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Relationship Between Deuterium Inhibition of Growth and Glucose Catabolism in *Saccharomyces cerevisiae*.* (27987)

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The biological effects of deuterium have been ably reviewed by Morowitz and Brown (1) and more recently by Katz(2). From these reports it is evident that almost without exception cell division is partially or completely inhibited by deuterium. Sensitivity to deuterium varies considerably among different types of cells and apparently is related to the mechanism by which cell division is accomplished. Mitosis is completely inhibited at all stages in 50 to 75% deuterium oxide (D_2O)(3,4), whereas bacteria, yeast, and some algae, which accomplish cell division by other means, can be grown in 99+% D_2O(5). Deuterium inhibition of microbial cell division (growth) is thought to be due to effects on the metabolic state of the cell(3), although direct experimental evidence for this is lacking. In view of this, the effects of deuterium on growth and glucose catabolism in yeast were compared.

Methods. A haploid strain of *Saccharomyces cerevisiae* was used for these studies. Stock cultures were maintained in peptone, yeast extract, glucose agar slants and were transferred at monthly intervals. For experiments cells were cultured at 25°C in the defined medium of Burkholder, McVeigh and Moyer(6). This medium supported growth when made with D_2O or water; however, for comparative purposes in some experiments the medium was supplemented with 1% peptone and 0.5% yeast extract. Before use D_2O was distilled with permanganate and the final concentration as determined by specific gravity was 99-99.5%. Growth was followed routinely by determining the optical densities of cultures at 640 mμ on a Coleman spectrophotometer and by periodic dry weight determinations. Standard manometric technics (7) were employed in gas exchange studies. All experiments were repeated at least 5 times and results are reported as mean ± standard deviation.

Results. Growth studies. In Fig. 1 typical results of growth experiments are presented. Curve A shows the growth pattern seen with water grown cells in water media and serves as a control. Curve C illustrates the inhibitory effects of D_2O on growth of cells previously grown in water. The initial lag period was prolonged in D_2O media, in this case being about 30 minutes compared to 15 minutes in water media. The lag phase in D_2O was always about twice as long as in water regardless of composition of the medium. During log growth optical density increased 0.07 ± .01 O.D. units per hour in water media compared to .03 ± .01 units per hour in D_2O media. When media were supplemented with peptone and yeast extract growth rates increased; however, the relative deuterium effect $\left(\frac{\text{growth rate in } D_2O}{\text{growth rate in } H_2O} = 0.47 \pm .07\right)$ was the same. Attempts to follow growth by direct cell counting were unsuccessful due to clumping of the cells, particularly in D_2O cultures.

Also shown in Fig. 1 are results of experiments in which cells previously grown in D_2O were subcultured in D_2O and ordinary water media. Curve D shows the growth pattern of deuterated cells in D_2O, and by comparing this curve with Curve C (water grown cells in D_2O), it may be seen that growth rates were the same in each case (0.03 O.D.

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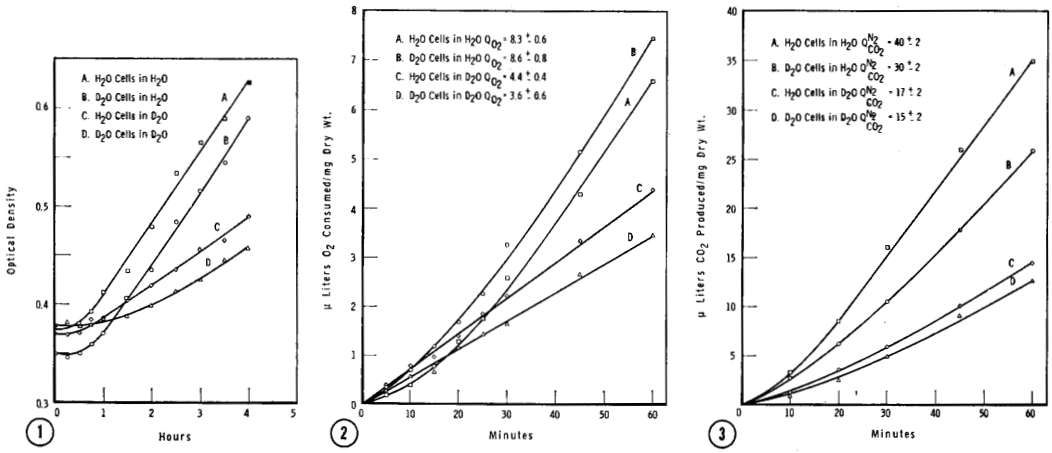

FIG. 1. Effect of D_2O on growth of *S. cerevisiae*. Cultures were inoculated with log phase cells and incubated with air as the gas phase at 25°C.

FIG. 2. Effects of D_2O on oxygen uptake. Vessels contained 0.2 ml 10% KOH in center well, 1.7 ml cells, and 0.1 ml glucose in sidearm. Final concentrations were 0.05 M glucose and 0.01 M phosphate buffer at pH (pD) 7.2. Temperature was 25.6°C and air was the gas phase. Q_{O_2} values are mean ± standard deviation.

FIG. 3. Effects of D_2O on fermentation of glucose. Experimental conditions were the same as in Fig. 1 except that water was substituted for KOH and gas phase was 95% N_2, 5% CO_2. Q_{CO_2} values are mean ± standard deviation.

units per hour). Thus, the ability of yeast cells to grow in a D_2O environment was not enhanced by prolonged culture in D_2O media. Curve B is typical of results obtained when D_2O grown cells were transferred directly to water media. The growth rate for this set of conditions was 0.07 ± .02 O.D. units per hour which was the same as for control cultures, showing that D_2O inhibition was readily reversed. It should also be noted that the initial lag periods of Curves A and B were the same, indicating that D_2O grown cells did not require an extended adaptation period before growth began in ordinary water. In contrast to this bacteria are reported to show an extended lag phase when transferred from water to D_2O or the reverse(8).

Manometric studies. In Fig. 2 the effects of D_2O on respiration are shown. Curve A shows results obtained with water grown cells in water environment and serves as a control. In D_2O respiration was inhibited about 50% with both water and D_2O grown cells (Curves C and D). Thus, as was the case with growth, prolonged culture on D_2O media had no effect on the magnitude of D_2O inhibition of respiratory activity. Inhibition was readily reversed when D_2O grown cells were suspended in ordinary water (Curve B). In these studies glucose was tipped into the flasks immediately and the first reading made 5 minutes later. At this time D_2O inhibition of respiration and reversal of inhibition were already evident. It is of interest that equilibration studies (unpublished data) indicated the half time for free cell water to exchange with the external environment was approximately 10 minutes.

Although growth studies were carried out under aerobic conditions, in which only about 10% of incorporated glucose was metabolized *via* the glycolytic pathway, it was of interest, nonetheless, to determine the effect of deuterium on glycolysis (Fig. 3). Curve A was obtained with water grown cells in water and serves as a control. As in the aerobic studies the inhibitory effects of deuterium on water and D_2O grown cells (Curves C and D) were of similar magnitude. When deuterated cells were suspended in ordinary water the rate of CO_2 evolution immediately increased (Curve B); although some residual inhibition remained after an hour.

The effects of deuterium on endogenous metabolism are shown separately in Table I. It can be seen that deuterium inhibition under aerobic conditions was similar to effects noted when glucose was present. Under

TABLE I. Effect of Deuterium on Endogenous Aerobic and Anaerobic Gas Exchange. Experimental conditions as in Figs 2 and 3.

Treatment	Q_{O_2}*	$Q^{N_2}_{CO_2}$
H_2O cells in H_2O	1.5 ± .2	.17 ± .10
" " " D_2O	.70 ± .05	.19 ± .11
D_2O " " D_2O	.73 ± .02	.19 ± .12
" " " H_2O	1.4 ± .2	.11 ± .09

* Results are expressed as mean ± standard deviation.

anaerobic conditions, due to low Q_{CO_2} values and variability between experiments, the effects of deuterium are less obvious. Low endogenous anaerobic metabolism is apparently characteristic of this yeast since starved and unstarved cells gave the same results.

Discussion. The data suggest that deuterium effects on growth and glucose catabolism were primarily due to the presence of D_2O rather than deuteration of cell protoplasm. This is supported by the following observations: First, when deuterated cells were transferred to water media there was no difference in duration of the initial lag phase or in subsequent growth rates during exponential growth. Second, inhibition of aerobic glucose catabolism was reversed within 5 minutes whereas a lag would be expected if recovery was dependent on cellular deuterium being exchanged. There was some evidence of latent damage to glycolysis since full recovery was not attained. At present no explanation of this finding will be attempted.

The striking similarity between deuterium effects on growth and glucose catabolism indicate that inhibition of the latter function is a primary factor in growth depression. Such a relationship is not surprising since growth activities are dependent on energy derived from the breakdown of glucose. In view of the effects of deuterium on endogenous metabolism it seems likely that inhibition of glucose catabolism is due to depression of rates at which certain enzyme reactions proceed. Studies on isolated enzyme systems have shown that enzymatically catalyzed dehydrogenation reactions are particularly sensitive to deuterium inhibition(9 and others).

The question remains as to what effect deuterium inhibition of energy metabolism has on other physiological functions such as DNA synthesis which have been reported to be suppressed by deuterium(3,10). Since macromolecule synthesis is energy dependent it is likely that at least part of the deuterium effects reported are due to inhibition of energy metabolism. In any event this possibility should be considered in future studies.

Summary. Deuterium reversibly inhibited yeast growth and glucose catabolism. The similarity between these effects indicated that growth rate depression was primarily due to suppression of glucose catabolism. The latter effect was probably due to inhibition of enzyme reaction rates.

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