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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1. "Lysis" refers to dissolution of cell wall as in blue-green algae, whereas "killing" refers to killing of green algae without lysis.
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3. Isolates of the following green algae were tested: *Chlamydomonas, Chlorosarcinopsis, Dictyochloris, Radiosphaera, Spirogyra, Spongiococcum, Tetracystis, Trichosarcina,* and *Zygnema.* Isolates of the following blue-green algae were tested: *Anabaena, Calothrix, Lyngbya, Nostoc, Oscillatoria, Phormidium,* and *Plectonema.*
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7. Gram-negative bacteria tested were: *Aerobacter aerogenes, Alcaligenes providence, Citrobacter* sp., *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhosa, Serratia marcescens,* and *Shigella flexneri.* Gram-positive bacteria tested were: *Bacillus subtilis, Gaffkya tetragena, Micrococcus lysodeikticus,* and *Staphylococcus aureus.*
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,

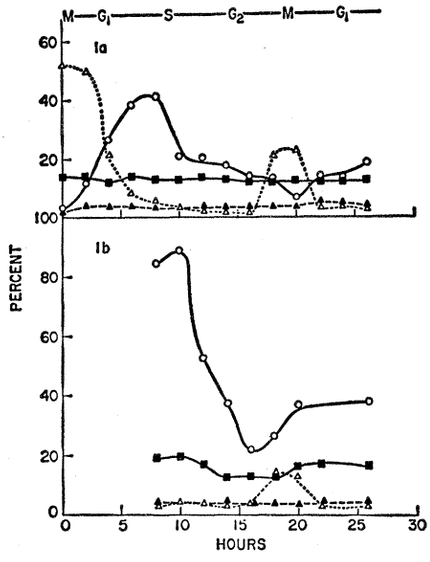

Fig. 1. Variations in immunoglobulin containing WiL2 cells during synchronous growth. Population arrested (a) at mitosis by thymidine and colcemid treatment and (b) at the onset of S phase by double thymidine treatment. Smears were made at intervals after synchronization and stained with fluorescent antiserum to human IgG (γ chain) and with Giemsa stain. The data were plotted so that mitosis corresponds in (a) and (b). ○, Fluorescing cells (%) in the synchronized culture; ■, fluorescing cells (%) in the control culture; △, percentage in mitosis, synchronized culture; and ▲, percentage in mitosis, control.

the cells were washed and suspended in fresh medium (10^6 cell/ml). Eight hours later, thymidine was added for a second 16-hour period. Cells were then washed and suspended in fresh medium with $10^{-6}M$ deoxycytidine.

When the cell cycle was arrested early in S phase by double thymidine blockade, 80 percent of the cell population contained IgG, as judged by immunofluorescence (Fig. 1b). This number rose to 91 percent, then fell sharply during G2 to 20 percent at mitosis. These results indicated differences in cell content of immunoglobulin G during the cell cycle.

The immunofluorescence technique did not distinguish between synthetic differences and differences in storage due to variations in the rate of secretion of IgG from the cell. Therefore, synchronous cultures were examined for variations of radioactive amino acid incorporation into specific protein. Synchronized WiL2 cells (4×10^7) were incubated in 2 ml of medium with 1 μc of H^3-leucine per milliliter for 2 hours. The extracellular medium was examined for H^3-labeled IgG and IgM by specific coprecipitation (*5*). Rates of DNA synthesis were determined by the incorporation of H^3-thymidine into acid precipitable (5 percent cold trichloroacetic acid) cellular material (*6*).

Appearance of IgG and IgM in the culture fluid of synchronized WiL2 cells was greatest during S and lowest during mitosis and the early G1 phase (Fig. 2c). Immunoglobulin synthesis correlated with the intracellular demonstration of γ, μ, and κ determinants by immunofluorescence (Fig. 2d), again confirming that immunoglobulin synthesis is restricted to a part of the cell cycle.

The demonstration that immunoglobulin synthesis occurs primarily in one part of the cell cycle is relevant to other observations on immunoglobulin synthesis in human lymphoid cell lines. Recent quantitative studies on human lymphoid lines inoculated into fresh medium reveal that IgG, IgA, and λ-chain synthesis is greatest when the cells are proliferating exponentially (*7*). Our results indicate that this finding may reflect the presence of relatively more cells passing through the late G1 and S periods, that is, periods during which immunoglobulin synthesis takes place.

The restricted expression of genes for immunoglobulins implies a limited period of gene transcription. Sequential gene transcription during the cell cycle has been demonstrated for bacterial (*8*) and yeast (*9*) enzyme synthesis. Tyrosine aminotransferase induction in hepatoma cells (*6*), histone synthesis in HeLa cells (*10*), and coordinated transcriptional and translational events prior to mitosis (*11*) represent similar phenomena in mammalian cells. Our observations indicate that limited gene transcription is also characteristic of the genes for immunoglobulins in human lymphoid cells. These observations clearly apply to genes for γ and μ polypeptide chains. Eludication of mechanisms controlling immunoglobulin gene transcription in lymphoid cell lines should lead to an understanding of similar events during active immune responses.

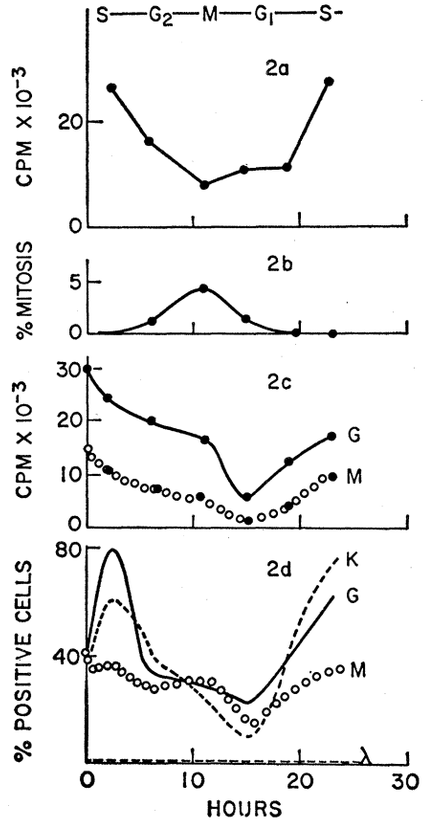

Fig. 2. Correlated events in synchronized WiL2 cells. (a) DNA synthesis is measured by 1-hour incorporation of H^3-thymidine (1 μc/ml for 10^6 cells); CPM, counts per minute. (b) Mitotic curve. (c) Incorporation of H^3-leucine into newly synthesized and released IgG and IgM; 2×10^7 cell/ml were incubated with H^3-leucine (10 μc/ml) for 2 hours. The culture fluid was analyzed for H^3-labeled IgG and IgM by specific coprecipitation; *G*, IgG; *M*, IgM. (d) Percentage of fluorescing cells. Smears were stained with fluorescein-conjugated antiserums to γ chain (*G*), μ chain (*M*), κ chain, and λ chain (λ).

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