found with cultured cells may also have been observed in a patient with Fabry's disease (*15*). Here it was found that two types of inclusion materials were present in biopsy specimens, one of which appeared to be a mucopolysaccharide on morphological grounds.

It is possible that the deficiency of an enzyme which participates in the degradation of both glycolipids and mucopolysaccharides may result in accumulation of both types of substance. A gal→gal linkage is present in the linkage region of a number of sulfated mucopolysaccharides (chondroitin-4-sulfate and -6-sulfate, dermatan sulfate, heparin and heparitin sulfates) (*16*) but has not been demonstrated in hyaluronic acid. It differs from that of the trihexosyl ceramide in that it is internal rather than terminal and is $\beta 1 \rightarrow 3$ rather than $\beta 1 \rightarrow 4$ (*17*). Whether the galactosidase can also act as an endoglycosidase is not known. Available evidence (*4*) suggests that the galactosidase missing in Fabry's disease is highly specific for GL-3.

In view of the accumulation of acid mucopolysaccharides in a number of diseases, it is possible that other types of mechanisms may be responsible for this phenomenon. The biosynthesis, accumulation, and excretion of acid mucopolysaccharides occur on membrane systems (*18*). The distribution of polysaccharides seems similar to normal in Fabry's disease, in contrast to the specific accumulation of dermatan sulfate in Hurler's disease and in the Hurler variant, and of hyaluronic acid in Marfan's disease. Interference in the metabolism of membrane components may secondarily influence mucopolysaccharide metabolism, though experimental evidence is lacking. Further information regarding the degradation of both glycolipids and mucopolysaccharides as well as the role of glycolipids in cell physiology are necessary for definitive answers to these questions. The demonstration of defects of both glycolipid and mucopolysaccharide metabolism in cultured fibroblasts opens new possibilities for the study of such genetic disorders as well as of normal cell physiology.

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Cytophaga That Kills or Lyses Algae

Abstract. *A myxobacterium* (Cytophaga N-5) *isolated from sewage kills or lyses an array of living green and blue-green algae. When assayed with* Nostoc muscorum *or* Plectonema boryanum, *plaques form like those caused by the blue-green algal virus LPP-1. This isolate lyses or inhibits mutually Gram-positive and Gram-negative eubacteria.*

In seeking new algal viruses from the waste stabilization ponds of Austin, Texas, we found a nonfilterable agent which killed or lysed (*1*) an array of living green and blue-green algae. This agent was distinguished from viruses by its wide host range and its ability to digest autoclaved algae.

This organism is Gram-negative, unicellular, rodlike with rounded ends (0.4 to 0.6 by 1.9 to 3.7 μ), and aflagellate (Fig. 1a). On agar it forms slimy, yellow colonies with thin spreading margins and, with age, produces a brown water-soluble pigment. The cells at the margins of colonies move in a gliding, flexuous manner. No fruiting bodies or microcysts are produced. Cellulose is digested; chitin is not. These characteristics relegate the organism to the order Myxobacterales and identify it as a new species of the genus *Cytophaga* (*2*) which is herein referred to as *Cytophaga N-5*.

A salient characteristic of *C. N-5* is its ability to kill or lyse a variety of green and blue-green algae (*3*). Sensitivity was determined by placing 0.1 to 0.2 ml of an actively growing culture of *C. N-5* on algal lawns. Killing or lysis occurred within 1 day to 2 weeks. Although most green and blue-green algae tested were killed or

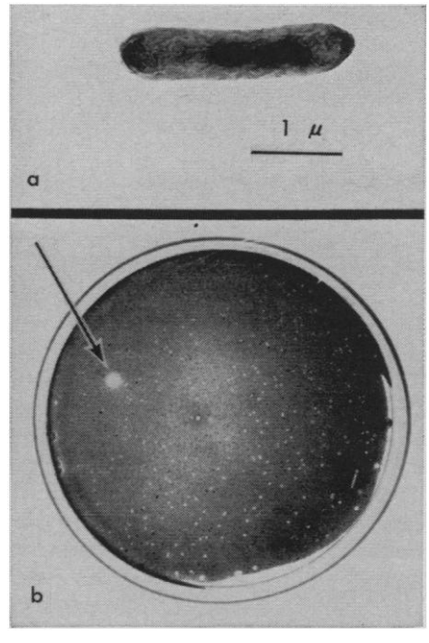

Fig. 1. (a) Electron micrograph of a cell in the log phase of growth negatively stained with 2 percent uranyl acetate. (b) Three-week-old overlay of *Cytophaga N-5* assayed with *Nostoc muscorum* in a 9-cm petri dish. Arrow indicates largest plaque, while 400 smaller plaques are visible at this dilution ($\times 10^{-7}$ cells per milliliter).

lysed, there were resistant organisms in both groups.

Plaque-forming units were assayed only on *Nostoc muscorum* (Fig. 1b) and *Plectonema boryanum* with the conventional double-agar overlay technique. Lysed areas are caused by dissolution of the algae after digestion of cell walls by the myxobacterium. Heterocysts and akinetes are more resistant to *C.N-5* than are vegetative cells.

When plaques form, isolated and discrete *Cytophaga* colonies appear among the algal overlay within 2 weeks. Little or no lysis of algae occurs before 2 weeks, when bacterial colonies are less compact and more flattened. After 3 weeks most *C. N-5* colonies are gone, but plaques of lysed algae evince the presence of *Cytophaga* cells. The ratio of viable bacterial cells to plaque-forming units in a culture of *C. N-5* is 1.28, as determined with *N. muscorum*.

In contrast to viral lysis, lysis by *C. N-5* is extracellular. However, *Cytophaga* and blue-green algal virus (LPP-1) plaques are microscopically similar in overall appearance, and both types of plaques can be enumerated. Selective dissolution of *Nostoc* vegetative cells, as opposed to heterocysts and akinetes, parallels the action of an unidentified bacterium (*4*) and an algal virus (*5*).

Cytophaga N-5 grows in chemically defined medium or on autoclaved or living algae in liquid or agar media. We believe this is the first account of growth of *Cytophaga* on green and blue-green algal substrates in which lysis is maintained regardless of culturing methods. Wu *et al.* (*6*) isolated a myxobacterium (probably *Myxococcus*) which lysed seven blue-green algae, but lytic activity was lost upon subculturing. We have tested nine Gram-negative and four Gram-positive eubacteria, and all are lysed or inhibited (or both) by *C. N-5* (*7*). The nature of the killing and lytic phenomena and whether *Cytophaga* could be used for biological control of algal blooms are unknown.

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8. Supported by U.S. Department of Interior grant 18050 DBR. We thank Dr. P. T. Kantz (Department of Biological Sciences, Sacramento State College) for a bacteria-free culture of *Nostoc muscorum* and K. McGahen (Department of Microbiology, University of Texas) for bacterial cultures.
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Limited Periods of Gene Expression in Immunoglobulin-Synthesizing Cells

Abstract. *In synchronized human lymphoid cell lines, production of immunoglobulins G and M is greatest during the late G1 and S phases of the cell cycle. Little immunoglobulin appears immediately before, during, and immediately after mitosis. The results indicate that transcription of immunoglobulin genes takes place during a limited part of the mitotic cycle.*

Cell proliferation and differentiation to immunoglobulin formation are essential elements of the antibody response. Established cultures of human lymphoid cells synthesize immunoglobulins (*1*). These cell lines provide an opportunity to study immunoglobulin synthesis in actively dividing cells. In exponentially growing lymphoid cultures usually about 15 percent of the lymphoid cell population contain detectable immunoglobulin when examined with specific fluorescein-conjugated antiserums (*1*). The majority of the cells, however, are negative. Positive cells might represent (i) clones of immunoglobulin-producing cells existing in culture with clones of nonimmunoglobulin-producing (fluorescence-negative) cells; (ii) cells which have differentiated to immunoglobulin-producing G_0 (nondividing) cells; or (iii) cells of the general population which synthesize immunoglobulins during a limited portion of their mitotic cycle. Our studies of several IgG-producing cell lines indicate that immunoglobulins are produced by the majority of cells in the population and that production is restricted to a portion of the cell cycle.

Cell line WiL2, derived from the spleen of a patient with hereditary spherocytic anemia (*2*) synthesizes immunoglobulin G (IgG) and immunoglobulin M (IgM) with κ-type light chains (IgG-κ and IgM-κ). Cell line IM-4, established in this laboratory from an American patient with Burkitt's lymphoma, produces IgG.

Synchronization in mitosis was achieved by thymidine followed by colcemid blockade (*3*). After exposure to thymidine for 16 hours, cells were grown in fresh medium with $10^{-6}M$ deoxycytidine and 0.02 μg of colcemid per milliliter for 8 hours. The culture was then allowed to grow in fresh medium. After synchronization, portions of cells were taken, and cover-slip smears were made every 2 hours. Controls consisted of cultures growing exponentially in standard medium. We detected immunoglobulin in cells by staining representative cover-slip smears with fluorescein-conjugated antiserums specific for human γ chains, for human μ chains, and for human κ chains (*1*).

A colcemid-synchronized WiL2 culture at mitosis had 3 percent immunofluorescing cells (Fig. 1a). The percentage of IgG-containing cells increased during the G1 phase, reaching 40 percent at 8 hours (S phase). The number of cells showing immunofluorescence fell during the G2 phase to 6 percent at mitosis. Thus the number of cells containing immunoglobulin is greatest in late G1 and S phases and low during mitosis. Control cultures consistently showed 10 to 15 percent fluorescing cells.

Similar results with immunofluorescence were obtained with cell line IM-4 when thymidine-colcemid synchronization was used. Cells containing immunoglobulin varied from 5 percent at mitosis to 39 percent at 10 hours (S phase) and then decreased to 7 percent at the time of the mitosis. Thus colcemid arrested the cells in a stage of their cycle where immunoglobulin synthesis was markedly limited (that is, during mitosis). Further studies were undertaken to investigate early S phase where immunoglobulin formation was marked.

Synchronization in early S phase was achieved by double thymidine blockade (*4*) as follows. After exposure to 2.5 mM thymidine for 16 hours,