

# Activities of Tricarboxylic Acid Cycle Enzymes, Glyoxylate Cycle Enzymes, and Fructose Diphosphatase in Bakers' Yeast During Adaptation to Acetate Oxidation

J. P. GOSLING AND P. F. DUGGAN

*Biochemistry Department, University College, Dublin, Ireland*

Received for publication 20 October 1970

Bakers' yeast oxidizes acetate at a high rate only after an adaptation period during which the capacity of the glyoxylate cycle is found to increase. There was apparently no necessity for the activity of acetyl-coenzyme A synthetase, the capacity of the tricarboxylic acid cycle, or the concentrations of the cytochromes to increase for this adaptation to occur. Elevation of fructose 1,6 diphosphatase occurred only when acetate oxidation was nearly maximal. Cycloheximide almost completely inhibited adaptation as well as increases in the activities of isocitrate lyase and aconitate hydratase, the only enzymes assayed. *p*-Fluorophenylalanine was partially effective and chloramphenicol did not inhibit at all. The presence of ammonium, which considerably delayed adaptation of the yeast to acetate oxidation, inhibited the increases in the activities of the glyoxylate cycle enzymes to different degrees, demonstrating noncoordinate control of these enzymes. Under the various conditions, the only enzyme activity increase consistently related to the rising oxygen uptake rate was that of isocitrate lyase which apparently limited the activity of the cycle.

It has often been reported that yeast does not oxidize acetate maximally without prior adaptation. Lynen (23) found that many substrates, when added in small concentrations along with acetate to starved yeast suspensions, considerably shortened the adaptation period. He concluded that they did so by providing energy necessary for the condensation of acetate and oxaloacetate or by providing oxaloacetate. Eaton and Klein (10) found that *Saccharomyces cerevisiae* grown aerobically with glucose could not oxidize acetate or ethanol during the lag phase of growth but could readily do so during the stationary phase. The activities of some tricarboxylic acid cycle enzymes were much greater in the stationary-phase yeast. This development of acetate oxidative ability after glucose exhaustion was later reported to be associated with the appearance of mitochondria in the yeast, as shown in photomicrographs (27).

The glyoxylate bypass or cycle was described by Kornberg and Krebs (20), who proposed that it was responsible for the formation of C<sub>4</sub> dicarboxylic acids during the growth of microorganisms on acetate. Polakis and Bartley (26) con-

cluded that the glyoxylate cycle, in conjunction with the tricarboxylic acid cycle, is necessary for maximal acetate oxidation by yeast, but they presented no direct experimental evidence for this. Witt et al. (29) examined the repressive effects of 2-deoxy-D-glucose and 2-amino-D-glucose on the synthesis of isocitrate lyase (EC 4.1.3.1), malate synthase (EC 4.1.3.2), and malate dehydrogenase (EC 1.1.1.37) by yeast and found that these compounds also repressed adaptation to the oxidation of acetate. They therefore concluded, because of the anaplerotic function of the glyoxylate cycle, that synthesis of these enzymes was responsible for the increasing acetate oxidation rate.

In fatty seedlings, the glyoxylate cycle was found (4) to be located in the glyoxysomes, which resemble peroxisomes (25), and a number of these enzymes are also peroxisomal in the protozoan, *Tetrahymena pyriformis* (25). Though Avers and Federman (2) reported the existence of peroxisomes in aerobically grown yeast, Duntze et al. (9) did not find the glyoxylate cycle of *S. cerevisiae* to be associated with any particles. They found isocitrate lyase and malate syn-

these activities only in the soluble fractions, and those of citrate synthase (EC 4.1.3.7), aconitate hydratase (EC 4.2.1.3), fumarate hydratase (EC 4.2.1.2), and malate dehydrogenase both in the mitochondrial and in the soluble fractions. They did not assay for any peroxisomal marker enzymes.

Bakers' yeast is grown commercially on crude sugar with vigorous aeration. It can readily oxidize ethanol and glucose (with a low RQ) but oxidizes acetate only at a high rate after a period of adaptation (8). In this paper we show that respiratory adaptation is not sufficient for maximal acetate oxidation by yeast. The glyoxylate cycle, which functions anaerobically in relation to the tricarboxylic acid cycle, appears to be necessary for acetate oxidation at a maximal rate. Preliminary accounts of this work have been published (12, 13).

### MATERIALS AND METHODS

**Treatment of the yeast.** The bakers' yeast (*S. cerevisiae*), produced by the Cork Yeast Co., Ltd. (Cork, Ireland), was obtained 2 to 4 days after it was harvested and was washed twice with distilled water before further use or treatment. Starvation of the yeast was performed by bubbling vigorously, with medical grade oxygen, a 10% (w/v, wet weight) suspension of the yeast in distilled water. It was found that 6 hr of oxygenation was as effective as overnight bubbling (16 hr) in extending the period of acetate adaptation. After starvation, the yeast was washed once with distilled water and stored as a thick paste at 4 C. Experiments for the determination of O<sub>2</sub> uptake rates and for sampling for later enzyme and protein assay were always performed within 5 days after the yeast was purchased. The yeast dry weight (which was 19 to 23% of the wet weight) was estimated by drying a weighed amount of the yeast overnight at about 100 C.

**Manometry.** Oxygen uptake rates were performed by conventional Warburg manometry.

**Comparison of cytochrome concentrations.** Spectra of the yeast were recorded by using a Unicam SP800 recording spectrophotometer with the sample and reference cuvettes mounted near the phototube to reduce light scattering. The yeast cells were suspended in 30% (v/v) glycerol at a concentration of 0.10 g of yeast (wet weight)/ml and reduced with a little sodium dithionite. The reference cuvette contained a suspension of flour (50 mg/ml) and soluble starch (5 mg/ml) in 30% (v/v) glycerol.

**Sampling for enzyme assay.** A rack fitted to the circular Warburg water bath held a maximum of eight 150-ml conical flasks and allowed the use of a small number of manometers at the same time. Each conical flask held 50 ml of 1.0% (w/v, wet weight) buffered yeast suspension, and 5 ml of substrate solution was added at time zero. On removal of a flask, its contents were immediately centrifuged in the cold for about 3 min at about 3,000 × g. The yeast was washed once with distilled water in the same way, frozen by partial immersion of the centrifuge tube in liquid N<sub>2</sub>, and stored at -15 C.

**Preparation of extracts.** The frozen yeast samples were added with twice their weight of alumina type 305 (Sigma Chemical Co., St. Louis, Mo.) to a prechilled mortar and ground vigorously for 1 min. The paste thus produced was suspended in 2 or 3 ml of 100 mM KH<sub>2</sub>PO<sub>4</sub>-KOH, pH 6.4, and centrifuged for 10 min at 750 × g in the cold. The supernatant fluid was used for the enzyme assays, which were performed on the day that the yeast was disrupted.

In experiments with chloramphenicol, *p*-fluorophenylalanine, and cycloheximide, these compounds were dissolved in the buffered yeast suspension for 30 min before the addition of acetate.

Protein was estimated by an automated procedure (22) or by the biuret method (21). Bovine serum albumin (Nutritional Biochemicals Corp., Cleveland, Ohio) was used as standard.

**Enzyme assays.** The following assays were performed by methods used and described by other workers: acetyl-coenzyme A (acetyl-CoA) synthetase, EC 6.2.1.1 (18); aconitate hydratase and fumarate hydratase (28); malate synthase (7); nicotinamide adenine dinucleotide phosphate (NADP)-linked isocitrate dehydrogenase, EC 1.1.1.42 (11); citrate synthase (11).

To measure isocitrate lyase, a variation of the method of Dixon and Kornberg (7) was used. Cell extract was added to 100 μmoles of imidazole-hydrochloride buffer (pH 6.6), 20 μmoles of MgCl<sub>2</sub>, 2 μmoles of ethylenediaminetetraacetic acid (EDTA), 10 μmoles phenylhydrazine-hydrochloride, and 5 μmoles of DL-isocitrate in a total volume of 2.5 ml. The reaction was started by addition of isocitrate, and the formation of phenylhydrazone was followed spectrophotometrically at 232 nm.

Malate dehydrogenase was assayed by following the decrease in optical density at 340 nm caused by the formation of nicotinamide adenine dinucleotide (NAD) from reduced NAD (NADH). The assay mixture consisted of 200 μmoles of triethanolamine-hydrochloride (pH 7.5), 0.3 μmole of NADH, and 9 μmoles of oxaloacetate, in a final volume of 3.0 ml.

Succinate dehydrogenase (EC 1.3.99.1) was assayed as succinate tetrazolium-oxidoreductase by using a continuous assay. The increase in the optical density at 490 nm caused by the succinate-dependant reductive formation of formazan from *p*-iodonitrotetrazolium violet was followed, and an increment in the optical density of 20.1 was taken to indicate the formation of 1.0 μmole of formazan per ml. In a final volume of 3.0 ml, there were 150 μmoles of KH<sub>2</sub>PO<sub>4</sub>-KOH (pH 7.4), 6 μmoles of EDTA, 150 μmoles of sucrose, 75 μmoles of succinate, and 0.1% *p*-iodonitrotetrazolium. The recorded specific activities were low, possibly because of a partial loss of activity during disruption of the yeast (9), but the variations in its activity under particular conditions were reproducible.

NAD-linked isocitrate dehydrogenase (EC 1.1.1.41) activity was measured by following the increase in optical density at 340 nm caused by the formation of NADH from NAD. The assay mixture consisted of 400 μmoles of tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, (pH 7.6), 10 μmoles of MgCl<sub>2</sub>, 0.8 μmole of adenosine monophosphate, 0.75 μmole of NAD, 22.5 μmoles of DL-isocitrate, and water to 3.0 ml.

Fructose diphosphatase (EC 3.1.3.11) activity was measured by means of a continuous linked assay. A final volume of 1.1 ml (1-cm light-path cuvettes were used) contained 75  $\mu$ moles of glycylglycine-hydrochloride (*pH* 7.5), 10  $\mu$ moles of  $MgCl_2$ , 1  $\mu$ mole of EDTA, 0.2  $\mu$ mole of fructose-1,6-diphosphate, 0.5  $\mu$ mole of NADP, and excess glucose-6-phosphate dehydrogenase and phosphohexose isomerase. The increase in the optical density at 340 nm due to the formation of NADPH was recorded.

The continuous enzyme assays were carried out semiautomatically by using a Unicam SP 800 recording spectrophotometer fitted with an automatic cell changer, or manually by using a Beckman DU spectrophotometer. All the enzyme assays were performed at 25 C, except that of acetyl-CoA synthetase, which was performed at 37 C.

Most of the biochemicals used were purchased from Sigma Chemical Co. Glucose-6-phosphate dehydrogenase, phosphohexose isomerase, NADP, and adenosine monophosphate were obtained from C. F. Boehringer and Sohne (Mannheim, Germany). Acetyl-CoA was obtained from P-L Biochemicals, Inc., Milwaukee, Wis.

## RESULTS

Glucose and ethanol are readily oxidized by the bakers' yeast used in this study, but with 9 mM acetate at *pH* 7.5 on adaptation period of about 60 min was required before a maximal rate of oxidation was attained (Fig. 1). Starvation of the yeast by prior oxygenation for 16 hr extended this adaptation period to 2 hr without affecting the final rate. The oxidation of glucose and ethanol was not affected by this treatment.

**Adaptation to acetate oxidation and changes in the activities of relevant enzymes.** The first step in the utilization of acetate is its condensation with CoA catalyzed by acetyl-CoA synthetase; therefore the possible involvement of this enzyme was investigated. In Fig. 2 are plotted the specific activities of isocitrate lyase and acetyl-CoA synthetase in yeast as it adapted to acetate oxidation while suspended in buffer at *pH* 7.5. Whereas the activity of isocitrate lyase increased in synchrony with the increasing  $O_2$  uptake rate, the increase in acetyl-CoA synthetase activity was insignificant at 120 min, at which time the acetate oxidation rate was nearly maximal.

The activities of other enzymes relevant to acetate metabolism were also measured as yeast adapted to acetate oxidation. Malate synthase, the other enzyme participating in the glyoxylate but not in the tricarboxylic acid cycle, and malate dehydrogenase increased in activity along with the increasing  $O_2$  uptake rate (Fig. 3). Activities of citrate synthase and aconitate hydratase but not of fumarate hydratase and succinate dehydrogenase increased during the increasing acetate oxidation rate (Fig. 4). The activities of

NAD- and NADP-linked isocitrate dehydrogenases (*not shown*) were found to decrease slowly when the yeast was incubated with acetate at *pH* 7.5.

Fructose diphosphatase is necessary for gluconeogenesis from acetate, and its activity was therefore measured in yeast as it adapted to acetate oxidation. Its activity did indeed increase during adaptation, but there was invariably a delay, relative to the glyoxylate cycle activities, before the rate of increase became large (Fig. 3 and 4).

**Effects of ammonium of adaptation to acetate oxidation and the activities of various enzymes.** When ammonium is added to starved yeast, the induction of  $\alpha$ -glucosidase (EC 3.2.1.20) by maltose is accelerated due to expansion of the amino acid pool (15). However, when ammonium was added to incubation medium along with acetate, the adaptation to acetate oxidation was greatly delayed in either starved or unstarved yeast. In yeast starved for 6 hr, the time before maximal oxidation was achieved was extended from 90 min (Fig. 4) to 190 min (Fig. 5) with 1 mM ammonium chloride. Although aconitate hydratase increased slightly towards the end of acetate adaptation (Fig. 5), the increase in the acetate oxidation rate was now dissociated from any increase in the activities of fumarate hydratase (Fig. 5), malate synthase, and malate dehydrogenase (Fig. 6).

In contrast, the activity of citrate synthase was not much affected by the presence of ammonium (compare Fig. 4 with Fig. 5 and 6), whereas the rise in activity of isocitrate lyase was closely associated with the increase in the acetate oxidation rate in the presence as well as in the absence of ammonium (Fig. 4 and 5). Increase in the activity of fructose diphosphatase was also delayed and the activity of succinate dehydrogenase increased slightly when ammonium was added (Fig. 5).

**Effects of inhibitors of protein synthesis on adaptation to acetate.** To determine whether de novo protein synthesis or activation of existing enzyme molecules was responsible for the increasing oxygen uptake, several well-characterized inhibitors were used. Cycloheximide inhibits yeast cytoplasmic protein synthesis (5), chloramphenicol inhibits mitochondrial protein synthesis but does not affect the growth of yeast unless respiratory adaptation is necessary (5), and *p*-fluorophenylalanine replaces phenylalanine in proteins (17). The effects of these three agents on the adaptation of the yeast to acetate oxidation and on the increases in activities of two enzymes were examined (Fig. 7). Cycloheximide at a concentration of 1  $\mu$ g/ml (3.7  $\mu$ M) completely inhib-

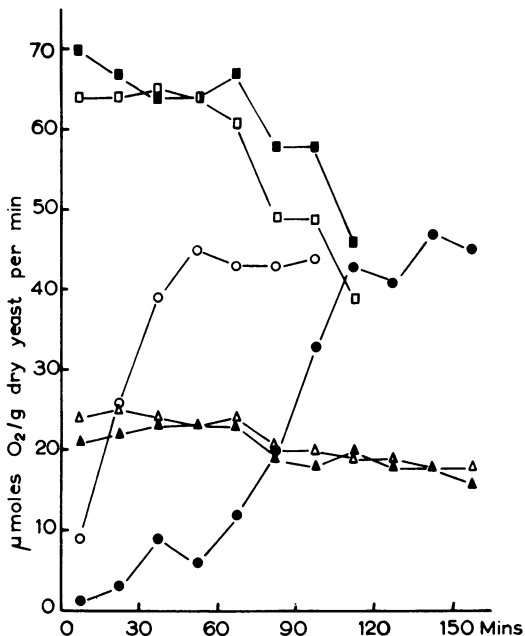


FIG. 1. Effect of prior starvation of the yeast on its ability to oxidise glucose, ethanol, and acetate. The  $\text{O}_2$  uptake rates were measured by using unstarved yeast (open symbols), or starved yeast (closed symbols), suspended at a concentration of 0.45% (w/v, wet weight) in 45 mM Tris-hydrochloride (pH 7.5) and 4.5 mM glucose ( $\Delta$ ,  $\blacktriangle$ ), 9 mM ethanol ( $\square$ ,  $\blacksquare$ ), or 4.5 mM Mg (acetate)<sub>2</sub> ( $\circ$ ,  $\bullet$ ).

ited adaptation with acetate oxidation not exceeding 6  $\mu\text{moles of O}_2/\text{g}$  of dry yeast per min, with no increases in the activities of aconitate hydratase and isocitrate lyase. *p*-Fluorophenylalanine (10 mM) only partially prevented adaptation and incompletely prevented increases in the activities of aconitate hydratase and isocitrate lyase. After 195 min, increases in both the acetate oxidation rate and the activity of aconitate hydratase were inhibited by about 50%, whereas that of isocitrate was reduced by nearly 80%. This was the only condition under which the measured activity of isocitrate lyase was not more closely related to the increasing acetate oxidation rate than that of aconitate hydratase. Chloramphenicol, even at the relatively high concentration of 5 mg/ml (15 mM), never significantly inhibited adaptation, and in this experiment it actually caused a slight stimulation of the oxygen uptake rate.

**Adaptation to acetate at pH 4.5.** It seemed possible that the penetration of acetate rather than the isocitrate lyase activity was rate limiting; therefore some experiments were performed at pH 4.5, thus increasing the concentration of the very permeable acetic acid about 500-

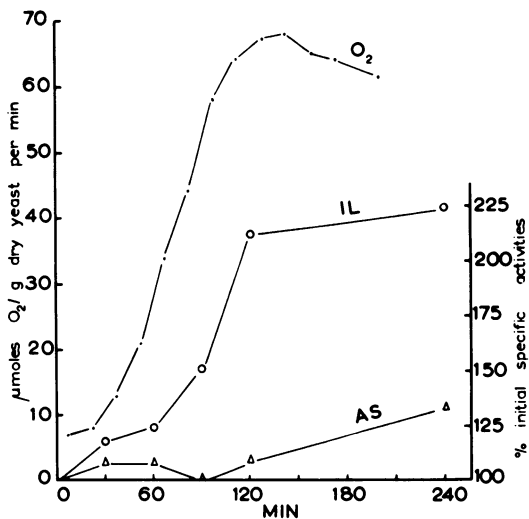


FIG. 2. Activities of acetyl-CoA synthetase and isocitrate lyase in bakers' yeast during its adaptation to the oxidation of acetate at pH 7.5. Sampling for enzyme assay and measurement of the  $\text{O}_2$  uptake rate ( $\text{O}_2$ ) were performed by using 0.9% (w/v, wet weight) suspensions of 16-hr starved yeast in 45 mM Tris-hydrochloride, pH 7.5, and 10 mM Mg (acetate)<sub>2</sub> at 25 C. The initial enzyme activities were: isocitrate lyase (IL), 17; acetyl-CoA synthetase (AS), 12 nmoles of substrate transformed per mg of protein per min.

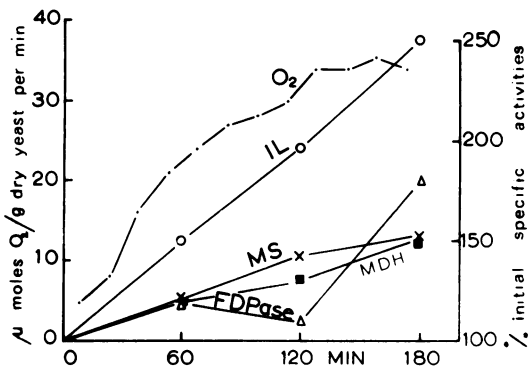


FIG. 3. Activities of isocitrate lyase, malate synthase, fructose diphosphatase, and malate dehydrogenase in yeast during adaptation to acetate oxidation at pH 7.5. Yeast, starved for 16 hr, was used as 0.9% (w/v, wet weight) suspensions in 45 mM Tris-hydrochloride, pH 7.5, and 10 mM Mg (acetate)<sub>2</sub> at 25 C. Samplings for enzyme assay were in duplicate. Average initial activities were: isocitrate lyase (IL), 19; malate synthase (MS), 82; fructose diphosphatase (FDPase), 20; malate dehydrogenase (MDH), 315 nmoles of substrate transformed per mg of protein per min.

fold over that at pH 7.5. However, the same curves of increasing oxygen uptake and increasing enzyme activities were found as at pH 7.5. Also, although acetyl-CoA synthetase, fumarate hydratase, malate dehydrogenase, and

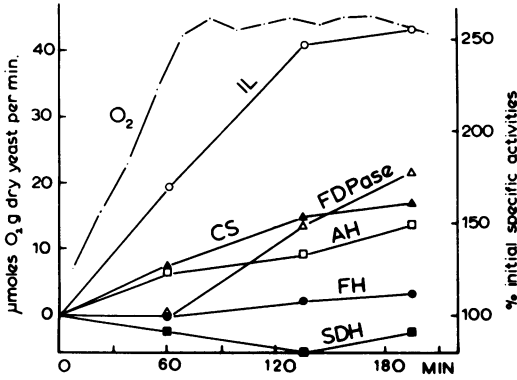


FIG. 4. Activities of enzymes concerned with acetate metabolism during adaptation. Yeast starved for 6 hr was used as 0.9% (w/v, wet weight) suspensions in 45 mM Tris-hydrochloride, pH 7.5, and 10 mM Mg (acetate)<sub>2</sub>. Initial activities were: isocitrate lyase (IL), 21; citrate synthase (CS), 877; fructose diphosphatase (FDPase), 18; aconitate hydratase (AH), 340; fumarate hydratase (FH), 86; succinate dehydrogenase (SDH), 1.3 nmoles of substrate transformed per mg of protein per min.

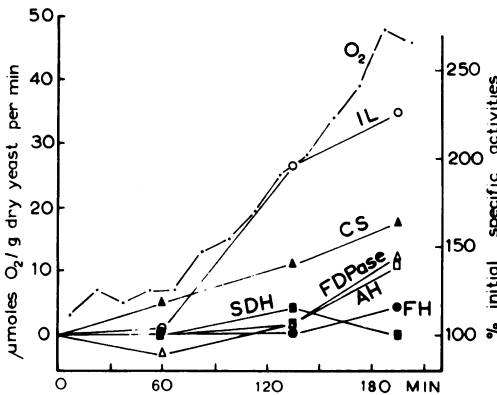


FIG. 5. Activities of isocitrate lyase and other enzymes related to acetate metabolism in bakers' yeast adapting to acetate oxidation in the presence of 1 mM ammonium chloride. This experiment was performed in parallel with that of Fig. 4. For further details, see legend of Fig. 4.

succinate dehydrogenase increased to a greater extent at pH 4.5 than at pH 7.5, the experiments of Fig. 2, 4, and 5 had already shown that adaptation to acetate does not necessitate any increase in the activities of these enzymes.

**Cytochrome levels in bakers' yeast adapting to acetate oxidation.** If synthesis of the components of the electron transport chain were necessary for adaptation to acetate oxidation, the concentrations of respiratory pigments would be expected to increase. With the unstarved yeast, all of the cytochrome peaks increased a little in intensity and chloramphenicol partially inhibited these

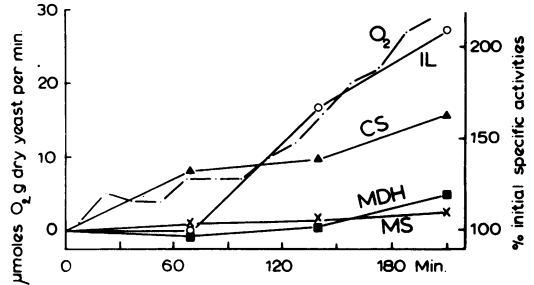


FIG. 6. Activities of additional enzymes associated with acetate metabolism during adaptation to acetate oxidation in the presence of 1 mM ammonium chloride. Conditions were the same as in the experiment of Fig. 3. The average initial activities were: isocitrate lyase (IL), 10; malate synthase (MS), 117; citrate synthase (CS), 494; malate dehydrogenase (MDH), 151 nmoles of substrate transformed per mg of protein per min.

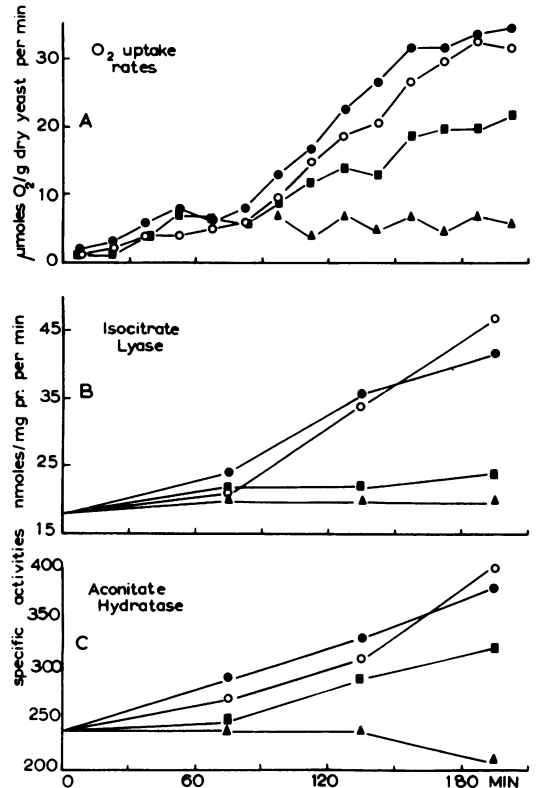


FIG. 7. Effects of chloramphenicol, *p*-fluorophenylalanine, and cycloheximide on the adaptation of bakers' yeast to acetate oxidation (A) and on increases in the activities of isocitrate lyase (B) and aconitate hydratase (C). The O<sub>2</sub> uptake rates were measured and sampling for enzyme assay was performed by using 0.9% (w/v, wet weight) suspensions of 16-hr starved yeast in 45 mM Tris-hydrochloride, pH 7.5, and 10 mM Mg (acetate)<sub>2</sub>. Further additions: none (○); 5 mg of chloramphenicol per ml (●); 10 mM *p*-fluorophenylalanine (■); 1 μg of cycloheximide per ml (▲).

increases, including those characteristic of cytochrome *c* (Fig. 8A). With yeast previously starved for 6 hr, adaptation in the presence and absence of chloramphenicol had no detectable effect on the height of any of the cytochrome peaks (Fig. 8B). The effect of starvation itself was to cause a slight decrease in the yeast cytochrome concentrations. Overall, therefore, no gross changes in cytochrome spectra were observed during acetate oxidation.

### DISCUSSION

The bakers' yeast used in this investigation is an aerobic yeast. It oxidizes ethanol immediately at a high rate, and prior oxygenation of the yeast does not affect this rate, nor was the oxidation of glucose affected. However, in fresh yeast maximal oxidation of acetate at pH 7.5 and 25 C occurred only after an adaptation period of about 1 hr, and oxygenation for 6 to 16 hr doubled this time without affecting the final rate reached. Of the various enzyme activities measured, consistent increases were found with citrate synthase, aconitate hydratase, isocitrate lyase, malate synthase, and malate dehydrogenase. These enzymes have been found by Duntze et al. (9) in the soluble fractions of yeast adapted to acetate and were considered by them to mediate a cytoplasmic glyoxylate cycle. In this investigation, enzyme activities were assayed in unfractionated yeast extracts. There was very little change in the activities of succinate dehydrogenase, fumarate hydratase, acetyl CoA synthetase, fructose 1,6 diphosphatase, and NAD- and NADP-linked isocitrate dehydrogenases. Fructose 1,6 diphosphatase did increase after adaptation, thus emphasizing its role in gluconeogenesis and showing that its synthesis is controlled differently from that of the glyoxylate cycle enzymes.

Ammonium ions were added to a yeast suspension to see whether, by enlarging the amino acid pool, the time taken for adaptation to acetate oxidation might be shortened. Several effects were obtained. (i) The adaptation period was considerably extended without affecting the final rate of acetate oxidation. (ii) The increase in citrate synthase was not altered. (iii) Aconitate hydratase, malate synthase, and malate dehydrogenase did not increase over the basal level during adaptation. (iv) Only in the case of isocitrate lyase was the delay in activity increase synchronous with the delay in oxygen uptake. Consequently, regulation of the activities of the glyoxylate cycle enzymes in yeast is not coordinate, in agreement with the conclusion of Duntze et al. (9), and isocitrate lyase is apparently the limiting activity. This was suggested before by

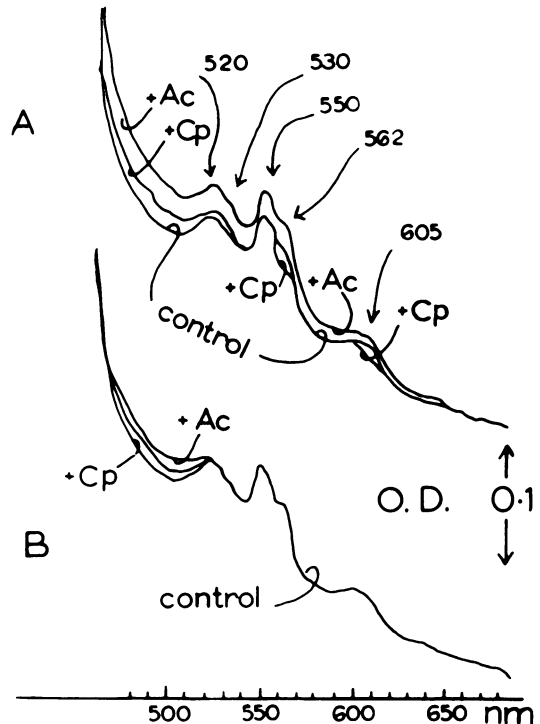


FIG. 8. Adaptation to acetate oxidation and the concentration of cytochromes in bakers' yeast. Yeast was suspended at a concentration of 0.9% (wet wt/vol) in 45 mM Tris-hydrochloride, pH 7.5, alone (control), plus 10 mM Mg (acetate)<sub>2</sub> (+Ac), or plus 10 mM Mg (acetate)<sub>2</sub> and 5 mg of chloramphenicol per ml (+Cp), and was then incubated with shaking at 25 C for 3 hr. The spectra of the yeast samples were recorded as described in Materials and Methods. Cytochromes *a* + *a*<sub>3</sub> absorb at 605, cytochrome *b* at 562 and 530, and cytochrome *c* + *c*<sub>1</sub> at 550 and 520 nm. A, Unstarved yeast, B, same yeast when starved for 6 hr. The oxygenation reduced the cytochrome peaks by 20%.

Barnett and Kornberg (3) and is supported by the fact that the specific activity of isocitrate lyase is much lower than those of the other glyoxylate cycle enzymes.

The mechanism by which ammonium delays the increases in enzyme increases is not known, but it is probably related to its effect in inhibiting sporogenesis of yeast (24), since only yeast adapted to acetate oxidation forms spores (6). Ammonium has been found to stimulate the activity of phosphofructokinase (E.C. 2.7.11; see reference 1) and, as a consequence, glycolysis. Alternatively, incorporation of ammonium into glutamate, which is catalyzed by the NADP-linked glutamate dehydrogenase, may stimulate the direct oxidative pathway of the yeast (16). Intermediates of both these pathways have been proposed as corepressors of isocitrate lyase (14, 19).

The experiments with cycloheximide, a potent inhibitor of cytoplasmic protein synthesis in yeast (5), and adaptation to acetate demonstrate that new enzymes must be synthesized during acetate oxidation. *p*-Fluorophenylalanine was much less effective, but its inhibitory action is very time dependent, as it functions by incorporation into proteins in place of phenylalanine (17), thus rendering them inactive. Chloramphenicol was without effect and may not have been taken up by the yeast cell in any quantity. In fact, a slight increase in the oxygen uptake rate was obtained, associated with consistent small elevations in the activities of the only enzymes assayed, isocitrate lyase and aconitate hydratase.

In conclusion, under the varying conditions of exposure of bakers' yeast to acetate, the increasing oxygen uptake rate was, of the various enzyme activities of the tricarboxylic acid and glyoxylate cycles assayed, consistently related only to the augmentation of the activity of isocitrate lyase which was initially low and apparently rate limiting.

#### ACKNOWLEDGMENTS

We express our appreciation to V. E. Chester (Guinness Research Laboratories, Dublin) for fruitful discussions, to M. G. Harrington for continuous help and cooperation, and P. O'Carra (University College, Galway) for his critical comments on the manuscript.

This study is part of the work undertaken by J. P. G. in partial fulfillment of requirements for the Ph.D. degree from the National University of Ireland.

#### LITERATURE CITED

- Atzpodien, W., and W. Bode. 1970. Purification and properties of ATP-sensitive phosphofructokinase from yeast. *Eur. J. Biochem.* **12**:126-132.
- Avers, C. J., and M. Federman. 1968. The occurrence in yeast of cytoplasmic granules which resemble microbodies. *J. Cell Biol.* **37**:555-559.
- Barnett, J. A., and H. L. Kornberg. 1960. The utilization by yeasts of acids of the tricarboxylic acid cycle. *J. Gen. Microbiol.* **23**:64-82.
- Breidenbach, R. W., and H. Beevers. 1967. Association of the glyoxylate cycle enzymes in a novel subcellular particle from castor bean endosperm. *Biochem. Biophys. Res. Commun.* **27**:462-469.
- Clark-Walker, G. D., and A. W. Linnane. 1966. In vivo differentiation of yeast cytoplasmic and mitochondrial protein synthesis with antibiotics. *Biochem. Biophys. Res. Commun.* **25**:8-13.
- Croes, A. F. 1967. Induction of meiosis in yeast. II. Metabolic factors leading to meiosis. *Planta (Berl.)* **76**:227-237.
- Dixon, G. H., and H. L. Kornberg. 1959. Assay methods for key enzymes of the glyoxylate cycle. *Biochem. J.* **72**:3P.
- Duggan, P. F. 1964. Acetate and ethanol oxidation by yeast. *Irish J. Med. Sci.* **457**:19-30.
- Duntze, W., D. Neumann, J. M. Grancedo, W. Atzpodien, and H. Holzer. 1969. Studies on the regulation and localization of the glyoxylate cycle enzymes in *Saccharomyces cerevisiae*. *Eur. J. Biochem.* **10**:83-89.
- Eaton, N. R., and H. P. Klein. 1954. The oxidation of glucose and acetate by *Saccharomyces cerevisiae*. *J. Bacteriol.* **68**:110-116.
- Flavell, R. B., and J. R. S. Ficham. 1968. Acetate-nonutilizing mutants of *Neurospora crassa*. II. Biochemical deficiencies and the roles of certain enzymes. *J. Bacteriol.* **95**:1063-1068.
- Gosling, J. P., and P. F. Duggan. 1969. The adaptation of baker's yeast to the oxidation of acetate. *Biochem. J.* **110**:19P.
- Gosling, J. P., and P. F. Duggan. 1969. The oxidation of acetate, ethanol, and pyruvate by baker's yeast. *Biochem. J.* **115**:8P.
- Guerritore, A., G. M. Hanozet, and M. C. Cocucci. 1969. Regulation of isocitrate lyase level in yeast growing on external carbon sources or on lipid reserves. *Experientia* **25**:131-132.
- Halvorson, H. O., and S. Spiegelman. 1953. The effect of free amino acid pool levels on the induced synthesis of enzymes. *J. Bacteriol.* **65**:496-504.
- Holzer, H., and I. Witt. 1960. Beschleunigung des oxydativen Pentosephosphatzyklus in Hefezellen durch Ammoniumsalze. *Biochim. Biophys. Acta* **38**:163-164.
- Kempner, E. S., and D. B. Cowie. 1960. Metabolic pools and the utilisation of amino acid analogs for protein synthesis. *Biochim. Biophys. Acta* **42**:401-408.
- Klein, H. P., and L. Jahnke. 1968. Cellular localization of acetyl-coenzyme A synthetase in yeast. *J. Bacteriol.* **96**:1632-1639.
- Kornberg, H. L. 1966. The role and control of the glyoxylate cycle in *Escherichia coli*. *Biochem. J.* **99**:1-11.
- Kornberg, H. L., and H. A. Krebs. 1957. Synthesis of cell constituents from C2 units by a modified tricarboxylic acid cycle. *Nature (London)* **179**:988-991.
- Layne, E. 1957. Spectrophotometric and turbidimetric methods for measuring proteins. III. Buiet method, p. 450-454. *In* S. P. Colowick and N. O. Kaplan (ed.), *Methods in enzymology*, vol. 3. Academic Press Inc., New York.
- Leighton, F., B. Poole, H. Beaufay, P. Baudhuin, J. W. Coffey, S. Fowler, and C. de Duve. 1968. The large scale separation of peroxisomes, mitochondria, and lysosomes from the livers of rats injected with Triton WR-1339. *J. Cell Biol.* **37**:482-513.
- Lynen, F. 1942. Zum biologischen Abbau der Essigsäure. I. Über die "Induktionszeit" Bei veramerter Hefe. *Liebig's Ann. Chem.* **552**:270-306.
- Miller, J. J. 1963. Determination by ammonium of the manner of yeast nuclear division. *Nature* **196**:214-215.
- Muller, M., J. F. Hogg, and C. de Duve. 1968. Distribution of tricarboxylic acid cycle enzymes and glyoxylate cycle enzymes between mitochondria and peroxisomes in *Tetrahymena pyriformis*. *J. Biol. Chem.* **243**:5385-5395.
- Polakis, E. S., and W. Bartley. 1965. Changes in the enzyme activities of *Saccharomyces cerevisiae* during aerobic growth on different carbon sources. *Biochem. J.* **97**:284-297.
- Polakis, E. S., W. Bartley, and G. A. Meek. 1964. Changes in the structure and enzyme activity of *Saccharomyces cerevisiae* in response to changes in the environment. *Biochem. J.* **90**:369-374.
- Racker, E. 1950. Spectrophotometric measurements of the enzymatic formation of fumaric and cis-aconitic acids. *Biochim. Biophys. Acta* **4**:211-214.
- Witt, I., R. Kronau, and H. Holzer. 1966. Repression von Alkoholdehydrogenase, Malatdehydrogenase, Isocitratlyase, und Malatsynthase in Hefe durch Glucose. *Biochim. Biophys. Acta* **118**:522-537.