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Inhibition of Yeast Growth by 2-4 Dinitrophenol.*

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The undissociated acid form of 2-4 dinitrophenol (alpha-DNP) has been shown by Field, Martin and Field[1,2] to stimulate both the rate of oxygen consumption and of fermentation by yeast.

Is this heightened metabolic level reflected in an increase in the growth rate? To test this we have grown yeast in the L-shaped rocker tubes designed by Fraser,[3] rocking 42 times per minute through an angle of 50°, in a water bath with temperature maintained at 25° C. The medium used was 10% yeast autolysate[4] in 0.2 M phosphate buffer at pH 6.8. The solution contained 2% to 5% glucose. Adequate precautions were taken to prevent contamination during inoculation and sampling, and our pure strain[1,2] of *Saccharomyces cerevisiae,* race F, was used. Under these conditions there was little or no change in pH over a period of 4 days. Use of such yeast, tubes and media gave good checks (within 5%) in the control tubes.

The concentration of the sodium salt of alpha-DNP which gave maximum stimulation of respiration at pH 6.8 was 400 mg. per liter or 1.785×10^{-3} molar.[1,2] This gives a concentration of 2.87×10^{-6} molar for the free acid form. Instead of a stimulation of growth, a marked inhibition occurred with this dosage, there was an increase in duration of the lag and log phase (nomenclature of Buchanan[5]) and a decrease in the total population attained, as shown in Fig. 1. All of the free acid concentrations tried by us, ranging from 7.18×10^{-8} to 7.18×10^{-6} molar, had a more or less inhibitory effect on growth.

Hopkins tube readings agreed with the cell counts in showing that at no period did the tubes containing alpha-DNP attain either

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[1] Field, J. II, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 56.

[2] Field, J. II, Martin, A. W., and Field, S. M., *J. Cell. and Comp. Physiol.,* 1934, **4**, 405.

[3] Fraser, C. G., *J. Physical Chem.,* 1921, **25**, 1.

[4] Orla-Jensen, S., "The Lactic Acid Bacteria," Copenhagen, 1919.

[5] Buchanan, R. E., *J. Infect. Dis.,* 1918, **23**, 109.

the number of cells or the quantity of protoplasm of the control tubes.

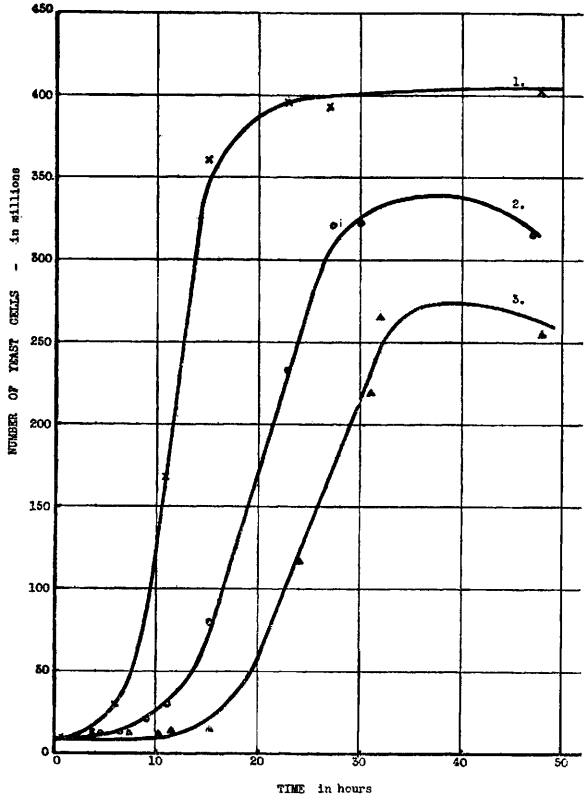

FIG. 1.

Comparison of lag and log phases of yeast growth from identical seedings. All tubes contain 5% glucose and 10% yeast autolysate made up in 0.2 M phosphate buffer, pH 6.8. Concentration of free acid alpha-DNP is 0 in tube 1, 1.795×10^{-6} molar in 2, 2.87×10^{-6} molar in 3.

Testing the yeast grown in this manner in the Warburg respirometers we found that the alpha-DNP optimum for respiration has been shifted in each case to a lower value. This can probably be explained by residual alpha-DNP in the cells in spite of the repeated washings.

A striking effect, perhaps due to acclimatization or selection of yeast cells, or both (compare Fulmer[6]) is the greatly increased respiratory rate per 10^8 cells observed with yeast grown in tubes containing alpha-DNP. This may reach 300% of the control level.

Further work is in progress.

[6] Fulmer, E. I., *J. Physical Chem.*, 1921, **25**, 455.