motic gradient within a cell under conditions of "idling"—that is, zero water transport. The term *solute flux* means the rate of diffusion of solutes per unit time, per unit area of cell, or per unit area of gradient-maintaining tissue. Intensity of respiration means the rate of energy expenditure per unit time, per unit mass of gradient-containing tissue.

Reduction of the surface area available for water permeation while fixing the cell width across which the concentration difference exists will reduce the total energy expenditure per unit time but *will not affect* the energy expenditure per unit mass of gradient-maintaining tissue, per unit time. Furthermore, such a reduction of "available" area demands that pores of cross-sectional area equal to that of the "available" surface extend across the fixed cell width like microcapillary tubes.

Incidentally, such a structural arrangement differs markedly from the Franck-Mayer scheme, where the gradient existed in an entire cell [*Arch. Biochem.* **14**, 297 (1947)]. Since the gradient would have to be maintained within the pore boundaries, a region of cell fluid would have to be insulated against diffusion between itself and the ambient intracellular fluid. Moreover, there must be a cellular device for funneling a significant fraction of energy from the surrounding cell fluid into the pore, since the energy expenditure per mass of gradient-maintaining region is much greater than that of the rest of the cell. Even if the tubular surface area were reduced to zero, the problem of maintaining a fixed gradient within a localized region (pore) of cell fluid would be the same as that reported previously [*Science* **121**, 302 (1955)]. If the pore extends through the cell membrane only, the osmotic gradient would be increased a 1000-fold, thereby offsetting the reduction of energy expenditure resulting from a decreased surface area.

The effect of reducing "available" surface area during the active transport of a finite volume of water is one of reducing the total energy expenditure while *increasing* the intensity of energy expenditure of the gradient containing tissue. Thus, selection of the appropriate surface area will yield reasonable rates of energy expenditure, but the aforementioned restrictions on pore geometry, funneling of energy, and intensity of respiration still apply. The velocity of flow of transported water increases as the permeation area is decreased, and the energy expenditure over and above that required for gradient maintenance during "idling" can be a small value. This means, as stated previously [*Science* **121**, 302 (1955)], that one cannot exclude rigorously any type of osmotic-gradient hypothesis for the active transport of water. It is pertinent to note that this analysis assumes no frictional resistance to the flow of transported water, no matter how high the velocity, and 100-percent thermodynamic efficiency of the gradient-maintaining mechanism. The minimum value for intensity of cell respiration during water transport is always greater than the value of 21,000 kcal/kg hr, reported previously for the condition of no water transport.

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Ultrafractionated Ultraviolet Irradiation of Yeast Cells

The biological effect of a certain dose of ultrafractionated ionizing radiation was recently studied by Witte (*1, 2*), Witte and Sigmund (*3*), and by Hofmann *et al.* (*4, 5*). Using x- and beta-rays, they found definite minima in the effect on *Drosophila* pupae and eggs and *Escherichia coli* for certain selected frequencies, depending on the ratio of single exposure time (up to 10 μsec) to pause time, which varied from 1/2 to 1/9. This paper presents studies on the effects of ultrafractionated ultraviolet radiation on yeast cells.

The experimental arrangement consisted of a Hanovia quartz lamp (143 v, 1.3 amp direct current, no filter, 7-cm distance) with a motor-driven, aluminum strobic disk (8 circular apertures, each 7/8 in. in diameter, ratio of exposure time to pause time of 1/2). A powerstat provided regulation of motor speeds up to 2100 rev/min; disk speeds were measured with a strobotac. The test specimen was a strain (*6*) of Saccharomyces cerevisiae (*7*). Stock cultures of yeast were grown for 1 to 2 weeks on slants having the following composition: 1 percent dextrose, 0.5 percent yeast extract, 0.5 percent peptone, and 2 percent agar. After incubation at room temperature for 36 to 48 hours, the fresh slants were stored at 5°C. Cells washed from a stored slant into Sorenson's phosphate buffer at *p*H 5.4 served as inoculum for the liquid culture to be used in the experiments. By suitable dilution, the inoculum was brought to a standard optical density of 0.60 to 0.67 (compared with 0.00 for distilled water) as read on a Coleman spectrophotometer at a wavelength of 6000 A. Three 20-ml liquid cultures (media composition: 5 percent dextose, 0.5 percent yeast extract, and 0.5 percent peptone) were inoculated with 0.5 ml of cell suspension and then incubated without shaking at 30°C for 16 hours. After incubation, the pooled cultures were centrifuged at 200g for 5 minutes. The supernatant was discarded, and the residue was resuspended in an equivalent volume of Sorenson's phosphate buffer at *pH* 5.4. After the suspension had been centrifuged and resuspended twice more, the optical density of the suspension was brought to a standard of 0.60 to 0.67.

One-milliliter aliquots of the cell suspension contained in glass planchets (suspension depth of 0.4 mm) were used for radiations. The suspension was magnetically stirred to provide a more homogeneous dose. Times of 1 minute for

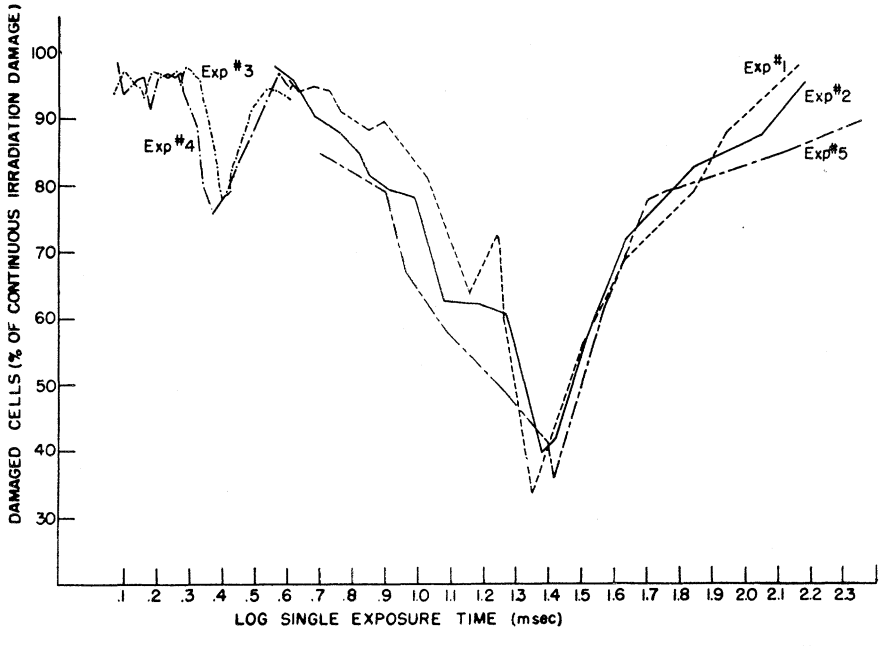

Fig. 1. Effects of ultrafractionated ultraviolet irradiation on yeast cells.

continuous radiation and 3 minutes for ultrafractionated radiation were used.

Immediately after irradiation, a drop of cell suspension was placed on each counting area of a hemocytometer slide. A drop of 0.5-percent aqueous eosin B (*8*) was mixed with each. The slide with cover slip was allowed to stand in subdued light for 10 minutes. Two hundred cells were then counted from each area. Stained and unstained cells were tabulated, and the percentage of stained cells was calculated. Maximum variation between the two 200-cell samples from the slide was 3 percent. The percentage of stained cells for 1 minute of continuous radiation served as a basis for testing the reproducibility of the method. The staining method was selected to indicate the early effects of, or damage by the radiation, or both.

The results of five experiments, covering single exposure times from 0.1516 sec to 0.0012 sec, are shown in Fig. 1. Starting at a single exposure time of 0.1107 sec, there was a decrease in the percentage of damage with decreasing single exposure time. A minimum in percentage of damage, averaging 36.7 percent of the continuous irradiation damage, was found at an average single exposure time of 0.024 sec. When single exposure times were further shortened, the percentage of damage approached the continuous irradiation value. A second minimum of less magnitude, averaging 77 percent of the continuous irradiation damage, occurred at an average single exposure time of 0.0025 sec. For further decrease in the single exposure time, the percentage of damage again approached the continuous irradiation damage. The straight line at 100 percent indicates the damage with continuous irradiation—that is an average of 65.2 percent of the total cells counted.

Similar to the findings of Witte (*1, 2*), Witte and Sigmund (*3*), and Hofmann *et al.* (*4, 5*) for ionizing radiation, ultrafractionated ultraviolet radiation also shows in its biologic effect a dependency on the frequency (single exposure time) of the incident radiation. Attempts have been made to interpret these findings in terms of the space and time distribution of the radiation-produced ions (*1*), as well as in terms of reciprocal actions between the radiation impulse and the restitutional pause (*4*). A theoretical approach to the problem by Krohn and Michie (*9*) proposed a radiation-produced chemical substance, the mean level of which is a function of the single exposure time. A possible explanation could be based on the "time-existence" of the radiation-induced chemical reactions. Photochemical studies by Allmand and Style (*10*) and radiochemical studies by Hummel *et al.* (*11*) have shown the usefulness of pulsed-radiation techniques in studying these reactions. These conclusions for chemical systems can be extended to biological systems with the assumption that a biological system behaves as a complex chemical system. Therefore, it is suggested that the minima observed in these studies on ultrafractionated ultraviolet irradiation of yeast cells are related to the "time-existence" of specific radiation-induced reactions.

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Agronomic Use of an X-ray-Induced Mutant

In 1941, Genter and Brown (*1*) reported on the effects of varying x-ray dosages applied to the seed of *Phaseolus vulgaris* L. variety Michelite, a vine type of Navy or pea bean. Many different types of mutants were produced, and 90 percent of these were distinguishable within 5 weeks after emergence, 67 percent being chlorophyll abnormalities. No mention was made of any agronomic value being attached to any observed mutants. In 1941, progenies from this material without known mutants were planted in the regular breeding plots to insure against overlooking any valuable material. A mutant bush or nonvining type of bean that was smaller than Michelite and that matured about 12 days earlier than Michelite was found. Two years of testing proved the progeny of this mutant to be breeding true.

An attempt (*2*) was made to transfer the earliness of this mutant to the vine type by crossing and backcrossing to Michelite, followed by selection. The vine type of Michelite was found to be dominant to the bush and lateness was dominant to earliness. In this particular mutant, earliness and bush type were closely linked, so that no early vine type was obtained. However, during the process of selection, the size of the bush was increased considerably.

In 1948, the bean-breeding program of the Michigan Agricultural Experiment Station was made cooperative with the U.S. Department of Agriculture. At that time, a survey was made of the bean industry of Michigan to determine the priorities in bean improvement. It appeared desirable to develop a variety which might lend itself to harvesting by combine without pulling. It also appeared necessary that resistance to either bean anthracnose, which is caused by the fungus *Colletotrichum lindemuthianum* (Saec. and Magn.) Scrib., or to common bean mosaic, which is caused by *Phaseolus* virus 1 and its variant strain, be incorporated into any new variety which might be developed and released. The ultimate purpose of this would be to combine the resistance to these diseases in one commercial variety.

Several of the bush types that were recovered from the irradiated material were crossed to different anthracnose-resistant strains of beans belonging to a collection received from Cornell University. Four generations of back-crossing followed by five generations of selection resulted in a large number of anthracnose-resistant bush strains that were first tested in 1953. Twenty acres of one of these strains were grown in 1955. This strain has been named Sanilac and will be grown by foundation growers in 1956 for certification in 1957.

Table 1 compares the variety Sanilac with Michelite, the present variety which constitutes 95 percent of the 400,000 acres planted to Navy beans in Michigan.

The advantages of the new bush type, Sanilac, over the vine type, Michelite, can be enumerated as follows. (i) Sanilac is resistant to the alpha strain of bean anthracnose fungus, which attacks the Michelite and all other pea beans in Michigan. (ii) Because of better air movement resulting from the bush habit of growth, Sanilac has been found to be considerably less liable to injury by *Sclerotinia sclerotiorum* (Lib.) D By., the cause of sclerotinia wilt, or white mold disease, of beans. This is indicated by the differences in yield between the two varieties in 1953. (iii) The new variety is 6 days earlier than Michelite, indi-

Table 1. Yield comparisons and development rates of Sanilac versus Michelite (1953–55).

Variety	Yield (bu/acre)			Days to bloom	Days to harvest
	1953	1954	1955		
Sanilac	39.4	22.4	28.0	47	86
Michelite	22.0	24.6	23.1	53	92