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The inhibitory effect of 2-deoxy-D-glucose (2-DG) upon the metabolism of glucose and fructose by proliferating cells of Saccharomyces cerevisiae (OSU strain 2) has been examined. By the use of radiotracer methods, particularly radiorespirometric techniques, it has been possible to demonstrate that the rate of hexose assimilation by yeast cells is reduced significantly when 2-DG is present in the incubation medium. This can be attributed to the inhibitory effect of 2-DG upon permeation of either glucose or fructose into yeast cells. It appears that, when 2-DG and substrate hexose are in the medium, 2-DG is preferentially permitted into the cells and converted to 2-DG-6-P, thereby depleting most of the available ATP, which is of vital importance to the transport of hexoses into yeast cells.

When 2-DG is present in the medium at lower concentrations,

despite the fact that the rate of hexose transport is reduced, complete utilization of substrate hexose, administered in a single dose, can still be realized. By examining data of radiorespirometric experiments, one finds that substrate glucose or fructose has been routed to a relatively greater extent into catabolic pathways in the presence of 2-DG, and participation of the anabolic pathway is correspondingly reduced. However, it does not appear that the relative participation of individual pathways, for that portion of substrate glucose engaged in catabolism, has been altered to any great extent. When fructose is used as the sole carbon source, relative participation of catabolic pathways has been altered by the presence of 2-DG in the medium. This has been attributed to the inhibitory effect of 2-DG exerted on the enzymic reaction, catalyzed by phosphohexoisomerase, which is responsible for conversion of fructose-6-P to glucose-6-P. However, at low substrate levels of fructose, in the absence of 2-DG, the relative participation of catabolic pathways is also altered, presumably due to a necessity for saturation of glycolytic enzymes, before equilibration between fructose-6-P and glucose-6-P is achieved.

THE EFFECT OF 2-DEOXYGLUCOSE ON HEXOSE METABOLISM IN SACCHAROMYCES CEREVISIAE

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Typed by Joanne Wenstrom for Charles Wayland Conrad

TO MY WIFE

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INTRODUCTION

The inhibitory effect of the compound, 2-deoxy-D-glucose (2-DG) has been extensively studied, particularly relative to its therapeutic value in carcinogenesis. Early studies involved primarily the use of manometric techniques; however, recent works have made use of radiotracer methods. The effects of 2-DG upon carbohydrate metabolism have been noted in various biological systems, including microorganisms, intact animals, tissue homogenates, human cell cultures, and enzyme preparations. Whereas many of the findings have been verified, there still exist some conflicting reports in the literature.

Early workers in the field, Cramer and Woodward (1952) noted that with Saccharomyces cerevisiae cells the rate of anaerobic fermentation was reduced immediately upon addition of 2-DG to cell suspensions in concentration ratios (2-DG:glucose) of either 1:1 or 5:1. In sonicated cells, these authors noted, no immediate effect of 2-DG upon anaerobic fermentation could be demonstrated. Measurements of Km values of "cell wall enzyme" revealed that the affinity of this enzyme system for 2-DG is far greater than that for glucose, and hence displays the effect in the nature of a competitive inhibition.

The authors also demonstrated that 2-DG could be readily phosphorylated by hexokinase isolated from yeast; however, Woodward and Hudson (1955) later reported that glucose served as a far better substrate for hexokinase, in comparison to 2-DG.

Kipnis and Cori (1959) isolated 2-DG-6-phosphate (2-DG-6-P), but not 2-DG, from homogenized rat diaphragm cells previously incubated with 2-DG. They concluded that the rate of 2-DG phosphorylation was equal to or greater than that for the permeability of 2-DG into rat diaphragm cells. They also reported that transport and phosphorylation of 2-DG into rat diaphragm cells ceased when the concentration of 2-DG-6-P within the cells reached 0.02 M. When rat diaphragm cells were incubated with 2-DG along with either mannose or glucose, 2-DG was found to accumulate within the cells, while the level of 2-DG-6-P was observed to be much lower than in those cells which had been incubated with 2-DG as the only hexose in the medium.

In studies with hexokinase isolated from HeLa cells, Barban and Schulze (1961) could not demonstrate inhibition of the enzyme by either 2-DG or 2-DG-6-P when glucose was used as substrate. However, the rate of fructose phosphorylation was found to be reduced by 2-DG or 2-DG-6-P. On the other hand, Scharff (1961) showed inhibition of oxidation of glucose by 2-DG, but not by 2-DG-6-P, in both "acetone-dried" and resting yeast cells. Fermentation of

glucose in two Trypanosoma species was found to be inhibited by 2-DG, as reported by Seed, Baquero, and Duda (1964).

Work by Barban and Schulze (1961) showed that the growth of HeLa and human embryonic cells was inhibited by 2-DG at a concentration of 5 mM. It was further noted by these authors that the growth inhibition could be reversed if the cells, incubated with 2-DG up to three days, were separated from the medium, washed free of 2-DG, and resuspended in 2-DG-free cultural medium.

Heredia, de la Fuente, and Sols (1963), by measuring simultaneously the rates of growth and fermentation of yeast cells, found that the growth rate was reduced by the presence of 2-DG to a far greater extent in comparison with the rate of fermentation when the concentration ratio of glucose:2-DG was maintained at 5:1. With yeast cell extracts these authors demonstrated that the rate of galactose fermentation was reduced by 2-DG and interpreted their findings as due to the formation of a complex of 2-DG and uridine diphosphate (UDP), possibly an irreversible process.

Scharff and Montgomery (1965) demonstrated that, in resting yeast cells, endogenous glycogen was depleted when 2-DG was present in the medium. They further showed that one mole of CO_2 was evolved from these cells for every glucose unit derived from endogenous glycogen. Megnet (1965) found that Schizosaccharomyces pombe had very fragile cell walls when these cells had been grown

in the presence of 2-DG.

More recently, Biely and Bauer (1967) identified the presence of 2-DG-6-P, 2-DG-1,6-di P, 2-DG-1-P, and UDP-2-DG by means of paper chromatography, in lysed yeast cells previously incubated with 2-DG.

There are indications that 2-DG inhibits phosphohexoisomerase in animal tissues. Wick et al. (1956) showed that the enzymic reaction for the conversion of glucose-6-phosphate (glucose-6-P) to fructose-6-phosphate (fructose-6-P) in homogenized rat kidney preparations was inhibited by the presence of 2-DG. Similarly, Barban and Schulze (1961) showed that the activity of purified phosphohexoisomerase isolated from HeLa cells was inhibited by 2-DG-6-P, relative to the conversion of fructose-6-P to glucose-6-P. These workers also showed that 2-DG may exert an inhibitory effect upon the reaction catalyzed by glucose-6-P dehydrogenase. This conclusion was drawn from the observation that with dehydrogenase preparations from 2-DG-treated HeLa cells, the rate of nicotinamide adenosine dinucleotide phosphate (NADP) disappearance was noticeably reduced in comparison to that of preparations from cells grown in the absence of 2-DG.

Wick et al. (1956) indicated that 2-DG did not exert an inhibitory effect on enzymic reactions associated with the tricarboxylic acid (TCA) cycle in nephrectomized rabbits as evidenced by the

finding that the metabolism of acetate-1-^{14}C was not altered by the administration of 2-DG into the animals.

The literature cited illustrates well the complexity underlying the effects of 2-DG on hexose metabolism. Oxidation of hexoses appears to be slowed by inhibition at the sites of hexose permeation and phosphorylation. Growth of cells has also been seen to be inhibited by 2-DG, perhaps as a result of formation of a complex of UDP-2-DG; such a complex would make uridine nucleotides unavailable as catalysts for enzymatic reactions important in the biosynthesis of glycogen. Other enzymatic reactions which have been shown to be affected by 2-DG are those catalyzed by glucose-6-P dehydrogenase and phosphohexoisomerase.

The present work is designed to elucidate in more detail the inhibitory effect of 2-DG upon hexose metabolism in Saccharomyces cerevisiae (OSU Strain 2).

MATERIALS AND METHODS

Culture Conditions

Saccharomyces cerevisiae (OSU Strain 2), previously isolated from Fleischmann's bakers' yeast (Salman, 1966), was maintained on agar slants. Experimental cultures were then inoculated aseptically into flasks containing 100 ml of the incubation medium described by Salman (1966). Cells were incubated under aerobic conditions at 30°C on a rotary shaker prior to experiments.

Radiochemical Substrates

Specifically labeled compounds used in this work were glucose, labeled with ^{14}C at C-1, C-2, C-3(4), or C-6; fructose, labeled with ^{14}C at C-1 or C-6; and 2-DG-1-^{14}C, all of which were obtained from New England Nuclear Corporation.

Radiorespirometric Experiments

Radiorespirometric studies involving single-dose substrate administration were carried out according to the methods described by Wang (1967). Each 50 ml respirometer flask contained 10 mg of yeast cells suspended in 10 ml of incubation medium containing no glucose. Flasks were placed in a water bath at 30°C and aerated with

air at the rate of 40 ml/minute. Whenever 2-DG was involved in the experiment, the compound was added to the medium at the time of preparing the cell suspension. The cell suspension was equilibrated with the experimental environment for a period of 90 minutes before specifically labeled hexoses were tipped into the cell suspension. Respiratory $^{14}CO_2$ was trapped in a solution of ethanolamine:ethanol:: 1:2 (v:v), and the resulting solution was prepared for liquid scintillation counting according to the method of Wang (1967). At the end of the experiment, cells and medium were separated by centrifugation and counted in thixotropic gel as described by White and Helf (1956). The efficiency of liquid scintillation counting was determined by the use of internal standards, and all counting was done to a standard deviation of one percent.

In high resolution radiorespirometric studies involving continuous feeding of the substrate to cells, shake cultures of yeast were directly used for experiments, as described by Jacobsen (1968). With this type of experiment, glucose-3(4)-^{14}C and 2-DG were added to the radiorespirometer flask by syringe. Respiratory $^{14}CO_2$ was measured with the high-resolution radiorespirometric apparatus also described by Jacobsen (1968).

Determination of the Amount of Substrate Glucose Converted to Di- and Polysaccharides

Cells were incubated as in the radiorespirometric experiments involving single dose substrate administration; substrate used was 15 mg glucose-3(4)-^{14}C. At the end of the experiment, cells were separated from the medium by centrifugation, and the radioactivity of an aliquot of the medium fraction, suspended in thixotropic gel, was determined by liquid scintillation counting. From the cells, glycogen, trehalose, mannan, and glucan were separated according to the method of Berke and Rothstein (1957). Other cellular constituents were removed from the trehalose fraction according to the column chromatographic procedures outlined by Whistler and Durso (1950). Aliquots of aqueous solutions of isolated glycogen, trehalose, and mannan fractions were suspended in thixotropic gel for liquid scintillation counting. The glucan fraction, collected and dried on a millipore filter, was combusted according to the Schöniger method (Wang and Willis, 1965). The radioactivity of $^{14}CO_2$ derived from the combusted sample, trapped in ethanolamine-ethanol solution, was then counted by liquid scintillation techniques.

Determination of Glucose in Medium

Measurement of glucose present in the medium was done by first extracting with a syringe 0.5 ml or 1.0 ml of a cell suspension

from a radiorespirometer flask prepared as in the previous section, removing the cells from the medium by use of a millipore filter (0.45 μ pore size) apparatus, and determining colorimetrically the amount of glucose in the medium, by use of the Glucostat method (Worthington, 1963).

Determination of 2-DG-1-^{14}C in Medium and Cells

Cells were incubated in radiorespirometer flasks in the presence of 2-DG-1-^{14}C. A syringe was used to extract 0.5 ml or 1.0 ml of the cell suspension; cells in this aliquot were removed from the medium by use of millipore filters (0.45 μ pore size). Radioactivity in the medium was determined by liquid scintillation counting using the thixotropic gel method. Cells attached to millipore filters were dried and combusted according to the Schöniger method.

RESULTS AND DISCUSSION

Catabolic Pathways of Glucose and Fructose in Yeast Cells

In order to assess the effect of 2-DG upon carbohydrate metabolism in yeast cells, it is essential to first examine the catabolic pathways of glucose and fructose in the absence of 2-DG. Such a study has been carried out by means of radiorespirometry.

Radiorespirometric patterns for yeast cells utilizing 15 mg of glucose, labeled with ^{14}C at C-1, C-2, C-3(4), or C-6, are shown in Figure 1. These findings verified those reported by Salman (1966). It is noted that the time-course for complete substrate utilization was four hours after substrate administration. Similar radiorespirometric results for yeast cells utilizing 15 mg of fructose are presented in Figure 2.

Effect of Concentration of 2-DG on Glucose Metabolism

It has been reported (Barban and Schulze, 1961; Scharff, 1961; Heredia, de la Fuente, and Sols, 1963; Megnet, 1965) that 2-DG, in high concentrations, inhibits the growth and respiration of cells; however, the effect of 2-DG at lower concentrations, if any, has not been extensively studied. Radiorespirometric patterns from cells grown in medium containing 15 mg of glucose-1-^{14}C and various

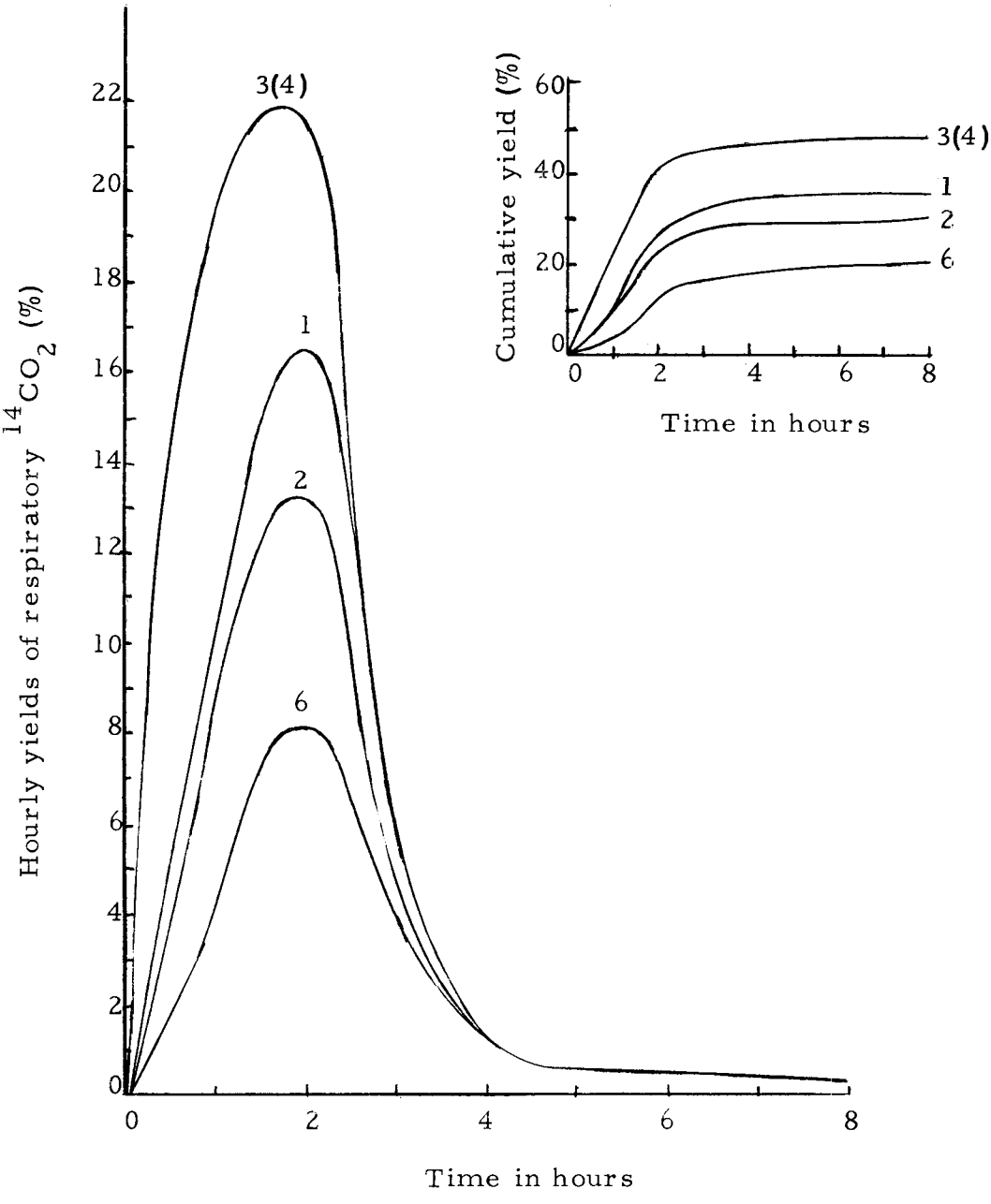

Figure 1. Radiorespirometric patterns for utilization of glucose by Saccharomyces cerevisiae cells, single dose experiment. Experimental conditions: cell weight, 10 mg; volume of medium, 10 ml; substrate, 15 mg glucose. Numerals refer to position of carbon atom of glucose labeled with ^{14}C.

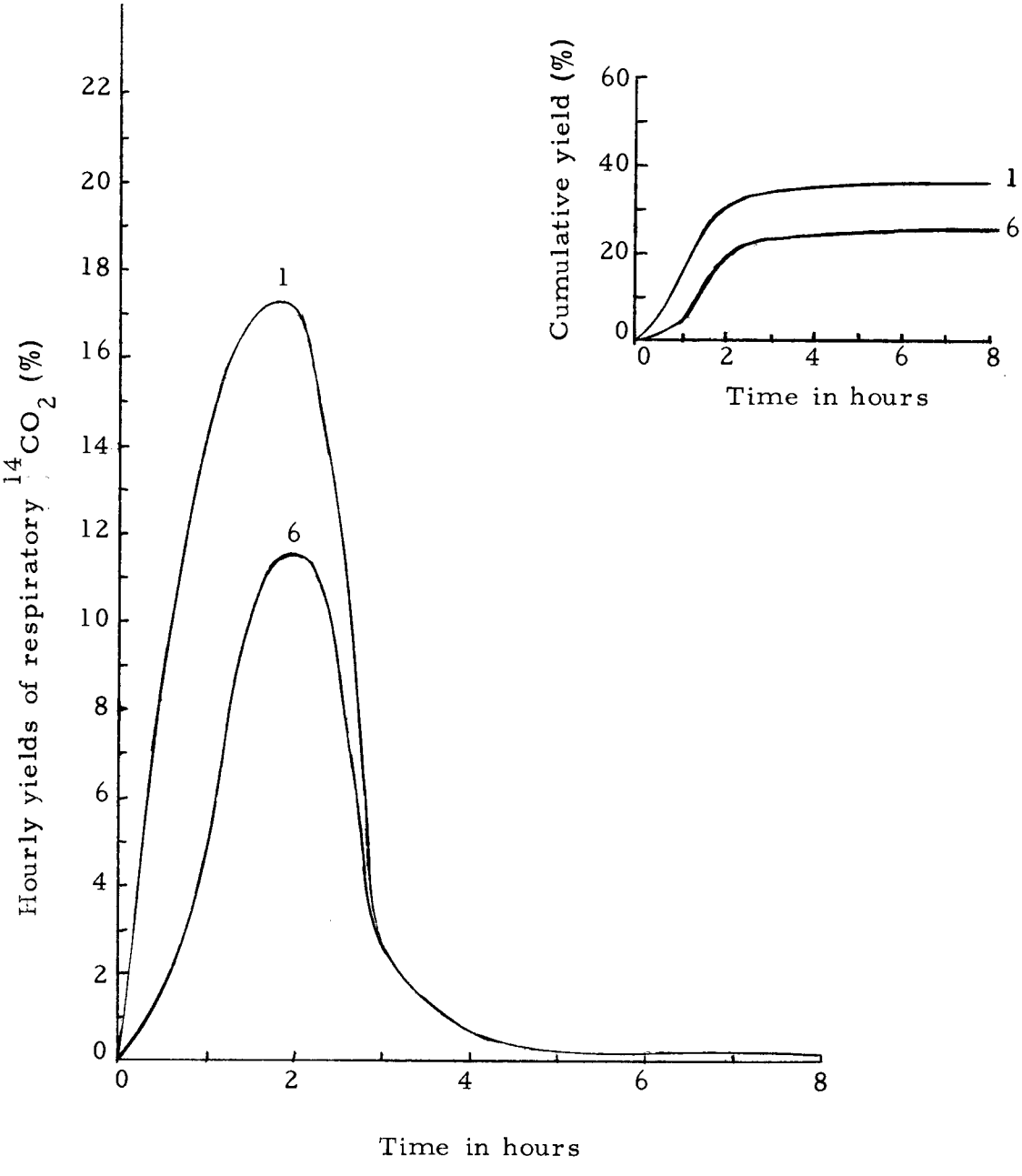

Figure 2. Radiorespirometric patterns for utilization of fructose by Saccharomyces cerevisiae cells, single dose experiment. Experimental conditions: cell weight, 10 mg; volume of medium, 10 ml; substrate, 15 mg fructose. Numerals refer to position of carbon atom of fructose labeled with ^{14}C.

amounts of 2-DG are shown in Figure 3. It is immediately obvious that as the concentration of 2-DG increased, the rate for substrate assimilation decreased accordingly. However, complete substrate utilization was realized even when 2-DG concentration was as high as 1.64 mg (0.01 mmole)/10 ml incubation medium.

Cumulative yields of $^{14}CO_2$, at the end of the time-course for complete substrate utilization, from yeast cells metabolizing 15 mg of glucose-1-^{14}C or fructose-1-^{14}C, in the presence of various amounts of 2-DG, are shown in Table 1. It is of great interest to note that, although the rate of glucose catabolism was evidently reduced in the presence of even very small amounts of 2-DG, the cumulative yields of $^{14}CO_2$ from C-1 of glucose, at the time when the substrate had been completely utilized, were greater than those observed in the control experiments. It appears that, when the concentration of 2-DG was between 0.01 mmole and 0.05 mmole/10 ml incubation medium, the effect of 2-DG upon catabolism was most pronounced, insofar as production of respiratory $^{14}CO_2$ was concerned. When the concentration of 2-DG reached 0.10 mmole/10 ml, the cumulative yield of respiratory $^{14}CO_2$ was only slightly greater than that observed in the control experiment. This fact indicates that 2-DG may have incurred other effects to yeast physiology, since it is known that 2-DG in high concentrations inhibits the growth and respiration of cells. In the case of fructose

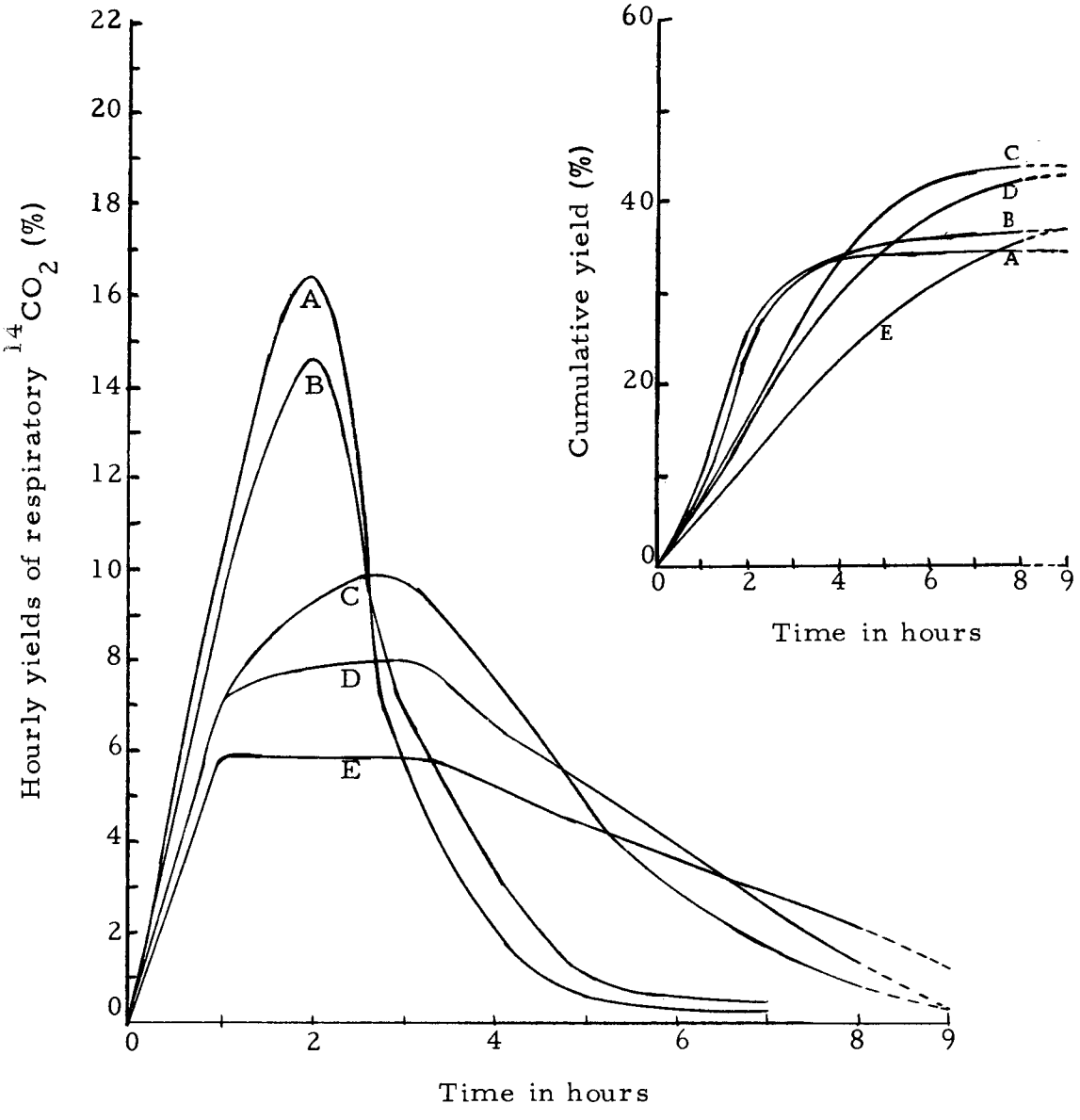

Figure 3. Radiorespirometric patterns for utilization of glucose by Saccharomyces cerevisiae cells in the presence of various amounts of 2-DG, single dose experiment. Experimental conditions: cell weight, 10 mg; volume of medium, 10 ml; substrate, 15 mg glucose-1-^{14}C. The following amounts of 2-DG were added to the incubation medium: A -- 0.00 mg; B -- 0.16 mg (0.001 mmole); C -- 1.64 mg (0.01 mmole); D -- 8.20 mg (0.05 mmole); E -- 16.4 mg (0.10 mmole).

Table 1. The inhibitory effect of 2-DG upon catabolism of glucose-1-^{14}C or fructose-1-^{14}C by Saccharomyces cerevisiae cells.

Substrate	2-DG administered		Yield of $^{14}CO_2$
	mg	mmoles	%
Glucose-1-^{14}C	0.00	0.000	35
	0.16	0.001	37
	1.64	0.01	44
	8.20	0.05	43
	16.4	0.10	37
Fructose-1-^{14}C	0.00	0.00	37
	1.64	0.01	43

Experimental conditions: cell weight, 10 mg; volume of medium, 10 ml; substrate, 15 mg glucose-1-^{14}C or fructose-1-^{14}C; 2-DG, various amounts as shown.

utilization, findings similar to those of glucose experiments were observed when 2-DG concentration in the incubation medium was 1.64 mg (0.01 mmole)/10 ml.

Effects of 2-DG on Catabolic Pathways of Hexoses in Yeast Cells

In view of the findings shown in Table 1, the effects of 2-DG at the concentration of 1.64 mg (0.01 mmole)/10 ml medium upon catabolic pathways of hexoses in yeast cells have been examined in a series of radiorespirometric experiments. Substrates used were 15 mg of glucose, labeled with ^{14}C at C-1, C-2, C-3(4), or C-6, or 15 mg fructose, labeled at C-1 or C-6. It is noted that the time-courses for complete substrate utilization were eight hours and ten hours in the glucose and fructose experiments, respectively. This is to be compared with a time-course of four hours observed in similar experiments in the absence of 2-DG. The findings of this series of experiments are shown in Figures 4 and 5, and the substrate inventory for these experiments, as well as that of the control experiments, is given in Table 2.

It is obvious from these findings that, although the rate of assimilation of either glucose or fructose by yeast cells was reduced, conversions of all the labeled carbon atoms of either substrate to respiratory $^{14}CO_2$ were enhanced, resulting in significantly greater yields at the end of the time-course for complete

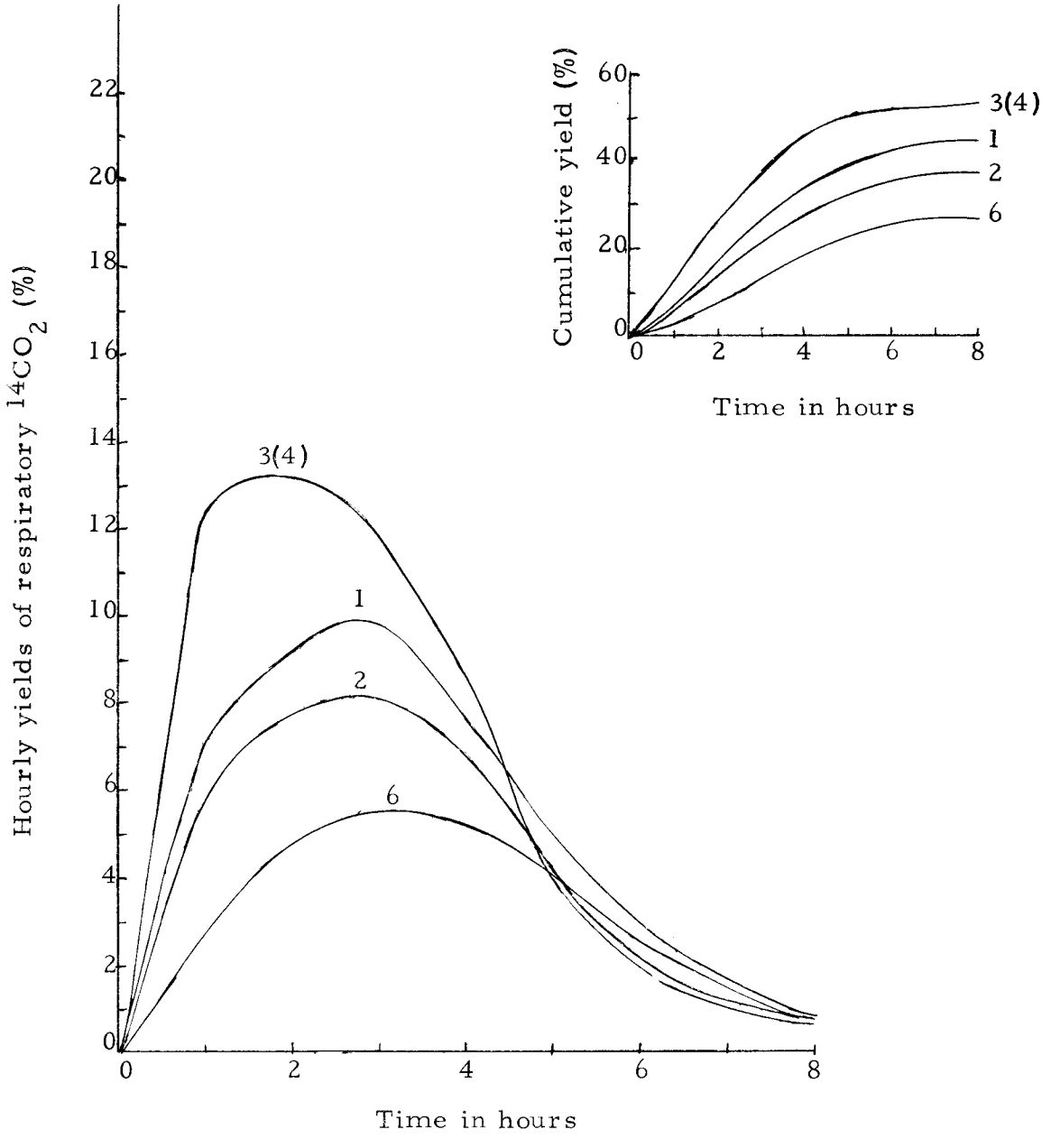

Figure 4. Radiorespirometric patterns for utilization of glucose by Saccharomyces cerevisiae cells in the presence of 1.64 mg 2-DG, single dose experiment. Experimental conditions: cell weight, 10 mg; volume of medium, 10 ml; substrate, 15 mg glucose. Numerals refer to position of carbon atom of glucose labeled with ^{14}C.

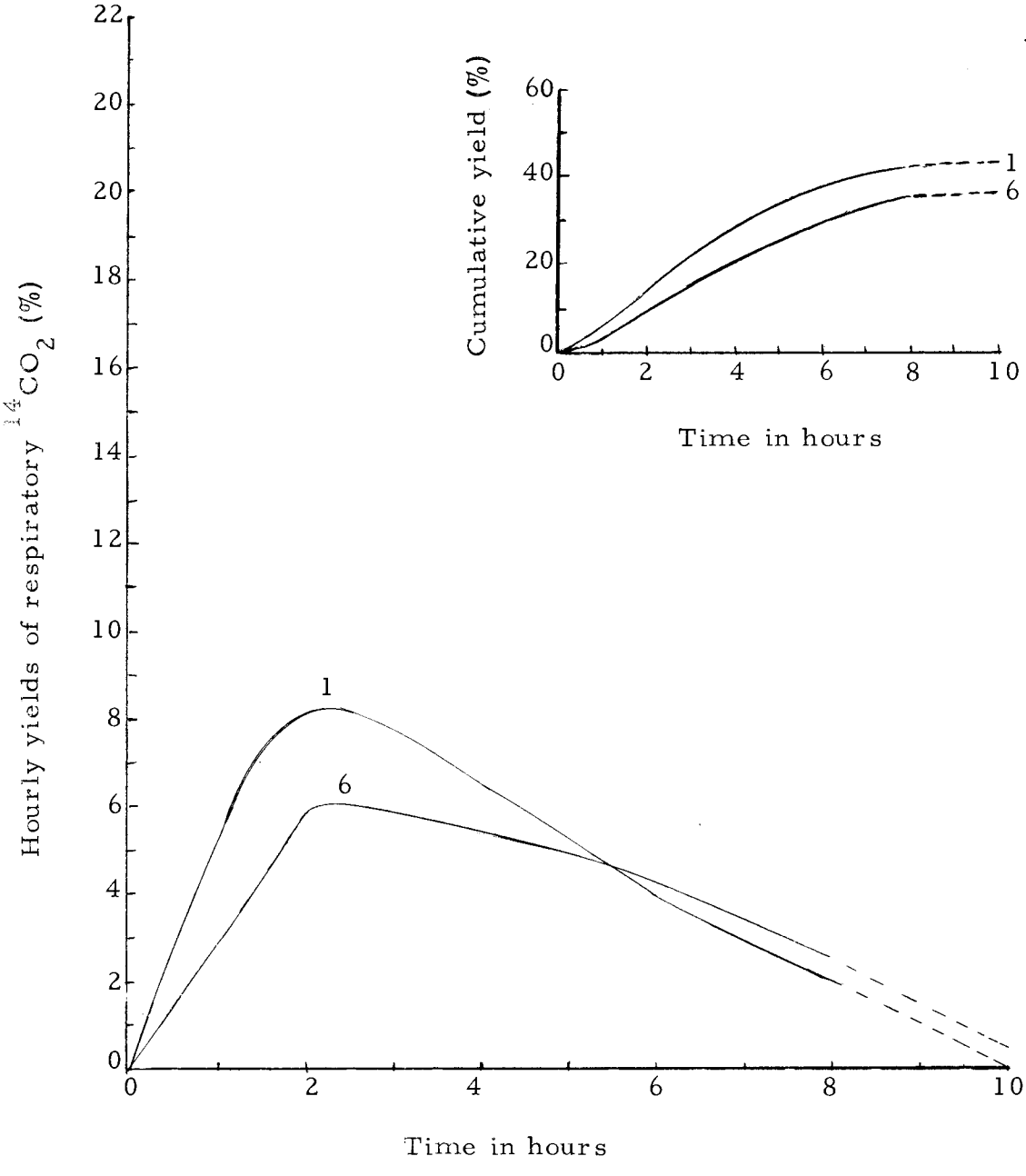

Figure 5. Radiorespirometric patterns for utilization of fructose by Saccharomyces cerevisiae cells in the presence of 1.64 mg 2-DG, single dose experiment. Experimental conditions: cell weight, 10 mg; volume of medium, 10 ml; substrate, 15 mg fructose. Numerals refer to position of carbon atom of fructose labeled with ^{14}C.

Table 2. Fate of substrate glucose and fructose metabolized by Saccharomyces cerevisiae cells in the presence or absence of 2-DG.

Substrate	CO_2		Medium		Cells		Total	
	2-DG	control	2-DG	control	2-DG	control	2-DG	control
Glucose-1	44	35	16	14	34	44	94	93
Glucose-2	37	30	15	12	39	50	91	92
Glucose-3(4)	54	47	7	5	26	35	87	87
Glucose-6	27	18	14	10	43	56	84	84
Fructose-1	43	37	16	9	30	46	89	92
Fructose-6	37	25	14	7	40	64	91	96

Experimental conditions: cell weight, 10 mg; volume of medium, 10 ml; substrate, 15 mg glucose or fructose labeled with ^{14}C at carbon position indicated; 2-DG, 1.64 mg in 2-DG experiments, none in control experiments. Values expressed as percent of administered substrate.

substrate utilization. It is also noted that the incorporations of ^{14}C labeled carbon atoms of either glucose or fructose into cellular constituents were noticeably reduced when 2-DG was present in the medium. These findings are interpreted as reflecting that 2-DG exerts an effect on the relative participation of anabolic and catabolic pathways, or on the catabolic pathways alone.

In order to estimate the relative participation of catabolic pathways in yeast cells, the method described by Wang and Krackov (1962) can be readily used. The equations derived by these authors are given in the following:

$$Gp = \frac{G_1 - (G_6 - A_6 Gp)}{G_T - G_{T'}} \qquad (1)$$

$$Ge = 1 - Gp \qquad (2)$$

where: Gp = fraction of glucose catabolized via the pentose phosphate pathway

Ge = fraction of glucose catabolized via the glycolytic pathway

G_1 = cumulative yield, at the end of the time-course for complete substrate utilization, of respiratory $^{14}CO_2$, as fraction of administered glucose-1-^{14}C

G_6 = cumulative yield, at the end of the time-course for complete substrate utilization, of respiratory $^{14}CO_2$, as fraction of administered glucose-6-^{14}C

A_6 = cumulative yield, at the end of the time-course for complete substrate utilization, of respiratory $^{14}CO_2$, as fraction of administered gluconate-6-^{14}C

G_T = total activity of labeled substrate, taken as unity

$G_{T'}$ = fraction of administered glucose which undergoes anabolism.

Equation 1 can be simplified to:

$$Gp = \frac{G_1 - G_6}{G_T - G_{T'} - A_6}. \qquad (3)$$

Since yeast cells cannot utilize gluconate, the value of A_6 cannot be ascertained in this case. Nevertheless, the term G_6 may be substituted for A_6, as suggested by the CO_2 yield method (Katz and Wood, 1963), and hence Equation 3 can be expressed as follows:

$$Gp = \frac{G_1 - G_6}{G_T - G_{T'} - G_6}. \qquad (4)$$

A method for the estimation of relative participation of catabolic pathways for fructose utilization has not yet been developed; however, if one visualizes that fructose, upon entry into yeast

cells, is converted to fructose-6-P via phosphorylation, it is justifiable to consider that fructose-6-P may assume a very rapid isotopic equilibrium with glucose-6-P (Landau et al., 1964; Landau and Katz, 1964). If such is the case, it is possible to make use of the equations derived for estimation of glucose pathways to estimate the relative participation of catabolic pathways for fructose utilization. Such equations are given in the following:

$$Fp = \frac{F_1 - F_6}{F_T - F_{T'} - F_6} \qquad (5)$$

$$Fe = 1 - Fp, \qquad (6)$$

where Fp, Fe, F_1, F_6, F_T, and $F_{T'}$ are analogous in definition to the terms Gp, Ge, G_1, G_6, G_T, and $G_{T'}$ respectively.

It is evident that in applying Equations 2, 4, 5, and 6 for pathway estimations, it is necessary to have information on the values of $G_{T'}$ and $F_{T'}$. The assessment of the amount of substrate hexose that has engaged in anabolic activities is a difficult task. It has been suggested by Salman (1966) that anabolism may account for a significant portion of the total metabolism of hexoses in yeast. In order to determine the value of $G_{T'}$ in yeast cells grown in the presence of 1.64 mg of 2-DG or in the absence of 2-DG, the ^{14}C labeling of endogenous carbohydrates isolated from yeast cells

grown in medium containing 15 mg of glucose-3(4)-^{14}C was studied. The substrate, glucose-3(4)-^{14}C, was used for the reason that C-3 and C-4 of glucose are extensively converted to respiratory CO_2 when glucose is catabolized via all known pathways. In contrast, these two carbon atoms of glucose are likely to be preserved when glucose is engaged in anabolic pathways. The amounts of endogenous carbohydrates derived from glucose-3(4)-^{14}C, expressed as percent of administered substrate activity, are presented in Table 3.

Thus, one finds that approximately 26 percent of the administered glucose, represented by glucose-3(4)-^{14}C, has been incorporated into di- and polysaccharides in cells grown in the absence of 2-DG, in comparison to 14 percent in cells grown in the presence of 1.64 mg (0.01 mmole) of 2-DG. Fructose-3(4)-^{14}C is not readily available for a similar experiment; however, if one assumes that the metabolic behavior of fructose in yeast is similar to that of glucose, information collected in the glucose-3(4)-^{14}C experiment can be applied, by approximation, to the analysis of data involving fructose as substrate. The observed inhibitory effect of 2-DG upon anabolic pathways of hexoses in yeast is presumably due to the conjugation of 2-DG-1-P with UTP. Previously, Biely and Bauer (1966; 1967) reported that UDP-2-DG is formed in yeast.

The information gained in the glucose-3(4)-^{14}C experiment, relative to the extent of substrate hexose engaged in anabolic

Table 3. Inhibitory effect of 2-DG upon anabolic pathways in Saccharomyces cerevisiae cells metabolizing glucose-3(4)-^{14}C.

Fraction	Percent of administered ^{14}C in fraction	
	2-DG	control
Glycogen	11	20
Trehalose	2	4
Mannan	< 1	1
Glucan	< 1	1
Total	14	26

Experimental conditions: cell weight, 10 mg; volume of medium, 10 ml; substrate, 15 mg glucose-3(4)-^{14}C; 2-DG, 1.64 mg in 2-DG experiments, none in control experiments.

pathways, i. e. the values for $G_{T'}$ and $F_{T'}$, makes it possible to estimate the relative participation of catabolic pathways for glucose and fructose and the effect of 2-DG, if any, upon catabolic mechanisms in yeast cells. The results calculated on the basis of Equations 2, 4, 5, and 6 are given in Table 4, making use of yield data observed in experiments with hexose concentration of 15 mg/10 ml incubation medium.

Several interesting conclusions can be drawn from the information given in Table 4. First, of cells under normal physiological conditions, relative participation of the glycolytic and pentose phosphate pathways for glucose utilization is slightly different from that for fructose utilization. The glycolytic pathway assumes a slightly more important role in the catabolism of fructose. This effect may reflect that fructose-6-P derived from substrate fructose is not in perfect isotopic equilibrium with glucose-6-P. Of more interest is the finding that, whereas 2-DG does not exert a significant effect upon the relative participation of catabolic pathways for glucose utilization, the fraction of fructose routed through the pentose phosphate pathway has been significantly reduced, and the participation of the glycolytic pathway has been correspondingly enhanced, when 2-DG is present in the medium. This finding reveals that 2-DG may exert an inhibitory effect upon the enzyme responsible for conversion of fructose-6-P to glucose-6-P, phosphohexoisomerase.

Table 4. Inhibitory effect of 2-DG upon relative participation of catabolic pathways for the utilization of glucose and fructose by Saccharomyces cerevisiae cells.

Glucose experiment			Fructose experiment		
Expression	2-DG	control	Expression	2-DG	control
G_1	.44	.35	F_1	.42	.36
G_6	.27	.18	F_6	.35	.24
G_T	1.00	1.00	F_T	1.00	1.00
$G_{T'}$	.14	.26	$F_{T'}$	.14	.26
Gp (Equation 4)	.28	.29	Fp (Equation 5)	.14	.24
Ge (Equation 2)	.72	.71	Fe (Equation 6)	.86	.76

Experimental conditions: same as those described for Table 2. Values expressed as fraction of administered substrate.

In order to verify the foregoing conclusion, a series of experiments has been carried out, with various amounts (10 mg to 30 mg) of glucose or fructose, labeled with ^{14}C at C-1 or C-6, used as substrate. The results obtained, along with calculated values of Gp, Ge, Fp, and Fe, are shown in Table 5. These results verify that the value of Gp is not dependent upon the presence of 2-DG, nor upon the amount of glucose present in the medium. Also verified is the previous observation that the presence of 2-DG causes a decrease in the value of Fp from that observed in the control experiments. It is also of interest to note that, as the amount of substrate fructose is increased, a corresponding increase is seen in the value of Fp until it equals the value of Gp. This observation leads one to the conclusion that a certain level of fructose-6-P, derived from substrate fructose, must be reached within the cell in order to saturate the enzyme phosphofructokinase; otherwise, the fructose-6-P is converted to fructose-1, 6-diP preferentially, without ever having a chance to equilibrate with glucose-6-P via the phosphohexoisomerase reaction. It is visualized that the observed resemblance of catabolic behavior of substrate fructose and glucose at high substrate level, i. e. 30 mg/10 ml medium, may indicate that fructose-6-P is in isotopic equilibrium with glucose-6-P.

Table 5. Inhibitory effect of 2-DG upon relative participation of catabolic pathways for the utilization of various amounts of glucose and fructose by Saccharomyces cerevisiae cells.

Expression	10 mg		15 mg		30 mg	
	2-DG	control	2-DG	control	2-DG	control
G_1	.43	.34	.44	.35	---	.36
G_6	.26	.17	.27	.18	---	.18
G_T	1.00	1.00	1.00	1.00	---	1.00
$G_{T'}$	.14	.26	.14	.26	---	.26
Gp (Equation 4)	.28	.28	.28	.29	---	.31
Ge (Equation 2)	.72	.72	.72	.71	---	.69
F_1	.49	.33	.42	.36	.52	.40
F_6	.42	.24	.35	.24	.44	.23
F_T	1.00	1.00	1.00	1.00	1.00	1.00
$F_{T'}$	.14	.26	.14	.26	.14	.26
Fp (Equation 5)	.16	.18	.14	.24	.19	.33
Fe (Equation 6)	.84	.82	.86	.76	.81	.67

Experimental conditions: cell weight, 10 mg; volume of medium, 10 ml; substrate, indicated amounts of glucose or fructose labeled with ^{14}C at C-1 or C-6; 2-DG, 1.64 mg in 2-DG experiments, none in control experiments.

Effect of 2-DG Upon Transport of Hexoses

As indicated by results obtained in radiorespirometric experiments, using ^{14}C labeled glucose as substrate (Figures 3 and 4), 2-DG exerts an effect in reducing the rate of glucose utilization. This effect is visualized as due to an inhibition of 2-DG upon such processes as glucose permeation or phosphorylation. In order to verify this contention, the rate of assimilation of 15 mg of unlabeled glucose in the presence and absence of 2-DG has been examined by periodic determination of the amount of substrate glucose in the incubation medium by means of the Glucostat method (Worthington, 1963). The results of this experiment, shown in Figure 6, support the hypothesis that permeation of glucose into yeast cells has been hindered by the presence of 2-DG.

The case is further examined by the use of high-resolution radiorespirometry (Jacobsen, 1968), since the newly-developed method permits one to gain more insight on the kinetics of the inhibitory effect of 2-DG. In this series of experiments, unperturbed shake cultures of yeast cells were used. When yeast cells reached the late logarithmic phase of growth, the glucose concentration in the medium was approximately 60 mg (0. 33 mmole)/10 ml incubation medium. A tracer amount of glucose-3(4)-^{14}C, with high specific activity, was added to the culture, and 2-DG of various amounts was

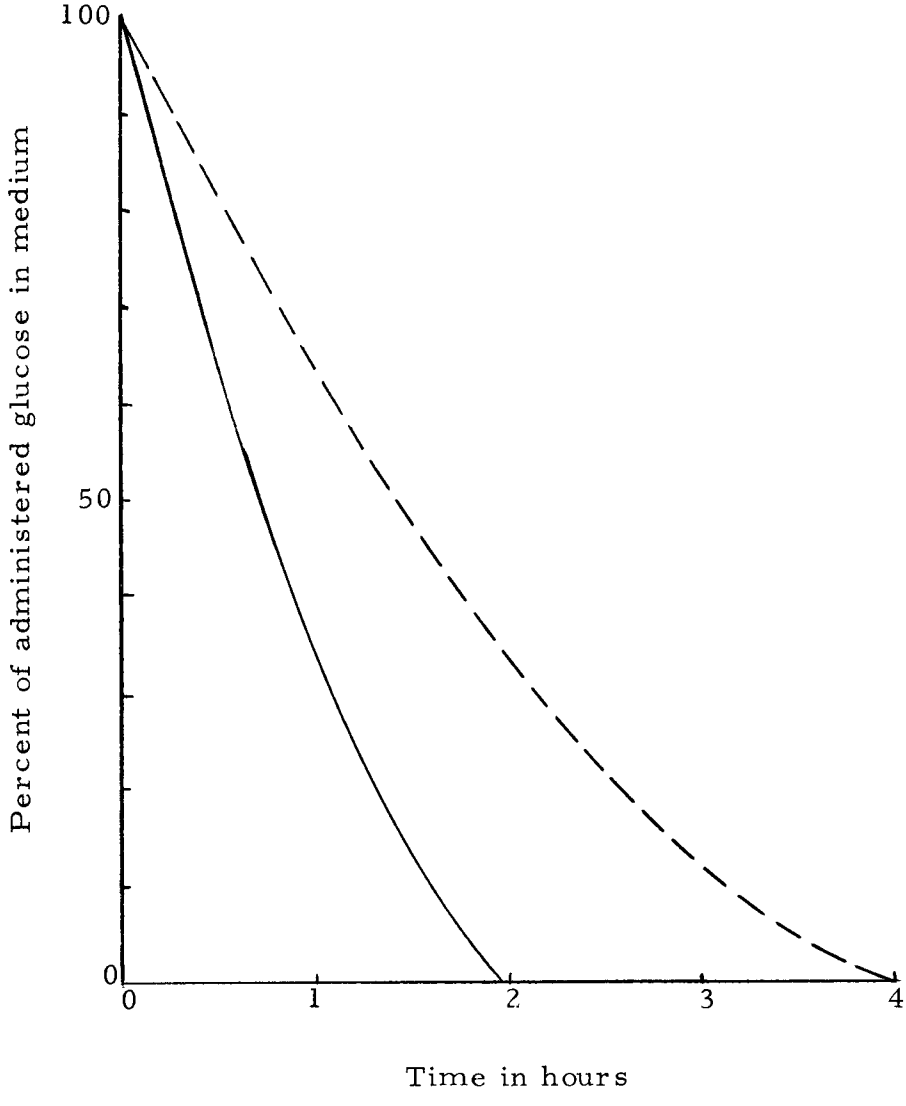

Figure 6. Rate of utilization of glucose by Saccharomyces cerevisiae cells in the presence and absence of 2-DG, single dose experiment. Experimental conditions: cell weight, 10 mg; volume of medium, 10 ml; substrate, 15 mg glucose-U-^{14}C; 2-DG, 1.64 mg in 2-DG experiment, none in control experiment.

_____ without 2-DG (control)

---------- with 2-DG

introduced soon afterward into the incubation medium. The results of this series of experiments are given in Figure 7. It is noted that, without the presence of 2-DG, the rate plot of $^{14}CO_2$ production (Curve A, Figure 7) displayed an upward slope, presumably reflecting increase in cell population. In the presence of 2-DG at 1.64 mg (0.01 mmole)/10 ml incubation medium (molar ratio, glucose:2-DG:: 33:1), the profile of the rate curve for $^{14}CO_2$ production (Curve B) resembles that of Curve A. Although the rate is lower in magnitude, the time-course for complete substrate utilization is extended beyond that of Curve A. This finding is in line with that observed in the single-dose experiment (Figure 4).

Of more interest are findings observed when the level of administered 2-DG was set at 16.4 mg (0.10 mmole) or 164 mg (1.0 mmole)/10 ml incubation medium (Curve C and Curve D respectively, Figure 7), and the respective molar concentrations in these experiments (glucose:2-DG) were 3.3:1 and 1:3. Thus, when the amount of 2-DG present in the medium was 1 mmole, glucose permeation into yeast cells was seriously hindered immediately upon addition of 2-DG into the incubation medium.

These findings lead one to believe that 2-DG exerts an effect, in the nature of a competitive inhibition, upon glucose permeation into cells, and the findings support the conclusions of Cramer and Woodward (1952). However, yet to be ascertained is whether the

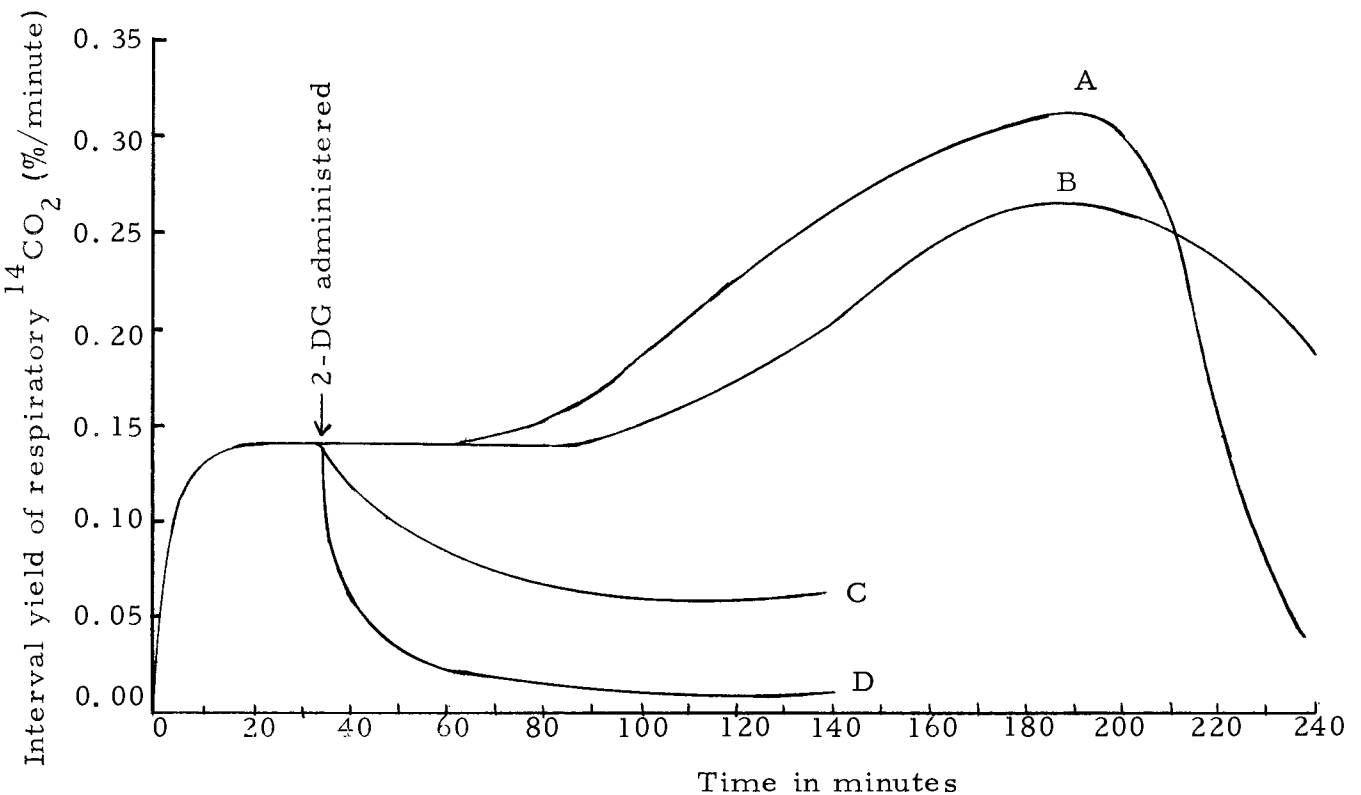

Figure 7. Radiorespirometric patterns for utilization of glucose by Saccharomyces cerevisiae cells in the presence of various amounts of 2-DG, continuous feeding experiment. Experimental conditions: cell weight, 13 mg; volume of medium, 10 ml; substrate, 60 mg (0.33 mmole) glucose; tracer amount of glucose-3(4)-^{14}C added at time 0. The following amounts of 2-DG were added at time indicated: A -- 0.00 mg; B -- 1.64 mg (0.01 mmoles); C -- 16.4 mg (0.10); D -- 164 mg (1.00 mmole).

inhibition involves competition for the entry sites or competition for the availability of ATP in cells, of vital importance to phosphorylation of substrate glucose.

Permeation of 2-DG Into Cells

The kinetic aspect of the permeation of 2-DG into yeast cells has been examined by the use of 2-DG-1-^{14}C as the tracer. The results in Table 6 show clearly that the amount of 2-DG transported into the cells increased correspondingly with an increase of the amount of 2-DG in the medium. However, of more interest is the finding that the net amount of 2-DG which entered yeast cells reached a maximum when 2-DG concentration was 0.82 mg (0.005 mmole)/10 ml medium. In fact, when the 2-DG concentration was increased to 1.64 mg/10 ml medium, the amount of 2-DG assimilated was only slightly higher than that observed in the 0.82 mg experiment. These results lead one to believe that assimilation of 2-DG into yeast cells is limited by the amount of available ATP within the cells. The ready phosphorylation of 2-DG, giving rise to 2-DG-6-P, in rat diaphragm cells has been reported by Kipnis and Cori (1959). In fact, the findings obtained with this series of experiments (Table 6) may prove to be useful in determining the amount of ATP available for phosphorylation of hexoses.

Kinetic information pertaining to assimilation of 2-DG by

Table 6. Permeation of 2-DG into Saccharomyces cerevisiae cells.

2-DG administered (mg)	2-DG assimilated (mg)
0.1	0.04
0.2	0.10
0.41	0.14
0.82	0.20
1.64	0.23

Experimental conditions: cell weight, 10 mg; volume of medium, 10 ml; substrate, various amounts of 2-DG-1-^{14}C; no other hexoses present in the medium.

Table 7. Time-course of permeation of 2-DG into Saccharomyces cerevisiae cells.

Time in minutes	2-DG assimilated (mg)
1	0.23
30	0.23
60	0.23
90	0.23
120	0.23

Experimental conditions: cell weight, 10 mg; volume of medium, 10 ml; substrate, 1.64 mg 2-DG-1-^{14}C; no other hexoses present in the medium.

yeast cells is provided in results given in Table 7. The fact that the net amount of assimilation of 2-DG does not increase with prolonged time of incubation renders support to the idea that transport of 2-DG across cell walls involves a phosphorylation step. Once the available ATP within the cells is depleted, further permeation of 2-DG into cells is not possible. These findings also support the contention that the inhibitory effect of 2-DG upon glucose permeation involves the competition of 2-DG and glucose for available ATP within the cells. The phosphorylation of 2-DG is evidently a more rapid process, in comparison to phosphorylation of substrate glucose. As indicated by results given in Table 7, despite the fact that the concentration of 2-DG-1-^{14}C is 1.64 mg/10 ml medium, the amount of 2-DG assimilated by the cells was observed at the level of 0.23 mg within one minute after 2-DG administration and throughout the entire duration (120 minutes) of the experiment. These findings demonstrate that the entry of 2-DG is a very rapid process, and that there is an upper limit to the amount of 2-DG which can be transported into cells. The latter may indicate that this amount of 2-DG is limited by the amount of ATP available in the cell. The conversion of 2-DG to 2-DG-6-P in rat diaphragm cells has been demonstrated by Kipnis and Cori, 1959. It is evident, therefore, that the reserve of ATP in cells is probably depleted upon entry of 2-DG.

SUMMARY

The inhibitory effect of 2-DG upon hexose metabolism in Saccharomyces cerevisiae cells has been examined in detail. The findings lead one to conclude that: (a) the permeation of glucose into yeast cells is noticeably hindered by the presence of 2-DG in medium. It appears that 2-DG is preferentially phosphorylated by available ATP within the cells; (b) the anabolic assimilation of hexose has been significantly inhibited by 2-DG at concentrations as low as 0.16 mg/10 ml. In contrast, substrate hexose, such as glucose or fructose, is routed to a greater extent through catabolic processes; however, relative participation of individual pathways for catabolic breakdown of substrate glucose does not appear to be altered; (c) 2-DG also exerted an effect on conversion of fructose-6-P to glucose-6-P via action of the enzyme, phosphohexoisomerase.

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