J. gen. Microbiol. (1970), **60**, 107–116
Printed in Great Britain

The Effect of Temperature on the Metabolism of Baker's Yeast growing on Continuous Culture

By R. C. JONES* AND J. S. HOUGH
Department of Biochemistry, University of Birmingham

(*Accepted for publication 10 October 1969*)

SUMMARY

Glucose-limited cultures of baker's yeast growing at 25° had a maximum growth rate, saturation constant and yield constant of 0·22 hr^{-1}, 129 μg./ml. and 0·225, respectively, whereas when growing at 38° the corresponding values were 0·25 hr^{-1}, 300 μg./ml. and 0·204. In continuous culture, with the dilution rate fixed at 0·1 hr^{-1} there were no differences observed in viability, incidence of respiratory deficient mutants, cytochrome spectra or mean cell dry weights, between cultures grown at 25 and 38°. The culture grown at 25° had a smaller mean cell volume, greater yield value and nitrogen utilization. Ethanol, pyruvate and α-ketoglutarate were secreted to a greater degree in cultures grown at 38°. Yeast grown at 25° had a smaller capacity to produce carbon dioxide but greater ability to take up oxygen. Enzymes associated with glycolysis, alcohol production, tricarboxylic acid cycle and respiratory chain in organisms cultured continuously at 25 and 38° showed few important differences. The most obvious ones were those involving α-ketoglutarate as a substrate, especially α-ketoglutarate dehydrogenase. There were only small differences in adenosine phosphates and nicotinamide nucleotides. At 25° the ratio NAD/NADH was 1·5 but for organisms grown at 38° the ratio was 1·1.

INTRODUCTION

The growth and metabolic activities of micro-organisms are profoundly affected by the temperature at which they grow. For example, with Saccharomyces it has long been known that the rate of alcohol production increases with temperature up to 40° (Brown, 1914). Again fusel alcohol production is stimulated by increasing temperature with top-fermenting yeasts but not with bottom-fermenting yeasts (Hough & Stevens, 1961) and Wolter, Lietz & Beubler (1966) showed an increase in ethyl acetate production with increases in incubation temperature.

There are many reports that the synthesis of enzymes are affected by the growth temperature (Knox, 1955). Christopherson (1967) demonstrated with *Candida pseudotropicalis* that the activity of glucose-6-phosphate dehydrogenase was lower when grown at 37° than when grown at 20°. In a similar experiment the alcohol dehydrogenase activity of *Saccharomyces cerevisiae* was two- to three-fold different between organisms grown at 15 and 37°. The induction of the enzyme catalase in *S. cerevisiae* also has been reported to be temperature sensitive (Sulebele & Rege, 1967).

In continuous culture the metabolic activities of a micro-organism vary with growth rate. Thus Tempest & Herbert (1965) demonstrated with glucose-limited continuous

* Present address: The Distillers Co. Ltd. Menstrie, Clackmannan.

cultures of *Candida utilis* that growth rate markedly affected respiration rate. But the growth rate in batch culture varies with temperature and thus there is always doubt as to whether reported effects of temperature on metabolism are direct or arise from an alteration in growth rate. An assessment of the effect of culture temperature on the metabolism of a micro-organism, therefore, is best carried out under conditions where the growth rate is constant and independent of temperature. This is readily achieved with continuous culture.

In the present study, a detailed comparison of the metabolism of baker's yeast has been carried out with continuous cultures maintained at 25 and at 38°, but with the rate of growth kept constant. The object was to obtain information which would explain why, at higher temperatures of cultivation, cell production is decreased but the rate of ethanol production is enhanced (Hough & Rudin, 1958).

METHODS

Organism and media. The yeast used was a strain of *Saccharomyces cerevisiae* isolated from a commercial sample of baker's yeast and employed in earlier studies (Brown & Hough, 1965, 1966). It was maintained on a solidified malt extract medium (Wickerham, 1951) and subcultured monthly. For experimental work the liquid synthetic medium of Cutts & Rainbow (1950) was modified in that the lactate buffer was replaced by citric acid monohydrate (1·13 g./l.) plus trisodium citrate dihydrate (4·44 g./l.).

Culture conditions. Batch cultures (50 ml.) were grown in 150 ml. conical flasks in a thermostatically-controlled incubator at temperatures from 25 to 40°. The flasks were shaken at 100 strokes/min. with an amplitude of 4·5 cm. Continuous cultures were established in a single-stage glass culture vessel of 150 ml. working volume. The dilution rate was controlled, generally at 0·1 hr^{-1} (equivalent to a residence time of 10 hr) using a peristaltic metering pump and the temperature regulated by pumping water from a thermostatically-controlled water-bath through a jacket surrounding the vessel. Filtered sterilized air was injected below liquid level in the vessel, at a rate of 50 ml./min. This procedure gave reasonably high levels of dissolved oxygen, indicated by similar yields when various oxygen and air mixtures at the same flow rates were used. The culture was inoculated and sampled by means of a device described by Heatley (1950). When the culture optical density (measured at 625 nm., using a Unicam SP500 spectrophotometer) was constant for 3 days, equilibrium was established and samples taken for detailed analyses.

Growth constants. Yeast, previously grown in glucose-limited continuous culture, was inoculated into shake flasks containing a complete medium but with glucose concentration ranging from 0·1 to 10 mg./ml. In each series of experiments the inoculum yeast was grown at a corresponding temperature. Readings of absorbance, at 625 nm., were taken at short intervals during early stages of growth of the yeast Results were calculated from the ratio of $\log_2$ O.D. to time.

Viability measurements. Both a staining method (Lindegren, 1949) and slide-culture technique (Gilliland, 1959) were employed.

Respiratory-deficient mutants. The method of Ogur, St. John & Nagai (1957) was used for detection of the respiratory deficient organisms.

Distribution of yeast cell sizes. Organisms suspended in sodium chloride solution

(0·9%), containing maltose (10%) to minimize aggregation, were examined in a Coulter electronic particle counter fitted with 70 μm. orifice.

Growth factor requirements. These were determined by the method of Shultz & Atkin (1947).

Manometry. Organisms were separated from the cultures by centrifugation washed with citrate buffer (50 mM) plus potassium dihydrogen phosphate (5 mM) at pH 5·0 and resuspended in the same buffer solution at a known concentration of about 2 mg./ml. Oxygen uptake and carbon dioxide output under aerobic conditions were measured using a Braun Rotary Warburg Manometer.

Samples for analyses. Samples were transferred to ice-cooled tubes and centrifuged (3000 *g* for 3 min.) at 2°. The samples for cytochrome spectra and assays of enzyme and coenzyme estimations were then treated as indicated below. For all other analyses, the yeast pellets were washed with water (at 2°) and then dried under reduced pressure until of constant weight. They were then stored until required in a desiccator (at 4°).

Cytochrome spectra. The method of Linnane, Biggs, Huang & Clark-Walker (1968) was used.

Table 1. *List of enzymes and coenzymes investigated*

Enzyme or coenzyme	E.C. number	Reference for assay
Phosphofructokinase	2.7.1.11.	Viñuela, Sales & Sols, 1963
Pyruvate kinase	2.7.1.40	Rose, 1960
Pyruvate dehydrogenase enzyme system	—	Alvarez, Vanderwinkel & Wiame, 1958
Pyruvate decarboxylase	4.1.1.1.	Holzer & Goedde, 1957
Alcohol dehydrogenase	1.1.1.1.	Bücher & Redetzki, 1951
Aconitate hydratase	4.2.1.3.	Racker, 1950
Isocitrate dehydrogenase (NAD specific)	1.1.1.41.	Kornberg, 1955*a*
Isocitrate dehydrogenase (NADP specific)	1.1.1.42.	Kornberg, 1955*b*
Ketoglutarate dehydrogenase enzyme system	—	Holzer, Hierholzer & Witt, 1963
Succinate dehydrogenase	1.3.99.1.	Hauber & Singer, 1967
Fumarate hydratase	4.2.1.2.	Racker, 1950
Malate dehydrogenase	1.1.1.37.	Bergmeyer, 1963
NADH oxidase enzyme system	—	Green & Ziegler, 1963
NADH: cytochrome-*c* oxidoreductase	1.6.2.1.	Polakis, Bartley & Meek, 1964
Cytochrome-*c*: oxygen oxidoreductase	1.9.3.1.	Polakis, Bartley & Meek, 1964
Succinate:cytochrome-*c* oxidoreductase	—	Mackler *et al.* 1962
Glutamate:NAD oxidoreductase	1.4.1.2/3.	Holzer & Schneider, 1957
Glutamate:NADP oxidoreductase	1.4.1.3/4.	Holzer & Schneider, 1957
Aspartate:ketoglutarate aminotransferase	2.6.1.1.	Holzer, Gerlach, Jacobi & Gnoth, 1958
ATP, ADP, AMP	—	Bergmeyer, 1963
NAD	—	Bergmeyer, 1963
NADH	—	Polakis & Bartley, 1965

Assays for enzyme and coenzyme estimations. Yeast samples (100 to 200 mg. dry weight) were washed twice with 50 mM-potassium phosphate buffer (pH 7·0), resuspended in 6 ml. of this buffer and disintegrated in a Mickle tissue disintegrator (15 min. using 1 mm. diam. glass beads). After centrifugation (10 min. at 3000 *g*, 2°) the clear supernatant fraction contained the soluble enzymes and enzymes of the mitochondria which were then assayed immediately. All stages in the extraction were carried out at 2°. The assays used are given in Table 1. Protein was estimated by the Folin–Lowry method (Lowry, Rosebrough, Farr & Randall, 1951).

Keto-acids. Pyruvate and α-ketoglutarate were estimated enzymically by the methods described by Bergmeyer (1963).

RESULTS

The effect of temperature on the growth characteristics of Saccharomyces cerevisiae

The growth factor requirements of this yeast when growing at 25° or at 38° were identical. There was an absolute requirement for biotin, pantothenate, inositol and pyridoxine and a partial requirement for nicotinic acid and thiamine. Comparison of growth constants of yeast cultured at 25 and 38° are, therefore, not complicated by any changes in growth factor requirements. The saturation constants (K_s) and max. growth rates (μ_{max}) for glucose-limited cultures at these temperatures were calculated from experimental results obtained with batch cultures containing a range of glucose concentrations (Herbert, Elsworth & Telling, 1956). For cultures incubated at 25° the μ_{max} value was 0·22 hr^{-1} and K_s value 129 μg./ml. whereas for cultures incubated at 38°, μ_{max} was 0·25 hr^{-1} and K_s equal to 300 μg./ml. (Fig. 1). The yield constants for these cultures grown at 25 and 38° were 0·225 and 0·204, respectively.

Table 2. *Growth characteristics of S. cerevisiae*

Temperature of continuous culture	Glucose utilized (%)	Cell yield mg./ml. medium	Ethanol produced μg./mg. yeasts	Nitrogen utilized (%)
25°	100	3·2	193	41
38°	99	2·6	468	37

The viability of the yeast populations were measured over a range of dilution rates from 0·07 to 0·20 hr^{-1} when growing in a glucose-limited chemostat. With the growth temperature at 25° the percentage of viable cells ranged from 92 to 98, while with cultures maintained at 38°, viability varied from 90 to 98. The incidence of respiratory deficient mutants was less than 0·2 % irrespective of the growth temperatures and, therefore, like viability could be ignored when interpreting subsequent results.

Again, the cytochrome spectra of organisms grown continuously (glucose-limited, D = 0·1 hr^{-1}) were similar and showed peaks at 524, 530, 551, 562 and 600 nm. These probably were due to the presence of cytochromes $c+c_1$, $b+b_2$ and $a+a_3$ respectively. There was a difference, however, between the cultures grown at 25 and 38° with respect to their mean cell volumes; organisms grown at 38° were slightly larger than those grown at 25° although their mean cell dry weights were identical.

Cultures grown at 25 and 38° in a glucose-limited medium (D = 0·1 hr^{-1}) were found to differ in several respects (Table 2). The yield of organisms was greater at the lower growth temperature and slightly more nitrogen was utilized. In contrast, the ethanol production was less than half that observed with cultures grown at 38°, despite the complete utilization of glucose.

A further difference was the higher levels of α-keto acids excreted into the medium by cultures grown continuously at 38°. The steady state levels of the pyruvate and α-ketoglutarate attained in glucose-limited (D = 0·1 hr^{-1}) when the temperature was varied from 20 to 42° are shown in continuous culture (Fig. 2). The level of α-ketoglutarate was higher than that of pyruvate at all temperatures below 40°. Between the growth temperature of 30 and 35° the level of α-ketoglutarate in the medium rose from 3 to 11 μg./ml. At 35° the rate of excretion of α-ketoglutarate was maximal. At higher temperatures the level of pyruvate in the medium rose sharply and at 41°

exceeded 150 μg./ml. Other metabolities of the tricarboxylic acid cycle (malate, oxaloacetate and isocitrate) could not be detected in the medium, nor were amino acids, fatty acids and lactic acid excreted.

Respiratory activities. Manometric studies were carried out in order to examine the effect of various growth temperatures upon the respiratory activities of the yeast. In one experiment, the growth-limiting substrate was glucose (10 mg./l.) and in another DL-lactate (15 mg./l.). The dilution rate was held constant (0·1 hr^{-1}) and the temperature raised progressively in small steps from 25 to 39°. Organisms were removed

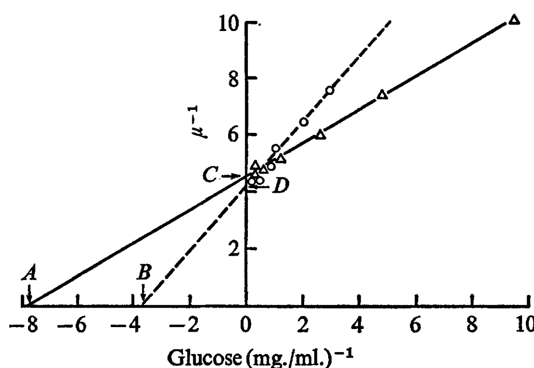

Fig. 1

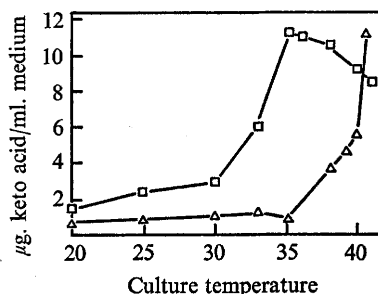

Fig. 2

Fig. 1. Plot of reciprocal of specific growth rate (μ) against the reciprocal of the glucose concentration for a series of shaken batch cultures of *S. cerevisiae*. Solid line and triangles refer to 25° cultures, broken line and circles to 38° cultures. A and B indicate values for $-1/K_s$ from which the values of the saturation constant for 25 and 38° respectively were calculated. *C* and *D* indicate values for maximum specific growth rates for 25 and 28° respectively. Growth was limited by glucose concentrations which ranged from 0·1 to 10 mg./ml.

Fig. 2. Levels of α-ketoglutarate (square symbols) and pyruvate (triangular symbols) present in the effluent from a glucose-limited chemostat culture of *S. cerevisiae* maintained at various temperatures at a dilution rate of 0·1 hr^{-1}. Glucose concentration in feed 10 mg./ml.

from the chemostat cultures when equilibrium conditions prevailed and were washed and transferred to Warburg flasks with an excess of glucose as substrate and incubation at 25°. With glucose as growth-limiting substrate in the chemostat and aerobic conditions in the respirometer, the carbon dioxide output was found to be higher as temperature at which the organisms were cultured increased from 25 to 39°: in contrast, the oxygen uptake remained fairly constant between 25 and 32° and then fell steadily with increasing temperature (Fig. 3). The respiratory quotient at 25° was 1·15 but at 39° it was 8·0 indicating a substantial change in the metabolic pattern of the yeast.

With DL-lactate (15 mg./ml.) as growth-limiting substrate in the chemostat and glucose in excess in the respirometer the oxygen uptake increased as the temperature of the culture was increased from 25 to 34°. With cultures grown at 34 to 39° the Q_{O_2} (glucose) declined. When lactate was substituted for glucose in the respirometer the Q_{O_2} results were generally similar although slightly greater in the range 25 to 35° (Fig. 4).

In order to determine whether, at higher temperatures, the synthesis of respiratory

enzymes was inhibited or their activity impaired the following experiments were performed. Yeasts grown in a glucose-limited continuous culture at 25° were transferred to a respirometer maintained at various temperatures between 22 and 45°. The oxygen uptake increased with temperature up to 42°. Above this temperature (which incidentally, is the maximum permitting growth of the yeast) the respiratory ability fell sharply. This contrasts strongly with the effect of temperature or growth and suggests that it is the synthesis of respiratory enzyme that is inhibited by elevated growth temperatures rather than the activity of the preformed enzymes.

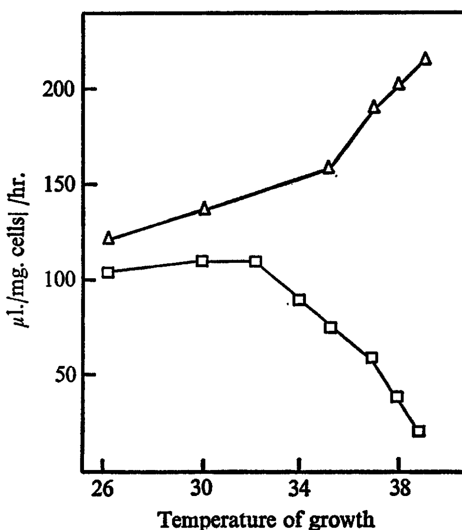

Fig. 3

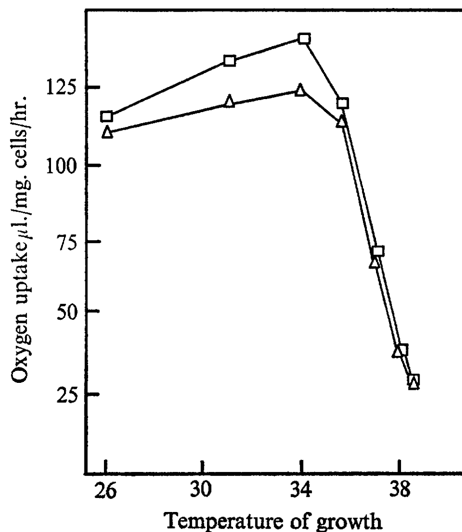

Fig. 4

Fig. 3. Relationship between oxygen uptake (square symbols), carbon dioxide output (triangular symbols) by *S. cerevisiae* and the temperature of growth using continuous culture and a dilution rate of 0·1 hr^{-1}. Glucose concentration in the feed was 10 mg./ml. and growth-limiting. Gas exchange was measured at 25° after samples of yeasts from the chemostat were withdrawn, washed and placed in Warburg respirometers with glucose as substrate.

Fig. 4. Relationship between oxygen uptake by washed suspensions of *S. cerevisiae* and temperature of growth in continuous culture. Dilution rate was 0·1 hr^{-1}, DL-lactate concentration in the feed was 15 mg./ml. and growth-limiting. Gas exchange was measured as for Fig. 3 with glucose (triangular symbols) and lactate (square symbols) as substrate.

Intracellular enzyme and metabolite levels

A comparison of the enzyme complements of yeast cells grown at 25 and 38° (Table 3) revealed little difference, particularly for the enzymes concerned with glycolysis and alcohol production. Yeasts grown at 38° had a slightly smaller content of TCA cycle enzymes: exceptions were succinate dehydrogenase and fumarate hydratase which were similar in the two types of yeast. Activities of most of the respiratory enzymes measured were slightly greater in the organisms grown at 38° but succinate: Cytochrome-*c* oxidoreductase was different in that it was lower.

The greatest differences in enzyme contents were found with enzymes reacting with α-ketoglutarate (Table 4). Only one of the enzymes in this group (aspartate: α-ketoglutarate aminotransferase) was unaffected by the temperature at which the

organisms were grown. The enzyme most influenced was α-ketoglutarate dehydrogenase; the levels within organisms grown at 25° were low but the extracts of the organisms grown at 38° had no detectable activity. The yeast cultured at 38° had slightly greater levels of α-keto acids. Pyruvate and α-ketoglutarate were 7·5 ± 0·2 and 3·1 ± 0·2 nmole/mg. yeast material. For yeasts grown at 25° the corresponding values were 5·8 ± 0·2 and 2·1 ± 0·2.

Table 3. *Comparison of activities of enzymes associated with glycolysis, alcohol production, tricarboxylic acid cycle and respiratory chain*

Enzyme	E.C. number	Yeasts grown continuously at 25°	Yeasts grown continuously at 38°
Phosphofructokinase	2.7.1.11.	73 ± 3·0 (3)	77 ± 3·7 (3)
Pyruvate kinase	2.7.1.40.	6·1 ± 0·5 (3)	6·3 ± 0·4 (3)
Pyruvate dehydrogenase enzyme system	—	3·0 ± 0·4 (5)	2·7 ± 0·3 (4)
Pyruvate decarboxylase	4.1.1.1.	734 ± 5·0 (4)	509 ± 4·4 (4)
Alcohol dehydrogenase	1.1.1.1.	224 ± 3·7 (4)	224 ± 5·7 (4)
Aconitate hydratase	4.2.1.3.	115 ± 1·4 (4)	93·0 ± 1·7 (4)
Isocitrate dehydrogenase (NAD specific)	1.1.1.41.	6·6 ± 0·2 (3)	5·6 ± 0·3 (4)
Isocitrate dehydrogenase (NADP specific)	1.1.1.42.	15·9 ± 0·5 (4)	12·2 ± 0·5 (3)
Succinate dehydrogenase	1.3.99.1.	34·2 ± 1·0 (4)	35·7 ± 0·6 (4)
Fumarate hydratase	4.2.1.2.	27·8 ± 0·6 (3)	28·0 ± 1·5 (3)
Malate dehydrogenase	1.1.1.37.	613 ± 8·0 (4)	517 ± 3·5 (3)
NADH oxidase enzyme system	—	13·7 ± 0·3 (4)	16·4 ± 0·4 (4)
NADH: cytochrome-*c* oxidoreductase	1.6.2.1.	41·5 ± 0·8 (3)	52·8 ± 0·5 (3)
Cytochrome-*c*: oxygen oxidoreductase	1.9.3.1.	18·3 ± 0·4 (3)	22·7 ± 0·5 (3)
Succinate: cytochrome-*c* oxidoreductase	—	12·1 ± 0·2 (3)	7·9 ± 0·2 (4)

Levels of enzyme activities in yeast grown in glucose-limited continuous culture. (Glucose concentration 10 mg./ml.; dilution rate 0·1 hr^{-1}.) Results are mean values expressed as mμmole substrate utilized/min./mg. protein ± S.E.M. The numbers of observations are given in parenthesis.

Table 4. *Comparison of activities of enzymes in yeast which involve ketoglutarate as a substrate*

Enzyme	E.C. number	Yeasts grown continuously at 25°	Yeasts grown continuously at 38°
α-Ketoglutarate dehydrogenase enzyme system	—	2·2 ± 0·4 (8)	0·3 (14)
Glutamate: NAD oxidoreductase	1.4.1.2/3.	13·5 ± 0.3 (4)	27·5 ± 0·3 (3)
Glutamate: NADP oxidoreductase	1.4.1.3/4	668 ± 8·2 (4)	532 ± 7·6 (4)
Aspartate: ketoglutarate aminotransferase	2.6.1.1.	13·7 ± 0·2 (3)	13·1 ± 0·3 (3)

Total adenosine phosphate levels for yeasts grown at 38 and 25° were, respectively, 6·4 and 7·1 nmole/mg. yeast material. This small difference was reflected in the corresponding values for ATP and AMP; levels of ADP were slightly greater for the 38° culture. There were no major differences between the levels of NAD at the two

temperatures and of reduced NAD. In each case the value was in the range 1·1 and 1·6 nmole/mg. yeast material. NAD:NADH ratios were 1·1 for yeasts grown at 38° and 1·5 for those cultured at 25°.

DISCUSSION

Many reports on the effect of temperature upon micro-organisms are complicated by the fact that not all the other environmental parameters were controlled. This is particularly true for batch culture in which the environment invariably changes continuously, leading to changes in enzymic composition of the organisms. For instance, the level of succinate-cytochrome C oxidoreductase in yeast cells varied 100-fold during growth in a batch culture (Ephrussi, Slonimski, Yotsuyanagi & Tavlitski, 1956). The effect of temperature on the metabolism of growing organisms is, therefore, more easily studied when they are growing in a constant environment with their growth rate determined by the rate of supply of growth-limiting nutrient; that is, in continuous culture.

The chemical environments provided by the media of the yeast culture growing in glucose-limited chemostat 25 and 38° were very similar. This was emphasized by the overall constancy in the levels of both the tricarboxylic acid cycle enzymes and the enzymes of the respiratory chain between the cells grown at the two temperatures. However, a marked decrease occurred in the respiratory capacity of the yeast grown at the higher temperature. Yeast grown in lactate-limited cultures was affected to the same extent indicating that the effect was not restricted to organisms metabolizing glucose as the sole carbon source. Neither was the effect due to an enrichment of the population with respiratory-deficient mutants nor to a decrease in viability nor to a significant change in the level of aeration. The decrease in the respiratory capacity of yeast grown at the higher temperature was accompanied by a decrease in yield and by an increase in the amount of ethanol produced. These effects were accompanied by an increase in the levels of α-keto acids in the growth medium. With α-ketoglutarate the levels rose in that range of culture temperature (30 to 35°) in which the respiratory capacity began to decline. At growth temperatures above 35° the level of pyruvate in the medium increased sharply as the respiratory activity continued to fall (compare Fig. 2 with Fig. 3, 4). In contrast the intracellular levels of α-keto acids showed a much smaller increase at the higher growth temperature. A similar situation has been reported by Suomalainen & Ronkainen (1963) for baker's yeast grown first under aerobic and then anaerobic conditions. In their system, despite a considerable increase in the levels of α-keto acids in the growth medium, the intracellular levels remained relatively constant.

The increase in the level of pyruvate from 5·8 nmole/mg. in the organisms grown at 25° to 7·5 nmole/mg. in those grown at 38° may well favour fermentation without any change in the rate of oxidation of pyruvate. Taking results of Polakis & Bartley (1965) for internal cell volume, the corresponding internal mean concentrations were about 1·9 and 2·5 mM. Holzer (1961) reported that a yeast pyruvate oxidase system became saturated at the level of 1 mM-pyruvate whereas pyruvate decarboxylase was only saturated at 20 mM-pyruvate.

A comprehensive enzymic survey of this yeast revealed that the increased level of α-ketoglutarate in the growth medium may be correlated with the level of α-ketoglutarate dehydrogenase present in the cells. In cell-free extracts of organisms grown

at 25° the activity of this enzyme was low but in extracts of organisms grown at 38° the level was greatly reduced. Vitols & Linnane (1961) also found a low level of this enzyme in a commercial sample of baker's yeast, presumably cultured at temperatures below 25°. Cell-free extracts of their yeast quickly oxidized citrate and pyruvate plus malate but accumulated α-ketoglutarate.

It is of interest that one enzyme, NAD-specific glutamate dehydrogenase, showed a significant increase in complement (100%) at the higher temperature of culture. In contrast the complement of NADP-specific glutamate dehydrogenase decreased by 20%. Chapman & Bartley (1968) demonstrated a similar reciprocal relationship between the changes in levels of the two glutamate dehydrogenases of yeast during a change from aerobic to anaerobic conditions. The reason for such a reciprocal relationship is not known but may be related to the intracellular compartmentation of α-ketoglutarate. It is suggested that the primary effect on the yeast of the increase in growth temperature was to reduce the level of α-ketoglutarate dehydrogenase within the organisms, probably arising from the inhibition of the synthesis of the enzyme by the elevated temperature. The changes in respiratory capacity, metabolite levels and levels of other enzymes were probably of secondary importance but acted to stimulate fermentative metabolism at the higher temperatures of growth.

REFERENCES

ALVAREZ, A., VANDERWINKEL, E. & WIAME, J. M. (1958). Oxidation of pyruvic acid in yeast. *Biochimica et Biophysica Acta* **28**, 333.

BERGMEYER, H.-U. (1963). *Methods of Enzymatic Analysis*. New York: Academic Press.

BROWN, C. M. & HOUGH, J. S. (1965). Elongation of yeast cells in continuous culture. *Nature, London* **206**, 676.

BROWN, C .M. & HOUGH, J. S. (1966). Protein–disulphide reductase activity in yeast. *Nature, London* **211**, 201.

BROWN, H. T. (1914). Some studies on yeast. *Annals of Botany* **28**, 197.

BÜCHER, T. & REDETSKI, H. (1951). Eine spezifische photometrische Bestimmung von äthylalkohol auf fermentativem Wege. *Klinische Wochenschrift* **29**, 615.

CHAPMAN, C. & BARTLEY, W. (1968). The kinetics of enzyme changes in yeast under conditions that cause the loss of mitochondria. *Biochemical Journal* **107**, 455.

CHRISTOPHERSON, J. (1967). In *Molecular Mechanisms of Temperature Adaption*. Ed. by Ladd Prosser. American Association for the Advancement of Science, Washington.

CUTTS, N. S. & RAINBOW, C. (1950). Studies of a yeast exacting towards *p*-aminobenzoic acid. *Journal of General Microbiology* **4**, 150.

EPHRUSSI, B., SLONIMSKI, P. P., YOTSUYANAGI, Y. & TAVLITSKI, J. (1956). Variations physiologiques et cytoliques de la levure au cours du cycle de la croissance aerobie. *Compte Rendu des Travaux du Laboratoire Carlsberg: Série Physiologique* **26**, 87.

GILLILAND, R. B. (1959). Determination of yeast viability. *Journal of the Institute of Brewing* **65**, 424.

GREEN, D. E. & ZIEGLER, D. M. (1963). Electron transport particles. *Methods in Enzymology* **6**, 416.

HAUBER, J. & SINGER, T. P. (1967). Studies on succinate dehydrogenase—intracellular distribution, catalytic properties & regulation of fumarate reductases in yeasts. *European Journal of Biochemistry* **3**, 107.

HEATLEY, N. G. (1950). A versatile fermentation sampling arrangement. *Journal of General Microbiology* **4**, 410.

HERBERT, D., ELSWORTH, R. & TELLING, R. C. (1956). The continuous culture of bacteria; a theoretical and experimental study. *Journal of General Microbiology* **14**, 601.

HOLZER, H. (1961). Regulation of carbohydrate metabolism by enzyme competition. *Cold Spring Harbor Symposia on Quantitative Biology* **26**, 277.

HOLZER, H., GARLACH, V., JACOBI, G. & GNOTH, M. (1958). Anreicherung und Eigenschaften einer Transaminase aus Bierhefe. *Biochemische Zeitschrift* **329**, 529.

HOLZER, H. & GOEDDE, H. W. (1957). Oxydation von α-Ketosauren und einigen Aldehyden mit Pyruvat-decarboxylase aus Hefe. *Biochemische Zeitschrift* **329**, 192.

HOLZER, H., HIERHOLZER, G. & WITT, I. (1963). α-Ketoglutaratoxydase der Hefe. *Biochemische Zeitschrift* **337**, 115.

HOLZER, H. & SCHNEIDER, S. (1957). Anreicherung und Trennung einer D.P.N.-spezifischen und einer T.P.N.-spezifischen Glutaminsäure-dehydrogenase aus Hefe. *Biochemische Zeitschrift* **329**, 361.

HOUGH, J. S. & RUDIN, A. D. (1958). Experimental production of beer by continuous fermentation. *Journal of the Institute of Brewing* **64**, 404.

HOUGH, J. S. & STEVENS, R. (1961). Beer flavour. IV. Factors affecting the production of fusel oil. *Journal of the Institute of Brewing* **67**, 488.

KNOX, R. (1955). The effect of temperature on enzymic adaptation, growth and drug resistance. *Symposium of the Society for General Microbiology* **3**, 184.

KORNBERG, A. (1955*a*). Isocitric dehydrogenase of yeast (TPN). *Methods in Enzymology* **1**, 705.

KORNBERG, A. (1955*b*). Isocitric dehydrogenase of yeast (DPN). *Methods in Enzymology* **1**, 707.

LINDEGREN, C. C. (1949). *The Yeast Cell, its Genetics and Cytology*. St. Louis: Education Publishers.

LINNANE, A. W., BIGGS, D. R., HUANG, M. & CLARK-WALKER, G. D. (1968). The effects of chloramphenicol on the differentiation of the mitochondrial organelle. In *Aspects of Yeast Metabolism.* Ed. by A. K. Mills and H. Krebs, p. 222. Oxford: Blackwell.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265.

MACKLER, B., COLLIP, P. J., APPAJI-RAO, N., DUNCAN, H. M. & HUENNEKENS, F. M. (1962). An electron transport particle from yeast: purification & properties. *Journal of Biological Chemistry* **237**, 2968.

OGUR, M., ST. JOHN, R. & NAGAI, S. (1957). Tetrazolium overlay technique for population studies of respiration deficiency in yeast. *Science, New York* **125**, 928.

POLAKIS, E. S. & BARTLEY, W. (1965). Changes in the enzyme activities of *S. cerevisiae* during aerobic growth on different carbon sources. *Biochemical Journal* **97**, 284.

POLAKIS, E. S., BARTLEY, W. & MEEK, G. A. (1964). Changes in the structure and enzyme activity of *S. cerevisiae* in response to changes in the environment. *Biochemical Journal* **90**, 369.

RACKER, E. (1950). Spectrophotometric measurement of the enzymic formation of fumaric and cis-aconitic acids. *Biochimica et Biophysica Acta* **4**, 211.

ROSE, I. A. (1960). Studies on the enolization of pyruvate by pyruvate kinase. *Journal of Biological Chemistry* **235**, 1170.

SHULTZ, A. S. & ATKIN, E. (1947). The utility of bios response in yeast classification and nomenclature. *Archives of Biochemistry* **14**, 369.

SULEBELE, G. A. & REGE, D. V. (1967). Temperature sensitivity of catalase induction in *S. cerevisiae. Enzymologia* **33**, 354.

SUOMALAINEN, H. & RONKAINEN, P. (1963). Keto-acids in bakers yeast and in fermentation solution. *Journal of the Institute of Brewing* **69**, 478.

TEMPEST, D. W. & HERBERT, D. (1965). Effect of dilution rate and growth limiting substrate on the metabolic activity of *Torula utilis* cultures. *Journal of General Microbiology* **41**, 143.

VIÑUELA, E., SALAS, M. L. & SOLS, A. (1963). End product inhibition of yeast phosphofructokinase by ATP. *Biochemical and Biophysical Research Communications* **12**, 140.

VITOLS, E. & LINNANE, A. W. (1961). Studies on the oxidative metabolism of *S. cerevisiae. Journal of Cell Biology* **9**, 701.

WOLTER, H., LIETZ, P. & BEUBLER, A. (1966). Influence of temperature and yeast strain on the formation of amyl alcohol, isobutanol and ethyl acetate in fermenting malt wort. *Folia Microbiologica, Praha* **11**, 210.

WICKERHAM, L. J. (1951). *Taxonomy of Yeasts. Bulletin. United States Department of Agriculture* No. 1029.