ORGANIC AND BIOLOGICAL CHEMISTRY

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND SCIENCE RESEARCH INSTITUTE, OREGON STATE COLLEGE]

Carbohydrate Metabolism in Bakers' Yeast.[1,2] I. Time Course Study of Glucose Utilization

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The time course study of the utilization of specifically labeled glucose by yeast has been carried out under proliferating conditions. The rate of change of specific activity in the respiratory CO_2 from the respectively labeled substrate agrees with the known breakdown pathways of glucose in this organism. Calculations were made on the basis of cumulative radiochemical recoveries of metabolic CO_2 from each labeled glucose which revealed that approximately 87% of the administered glucose was metabolized by way of the Embden–Meyerhof pathway in combination with the tricarboxylic acid cycle, and 13% was consumed through phosphogluconate decarboxylation. The efficiency of the utilization of each glucose carbon atom in biosynthesis also was estimated.

Recent reports[4,5] on glucose metabolism in bakers' yeast have shown that the direct oxidative pathway may consume up to as much as 30% of the total glucose utilized by resting cells under aerobic conditions. The nature as well as the function of the latter pathway in this organism, however, is not yet fully understood, although some of the key pentose cycle enzymes have been demonstrated to be present in yeast.[6,7]

In the present work, the utilization with time of specifically C^{14}-labeled glucose has been followed using bakers' yeast in a medium that permitted cell proliferation. The specific activity and the recovery of respiratory CO_2 has been observed as well as the incorporation of activity into cellular constituents. The respiratory patterns point to extensive operation of glycolysis and the tricarboxylic acid cycle. Calculations indicated that about 13% of the administered glucose was metabolized by phosphogluconate decarboxylation. Up to 10% of the pyruvate originating from glucose was involved in CO_2 fixation. Efficiency of the utilization of acetate methyl and carboxyl carbon atoms in biosynthesis also was estimated.

Experimental

The apparatus used in the time course studies consisted essentially of a 3-necked round-bottom 500-ml. flask provided with a gas sparger, a sampling tube and a gas outlet tube which was connected to a gas scrubber containing 1 *N* CO_2-free NaOH solution for the collection of respiratory CO_2. In a typical experiment, 100 mg. (dry weight) of yeast cells previously grown on malt medium were resuspended in 200 ml. of salt medium and transferred into each of a set of growth flasks maintained at 30°. The cells were starved for two hours under vigorous aeration. To each flask was added a prescribed amount of glucose-1,2,6, or u-C^{14} having equal specific activity. One hundred and fifty ml. of sterile CO_2-free air per minute was introduced into the flask through the inlet tube. The respiratory CO_2 was thus swept through outlet tube into NaOH trap. The glucose content of the medium as well as the activity incorporated into the cells was determined occasionally by removing a 2-ml. aliquot of the yeast suspension through the sampling tube. The NaOH solution was replaced every half hour in the earlier phase and every hour after the administered glucose was exhausted from the medium. Usually a battery of five flasks was used in each series and a flowmeter was used in the sweeping train to ensure a uniform air flow rate through each flask. Respiratory CO_2 was recovered from the NaOH trap as $BaCO_3$ by means of $BaCl_2$–NH_4Cl precipitation, followed by quantitative plating (centrifuging) on aluminum planchets. The activities of the $BaCO_3$ plates were counted using an end-window GM counter. Corrections for background and self-absorption were applied in the conventional manner.

The yeast samples collected during the experiment were counted by direct-plating technique and subsequently dried *in vacuo*. Each sample was hydrolyzed by autoclaving with 6 *N* HCl for 10 hours in a sealed tube. The hydrolyzed samples thus obtained were filtered to remove humin, dried in a vacuum desiccator in the presence of P_2O_5 and KOH pellets, and the residue dissolved in a prescribed amount of water. Aliquots of this solution were taken for radioactivity assay, paper chromatographic separation and radioautography of amino acids.

Results and Discussion

Nature of Catabolic Pathways in Glucose Breakdown.—The time course plot of chemical and radiochemical recoveries of carbon (as $BaCO_3$), and interval specific activities of CO_2 from C^{14} specifically labeled glucose metabolized by proliferating yeast cells are given in Sections A, B and C of Fig. 1. The dotted line represents the rate of disappearance of glucose from the medium as determined by chemical assay. Inasmuch as the administered glucose was exhausted from the medium in slightly over four hours in these experiments, the fraction of each curve covering the remainder of the experiment is termed "depletion" under the prevailing conditions.

During the active assimilation period (0–5 hours), it is evident that an appreciable amount of glucose C-1 activity has appeared in the respiratory CO_2, in contrast to much lower labeling from C-2 of glucose and practically none from C-6. This is apparently the result of 6-phosphogluconate decarboxylation, which converts C-1 of glucose directly to CO_2. The pathway which is responsible for the conversion of C-2 of glucose to CO_2 in this period cannot be definitely identified in the present experiment, since

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(2) Presented in part at the 128th Meeting of the American Chemical Society, Minneapolis, Minnesota, September, 1955.

(3) Taken in part from the M.S. thesis of C. T. Gregg, Oregon State College, 1955.

(4) J. J. Blumenthal, K. F. Lewis and S. Weinhouse, THIS JOURNAL, **76**, 6093 (1954).

(5) H. Beevers and M. Gibbs, *Nature*, **173**, 640 (1954).

(6) E. Racker, "Advances in Enzymology," Interscience Publishers, Inc., New York, N. Y., 1954, pp. 141–178.

(7) I. C. Gunsalus, B. L. Horecker and W. A. Wood, *Bact. Rev.*, **19**, 79 (1955).

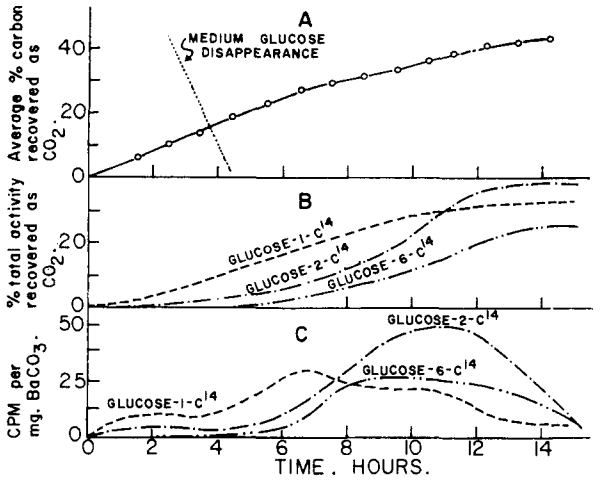

Fig. 1.—Chemical, radiochemical and interval specific activities of respiratory $C^{14}O_2$.

this carbon atom can be burned to CO_2 by way of either the pentose cycle or the citric acid cycle following the Embden–Meyerhof pathway. However, in view of the low specific activity of CO_2 in the early phase of glucose-2-C^{14} experiment, the operation of the pentose cycle could not have been extensive. As would be expected, C-6 of glucose is conserved during active growth; this reflects the preferential utilization of C-3 of pyruvate in the biosynthesis of amino acids, and also indicates that the formation of hexose through recombination of trioses is limited.

The specific activity of respiratory CO_2 in the glucose-1-C^{14} experiment remained at a constant level throughout the assimilation period and gradually increased during depletion to a peak value at 6.5 hours. The rise in CO_2 specific activity was evidently the result of extensive glycolysis and citric acid cycle oxidation, since a parallel rise appeared in the specific activities of CO_2 from glucose-1-C^{14}, glucose-2-C^{14} and glucose-6-C^{14}.

A comparison of the specific activities of CO_2 from glucose-2-C^{14} and glucose-6-C^{14} in the early phase of the depletion period (*i.e.*, 5–8 hours), also provides some interesting information on terminal respiration processes in yeast. Since the bulk of the glucose was metabolized by way of glycolysis, it is reasonable to visualize that the key intermediate, pyruvate, would be labeled in the methyl carbon atom from C-1 and 6; the carbonyl carbon atom from C-2 and 5; and the carboxyl carbon atom from C-3 and 4 of glucose. Similarly, acetate would derive its methyl carbon from glucose C-1 and 6 and its carboxyl carbon from C-2 and 5 of glucose. Ehrensvärd and co-workers,[8] using doubly labeled acetate as the sole carbon source in yeast, reported that the relative contribution of the carboxyl carbon and the methyl carbon to respiratory CO_2 was approximately 2:1, again reflecting conservation of C-1 and 6 (acetate methyl). In the present study, if pyruvate were channeled into the citric acid cycle *via* acetate during the depletion period, one would thus have expected a ratio of 2:1 from glucose-2-C^{14} and glucose-6-C^{14}, respectively, in the formation of $C^{14}O_2$. The ratio of 1.2:1 observed up to the eighth hour (section B, Fig. 1) points to a partial conservation of C-2 of glucose and renders support to our previous finding that instead of exclusive decarboxylation of the pyruvate to acetate, some of the pyruvate derived from glucose could have participated in C_4 acid formation by way of CO_2 fixation. The C_4 acid (oxalacetate or malate) thus formed would then have its two center carbon atoms derived from glucose C-2 and 5 and C-1 and 6 which are equivalent to each other in the operation of a Krebs cycle. On the other hand, during extensive depletion of the cells (8–11 hours, Fig. 1) the previously formed C_4 acids could have formed acetate units by way of double decarboxylation of oxalacetate. Such acetate would be similar in labeling pattern to that derived from glucose directly and hence would give rise to higher specific activity in CO_2 from glucose-2-C^{14} than from glucose-6-C^{14}. Eventually the ratio of 2:1 would be reached as discussed previously. In the present experiment, the specific activity of CO_2 from glucose-6-C^{14} began to level off at the eighth hour while that from glucose-2-C^{14} continued to rise to its peak value shortly after 11 hours. At this point the ratio of these two activities was indeed very close to a value of 2:1.

It is interesting to note that the specific activities of respiratory CO_2 in all experiments fell to a very low value at 14 hours. At this point, the cumulative radiochemical yield of metabolic CO_2 was 38.6, 33.0 and 25.2%, respectively, from glucose-2,1, and 6-C^{14}. The relatively low recovery of activity indicates that much of the substrate carbon was incorporated into cellular constituents which were not readily available for respiration.

Estimation of Pathways of Glucose Catabolism.—In order to determine the contribution of individual pathways in the over-all glucose catabolism, it is desirable to know the time required to consume all of the substrate glucose within the cells. This should coincide with the disappearance of C-3 of glucose in the respiratory CO_2. Values for C-3 are not available directly, but they can be calculated if the following assumptions are made.

(1) The administered glucose is metabolized under the experimental conditions either by phosphogluconate decarboxylation or the Embden–Meyerhof pathway, in combination with the citric acid cycle.[4]

(2) The incorporation of glucose in the polysaccharide fraction of cells is limited.[9]

(3) The removal of C-1 from glucose through phosphogluconate decarboxylation is a rapid process.

(4) The pyruvate formed in glycolysis is decarboxylated promptly, except for the portion involved in CO_2 fixation.

(5) The pentose formed from phosphogluconate decarboxylation is only slightly metabolized, by way of either the pentose cycle or degradation to C_2 and C_3 intermediates.

(6) The trioses formed in the glycolytic process are equivalent to each other in respect to further metabolic reactions.

(8) J. Baddiley, G. Ehrensvärd, R. Johansson, L. Reio, E. Saluste and R. Stjernholm, *J. Biol. Chem.*, **183**, 771 (1950).

(9) J. C. Sowden, S. Frankel, B. H. Moore and J. E. McClary, *ibid.*, **206**, 547 (1954).

TABLE I

UTILIZATION OF GLUCOSE CARBON ATOMS IN RESPIRATORY AND BIOSYNTHETIC FUNCTIONS

110 mg. of yeast cells incubated at 30° in NH_4-salt medium containing 400 mg. of labeled glucose (sp. act. 5.4 × 10^4 c.p.m. per mM of glucose).

Substrate		Glucose-1-C^{14}		Glucose-2-C^{14}		Glucose-6-C^{14}		Glucose-u-C^{14}		Glucose-3-C^{14}[a]	
		Sp. act., c.p.m./ mM carbon × 10^3	Recovery, c.p.m. × 10^3	Sp. act., c.p.m./ mM carbon × 10^3	Recovery, c.p.m. × 10^3	Sp. act., c.p.m./ mM carbon × 10^3	Recovery c.p.m. × 10^3	Sp. act., c.p.m./ mM carbon × 10^3	Recovery, c.p.m. × 10^3	Sp. act., c.p.m./ mM carbon × 10^3	Recovery, c.p.m. × 10^3
	Hr.										
Respiratory CO_2	5[b]	4.68	20.1	5.39	19.6	1.71	5.82	8.62	38.6	21.7	87.5
	9[c]	5.35	27.5	6.51	31.3	3.01	12.6	8.70	48.8	19.3	95.1
	13.5[d]	5.68	32.0	8.68	46.8	3.98	19.9	8.64	53.3	19.3	95.1
	Hr.	Sp. act., c.p.m./mg.		Sp. act., c.p.m./mg.		Sp. act., c.p.m./mg.		Sp. act., c.p.m./mg.		Sp. act., c.p.m./mg.	
Cells	5[b]	126		118		142		46			
	13.5[d]	213		152		254		154			

[a] Calculated values—equation 1. [b] Glucose exhausted from the medium. [c] Glucose exhausted from the cells. [d] Termination of experiment.

(7) The extent of formation of hexose from trioses is slight.

Assumptions (3), (5) and (7) are supported by the specific activity curves given in Fig. 1C as discussed in the previous sections.

On the basis of these assumptions, it is then possible to calculate the interval recovery of C-3 of glucose in metabolic CO_2 according to the equation

$$G_3 = \frac{6G_u - (G_1 + 2G_2 + G_6)}{2} \qquad (1)$$

where G_u, G_1, G_2 and G_6 represent the activity recovered in metabolic CO_2 from equal levels of glucose-1,2,6, and uniform-C^{14}, respectively.

It is reasonable to expect that the exhaustion of intact glucose in the cells would be reflected directly by the disappearance of C-3 of glucose in the respiratory CO_2. This disappearance would be registered as a maximum on the time course curves of cumulative values of G_3, calculated from equation 1.

In Fig. 2 are given the typical rates of radiochemical recoveries of carbon as $BaCO_3$ from various C^{14}-labeled glucose samples. The recoveries of C-3 of glucose are calculated according to equation 1. The dotted line in the figure represents the rate of disappearance of glucose from the medium in these experiments.

The calculation of the relative contribution of the metabolic pathways is derived on the previously given assumptions and expressed in the following manner:

Let T = total activity of glucose-1,2,6 or u-C^{14} administered to the medium. G_1', G_2', G_3' and G_6' = total activity recovered in metabolic CO_2 of yeast cells utilizing the respectively labeled glucose up to the time of complete exhaustion of administered glucose (Fig. 2). G_3' values are calculated according to equation 1.

Contribution of Phosphogluconate Decarboxylation.—Net activity recovered as CO_2 from glucose-1-C^{14} by phosphogluconate decarboxylation = $G_1' - G_6'$. Fraction of glucose metabolized by the phosphogluconate decarboxylation, designated G_p

$$G_p = \frac{G_1' - G_6'}{T} \qquad (2)$$

Contribution from Embden–Meyerhof Pathway.—Fraction of glucose metabolized by the glycolytic pathway, designated as G_e, is

$$G_e = 1 - G_p \qquad (3)$$

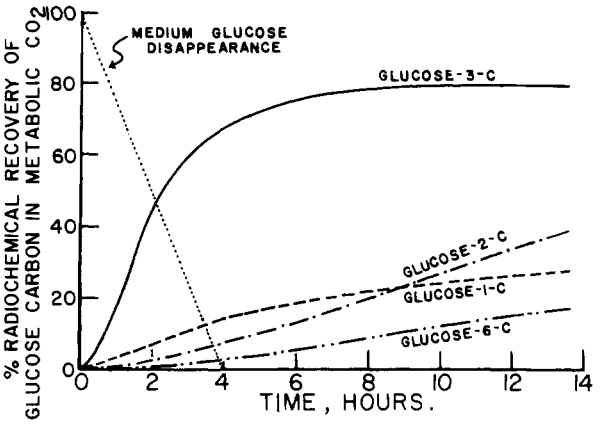

Fig. 2.—Cumulative radiochemical recovery of labeled glucose carbon atoms in metabolic CO_2.

Fate of Pyruvate.—Pyruvate decarboxylated (fraction of pyruvate decarboxylated oxidatively to acetate) = P_d

$$P_d = G_3'/TG_e \qquad (4)$$

Pyruvate fixation (fraction of pyruvate participating in CO_2 fixation) = P_f

$$P_f = 1 - P_d \qquad (5)$$

Fate of Acetate.—Respiration due to carboxyl group (fraction of acetate carboxyl converted to CO_2) = R_c

$$R_c = G_2'/G_3' \qquad (6)$$

Synthesis from carboxyl group (fraction of acetate carboxyl utilized in biosynthesis) = S_c

$$S_c = 1 - R_c \qquad (7)$$

Respiration due to methyl group (fraction of acetate methyl converted to CO_2) = R_m

$$R_m = G_6'/G_3' \qquad (8)$$

Synthesis from methyl group (fraction of acetate methyl utilized in biosynthesis) = S_m

$$S_m = 1 - R_m \qquad (9)$$

In Table I is given a typical recovery of activity in the respiratory CO_2 and cells, at the time of complete breakdown of the administered glucose defined previously. Using these data, one may estimate that

TABLE II

RELATIVE RADIOACTIVITIES OF YEAST AMINO ACIDS[a]

Amino acids	Time in minutes 7	15	30	60	120	180	240	360	480
Aspartic acid		+	+++	++	+	+	+	+	+
Glutamic acid		+	+++	+++	+++	++++	++++	++	+
Proline		+	+	+	+	+	+	+	+
Glycine–serine		+	+	+	+	+	+	++	++
Alanine		+	+	+	+	+	+	+	+
Threonine				+	+	+	+		
Phenylalanine		+	+	+	+	+	+	+	+
Tyrosine		+	+	+	+	+	+	+	+
Valine					+	+	+		
Arginine				+	+	+	+	+	+
Lysine			+	+	+	++	+++	+++	+++

[a] Total radioactivity as determined by radioautography: ++++, very heavily labeled; +++, heavily labeled; ++ moderately labeled; +, detectable; substrate: glucose-u-C^{14} in NH_4-salt medium.

fraction of glucose metabolized glycolytically	= 87%
fraction of glucose metabolized through phosphogluconate decarboxylation	= 13%
fraction of pyruvate degraded to acetate	= 90%
fraction of pyruvate utilized in C_4 synthesis	= 10%
fraction of acetate carboxyl carbon utilized in biosynthesis	= 72%
fraction of acetate methyl carbon utilized in biosynthesis	= 88%

The value of G_e so obtained is in good agreement with that observed by Blumenthal, Lewis and Weinhouse,[4] using a different method. In view of the simplicity, reproducibility and the kinetic information provided by the present approach it is believed that it will prove useful in identifying and estimating catabolic pathways of carbohydrates in various species of microörganisms.

The Incorporation of Glucose Carbon into Amino Acids.—In Table II are given the relative total radioactivities of yeast amino acids derived from glucose-u-C^{14}. Activity appeared in seven amino acids, particularly aspartic and glutamic acid and alanine, 15 minutes after the administration of the labeled substrate. This is indicative of the rate of a sequence of reactions including glycolysis, the citric acid cycle and transamination. The much heavier labeling level in glutamic acid supports the previous finding[10] that carbon reserves may be accumulated as glutamic acid in yeast cells during glucose assimilation. The labeling in these amino acids appeared in line with the respective known biosynthetic pathways in this organism.

(10) C. H. Wang, B. E. Christensen and V. H. Cheldelin, *J. Biol. Chem.*, **201**, 683 (1953).

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[CONTRIBUTION NO. 2043 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

The Effect of Added Sucrose Upon the α-Chymotrypsin-catalyzed Hydrolysis of Chloroacetyl-L-tyrosinamide in Aqueous Solutions at 25° and *p*H 7.75[1]

BY HENRY J. SHINE AND CARL NIEMANN[2]

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The initial rate of the α-chymotrypsin-catalyzed hydrolysis of chloroacetyl-L-tyrosinamide in aqueous solutions at 25° and *p*H 7.75 and 0.02 *M* in the THAM component of a THAM-HCl buffer is increased by the addition of 0.5 *M* sucrose and it has been shown that the behavior of the above system in the presence of 0.5 *M* sucrose is qualitatively the same as that observed previously for the same system in the presence of 0.3 or 0.4 *M* sodium chloride.

In a recent communication from these laboratories[3] it was shown that the initial rate of the α-chymotrypsin-catalyzed hydrolysis of chloroacetyl-L-tyrosinamide in aqueous solutions at 25° and *p*H 7.75 and 0.02 *M* in the THAM[4] component of a THAM–HCl buffer is increased by the addition of either sodium or potassium chloride and that the increase in initial velocities is given by the relation $\log (v_0/v_0^0) = 0.30 \pm 0.01\sqrt{M}$ for values of *M*, *i.e.*, the molarity of the reaction system with respect to added sodium or potassium chloride, up to and possibly exceeding 1.5 *M*. Furthermore, from a study of the effect of 0.3 and 0.4 *M* sodium chloride in systems in which the concentration of the specific substrate was varied, it was found that the value of K_S was essentially independent of the concentration of the added sodium chloride at the two levels investigated, whereas the value of k_3 was increased by the amount given by the relation $\log (k_3/k_3^0) = 0.30 \pm 0.01\sqrt{M}$. Since it was not obvious that the effects noted above were to be interpreted solely in terms of the properties of electrolytes, it was decided to examine the consequences of the addition of several non-electrolytes such as sucrose, glucose and fructose to the same basic reaction system. The results of this latter investigation are the subject of this communication.

(1) Supported in part by a grant from Eli Lilly and Co.

(2) To whom inquiries regarding this article should be sent.

(3) H. J. Shine and C. Niemann, THIS JOURNAL, **77**, 4275 (1955).

(4) Tris-(hydroxymethyl)-aminomethane.