

**ACTION OF DINITROCRESOL ON YEAST  
FERMENTATION AND OXIDATION**

*Sir:*

The details of a study of the effect of 4,6-dinitro-*o*-cresol (DNC) on the metabolic activities of yeast will be presented in a paper now being prepared for publication. The present communication gives a summary of certain of these experiments conducted with normally fermenting yeast which bear on the mechanism of DNC action. These experiments were conducted in January, February and March, 1935, with yeast obtained from Anheuser-Busch, Inc.

(1) The rate of anaerobic carbon dioxide production by yeast in a glucose-phosphate medium (*pH* 4.45) was increased by DNC in concentrations from  $10^{-6}$  to  $10^{-4}$  molar. Greater concentrations of DNC caused a subnormal anaerobic carbon dioxide production. Maximum stimulation occurred at  $10^{-5}$  to  $2 \times 10^{-5}$  *M* DNC with yeast in a concentration of 6 mg. per cc. In a normal system  $Q_{CO_2}^{N_2}$  was, in two experiments, 74.5 and 78; after addition of  $2 \times 10^{-5}$  *M* DNC  $Q_{CO_2}^{N_2}$  was increased to 159 and 157, respectively. Analysis showed that an amount of alcohol equimolar to the anaerobic carbon dioxide was produced in the presence of optimum concentration of DNC.

(2) The rate of oxygen consumption by buffer washed yeast was increased by DNC in concentrations from  $10^{-6}$  to  $2 \times 10^{-5}$  molar. Greater concentrations of DNC caused a subnormal oxygen consumption. Maximum stimulation (50 to 100%) occurred at  $10^{-5}$  *M* DNC. With 1 to 6 mg. of yeast per cc., oxygen consumption was stimulated by DNC in a medium containing glucose or alcohol as substrate but not in a medium containing pyruvate as substrate.

(3) The rate of reduction of cytochrome (549 *mμ* band) in buffer washed yeast was increased by DNC in concentrations from  $10^{-6}$  to  $2 \times 10^{-5}$  *M*. Greater concentrations of DNC lengthened the reduction time. The greatest shortening of reduction time (from one hundred and five to thirty-two seconds) was effected by  $10^{-5}$  *M* DNC.

(4) With a concentration of DNC ( $10^{-5}$  *M*), which caused the optimum rise in oxygen consumption of yeast in a glucose medium, an increased aerobic alcohol production occurred only at high glucose concentrations and for limited times. At low glucose concentrations, and over extended time periods, DNC effected no increase

in amount of alcohol produced aerobically; in fact, any alcohol initially present was oxidized.

(5) Since part of the carbon dioxide produced aerobically resulted from fermentation, an amount of carbon dioxide equimolar to the alcohol produced aerobically was subtracted from the total to get the oxidative carbon dioxide. In several experiments where this was done the ratio of oxidative carbon dioxide to oxygen consumed was not significantly influenced by the presence of  $10^{-5}$  *M* DNC.

(6) At  $10^{-5}$  *M*, DNC increased not only the rate of aerobic oxygen consumption and anaerobic carbon dioxide production, but also the total amount of glucose oxidized or fermented as determined from the products of reaction.

(7) The amount of glucose disappearing, as determined by analysis, was greater than that accounted for by the products of oxidation and/or fermentation. DNC had no effect on the amount of glucose which disappeared.

(8) Actual count showed no increase in number of yeast cells during the period of the experiment.

(9) At optimum concentration, DNC stimulated anaerobic carbon dioxide and alcohol production to a greater degree than it stimulated oxygen consumption in a corresponding aerobic system, and caused increased anaerobic utilization of sufficient sugar to account for excess oxygen consumed and excess alcohol produced in the aerobic system.

These results are not incompatible with the view that the principal metabolic effect of DNC is to accelerate one of the anaerobic processes occurring early in the series of reactions concerned with the formation of (a) substances subsequently oxidized by the cell, and (b) products of anaerobic fermentation.

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**THE RELATIVE RATES OF COMBINATION OF  
LIGHT AND HEAVY HYDROGEN WITH ETHYLENE**

*Sir:*

Preliminary determinations of the rate of combination of heavy hydrogen with ethylene have been made. The reaction has been carried out both on a copper catalyst and homogeneously, the rate of these reactions being compared with the