CARBOHYDRATE ASSIMILATION IN ACTIVELY GROWING YEAST, *SACCHAROMYCES CEREVISIAE*

I. METABOLIC PATHWAYS FOR [^{14}C]GLUCOSE UTILIZATION BY YEAST DURING AEROBIC FERMENTATION

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SUMMARY

The metabolic pathways for [^{14}C]glucose utilization by the actively growing yeast, *Saccharomyces cerevisiae*, was investigated in a shaker flask fermentation system, which permitted maximum efficiency of yeast growth. A truly aerobic fermentation can be obtained only above an effective aeration rate of 50 mmoles O_2/l/h under the experimental conditions employed. Various ^{14}C-labeled glucose substrates were fed continuously into the fermentation flasks. The existence of a uniform glucose metabolism pattern throughout the entire course of aerobic fermentation has been demonstrated. In other words, the true metabolism pattern, not the overall pattern, was investigated.

During the aerobic fermentation (*i.e.* 69 mmoles O_2/l/h) by the actively growing yeast cells, 58 to 60 % of the glucose carbon was assimilated into the yeast cells, while 40 to 42 % was metabolized into CO_2. About 6 % of the total metabolic CO_2 produced was estimated to be derived via the hexose monophosphate shunt, 51 % derived from the decarboxylation of pyruvate and 43 % from the TCA-oxidative pathway. The effect of aeration on the quantitative significance of these pathways was also investigated. The fractions of the metabolic CO_2 derived by hexose monophosphate shunt remain quite constant at lower aeration levels, *i.e.* 4.4 % at 15 mmoles O_2/l/h and 4.3 % at 7 mmoles O_2/l/h, while that produced by pyruvate decarboxylation increases substantially, namely 80 % and 95 %, respectively. As expected, more ethanol was produced at the lower aeration levels. However, the specific radioactivity of the ethanol produced at these two aeration levels was about the same. The application of WEINHOUSE's method, based upon the relative specific radioactivity of the isolated ethanol, for the evaluation of the quantitative significance of various catabolic pathways of glucose was discussed.

INTRODUCTION

A number of papers have recently been published on the pathway of glucose catabolism by yeast[1-4]. However, most of the experiments reported were either performed under

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conditions where little growth could have taken place or with resting cells. In this study, the metabolic pattern for glucose utilization by actively growing yeast cells was investigated.

In order to achieve maximum efficiency in yeast propagation, an adequate supply of nutrients, proper pH control and other generally recognized conditions have to be provided. However, it is also essential to meet the following two requirements: (1) adequate aeration and (2) low sugar content in the medium[5,6]. Failure to fulfil these requirements favors anaerobic fermentation and thereby decreases the efficiency of yeast growth. To observe the first requirement, the amount of oxygen actually dissolved in the liquid medium must be measured and controlled. Frequently, only air flow rate was reported as a measure of the aeration level in the growth medium. This is inadequate, since the amount of oxygen dissolved depends also upon the air bubble size, volume and height of the growth medium in the fermenter, etc. The second requirement may be fulfilled by feeding sugar continuously into the fermentation vessel at a rate proportional to the yeast population in the medium so that no excess sugar is accumulated at any time during the course of aerobic fermentation. It is under these conditions that the metabolic pathways for glucose utilization by the actively growing yeast were investigated and reported in this communication.

METHODS

Fermentation system

In order to avoid passing through the growth medium a large volume of air required to achieve the desirable aeration levels, a shaker flask fermentation system was used[7]. With this system, different aeration levels were readily obtained by changing the number and the position of the indentations on the flask wall. Owing to the vigorous agitation of the medium in the flasks, only a moderate rate of air flow through the stoppered flasks was required for proper aeration.

The yeast cells were grown in 1-liter Florence flasks. Each flask was charged with 175 ml of growth medium, the composition of which was modified from PEPPLER[8] as follows: glucose 160 mg, diammonium citrate 1250 mg, sodium succinate ($Na_2C_4H_4O_4 \cdot 6H_2O$) 772 mg, succinic acid 71 mg, $(NH_4)_2SO_4$ 98 mg, KCl 81 mg, $MgSO_4 \cdot 7H_2O$ 103 mg, $NH_4H_2PO_4$ 46 mg, $ZnSO_4$ 1.6 mg, $FeSO_4(NH_4)_2SO_4$ 880 μg, $CuSO_4$ 65 μg, $MnSO_4 \cdot H_2O$ 65 μg, inositol 8 mg, pyridoxine·HCl 1 mg, thiamine·HCl 1 mg, calcium pantothenate 1 mg, biotine 25 μg. The final pH of the medium was 5.15. Each of these shaker flasks was then seeded with 0.5 g of yeast (*Saccharomyces cerevisiae*, R.S. No. 53) and the experiment started. After shaking for 30 min, various ^{14}C-labeled glucose substrates were continuously fed into these flasks by means of cam-driven syringes according to a predetermined rate, which was proportional to the yeast population in the flasks. During a 12 h fermentation period, a total of 24.0 ml of sugar solution, containing 21.27 mmoles of [^{14}C]glucose was fed into each flask.

Absorption of metabolic CO_2

The metabolic CO_2 produced during aerobic fermentation was absorbed through fritted gas dispersion cylinders (Corning No. 39533) in NaOH solution. The absorber, which contained 30 ml of 5 *N* NaOH, was changed at hourly intervals. A constant air flow rate of 180 ml/min through the stoppered fermentation flasks was maintained during the fermentation period both for aeration and for the removal of metabolic

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CO_2 from the flasks. The collected CO_2 was determined gravimetrically following NH_4Cl–$BaCl_2$ precipitation.

Effective aeration of the growth medium

The effective aeration rate was measured by the sulfite oxidation method of COOPER *et al.*[9].

Carbohydrate determination

Carbohydrates were determined by an anthrone method modified from FALES[10] as follows: Fresh anthrone reagent was prepared each day by dissolving 0.4 g of anthrone in 200 ml of concentrated H_2SO_4. The acid solution was carefully added to 60 ml of distilled water in an ice bath. To 1 ml of sample solution, containing not more than 200 μg of glucose equivalent, 10 ml of anthrone reagent was added and mixed immediately. The mixture was heated in a boiling water bath for exactly 10 min, at the end of which it was cooled in an ice bath for another 10 min and read at 620 mμ[11].

Glucose determination

Glucose was determined by the method of SOMOGYI[12].

Ethanol determination

Ethanol, which was collected from the distillate of the neutralized fermentation medium and from the concentrated H_2SO_4-trap along the CO_2-absorption train, was determined by a $K_2Cr_2O_7$–H_2SO_4 oxidation method[6].

Carbon determination in yeast

The carbon content in the yeast samples was determined both by a wet combustion method with VAN SLYKE–FOLCH combustion mixture[13] and a persulfate oxidation method[14].

Radioactivity measurement

Radioactivity was measured with a thin end-window Geiger counter. All samples were measured in the form of $BaCO_3$. Self-absorption was corrected with a predetermined correction curve.

Specific radioactivity of hexoses and ethanol

The specific activities of these compounds were determined after their complete oxidation to CO_2 by the persulfate oxidation method of KATZ *et al.*[15].

Preparation of [3,4-^{14}C]glucose

[3,4-^{14}C]Glucose was prepared from rat liver glycogen[16,17]. Two white rats (218 g and 228 g) were fasted for 48 h before each was given 400 mg of glucose by stomach tube. One-half ml of $NaH^{14}CO_3$ was then injected intraperitoneally at 30-min intervals. A total of six injections, equivalent to 0.5 mC, were given to each rat. Half an hour after the last injection, the animals were sacrificed. Livers were removed and dissolved in 50 ml of boiling 30 % KOH solution. After boiling for 3 h, the solution was filtered through glass wool. Rat liver glycogen was precipitated with 1.2 vol. of ethanol. The precipitate was then removed by centrifugation and extracted with 10 % trichloro-

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acetic acid. Glycogen was again precipitated from the acid solution with 1.2 vol of ethanol. The purified glycogen was removed by centrifugation and dissolved in distilled water. Undissolved matter was filtered off. Glycogen was then hydrolyzed to glucose with 0.5 N H_2SO_4 for 24 h at 100°. The sulfate ions were removed from the hydrolysate with $Ba(OH)_2$.

EXPERIMENTAL RESULTS

Effect of aeration on the growth of yeast

As previously stated, the growth efficiency of yeast is greatly influenced by the effective aeration in the medium. It is, therefore, necessary to establish quantitatively the effect of aeration on the yeast growth under the experimental conditions employed. Different aeration levels were achieved by changing the number and the position of the indentations on the shaker flask wall, while keeping the air flow rate constant, *i.e.*

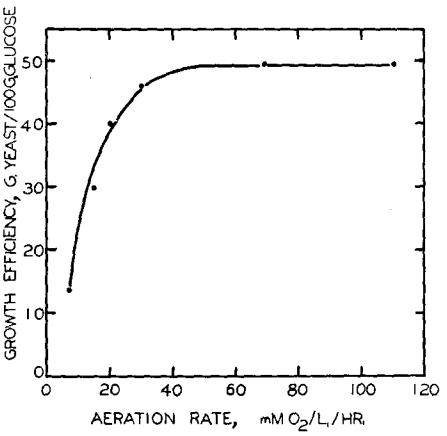

Fig. 1. Effect of aeration rate on the growth efficiency of yeast in shaker fermentation.

180 ml/min. The effect of aeration on the growth efficiency is shown in Fig. 1. Under our experimental conditions, maximum growth efficiency is obtained above an aeration rate of 50 mmoles O_2/l/h. In other words, a truly aerobic fermentation takes place only above this aeration level.

Uniformity of glucose metabolism pattern during aerobic fermentation

In order to ascertain that we are investigating the true metabolic pattern, not the overall pattern, it is necessary to know if one definite pattern prevails throughout the entire course of aerobic fermentation. Such information may be provided by determining the specific radioactivity of the metabolic CO_2 collected at regular time intervals and also the molar ratio of metabolic CO_2/glucose fed. In a typical aerobic fermentation experiment (effective aeration rate = 69 mmoles O_2/l/h), fed continuously with [1-^{14}C]glucose solution as previously described, the specific radioactivity of the metabolic CO_2 collected at hourly intervals was quite constant, 3606* ± 252

* Owing to the presence of a small quantity of unlabeled glucose originally in the growth medium, the specific radioactivity of CO_2 collected during the first hour and a half was omitted in this calculation.

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counts/min/mmole CO_2, and so was the molar ratio of metabolic CO_2/glucose fed, 2.40 ± 0.16. These results indicate the existence of a definite and constant metabolic pattern under conditions of the continuously-fed aerobic fermentations.

Re-absorption of metabolic CO_2 by yeast during aerobic fermentation

Since our interpretation of the metabolic pathways for glucose utilization is based mainly upon the radioactivity of the metabolic CO_2 liberated, it is essential to know to what extent metabolic CO_2 is re-absorbed by the yeast cells during aerobic fermentation. This problem was investigated in an experiment similar to the one described in the last section, except that the ammonium citrate in the growth medium was replaced with [^{14}C]urea. A total of 5.50 mmoles of [^{14}C]urea, containing a radioactivity equivalent to 463,000 counts/min, was added both continuously and to the medium in each flask. Unlabeled glucose was fed in this experiment. At the end of the experiment, over 98% (*i.e.* 457,000 counts/min) of the radioactive carbon from [^{14}C]urea was recovered in the metabolic CO_2. Only 1% of the supplied radioactivity was found in the yeast cells. These results show that during aerobic fermentation, the re-absorption of metabolic CO_2 and hence, its effect on the interpretation of the metabolic pathway for glucose utilization, was negligible.

The origin of metabolic CO_2

The origin of the metabolic CO_2 produced by yeast during aerobic fermentation was investigated by feeding continuously various [^{14}C]glucoses at a predetermined rate as previously described. In the case of [1-^{14}C]glucose and [6-^{14}C]glucose, a total of 21.27 mmoles of the labeled sugar, containing a similar amount of radioactivity (517,000 and 521,000 counts/min respectively), was fed into each shaker flask over a period of 12 h. The metabolic CO_2 liberated was collected hourly and analyzed. At the end of the experiment, yeast was harvested. The ethanol in the medium and in the H_2SO_4-trap was also collected and analyzed. For [3,4-^{14}C]glucose, the experiment was scaled down to one-tenth of the regular level, due to the limited supply of this compound.

TABLE I

AEROBIC FERMENTATION OF [^{14}C]GLUCOSE BY YEAST: CARBON BALANCE

Expt.	*[^{14}C]Glucose fed*	*Growth* efficiency*	*Carbon recovery*					
			*mmoles C** fed as glucose*	*mmoles CO_2 liberated*	*mmoles C in yeast*	*mmoles C in EtOH*	*mmoles C recovered*	*% recovery*
1	[1-^{14}C]Glucose	49.8	132.96	53.93	79.13	0.46	133.52	100.4
2	[6-^{14}C]Glucose	48.2	132.96	55.56	76.43	0.30	132.29	99.5
3	[3,4-^{14}C]Glucose	45.2	13.30	5.69	7.24	—	12.93	97.3

* g of yeast solids produced/100 g glucose fed. The effective aeration rate was 69 mmoles O_2/l/h.
** For Expts. 1 and 2, 0.89 mmoles unlabeled glucose was added to the medium at the beginning of the experiment. 21.27 mmoles of ^{14}C-labeleled glucose was then fed continuously into the fermentation flask during fermentation. For Expt. 3, one-tenth of these quantities was used.

The carbon and the radioactivity balances in these experiments are summarized in Tables I and II respectively. Since neither succinate nor citrate in the medium

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could be metabolized by the yeast cells[18,19], the presence of these compounds did not interfere with the calculations of the carbon balance. The results of Table I showed that the carbon balance in all experiments was satisfactory, *i.e.* 97–100 %. About 40 to 42 % of the glucose carbon was metabolized into CO_2, while 58 to 60 % was assimilated into the yeast cells. Very little ethanol was produced. The radioactivity recoveries in these experiments were also satisfactory, *i.e.* 97 to 100 % (Table II).

TABLE II

AEROBIC FERMENTATION OF [^{14}C]GLUCOSE BY YEAST: RADIOACTIVITY BALANCE

Expt.	[^{14}C]*Glucose fed*	*Radioactivity recovery*					
		counts/min fed in glucose	*counts/min in CO_2*	*counts/min in yeast*	*counts/min in EtOH*	*Total counts/min recovered*	*% recovery*
1	[1-^{14}C]Glucose	517,000	192,940	308,800	2,000	503,740	97.3
2	[6-^{14}C]Glucose	521,000	123,580	395,000	1,660	520,240	99.8
3	[3,4-^{14}C]Glucose	22,000	13,670	8,230	—	21,900	99.5

In a typical aerobic fermentation experiment, such as Expts. 1 and 2 of Table I, a total of 133 mmoles of carbon in the form of glucose was supplied to each fermentation flask. Thus, each carbon atom of the glucose molecule accounted for 133/6 = 22.2 mmoles. The total quantity of the metabolic CO_2 produced in these experiments was found to be 54 to 55 mmoles (Table I). In Expt. 1 with [1-^{14}C]glucose substrate, 38.4 % of the C^1 of the glucose molecule was recovered in the metabolic CO_2 (Table III), and thus accounted for $22.2 \cdot 0.384 = 8.55$ mmoles of the metabolic CO_2 produced. The results in the same table show that in Expt. 2 with [6-^{14}C]glucose substrate, 23.6 % of the C^6 was recovered as CO_2, and hence accounted for $22.2 \cdot 0.236 = 5.24$ mmoles of the total CO_2 liberated. From Expt. 3 with [3,4-^{14}C]glucose substrate, we found that 62.4 % of C^3 and C^4 was recovered as metabolic CO_2. Thus, either C^3 or C^4 accounted for $22.2 \cdot 0.624 = 13.85$ mmoles of the metabolic CO_2, since this sugar was symmetrically labeled[17]. Altogether, these four carbon atoms of the glucose molecule accounted for 41.49 mmoles of the total metabolic CO_2 produced. The difference, *i.e.* $54 - 41.49 = 12.51$ mmoles CO_2, was contributed by the C^2 and C^5. On the assumption that two identical trioses were formed during glycolysis, C^2 and C^5 were expected to be identically metabolized. Thus, each of these two carbon atoms accounted for 6.25 mmoles of the metabolic CO_2 produced.

TABLE III

RADIOCHEMICAL YIELD AND SPECIFIC RADIOACTIVITY IN METABOLIC CO_2 PRODUCED DURING AEROBIC FERMENTATION OF [^{14}C]GLUCOSE BY YEAST

Expt.	[^{14}C]*Glucose fed*	*Radiochemical* yield in CO_2*	*Spec. act. per mmole C in glucose counts/min*	*Spec. act. per mmole CO_2 counts/min*
1	[1-^{14}C]Glucose	38.4	4050	3580
2	[6-^{14}C]Glucose	23.6	4070	2210
3	[3,4-^{14}C]Glucose	62.4	1720	2400

* % of total radioactivity recovered in metabolic CO_2.

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Metabolism of glucose during sub-aerobic fermentation

As shown in a previous section, lower aeration rates resulted in lower growth efficiency in yeast (Fig. 1). As expected, the amount of ethanol produced increased correspondingly. Some of the results on the sub-aerobic fermentation by yeast are summarized in Table IV. It may be pointed out that the specific radioactivity of the metabolic CO_2 produced with both [1-^{14}C]glucose and [6-^{14}C]glucose substrates decreased rapidly with the aeration rates. In other words, during sub-aerobic fermentation, less metabolic CO_2 was derived from C^1 and C^6 of the glucose molecule. It is also interesting to note that the specific radioactivities of the ethanol produced under different aeration levels were relatively constant, about 9,700 counts/min/mmole EtOH with [1-^{14}C]glucose (Expts. 4 and 5, Table IV) and 11,500 counts/min/mmole EtOH with [6-^{14}C]glucose substrate (Expts. 6 and 7, Table IV).

TABLE IV

METABOLISM OF [^{14}C]GLUCOSE BY YEAST DURING SUB-AEROBIC FERMENTATION

Expt.	[^{14}C]*Glucose fed**	*Effective aeration (mmoles O_2/l/h)*	*Growth efficiency*	*Metabolic** CO_2 *(mmoles)*	*Spec. act. per mmole CO_2 counts/min*	*Radiochemical yield in CO_2*	*EtOH produced (mmoles)*	*Spec. act. per mmole EtOH counts/min*
4	[1-^{14}C]Glucose	15.0	30.6	48.60	1886	17.70	13.82	9,798
5	[1-^{14}C]Glucose	7.0	13.7	34.07	1025	6.77	28.60	9,626
6	[6-^{14}C]Glucose	15.0	32.3	47.80	885	8.19	13.76	11,900
7	[6-^{14}C]Glucose	7.0	12.7	33.50	46	0.29	28.07	11,460

* For the quantity of [^{14}C]glucose fed, see footnote Table I.

** Specific radioactivity of [1-^{14}C]glucose substrate: 4,050 counts/min/mmole carbon in glucose or 24,300 counts/min/mmole glucose. Specific radioactivity of [6-^{14}C]glucose substrate: 4,070 counts/min/mmole carbon in glucose or 24,420 counts/min/mmole glucose.

DISCUSSION

It was shown in Table I that during aerobic fermentation, about 58 to 60 % of the glucose carbon supplied was assimilated into the yeast cells, while 40 to 42 % was metabolized into CO_2 to furnish energy for various assimilation processes. Glucose substrate was known to be metabolized into CO_2 via the following pathways: (a) hexose monophosphate shunt[20], in which C^1 of the glucose molecule is liberated directly into metabolic CO_2. The fact that the radiochemical yield in the metabolic CO_2 was greater with [1-^{14}C]glucose substrate than that with [6-^{14}C]glucose substrate (Table III) indicated the participation of the hexose monophosphate shunt; (b) decarboxylation of pyruvate in which the C^3 and C^4 of the glucose molecule are liberated as CO_2[21,22]. A radiochemical yield of 62.4 % with [3,4-^{14}C]glucose substrate (Expt. 3, Table III) was in agreement with the occurrence of this pathway; (c) oxidation via the tricarboxylic acid cycle[23]. A radiochemical yield of 23.6 % with [6-^{14}C]glucose substrate was consistent with the operation of this pathway (Table III, Expt. 2).

While the demonstration of the participation of these pathways is rather straight forward, any attempt to assess their quantitative significance is subject to many sources of error, since many assumptions have to be made[24]. With these limitations in mind, an interpretation of the results is attempted so as to provide an approximation of the glucose metabolism pattern by actively growing yeast.

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On the assumption that in the hexose monophosphate shunt, CO_2 is formed only from the C^1 of the glucose molecule, the fraction of C^1 in the metabolic CO_2 derived via the "shunt" may be estimated as follows:

$$F_s = \frac{G_1 - G_6}{G_1} = \frac{38.4 - 23.6}{38.4} = 0.385 \text{ or } 38.5\,\%$$

where F_s = fraction of C^1 in the metabolic CO_2 derived via the shunt, G_1 and G_6 = radiochemical yield in the metabolic CO_2 with [1-^{14}C]glucose and [6-^{14}C]glucose substrate respectively.

It has been shown that in a typical aerobic fermentation with [1-^{14}C]glucose substrate (Table I, Expt. 1), the total amount of the metabolic CO_2 produced was 54 mmoles, of which the C^1 of the glucose molecule accounted for 8.55 mmoles or 15.8 %. Of this fraction, 38.5 % was shown above to be derived via the "shunt". Thus, the total metabolic CO_2 produced via the "shunt" is

$$CO_2^S = 0.158 \cdot 0.385 \cdot 100 = 6.1\,\%.$$

Assuming further that the hexose monophosphate shunt and the EMP-TCA* pathway account for all the metabolic CO_2 formed, the total metabolic CO_2 produced via the EMP-TCA pathway is

$$CO_2^E = 1 - CO_2^S = 93.9\,\%.$$

Judging from the results of the Expt. 2 (Table III) with [6-^{14}C]glucose substrate, it is obvious that only a fraction of the glucose molecules metabolized via the EMP-TCA pathway was completely oxidized. Considering the fraction of the metabolic CO_2 accounted for by the C^3 and C^4 is due to pyruvate decarboxylation, the fraction of the metabolic CO_2 produced via the TCA oxidative pathway may be calculated. For example, in Expt. 3 (Table III) with [3,4-^{14}C]glucose substrate, the radiochemical yield was 62.4 %. C^3 or C^4 accounted for 13.85 mmoles of the total metabolic CO_2. Thus, the metabolic CO_2 produced by pyruvate decarboxylation, CO_2^P, amounted to

$$CO_2^P = \frac{2 \cdot 13.85}{54} \cdot 100 = 51.3\,\%.$$

The difference was due to that derived from the TCA oxidative pathway. Thus,

$$CO_2^O = 100 - (6.1 + 51.3) = 42.6\,\%.$$

These results indicated that the CO_2 derived from pyruvate decarboxylation accounted for one-half of the metabolic CO_2 produced under highly aerobic conditions. As the aeration rate decreases, this pathway may assume an even greater significance. In attempting to estimate the quantitative significance of various metabolic pathways under sub-aerobic conditions, a rough approximation will have to be made, because no direct measurement has been performed with [3,4-^{14}C]glucose substrate at the lower aeration levels. For estimating the fraction of metabolic CO_2 derived via the TCA oxidative pathway at the lower aeration levels, it was assumed that, in this pathway, C^1, C^2 and C^5 were metabolized to CO_2 to a similar extent as C^6. Strictly speaking, this assumption might not be justified, but the error introduced was not excessive. For example, in the aerobic fermentations, CO_2^O, as shown above, was calculated to be 42.6 %. Based upon the assumption previously stated, this value could be estimated from the results of the experiment with the [6-^{14}C]glucose substrate alone. Thus, in Expt. 2 (Table III), the radiochemical yield in the metabolic CO_2 was

* EMP-TCA = Embden-Meyerhof-Parnas-Tricarboxylic acid cycle pathway.

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23.6 % for C^6, which accounted for 5.24 mmoles of the total metabolic CO_2 produced. From this figure, the fraction of metabolic CO_2 produced via the TCA oxidative pathway was estimated to be:

$$\text{Estimated } CO_2^O = \frac{4 \cdot 5.24}{54} \cdot 100 = 38.8\,\%$$

which agreed reasonably well with the calculated value of 42.6 %.

The effect of aeration on the quantitative significance of various metabolic pathways for glucose utilization by yeast is summarized in Table V. It is apparent from this table that as the aeration rate decreases, a greater percentage of the metabolic CO_2 is derived from the decarboxylation of pyruvate, while that derived from the TCA oxidative pathway rapidly declines. However, the fraction of the metabolic CO_2 derived via the hexose monophosphate shunt remains relatively constant, *i.e.* 4 to 6 %. Undoubtedly, at the lower aeration levels, part of the CO_2 derived from pyruvate decarboxylation is glycolytic CO_2. On the assumption that the quantity of glycolytic CO_2 produced is equivalent to that of ethanol, we have about 13.8 mmoles and 28.3 mmoles of glycolytic CO_2 produced at the following aeration levels, namely 15 mmoles O_2/l/h and 7 mmoles O_2/l/h respectively (Table IV). Thus it may be calculated that about 35 % and 88 %, respectively, of the CO_2 derived from pyruvate decarboxylation at these two aeration levels are due to glycolysis.

TABLE V

EFFECT OF AERATION ON THE QUANTITATIVE SIGNIFICANCE OF DIFFERENT PATHWAYS FOR GLUCOSE METABOLISM BY YEAST

Aeration (mmoles O_2/l/h)	*Growth efficiency*	*% metabolic CO_2 via different pathways*		
		"Shunt"	*TCA oxidation*	*Pyruvate decarboxylation*
69	49.0	6.1	42.6	51.3
15	31.0	4.4	15.1	80.5
7	13.0	4.3	0.7	95.0

Another method, based upon the determination of the relative specific radioactivities of the isolated C_2 and C_3 intermediates, has been proposed by WEINHOUSE and co-workers for the assessment of the quantitative significance of different pathways for glucose catabolism in yeast and in animal tissues[2,25]. While this method may prove to be quite convenient, its application has some inherent limitations. Firstly an evaluation based upon this method is usually a measure of the overall metabolic pattern. This is especially true in those fermentation experiments where all the labeled glucose was added at the beginning of the experiment[1-4]. When the glucose substrate was present in excess, the yeast cells assumed one metabolic pattern. But as the fermentation proceeded, sugar became depleted and another metabolic pattern might prevail. Unless the existence of a uniform glucose metabolism pattern has been demonstrated, as described in this paper, the overall metabolic pattern may deviate from the true picture. Secondly, while this method may provide information on the fraction of the C_2 compounds, such as acetate and/or ethanol, produced via the hexose monophosphate shunt as against the EMP-pathway, it does not permit any evaluation of the TCA-oxidative pathway. For example, in Expts. 4 and 5 (Table IV) with [1-^{14}C]glucose substrate, the specific radioactivity of the ethanol produced at two

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different aeration levels, *i.e.* 15 and 7 mmoles O_2/l/h, were about the same. Similar results were obtained in Expts. 6 and 7 of the same table with [6-^{14}C]glucose substrate. Based upon this method, one would conclude from these data that the fraction of the total C_2 intermediates produced via the hexose monophosphate shunt, and hence that via EMP-pathway, were about the same at both aeration levels. This conclusion is in good agreement with the results shown in Table V, in which the fractions of the metabolic CO_2 produced via the hexose monophosphate shunt were identical, *i.e* 4.4 and 4.3 % respectively, for these two aeration levels. However, the glucose catabolism pattern in these two sets of experiments was quite different. At the aeration level of 15 mmoles O_2/l/h, about 80 % of the metabolic CO_2 was shown to be derived from pyruvate decarboxylation, of which one-third was due to glycolysis, while at the lower aeration level of 7 mmoles O_2/l/h, 95 % of the metabolic CO_2 was found to be derived via this pathway (about 88 % due to glycolysis). Thus, a similar relative specific radioactivity in the C_2 and C_3 intermediate compounds does not necessarily indicate a similar glucose catabolism pattern in micro-organisms or in animal tissues, neoplastic or normal[26].

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