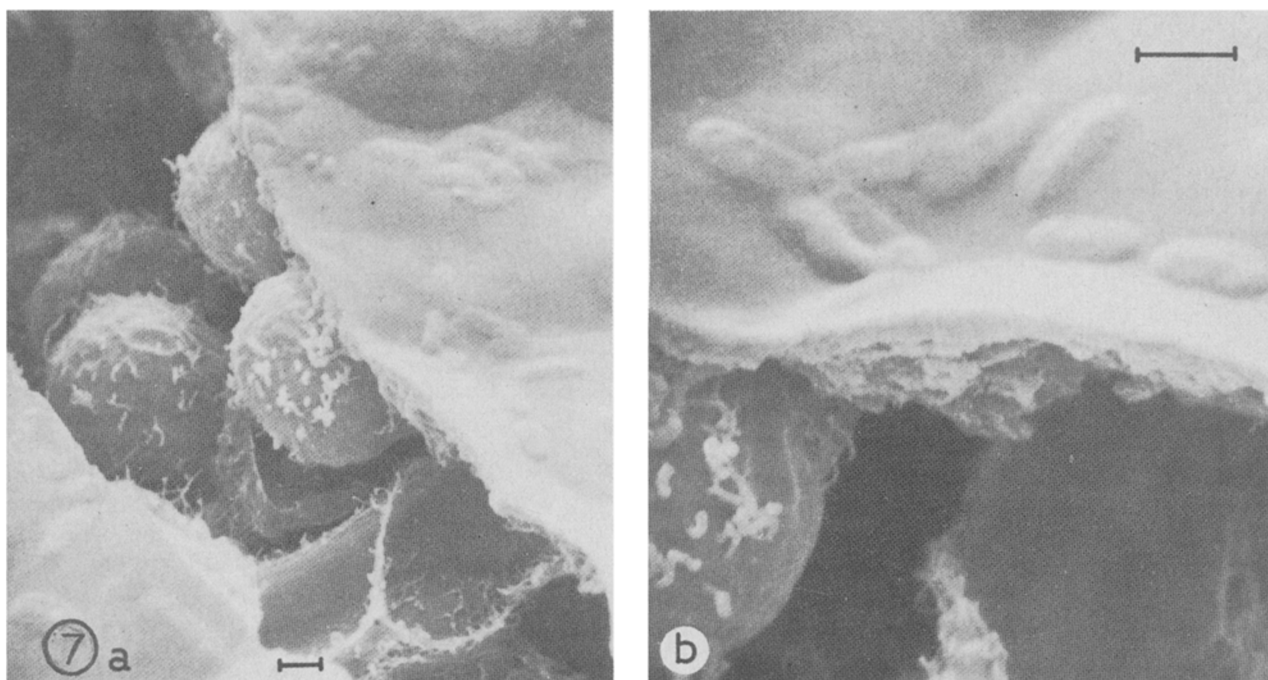

Fig. 7a and b. Scanning electron micrograph of fixed, freeze-dried mature pond *Microcystis* colony [scale (a) equals 10 μm, in (b) 1 μm]

extends to accomodate the increasing number of cells. The multicellular structure comprising the *M. marginata* colony shows no cellular differentiation, but there is evidence of an intrasheath environment differing from the external milieu. The different inner and outer aspects observed in the ultrastructure of the colonial sheath (see Figs. 4 and 7) suggest differences in the environments. That the inner environment is a function of the presence of an intact colonial sheath was demonstrated by the formation of new distinct sheaths around individual cells immediately after rupture of the original colonial sheath (see Fig. 3).

The colonial form of *M. marginata* contrasts with the situation observed in *Gloeocapsa* colonies. In *Gloeocapsa*, multilaminate sheaths are formed around individual cells after cell division, giving rise to a series of sheaths within sheaths, each sheath containing four cells (Stanier *et al.*, 1971). In this case, the intrasheath environment does not prevent sheath formation as it apparently does in the case of our *Microcystis*.

The outer aspect of this colonial sheath shows fibrils (2–4 μm long) extending from the sheath surface when examined after thin sectioning (Fig. 6), and seen as a flattened network of fibrils when examined by shadow casting (Fig. 7). Drews (1973) pointed out that the removal of water may change the fibrillar layer. Staining by ruthenium red confirms that this sheath's composition is similar to that described for

Anabaena sp. (Leak, 1967) and consists of mucopolysaccharides and pectates.

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Dr. Martin Kessel
Department of Microbiological Chemistry, The Hebrew University—Hadassah Medical School
Jerusalem, Israel