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Some Characteristics of the Stimulating Effects of 2-Deoxy-D-Glucose On Endogenous Fermentation in Yeast.* (30808)

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It was shown earlier(1) that 2-deoxy-D-glucose (2DG) elicits a dissimilation of glycogen in yeast, with one mole of CO_2 formed for each glucose equivalent of glycogen lost. These data were consistent with a proposed mechanism of cyclic fermentation reported earlier(2) from studies made on dialyzed yeast extracts. In the present paper certain characteristics of the 2DG-stimulated fermentation in cells are presented along with possible implications of such findings.

Methods. All experiments were done at 30°C under nitrogen on nonproliferating baker's yeast previously washed and aerated(3). Tris-succinate-tartrate adjusted to appropriate pH served as buffer. CO_2 production was measured in a Warburg apparatus. Glycogen was determined using the method of Berke and Rothstein(4) after washing the yeast 3 times with distilled water.

Results. Specificity of the stimulatory effect of 2DG; absence of effects of KCl. In Fig. 1 and Table I are shown cumulative CO_2 productions by yeast suspensions for various sugars added under anaerobic conditions. As reported by Brady *et al*(5) and later, by us (1), it is seen that a low but significant rate of endogenous fermentation occurred. Addition of 2DG at pH 5.7 resulted in a rate of CO_2 production about 4.5 times that of the endogenous control. This phenomenon had been reported earlier(1). On the other hand, KCl, 3-0-methyl-D-glycopyranose, L-(-)rhamnose, d-sorbitol and galactose yielded rates not appreciably different from the endogenous rate (CO_2 formed in the presence of 3-0-methyl glucose, sorbitol and galactose were not included in Fig. 1 to maintain clarity. Actual values for CO_2 produced in 130 minutes lie between those in the presence of KCl and rhamnose.) Results with L-sorbose were equivocal. In contrast to the results obtained in the presence of acetic acid(7), KCl had no effect on either endogenous (Fig. 1) or 2DG-induced anaerobic CO_2 production (Table I), at pH 5.7. Furthermore, at pH 2.5 no effects of KCl were found on either endogenous or on 2DG-induced fermentation.

Endogenous and 2DG-induced fermentations vs *pH.* Fig. 2 shows that anaerobic endogenous metabolism reached a maximum in

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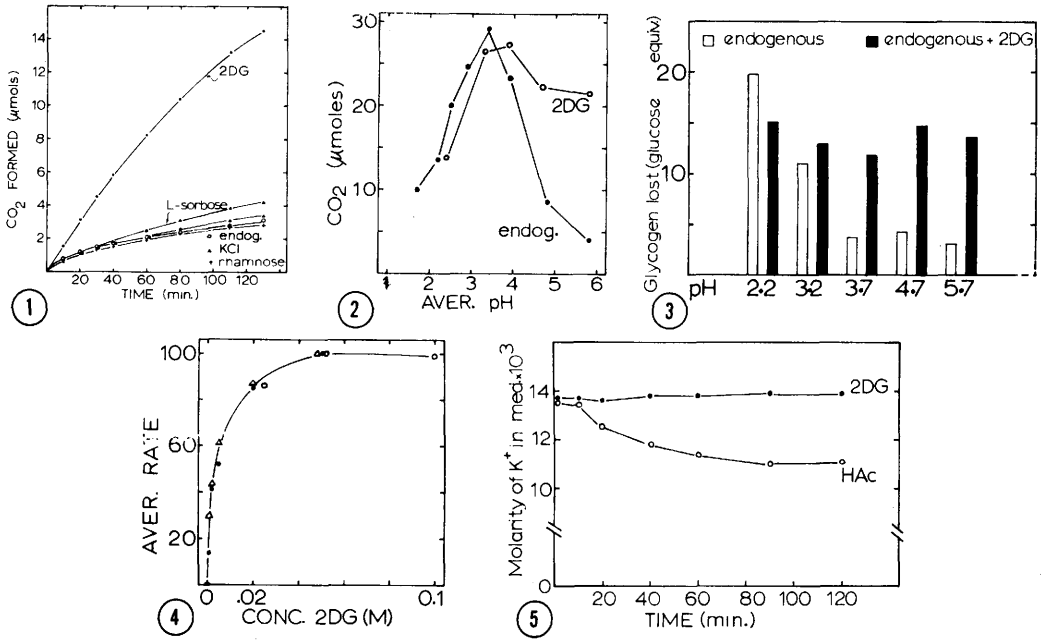

FIG. 1. Effcts of Various Sugars and of KCl on Anaerobic Endogenous Metabolism in Yeast. Yeast 100 mg/ml in 3 ml volume. Sugars, 0.05 M. KCl, where used, 0.02 M. Tris-succinate-tartrate buffer, 0.02 M, pH 5.7. Effects of sorbitol, 3-0-methyl glucose, and galactose are not shown in graph—see text. Results are average of 2 experiments. ffl

FIG. 2. Endogenous and 2DG-Induced Anaerobic Fermentation *versus* pH. Yeast 300 mg in 3 ml suspension; 0.06 M Tris-succinate-tartrate buffer; 0.05 M 2DG where used. Time, 4 hr. Average of 3 experiments.

FIG. 3. Glycogen Depletion *vs* pH for Anaerobic Endogenous Metabolism and 2DG-Induced Metabolism. 150 mg of yeast in 3 ml Tris-succinate-tartrate buffer, 0.04 M, adjusted to appropriate pH. 2DG, 0.05 M. 2 experiments.

FIG. 4. Average Rate of Anaerobic CO_2 Production *vs* Concentration of 2DG. Yeast, 50 mg/ml in 3 ml volume. Tris-succinate-tartrate buffer, 0.06 M, pH 5.6. Time of experiments, 3hr. Results corrected for endogenous rate of CO_2 production at pH 5.6. 3 experiments.

FIG. 5. Net K^+ Ion Uptake in Presence of 2DG or Acetic Acid. 300 mg of yeast in 3 ml suspension; KCl, 0.01 M initially; 0.05 M acetic acid at pH 4.8 or 0.05 M 2DG in 0.02 M Tris-succinate-tartrate of pH 4.8. 1 experiment.

the pH range 3-4, with a marked decline on either side of the maximum. In the presence of 2DG a similar decline in CO_2 production was seen on the acid side of the maximum; but on the side toward alkalinity the 2DG rate, as expected from previous experiments (1), was considerably higher than the endogenous rate at the same pH. Fig. 3 shows glycogen losses (in glucose equivalents) at various values of pH of the medium. Increasing acidity down to pH 2.2 resulted in increasing loss of glycogen in the absence of 2DG. Glycogen loss in the presence of 2DG, however, was consistently high throughout the range of pH used. In addition, the maximum quantities of CO_2 produced (pH 3-4, Fig. 2) were approximately equal in either the presence or absence of 2DG. It was noted in these experiments that relatively high con-

TABLE I. Effects of Various Compounds on Anaerobic Endogenous Metabolism of Yeast.

Compound	CO_2 produced per 130 min	Avg
endogenous	2.7, 3.1	2.9
L-sorbose	3.6, 4.2	3.9
KCl	2.8, 3.4	3.1
L-(-)rhamnose	2.8, 2.8	2.8
d-sorbitol	2.5, 2.9	2.7
galactose	3.3, 3.1	3.2
3-0-methyl-D-glucopyranose	1.9, 3.3	2.7
2DG	11.5, 14.5	13.0
2DG + KCl	11.0, 13.6	12.3

Avg of 2 experiments. 300 mg of yeast in 3 ml suspension containing 0.05 M sugar (0.02 KCl, when used) 0.02 M Tris-succinate-tartrate buffer at pH 5.7.

centrations of buffer could not maintain the pH at the low values, the cells raising the external pH as much as 0.7 (usually less) of a pH unit. Hence pH is expressed as "average pH" in the graph.

CO_2 production vs *2DG concentration at pH 5.7.* Data from three separate experiments involving different concentrations of 2DG are shown in Fig. 4. Because of the variability of CO_2 production from experiment to experiment, the CO_2 produced in the presence of 0.05 M 2DG was assigned an arbitrary value of 100. Amounts of CO_2 for other 2DG concentrations were expressed relative to this arbitrary value. The average half maximal rate occurred (Fig. 4) at about 0.002-0.005 M 2DG.

Inability of 2DG to elicit increased uptake of K^+. Under anaerobic conditions organic acids which penetrate the yeast cell bring about both fermentation and a net uptake of potassium ion added to the medium(7). The K^+ uptake is accompanied by an inhibition of the induced fermentation(7). Fig. 5 shows that no appreciable net uptake of K^+ occurred upon addition of 2DG, whereas acetic acid at the same concentration and pH (4.8) brought about an uptake of K^+.

Discussion. Rothstein(8) postulated the existence of fermentation "bundles" in yeast. This postulate was extended by us(9) to include a transport component in the "bundle." Since we found in the present experiments that CO_2 production or glycogen loss in the presence of 2DG never exceeded the highest endogenous rates at acid pH *externally* (Fig. 2, 3), and since 2DG is phosphorylated by hexokinase to the acidic 2-deoxyglucose-6-phosphate(13,10) with no further conversion of the latter known to occur anaerobically, it appears that the stimulatory effects of 2DG on endogenous metabolism may be related to an increased acidity in a localized region of the cell, perhaps the "bundles."

The CO_2 produced compared to glycogen lost (in glucose equivalents) is in a ratio of 1, a ratio unexpected but nevertheless seen in 2DG-treated cells(1). These effects are different from those produced when carbohydrate stores are dissimilated by *totally penetrating acids* which also appear to produce their effects through acidification(12,7). In the latter case the CO_2/glycogen ratio is 2. The possibility has not been ruled out that the CO_2/glycogen ratio of 1 brought about by 2DG-induced dissimilation of glycogen results from an incomplete (inhibited) fermentation with a resulting accumulation of one or more fermentative intermediates. One such possibility has already been presented(1).

The observations of (a) an increased loss of glycogen from the cells with increasing external acidity (Fig. 3) and (b) an intermediate pH range (pH 3-4) where maximal CO_2 production occurs (Fig 2) are consistent with the possibility that more energy is required to maintain homeostasis as greater external stress (acidity) is encountered. Hence, a greater depletion of the carbohydrate stores would occur to supply the necessary energy. CO_2 production, however, decreased as the lower limits of pH were encountered (Fig. 2), possibly because of the marked sensitivity of fermentation to low external pH(6). It may be speculated that, although glycogen breakdown provides a source of energy to maintain cellular integrity at *very low pH,* in this pH region further metabolism of the glycogen is inhibited by high concentrations of hydrogen.

It had been reported by us(9) and later by Augustin and Hofmann(11) that the binding affinity between 2DG and the carrier for hexose in the yeast cell was expressed by a K_I of 1.2-1.7 $\times$ 10^{-3} M. It is of interest that the concentration of 2DG required to produce half-maximal stimulation of glycogen breakdown is very roughly the same (2-5 $\times$ 10^{-3} M) as the concentration of 2DG required to half-saturate the glucose carrier. These may be coincidental findings, but the possibility is raised that a common locus exists for both the inhibitory effects of 2DG on sugar transport and the stimulatory effects of 2DG on glycogen dissimilation.

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Activity Patterns of Glucose-ATP Phosphotransferases in Livers and Mammary Glands of Virgin, Pregnant, Lactating and Postlactating Mice.* (30809)

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Recent studies have shown that rat(1-7) and guinea pig livers(8) and rat epididymal fat pads(9) contain 2 molecularly distinct glucose-ATP phosphotransferases. These two enzymes have been separated from rat liver by ammonium sulfate fractionation(1,5), and from epididymal fat pads by starch-gel electrophoresis(9). One of them, glucokinase, has a low affinity for glucose, with a K_m of 10 mM; the other, hexokinase, has a high affinity for glucose, with a K_m of 0.01 mM (1). The introduction by Viñuela *et al*(1) of a simple method for assay of these 2 enzymes has facilitated study of their activities in crude homogenate fractions.

The activity of the 2 glucose-ATP phosphotransferases in the particle-free supernatant fraction (100,000 $\times$ *g* for 60 minutes) of rat liver homogenates accounts for practically all of the glucose phosphorylating activity of the whole liver homogenate(5,10). In other tissues, such as brain(5,11), small intestine(5), heart(5), thyroid(12) and lactating rat mammary gland(5,13), glucose phosphorylation has been observed in both the particulate and the particle-free supernatant fraction; but in both cellular locations in these tissues, hexokinase appeared to be the only enzyme concerned with this phosphorylation(5,12,13).

It is well established that the activity of glucokinase in liver, but not that of hepatic hexokinase, is altered by dietary changes, diabetes and insulin administration(1-8). Since other hepatic enzymes that adapt to changes in diet also change with lactation (14,15), we became interested in studying the patterns of activity of glucose-ATP phosphotransferases in the livers and the mammary glands of virgin, pregnant, lactating and postlactating mice.

Experimental. Female mice of the C_3H strain that had been raised and maintained on a nutritionally adequate diet (Purina Lab Chow) were used. Livers and mammary glands were excised from mice in the virgin state; in the prepartum state (17th and 18th day of gestation); in the postpartum state while each mouse was suckling 6 pups (17th and 18th day of lactation); and in the postlactating state (2 days after the pups had been weaned) during which the glands were undergoing regression. The mice were killed by cervical fracture, and their mammary glands and livers were rapidly excised and

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