Adaptive Variation in the Level of Oxidative Activity in *Saccharomyces cerevisiae*

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SUMMARY: After growth in a medium containing 2 % (w/v) galactose, a strain of *Saccharomyces cerevisiae* showed greater oxidative activity, an essentially unchanged anaerobic fermentation rate and an increase in total growth, compared with yeast grown in the same medium but containing instead 2 % (w/v) glucose as major carbon source. The galactose-grown yeast possessed a generally higher level of oxidative metabolism, with enhanced $Q_{O_2}^{air}$ values (up to 500 %) with glucose and other oxidizable substrates, a higher cytochrome content and a greatly increased cytochrome oxidase activity. These differences were associated with an inhibitory effect of glucose on the formation of oxidative enzymes when this sugar was present in the growth medium at concentration greater than 0.05 % (w/v). Changes in the composition of the basal growth medium greatly altered the magnitude of the differences between the glucose-grown and galactose-grown organisms and the differences were not apparent with certain growth media. Possible interpretations of these findings are discussed.

The adaptation of yeast and other micro-organisms to the utilization of galactose or other saccharides has been widely employed as the test system in studies on the mechanisms of enzymic adaptation (Stephenson & Yudkin, 1936; Monod, 1942; Spiegelman, 1946). Such adaptations involve the formation of enzymes for the conversion of these sugars into intermediates of glucose or ribose metabolism; the existence of independent routes for metabolism of other sugars has not been clearly demonstrated. Thus, in the adaptive utilization of galactose in yeast a specific galactokinase is formed for conversion of galactose to galactose-1-phosphate (Trucco, Caputto, Leloir & Mittelman, 1948), and a second adaptive enzyme, galactowaldenase, converts galactose-1-phosphate to glucose-1-phosphate (Caputto, Leloir, Cardini & Paladini, 1950). The glucose-1-phosphate is assumed to be then metabolized by the usual fermentative and oxidative routes of glucose metabolism. Adapted yeast can utilize galactose either oxidatively or fermentatively (Sheffner & Lindegren, 1952). In growth experiments with *Streptococcus pyogenes*, White, Steele & Pierce (1955) observed a partial shift from homolactic metabolism with glucose as carbon source to a heterolactic metabolism with galactose. However, the possibility of the development of alternate pathways or of alterations in the balance of existing metabolic patterns as a result of adaptation appears not to have been thoroughly examined in yeasts.

It therefore seemed of interest to study further the observation made in this laboratory (Dr June Lascelles, personal communication) that after growth on galactose a strain of *Saccharomyces cerevisiae* oxidized glucose more rapidly

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than did the unadapted yeast grown on the same type of medium but utilizing glucose as major carbon source. The present work was an attempt to define this phenomenon more precisely. A preliminary report of this study has been presented previously (Strittmatter, 1955).

METHODS

Saccharomyces cerevisiae, strain no. 77 of the National Collection of Yeast Cultures (Brewing Industry Research Foundation, Nutfield, Surrey, England), was used in this work. The organism has been maintained in our laboratory on agar slopes, the medium containing inorganic salts, 2.25 % (w/v) Difco malt extract, 0.05 % (w/v) Difco yeast extract and 0.5 % (w/v) sucrose.

Preparation of yeast suspensions

An 18 hr. agar slope culture of the organism on the maintenance medium was harvested by centrifugation, washed once with water and resuspended in water to yield a suspension containing the equivalent of 0.3 mg. dry wt. organisms/ml. Samples (0.3 ml.) of this suspension were added to 50 ml. samples of liquid growth medium in vessels shaped like an inverted T, similar to those used by Monod, Cohen-Bazire & Cohn (1951) except that the horizontal limbs of the vessels had a volume of 140 ml. Except where otherwise specified, the liquid growth medium contained 17.5 % (v/v) of a 10 % (w/v) tryptic digest of casein prepared by the method of Cole & Onslow (1916), 0.05 % (w/v) Marmite (a commercial autolysate of yeast), and either 2 % (w/v) glucose or 2 % (w/v) galactose; all growth media were initially adjusted to pH 5.3. The vessels were incubated at 30° and were shaken at 36 oscillations/min. to ensure aeration of the medium. Unless otherwise indicated, the organisms were harvested by centrifugation at equivalent physiological ages, 1 hr. before the end of the period of logarithmic growth; this time of harvest ranged from 18 to 40 hr., depending upon the medium employed. The organisms were washed three times with cold distilled water (10 ml./50 ml. culture medium) and were resuspended in distilled water immediately before use, unless otherwise indicated. The washed organisms were generally used at once, but might be stored at 2° for at least 2 days without appreciable change in activity. Culture densities and the dry weight of harvested suspensions were estimated with an EEL photoelectric colorimeter (Evans Electroselenium Ltd.) or a Spekker Absorptiometer (Hilger and Watts Ltd.) which were calibrated for the organism.

Preparation of organisms for assay of cytochrome oxidase

Heated suspensions (Keilin, 1929). A 2 % (w/v) suspension of organisms in 0.05 M-phosphate (pH 7.1) was warmed quickly to 50° in a water bath, kept at that temperature for 90 min., cooled at 25° and diluted appropriately for assay.

Cell-free extracts. A 50 % (w/v) suspension (4 ml.) of the yeast in 0.1 M-phosphate (pH 7.1) was mixed with 10 g. glass balls (Ballotini no. 13) and shaken in

the Mickle disintegrator (Mickle, 1948) at 2° until microscopic examination indicated that at least 90 % of the organisms were disrupted. The glass balls, unbroken organisms and cellular debris were removed by centrifugation in the cold, and the opalescent, cell-free supernatant solution used as enzyme preparation.

Manometric determinations

Rates of oxygen uptake in air ($Q_{O_2}^{air}$) and of carbon dioxide evolution in nitrogen ($Q_{CO_2}^{N_2}$) were determined by conventional techniques in the Warburg respirometer at 30°. Suitable amounts of the yeast preparations, generally 2–10 mg. dry wt., were suspended in 0.033 M-phosphate buffer (pH 6) containing any additional substances as noted in the text. Substrates were added from the side-arm after equilibration to give a final concentration of either 0.01 M (for hexoses) or 0.02 M (for other substrates). In oxygen uptake measurements, 20 % (w/v) KOH was placed in the centre well; for measurements on cyanide inhibition of respiration, this was replaced with appropriate KOH-KCN mixtures (Umbreit, Burris & Stauffer, 1949).

For cytochrome oxidase assays on heated suspensions, the substrate was 0.02 M-*p*-phenylenediamine and the vessels contained 0.033 M-phosphate buffer (pH 7.1); in the assays on cell-free extracts, the substrate was 0.02 M-ascorbate and the vessels contained 0.033 M-phosphate buffer (pH 7.1), 0.01 M-NaCl and 3×10^{-5} M-cytochrome *c*. The recorded values have been corrected for autoxidation of the substrates; in the assays with ascorbate the autoxidation rate was measured by extrapolating to an enzyme concentration of zero from a series of different enzyme concentrations (see Umbreit *et al.* 1949).

The $Q_{O_2}^{air}$ and $Q_{CO_2}^{N_2}$ values were calculated for a period of linear activity, generally for the first hour, and are expressed as μ l. gas/mg. dry wt. yeast/hr. Except where otherwise specified, all $Q_{O_2}^{air}$ and $Q_{CO_2}^{N_2}$ values have been corrected for endogenous values; the trends of the results are not altered by omitting these corrections but the corrected values are felt to be a better expression of the activities studied. The endogenous values did not vary appreciably in the different preparations and, with exceptions indicated in the text, were only a small percentage of the values obtained with added substrate. The endogenous $Q_{O_2}^{air}$ value was 2–3.5 for organisms grown on the standard glucose-containing media and 3–5 for those grown on galactose-containing media, while the endogenous $Q_{CO_2}^{N_2}$ value was 0–2 and may be considered negligible.

Determination of cytochrome c

Quantitative estimation of cytochrome *c* content in yeast suspensions was made with a microspectroscope assembly (kindly made available by Dr W. E. van Heyningen). A solution of commercial cytochrome *c* was used as a standard, and sodium hydrosulphite (dithionite) was added to both suspensions and standard to maintain the cytochrome in the reduced state.

RESULTS

Characteristics of organisms grown in the standard glucose and galactose media

Growth. The nature of the sugar added to the basal casein digest + Marmite growth medium influenced both the kinetics of growth and the maximum yields of organisms obtained (Fig. 1). In the presence of glucose, growth began without appreciable delay and the final yield was equivalent to 1.1 mg. dry wt. organisms/ml. medium. With galactose there was a lag period of about 6 hr.,

Fig. 1. Effect of sugar in growth medium on growth of *Saccharomyces cerevisiae*. Unadapted yeast was grown in the basal casein digest + Marmite medium to which was added: (a) 2% (w/v) glucose; (b) 2% (w/v) galactose; (c) 2% (w/v) glucose + 2% (w/v) galactose; (d) no sugar added.

reflecting the need for this organism to adapt to utilization of galactose, but the final growth obtained was twice as great as that obtained with the glucose medium. Mixtures of glucose and galactose produced growth curves approximating that obtained with glucose alone, while omission of any added sugar resulted in only poor growth after a long and variable lag period of 18–24 hr.

Oxidation and fermentation of glucose. Organisms growing in the standard casein digest + Marmite medium containing either glucose or galactose were harvested at various ages and their ability to oxidize and ferment glucose was determined. For any given growth time, galactose-grown organisms oxidized glucose much more rapidly than did glucose-grown organisms (Fig. 2a); this difference was of the same magnitude whether or not a correction for endogenous oxygen uptake was made. Except for organisms from very young cultures (12 hr.), galactose-grown organisms also fermented glucose at a greater rate than did the glucose-grown ones (Fig. 2b).

Since both oxidative and fermentative capacities decreased with age in both

types of yeast, further experiments in this study were carried out with organisms of the same physiological age, harvested 1 hr. before the end of the logarithmic phase of growth. As can be seen from Fig. 1, this age for harvesting was at about 18 hr. for glucose-grown organisms and at about 24 hr. for galactose-grown organisms. When compared at these equivalent physiological ages, the galactose-grown organisms oxidized glucose 5.7 times as rapidly as the glucose-grown ones (Fig. 2*a*); in a series of ten preparations, the average (corrected) values of $Q_{O_2}^{air}$ (glucose) were $50.7 (\pm 2.8)$ and $8.8 (\pm 0.9)$, respectively. The $Q_{CO_2}^{N_2}$ (glucose) level was about 150 for both types of organism (Fig. 2*b*).

Fig. 2*a, b*. Effect of age of culture on ability of harvested *Saccharomyces cerevisiae* to (*a*) oxidize glucose, and (*b*) ferment glucose anaerobically. Organisms were harvested at indicated times from standard casein digest + Marmite medium containing either 2% (w/v) glucose (●—●) or 2% (w/v) galactose (+—+) and were assayed manometrically with 0.01 M-glucose as substrate. In Fig. 2*a*, the lower of each pair of points represents the activity corrected for endogenous respiration; the upper point is uncorrected.

Oxidation and fermentation of galactose. The unadapted glucose-grown yeast did not metabolize galactose appreciably, while the galactose-grown organisms both oxidized and fermented this sugar (Table 1). It is of interest that the

Table 1. *Metabolism of glucose and galactose by Saccharomyces cerevisiae grown in media containing either glucose or galactose*

Assay	Growth conditions		
	Glucose (18 hr.)	Galactose (24 hr.)	Galactose (44 hr.)
$Q_{O_2}^{air}$ (glucose)	9.5	52.4	17.6
$Q_{O_2}^{air}$ (galactose)	0	15.8	17.9
$Q_{CO_2}^{N_2}$ (glucose)	148.0	151.0	52.0
$Q_{CO_2}^{N_2}$ (galactose)	1.0	138.0	49.5

Organisms were harvested at the times indicated and assayed manometrically with 0.01 M-glucose or galactose as substrate.

24 hr. galactose-grown organisms oxidized galactose at only one-third the rate observed with glucose although both sugars were fermented at similar rates; in older cultures the oxidative rates for the two sugars approached equality. Since, however, the rates of both oxidation and fermentation of glucose were decreasing markedly with age (Fig. 2), it is difficult to deduce evidence for changing metabolic patterns from such data.

Oxidation of other substrates. The high rate of glucose oxidation by the galactose-grown yeast reflected a generally higher level of oxidative activity in these organisms, compared with those grown on glucose. Of the substrates tested, all those attacked by the yeasts were oxidized more rapidly by the galactose-grown organisms (Table 2).

Table 2. *Respiration of glucose-grown and galactose-grown Saccharomyces cerevisiae in the presence of various substrates*

Harvested organisms were assayed by conventional manometric techniques at 30° in 0.033 M-phosphate, pH 6.0. Acid substrates were neutralized with sodium hydroxide to pH 6.0 before addition. The endogenous respiratory rate was not subtracted in calculating the $Q_{O_2}^{air}$ values in this table.

Substrate	$Q_{O_2}^{air}$	
	Glucose-grown yeast	Galactose-grown yeast
None	3.0	4.5
Glucose, 0.01 M	13.2	54.7
Ethanol, 0.02 M	13.3	52.1
Lactate, 0.02 M	5.4	31.9
Pyruvate, 0.02 M	3.6	19.2
Acetate, 0.02 M	2.6	4.5
Succinate, 0.02 M	3.0	6.0
Fructose diphosphate, 0.01 M	2.6	4.5

There is no evidence that these parallel differences between the two kinds of organism derived from a generally greater permeability of the galactose-grown ones to the substrates. Glucose freely entered both types, as indicated by the same high rates of anaerobic fermentation in both (Fig. 2*b*). On the other hand, succinate and fructose diphosphate, the two test substances which are known to be seriously affected by a permeability barrier at the pH value of the assay system, were not rapidly attacked by either type. Of the other substrates, pyruvate is known to permeate the yeast cell wall, though less readily than at an acid pH value, and the remaining substances pass freely (see Barron, Ardao & Hearon, 1951; Eaton & Klein, 1954).

Electron transport system. The generally higher level of oxidative activity in the galactose-grown organisms suggested a greater capacity of its terminal respiratory system for transport of electrons to oxygen. If grown aerobically, most strains of *Saccharomyces cerevisiae* develop the cyanide-sensitive cytochrome-cytochrome oxidase system as the major pathway of electron transport (Warburg, 1927; Keilin, 1929). This system also predominated in the yeasts used in the present study, since cyanide (10^{-3} M) produced 85–90% inhibition of glucose oxidation and about 95% inhibition of lactate oxidation

by suspensions of both glucose-grown and galactose-grown organisms. The concentration of components of the cytochrome-cytochrome oxidase system in the two kinds of yeast was therefore compared (Table 3).

Table 3. *Cytochrome oxidase activity and cytochrome c content of glucose-grown and galactose-grown Saccharomyces cerevisiae*

Preparation used	Assay	Activity	
		Glucose-grown yeast	Galactose-grown yeast
Heated yeast suspension	$Q_{O_2}^{air*}$ (endogenous)	0.1	0.4
	$Q_{O_2}^{air*}$ (<i>p</i> -phenylenediamine)	0.9	5.7
Cell-free extract	$Q_{O_2}^{air*}$ (endogenous)	0	0
	$Q_{O_2}^{air*}$ (ascorbate)	0.9	9.1
	$Q_{O_2}^{air†}$ (ascorbate)	3.0	26.3
Untreated yeast suspension	Cytochrome <i>c</i> . (μ mole/mg. dry wt.)	0.64×10^{-4}	1.08×10^{-4}

The $Q_{O_2}^{air}$ values are corrected for autoxidation of substrate but not for endogenous respiration.

* μ l. O_2 /mg. dry wt. of original yeast suspension/hr.

† μ l. O_2 /mg. dry wt. of cell-free extract/hr.

Cytochrome oxidase activity was determined manometrically both on heated suspensions with *p*-phenylenediamine as substrate and on cell-free extracts with ascorbate as substrate, as described in Methods. The ratio of activities for galactose-grown against glucose-grown organisms was about 6.5:1 by assay of heated organisms and 10:1 by assay of the cell-free extracts. The activities of the cell-free extracts were calculated both on the basis of dry weight of the original suspensions and on the basis of 'dry weight' of the extracts, determined by drying the material precipitable from the extracts by trichloroacetic acid. The ratio of activities was nearly the same by both methods of calculation, indicating that both cell-free extracts represented approximately the same proportion (about one-third) of the original whole cell substance.

The $Q_{O_2}^{air}$ values obtained by either assay fall below the minimum physiological capacity of the cytochrome oxidase system (presumably represented by the $Q_{O_2}^{air}$ values obtained with glucose or ethanol as substrate). These low activities probably reflect non-optimal conditions of the assay procedures and loss of enzyme activity during preparation of extracts or heated suspensions. However, the fair agreement of the activity ratios obtained by independent assay systems supports the conclusions that the cytochrome oxidase activity of galactose-grown organisms is markedly greater than that of glucose-grown ones.

The cytochrome *c* content, as determined on suspensions, was only 70% higher in galactose-grown organisms. This small increase in cytochrome *c*, in contrast to the great increase in cytochrome oxidase activity, may be compared with observations of Slonimski (1955) on anaerobically-grown yeast: exposure to oxygen induced a 200-fold increase in cytochrome oxidase activity, but only a 20-fold increase in cytochrome *c* content.

Adaptive (inductive) nature of the phenomenon

Two types of experiments were employed to test whether the differences in oxidative capacities between glucose-grown and galactose-grown organisms result from adaptive (inductive) changes or from selection of different strains (or mutants) in the growth media.

First, following seven subcultivations in the standard galactose-containing liquid medium, the harvested organisms had a $Q_{O_2}^{air}$ (glucose) value of 52, typical of galactose-grown organisms. If after such repeated galactose cultivation the yeast was grown once in the standard glucose medium, the $Q_{O_2}^{air}$ (glucose) value of harvested organisms was 9.

Secondly, an inoculum from the stock yeast culture was streaked on agar plates of the same composition as the maintenance medium but with 2 % (w/v) galactose replacing 0.5 % (w/v) sucrose as chief carbon source. After growth a single colony was chosen to streak a new plate, and after ten such transfers a single colony was used as inoculum for growth in casein digest + Marmite liquid medium containing 2 % (w/v) glucose or 2 % (w/v) galactose. The organisms were harvested and assayed manometrically. In three independent experiments, the organisms thus grown in galactose medium possessed an average $Q_{O_2}^{air}$ (glucose) value of 53.2 (51.2, 53.6, 54.9); the glucose-grown organisms possessed a $Q_{O_2}^{air}$ (glucose) value of 9.0 (8.6, 8.9, 9.5).

In both types of experiments, therefore, repeated exposure to conditions favouring selection of highly oxidative strains did not alter the oxidative capacity of the galactose-grown yeast, while a single passage through glucose-containing medium reduced the oxidative level to that normally observed with glucose-grown organisms. The results strongly suggest that the differences in oxidative levels between these glucose-grown and galactose-grown yeasts are adaptive (inductive) in origin.

Factors which affect the differences in growth and oxidative capacity

Strain of yeast. The differences between glucose-grown and galactose-grown yeast with respect to growth and oxidative capacity were not unique to *Saccharomyces cerevisiae* strain no. 77, but were also observed with the 'Yeast Foam' strain of *S. cerevisiae* (kindly supplied by Dr P. Slonimski). However, the differences were not as great with the 'Yeast Foam' strain.

Concentration of sugar in the growth medium. The oxidative activity of harvested organisms was markedly decreased when the initial concentration of glucose in the growth medium exceeded a very low optimal concentration, while high initial concentrations of galactose caused a relatively slight decrease of oxidative activity (Table 4). The anaerobic fermentation rates were not appreciably altered by these manipulations. With the lowest concentration of glucose tested, 0.5 mg./ml. growth medium, the organisms possessed a $Q_{O_2}^{air}$ (glucose) value approaching that found with galactose-grown organisms, while with concentrations of 10 mg./ml. or more, the oxidative rate was less than 20 % of that of galactose-grown organisms. With galactose, increasing the concentration from 1 to 200 mg./ml. decreased the $Q_{O_2}^{air}$ (glucose) value of the

resultant organisms by only about 20 %. The sugar concentrations required to produce maximum oxidative activities may be lower than the minimum concentrations employed. However, this possibility could not be tested since appreciably lower concentrations had the same effect as entirely omitting

Table 4. *Effect of varying the sugar concentration of the growth medium on metabolic activity of Saccharomyces cerevisiae*

Glucose or galactose was added to the basal casein digest + Marmite medium in the concentrations indicated. Organisms were grown and harvested in the standard manner and assayed manometrically with 0.01 M-glucose as substrate.

Initial concentration of sugar in growth medium (mg./ml.)	Activity			
	Glucose-grown yeast		Galactose-grown yeast	
	$Q_{O_2}^{air}$	$Q_{CO_2}^{N_2}$	$Q_{O_2}^{air}$	$Q_{CO_2}^{N_2}$
0.5	52.0	163	—	—
1.0	39.5	174	60.9	180
2.0	30.1	160	59.8	162
10.0	10.5	153	—	—
20.0	8.8	148	51.3	156
50.0	7.9	150	49.7	160
200.0	—	—	50.4	163

added sugar from the basal growth medium: there were long lag periods and poor growth (Fig. 1), and the organisms obtained had low metabolic activities (Table 5).

Table 5. *Effect of varying the type of sugar in the growth medium on growth and metabolic activity of Saccharomyces cerevisiae*

Sugars as indicated were added to the basal casein digest + Marmite growth medium; growth, harvesting and assays were carried out by the standard procedures. The $Q_{O_2}^{air}$ and $Q_{CO_2}^{N_2}$ values were obtained with the harvested organisms using 0.01 M-glucose as substrate. Growth values represent the levels reached if the cultures were permitted to grow to maximum density. Values given are the average of results from four to ten experiments.

Sugar added to growth medium	Activity of harvested organisms		Maximum growth (mg./ml.)	Increase in lag phase* (hr.)
	$Q_{O_2}^{air}$	$Q_{CO_2}^{N_2}$		
2 % (w/v) glucose	8.8	148	1.10	(0)
2 % (w/v) galactose	50.7	153	2.20	6
2 % (w/v) glucose + 2 % (w/v) galactose	14.3	135	1.15	0
2 % (w/v) fructose	9.0	166	1.18	0
2 % (w/v) mannose	15.0	161	1.25	2
None added	< 5	< 25	0.30	18–24

* Increase in duration of lag phase over that obtained with glucose.

These results suggest that the relatively low oxidative activity of the glucose-grown organisms derives primarily from an inhibitory effect of glucose on the development of oxidative capacity in the growing organisms, i.e. on the formation of the enzymes involved in oxidative metabolism. However, the

possibility of an additional stimulatory effect of galactose is not thereby ruled out.

Type of sugar in growth medium. Exploratory experiments showed that the glucose-grown yeast could, without preliminary adaptation, ferment or oxidize fructose and mannose approximately as fast as it did glucose. Yeast hexokinase is known to phosphorylate fructose and mannose readily (Berger, Slein, Colowick & Cori, 1946) and the phosphorylated derivatives enter the main stream of glucose metabolism. It is therefore significant that when used as growth substrates (Table 5) these sugars produced organisms similar in their low oxidative activities to those grown in glucose medium; in addition, the growth curve and yield of organism with these sugars were similar to those obtained with glucose.

Organisms grown in a mixture of glucose and galactose also were similar in their growth characteristics and low oxidative activity to those grown in glucose alone (Table 5). Apparently, these organisms produced preferentially the balance of enzyme constituents required for the less oxidative metabolic pattern evoked by high concentrations of the normal 'constitutive' substrate, glucose. These glucose + galactose-grown organisms were partially adapted to utilization of galactose at the standard age of harvest (18 hr.): the $Q_{O_2}^{sr}$ and $Q_{CO_2}^{N_2}$ values with galactose as substrate were 5.4 and 7.0, respectively. Adaptation increased with age of culture, e.g. at 24 hr. $Q_{CO_2}^{N_2}$ (galactose) was 27, but the decrease of metabolism activities with age of culture became the more significant factor, e.g. at 24 hr. the $Q_{O_2}^{sr}$ (galactose) and $Q_{O_2}^{sr}$ (glucose) values, while now nearly equal, had dropped to 4.2 and 5.3, respectively.

Changes in medium during growth. Since the acidity of the environment may determine what type of metabolism develops in a micro-organism (White *et al.* 1955), the pH value of the glucose and galactose growth media was determined at various ages of culture. The acidity increased in both media in proportion to the amount of growth. At the standard times of harvest the pH value of the glucose medium was 4.5 and that of the galactose medium, 4.2. This parallel drop of pH value and lack of gross difference in acidity between the two cultures appears to rule out acidity as a significant factor in the different metabolic patterns of glucose-grown and galactose-grown yeasts.

The decline in the concentration of added sugar in the medium with time was also examined, since exhaustion of hexose might stimulate increased development of oxidative metabolism (Swanson & Clifton, 1948). The remaining sugar of the growth media was separated by paper chromatography, eluted and assayed by an anthrone method (Koehler, 1952). More than one-fourth of the glucose or galactose initially present remained at the standard ages of harvest, hence the increased oxidative activity of the galactose-grown organisms cannot be attributed to an early exhaustion of galactose from the growth medium.

Composition of the basal growth medium. The difference between the glucose-grown and galactose-grown yeasts with respect to oxidative activity and maximum growth were profoundly affected by altering the composition of the basal growth medium (Table 6). For organisms of equivalent physiological ages the

oxidative activity was similar in galactose-grown organisms harvested from different media (column 6), while the oxidative activity of glucose-grown organisms was greatly altered by change of growth medium (column 3). Variation in the ratio of oxidative activities in the two types of yeast therefore largely reflects the effect of the medium on the inhibition by glucose of oxida-

Table 6. *Influence of composition of growth medium on growth and metabolic activity of Saccharomyces cerevisiae*

Organisms were grown in the various basal media to which were added either 2% (w/v) glucose or 2% (w/v) galactose; all media were initially adjusted to pH 5.3. The $Q_{O_2}^{air}$ and $Q_{CO_2}^{N_2}$ values with glucose as substrate were obtained with organisms harvested at equivalent physiological ages late in the period of logarithmic growth. Growth values represent the levels obtained when the cultures were permitted to grow to maximum density. Values given in this table represent the average of results from three to ten experiments.

Medium no.	Composition of basal medium (1)	Glucose-grown yeast			Galactose-grown yeast		
		$Q_{O_2}^{air}$	$Q_{CO_2}^{N_2}$	Yield (mg./ml.)	$Q_{O_2}^{air}$	$Q_{CO_2}^{N_2}$	Yield (mg./ml.)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1	Casein digest + 0.05% (w/v) Marmite	8.8	148	1.10	50.7	153	2.20
2	Casein digest + 0.025% (w/v) Marmite	6.5	166	0.38	43.7	163	0.78
3	Casein digest + 0.2% (w/v) Marmite	15.8	174*	1.93	56.8	164*	3.44
4	Casein digest + 0.5% (w/v) Marmite	45.6	250*	5.28	73.7	175*	5.32
5	0.05% (w/v) Marmite + 0.1 M-phosphate	36.3	165	0.58	42.7	157	0.65
6	0.4% (w/v) NH_4Cl + 0.05% (w/v) Marmite + 0.1 M- phosphate	28.2	183	0.65	67.3	191	0.81
7	Chemically defined	22.4	215*	1.99	55.4	243*	1.79
8	Yeast extract-malt extract-salts	72.9	195	11.0	74.7	163	11.5

* Not corrected for endogenous activity.

tive enzyme formation. The maximum growth for both glucose-grown and galactose-grown organisms varied with the different media (columns 5 and 8, respectively), but variation in the relative growth of the two types again reflects the influence of the medium on the inhibitory effect of glucose. The anaerobic fermentation rate was of the same order of magnitude in both types for all growth media tested (columns 4 and 6, respectively). This constancy of fermentative capacity tends to confirm that the various yeast preparations were of comparable physiological ages.

Of various basal media examined, the most marked differences between glucose-grown and galactose-grown organisms were observed with the standard casein digest + Marmite medium; this medium was consequently used as a basis for the experiments reported in this paper. The results of varying the

concentration of Marmite (media 2, 3, 4) from that in the standard basal medium (medium 1) indicate that an increased Marmite concentration reduces the differences in oxidative activity. Since the Marmite concentration was the limiting factor in the amount of growth obtained, attempts to increase the inhibitory effect of glucose by drastic decrease of Marmite concentration proved impractical.

Omission of casein digest (medium 5) from the standard basal growth medium also resulted in glucose-grown organisms with maximum growth and oxidative activity approaching the levels found in galactose-grown ones. These results suggest that the presence of casein digest permits or potentiates the appearance of the differences between the two types of organism. The phosphate was added to medium 5 to provide buffering capacity; it had no specific effect, as indicated by the fact that addition of 0.1 M-phosphate to the standard growth medium (results not tabulated) produced no significant alterations in metabolic activities or growth.

To test further the effects of varying the major nitrogenous constituents, casein digest was replaced by ammonium chloride (medium 6) or ammonium sulphate was used as sole major nitrogen source (medium 7). In the latter case, the chemically defined medium contained, per litre, 2 g. $(\text{NH}_4)_2\text{SO}_4$, 7.5 g. KH_2PO_4 , 0.5 g. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.15 g. CaCl_2 , 2.8 mg. FeSO_4 , 0.4 mg. thiamine, 5 mg. inositol, 4 mg. biotin, 0.4 mg. pantothenate and 0.14 mg. *p*-aminobenzoic acid. With either medium, a partial decrease of the glucose inhibition of oxidative activity was obtained, while the maximum growth obtained was nearly the same for glucose-grown and galactose-grown organisms.

The fact that the differences between glucose-grown and galactose-grown organisms were least evident when Marmite was the major nitrogenous component of the growth medium suggests that a balance of substances in the growth medium which resembles the composition of the yeast aids in suppressing the inhibitory effects of glucose.

For comparison, the yeast was also grown in a complex medium (medium 8) identical with that used for the maintenance-agar slopes except for omission of agar and substitution of 2% (w/v) glucose or 2% (w/v) galactose for 0.5% (w/v) sucrose. This rich medium, on which the organisms had been maintained for a long period, produced high yields of yeast and high oxidative activity, with no appreciable differences between glucose-grown and galactose-grown organisms in these respects.

DISCUSSION

The ultimate mechanism by which glucose inhibits the development of oxidative capacity with this *Saccharomyces cerevisiae* strain is not certain, but several possible factors suggest themselves. First, the results obtained by varying the nature of the sugar present in the growth medium (Table 5) indicate that oxidative pathways are relatively little developed in the presence of high concentrations of a sugar readily and immediately fermentable without need for preliminary adaptation. Slonimski (1955) found that high concentrations of

glucose result in a decreased rate of oxygen-induced formation of certain oxidative enzymes in non-proliferating yeast suspensions; this retardation was associated with a heightened aerobic fermentation rate. Glucose is known to inhibit the formation of a number of enzymes in various organisms (Epps & Gale, 1942). In at least some instances, this glucose effect may be interpreted as a homœostatic mechanism whereby products formed by metabolism of glucose by 'constitutive' enzyme systems can inhibit the formation of 'adaptive' (inducible) enzymes whose activity would merely increase the metabolic pool of these products (Neidhardt, 1956). Glucose inhibition of oxidative enzyme formation in yeast may similarly reflect an intracellular regulatory mechanism; the 'constitutive' enzyme systems readily fill the energy requirements of the organism by fermentative breakdown of glucose when this sugar is present, and the presence of this metabolic energy pool may partially inhibit the formation of 'adaptive' energy-yielding oxidative enzyme systems.

Secondly, the results obtained by varying the composition of the growth medium (Table 6) suggest that glucose inhibition may involve depletion or imbalance of essential constituents required for formation of oxidative enzymes. Such deficiencies may result from increased utilization of essential components for other purposes or in other reactions not as prominent in a more oxidative metabolic milieu. The synthesis of enzymic protein requires the presence of a complete array of the component amino acids in sufficient quantity (Spiegelman & Halvorson, 1953), and for optimal utilization by the organism the various amino acids must be presented in correct proportions (Almquist, 1953). Various other substances, including nucleic acids, are also essential components of the protein synthesis mechanism (Gale & Folkes, 1955).

The reasons for the differences between glucose-grown and galactose-grown organisms reported here are obscure. Gross differences of pH value in the media during growth or an early depletion of galactose appear to be ruled out as significant factors in causing these differences. Further, organisms grown in glucose + galactose mixtures possess low oxidative activity and give a yield of organisms characteristic of glucose-grown ones (Table 5), despite their ability to metabolize galactose. It therefore seems improbable that galactose or some metabolic derivative of galactose required for essential structural or functional roles exerts a specific stimulatory effect on development of oxidative enzyme activity. The apparent stimulatory effect of galactose appears rather to arise from a relative lack of the glucose type of inhibition. The organism studied must adapt to utilization of galactose and then actively maintain the adaptive enzyme system; the consequent lower facility and capacity for fermenting this substrate, particularly in newly adapting cultures, may permit development of efficient oxidative pathways for more complete utilization of the available supply of substrates for energy production. Galactose may also evoke development of an alternate oxidative pathway perhaps characteristic of galactose metabolism but also utilizable by glucose in the adapted yeast; however, no direct evidence for such a pathway has been obtained.

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