dence of kidney degeneration. From the experiment it appears that the chemical substances injected had a toxic effect on the mesonephros but not on the metanephros.

1. Landauer, Walter, *J. Exp. Zool.*, 1945, v98, 65.
2. Moseley, Helen M., *J. Exp. Zool.*, 1947, v105, 279.
3. Landauer, Walter, *J. Exp. Zool.*, 1948, v109, 283.
4. Landauer, Walter, and Bliss, C. I., *J. Exp. Zool.*, 1946, v102, 1.
5. Hansborough, L. A., *Growth*, 1947, v11, 177.
6. Gray, Peter, *Arch. Entwickmech.*, 1939, v139, 732.
7. Hammett, F. S., and Wallace, V. L., *J. Exp. Med.*, 1928, v48, 659.
8. Dilling, W. J., *Brit. Med. J.*, 1926, No. 3437, 924.
9. Ridgway, L. P., Patterson, P. A., and Karnofsky, D. A., *Fed. Proc.*, 1950, v9, 341.
10. Eakin, Richard M., *Sci.*, 1950, v111, 281.
11. Machlin, L. J., Denton, C. A., and Pearson, P. B., unpub. data, 1951.
12. Patten, Bradley M., Early embryology of the chick, Blakiston, Philadelphia, Pa., 1951.
13. Lillie, Frank R., The development of the chick, Henry Holt, New York City, 1912.
14. Needham, Joseph, Chemical embryology, University Press, Cambridge, England, 1931.

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Effect of Ploidy in Photoreactivation.* (19347)

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This article will present and discuss some preliminary results of measurements of the relations between ultraviolet dose and survival of haploid and diploid strains of a common species of yeast, both in the dark, and with subsequent irradiation by visible light following initial ultraviolet irradiation. While much work on the effect of ultraviolet light on survival of microorganisms has been published (1), only very limited quantitative studies have been made on the yeast cell in particular; one of the purposes of this work is to emphasize the advantages of the yeast as a test organism in the study of radiation damage. That is, there are available strains differing only in ploidy,‡ making studies on genetic effects relatively easy. The cells are large (5 μ diameter for diploid), easily grown, and give rise to several mutant strains, which are often colored. The present work gives survival curves of the diploid and haploid strains of *Saccharomyces cerevisiae* (SC6 and 7 respectively) both with and without subsequent photoreactivation, with a view to testing the Novick and Szilard theory of the reactivation phenomenon(2).

Procedure. For the initial radiation, 10 ml suspensions of yeast cells in sterile distilled water contained in 50 ml short beakers, of concentrations convenient for plating, were placed on a Gump shaker about 50 cm below the center of a G.E. Sterilamp; after doses up to 220 sec. exposure, aliquots were taken and spread on the surfaces of the growth medium (Difco potato-dextrose agar) in petri dishes. The remainder of the irradiated batch was then used for photoreactivation. The visible light source consisted of four photoflood lamps, arranged such that the light intensity, as measured with a photometer, was uniform over a large area of a transparent plastic shelf supported about 20 cm above the lamps; a glass tray containing 0.05 M $CuSO_4$, interposed between the yeast suspensions and the lamps, effectively kept the temperature rise at the position of the yeast less than 3°C/hr. The concentration of the suspensions varied from 3×10^3 to 5×10^5 cells/ml; within these limits the survival for a given dose did not, as shown by auxiliary experiments, depend on

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‡ Genetic structure of yeast is described at length in C. C. Lindegren, Genetics and Cytology of the Yeast Cell (Carbondale, 1950).

the concentration. Greater concentrations resulted in too strong absorption of the radiation. The survival probability was then obtained in the usual way, as the ratio of survivors on a plate poured from an irradiated sample, to those poured from the control sample. However, several complicating factors make it necessary to consider the criterion for the death of a yeast. Some of these complications are not so apparent in the case of *E. coli*, for example. Thus, the yeast cells may sporulate; they can metabolize quite actively, even though unable to divide; storage of essential nutrients can strongly affect the division rate, even to the extent(2) that cells may lie dormant (glycogen storage) in a sated condition, if healthy, or furnish enough material to produce as many as, say, 10 daughters from a severely damaged parent. Further, since "death" due to ultraviolet irradiation is apparently not a well defined state, some cells, which might eventually divide but whose division has been delayed greatly, might, because of depletion of the medium by the cells with shorter delays, never appear as viable. Finally, as in any experiment with U.V.(3) there is generally a large spread in radiation resistance in the original population due to a spread in the ages of individual cells, in physiological state, and even, apparently, in strain, *i.e.*, cells apparently alike in every other way (age, state) may still differ strongly in their resistance to the radiation. Therefore, prior to irradiation, the cells were transferred from slant cultures on PD agar to a medium containing only inorganic salts(4), and left for 36 hours, while the air was bubbled through; just before the run, this suspension was centrifuged, the cells washed in isotonic salt solution, resuspended in sterile water and filtered through 2 layers of sterile absorbent cotton. Microscopic examination showed the absence of any significant number of clumps of cells, and by comparison of the actual microscopic count and the colony count, all cells taken from the fern spore medium were viable. After this treatment, cells in a sample of this suspension should be of about the same age and physiological state, with a small amount of stored material.§ The last point was checked, qualitatively, by comparing the survival obtained by microscope count (scoring microcolonies of n cells or more as produced by "living" cells, of less than n cells as "dead") with the survival obtained from colony count in a petri dish. Without the above pretreatment of the cells the "microscopic" survival was much greater than the "macroscopic" unless $n \sim 10$; with the treatment, the two counts agreed fairly well with $n = 3$. Further, to check on the effect of the growth medium, aliquots of suspensions irradiated to about 10%, and 1%, survival were spread on 4 pairs of plates containing (a) standard PD agar, (b) PD agar enriched with 2% yeast extract, (c) PD agar and 2% casein hydrolysate, (d) PD agar on which about 300 large colonies had grown, the colonies removed, the agar washed in distilled water and then resterilized. No significant difference in survival was observed between any of these, although a difference in delay (time to reach full growth) was apparent, and the average size of individual colonies differed somewhat from one pair to another. All yeast stocks were subcultured every 48 hours on agar slants, and to check that the haploid had not become contaminated, single colony isolates, taken from the survivors of an X-ray irradiation, were grown to large cultures and samples again X-irradiated. Since the exponential curve, with the LD50 measured by Zirkle and Tobias(5) was obtained, it was assumed that this was indeed the SC7 strain; the average U.V. curves obtained with this material agreed with the average curve on the stock material. Runs made just as above but with the haploid cells taken from 6- to 8-day-old cultures often showed a greatly increased resistance in the haploid, with the semilog curve sometimes concave upwards. Presumably this was due to contamination of the haploid stock with diploid because of the tendency for the haploid to undergo illegitimate conjugation (this was a complicating factor in the work of Zirkle and Tobias).

§ Work by T. H. Wood, to be published, indicates that this procedure should be used with caution, if at all, if the aim is to obtain a homogeneous population. However, while the criticism is valid, the essential results of this work are probably correct.

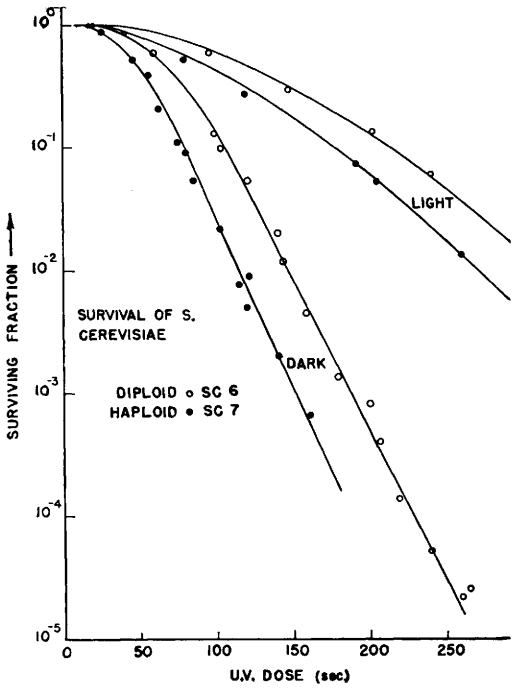

FIG. 1. Survival of diploid and haploid yeast under the action of ultraviolet light ("dark") and with irradiation by visible light subsequent to the initial irradiation by U.V. ("light").

Fresh cultures showed no such enhanced resistance, and the diploid runs were quite reproducible, almost independently of the age of the culture, for ages from 2 to 9 days.

Results and discussion. The dark survival of the two strains is shown in Fig. 1, carried to a survival probability of about 10^{-4}. (Each point on the curves represents at least 500 macrocolonies.) About half of the points on each of the curves represent runs in which the deviations could be explained by changes in output of the lamp; the diploid (SC6) survival, known from 3 consistent runs made earlier, was used as an indicator of the lamp's intensity in a statistically significant comparison between haploid and diploid strains. While, therefore, the measured survival of the haploid is not accurate, and therefore unsatisfactory, it is definitely not exponential in these experiments. This differs from the conclusion of Caldas and Constantin(6), who obtained not only an exponential survival for the haploid but a greater haploid survival than diploid for survival below about 17%. Their diploid curve was similar to the one shown here. Possibly their haploid strain had become contaminated with diploid, as mentioned above, although this could not result in an exponential curve; however, since their observed points are not published it is difficult to determine the source of the difference.

While the two dark survival curves are not exponential, they become straight at high dose, and asymptotically parallel. On the basis of the target theory, this suggests that part of the action of the radiation is on the genetic structure, with 2m targets inactivated in the diploid and m in the haploid; from the curves, m would be of the order 3. If this mechanism is accepted, and the multiple-hit theory ruled implausible, one must then suppose that there is, superimposed on the direct action, an indirect action which becomes less important as the dose becomes great, since the haploid curve is sigmoid. One must further suppose that this indirect action is not significant at any dose when X-rays are used since the X-ray survival curve is exponential (5,6). It would be interesting to verify these speculations by, for example, studying the relative temperature dependence of the low and high survival parts of the two curves, and by an additivity study of X- and U.V. radiations.

The photoactivation is as striking as in the case of other organisms. Here only the curves for maximum reactivation (about 30 min.) are shown (Fig. 1), although rough data for intermediate curves (5, 10, 15 min.) were also taken. If one applies the reduced-dose principle(7) to these data, then (with P_o = survival for zero light ("dark"), P_{oo} = survival for infinite light ("light"), $P_o(qx) = P_{oo}(x)$ with $q<1$, x = dose. That is, the light survival at any dose is obtained from the dark survival at that dose reduced by a constant fraction q.) These data satisfy the relation quite well. By the theory of Novick and Szilard(3) this relation can be obtained by assuming that the effect of the radiation is to produce a poison, of which the fraction q can be decomposed by the visible light, as in a monomolecular reaction. That is, q appears as a characteristic parameter of the poison, for which the organism has a characteristic

sensitivity. Therefore it is highly suggestive that the q values for the haploid and diploid curves are 0.43, 0.48 respectively, *i.e.*, very close to each other (always noting, however, that these numbers are subject to about 20% error) and also close to the value obtained for *E. coli* by Novick and Szilard. It is also interesting to inquire whether in reactivation the initial effect is direct (*e.g.* by the induced decay of a previously excited metastable state) or "indirect" (as in the poison theory). First, it can be shown, in a purely formal way, that once the dose-reduction principle is accepted, then the initial lethal action of the radiation can not be through purely target or direct effects.‖ If the reactivation is through a metastable state, with or without superimposed chemical action that takes no part in the reactivation, then it can be shown that $P(x,t) = P_o(x) f(t) + P_{oo}(x) [1-f(t)]$ for ultraviolet dose x, light dose t, and where 1-f is the probability of inducing a transition to a "live" state from a dormant state; for a single metastable, for example, $f = e^{-at}$. In any event, the survival at an intermediate value of visible light dose is a linear interpolation between the extreme curves of dark, and maximum light, survival. The theory of Novick and Szilard, on the other hand results in $P = P(L)$, $L = xg(t)$ where $g(O) = 1$, $g(oo) = q$. While neither the intermediate values taken here (not shown), nor the published data of Novick and Szilard, fit the metastable state picture, these measurements are consistent with the poison theory, as is the work of the last named authors.

Summary. The survival fractions of diploid and haploid strains of *S. cerevisiae* after ultraviolet irradiation (2537 A.U.) have been obtained, compared, and modified by photoreactivation following the initial irradiation. The haploid survival follows a sigmoid curve and is smaller than the diploid at the same dose. The dose reduction principle (which can be successfully applied to the data) yields a reduction factor that appears substantially the same for photoreactivation of both strains. The data appear consistent with the model proposed by Novick and Szilard.

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‖ This can be shown by an examination of the class of functions that result from the target theory, (see Timoféeff-Ressovsky, N. W., and Zimmer, K. G., Das Trefferprinzip in der Biologie, *Biophysik* I, (S. Hirzel, Leipzig, 1947)) and noting that if one of these represents the dark survival, then the light survival function cannot belong to the same class, except in the special case where both light and dark survival are exponential.

1. For a recent review, see A. Giese, *Physiol. Rev.*, 1950, v30, 431.

2. Novick, A., Szilard, L., *Proc. Nat. Acad. Sci. U.S.*, 1949, v35, 541.

3. Rentschler, H. G., Nagy, R., and Mouromseff, G., *J. Bact.*, 1941, v41, 745.

4. Zirkle, R. E., *Am. J. Roentgenol.*, 1936, v35, 230.

5. To be published. The designation SC6, SC7 are those workers' notations for strains supplied by C. C. Lindegren, whose original designation was #93 and #93.1C(*a*) respectively.

6. Caldas, L. R., and Constantin, T., *Compt. rend.*, 1950, v252, 2356; Latarjet, R., and Ephrussi, B., *Compt. rend.*, 1949, v229, 306.

7. Kelner, A., *J. Bact.*, 1949, v58, 511.

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