der the conditions used in the experiments reported here, just under half the product is oxidized as far as carboxylic acid (*16*).

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Inhibition of Cell Division of Escherichia coli by Low Doses of Ultraviolet Light

Several workers have observed and worked with the division inhibition of *Escherichia coli B* by ultraviolet light (*1, 2*). However, little quantitative work has been done on the division inhibition as measured microscopically. Ultraviolet light inhibits the cellular division and causes the cells to grow into long "filaments," or "snakes," of about the same diameter as the normal cells but of many times the normal length. The filaments, as they will be called in this report, have no constrictions and appear as long, continuous structures under a phase-contrast microscope.

Cultures of *Escherichia coli B*, carried on nutrient agar slants, were inoculated into C-1 minimal medium (*3*) and grown overnight at 37°C with aeration. By morning, the cells were adapted to growth in the minimal medium and were growing exponentially at a concentration of about 10^9 cells per milliliter. These were diluted to about 10^8 cells per milliliter into fresh C-1 medium and grown to a concentration of about 2×10^8 cells per milliliter. These cultures were then transferred to quartz cuvettes, kept at 37°C, and stirred during the subsequent irradiation. Irradiation was done with a water-prism ultraviolet monochromator at the desired wavelength. Incident intensities were measured with a calibrated photocell, and the average intensity through the sample was obtained by the method of Morowitz (*4*). Irradiation times were of the order of 10 to 100 seconds, and reciprocity held over a factor of at least 25 in intensity. Following irradiation, the cultures were grown at 37°C in C-1 medium in subdued light for the desired time.

For doses up to about 100 erg/mm² at 2652 A, the rate of total mass increase of the irradiated cultures as determined by the turbidity at 650 mμ was indistinguishable from the rate of increase of unirradiated cultures. Microscopic observation and Petroff-Hauser counts of the irradiated cells during growth indicated that there were two important classes of cells: (i) those that continued to divide and grow at the usual rate, and (ii) those which increased in length but did not undergo division. During growth after irradiation, the normally dividing cells increased in number according to the division rate. The nondividing cells increased in length, so they were easily distinguishable from the normal cells. By phase microscopy, the relative number of normal cells and filaments was then determined. Knowing the factor by which the normal cells had increased since irradiation, the ratio of dividing cells to nondividing cells could be calculated for the culture as it existed immediately after irradiation.

This microscopic technique does not have the complications that arise in the determination of colony-forming ability, which is highly sensitive to the plating conditions. The cells are irradiated at 37°C during growth in the log phase with no significant interruption of growth conditions before, during, or after irradiation. The only stress being imposed on the system is the action of the ultraviolet light.

The logarithm of the survival of the ability to divide, determined as outlined, is proportional to the ultraviolet dose down to about 10-percent survival, as is shown in Fig. 1. An ultraviolet cross section for the ability to divide can thereby be found from the relation, $N/N_0 = e^{-\sigma D}$, where N/N_0 is the division survival ratio, D is the dose of ultraviolet, and σ is the sensitivity parameter called the cross section for division inhibition.

The ultraviolet doses to inhibit the division in this strain of *E. coli* relative to those needed to alter appreciably other metabolic processes in the same strain have been determined. These results, also shown in Fig. 1, are for random, nonsynchronized cultures. Irradiations were done at 2652 A on C-1 cultures at 37°C.

For determination of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), the cells were extracted with 70 percent ethanol and hot ethanol-ether to remove lipid material, cold 5 percent trichloroacetic acid to remove acid-soluble small compounds, and then hot trichloroacetic acid (5 percent) to remove the RNA and DNA. The hot trichloroacetic acid fraction was assayed for RNA by the orcinol reaction (*5*) and for DNA by the Keck modification of the Ceriotti reaction (*6*).

The doses which inhibit the microscopically observable division of almost all the cells have no detectable effect on the rate of DNA and RNA synthesis. Thus, the DNA and RNA content of the filaments is the same per unit cell mass as for the normally dividing cells. With

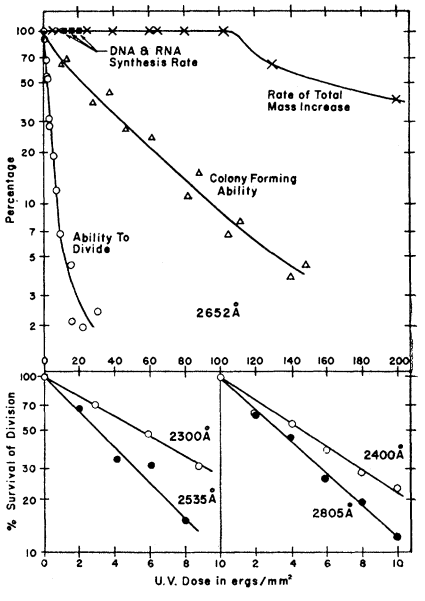

Fig. 1. (Top) Curves of log survival ratio for the given cellular property versus incident ultraviolet dose: ○, ability to divide within 3 hours in C-1 medium during continuous growth after irradiation; △, colony-forming ability on nutrient agar (plating done at about 23°C within 1 hour after irradiation; dilution series done in ice cold C-1 medium without glucose; 37°C incubation); ×, ratio of the rate of turbidity increase after irradiation to the rate for unirradiated samples; ■, ratio of the rate of RNA and DNA increase in irradiated to that in controls (this ratio is 1.00 to within experimental error of 5 to 10 percent for at least 2 hours after irradiation). (Bottom) Microscopically determined survival curves for the division versus ultraviolet dose at several wavelengths. These curves have been corrected to account for the spontaneously arising filaments in unirradiated cultures.

much higher doses of ultraviolet, Kelner (*7*), and Kanazir and Errera (*8*) found DNA synthesis to be inhibited.

Even though the cells do not divide within at least 3 hours after irradiation when grown at 37°C in the C-1 medium, most of them apparently recover their ability to divide at a later time and give rise to a normal macroscopic colony.

Further results relating to division inhibition mechanism follow. (i) The action spectrum for division inhibition, for which some data are shown in the lower part of Fig. 1, has a maximum at 2652 A and a minimum at 2300 A. (ii) As is shown by the work of Errera (*2*), the division inhibition can be reversed by visible light if the light is given within 15 minutes after the ultraviolet. (iii) The procedure of Robinow (*9*) for staining cytoplasmic cell boundaries discloses dark-staining bands at intervals along the filament of about the same distance as the length of normal cells. In old filament cultures, fission was occasionally seen at the dark-staining regions. Microscopic observation of the manner of lysis of the filaments by T-1 phage also indicates some sort of transverse cell boundaries.

Some preliminary work has been done on division inhibition in synchronized cultures. Synchronization was done by a slight modification of the technique of McNair Scott (*10*). There appears to be some variation in ultraviolet sensitivity as the cells go through the different stages of division. However, the change in sensitivity is probably not over a factor of 2. No definite correlation of this change with steps of division can be made yet, except that division seems to be more easily inhibited either just before or just after cytoplasmic division.

Since most of the filaments described here give rise to a normal-appearing colony when plated on nutrient agar, the effect measured by the microscopic technique is more correctly described as an extended division delay rather than a permanent inhibition. The colony formation is thus a result of a recovery of the ability to divide superimposed upon the original inhibition. The extent of recovery depends on the plating conditions. The initial inhibition seems to be very specific, and DNA, RNA, and protein synthesis continues at the usual rate (*11*).

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Respiratory Competence in the Diagnosis of Gene-Controlled Phenotypes in Saccharomyces

Respiratory deficiency (*1–6*) in yeast diminishes the phenotypic expression of many genes controlling the synthesis of enzymes. Comparison of the gene-controlled, adaptive fermentation of different sugars by respiration-deficient (aer) and respiration-sufficient (AER) strains indicates a slowed or delayed expression of fermentative ability. Studies of the adaptation of yeast to galactose in defined atmospheres suggest dependence of the rate of adaptation on the availability of oxygen. Thus, both a functioning respiratory mechanism and aerobic conditions are implicated in the rapid synthesis of enzymes. Respiration-deficient yeasts grow slowly on the surface of complete nutrient agar containing 2 percent of glucose and even more slowly on synthetic nutrient agar. When respiration-deficient strains carrying the appropriate dominant genes for fermentative ability are tested by replica plating or streaking on a synthetic medium for ability to ferment melezitose or galactose, or to synthesize adenine, they often resemble cultures carrying the recessive gene when they are scored after the usual period of from 1 to 2 days required for the respiration-sufficient dominant strain to form confluent growth. An example of the effect of respiratory competence on the diagnosis of phenotypes follows.

A hybrid (15177-uv4 × 8256) was made between two cultures, both of which were apparent nonfermenters of melezitose (one had been obtained by ultraviolet irradiation). The diploid hybrid was a rapid fermenter of melezitose, capable of rapid growth on solid synthetic medium or in broth tubes containing melezitose. This phenomenon might have been the result of the synergistic effect of two pairs of complementary genes controlling melezitose fermentation. Four tetrads segregated 2 AER melezitose-fermenters : 2 AER melezitose-nonfermenters. Further tests showed that the apparent nonfermenter parent (15177-uv4) was respiration-deficient, carrying the dominant gene controlling melezitose fermentation, while the other (8256) was respiration-sufficient, carrying the recessive allele for melezitose fermentation. It is clear that synergistic or complementary melezitose genes were not involved. Only a single pair of genes (*7*) controlling melezitose fermentation was concerned, the diminished fermentative ability of one parent being under cytoplasmic control. The types of progeny from the hybrid depend on whether or not the respiratory deficiency is under cytoplasmic or genic control (*8*). The effect of respiratory damage in obscuring the genotype of cultures is particularly significant, since many biochemical mutants used in genetic studies are derived from ultraviolet radiation, which is an inducer of respiratory deficiency in yeast (*9*).

It is reasonable to suppose that many different kinds of defects, both in genes and in cytoplasmic granules, may lead to respiratory deficiency. It is possible that (in addition to *bona fide* recessives) defects expressed by the slowing of adaptation to galactose- or maltose-fermentation which segregate regularly may be attributable to gene-controlled respiratory defects. This is especially true of delays in Durham-tube fermentation involving periods of less than 5 days (*10*) (Table 1). Delays of more than 5 days often involve mutation and selection. Reports of isomeric genes con-

Table 1. The effect of differences in respiratory ability of fermenters (GA) and nonfermenters of galactose (ga). AER signifies the phenotype for respiratory sufficiency; aer signifies the phenotype for respiratory deficiency. GA and ga indicate genotype. Plus sign denotes rapid growth of cells and gas evolution in inserts of Durham tubes and confluent growth of cells when streaked on the surface of agar. Minus sign denotes no appreciable growth and a lack of gas evolution in inserts of Durham tubes and no appreciable growth of cells streaked on the surface of agar. Mutant colonies usually are detected at frequencies of from 10^{-6} and 10^{-10} nonfermenter cells plated.

Genotype	Respiratory phenotype	Galactose Durham tube		Galactose agar streak	
		2 days	4 days	2 days	6 days
GA	AER	+	+	+	+
ga	AER	−	−	−	Mutant colonies
GA	aer	−/+	+	−	+
ga	aer	−	−	−	Mutant colonies