bilirubin since mitotic suppression was not evident in cultures inoculated with serum from patients with other forms of liver disease. The inhibition of mitosis may be related to damage of leukocytes by the virus of hepatitis, which may multiply in the leukocytes with resultant cell injury or interference with cell replication. Indeed, infectious hepatitis is characterized by viremia and by a prominent leukopenia during the late period of incubation and early acute illness (*2*).

These results do not provide definite evidence that mitotic suppression reflects the action of virus. Other, as yet undefined, factors may be responsible for these effects. Inhibition of leukocyte mitosis for which there has been no explanation has been frequently observed. Rubella virus has been associated with suppression of mitotic activity in several monolayer tissue cultures (*3*). Leukocytes from patients with Hodgkin's disease are affected similarly (*4*).

Chromosomes of leukocytes taken from patients with hepatitis during convalescence had an increased incidence of breaks, stickiness, and abnormal numbers (deletions or additions) that were not present previously. Similar changes have been seen in association with other viral infections (*5*). Chromosome breaks have been reported in association with acute infectious hepatitis (*6*). Suppression of mitosis and aneuploidy have not been observed. The appearance of chromosomal aberrations following hepatitis is of interest in light of recent observations suggesting an epidemiologic association of hepatitis and Down's Syndrome (*7*).

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30 November 1966

Bacterial Growth Rate in the Sea: Direct Analysis by Thymidine Autoradiography

Abstract. *Autoradiography with tritiated thymidine was used to study microbial growth directly in nature. The epiphyte* Leucothrix mucor *was used since it is a large filamentous bacterium with a characteristic morphology making it recognizable in natural collections. The technique was developed initially with pure cultures. The relation between growth rate and the rate of accumulation of radioactive cells permitted derivation of a constant for use in calculating growth rate in natural material and in two-membered cultures of* L. mucor *growing epiphytically on pure cultures of marine algae. The growth rate (generation time) in two-membered cultures with the red alga* Antithamnion sarniense *was 94 minutes under the conditions used. In nature the growth rate of a sample from Iceland was 685 minutes; that of a sample from Long Island Sound was 660 minutes. There was no evidence of preferential growth in the basal portion of bacterial filaments nearest the algal surface. However, filamentous growth in nature, but not in pure or two-membered culture, was nonrandom, growth being clustered in some regions.*

In many situations it is desirable to measure the growth rate of microorganisms directly in natural environments. Since microorganisms are small, their environments are also small, and thus microorganisms can usually be studied directly in nature only with the microscope. Microscopic studies of many characteristics which are of greatest ecological interest (such as nutritional requirements, responses to environmental changes, production of metabolites, growth rate, and so forth) have not been examined. Studies have been made only of pure cultures, the behavior of the parent organisms in nature being extrapolated from these findings. Since an organism undoubtedly adapts to the conditions of culture, the behavior of a pure culture reveals only what the organism can do in nature, not what it was actually doing. Even if the environment of the pure culture were to mimic precisely the physical and chemical aspects of the natural environment, the laboratory environment could not precisely reproduce those aspects of the natural environment which involve competition and cooperation with other organisms.

The present report describes autoradiographic procedures with tritiated thymidine which permit estimation of the growth rate of a microorganism living in nature. The method, used by cell biologists and immunologists to measure the growth rate of specific cell types in the animal body (*1*), depends on the following: (i) Only dividing cells synthesize DNA; (ii) tritiated thymidine is incorporated into acid-insoluble material only during DNA synthesis; (iii) cellular incorporation of tritiated thymidine can be detected autoradiographically.

The marine organism *Leucothrix mucor* is an excellent model, since it is a large filamentous bacterium with characteristic morphological features recognizable in natural collections. This organism occurs generally as an epiphyte on marine algae, its filaments projecting perpendicularly from the surface of algal fronds, permitting easy microscopic study (*2*). Moreover, on marine algae growing in areas with much wave action or tidal current, *L. mucor* is often the only bacterium visible, and its consistent presence in such habitats assures that any algal frond will have at least some *L. mucor* filaments. In addition, strains of *L. mucor* from geographically diverse areas have remarkably similar characteristics in the laboratory, showing similar nutritional requirements, temperature responses, salinity requirements (*3*), and DNA-base-compositions (*4*). Finally, *L. mucor* readily incorporates tritiated thymidine during growth.

Cultures, culture media, and growth conditions for *L. mucor* have been described (*2, 5*). The growth rate (generation time) of *L. mucor* in pure culture was measured. I determined the rate of incorporation of tritiated thymidine by incubating growing cultures in synthetic sea-water medium with tritiated thymidine (1 μc/ml, 6.7 c/mmole), by preparing autoradiograms at different incubation times (*6*), and by determining the percentage of radioactive cells. (No attempt was made to count the number of silver grains per cell; a cell was scored as radioactive or nonradioactive.) The number of radioactive cells was directly proportional to the length of the incubation period until at least 80 percent of the cells were radioactive. Under the

Table 1. Distributions of silver grains in autoradiographs of *Leucothrix mucor* growing epiphytically in Flaxafloi Fjord, Sudurnes, near Reykjavik, Iceland.*

Filament number	Tip — Base
1	2 9 4 1 1 1 5 1 5 1 3 5 2 3 4 2 2 1 1 1 5 1 1 1 9
2	4 3 1 12 12 2 1 1 2 1
3	4 1 1 1 2 4 1 1 1 7 1 2 2 3 2 1 1 1 6
4	1 6 1 3 1 11 1 1 1 1 2 5 3 5 3
5	2 1 2 1 1 4 4 9 6 1 3 4
6	4 2 4 1 2 1 1 1 6 2 1 1 1 7 1 2 6 2
7	7 1 12 1 3 1 2 1 2 2 1 3 2 1 2 2 11 1 1 3 3 2 1 2 1 4
8	5 1 3 1 3 2 2 1 2 2 3 8 5 1 1 3
9	7 2 2 2 2 4 2 1 4 3 1 2 3
10	7 3 2 3 2 1 1 3 1 2 1 5 1 9 1 3
11	2 2 1 6 3 2 2 2 2 1 3 1 3 1 9 4 1 2 1 1 1 2 4 9
12	3 1 4 12 5 2 2 1 1 2 3 3 4 3 2 1 3 1
13	1 3 4 4 3 1 3 1 2 1 2 9 3 2 1 3 2 5 1 2 1 2 2 25 2 1 2 1 2 3 1 1 6 5 3 1 1 1 2 1
14	1 6 2 4 1 2 1 1 1 5 2 2 3 3 1 9
15	2 1 5 1 5 1 4 2 2 2 2 1 1 1 2 3 1 6 2 2 2 3 3
16	4 2 3 5 7 1 5 4 6 3 8 2 2 2 3 2 1 4 4 1 1 3
17	2 1 4 2 1 3 6 3 1 2 1 3 1 12 2 1 1 2 2 2 3 2 1 4 2 1 2 2 1 1 3 4 1 7 1 2 1 2 1 1 2 3 2 6 6 6 2
18	1 6 5 1 3 3 2 7 2 2 4 1 1 6 5 1 2 1 1 1 4 5 1 3 1 6 12 1 6
19	1 2 1 3 2 3 1 3 2 5 1 3 1 3 1 8 2 1 7 2 2 1 1 7 1
20	2 14 1 1 1 1 1 4 1 1 1 3 1 2 1 1 1 1 2
21	5 1 2 1 2 1 3 17 1 2 1 1 2 1 1 3 2 2 1 2 1 3 9 1
22	4 1 1 2 1 1 2 1 1 1 2 2 3 1 2 6 1 2 1 1 2 1 1 6 4 2
23	2 1 11 1 2 1 3 2 3 7 1 1 2 6 4 1 2 2
24	4 1 1 4 5 2 1 2 3 3 1 5 2 6 2 5 6 2 1 2 4 2 1 3 4 1 6 2 11 3 3

*The numbers in italics indicate the number of radioactive cells; the other numbers indicate the number of nonradioactive cells. For example, 2 9 4 1 indicates two nonradioactive cells, nine radioactive cells, four nonradioactive cells, and so forth. Incubation: 4 August 1965, low tide, 5:00 to 6:00 p.m. Sea temperature 13.2°C. Filamentous red alga growing attached to rocks below water level at low tide. Tritiated thymidine, 1 μc/ml. Total water volume per vial, 1.0 ml.

conditions used in the synthetic seawater medium at 25°C, the generation time of the organism was 170 minutes, and the rate of accumulation of radioactive cells was 3 percent per minute. Since in 1 minute there is 1/170 or 0.006 generation, 3 percent of the cells would be radioactive in 0.006 generation or 1 percent of the cells would be radioactive in 0.002 generation. Under similar conditions, but at 10°C (generation time, 410 minutes), 1 percent of the cells became radioactive in 0.0019 generation. These studies were repeated several times with similar results. The rate of accumulation of radioactive cells was proportional to the growth rate over a wide range of growth rates.

This relationship, 1 percent of cells radioactive in 0.002 generation, can be used as a constant for estimation of the growth rate in nature. Natural material can be incubated with tritiated thymidine, and samples taken at several incubation times. Autoradiograms can be prepared, and the percentage of radioactive cells estimated. Assume that 10 percent of the cells are radioactive after a 30-minute incubation. Since a 1-percent increase in radioactive cells represents 0.002 generation, a 10-percent increase represents 0.02 generation. Thus in 30 minutes there would be 0.02 generation, and the generation time of the unknown would be 30 divided by 0.02, or 1500 minutes.

In these calculations, one assumes that the rate of accumulation of radioactive cells is linear and begins without a lag, just as it does in pure cultures, and that nonradioactive thymidine, which might dilute the radioactive material, is absent from the sea water. With the specific activity of the tritiated thymidine used in these experiments, the final concentration of thymidine in the experimental samples is 36 μg/liter of water. Since the concentration of dissolved organic carbon (mostly as amino acids, sugars, organic acids, and other compounds derived from seaweeds and phytoplankton) in sea water is 2 to 20 mg/liter (*7*) it is unlikely that the amount of thymidine in natural waters approaches that added. Thus it can be reasonably assumed that the specific activity of the tritiated thymidine is the same in sea water as it is in culture media.

Since *L. mucor* can also be grown epiphytically on marine seaweeds in the laboratory (*2*), I examined two-membered cultures in the laboratory before performing field experiments. The synthetic culture medium used for the algae does not support growth of *L. mucor*, but *L. mucor* will grow attached to the alga in much the way it grows in nature. In an experiment with combined pure cultures of *L. mucor* and the red alga *Antithamnion sarniense, a* 30-minute exposure to tritiated thymidine at 15°C was used, and 42 separate filaments of various lengths of from 4 to 42 cells were analyzed. Fifty-four percent of the cells were radioactive; thus, in the 30-minute incubation time 0.32 generation occurred (based on the constant presented earlier). Hence the average generation time under these conditions was 94 minutes, which is a rate even faster than that of individual pure cultures of *L. mucor*. Besides providing information on growth rate, the autoradiographic technique can be used for the determination of any regions along a *L. mucor* filament showing preferential growth. Statistical analysis, with the nonparametric, One-Sample Runs test (*8*) of the distribution of radioactive cells along the filaments gave no indication of any preferential growth in the basal, apical, or central regions.

Experiments in nature with tritiated thymidine have been done in: (i) Late April 1965 at Long Island Sound, Connecticut, and Narragansett Bay, Rhode Island; (ii) mid-July 1965 at Narragansett Bay; (iii) late July and early August 1965 in Iceland at two locations, Cape Reykjanes and Sudurnes near Reykjavik; (iv) early March 1966 at Ischia Ponte, Bay of Naples, Italy; and (v) mid-April 1966 at Loch Ewe, Scotland. Small samples of seaweed were collected at low tide and placed in screw-cap vials containing 1 ml of natural sea water; tritiated thymidine was added to a final concentra-

tion of 1 μc/ml. Incubation was carried out at the site from which the algae were collected, with the vials floating naturally in the water. Several incubation times were used, but because of the slow division rates the longer period of incubation (60 minutes) yielded the best autoradiograms for suitable analysis. The distribution of radioactive and nonradioactive cells along a number of filaments can be seen (Table 1). As in the two-membered cultures, there is no evidence for preferential growth at either base or tip of a filament, although radioactive cells occurred in clusters of four to eight or more cells interspaced with clusters of nonradioactive cells. This nonrandom distribution was verified by statistical analysis (*8*) and is seen only in natural situations. From the data, according to the assumptions discussed earlier and using the average percentage of radioactive cells throughout all of the filaments studied, one can estimate the average growth rate of *L. mucor* in nature. Since 43.8 percent of the cells are radioactive, the estimated generation time is 685 minutes. In a similar situation for Long Island Sound, the estimated generation time is 660 minutes. Similar generation times were found in the other habitats studied. These generation times are considerably longer than those determined for two-membered or pure cultures. This difference in generation times is not surprising in view of differences between a natural environment and laboratory cultures with respect to macro- and micro-environmental factors.

My technique should be adaptable to the estimation of the growth rate of any microorganism that can incorporate tritiated thymidine, for which pure cultures are available, and that can be recognized in nature microscopically. Because a wide variety of microorganisms meet these requirements, this technique may have wide application in microbial ecology.

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28 September 1966

Tryptophan Deficiency in Rabbit Reticulocytes: Polyribosomes during Interrupted Growth of Hemoglobin Chains

Abstract. *Of several amino acids essential for optimum hemoglobin synthesis by the rabbit reticulocyte, only omission of tryptophan results in polyribosome disaggregation. This disaggregation is prevented by the omission of both tryptophan and an amino acid that is relatively more essential than tryptophan for hemoglobin synthesis. Since tryptophan is located only near the amino-terminal ends of both chains of rabbit globin, the results indicate that single ribosomes and those in polyribosomes are in a dynamic state in the intact cell.*

The synthesis of proteins in a variety of cell types occurs on polyribosomes (*1*). It has been suggested that single ribosomes (or their subunits) become associated with the end of messenger RNA (mRNA) coding for the amino-terminal end of a peptide chain, and then, in concert with aminoacyl transfer RNA (tRNA), enzymes, and cofactors, travel along the mRNA to translate the nucleotide code into an amino acid sequence (*2*). After completion of one round of protein synthesis, the single ribosomes become detached from the mRNA and remain as single ribosomes until they initiate the synthesis of a new protein molecule. Experimental evidence in support of this model has come principally from studies with cell-free preparations (*3*), and it is therefore of interest to ascertain whether recycling of free ribosomes with those in polyribosomes takes place during protein synthesis in the intact cell (*4*, *5*).

The α- and β-chains of rabbit globin contain the common amino acids nearly all of which are distributed at a number of different sites throughout the protein molecule (*6*). A unique characteristic of tryptophan is its location near the amino-terminal end of rabbit hemoglobin, at position 14 of the α-chain and in positions 15 and 37 of the β-chain (*6*). Growth of the peptide chains proceeds from the amino-terminal end (*7*), and therefore during a relative deficiency of tryptophan the rate of

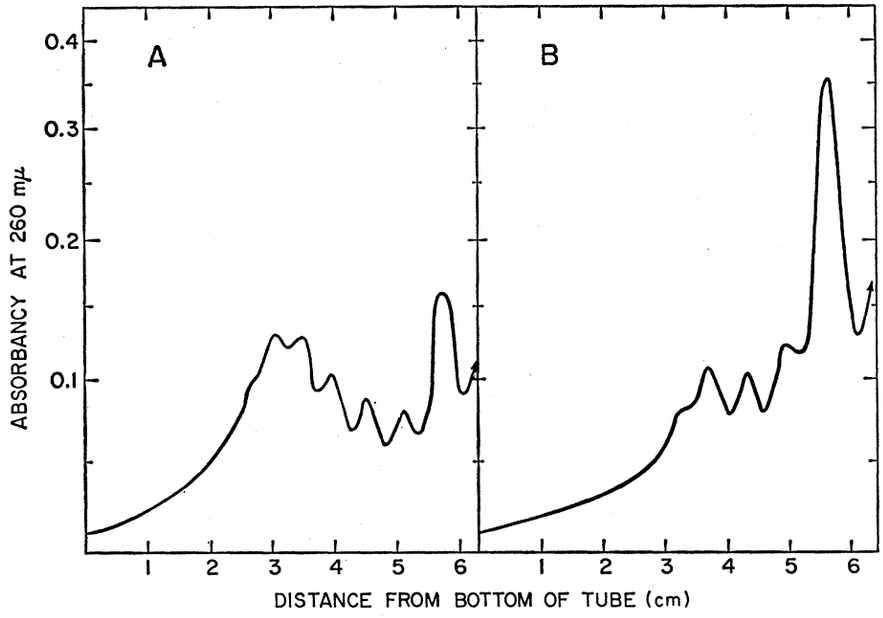

Fig. 1. Reticulocyte polyribosomes during amino acid deficiencies. Conditions corresponding to (A) and (B) are described in Tables 1 and 2.