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The catabolism of glucose by proliferating and resting cells of two strains of Saccharomyces cerevisiae has been examined, with respect to the nature and participation of concurrent glucose pathways by means of the radiorespirometric method. The nicotinamide nucleotide content, and nicotinamide nucleotide oxidase activities in these cells were also examined.

Data from the radiorespirometric experiments indicated that both strains of S. cerevisiae catabolized glucose by way of the EMP-TCA and the PP pathway although relative participation of these two pathways were different. The pentose phosphate pathway is playing a much more important role in strain 2 of the bakers' yeast. Examination of the radiorespirometric pattern as well as the data on the incorporation of substrate carbon atoms into cellular constituents revealed that when bakers' yeast of strain 2 is permitted to utilize glucose under resting conditions, the data observed on

the yields of respiratory $C^{14}O_2$ cannot be used for the purpose of pathway estimation. This is true since under resting conditions, the slow rate of glucose utilization permits considerable interaction of glucose intermediates, thereby resulting in randomization of the two labeled carbon atoms.

The contents of nicotinamide nucleotides as well as the activities of nicotinamide nucleotide oxidases in these cells were determined and found that they cannot be correlated with relative participation of glucose pathways.

THE NATURE OF THE PENTOSE PHOSPHATE PATHWAY IN STRAINS OF *SACCHAROMYCES CEREVISIAE*

by

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TO MY PARENTS AND WIFE

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THE NATURE OF THE PENTOSE PHOSPHATE PATHWAY IN STRAINS OF SACCHAROMYCES CEREVISIAE

INTRODUCTION

In recent years much interest has been focused on the nature of catabolic pathways functioning in biological systems. These studies have been concerned with the identification of pathway mechanisms, estimation of relative participation of concurrent pathways, and the evaluation of the function of each pathway. Radiotracer methods have been advantageously used for these studies.

Insofar as the problem of pathway estimation is concerned, work done in the earlier 1950's involved the use of the data on the specific activities of catabolic products, such as respiratory $C^{14}O_2$ or acetate, derived from glucose-1-C^{14} and glucose-6-C^{14}. Thus, Beevers and Gibbs (3), and particularly Bloom and Stetten (4) have devised equations to estimate pathway participation making use of data on the specific activities of respiratory $C^{14}O_2$ from the labeled glucose substrates. Similarly, Blumenthal, Weinhouse, and co-workers (5) have devised equations for pathway estimation making use of specific activities of acetate, derived from C^{14} specifically labeled glucose substrate. Later Abraham, Katz, and Chaikoff (1) devised another method making use of the amount of

C^{14} specifically labeled glucose that has been incorporated into fatty acids of animal tissue. Meanwhile, Wang (20) and his co-workers made use of radiochemical yields of respiratory $C^{14}O_2$ derived from specifically labeled glucose substrates for the estimate of pathway participation in various biological systems.

The foregoing cited papers all involved a key assumption, i.e. that pentose phosphate derived from glucose via the pentose phosphate (PP) pathway does not participate significantly in further catabolism. However, later experimental evidences revealed that pentose phosphate can be indeed catabolized by various biological tissues via the pentose cycle or other similar pathways giving rise to the formation of respiratory CO_2. It should be stressed that the fate of pentose phosphate, derived from glucose in biological systems, was elucidated extensively during that period through the effort of Racker, Horecker, and other biochemists.

With the information on pentose phosphate metabolism, particularly those in the nature of the pentose cycle pathway on hand; Katz and Wood have derived mathematical relationships relative to the participation on the pentose cycle pathway in glucose catabolism. It is noted, however, that in their derivation, the pentose cycle was defined to represent the net conversion of: 1 G-6-P $\longrightarrow$ 3 CO_2 + 1 glyceraldehyde-3-phosphate. Such a definition calls for a prerequisite assumption that the pentose phosphate derived from glucose is

not engaged in any biosynthetic functions which may cause net drainage of the pentose species or their intermediates from the metabolic glucose pool.

The participation of the pentose cycle pathway in glucose catabolism was also examined by Dawes and Holmes (9). They recognized that the pentose phosphate can give rise to the formation of fructose-6-P which in turn can either be isomerized to glucose-6-phosphate or be phosphorylated to yield fructose 1, -6-diphosphate, the latter can naturally be metabolized further via the glycolytic pathway.

In a revised version (22) of the equation earlier devised by Wang and his co-workers, the catabolism of pentose phosphate was also taken into consideration. However, it should be stressed that in this work the treatment of Wang and his co-workers of pathway participation was made on the basis of fractions of glucose channeled initially into concurrent pathways. Such treatment is not only more realistic but also caused no confusion since the drainage of pentose phosphate or any other secondary catabolic processes would not effect the pathway participation picture if one is concerned with the initial fate of glucose.

In this connection, it is noted that the problems of pentose phosphate drainage and other similar problems are of more importance when one studies the biological systems under proliferating

conditions. Earlier glucose pathway studies have dealt primarily with the use of resting cells despite the fact that the pathway information so obtained represents that observed with biological systems in a non-physiological state. Consequently, there is an urgent need to investigate the effect of environment, particularly the environmental conditions upon the pathway participation for the utilization of glucose by biological systems.

In order to compare directly the pathway participation for glucose utilization by resting cells and that by proliferating cells, the present work was undertaken with bakers' yeast, (Saccharomyces cerevisiae) chosen as the test system. It is a well-known fact that yeast metabolism resembles much that of mammalian tissues and, moreover, the glucose catabolism in bakers' yeast has been extensively studied in several laboratories.

Early work on glucose pathway participation in S. cerevisiae indicates that glucose is catabolized mainly by the Embden-Mayerhof-Parnas (EMP) pathway, and to a limited extent via the pentose phosphate HMP pathway. Thus Beevers and Gibbs (3) showed a preferential conversion of C-1 of glucose to CO_2 over that of C-6 of glucose providing evidence for the occurrence of the PP pathway. Blumenthal, et al. (5) examined the relative specific activities of acetate derived from glucose-1-C^{14} and glucose-6-C^{14} and concluded that the PP pathway may have accounted for as much as 30 percent

of the glucose catabolized. Wang, et al. (21) estimated that the fraction of glucose catabolized via the pentose phosphate pathway is approximately 13 percent using the radiochemical yields of respiratory $C^{14}O_2$ from specifically labeled glucose samples as the basis of calculation. Chen (7) has shown that in S. cerevisiae the aeration rate does not affect the amount of CO_2 produced by the PP pathway, but does change the amount of CO_2 produced by pyruvate decarboxylation.

Insofar as the metabolism of glucose intermediates are concerned, the tricarboxylic acid cycle (TCA) has been previously demonstrated as to play a predominant role in yeast metabolism. It is also recognized that the TCA cycle in conjunction with a mechanism of C_4 synthesis provides a convenient means for the synthesis of carbon skeleton for various amino acids.

The function of the pentose phosphate pathway or the HMP pathway in yeast metabolism is presumably concerned with the provision of intermediates for the synthesis of aromatic amino acids, nucleic acid, and others. Meanwhile, not to be ignored is the fact that the operation of the pentose phosphate pathway also plays an important role in the formation of NADPH which is of primary importance in the biosynthesis of fatty acids.

The role played by the co-enzymes NAD, essential for the glycolytic pathway and NADP, essential for the pentose phosphate

pathway has attracted much attention in recent studies on metabolic control. Thus, McLean (15) and Cahill et al. (6) have demonstrated that in various mammalian tissues, the PP pathway is regulated by the supply of NADP. The results of Glock and McLean (14) showed that, in most mammalian tissues, NAD is present mostly in the oxidized form, while NADP is present mostly in the reduced form. Some effort has been made (10) to correlate the activity of the PP pathway to the total NADP content in mammalian tissues.

However, in microorganisms, the relationship between total NADP content and the PP pathway has not been extensively studied. Eagon (12) has related pathway participation in various microorganisms with the activities of NADH-oxidase, NADPH-oxidase, and pyridine nucleotide transhydrogenase prevailing in the respective organisms. He concludes that the catabolic fate of glucose via the PP pathway is limited by the rate of NADP supply in microorganisms. In organisms equipped with both the glycolytic and the PP pathways for glucose catabolism, Eagon (11) proposed that NADPH is extensively oxidized by coupling to reductive biosynthetic reactions.

More recently, it has been found in this laboratory that bakers' yeast, isolated from the commercially available source (Fleishmann's Yeast cake), displays a noticeably different catabolic behavior than that of the ATCC culture (ATCC # 7754). Consequently, in the present work, both strains of yeast have been used as test systems. The

study includes an evaluation of the participation of glucose pathways under proliferating and resting conditions. A critical examination of complexities underlying the methods for pathway estimation shows the relationships existing among the contents of co-enzymes, NAD-NADH and NADP-NADPH; the activities of NADH oxidase and NADPH oxidase; the relative participation of glucose pathways in both strains of yeast has also been critically examined

MATERIALS AND METHODS

Culture Conditions

Two strains of Saccharomyces cerevisiae, one ATTC 7754 (strain 1) and the other, isolated from a cake of Fleischmann's bakers' yeast (strain 2), were maintained on agar slants. The cells were grown aseptically in a medium containing: $(NH_4)_2SO_4$, 2.5 g; NaCl, 2 g; KH_2PO_4, 2 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; $CaCl_2 \cdot 2H_2O$, 0.25 g; H_3BO_3, 1.0 mg; $ZnSO_4$, 1.0 mg; $MnSO_4 \cdot 4H_2O$, 1.0 mg; $FeCl_3$, 1.0 mg; $CuSO_4 \cdot 5H_2O$, 0.1 mg; KI, 0.1 mg; Bacto yeast extract, 0.1 g; glucose, 20 g; all dissolved in one liter of distilled water (18). The pH of the medium was adjusted to 4.5 with HCl. Incubation was carried out aerobically at 30° C on a rotary shaker.

Radiorespirometric Experiments and Radioactivity Measurements

The radiorespirometric studies were carried out according to the method of Wang and Krackov (22). Each incubation flask contained ten mg of cells in ten ml of glucose-free medium, proliferating cells, or in ten ml of 0.067 M KH_2PO_4, resting cells, to which was added 30 mg of specifically labeled glucose. The flasks were placed in a 30° C bath and aerated with air at the rate of 40 ml per minute with shaking. Respiratory $C^{14}O_2$ was trapped in

ten ml of 2 - aminoethanol:absolute ethanol (1:2) solution which was removed hourly. To the trap solution was added five ml of absolute ethanol making the final volume 15 ml. A five ml aliquot was taken and mixed with ten ml of toluene containing terphenyl (three g/l) and 1, 4-bis-2'(5'-phenyloxazolyl)-benzene (30 mg/l) in a 20 ml glass counting vial. Countings were carried out according to Wang (22). At the termination of the experiments, the cells were separated from the medium by centrifugation and aliquots of each were counted as described by White and Helf (23). The efficiency of liquid scintillation counting in the samples was determined by the use of internal standards. All counting was carried out to a relative standard deviation of no greater than two percent.

Radiochemical Substrates

The radiochemical substrates used in this work were glucose-1, 2, 3(4), or 6-C^{14} obtained from New England Nuclear.

Preparation of Cellular Extracts

Cell free extracts from both strains of Saccharomyces cerevisiae were prepared according to the procedure of Eagon and Wang (13). The cells were harvested during log phase of growth by centrifugation, washed with .052 M $MgCl_2$, and resuspended in 0.1 M tris-HCl buffer (pH 7.5) to a cell concentration of six mg/ml.

Fifteen ml aliquots were sonified for 1-5 minutes at 2-5° C in a Branson sealed-atmosphere chamber. The resulting suspension was centrifuged at 12,000 x g for 30 minutes at 0° C. The supernatant was dialized against .1 M tris-HCl buffer (pH 7.5) for 24 hours prior to the analysis of NADH and NADPH-oxidase.

Determination of NADH and NADPH Oxidase Activity

NADH and NADPH oxidase activities were determined essentially according to the method of Eagon and Wang (13). Two ml of the cellular extract, and 0.5 μmole of NADH or NADPH in one ml containing 100 μmoles of tris-HCl buffer (pH 7.65), were placed in a Beckman DB spectrophotometer. The rate of decrease in absorbancy at 340 mμ was measured. A five ml aliquot was also taken for a protein determination according to the procedure of Robinson and Hogden (16).

Determination of Nicotinamide Nucleotide Coenzymes

Both strains of Saccharomyces cerevisiae were harvested during log phase of growth, washed with .067 M phosphate buffer (pH 7.0), and resuspended in a small volume of distilled water so that 0.5 ml contained approximately 30 mg of cells in dry weight.

Extraction and determination of oxidized and reduced forms of NAD and NADP were performed according to the methods of

Takebe and Kitahara (17), and Bassham (2). Fluorescence was measured with an Aminco-Bowman spectrophotofluorimeter at 460 mμ, the activation wavelength being 360 mμ. The efficiency of extraction and assay of the respective coenzymes was determined according to the procedure of Takebe (17). Recovery values were 96 percent for NAD, 80 percent for NADP, 91 percent for NADH, and 69 percent for NADPH. The respective coenzymes used for standardization and recovery determinations were purchased from Sigma Company.

RESULTS

The radiorespirometric patterns, representing the average values of three replicate experiments, for the utilization of specifically labeled glucose by cells of S. cerevisiae (strain 1) under proliferating and resting conditions are given in Figures 1 and 2, respectively. Similarly, the patterns for strain 2 under proliferating and resting conditions are given in Figures 3 and 4, respectively. The average deviation from the mean with respect to $C^{14}O_2$ yields was calculated to be ± two percent. The distribution of the substrate activity in the respiratory $C^{14}O_2$, cells, and media at the termination of the respective radiorespirometric experiments is given in Table 1.

The NADH and NADPH-oxidase activities, and the content of nicotinamide nucleotide coenzymes of both strains of S. cerevisiae under proliferating and resting conditions are given in Tables 2 and 3, respectively.

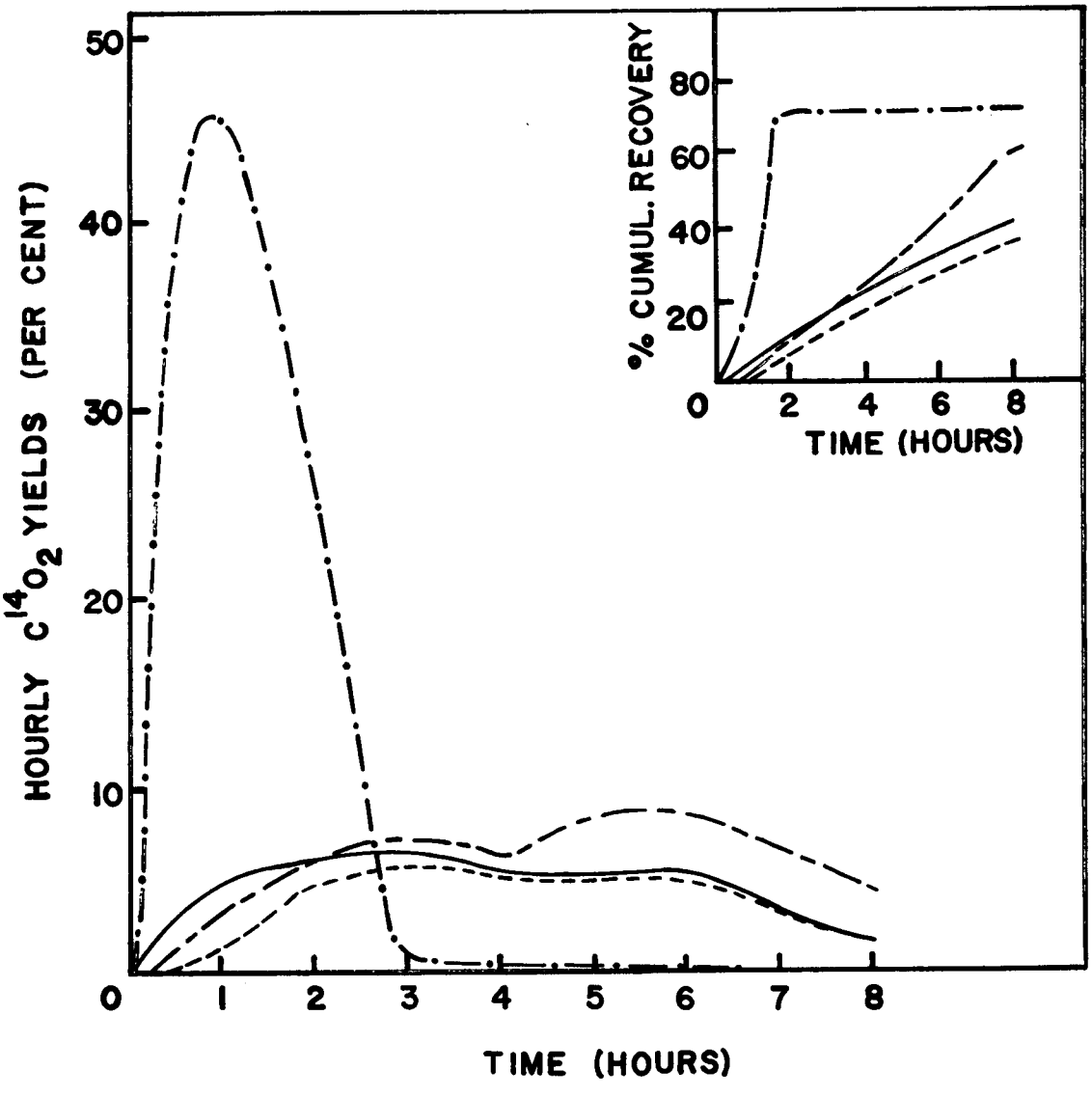

Figure 1. Radiorespirometric patterns for the utilization of specifically labeled glucose by proliferating cells of Saccharomyces cerevisiae (strain 1).

Glucose-1-C^{14} ——————

Glucose-2-C^{14} — — — — —

Glucose-3(4)-C^{14} —•—•—•—•

Glucose-6-C^{14} - - - - - - - - - -

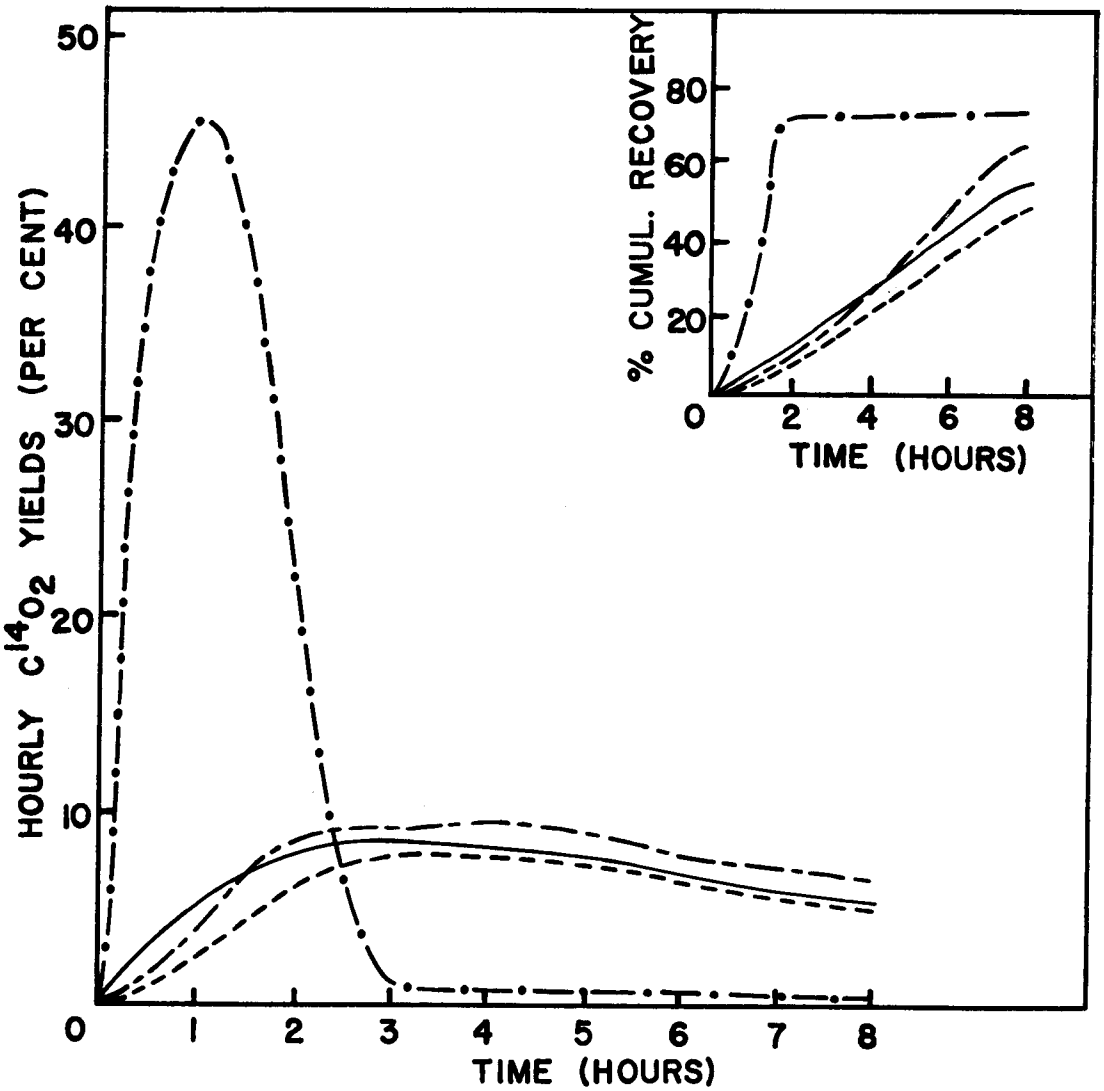

Figure 2. Radiorespirometric patterns for the utilization of specifically labeled glucose by resting cells of Saccharomyces cerevisiae (strain 1).

Glucose-1-C^{14} ——————

Glucose-2-C^{14} — — — —

Glucose-3(4)-C^{14} —·—·—

Glucose-6-C^{14} - - - - - - -

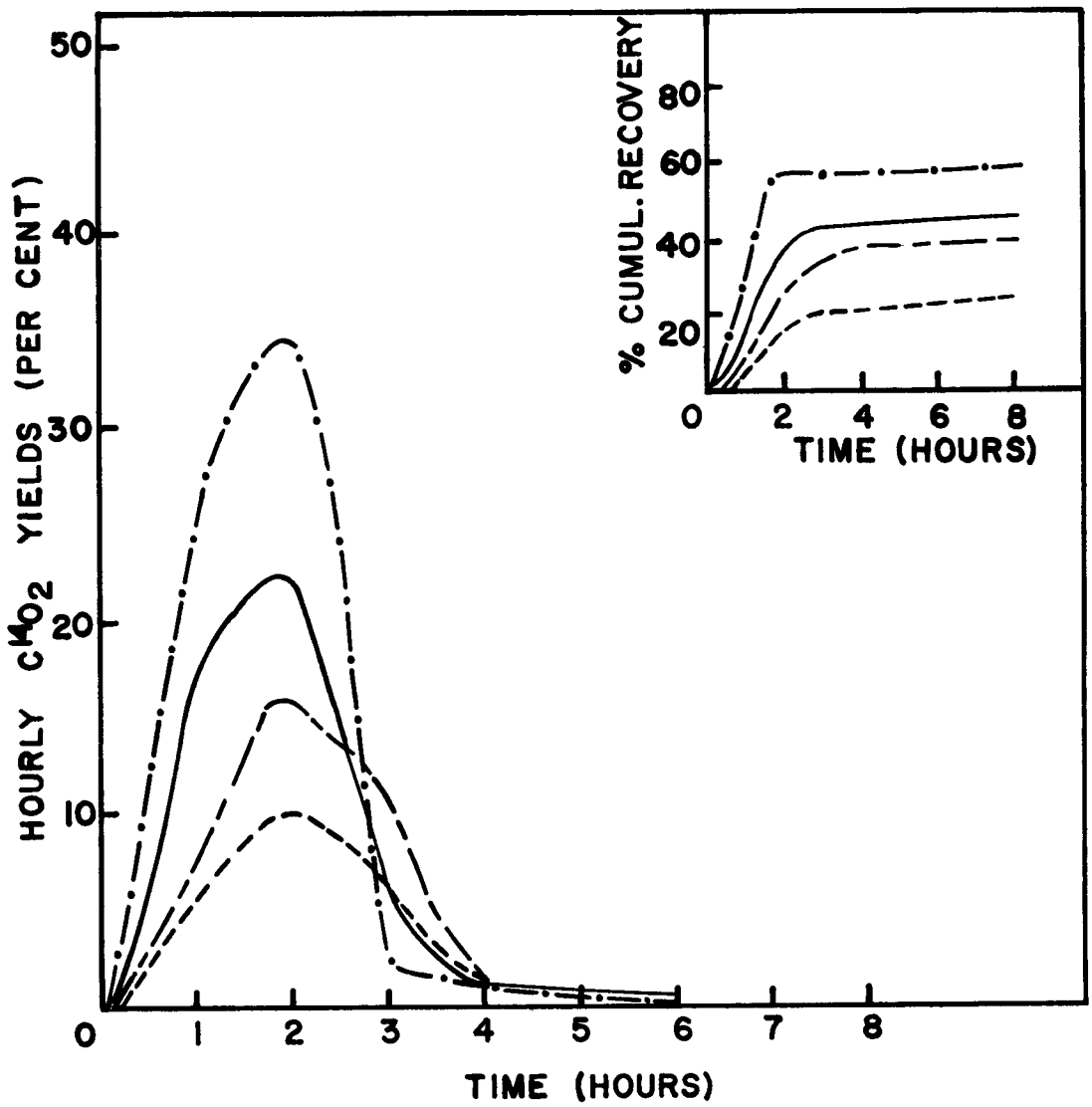

Figure 3. Radiorespirometric patterns for the utilization of specifically labeled glucose by proliferating cells of Saccharomyces cerevisiae (strain 2).

Glucose-1-C^{14} ——————

Glucose-2-C^{14} — — — —

Glucose-3(4)-C^{14} — • — • —

Glucose-6-C^{14} — — — — — —

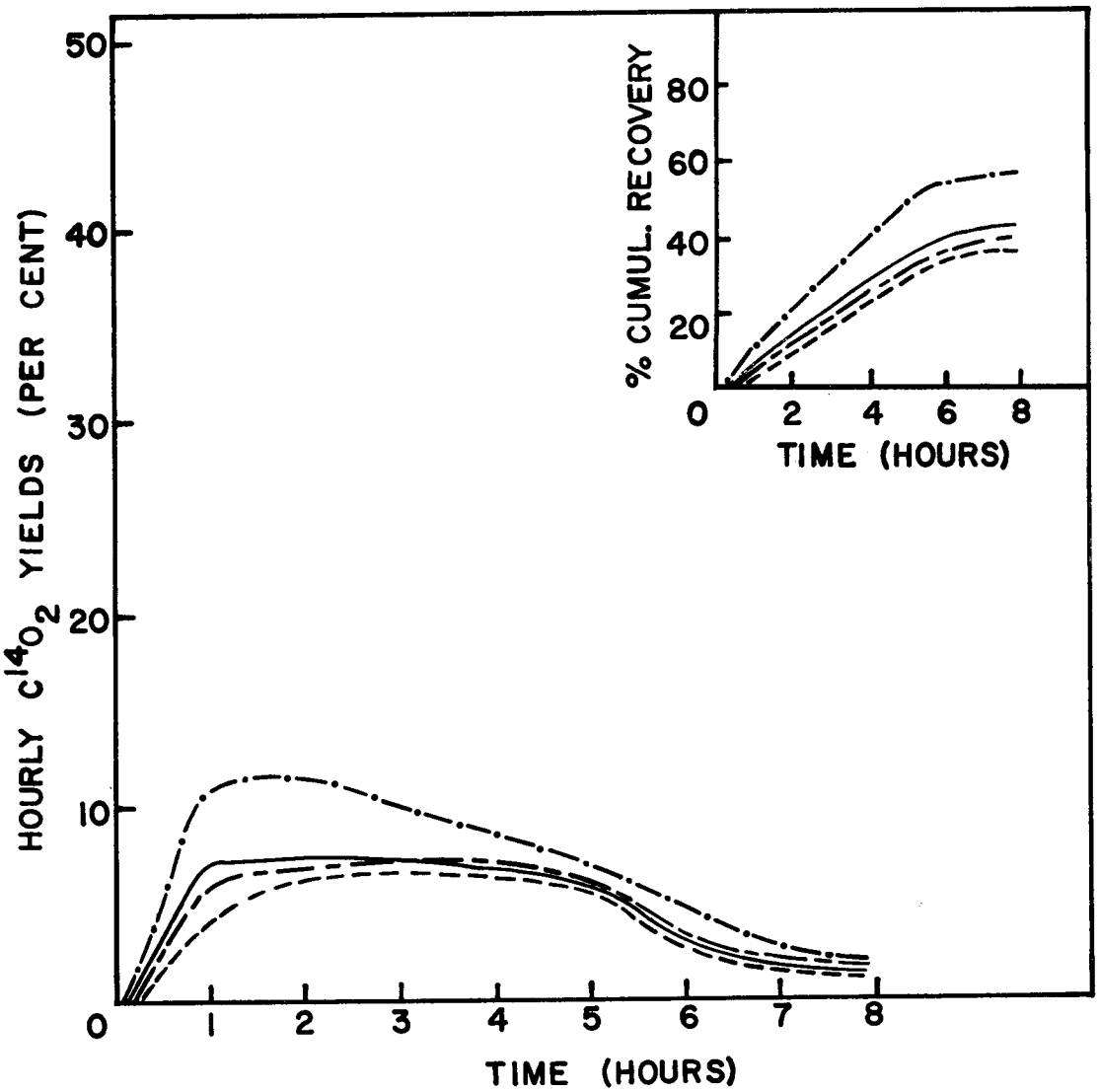

Figure 4. Radiorespirometric patterns for the utilization of specifically labeled glucose by resting cells of Saccharomyces cerevisiae (strain 2).

Glucose-1-C^{14} ——————

Glucose-2-C^{14} — — — — — —

Glucose-3(4)-C^{14} —·—·—·—

Glucose-6-C^{14} - - - - - - - - - -

Table 1. Utilization of C^{14} Specifically Labeled Glucose Substrates by Two Strains of Bakers' Yeast (S. cerevisiae).

Strain and Cultural Conditions	Substrate	Substrate Level		Radiochemical Yield (percent) in			
		μc	mg	CO_2	Cells	Medium	Total
Strain 1 Proliferating Cells	Glucose-1-C^{14}	.25	30	43	43	7	93
	Glucose-2-C^{14}	.25	30	60	29	6	95
	Glucose-3(4)-C^{14}	.03	30	76	10	5	91
	Glucose-6-C^{14}	.25	30	38	44	13	95
Strain 1 Resting Cells	Glucose-1-C^{14}	.25	30	54	27	10	91
	Glucose-2-C^{14}	.25	30	63	22	10	95
	Glucose-3(4)-C^{14}	.03	30	72	8	5	85
	Glucose-6-C^{14}	.25	30	49	24	15	88
Strain 2 Proliferating Cells	Glucose-1-C^{14}	.25	30	46	44	9	99
	Glucose-2-C^{14}	.25	30	38	51	6	95
	Glucose-3(4)-C^{14}	.03	30	60	32	4	96
	Glucose-6-C^{14}	.25	30	24	61	6	91
Strain 2 Resting Cells	Glucose-1-C^{14}	.25	30	43	47	10	100
	Glucose-2-C^{14}	.25	30	41	46	7	94
	Glucose-3(4)-C^{14}	.03	30	58	34	6	98
	Glucose-6-C^{14}	.25	30	38	46	8	92

Table 2. NADH-oxidase and NADPH-oxidase Specific Activities in Extracts from Strains of Saccharomyces cerevisiae.

Strain and Cultural Conditions	E_{340}/min/mg Protein X 10^3	
	NADH	NADPH
Strain 1 Proliferating Cells	126	24
Strain 2 Proliferating Cells	310	10
Strain 2 Resting Cells	105	9

Table 3. Nicotinamide Nucleotide Contents in Strains of Saccharomyces cerevisiae.

Coenzyme	Strain 1 (proliferating cells) mg/g dry weight	Strain 2 (proliferating cells) mg/g dry weight	Strain 2 (resting cells) mg/g dry weight
NAD	1.68 ± .22*	2.36 ± .30	2.11 ± .06
NADH	.48 ± .05	.92 ± .15	.87 ± .13
NADP	.28 ± .04	.32 ± .05	.33 ± .02
NADPH	.41 ± .02	.41 ± .08	.49 ± .12
NAD + NADH	2.16 ± .24	3.28 ± .24	2.98 ± .09
NADP + NADPH	.71 ± .06	.73 ± .09	.82 ± .02
$\frac{\text{NADP + NADPH}}{\text{NAD + NADH}}$	.32	.24	.27

*mg/g dry weight ± s.e.m.

DISCUSSION

The radiorespirometric pattern for the utilization of glucose by proliferating cells of S. cerevisiae (strain 1), as depicted in Figure 1, can be recognized as to consist of two phases as described previously (20, 21). The assimilation phase corresponds to a rapid appearance in $C^{14}O_2$ from C-3(4) of glucose reflecting the important role played by the EMP pathway. Concurrently, a preferential conversion of C-1 of glucose to CO_2 over that of C-6 and C-2 of glucose, provides evidence for the operation of the PP pathway, since there exists no evidence for the operation of the Entner Doudoroff (ED) pathway in yeast. In the second phase, that of depletion, one finds that the conversion of glucose carbon atoms to CO_2 follows the order C-2>C-1 = C-6>C-3(4). This is explainable since substrate glucose was presumably converted to biosynthetic intermediates via mainly the EMP-TCA cycle sequence during the assimilation phase; the reverse of these processes into the TCA cycle during the depletion phase results in the observed order. Since incorporation of C-3(4) of glucose into cellular constituents is very low, one can assume that very little substrate glucose was engaged in anabolic processes.

In contrast, one finds that the radiorespirometric pattern of strain 2 for the utilization of glucose under proliferating conditions

(Figure 3), consists essentially of one phase. Here again, the rapid appearance in $C^{14}O_2$ from C-3(4) of glucose reflects conversion of substrate glucose by way of the EMP pathway. Analysis of the cumulative $C^{14}O_2$ recovery (Table 1) shows the conversion of substrate glucose to CO_2 follows the order of C-3(4)>C-1>C-2>C-6. The fact that the yields of C-1 of glucose in respiratory CO_2 are considerably higher than that of C-2 and C-6 provides evidence for an extensive operation of the PP pathway in this organism. This interpretation is supported also by the fact that the incorporation of C-6 and C-2 of glucose into the cells is much higher in extent as compared to that of C-1 of glucose, reflecting significant conversion of glucose intermediates formed via the PP pathway into biosynthetic products.

Although the nature of major glucose pathways functioning in these two strains of yeast is basically the same, there exist some noticeable differences.

Firstly, the absence of a depletion phase, with regard to production of respiratory $C^{14}O_2$ from glucose carbon atoms, in strain 2 may indicate that in this strain endogenous reserve of carbon source is not in the nature of cellular amino acids, such as glutamic acid (19). The accumulation of cellular glutamic acid in yeast has been previously reported. The contention is also supported by the fact that in strain two a significant amount of C-3(4)

of glucose was found in the cells. This fact may indicate that a sizeable amount of substrate glucose has been converted to cellular polysaccharides which could readily serve as reserve carbon sources.

It is also of interest to examine pathway participation for glucose utilization in both strains of yeast cultured under resting conditions. Despite the fact that resting conditions constitute a non-physiological state for biological systems, the bulk of the work done on microbial metabolism involved the use of resting cells. This is primarily due to the extensive use of manometric techniques in the studies of microbial respiration in the past decades. It should be stressed that under resting conditions one would expect that the biosynthetic activities of the cells are reduced drastically, thereby altering the functions of the glucose pathways particularly those involved in the provision of biosynthetic intermediates.

The radiorespirometric pattern of strain 1 for the utilization of glucose under resting conditions, shown in Figure 2, does not display a sharp demarkation of two phases. It is also noted that the conversion of carbon atoms of glucose to respiratory $C^{14}O_2$ is generally greater in extent and the incorporation of glucose carbon atoms into cellular constituents is drastically reduced in extent, when the data with resting cells are compared with that of the proliferating cells. This is understandable inasmuch as biosynthesis is very much limited in cells under resting conditions.

In the case of strain 2 yeast cells, the radiorespirometric pattern for the glucose utilization by resting cells (Figure 4) is significantly different from that observed with proliferating cells. Firstly, the rate of glucose utilization is drastically reduced in the case of resting cells. More important is the finding that under resting conditions, the incorporation of glucose carbon atoms into cellular constituents remains approximately the same in magnitude as that of proliferating cells except that of C-6 of glucose. The production of respiratory $C^{14}O_2$ from the labeled carbon atoms of glucose is about the same in magnitude, regardless of cultural conditions, except that the conversion of C-6 of glucose to CO_2 is impressively increased in the case of resting cells.

These findings can be interpreted if one recognizes that the heavy incorporation of C-3(4) of glucose into resting cells implies that the change in cultural conditions does not alter the extent of conversion of glucose to cellular polysaccharides. If this were the case, one could conclude that the major change in catabolic behaviors when proliferating cells were cultured under resting conditions, is a sharp reduction in the use of some carbon atoms for biosynthetic functions. Such a reduction is particularly noticeable with C-6 of glucose and to some extent with C-2 and C-3(4) of glucose. This would also imply that a considerable amount of pentose phosphate derivatives, as derived from glucose via the

PP pathway, had been converted to respiratory CO_2 instead of channeled into cellular constituents when cells are incubated under resting conditions. Since the rate of $C^{14}O_2$ production from C-1 of glucose is closely followed by that of C-2 of glucose (Figure 4), it may be inferred that these cells convert more re-formed hexose to CO_2 by way of the pentose cycle pathway.

With the foregoing given qualitative analysis of data in mind, it is also of interest to estimate quantitatively the relative participation of the EMP and PP in the respective strains of yeast cells under proliferating and resting conditions. The equation used for such an estimation is much the same as that described by Wang and Krackov (22). Thus,

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

where: G_p = Fraction of glucose catabolized via the PP pathway

G_1 and G_6 = Cumulative radiochemical yields of respiratory $C^{14}O_2$ from equal amounts of respectively labeled glucose substrates.

A_6 = Cumulative radiochemical yield of respiratory $C^{14}O_2$ from gluconate-6-C^{14} as substrate.

G_T = Total radioactivity of each labeled glucose administered taken as unity.

$G_{T'}$ = Fraction of the administered labeled glucose which undergoes anabolic processes.

When substrate glucose is completely catabolized, the value $G_{T'}$ approaches zero and $G_T - G_{T'} = 1$. Simplification of Equation (1) gives rise to:

$$G_p = \frac{G_1 - G_6}{1 - A_6} \tag{2}$$

Moreover, since gluconate cannot be utilized by yeast, the present data for the term A_6 are therefore not available. From the concluded approximation, one can use the term G_6 to substitute for the term A_6 and,

$$G_p = \frac{G_1 - G_6}{1 - G_6} \tag{3}$$

However, in the present case, it is known that in strain 2 a significant amount of glucose was engaged in anabolic processes and hence the term $G_{T'}$ cannot be ignored. Equation 1 is therefore revised to read:

$$G_p = \frac{G_1 - G_6}{G_T - G_{T'} - A_6} \tag{4}$$

Since G_6 is substituted for A_6, G_p becomes:

$$G_p = \frac{G_1 - G_6}{G_T - G_{T'} - G_6} \tag{5}$$

The data for the term $G_{T'}$ are not readily available, nevertheless the maximum amount of substrate glucose engaged in anabolic processes can be represented by the extent of incorporation of C-3(4) of glucose into cellular materials. This is true since an examination of existing information on the pathways that could have incorporated these two carbon atoms of glucose into cellular constituents revealed that the most important one is that of polysaccharide formation from glucose (8).

Using equations 3 and 5 for pathway estimation, calculations have been made for both strains of yeast under proliferating and resting conditions making use of the radiorespirometric data collected at the end of the time-course for glucose utilization. The results are given in Table 4.

A close examination of the estimated pathway participation for glucose utilization by two strains of yeast revealed many interesting facets. In the case of strain 1, it can be readily seen that there exists no significant difference in the relative participation of glucose pathways whether the culture is incubated under proliferating conditions or resting conditions, although the conversion of carbon atoms of glucose to respiratory CO_2 is much greater in extent when cells are incubated under resting conditions. It is noted that with this strain of yeast, the rate of glucose utilization remains approximately the same regardless of culture environment.

Table 4. Estimation of Pathway Participation for Utilization of Glucose by Two Strains of Bakers' Yeast (S. cerevisiae).

I	II	III	IV *	V	VI	VII
Strain and cultural conditions	G_1 $C^{14}O_2$ Yield with Glucose-1-C^{14} (percent)	G_6 $C^{14}O_2$ Yield with Glucose-6-C^{14} (percent)	$G_{T'}$ Fraction of substrate glucose engaged in anabolic processes	$G_T - G_{T'}$ Fraction of substrate glucose catabolized	G_p ** Equation 3 $\frac{G_1 - G_6}{1 - G_6}$	G_p ** Equation 5 $\frac{G_1 - G_6}{G_T - G_{T'} - G_6}$
Strain 1 Proliferating Cells	43	38	0.10	0.9	8	10
Strain 1 Resting Cells	54	49	0.08	0.92	10	12
Strain 2 Proliferating Cells	46	24	0.32	0.68	30	50
Strain 2 Resting Cells	43	38	0.34	0.66	8	18

* The term $G_{T'}$ is represented by the extent of incorporation observed in respective glucose-3(4)-C^{14} experiments.

** The term G_p is the fraction of substrate glucose catabolized via PP pathway.

This fact implies that equation 3 is valid in estimating the relative participation of glucose pathways since one of the key assumptions underlying the derivation of equation 1 (22) is that substrate glucose is promptly catabolized in the organism via individual pathways hence, eliminating any complication resulting from interaction of glucose intermediates.

In sharp contrast to the case of strain 1, one finds that with strain 2 of bakers' yeast not only the participation of the pentose phosphate pathway is much greater in extent but also the estimated value of participation of the pentose phosphate pathway is drastically different when one compares the values of G_p calculated for proliferating cells and that for resting cells.

A close examination of the radiorespirometric data obtained with strain 2 provides one with valuable leads in accounting for the observed discrepancies. Firstly; the rate of glucose utilization by resting cells was found to be considerably slower than that with proliferating cells. This fact implies that there could be an accumulation of glucose intermediates in the resting cells and the interaction of these intermediates may introduce complications resulting from the randomization of the labeled carbon atoms of glucose. Secondly; one notes that although the $C^{14}O_2$ yields from C-1 of glucose are approximately the same in magnitude with cells under either proliferating or resting conditions, the $C^{14}O_2$ yield

from C-6 of glucose is much higher in the case of resting cells. This results in a drastic reduction of the value of G_p as calculated according to equation 3.

Inasmuch as the catabolism of C-6 of glucose to CO_2 via the pentose phosphate pathway has been taken into consideration in the derivation of equation 1, it appears that the low value of G_p calculated for the resting cells represents an artifact resulting from some other complicating factors. One of the most probable factors in this regard is the re-combination of triose phosphate, derived from the pentose phosphate pathway or pentose cycle pathway, giving rise to the formation of hexose monophosphate with C-1 of the re-formed hexose originating from C-6 of the substrate glucose. The catabolism of the hexose monophosphate so formed via the pentose phosphate pathway would give rise to a preferential conversion of C-6 of glucose to respiratory CO_2. The findings on pathway participation for glucose utilization by strain 2 of bakers' yeast consequently point to an important conclusion that only the radiorespirometric data obtained with proliferating cells are valid for pathway estimations according to equation 3.

A cross-comparison of the pathway information obtained with both strains of bakers' yeast also leads one to recognize the importance of pathway information in microbial taxonomy despite the classification of these two strains under the name of bakers' yeast.

The catabolic behaviors are indeed very much different in several important aspects including nature of endogenous reserve and the participation of concurrent pathways.

Eagon (12), upon examination of the activities of NADH-oxidase and NADPH-oxidase and pyridine nucleotide transhydrogenase of a great number of microorganisms, concluded that the operation of the pentose phosphate pathway in microorganisms is rate limited by the rate of NADP supply. The findings are similar to that observed with mammalian tissue (6, 15). In the light of these works, the specific activities of NADH-oxidase and NADPH-oxidase have been examined in both strains of the bakers' yeast cultured under proliferating conditions and resting conditions, respectively. As shown by the results given in Table 2, the NADPH-oxidase activity is greater in strain 1 as compared to that of strain 2. The reverse is true when one examines the activity of NADH-oxidase in these two strain.

These findings do not support the work of Eagon. In fact, one is inclined to conclude that it is not possible to correlate the information on pathway participation with that of pyridine nucleotide oxidases. However, it is interesting to note that with strain 2, the activity of NADH-oxidase is drastically reduced when cells are permitted to oxidize glucose under resting conditions.

It also appears impossible to correlate the net content of

nicotinamide nucleotides, in the oxidized form and the reduced form with information on pathway participation. As shown by the results given in Table 3, the NADP contents are similar in magnitude with either strain of yeast, yet pathway estimation revealed that the pentose phosphate pathway or the pentose cycle pathway, is playing a much more important role in the glucose catabolism of strain 2. Moreover, in the case of strain 2, the net contents of nicotinamide nucleotides do not appear to be altered by a change in environmental conditions.

These findings lead one to conclude that the participation of the pentose phosphate pathway in glucose metabolism is not rate limited by the specific activities of NADPH-oxidases nor the net contents of NADP or NADPH. It remains possible that NAD may play an important role in the oxidation of glucose via the pentose phosphate pathway in these organisms.

SUMMARY

The pathways of glucose catabolism in two strains of S. cerevisiae have been examined by means of the radiorespirometric method. Qualitatively, both organisms rely on the glycolytic and the pentose phosphate pathway for glucose utilization. However, the pentose phosphate pathway is playing a much more important role in strain 2 of the bakers' yeast.

Pathway estimation studies were demonstrated to be not feasible when radiorespirometric data were collected from strain 2 of yeast cells, incubated under resting conditions. The slow rate of glucose utilization under resting conditions created complications, derived from the randomization of the labeled carbon atoms in glucose.

The contents of nicotinamide nucleotides as well as the activities of nicotinamide nucleotide oxidases in these cells were determined and found that they cannot be correlated with relative participation of glucose pathways.

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