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Studies on the Aerobic Degradation of Glucose by *Saccharomyces cerevisiae*

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The anaerobic degradation of glucose by yeasts has been thoroughly studied (Lardy, 1949; Meyerhof, 1951). However, much less is known about the metabolic pathways involved in the oxidation of glucose by yeasts. Based on studies of various strains of yeast and also by analogy with mechanisms discovered in other organisms, two major routes have been described for glucose oxidation: (1) direct oxidation, i.e. oxidation of the C-1 of glucose as the primary step, with or without preliminary phosphorylation of the molecule, and (2) oxidation

only after an obligatory stage of fermentation via the classical Embden-Meyerhof pathway.

The presence in *Saccharomyces cerevisiae* of enzymes involved in fermentation can be readily demonstrated, and the enzymic equipment necessary for the oxidation of glucose via a hexose monophosphate (HMP) pathway is almost as well documented (Horecker & Mehler, 1955). Recent attempts to assess the relative importance in yeast of these two routes during glucose oxidation have been performed by allowing intact cells to oxidize [¹⁴C]glucose labelled in different positions and analysing the products. For example, Blumenthal, Lewis & Weinhouse (1954) collected the acetate formed during oxidation of labelled glucose and,

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from the distribution of labelling, concluded that in *S. cerevisiae* the HMP pathway was responsible for the oxidation of 0–30 % of the glucose oxidized. On the other hand Beavers & Gibbs (1954) collected the carbon dioxide formed during the utilization of [$6\text{-}^{14}\text{C}$]glucose and of [$1\text{-}^{14}\text{C}$]glucose and concluded that a large proportion of glucose is oxidized by this organism via the HMP route.

Previous work (Eaton & Klein, 1954) had indicated that the HMP shunt is not an important pathway of oxidation of glucose by 'young' cells of a strain of *S. cerevisiae*. It has also been shown that such cells, although they oxidize glucose rapidly and completely, do not readily oxidize ethanol, pyruvate or acetate (Eaton & Klein, 1954; Eaton, 1955). Consequently it was suggested that acetate is not an intermediate in glucose oxidation. However, further experiments described in this paper suggest that these cells convert glucose initially almost completely into ethanol and carbon dioxide, and that the ethanol is subsequently oxidized to acetate and the latter oxidized to carbon dioxide.

Whether the glucose skeleton is cleaved by direct oxidation or by fermentation pyruvic acid is postulated as a common intermediate; the ultimate route of the degradation of pyruvic acid has been the subject of several investigations (cf. Krebs, 1952). Various individual reactions of the tricarboxylic acid cycle have been shown to occur in yeast preparations (Lynen, 1943; Foulkes, 1951; Novelli & Lipmann, 1950), and Nossal (1954*a*) has shown that all of the acids of the tricarboxylic acid cycle can be oxidized by cell-free extracts of yeast and furthermore that labelled acetate is incorporated into these acids (Nossal, 1954*b*). Since the magnitude of oxygen uptake of these preparations was rather small, the importance of these reactions in the living cells might be questioned. However, De Moss, Swim & Krampitz (1955) and Eaton (1955) carried out similar experiments with intact cells and arrived at the conclusion that acetate oxidation proceeds via the tricarboxylic acid cycle. Evidence is presented in the present paper that the tricarboxylic acid cycle is involved in the oxidation of glucose.

METHODS

Growth of organism. *Saccharomyces cerevisiae*, strain LK2G12 (Spiegelman, 1945), was used throughout the study. Stock cultures of the yeast were kept on slants of the following composition: glucose, 2%; NH_4Cl , 0.1%; yeast extract (Difco), 0.1%; KH_2PO_4 , 0.1M; agar, 1.5%. These were subcultured at intervals of 1–2 weeks.

Cells used for experimental work were grown either in a medium of the above composition less agar (medium 1) or in Difco 'Yeast Nitrogen Base' medium with 1–2% of glucose added (medium 2). Aeration of the cultures was accomplished either by shaking on a rotary shaker or by passing a vigorous stream of air through a sintered-glass disk, which

was immersed in the medium. Unless otherwise stated, the cultures were incubated at 30°. 'Young' cells were obtained by harvesting the cultures before the stationary phase of growth; 'old' cells were harvested after logarithmic growth had ceased (Eaton & Klein, 1954).

In general, growth from a stock slant was used as the inoculum. The yeast was suspended in water and a volume equivalent to 4–5 mg. dry wt. was added/l. of medium.

Manometric methods. Oxygen uptake and production of CO_2 by resting-cell suspensions were measured in a conventional Warburg apparatus at 30°, with air as the gas phase. Carbon dioxide was determined by the 'direct' method (Umbreit, Burris & Stauffer, 1949). All determinations were carried out in 0.05M- KH_2PO_4 . Each flask contained a total volume of 2.2 ml.

Chromatographic separation of acids of the tricarboxylic acid cycle. For the isolation of acids of the tricarboxylic cycle a modification of the chromatographic method of Swim & Krampitz (1954) was used. Since the modified procedure differed in several important respects, it will be described here in detail.

Two major difficulties were encountered when Celite was used as the supporting material for the column: (1) packing the chromatography tube with Celite in the form of a slurry did not always result in a column which was even enough to ensure a good separation of the acids; (2) when an amount of aqueous phase sufficient to give good separation was added to the Celite, some of the aqueous phase was often forced out of the column along with the eluate. These difficulties have been overcome by using powdered cellulose as the supporting medium and packing the column 'dry' as described below.

The chromatography tube was made by sealing a stopcock to one end of a borosilicate tube of 15–16 mm. inside diameter and 35–40 cm. long. A disk of heavy blotting paper, slightly larger than the inside diameter of the tube, was pushed down against the constricted end of the tube to support the cellulose column.

Cellulose powder (5 g.; Whatman standard grade) was thoroughly mixed in a mortar with 2.5 ml. of 0.05N- H_2SO_4 . The mixture, which appeared dry, was transferred in portions (about 0.5 g.) to the chromatography tube. Each addition of cellulose powder was packed as firmly as possible with the end of a glass rod that had been flattened in such a way that it just fitted easily into the tube. After the last addition 50 ml. of chloroform, which had previously been equilibrated with 0.05N- H_2SO_4 , was added and forced through the column under sufficient air pressure to result in a flow rate of 80–100 ml./hr. When the solvent level reached 2–3 mm. above the level of the packed cellulose the air pressure was removed and the stopcock closed. The solvent level was never allowed to pass below the surface of the cellulose.

The mixture of organic acids was acidified with a drop of 10N- H_2SO_4 , taken up in not more than 0.25 ml. of water and thoroughly mixed with 0.5 g. of cellulose powder. The mixture was transferred quantitatively to the top of the prepared column and packed with a glass rod. The rod, beaker and spatula used for transferring the mixture were wiped with a plug of glass wool, which was subsequently pushed down on top of the column.

The column was developed by adding 50 ml. each of 0, 5, 10, 15, 20, 25, 30, 35, 40 and 50% (v/v) butanol in chloroform. The solvents were prepared for use by shaking with

10 ml. of 0.05N- H_2SO_4 and passing the organic phase through a dry filter paper to remove suspended droplets of water. Each succeeding solvent was added just before the previous solvent level passed into the column. Fractions of 2.7 ml. were collected in small test tubes containing 1 ml. of CO_2 -free water and a drop of phenol red indicator, and titrated with approx. 0.01N-NaOH, a microburette of the type described by Black (1949) being used. During the titration the samples were mixed by a stream of CO_2 -free air. A typical separation of several known acids is shown in Fig. 1.

Lactic and succinic acids are not separated by this solvent system, nor are fumaric and formic acids. However, these mixtures may be separated by evaporating to dryness the fractions containing the acids and rechromatographing the residue on a 10 g. cellulose column, with ether as the developing solvent (Phares, Mosbach, Denison & Carson, 1952). Separations of these mixtures are shown in Fig. 2.

Acids of the tricarboxylic acid cycle were extracted from yeast cells and prepared for chromatography in the following

Fig. 1. Chromatographic separation of known acids on a cellulose column.

Fig. 2. Chromatographic separation of mixtures of fumaric and formic and of succinic and lactic acids.

manner. Cells (10–15 g., wet wt.) were suspended in 5 ml. of water in a 250 ml. centrifuge bottle. The suspension was heated on a boiling-water bath for 5 min., centrifuged and the supernatant decanted into a 250 ml. beaker. The process was repeated three times, and the combined supernatants were neutralized (thymol blue end-point) with 4N-KOH. The solution was evaporated to dryness on a steam bath, after which the residue was acidified with 10N- H_2SO_4 , brought to a total volume of not more than 2.5 ml. and mixed well with 5 g. of cellulose powder. The mixture was packed into a chromatography tube and the acids were eluted with 200 ml. of 50% butanol in chloroform (Isherwood & Hanes, 1953). The eluate was neutralized with 4N-KOH, extracted four times with 10 ml. portions of water and the combined extracts were evaporated to dryness on a steam bath. The residue, containing a mixture of organic acids, was chromatographed as described above. A typical separation of the acids present in yeast cells is shown in Fig. 3. It should be mentioned that the α -oxo-acids (α -oxoglutarate, oxaloacetic, pyruvic) are destroyed by this method of preparation.

Radioisotope techniques. [3,4- C^{14}]Glucose was synthesized by the method of Zilversmit, Chaikoff, Feller & Masoro (1948), and was purified chromatographically (Putman & Hassid, 1952). Uniformly labelled [C^{14}]glucose was purchased from Tracerlab Co., Boston, Mass., U.S.A.

All compounds to be assayed for ^{14}C were first converted into BaCO_3 by the wet-oxidation method described by Calvin, Heidelberger, Reid, Tolbert & Yankwich (1949). The precipitated BaCO_3 was filtered on to small filter-paper disks, washed with 10 ml. of water and 5 ml. of ethanol, dried under an infrared lamp and counted with a Tracerlab Autoscaler, a windowless gas-flow counter being used. The activities were corrected for background and self-absorption in the usual manner.

Radioactive CO_2 released during manometric experiments was trapped in 0.2 ml. of CO_2 -free NaOH (a saturated solution of NaOH diluted 1:3 with CO_2 -free water), which was placed with a piece of filter paper in the centre well of the Warburg flask. At the appropriate time, 0.1 ml. of 10N- H_2SO_4 was tipped into the main compartment from a side arm to stop the reaction and to release bound CO_2 . The flask was allowed to continue shaking for 15 min., after which the

Fig. 3. Chromatographic separation of acids from *S. cerevisiae*.

filter paper was transferred to a clean test tube and the centre well was rinsed three times with CO_2 -free water. The filter paper was rinsed four times with 1–2 ml. of water, and this was added to the washings from the centre well, after which the CO_2 was precipitated with a solution of 0.25N- $\text{Ba}(\text{OH})_2$ in 0.3N- BaCl_2 , and the resulting BaCO_3 was filtered, washed, dried and counted as described above.

In those experiments where large amounts of cells were exposed to radioactive substrates before the isolation of the acids of the tricarboxylic acid cycle, the CO_2 produced was trapped in 15 ml. of a 1:3 dilution of saturated NaOH . This was accomplished by passing a stream of CO_2 -free air through the cell suspension and dispersing the air in the NaOH solution by means of a sintered-glass sparger. The hydroxide solution was made to a total volume of 25 ml. and portions were assayed for ^{14}C content as described above.

Chemical determinations. Ethanol was estimated by the rate of reduction of diphosphopyridine nucleotide (DPN) in the presence of alcohol dehydrogenase. The reaction mixture consisted of 0.33 mg. of the enzyme (obtained from Mann Research Laboratories) in a total volume of 0.2 ml. diluted with 0.05M-2-amino-2-hydroxymethylpropane-1:3-diol (tris) buffer at pH 8.5, 0.1 ml. of 0.1M-semicarbazide, 0.4 ml. of DPN solution (1 mg./ml.) and the sample, made to a total volume of 3 ml. with tris buffer, pH 8.5. The change in optical density at $340\text{ m}\mu$ was measured with a Beckman model DU spectrophotometer.

Acetate was determined by chromatography and titration as described above.

The absorption spectra of the yeast cytochromes were detected by means of a Hartridge reversion spectroscope mounted on a microscope (Slonimsky, 1953).

DPN was obtained from the Sigma Chemical Co., St Louis, Mo., U.S.A. and other chemicals used were either reagent or analytical grade.

RESULTS

It was first thought that the inability of 'young' cells to oxidize acetate, ethanol and pyruvate might in some way be due to a deficiency of cytochromes (Slonimsky, 1953), and that glucose oxidation might be mediated in this case by a flavin-carrier system. However, no differences could be found in the cytochrome spectra of 'young' and 'old' cells. (We are indebted to Dr H. Roman for assistance in comparing the cytochrome spectra of these cells.)

Determination of the extent of the HMP pathway. Evidence has already been presented (Eaton & Klein, 1954) that the HMP pathway is not important in the oxidation of glucose by this organism. More complete data were obtained in the present work. 'Young' cells were allowed to oxidize glucose, labelled in the 3 and 4 positions with ^{14}C , and assays were made for oxygen uptake and for total and labelled carbon dioxide released during the process (Fig. 4).

The total amount of glucose metabolized aerobically via the Embden-Meyerhof pathway by 'young' cells can be estimated from these data.

Within 30 min. after addition of the substrate, approx. 85% of C-3 and C-4 of the glucose was released. However, during the first 30 min. only a small fraction of the total oxygen was consumed. Therefore only an extremely small amount of C-3 and C-4 recovered in the carbon dioxide at this time could have arisen via an HMP mechanism. It should be noted also that little additional carbon from the 3 and 4 positions of glucose appeared in the carbon dioxide after the first 30 min. Thus about 85% of the added glucose was degraded to some C_2 compound before appreciable oxidation had occurred, with no indication that the HMP pathway played a significant role.

Formation of ethanol during glucose oxidation. The rapid initial release of C-3 and C-4 of glucose and the small amount of oxygen taken up during this period suggested that some ethanol might be formed aerobically. Surprisingly, large amounts of ethanol were found to accumulate in the supernatant fluid during the early stages, and this disappeared during the later stages of glucose oxidation by 'young' cells. Furthermore the amount of ethanol formed was found to be almost equivalent, on a molar basis, to the amount of carbon dioxide formed from C-3 and C-4 of glucose, as shown in Table 1. From these data it can be calculated that at least 85% of the glucose is degraded with ethanol as an intermediate. Since 60–70% of the total glucose carbon can ultimately be recovered as carbon dioxide, it is apparent that the ethanol formed from glucose must be oxidized, despite the fact that 'young'

Fig. 4. Oxidation of glucose by 'young' cells of *S. cerevisiae*. Each vessel contained $13.5\text{ }\mu\text{moles}$ of $[3,4\text{-}^{14}\text{C}]$ glucose and 11.3 mg. of cells previously grown in medium 1. A, Total carbon dioxide released; B, total oxygen taken up; C, carbon dioxide from C-3 and C-4 of glucose; D, endogenous oxygen uptake.

cells cannot oxidize this compound when it is present as the sole substrate. It can only be concluded that 'young' cells gain the ability to oxidize ethanol during the process of glucose oxidation.

It is also of interest that for the first hour of glucose oxidation the total amount of carbon dioxide increases only slightly more than the amount of carbon dioxide released from positions 3 and 4 of glucose. During this period oxygen is being taken up and the ethanol, which initially accumulates, begins to disappear. These observations suggest that for the first hour or more of glucose oxidation under these conditions the major portion of the oxygen consumed is used to oxidize ethanol to the level of acetaldehyde or acetate, but not further.

Acetaldehyde as an intermediate in glucose oxidation. A further investigation of the early stages of glucose breakdown brought to light certain subtle differences in the oxidative patterns of 'young' cells depending upon the conditions of growth. As shown in Fig. 5, 'young' cells grown in a completely synthetic medium (medium 2) release as carbon dioxide about 70 % of the carbon from positions 3 and 4 of glucose. However, only 44 % of the added glucose could be accounted for as ethanol. In contrast, with cells grown in medium 1, the amount of carbon dioxide from C-3 and C-4 of glucose was essentially equal to the amount of ethanol produced (Table 1). Thus about 26 % of the glucose was degraded to some C₂ compound that was not ethanol. Experiments bearing on the nature of this compound are reported below.

'Young' cells grown in medium 2 were allowed to oxidize uniformly labelled glucose. The oxidation was stopped after various periods of time; the ¹⁴C content of the carbon dioxide was determined, and the supernatant fluid was analysed for ethanol and acetate (Table 2). Since essentially all of the glucose carbon appearing as carbon dioxide within the first 30 min. is derived from positions 3 and 4 (Figs. 4 and 5), it can be deduced that 4.8 μ moles of glucose have been metabolized in this way to yield 9.6 μ moles each of carbon dioxide and of C₂ compounds. However, a total of only 7.1 μ moles of ethanol and acetate was recovered, leaving 2.5 μ moles of C₂ compound unaccounted for. The amount of oxygen required to produce the acetate actually found is 1.4 μ moles, leaving 1.1 μ moles in excess at that time. This amount is very close to that required to oxidize the glucose unaccounted for to 2.5 μ moles of acetaldehyde. Thus although no attempt was made to identify this C₂ compound further, its oxidation level makes it appear likely that the compound may be acetaldehyde.

In this connexion it should be noted that Wieland & Wille (1933, 1935) found traces of acetaldehyde accumulating during ethanol oxidation by yeast. One can therefore speculate that free acetaldehyde

Table 1. *Ethanol and carbon dioxide produced during the oxidation of [3:4-¹⁴C]glucose by 'young' cells*

Each flask contained 11.3 mg. of cells (from a culture grown for 9 hr. in medium 1), glucose (13.5 μ moles) and KH₂PO₄ (0.05M).

Time (min.)	CO ₂ formed (μ moles)		Ethanol formed (μ moles)
	Total	From C-3 and C-4	
15	19.1	19.5	17.5
30	21.9	23.0	21.6
60	23.6	22.0	13.7
225	48.7	23.2	0

Fig. 5. Oxidation of [3:4-¹⁴C]glucose and ethanol production by 'young' cells of *S. cerevisiae*. Each vessel contained 6.7 μ moles of [3:4-¹⁴C]glucose and 5 mg. of cells previously grown in medium 2. A, Total carbon dioxide; B, total oxygen taken up; C, carbon dioxide from C-3 and C-4 of glucose; D, endogenous oxygen uptake; E, ethanol produced.

Table 2. *Formation of ethanol, acetate and carbon dioxide from [¹⁴C]glucose by 'young' cells*

Each vessel contained 5.5 mg. of cells (from a culture grown for 10 hr. in medium 2), uniformly labelled [¹⁴C]glucose (5 μ moles) and KH₂PO₄ (0.05M).

Time (min.)	Glucose carbon (μ moles) recovered in			O ₂ used* (μ moles)
	CO ₂	Ethanol	Acetate	
30	9.6	5.7	2.5	2.5
90	13.7	0.3	12.2	12.2
180	20.6	0	21.9	21.9

* Endogenous subtracted.

represents a common intermediate in the formation of acetate either from glucose or from ethanol.

Effect of fluoroacetate. Evidence that these breakdown products of glucose might be oxidized terminally by the same pathway as acetate [i.e. via the tricarboxylic acid cycle (Eaton, 1955)] was obtained, in part, by carrying out the oxidation in the presence of fluoroacetate. This substance, a rather specific inhibitor of the tricarboxylic acid cycle, was found to inhibit completely acetate oxidation in this strain of yeast. The effect of fluoroacetate on glucose oxidation by 'young' cells is shown in Fig. 6. The total amount of oxygen consumed was 236 μ l., whereas 224 μ l. of oxygen is required to oxidize this amount of glucose to the level of acetate.

Relation of the acids of the tricarboxylic acid cycle to respired carbon dioxide. The contention that glucose is oxidized by way of the tricarboxylic acid cycle was further tested by allowing 'young' cells to oxidize uniformly labelled glucose, by isolating the acids of the tricarboxylic acid cycle and comparing

Fig. 6. Effect of fluoroacetate on glucose oxidation by 'young' cells of *S. cerevisiae*. Each vessel contained 5.1 mg. of cells, previously grown in medium 2, and the following; 0.005 M-fluoroacetate (A); 10 μ moles of acetate + 0.005 M-fluoroacetate (B); 5 μ moles of glucose + 0.005 M-fluoroacetate (C); 5 μ moles of glucose (D).

Table 3. Specific activities of carbon dioxide and acids of the tricarboxylic acid cycle formed from [14 C]-glucose by 'young' cells

Compound	Amount formed (μ moles)	$10^{-4} \times$ Sp. activity (counts/min./mg. of C)
CO $_2$ *	1560	2.3
Succinate	8.75	2.3
Malate	5.58	2.1
Citrate	2.17	2.8

* Carbon dioxide released between 45 and 135 min. Acids were analysed at 135 min.

the specific activity of these with that of the carbon dioxide produced, exclusive of the carbon dioxide derived by glycolysis. Since C-3 and C-4 of glucose are released early and before any significant oxidation takes place, only the 14 C content of that carbon dioxide that was produced after 45 min. was used for comparison with the specific activity of the acids. The results of an experiment of this type are shown in Table 3 and are entirely comparable to the results of similar experiments with acetate as the substrate (Eaton, 1955). The specific activity of the acids is, in all cases, as high as the specific activity of the carbon dioxide. Thus all of the carbon dioxide produced through respiration could have been formed from acids of the tricarboxylic acid cycle. In other experiments pairs of vessels were used, one containing [3:4- 14 C]glucose and the other uniformly labelled glucose; carbon dioxide arising from C-1, C-2, C-5 and C-6 was obtained by difference. Essentially the same results were obtained with this method.

It is interesting that in this case little if any carbon dioxide is produced from the oxidation of endogenous material by routes other than the tricarboxylic acid cycle. By contrast, cells oxidizing acetate ('old' cells) produce substantial amounts of carbon dioxide from endogenous sources by some pathway not involving the tricarboxylic acid cycle (De Moss *et al.* 1955; Eaton, 1955). This difference is probably explained by the fact that the endogenous respiration of 'young' cells is considerably lower than that of 'old' cells.

Adaptation to acetate oxidation during glucose oxidation. Since 'young' cells are not capable of oxidizing acetate rapidly when it is present initially as the sole substrate, the conclusion seems inescapable that an adaptation to acetate oxidation must occur during the process of glucose oxidation. (The term 'adaptation' is used here to denote the acquisition of an increased ability to oxidize certain compounds, with no implications as to the mechanism involved. However, recent preliminary experiments have shown that *p*-fluorophenylalanine, an inhibitor of protein synthesis (Halvorson & Spiegelman, 1952), prevents acetate oxidation, thus suggesting that enzyme synthesis is involved in this process.) According to this hypothesis, 'young' cells should be fully adapted to acetate oxidation after the oxidation of glucose.

In order to gain information on whether adaptation actually occurs, 'young' cells were removed from Warburg vessels at various times during the course of glucose oxidation and the ability of these cells to oxidize acetate was determined. The results of this experiment are shown in Fig. 7, from which it is evident that the ability of the cells to oxidize acetate does in fact increase during glucose oxidation.

Further evidence that the truly oxidative phase of glucose oxidation is an adaptive process came from studies with arsenate, a compound known to interfere with the generation of high-energy phosphate bonds (Meyerhof, Ohlmeyer & Mohle, 1938) and, consequently, with adaptive-enzyme formation (Reiner, 1948).

Fig. 8 shows that whereas 0.02M-arsenate completely prevented adaptation of 'old' cells to galactose oxidation glucose utilization was unaffected. The latter observation is not unexpected, since 'old' cells appear to be completely adapted to glucose utilization. With 'young' cells (Fig. 9) the same concentration of arsenate effectively inhibited glucose oxidation.

DISCUSSION

Although much significance has recently been attached to the hexose monophosphate pathway as a major route of glucose oxidation by yeasts, no evidence for such a mechanism as a major oxidative route was found in the strain of yeast used here. Virtually all of the glucose ultimately oxidized to carbon dioxide is first fermented, apparently via the Embden-Meyerhof pathway. This conclusion is based on the fact that 80-90% of C-3 and C-4 of the added glucose appears as carbon dioxide before any

significant amount of oxygen uptake has occurred. There is virtually no further release of C-3 and C-4 of glucose during the later stages of oxidation, and it must be concluded that glucose is oxidized only after it is initially degraded to C_2 compounds.

It has been shown that in 'young' cells harvested from a yeast extract-ammonium chloride medium, glucose is respired almost exclusively by way of ethanol. Cells grown in a synthetic medium, however, accumulate relatively large amounts of acetate and apparently some acetaldehyde, as well as ethanol, during the early stages of 'glucose oxidation', and it seems unlikely that in the latter case all of the C_2 units derived from glucose are first reduced to ethanol before being oxidized.

Regardless of these minor differences in the kinds of C_2 compounds formed initially from glucose, it can be stated that glucose (or its phosphate ester) is not oxidized directly by this strain of yeast. Data have been presented showing that after glucose is degraded to C_2 units, most, if not all, of these are

Fig. 7. Oxidation of acetate by 'young' cells of *S. cerevisiae* after oxidation of glucose for increasing periods of time. Cells were grown in medium 1 for 9.75 hr. After washing, 9.6 mg. of cells was suspended in each of several vessels and allowed to oxidize 5 μ moles of glucose or 10 μ moles of acetate. After 30, 60 and 135 min., the cells in glucose were removed, washed once and resuspended with 10 μ moles of acetate. A, Initial rate of acetate oxidation; B, acetate oxidation after 30 min. with glucose; C, acetate oxidation after 60 min. with glucose; D, acetate oxidation after 135 min. with glucose. Oxidation of glucose is given by curve E, and endogenous oxygen uptake by curve F.

Fig. 8. Effect of arsenate on glucose and galactose oxidations by 'old' cells of *S. cerevisiae* previously grown in medium 1. Each vessel contained 9.5 mg. of cells. A, Oxidation of 10 μ moles of glucose in the presence of 0.02M-arsenate; B, oxidation of 10 μ moles of glucose; C, oxidation of 1 μ mole of glucose + 10 μ moles of galactose; D, oxidation of 1 μ mole of glucose + 10 μ moles of galactose in the presence of 0.02M-arsenate; E, endogenous oxygen uptake; F, endogenous oxygen uptake in the presence of 0.02M-arsenate.

oxidized initially to free acetate. Weinhouse, Millington & Lewis (1948) presented evidence indicating that a large part of the glucose oxidized by *Saccharomyces cerevisiae* involves acetate as an intermediate. Our results confirm this observation and show further that at least part of the respired glucose may be oxidized by way of the tricarboxylic acid cycle. Moreover, since 'young' cells cannot oxidize acetate initially an adaptation to oxidation by way of the tricarboxylic acid cycle must occur during glucose oxidation.

Earlier experiments (Eaton & Klein, 1954) had indicated that adaptation to acetate oxidation did not occur during the oxidation of glucose. This interpretation was based on the fact that the oxygen-uptake curves with both glucose and acetate present showed a secondary lag or 'break' which corresponded approximately to the level of oxygen taken up with an equivalent amount of glucose alone. Re-examination of these data showed that this 'break' occurs only with cells grown in a synthetic medium (cf. Eaton, 1955). Further, an initial indistinct 'break' occurs after an amount of oxygen equivalent to the oxidation of glucose to the level of acetate, after correction for the low level of acetate oxidation has been consumed. After this 'break', the cells are able to oxidize acetate rapidly. The reason for the differences observed

with cells grown in a synthetic medium and in a 'natural' medium are not known.

At the present time, the most plausible explanation seems to be that the adaptation involves the synthesis of enzymes of the tricarboxylic acid cycle. This contention is based on the effect of arsenate in preventing glucose oxidation; on the observation reported earlier (Eaton & Klein, 1954) that cells unable to oxidize acetate show a markedly lower content of enzymes of the tricarboxylic acid cycle than cells that are capable of oxidizing this substrate; and also on the observed (Eaton, 1955) increases in certain of these enzymes in resting-cell suspensions during glucose oxidation.

SUMMARY

1. During the aerobic degradation of glucose by resting cells of *Saccharomyces cerevisiae* 80–90 % of the carbon from positions 3 and 4 of glucose was converted into carbon dioxide, and almost all of the remaining carbon was found as ethanol at a time when oxygen consumption had barely begun. It is concluded that the oxidation did not proceed by the hexose monophosphate pathway to any significant extent.

2. As oxygen uptake continued, ethanol disappeared and acetate accumulated. (With cells grown in a synthetic medium, material of the oxidation level of acetaldehyde also appeared as an intermediate in glucose degradation.)

3. The further oxidation of these C_2 compounds was inhibited by fluoroacetate, which suggests participation of the tricarboxylic acid cycle. This contention is confirmed by the finding that, when labelled glucose was oxidized, the specific activity of the carbon dioxide produced from C-1, C-2, C-5 and C-6 was comparable with that of cycle acids isolated from the cells.

4. The oxidation of accumulated acetate is adaptive in nature; its rate increased during glucose degradation. Arsenate inhibited the oxidation of glucose to carbon dioxide, presumably by preventing adaptation to acetate oxidation.

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Fig. 9. Effect of arsenate on glucose oxidation by 'young' cells of *S. cerevisiae*. Each vessel contained 6 mg. of cells previously grown in medium 1. A, Oxidation of 10 μ moles of glucose; B, oxidation of 10 μ moles of glucose in the presence of 0.02 M-arsenate; C, endogenous oxygen uptake; D, endogenous oxygen uptake in the presence of 0.02 M-arsenate.

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The Accumulation of Iodide by *Fucus ceranoides*

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The high concentrations of iodide found in certain marine algae suggest that these plants have mechanisms for accumulating iodide from sea water (e.g. Jacques & Osterhout, 1938). The uptake of radioactive iodide has been demonstrated in *Ascophyllum nodosum* (Kelly & Baily, 1951; Kelly, 1953), *Laminaria flexicaulis* (Roche & Yagi, 1952) and *Nereocystis luetkeana* (Tong & Chaikoff, 1955). In work described in the present paper the uptake of radioactive iodide by *Fucus ceranoides* from sea water appears to involve both a carrier mechanism and binding of the ion within the tissue.

EXPERIMENTAL

Special chemicals. Iodide, perchlorate, thiocyanate and nitrate were all used as solutions of their potassium salts. Carrier-free radioactive iodide ($^{131}\text{I}^-$) in 0.01 N-sodium thiosulphate from the Radiochemical Centre, Amersham, was diluted with water. Samples of 3-iodo-L-tyrosine and 3,5-di-iodo-L-tyrosine were dissolved in *n*-butanol for use as marker substances on paper chromatograms. Solutions of 2,4-dinitrophenol (DNP) in 0.025 N-NaOH and *p*-chloromercuribenzoate in 0.1 N-NaOH were neutralized with HCl before use. Methylthiouracil was dissolved in 0.1 N-NaOH and added to media containing equivalent amounts of HCl. Solutions of 2-mercapto-1-methylimidazole were made in

water. Sea water was filtered through Whatman no. 1 paper before use. It was assumed to have the usual iodide content of about 5 $\mu\text{g.}/100\text{ ml.}$ Artificial sea water was prepared according to Lyman & Fleming (1940).

Tissue preparation. *Fucus ceranoides* was collected at low tide from the shore of Southampton Water. Portions of the fronds from the region between 3 and 25 mm. behind the growing tip were cut into fragments of 10–20 mg. fresh wt. This tissue was washed eight times with filtered sea water, spread on filter paper for 30 sec. to remove excess of moisture and portions were weighed out for experiments. The dry weight of the tissue was 10–15% of the fresh weight.

Incubation procedures. All experiments (except measurements of oxygen uptake) were performed at room temperature (15–18°), which remained constant during individual experiments. The medium in all cases consisted of at least 90% (v/v) sea water. The concentration of thiosulphate due to $^{131}\text{I}^-$ added to the medium did not exceed 10 $\mu\text{M.}$ Even 1000 $\mu\text{M.}$ thiosulphate had no effect on uptake of $^{131}\text{I}^-$.

In experiments in which the radioactivity in the medium was measured at successive times (Tables 1 and 2) 2 g. of tissue was added to 50 ml. of medium in a 250 ml. flask. Except where inhibitors were allowed to act for longer periods, 2–5 $\mu\text{C.}$ of $^{131}\text{I}^-$ was added within 10 min. of mixing tissue and medium, and the flask placed on a shaker. Portions of the medium were decanted at intervals for measurement of radioactivity and then returned to the flask. In other experiments (except in Fig. 1, Expt. C) incubation was started by mixing the complete medium with the tissue. For comparison with uptake of thiocyanate, the uptake of $^{131}\text{I}^-$ by 3 g. of tissue was measured from 7.5 ml. of

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