Importance of the Pentose Phosphate Pathway for D-Glucose Catabolism in the Obligatory Aerobic Yeast *Rhodotorula gracilis*

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D-Glucose catabolism of a phosphofructokinase-deficient yeast *Rhodotorula gracilis* has been studied. By using D-glucose specifically ^{14}C -labelled at different positions and measuring the distribution of the label in various fractions of cell metabolism, the following results were found. 1. The pentose phosphate pathway, being the main pathway of D-glucose catabolism, simultaneously converts glucose molecules into pentose phosphates oxidatively by using two NADP-linked dehydrogenases and via the non-oxidative transketolase-transaldolase pathway. 2. From the correlation of the $^{14}\text{CO}_2$ liberation and the D-glucose consumption and from the fact that the pentose phosphate moiety in nucleic acids is almost equally labelled from D-[1- ^{14}C]- and D-[6- ^{14}C]-glucose, it is concluded that of the glucose utilized about 80% undergoes transformation via the non-oxidative pentose phosphate pathway. Only about 20% of glucose is directly decarboxylated to pentose phosphate. 3. For further degradation it is postulated that the pentose phosphates are split into C_2 fragments and glyceraldehyde 3-phosphates. 4. All three loci of oxidative decarboxylation appear to be effective in *Rh. gracilis*, the oxidative part of the pentose phosphate pathway, the decarboxylation of pyruvate in the later part of the glycolytic pathway as well as the oxidation in the tricarboxylic acid cycle. 5. D-Glucose molecules taken up are only partially oxidized to CO_2 : about four-fifths of each glucose molecule metabolized is incorporated into cell constituents. 6. The quantitative interrelations of the fluxes of D-glucose subunits along the catabolic pathways have been estimated and are discussed.

The red lipid-forming yeast *Rhodotorula gracilis* is a strictly aerobic micro-organism (Kleinzeller & Slechta, 1954). The report of Brady & Chambliss (1967) on the lack of phosphofructokinase activity in several species of *Rhodotorula* brought a possible explanation for the inability of the yeast to ferment. Our experiments (Höfer, Becker, Brand, Deckner & Betz, 1969a) confirmed the absence of phosphofructokinase in *Rh. gracilis*. Consequently, the pentose phosphate pathway appears to be the key metabolic pathway for sugar breakdown in this organism. The predominance of the pentose phosphate pathway for glucose catabolism has been discussed by Höfer (1968). However, the possibility of still another catabolic pathway was left open, primarily because of the considerable incorporation of radioactivity from D-[1- ^{14}C]glucose into the cell lipid fraction.

To account for this discrepancy we have postulated that xylulose 5-phosphate is formed partially via the transketolase and transaldolase reaction of

the pentose phosphate pathway (Höfer, Betz & Becker, 1970a). In this case the label on C-1 is maintained in the pentose phosphate molecule and can be further incorporated into the cell lipids by splitting xylulose 5-phosphate to glyceraldehyde 3-phosphate and a C_2 unit and by the action of the glyoxylate shunt.

The synthesis of pentose phosphates via the transketolase and transaldolase reaction in this pathway follows the equation:

To quantitate the fluxes of glucose molecules through the oxidative and the non-oxidative branches of the pentose phosphate pathway, *Rh. gracilis* cells were incubated with D-glucose labelled with ^{14}C at different positions and the labelling patterns of various fractions of the yeast cells were measured. The ratios of radioactivity incorporated in each fraction from [1- ^{14}C]-, [6- ^{14}C]- and [U- ^{14}C]-glucose were used for the estimation of

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the fluxes. The results of these experiments are reported in this paper.

MATERIALS AND METHODS

The collection strain used (*Rh. gracilis* 5/Fres/Harrison) was precultivated as described earlier (Kotyk & Höfer, 1965). The pre-culture, about 75 ml, was transferred into a Chemap incubator, containing 4800 ml of growth medium (see below), and further cultivated at 30°C. Cells were harvested after 24 h, washed twice with water and aerated overnight. A 50% (w/w) cell suspension in water was used for the experiments (corresponding to 108 mg dry wt./ml).

Incubation of yeast cells. The yeast cells were incubated in Warburg vessels containing 0.4 ml of the cell suspension (1.7×10^9 cells = 43 mg dry wt.) and 1.5 ml of Krebs-Ringer phosphate buffer, pH 5.0 (free of glucose and Ca^{2+} ; see below), in the main compartment, 0.1 ml of the appropriate radioactive glucose (200 μmol) in one side arm, 0.2 ml of 2.2 M- HClO_4 in the other side arm, and 0.2 ml of phenylethylamine in the central well. The incubation mixture was maintained at pH 5 and 30°C. The reaction was started by the addition of the appropriate ^{14}C -labelled D-glucose and stopped after 15, 30, 45 and 60 min by the addition of HClO_4 .

Fractionation of the HClO_4 extract. This extract was then centrifuged at 12000g. The precipitate containing the macromolecular cell fractions was washed with 0.2 M- HClO_4 and further fractionated by using the method of Schneider (1945) and Schmidt & Thannhauser (1945). The four main fractions contained lipids, alkaline extract of RNA, acid extract of DNA and proteins. Samples of these fractions were counted for radioactivity in Bray's (1960) solution by using a Packard liquid-scintillation counter.

Non-soluble residue. After this treatment the radioactivity of the non-soluble residue was also counted to get complete recovery of added ^{14}C radioactivity. For this measurement the residues were resuspended in methanol and portions of the suspensions were dispersed with agitation in 5% Aerosil gel, essentially as described by Gordon & Wolfe (1960).

Measurements of CO_2 . The $^{14}\text{CO}_2$ liberated during the incubation with various labelled glucoses was trapped in phenylethylamine and counted for ^{14}C radioactivity. The volume of the evolved CO_2 , used for the calculation of the specific radioactivity, was measured in a separate experiment by conventional manometry with a 1:10 diluted cell suspension. Each Warburg flask contained 0.7 ml of the cell suspension, 1.2 ml of Krebs-Ringer phosphate buffer, pH 5.0 (free of glucose and Ca^{2+}), in the main compartment, 0.1 ml of the appropriate radioactive D-glucose (200 μmol) in one side arm and 0.2 ml of 2.2 M- HClO_4 in the other side arm. When oxygen consumption was measured, 0.2 ml of phenylethylamine was present in the central well. The reaction, started by the addition of D-glucose, was stopped by the addition of HClO_4 after 60 min incubation at 30°C.

Analysis of the HClO_4 supernatant. This supernatant was neutralized with 2 M-KOH and assayed enzymically for glucose, ethanol and adenine nucleotides.

A sample of the neutralized HClO_4 supernatant was counted for the radioactivity yield in the acid-soluble cell extract (Table 1). The values were corrected for the

amount of the non-utilized D-glucose present in the incubation suspension.

Chemicals. [$1\text{-}^{14}\text{C}$], [$6\text{-}^{14}\text{C}$] and [$\text{U-}^{14}\text{C}$]-Glucose were products of New England Nuclear Chemicals G.m.b.H., Dreieichenhain, Germany. Enzymes, substrates and coenzymes for the assays of glucose, ethanol and adenine nucleotides were obtained from Boehringer und Soehne G.m.b.H., Mannheim, Germany. The calcium- and glucose-free Krebs-Ringer phosphate buffer, pH 5.0, contained: 123 mM-NaCl, 4.9 mM-KCl, 1.4 mM-MgSO₄ and 16 mM-Na₂HPO₄. This buffer was used for all preparations. Aerosil, a thixotropic gel powder (Cab-O-Sil), was obtained from Packard Instrument G.m.b.H., Frankfurt am Main, Germany. All other chemicals were of analytical-reagent grade. The solutions were prepared in deionized water. The growth medium consisted of: NH_4NO_3 , 0.066%; K_2HPO_4 , 0.1%; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01%; NaCl, 0.05%; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.033%; $\text{FeCl}_3 \cdot 2\text{H}_2\text{O}$, 0.005%; yeast extract, 0.03%; D-glucose, 4%; pH 5.4.

RESULTS

Fig. 1 presents the time-course of the incorporation of the radioactivity from specifically labelled D-glucose into all fractions analysed: CO_2 evolved, the cell lipid fraction, RNA, DNA, the alkaline-soluble protein fraction and the insoluble residue. Glucose consumption and ethanol production are also shown. It should be noted that the scales of the individual sections of the figure are not identical. For comparison of actual sizes of the various pools, see Table 1.

As expected from our earlier results (Höfer, 1968), there is a much greater release of radioactive CO_2 from [$1\text{-}^{14}\text{C}$]glucose than from [$6\text{-}^{14}\text{C}$]glucose, and the radioactivity in CO_2 from D-[$\text{U-}^{14}\text{C}$]glucose is between the two values. These results indicate that the oxidation at C-1 of glucose is mainly due to the decarboxylation in the oxidative pentose phosphate pathway of glucose catabolism. The radioactivity incorporated into the cell lipid fraction is about twice as much from D-[$6\text{-}^{14}\text{C}$]glucose as it is from D-[$1\text{-}^{14}\text{C}$]glucose, which, in turn, is almost identical with the radioactivity incorporated from D-[$\text{U-}^{14}\text{C}$]glucose. The curves indicate a lag phase in the incorporation of the label. The synthesis of lipids appears to start only after a certain concentration of intermediates or precursors is reached. The extrapolated time delay represents approx. 8 min.

The labelling of the RNA and DNA fractions reveals the most striking and important fact; that the radioactivity yields from both [$1\text{-}^{14}\text{C}$] and [$6\text{-}^{14}\text{C}$]glucose were almost identical in the two fractions, in spite of the fact that from D-[$1\text{-}^{14}\text{C}$]glucose approx. 6 times as much radioactivity was released into CO_2 as from D-[$6\text{-}^{14}\text{C}$]glucose. Since the ribose moiety of the nucleic acids is derived from the pentose phosphate pool, it appears evident that the pentose phosphate pool is nearly equally

Fig. 1. Time-course of radioactivity incorporation into various fractions of *Rh. gracilis* cells during 60 min incubation at 30°C with specifically labelled d-[¹⁴C]glucose: ●, [1-¹⁴C]glucose; ○, [6-¹⁴C]glucose; ⊙, [U-¹⁴C]glucose. (a) CO₂ evolved; (b) cell lipid fraction; (c) RNA fraction; (d) DNA fraction; (e) insoluble residue; (f) alkaline-soluble protein fraction; (g) glucose consumption (⊗) and ethanol production (●). For experimental details see the Materials and Methods section. The values on the ordinates, radioactivity incorporated, are expressed in nmol of glucose transformed into the particular fraction (calculated by dividing the radioactivity measured by the specific radioactivity of added glucose), except for (g), where the amounts of d-glucose consumed and ethanol produced were determined directly by means of enzymic analysis.

Table 1. *Distribution of radioactivity yields in various fractions of Rh. gracilis cell metabolism and recovery of the radioactivity after 60 min incubation at 30°C with [1-¹⁴C]-, [6-¹⁴C]- and [U-¹⁴C]-glucose*

The values in μmol express the amount of glucose transformed into the particular fraction (calculated by dividing the radioactivity measured by the specific radioactivity of added glucose).

	[1- ¹⁴ C]Glucose			[6- ¹⁴ C]Glucose			[U- ¹⁴ C]Glucose		
	d.p.m.	μmol	%	d.p.m.	μmol	%	d.p.m.	μmol	%
D-Glucose catabolized	1014300	27.6	100	1039285	27.1	100	1051200	29.2	100
Sp. radioactivity (d.p.m./ μmol of glucose)		36750			38350			36000 (6000 d.p.m./ μg -atom of C)	
Yields of radioactivity									
CO ₂	237588	6.480*	23.5	44959	1.170*	4.3	191585	5.32	18.2
0.2M-HClO ₄ cell extract	264124	7.195	26.1	373508	9.750	36.0	315410	8.755	30.0
Lipids	67823	1.835	6.7	118972	3.100	11.4	54862	1.530	5.2
RNA	6526	0.170	0.6	7385	0.195	0.7	5537	0.150	0.5
DNA	2895	0.079	0.3	3350†	0.087	0.3	2083	0.060	0.2
Proteins	2829	0.077	0.3	3557	0.093	0.3	2091	0.060	0.2
Insoluble residue	400213	10.90	39.5	496511	12.90	47.6	463505	12.40	42.5
Sum	981998	26.736	96.9	1048242	27.195	100.6	1035073	28.275	96.8

* Since the radioactivity of the specifically labelled D-glucose is localized at the single C-atom, these values represent the actual amounts of CO₂ evolved through the decarboxylation of C-1 or C-6, as well.

† This value was approximated from the 45 min incubation.

labelled regardless of the position of the label in the metabolized glucose molecules. The difference in the labelling of the pentose phosphate pool from [1-¹⁴C]- and [6-¹⁴C]-glucose is 15 and 10% calculated from the RNA and DNA fractions. It can therefore be concluded that the pentose phosphate pool is formed mainly by the non-oxidative transketolase-transaldolase pathway.

The labelling of the protein fraction exhibits a pattern similar to that of the lipid fraction. The activity incorporated from [1-¹⁴C]glucose is also approximately one-half of that from [6-¹⁴C]glucose. A lag phase even longer than that of the lipid fraction (approximately double) is obvious.

The amount of radioactivity incorporated into the insoluble residue is about 40–50% of the total taken up. This fraction represents the greatest sink for the radioactive intermediates of glucose catabolism. The time-course of the labelling of this fraction shows a trend to reach a steady state after 45–60 min incubation. The radioactivity yields from [6-¹⁴C]glucose are higher than those of the other two labelled sugars. We have not analysed the nature of the insoluble residue. It is very likely that it consists mainly of mucopolysaccharides of the cell walls. The uneven labelling of this fraction from [1-¹⁴C]- and [6-¹⁴C]-glucose shows that not glucose molecules themselves, but rather a product of their degradation, is involved in the construction of this fraction.

Table 1 summarizes the results of Figs. 1(a)–(g) obtained after a 60 min incubation period. It gives a survey of the distribution of radioactivity incorporated into the fraction analysed and the recovery. The values of D-[U-¹⁴C]glucose are the true representatives of the amount of utilized D-glucose transformed into the various cell constituents. The values of [1-¹⁴C]- or [6-¹⁴C]-glucose show the enrichment or impoverishment of the label-

ling of the various fractions due to the actual pathways of glucose catabolism.

To calculate the specific radioactivity of the CO₂ evolved, a separate experiment was carried out in which both the radioactivity of CO₂ and the volume of the CO₂ evolved were measured. The same yeast batch (1:10 diluted) was used for the experiment. The results are shown in Table 2 (for experimental conditions see the Materials and Methods section).

Normally, addition of D-glucose to a respiring *Rh. gracilis* cell suspension brings about a twofold increase in respiration (CO₂ evolution), and at the same time the utilization of endogenous substrates is inhibited by 50% (Höfer, Kotyk, Rihova, Janda & Betz, 1969b). This was determined by measuring the actual radioactivity of the CO₂ liberated by *Rh. gracilis* cells grown on ¹⁴C-labelled D-glucose. In the present experiment the endogenous CO₂ evolution was 5.0 μmol of CO₂/60 min incubation (Table 2). After addition of D-glucose about 9.9 μmol of CO₂ was evolved during 60 min incubation. Therefore, assuming that in the presence of D-glucose 50% of the endogenous CO₂ is inhibited, then 2.5 μmol of CO₂ is released from endogenous substrates and the net CO₂ evolution from the added D-glucose is 7.4 μmol/h. D-Glucose consumption was not measured directly in this experiment. It can, however, be estimated from the experiment shown in Table 1. The calculation leads to a value of 4.9 μmol of D-glucose consumed by 7.5 mg dry wt. of yeast. Thus the CO₂ evolved/D-glucose utilized ratio is 7.4/4.9 = 1.5.

The ratio can also be directly determined from the radioactivity yields in CO₂ evolved from D-[U-¹⁴C]glucose (see Table 1). In this case 28 μmol of D-glucose catabolized released 32 μmol of CO₂, and the CO₂ evolved/D-glucose utilized ratio is 1.14. Thus about 1.2 μmol of CO₂ is released through the oxidation of each D-glucose molecule catabolized.

Table 2. *Measurements of the respiratory parameters of Rh. gracilis and determination of the specific radioactivity of the evolved CO₂ after 60 min incubation at 30°C*

Conventional manometry was carried out with 7.5 mg dry wt. of cells in each Warburg flask. The amount of CO₂ evolved from D-glucose was determined after subtraction of CO₂ produced by oxidation of endogenous substrates (see the text for explanation).

	Endogenous	D-[1- ¹⁴ C]Glucose	D-[6- ¹⁴ C]Glucose	D-[U- ¹⁴ C]Glucose	Mean value
Q _{CO₂}	+25.1	+49.7	+54.3	+50.2	
Q _{O₂}	-29.2	-42.7	-44.8	-46.0	
Respiratory quotient	0.88	1.16	1.21	1.09	
Total CO ₂ evolved (μmol)	5.0	9.6	10.5	9.7	9.9
CO ₂ evolved from D-glucose (μmol)	—	7.1	8.0	7.2	7.4
Radioactivity of CO ₂ (d.p.m.)		66000	14859	47528	
Sp. radioactivity of CO ₂ evolved (d.p.m./μmol)		9300	1860	6590	
Sp. radioactivity of added D-glucose (d.p.m./μmol)		36750	38350	36000	

The specific radioactivity of CO_2 released through the oxidation of D-[U- ^{14}C]glucose was 6590 d.p.m./ μmol . This indicates the reliability of the measurements, since the value is one-sixth of the specific radioactivity of the glucose added (36000 d.p.m./ μmol). The specific radioactivities of CO_2 from [1- ^{14}C]- and [6- ^{14}C]-glucose correspond to a dilution by factors of 4 and 20 respectively, which are to be expected from the scheme of metabolic reactions (Scheme 1).

Table 1 also shows the recovery of the total radioactivity. The fact that only about one-fifth of the radioactivity from [U- ^{14}C]glucose is released as CO_2 indicates that the glucose molecules taken up are only partially oxidized to CO_2 . About two-fifths of each glucose molecule catabolized is incorporated into the insoluble cell residue.

The actual amount of $^{14}\text{CO}_2$ released from [U- ^{14}C]glucose and calculated from the specific radioactivity of each carbon atom of the glucose molecule is $32\mu\text{mol}$. The fact that only about $6.5\mu\text{mol}$ of CO_2 is released from $28\mu\text{mol}$ of [1- ^{14}C]-glucose in the oxidative pentose phosphate pathway confirms that most of the pentose phosphates

are formed in the non-oxidative transketolase-transaldolase pathway.

The ATP concentration decreased after the addition of D-glucose to the cell suspension and returned again to its original value during the incubation. This change was compensated by corresponding alteration of the ADP concentration. The sum of ATP, ADP and AMP remained unchanged throughout the experiment, amounting to about one-half of the total nucleotide pool, as measured spectrophotometrically at 260 nm.

DISCUSSION

The discrepancy between the inability of *Rh. gracilis* cells to catabolize D-glucose by glycolytic reaction, due to the lack of phosphofructokinase, and the incorporation of C-1 of added D-glucose into the cell lipid fraction led us to postulate that D-glucose molecules are degraded to pentose phosphates simultaneously through both the oxidative and the non-oxidative part of the pentose phosphate pathway (Höfer *et al.* 1970a). However, the quantitative correlation of the fluxes of D-glucose molecules through the two parts of the pentose

Scheme 1. Estimated fluxes of D-glucose subunits in the course of its breakdown. The numbers before the substances give the number of μmol used or formed during 60 min incubation. The numbers in the squares correspond to the distribution of the original position of the C atoms (in parentheses) in the glucose molecule.

phosphate pathway was lacking. The results presented in the present paper support our earlier hypothesis, since it was found that about 4 times more D-glucose is transformed through the transketolase-transaldolase reactions than is directly oxidized to pentose phosphate.

Quantitation of the fluxes through the two parts of the pentose phosphate pathway. Each D-glucose molecule catabolized in the oxidative part of the pentose phosphate pathway is decarboxylated at C-1. However, since the labelling of the RNA and DNA from [$1-^{14}\text{C}$]glucose was found to be only 15 and 10% less than that from [$6-^{14}\text{C}$]glucose it appears that most of the glucose metabolized is converted into pentose phosphate via the non-oxidative transketolase-transaldolase pathway. From the labelling patterns of the ribose moiety of the nucleic acids alone it is not possible to draw a definite conclusion about the contribution of the oxidative and non-oxidative pathways to pentose phosphate formation, since the equilibration constants of the different hexose, pentose and triose phosphates are not known.

Such an estimate can, however, be made by correlating the $^{14}\text{CO}_2$ liberation and the D-glucose consumption. Here it was found that $6.5\mu\text{mol}$ of $^{14}\text{CO}_2$ is oxidatively decarboxylated from $28\mu\text{mol}$ of D-[$1-^{14}\text{C}$]glucose catabolized. The amount of decarboxylation in the tricarboxylic acid cycle is difficult to determine. Nevertheless, since C-1 and C-6 are equally located in the C_2 fragments of glucose molecules, before they enter the tricarboxylic acid cycle, one can assume that the oxidation of C-1 and C-6 to CO_2 in the tricarboxylic acid cycle will also be equal. Thus of the $28\mu\text{mol}$ of [$1-^{14}\text{C}$]glucose catabolized $5.3\mu\text{mol}$ is directly oxidized to CO_2 through the oxidative part of the pentose phosphate pathway ($^{14}\text{CO}_2$ from [$1-^{14}\text{C}$]glucose minus $^{14}\text{CO}_2$ from [$6-^{14}\text{C}$]glucose, Table 1), i.e. 19%. The rest, i.e. 81% or $22.7\mu\text{mol}$, of glucose is transformed into pentose phosphates through the transketolase-transaldolase reactions of the non-oxidative part of the pathway.

By operation of both parts of the pathway $39.3\mu\text{mol}$ of pentose phosphate is formed, of which all include C-6 but only $34\mu\text{mol}$ contains C-1 from glucose (39.3 minus $5.3\mu\text{mol}$ of C-1 is oxidized directly). The difference in the labelling of the pentose phosphate pool amounts therefore to $(39.3 - 34)/39.3$, i.e. 13.5%. This value accords well with the experimentally obtained 10–15% from DNA and RNA labelling.

Of the $28\mu\text{mol}$ of glucose catabolized 42.5% is incorporated into the insoluble residue: 42.5% of $28\mu\text{mol}$ is $11.9\mu\text{mol}$ of glucose or correspondingly $14.3\mu\text{mol}$ of pentose phosphate. The compositions of the $25\mu\text{mol}$ of pentose phosphate that undergo further degradation to C_2 fragments and glyceraldehyde 3-phosphate are shown in the squares in

Scheme 1 (for this calculation see the equations in Scheme 2).

The results above indicate that the two parts of the pentose phosphate pathway operate simultaneously. In addition, the non-oxidative part of this pathway contributes to the overall catabolism of D-glucose to a greater extent than the oxidative part. The yeast *Rh. gracilis* proved to be a good subject for studying the quantitative fluxes of glucose through the two branches of the pentose phosphate pathway, since it lacks phosphofructokinase, and therefore the glyceraldehyde 3-phosphate molecules formed in this pathway cannot be diluted with glyceraldehyde phosphate from the splitting of fructose 1,6-diphosphate by aldolase.

The possibility that D-glucose could be, at least to a considerable extent, degraded in another metabolic route, such as the Entner-Doudoroff pathway (Entner & Doudoroff, 1952; Schlegel, 1969), was excluded, since (1) *Rh. gracilis* cells utilize pentose, e.g. D-xylose (Höfer *et al.* 1969b); (2) De Ley & Vandamme (1955) reported the inability of the yeast cells to grow on 2-oxogluconate or to consume oxygen in presence of this substrate; (3) yeast cell-free extract exhibits high 6-phosphogluconate dehydrogenase activity (Höfer *et al.* 1969a).

Sites of decarboxylation in the process of glucose catabolism. There are three loci of decarboxylation during glucose breakdown (see Scheme 1): one in the oxidative part of the pentose phosphate pathway, another in the later part of the glycolytic sequence and the last in the tricarboxylic acid cycle.

The problem of the determination of the quantitative participation of the different metabolic pathways in glucose breakdown by using specifically labelled substrates has frequently been discussed (see, e.g., Wood, Katz & Landau, 1963; Katz & Rognstad, 1967). The uniqueness of the metabolism of *Rh. gracilis* consists of the impossibility of phosphorylating fructose 6-phosphate to fructose 1,6-diphosphate and of dephosphorylating the latter to the former. Moreover, the recycling of the pentose phosphates to fructose 6-phosphate can hardly be assumed, since the oxidative and the non-oxidative parts of the pentose phosphate pathway operate simultaneously. This conclusion is further supported by the fact that only $6.5\mu\text{mol}$ of CO_2 is released from the C-1 position of $28\mu\text{mol}$ of D-glucose catabolized. If glucose were catabolized thoroughly by a recyclicalization in the oxidative pentose phosphate pathway, three carbon atoms from each glucose molecule catabolized should be converted into CO_2 (three into glyceraldehyde 3-phosphate), and we should find at least $28 + 2 \times 13.7 = 55.6\mu\text{mol}$ of CO_2 from $28\mu\text{mol}$ of [$1-^{14}\text{C}$]glucose (all glucose molecules should be once

oxidized in the cycle, then 14.3 μmol of pentose phosphate are incorporated and the rest, 13.7 μmol , should further recycle twice to give glyceraldehyde 3-phosphate). In the experiment, however, only 32 μmol of CO_2 was evolved. These results exclude a recycling of glucose 6-phosphate molecules in the pentose phosphate pathway. Therefore another pathway for pentose phosphate catabolism must be considered in this micro-organism, i.e. a splitting of the pentose phosphates formed in the oxidative and non-oxidative pathway into C_2 units, presumably glycollate, and glyceraldehyde 3-phosphate by the action of an aldolase. The former consists of the C-1 and C-2 atoms and partially of the C-3 atoms when coming from the non-oxidative part of the pentose phosphate pathway, or the C-2 and C-3 atoms when coming from the oxidative part of this pathway. The latter consists exclusively of the C-4, C-5 and C-6 atoms. Thus the CO_2 released through decarboxylation of pyruvate comes from the C-4 position of the glucose molecules.

The fission of pentose phosphate into C_2 fragments and glyceraldehyde 3-phosphate is a quite commonly occurring reaction in micro-organisms (Hurwitz, 1958; Hollmann, 1961). This reaction is catalysed by phosphoketolase and leads to acetyl phosphate and glyceraldehyde 3-phosphate. Although the formation of acetyl phosphate cannot be excluded the possibility of glycollate being the product of splitting xylulose 5-phosphate remains.

This problem is discussed by Höfer, Betz & Becker (1970a,b).

The distribution of the carbon atoms in the pentose phosphates formed through the two parts of the pentose phosphate pathway is represented by the equations in Scheme 2.

The specific radioactivities of CO_2 evolved from $[1-^{14}\text{C}]$ -, $[6-^{14}\text{C}]$ - and $[\text{U}-^{14}\text{C}]$ -glucose are 9300, 1900 and 6600 d.p.m./ μmol respectively (after subtraction of the CO_2 evolved from oxidation of endogenous substrates). The value for uniformly labelled D-glucose corresponds to the specific radioactivity of a single carbon atom of the original sugar molecule; that for D- $[6-^{14}\text{C}]$ glucose indicates a considerable dilution with unlabelled CO_2 .

Labelling of the lipid fraction. The lipid fraction has approximately twice as much label from $[6-^{14}\text{C}]$ -glucose as it has from $[1-^{14}\text{C}]$ glucose. This might seem to be contrary to the almost equal labelling of the pentose phosphate pool. However, the higher incorporation of the label from D- $[6-^{14}\text{C}]$ glucose can be accounted for by assuming that the acetyl-CoA used for fatty acid synthesis comes predominantly from glyceraldehyde 3-phosphate (consisting of C-4, C-5 and C-6). Further, the glycerol part of a lipid molecule becomes labelled only when D- $[6-^{14}\text{C}]$ glucose is metabolized.

Radioactivity incorporated into the insoluble residue. The insoluble residue left after all extractions has a surprisingly high radioactivity. During an in-

Oxidative part of the pentose phosphate pathway:

Non-oxidative part of the pentose phosphate pathway:

Scheme 2. Distribution of carbon atoms in the pentose phosphates formed through the two parts of the pentose phosphate pathway.

incubation period of 45 min, approx. 40–50% of the total radioactivity taken up is incorporated. The difference between the amounts of label incorporated from [1-¹⁴C]glucose and [6-¹⁴C]glucose might indicate that we are dealing here with some insoluble polypentosans, the monomeric units of which become labelled from the pentose phosphate pool. The calculations of the fluxes summarized in Scheme 1 depend on the assumption that the pentose phosphates or smaller units, and not glucose phosphate, build up the insoluble fraction. That means that the D-glucose taken up is catabolized at least to pentose phosphates before being incorporated into the insoluble fraction.

The other alternative, i.e. the hexose phosphates are incorporated into the insoluble residue, cannot be excluded on the basis of our results. However, if this were the case then there would be only 16 μ mol of glucose [28–12 μ mol (42.5% incorporated)] broken down in the pentose phosphate pathway. In this case the oxidative part of the pathway would amount to 5.3/16 (μ mol of CO₂/ μ mol of glucose), i.e. 33%; and the difference in the labelling of the pentose phosphate pool from [1-¹⁴C]-glucose and [6-¹⁴C]glucose (21.4–16.1)/21.4 ([1-¹⁴C]-pentose phosphates/[6-¹⁴C]pentose phosphates), i.e. 25%. Experimentally the difference was found to be 10–15%, therefore, the incorporation in the insoluble residue of the pentose phosphates according to Scheme 1 appears to be more likely.

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