Table 1. Powder data for pressure-induced fibrous sulfur-selenium ($S_{0.56}$ $Se_{0.44}$); CuKα radiation. Abbreviations: w, weak; m, medium; s, strong; v, very.

hkl	*d*(Å) Calc.	*d*(Å) Obs.	I_{rel}
100	6.80	6.80	vw
110	3.93	3.93	vs
111, $\bar{1}21$	2.99	2.99	vs
201, 021	2.74	2.73	s
210, 120	2.57	2.56	m
300	2.27		
211, $\bar{1}21$ / 231, $\bar{1}31$	2.25	2.25	ms
102, 012	2.19	2.19	m
301, 031	2.03	2.03	s
220	1.96	1.95	w
202, 022	1.91	1.91	w
310, $\bar{1}30$	1.885	1.879	w-m
221, $\bar{2}41$	1.806	1.803	m
131, $\bar{3}11$ / $\bar{1}41$, $\bar{3}41$	1.746	1.742	s
$\bar{2}12$, $\bar{1}22$ / 132, $\bar{2}32$	1.718	1.718	s
302, 032	1.618	1.615	w
401, 041	1.595	1.591	w
320, 230	1.560	1.555	m
103, 013	1.502	1.505	w-m
410, 140	1.483	1.477	w-m
$\bar{3}21$, 231 / $\bar{2}51$, $\bar{3}51$	1.478		
203, 023	1.403	1.404	vvw
402, 042	1.369	1.370	vvw
330	1.308	1.305	w
501, 051	1.304		
420, 240	1.285	1.283	w
$\bar{4}12$, $\bar{1}42$ / $\bar{1}52$, $\bar{4}52$	1.248	1.248	w
$\bar{5}11$, $\bar{1}51$ / $\bar{1}61$, $\bar{5}61$	1.180	1.177	vw
403, 043	1.141	1.141	vw
104, 014	1.139		
$\bar{4}22$, $\bar{2}42$ / $\bar{2}62$, $\bar{4}62$	1.123	1.122	vw
114, $\bar{1}24$	1.108	1.106	w-m
$\bar{5}21$, $\bar{2}51$ / $\bar{2}71$, $\bar{5}71$	1.060	1.056	w
$\bar{2}14$, $\bar{1}24$ / 134, $\bar{2}34$	1.053		

old and having been irradiated with 35-kv x-rays for about 1500 hours still remains unaltered in any observable manner. When examined about 3 months after it was made, the "single" crystal of the sulfur-selenium phase on which x-ray data had been collected had altered. It had gone partially to the fibrous sulfur (II) type phase.

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4. The values given for the helix radius and average S-Se distance should be taken as tentative Limits of error for these are now approximately ± 0.05 Å.
5. We thank P. B. Crandall for technical assistance.

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Leukocyte Mitosis: Suppression in vitro Associated with Acute Infectious Hepatitis

Abstract. *Inhibition of mitosis in vitro was observed in leukocytes from patients with acute infectious hepatitis. Similarly, in cultures of normal leukocytes, after the addition of small amounts of serum from patients with hepatitis, mitosis was suppressed. Although the incidence of mitosis became normal in leukocytes from convalescent patients, there were chromosomal abnormalities.*

The effect of infectious hepatitis on the chromosomes of cells in human peripheral blood was studied during a recent epidemic of this disease, in which more than 100 cases were recognized. Many were symptomatic; others were discovered during a survey of tests for liver functions [primarily for serum glutamic oxalacetic transaminase (SGOT)]. Specimens of blood and serum were obtained from 16 patients, some of whom had been previously karyotyped. Additional samples were obtained from patients at the Massachusetts General, Boston City, and St. Elizabeth's Hospitals. Serums from eight patients with noninfectious hepatic disease and comparable abnormalities of liver function served as controls. Normal specimens were obtained from healthy students and employees in the same institutions.

The standard method of Moorhead *et al.* (*1*) for culturing leukocytes and preparing chromosomes was used, with two modifications; eight drops of whole blood were added to the culture medium in place of the 1.0 ml of plasma, and the cells were exposed to colcemid for 2 hours instead of the 6 hours suggested by Moorhead. In every case all stained cells were studied. The percentage of leukocytes in metaphase was derived from a count of at least 200 cells.

The initial studies were performed with preparations of peripheral leukocytes obtained from patients with acute infectious hepatitis (hereafter referred to as the direct method). In another method (indirect) 0.1 ml of the serum to be tested was added to cultures of leukocytes obtained from healthy individuals. Preparations to which no serum was added served as culture controls.

No metaphase figures, as judged by the direct method, were seen in specimens obtained from 12 patients with acute infectious hepatitis. Most of the leukocytes present were contracted and deeply stained or macerated. Chromatin clumping occurred in a few cells, but there was no other sign of mitosis. Eight to 20 percent of cells taken from patients before they developed hepatitis had metaphase figures. Thirteen to 20 percent of leukocytes from convalescent patients after liver function tests had become normal had metaphase figures. However, these chromosomes showed an unusual sticky quality as well as multiple breaks, deletions, and additions (Fig. 1).

Serums from nine patients with infectious hepatitis repeatedly inhibited the development of metaphase figures in normal leukocytes. The incidence of metaphase figures in these preparations ranged from 0 to 0.5 percent of the cells examined. In contrast, control cultures revealed 8 to 20 percent of the leukocytes in metaphase.

Using the indirect method, dilutions of four serums that inhibited leukocyte mitosis were tested. In each case, the serums diluted up to one part in 1000 inhibited mitotic activity. Serums from normal, healthy individuals and eight patients with noninfectious hepatic disease did not suppress metaphase figures in leukocyte cultures; 12 to 20 percent of the leukocytes were in metaphase.

The blood and serum of patients with acute infectious hepatitis have a factor that inhibits leukocyte mitosis and mitosis of normal leukocytes in culture. The inhibition of leukocyte mitosis does not seem to be mediated by elevated concentrations of SGOT or

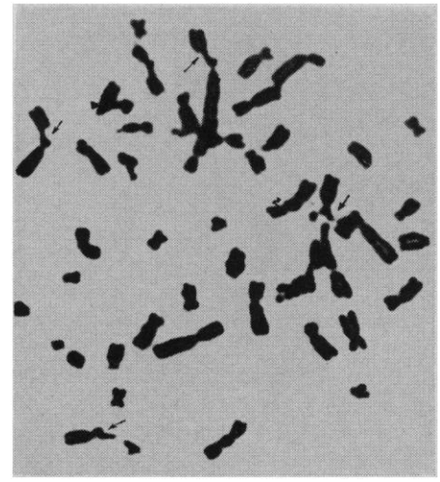

Fig. 1. Increased stickiness and other chromosomal aberrations. Several breaks are indicated by arrows (× 1200).

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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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