THE ACTION OF DEUTERIUM OXIDE IN LOW CONCENTRATION ON THE COURSE OF GAS-PRODUCTION BY BREWER'S YEAST

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Investigations have long indicated that the rate of cellular metabolism as measured by CO_2 production or oxygen consumption may be positively influenced by very low concentrations of toxic substances which generally serve as metabolism inhibitors. It has been shown by Cook (1926) that 0.0001M $AgNO_3$, for example, produces an increase in the respiration of fungi, while higher concentrations produce the characteristic toxic effect of a heavy metal salt. Brooks (1921), working with *B. subtilis,* indicated that sodium taurocholate causes an increase rather than a decrease in the respiratory rate when in very low concentrations. Branham (1929) has shown that the stimulating action of low concentrations of certain metallic salts, phenol, and a number of other antiseptics, is apparent during the course of gas-production by baker's yeast. A slight stimulating action of so toxic a substance as KCN when in quite low concentration has been demonstrated by Shoup and Boykin (1931) in the case of *Paramecium caudatum.*

There have been indications that the action of low concentrations of Deuterium Oxide on certain cellular processes may be excitatory rather than inhibitory. Richards (1933) grew a pure strain of *Saccharomyces cerevisiae* in Williams' medium with water containing the D_2O of S.G. 1.000061, and obtained an increase in cell size, weight, uniformity, and growth, as compared with controls in this medium with plain water. It has been shown that in high concentrations of D_2O definite adverse effects may occur to organisms (Taylor, Swingle, Eyring, & Frost, 1933 a, b.), but Macht and Davis (1934) used 1:2000 of D_2O in testing the germination and growth of seedlings with no definite stimulation. They also found no stimulation of gas-production by yeast. Barnes (1934) has been particularly interested in the action of dilute D_2O, and reports increased division and activity in certain protozoa in a medium containing the heavy water. Barnes and Jahn (1934) have pointed out that certain results of Harvey and Taylor (1934) with luminous bacteria, and of Taylor and Harvey (1934) with yeast, indicate that a concentration of D_2O of 0.18 per cent may bring about a slight increase in oxygen consumption.

We have had available for use some samples of a very active

brewer's yeast,[1] and have conducted the following experiments with the consideration that CO_2 production during fermentation of sucrose in Pasteur's solution with a D_2O content of 0.50 per cent, might aid in indicating the relation of the heavy isotope of hydrogen in dilute concentrations to reactions involved in fermentation by organisms. These experiments were expected also to indicate something regarding stimulation or inhibition of enzyme action, such as the recent

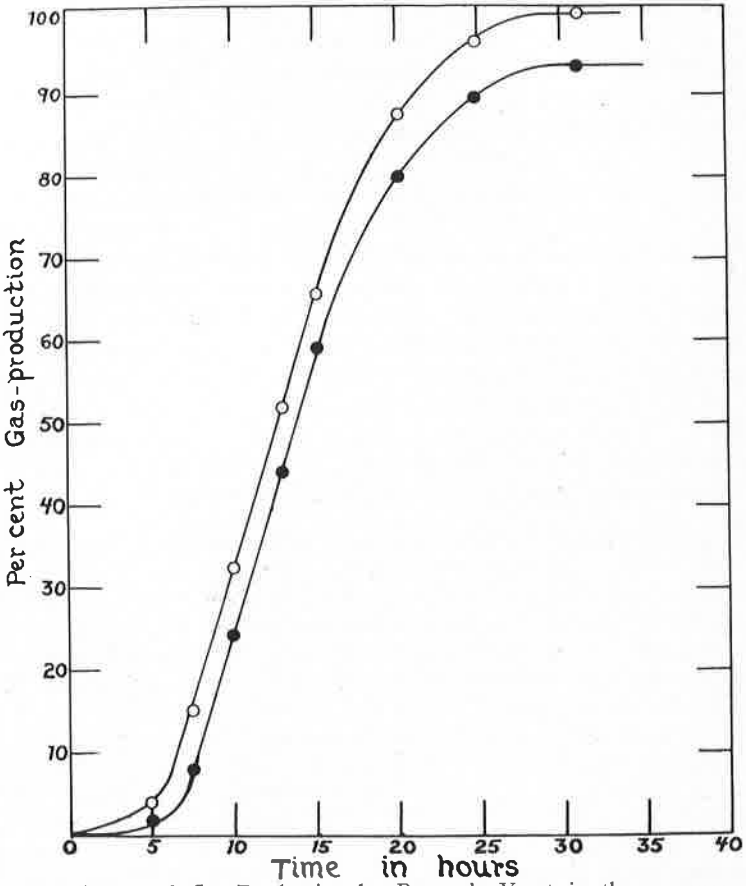

Fig. 1. Course of Gas-Production by Brewer's Yeast in the presence and absence of D_2O. Circles, Control in plain H_2O Pasteur's solution; solid dots, D_2O Pasteur's solution of 0.50 per cent D. Temperature, 30°C.

work of Barnes and Larsen (1933, 1934) where decreased CO_2 production during fermentation of glucose by zymin in dilute isotope water (0.60 per cent of D) indicated an inhibitory rather than a stimulating effect.

[1]We are deeply indebted to the William Gerst Brewing Company for kindly supplying samples of the brewer's yeast used.

Methods

In these tests on the fermentation of sucrose in Pasteur's solution with and without D_2O, 80 determinations were run in the incubator at a temperature of 30°C. Very uniform inoculations of fresh brewer's yeast were made into Pasteur's solution containing either 0.25 per cent, or 0.50 per cent of D_2O, and into control Pasteur's solution in ordinary water. Several cell counts were made with a Petroff-Hausser bacteria counter to assure uniformity of inoculation.

In the first series, Pasteur's solution with a concentration of 0.25 per cent D_2O was used. In a typical series of 18 determinations on the course of gas production, no significant difference in the rate was distinguished until after 45-50 hours of incubation, when in the last stages of fermentation the rate became slowed by an average of 2.6 per cent in D_2O as compared with the control.

In a second series of determinations, the concentraiton of D_2O of 0.50 per cent indicated a somewhat slowed course of gas production as compared with the control of H_2O. In the curve (Fig. 1) the average of 23 determinations shows a volume of gas produced at the end of fermentation of 6.5 per cent greater volume in H_2O. This was characteristic, and there is no significant difference in the rate of gas production during the initial stages of fermentation, but only the more pronounced slowing in the final stages when in D_2O. This may be due, as Barnes and Larsen (1934) have suggested, to the total inhibition of a certain amount of enzyme present or to a general reduction in the reactivity of a larger quantity.

Discussion

The results of these tests are in accord with the view of Richards (1934) that the heavy isotope of hydrogen may bring about an early aging of the cells, and a depression of cellular metabolism. Such an effect might well be expected to slow the rate of cellular processes involving enzyme action. These results are in line with the discussion recently presented by Curry, Pratt, and Trelease (1935) who point out that the action of dilute heavy water on certain biological processes is negligible. In our experiments there is no indication of a marked effect of the dilute D_2O of 0.50 per cent or less on the course of gas production by brewer's yeast.

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