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 (*p*H 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 2×10^{-5} *M* DNC with yeast in a concentration of 6 mg. per cc. In a normal system $Q^{N_2}_{CO_2}$ was, in two experiments, 74.5 and 78; after addition of 2×10^{-5} *M* DNC $Q^{N_2}_{CO_2}$ 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 2×10^{-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 2×10^{-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.

We wish to express our indebtedness to Miss A. K. Keltch for her coöperation.

LILLY RESEARCH LABORATORIES M. E. KRAHL
INDIANAPOLIS, INDIANA G. H. A. CLOWES

RECEIVED APRIL 11, 1935

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 corresponding light hydrogen reaction. Owing to uncertainties in the purity of the deuterium and as to the complete role of exchange reactions, full publication is being deferred.

The catalytic experiments using copper indicated at 0° a ratio of rates H_2/D_2 of 1.59. Since Farkas, Farkas and Rideal [*Proc. Roy. Soc.* (London), **A146,** 630 (1934)] found no exchange at low temperatures over nickel, it is likely that no exchange occurred in this reaction over the comparatively less reactive copper catalyst. However, this point is being investigated. At higher temperatures (184 to 306°) the observed ratio fell from 1.34 to 1.04. How much of this decrease is due to exchange will be determined.

In the homogeneous reaction at 524 and 560° the ratios were 2.10 and 2.0, respectively. This seems to point to a real difference in the activation energies of the homogeneous reactions, but again further investigation (which will be shortly undertaken) is required.

These preliminary results point to the likelihood that comparative rates of hydrogenation can be obtained at 0° on the copper catalyst and at 500–550° in the homogeneous reaction, without serious interference from exchange reactions. Such measurements are now being made with deuterium of known purity.

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RECEIVED APRIL 15, 1935

THE SEPARATION OF CERTAIN RARE EARTHS

Sir:

It has been shown by Yntema [THIS JOURNAL, **52,** 2782 (1930)] and by Ball and Yntema [*ibid.*, **52,** 4264 (1930)] that europium and ytterbium may be separated from other rare earths by precipitation as sulfate after electrolytic or other reduction to the divalent state. However, because of the small amounts of these elements found in rare earth mixtures, it has still been necessary to resort to fractional crystallization over a period of a year or more before the reduction method becomes applicable.

Using several samples of pure rare earths put at his disposal through the kindness of Professor B. Smith Hopkins of the University of Illinois, the writer has attempted to facilitate the separation of europium through the use of a co-precipitant. In a mixture of terbium group earths too dilute in europium to give any direct precipitate of europous sulfate, it has been found possible to obtain a substantially complete co-precipitation with barium sulfate of all the europium present. The europium is then readily removed from the filtered barium sulfate by repeated washing with a little hot concentrated nitric acid. There seems, therefore, no reason why europium, and probably ytterbium also, cannot be removed practically quantitatively from a crude rare earth mixture in as many days as it formerly took years.

The writer has also examined a possible separation of neodymium and samarium. The mixture of anhydrous chlorides is treated with hydrogen at 700°. This results in reduction of the samarium to $SmCl_2$. Treatment of the resultant mixture with water brings about a reaction probably represented by $6SmCl_2 + 3H_2O \longrightarrow 4SmCl_3 + Sm_2O_3 + 3H_2$, the neodymium trichloride simply dissolving. Part of the samarium is precipitated but seems always to be contaminated with some neodymium. Although the separation obtained is good, as rare earth separations go, it is nevertheless only fractional, and because of its difficulty probably cannot compete with the usual double nitrate fractional crystallization.

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RECEIVED APRIL 24, 1935

THE THERMAL REACTION BETWEEN CHLORINE AND FORMALDEHYDE

Sir:

Krauskopf and Rollefson [THIS JOURNAL, **56,** 2542 (1934)] have isolated formyl chloride from the photochemical interaction of chlorine and formaldehyde, and have suggested [*ibid.*, **57,** 590 (1935)] that this substance occurs as an intermediate in the corresponding thermal reaction [Spence and Wild, *Nature,* **132,** 170 (1933); *J. Chem. Soc.*, 1588 (1934)]. Furthermore, they suggest that the excess of carbon monoxide produced in our experiments over that calculated from the pressure change is due not to an induced polymerization of the formaldehyde, but to the presence of formyl chloride, which is produced without change of pressure. If the formyl chloride decomposed in the process of analysis giving HCl and CO, it is claimed that this explanation would account for our results equally well. Consideration of a number of