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Enhanced CO_2 Production by Yeast Exposed to Elevated Temperatures

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SUMMARY

After starvation, yeast exposed to elevated temperatures produced CO_2 twice as fast as unexposed organisms. The lag which preceded linear CO_2 production by starved yeast was essentially eliminated by heat treatment. Uptake and retention of sorbose was greater in heated yeast. Heating was accomplished by brief immersion of the organisms in heated solutions and by growth for 2 h. at 35°. Short heat treatments increased the production of CO_2 when glucose was included in the suspending medium, whereas heating in water or in growth medium without glucose resulted in a decreased production of CO_2.

INTRODUCTION

Temperature changes can markedly alter or completely inactivate biological processes (Wood, 1956; Ingraham, 1962; Farrell & Rose, 1967). Availability of nutrient (Sherman, 1959*a*; Begue & Lichstein, 1963) and age of culture (Rosenberg & Wood, 1957) have significant effects upon heat-induced changes in yeast, though the critically affected reactions have not been identified. Even small increases in temperature may affect control mechanisms and thus have far-reaching effects on organelle formation (Sherman, 1959*b*) and protein synthesis (Hartwell & McLaughlin, 1969; Schiebel, Chayka, DeVries & Rusch, 1969).

The effects of elevated temperatures on the anaerobic production of CO_2 by yeast were studied because this process and the enzymes involved are well known.

METHODS

Preparation of yeast samples. Cultures of *Saccharomyces cerevisiae* were grown at 28·5° as described previously (Spoerl & Doyle, 1968*a*). The organisms were harvested during exponential growth at 2×10^7/ml. and washed twice with distilled water by centrifugation. Samples for control tests were resuspended in distilled water for starvation or in one of the buffer solutions (see below) for measurement of CO_2 production or sorbose uptake. For starvation, suspensions (4×10^7 organisms/ml.) were shaken aerobically for 21 h. at 28·5°. This procedure was carried out as aseptically as was practicable; suspensions were examined microscopically and discarded if contaminated. For measurements of CO_2 production, sorbose uptake or sorbose efflux after starvation, samples were again washed twice with water and resuspended in the appropriate buffer solution.

Methods of heat treatment. Yeasts were exposed to increased temperatures for brief periods by resuspending in an appropriate fluid (*a*) at the desired temperature

in a water bath, or (*b*) at room temperature after which the samples were placed in a water bath at the desired temperature. In the latter case, yeast suspensions reached 45° in 3·5 min. The suspensions were then cooled rapidly to room temperature by a brief immersion in an ice bath, washed again with water (either by two centrifugations or after filtration onto a membrane filter (Millipore, type RA)) and finally resuspended either in a buffer solution for the measurement of CO_2 output and sorbose uptake, or in water for starvation.

Yeasts were also exposed to elevated temperatures by growing cultures at 35°. Culture flasks were moved from the normal shaker bath at 28·5° to one at 35° 2 h. before the yeast was due to be harvested. Other flasks were moved to a shaker bath at 21° for 2 h. After being harvested and washed, the yeasts were resuspended in buffer solution or in water as appropriate.

Manometric experiments. CO_2 production was measured at 30° under N_2 by standard Warburg procedures (Umbreit, Burris & Stauffer, 1949). Yeasts were suspended in 0·06 M buffer solution, pH 7·0 (except for Table 3), containing 0·1 M-glucose as substrate.

Uptake and efflux of sorbose. Yeasts were sampled from aerobic suspensions maintained at 30° and handled as described by Spoerl & Doyle (1968*c*). They were suspended in 0·02 M buffer solution (pH 5·6) containing 0·1 M-[^{14}C]-L-sorbose (uniformly labelled; 3 μCi/mole), a nonmetabolized sugar, for uptake measurements, and were washed and resuspended in 0·02 M buffer solution (pH 7·0) for efflux measurements. Efflux time for the second stage of exit was calculated from an initial value obtained by extrapolating the curve of efflux as a function of time back to zero time (Spoerl & Doyle, 1968*b*).

Buffer solutions. Solutions of mixtures of KH_2PO_4 and K_2HPO_4 were employed; they contained 2 mM-$MgCl_2$.

RESULTS

Effect of brief exposures of yeast to elevated temperatures. In the first experiments yeasts were rapidly resuspended in water or in growth medium at the desired temperature. CO_2 production was measured immediately after heat treatment and again after starvation (Table 1). For organisms which had been heated in growth medium at 40 or 45° and then starved, the Q_{CO_2} was increased. In contrast, heating in water at 45° decreased the Q_{CO_2} after starvation. Heating at 50° decreased CO_2 production by both starved and unstarved organisms. Starved yeast produces CO_2 at about 35 % the rate of unstarved controls (Spoerl & Doyle, 1967) so the heat-induced increases noted after starvation did not exceed the rate characteristic of fresh yeast.

In later experiments samples were heated at 45° in a variety of suspending media by immersing them in a water bath and holding them there for 1 min. after they reached 45° (total time 4·5 min.). The Q_{CO_2} of yeast heated in growth medium was doubled (Table 2) compared with unheated control yeast, and the lag in CO_2 output, characteristic of starved yeast (Spoerl & Doyle, 1967), was shortened. Heating in solutions of glucose (0·005 to 0·05 M) increased the Q_{CO_2} by 40 % compared with unheated organisms. Heating in 0·1 M-glucose, a concentration which reduces glycolysis and causes a loss, or 'excretion', of 260 nm. absorbing materials during incubation (Doyle & Spoerl, 1968), increased CO_2 production less than the lower concentrations of glucose, but the lag was more effectively shortened. Though a 21 h. incubation in

0·001 M-glucose increases CO_2 production (Spoerl & Doyle, 1968*a*), heating in such a glucose solution reduced the Q_{CO_2}, as did heating in water. Heating briefly in maltose and in mannitol solutions did not change the Q_{CO_2}, though incubation for 21 h. in 0·2 M-mannitol enhances CO_2 production (Spoerl & Doyle, 1968*a*). Heating in growth medium without glucose markedly reduced the Q_{CO_2}. The Q_{CO_2} also decreased when 0·01 M-glucose replaced the usual 0·22 M-glucose; 0·22 M-mannitol in the medium did not protect the yeast.

The Q_{CO_2} of yeast samples suspended in growth medium or glucose solutions and

Table 1. *CO_2 production by yeast heated at different temperatures and by yeast starved after heat treatment*

Samples of growing yeast cultures were washed and resuspended in buffer solution. CO_2 production was measured after heating and compared with unheated controls. Starved samples were starved for 21 h. before Q_{CO_2} measurements were made.

			Q_{CO_2} (% of control)							
			Period of heating (min.)							No. of
Test medium	Temperature	Type	0·5	1	2	4	8	16	32	experiments
H_2O	45°	Unstarved	103	99	102	105	—	—	—	2
H_2O	45°	Starved	54	35	36	9	—	—	—	3
Growth medium	40°	Starved	119	129	131	153	151	186	237	4
Growth medium	45°	Unstarved	103	96	99	96	84	—	—	3
Growth medium	45°	Starved	129	146	159	115	—	—	—	5
Growth medium	50°	Unstarved	78	73	64	—	—	—	—	3
Growth medium	50°	Starved	40	27	8	—	—	—	—	4

Table 2. *CO_2 production by starved yeast previously heated at 45° or held at 28·5° in various media*

After heat treatment, the yeast was starved for 21 h. before measuring Q_{CO_2} values. Lag period measured in min. The control Q_{CO_2} was 101 and its lag was 102 min. (average of 11 values). Values listed are averages of 3 to 6 separate measurements (more in the case of the 206% growth medium value).

Heated at 45° in	Q_{CO_2} (% of control)	Lag (% of control)
Growth medium	206*	3
K-phosphate buffer (0·06 M, pH 7·0)	71	79
H_2O	44*	100
Growth medium minus glucose	5*	—
Growth medium minus glucose + 0·22 M-mannitol	12*	—
Growth medium minus glucose + 0·01 M-glucose	83	30
0·1 M-Glucose	119	32
0·05 M-Glucose	138*	38
0·03 M-Glucose	143*	46
0·005 M-Glucose	141*	62
0·001 M-Glucose	66*	97
0·1 M-Maltose	110	100
0·2 M-Mannitol	92	90
Held at 28·5° in		
Growth medium	159*	20
0·1 M-Glucose	144*	43
0·01 M-Glucose	130*	48
0·005 M-Glucose	110	91

* Differs from 100 at the 5% level of significance.

held for 4·5 min. at 28·5° increased compared with samples resuspended directly in water and starved (Table 2). Heating at 45° enhanced the Q_{CO_2} more effectively, but evidently also produced some injury. That is, CO_2 production was significantly increased by suspending yeast in 0·1 M-glucose at 28·5° but not at 45°. The lower Q_{CO_2} of organisms heated at 45° may have resulted from an increased 'excretion' response (see above).

The Q_{CO_2} of yeast heated in growth medium and tested without starvation was 97% of the control. Heating did not increase immediate CO_2 production in any environment, and decreases in Q_{CO_2} were small except with some samples heated in glucose solutions. The excretion of 260 nm. absorbing materials caused by glucose (Lewis & Stephanopoulos, 1967; Doyle & Spoerl, 1968) may account for the lower Q_{CO_2} of organisms heated in glucose solutions.

Effect of growth at 35°. Yeasts suspended for 32 min. in medium at 40° produced CO_2 at high rates after starvation (Table 1). Because growth and a variety of syntheses could have occurred during this period of time, experiments were carried out in which the yeast was grown for 2 h. at 35°. The generation time decreased from 1·5 h. at 28·5° to 1·2 h.; the immediate Q_{CO_2} did not change (Table 3). After starvation, the Q_{CO_2} of yeast grown at 35° was double that of organisms grown at 28·5°, and the lag was shortened (Table 3). CO_2 production by yeast grown at 35° was similar at pH 4·5 and 7·0, whereas it was lower at pH 4·5 than at pH 7·0 when the yeast was grown at 28·5°. The Q_{CO_2} of organisms grown at 21° for 2 h. did not differ greatly from that of organisms grown at 28·5°.

Table 3. *CO_2 production by yeast grown at different temperatures*

The Q_{CO_2} of yeasts grown continuously at 28·5° or shifted for 2 h. of growth at 35° or 21° was measured at the listed pH immediately after harvesting and after 21 h. of starvation. No lag occurs with unstarved yeast. Values are averages of 4 to 9 separate measurements; the ratio column lists average values for Q_{CO_2} divided by the Q_{CO_2} at 28·5°.

		Starved yeast			Unstarved yeast	
Growth temperature	pH	Q_{CO_2}	Lag (min.)	Ratio	Q_{CO_2}	Ratio
28·5°	7·0	127	74	—	323	—
28·5°	4·5	84	72	—	324	—
35°	7·0	258	14	2·08*	340	1·03
35°	4·5	275	18	2·72*	334	1·03
21°	7·0	103	80	0·87	232	0·73*
21°	4·5	101	78	1·28	282	0·87

* Differs from 1·0 at the 5% level of significance.

Uptake and efflux of sorbose. The enhancement of CO_2 production by heat treatments is similar to an enhancement brought about by incubation of yeast in solutions of certain sugars and polyols (Spoerl & Doyle, 1968*a*). Sorbose uptake and efflux are also affected by incubation in sugar solutions (Spoerl & Doyle, 1968*b*), so the effect of heating yeast on these processes was examined.

Table 4 shows that more sorbose was taken up by starved organisms previously grown at 35°, and by starved organisms which had been heated at 45° in growth medium, than by those grown at 28·5°. Heating at 45° in mannitol did not increase sorbose

uptake and may have been deleterious. Uptake of sorbose by the yeast immediately after heat treatment did not differ significantly from that of unheated yeast.

Sorbose efflux from these yeasts initially occurs at a fast rate which then slows for a second stage of exit (Spoerl & Doyle, 1968*b*; Spoerl, 1969). Efflux during the second stage of exit was slower from heat-treated than from control yeast (Table 4). This difference in rate was not entirely consistent and did not occur every time. Initial efflux rates were alike (data not shown). Thus a slowed exit and an increased uptake of sorbose occurred in heat-treated yeast, though neither was as marked as those which occur in organisms incubated in sugar solutions (Spoerl & Doyle, 1968*b*).

Table 4. [^{14}C]-*L-Sorbose uptake and efflux from yeast exposed to elevated temperatures*

Percentages are averages of 3 to 9 measurements of μg. sorbose/mg. yeast dry wt after 90 min. of uptake and of the time for sorbose content to fall to one-half its initial value during the second stage of exit (see text). Controls were grown at 28·5°.

	Uptake (% of control)		Efflux (% of control)	
Treatment	Starved	Unstarved	Starved	Unstarved
Grown at 35° for 2 h.	116*	97	135	93
Heated at 45° in growth medium	126*	112	143	110
Heated at 45° in 0·01 M-glucose	109	108	144	99
Heated at 45° in 0·2 M-mannitol	88	106	—	—

*Differs from 100 at the 5% level of significance.

DISCUSSION

The temperatures employed in these experiments are not lethal and generally do not affect the appearance, growth or viability of *Saccharomyces cerevisiae* (Burns, 1956; Sherman, 1959*a*, *b*; Louderback, Scherbaum & Jahn, 1961). Starvation may influence various cellular processes (Mandelstam, 1960), and it functioned in these experiments to reveal heat-induced changes not observable in unstarved yeast.

Brief exposures to elevated temperatures, because they provide too limited a time for syntheses to occur, presumably caused a rearrangement of some constituents of the yeast so that the capacity to produce CO_2 was preserved. Because growth at 35° also resulted in an increased Q_{CO_2} after starvation, changes caused by such growth appear to be similar to those which occurred quickly when the yeast was exposed briefly to 45°. Exposure to higher temperatures or for longer times (Table 1) caused additional changes which resulted in less CO_2 production. If such additional effects occurred during brief exposures at 45°, they were overridden by the changes which produced the striking enhancement of CO_2 production.

Brief exposures to elevated temperatures enhanced the Q_{CO_2} when glucose was available, but not in mannitol or maltose solutions. Because use of maltose must be induced, this suggests that the changes at elevated temperatures which ultimately increase CO_2 production only occur quickly if an energy source is available. Moreover, heating in solutions which do not contain an energy source can reduce the Q_{CO_2}. Heating in growth medium which combines glucose with medium salts produced the highest Q_{CO_2}. The salts, perhaps by counteracting an 'excretion' response, prevented the decrease in the Q_{CO_2} which was caused by high concentrations of glucose alone, although the salts by themselves caused injury when glucose was not present.

Unheated yeast resuspended briefly in growth medium or in glucose solutions at 28·5° before starvation (Table 2) also showed an increased Q_{CO_2} compared with yeast taken directly from a growing culture at 28·5° and starved. The resuspension procedure, presumably by interrupting exponential growth and resupplying an energy source, affected metabolic processes so that the capacity to produce CO_2 did not decrease as usual during starvation. Though this procedure was involved in the results obtained with brief exposures, yeasts grown at 35° were not resuspended and the higher Q_{CO_2} was the result of the elevated temperature alone.

Both uptake and retention of sorbose are increased when CO_2 production is enhanced by incubation of yeast with glucose (Spoerl & Doyle, 1968*b*). The heat-treated organisms showed similar responses, but less consistently and to a lesser degree. Because maximal enhancement of CO_2 production did not differ greatly between incubation and heat experiments, the lesser uptake of sorbose in the heat experiments indicates that this response may be independent of the CO_2 response. Moreover, though heating in glucose solutions increased retention of sorbose, neither uptake nor CO_2 production was increased, as it was after heating in growth medium. Thus the mechanisms responsible for uptake and retention also may be affected independently. Other studies of sorbose retention have indicated that retention may be a function of the yeast vacuole (Spoerl, 1969); therefore, increased temperatures may affect vacuolar membranes.

Although a simple explanation for these heat effects is not at hand, studies of enhanced CO_2 production obtained by incubating yeast with sugars or polyols have indicated that the increased output of CO_2 did not involve changes in concentration of enzymes or cofactors, in viability, or in the loss of metabolites (Spoerl & Doyle, 1968*a*), and it was suggested that they might be due to changes in the yeast membranes. The capacities of membranes to transport and to bind sugars, as well as to form compartments for metabolites and reactions, include several functions which could be critically involved in the enhancement of CO_2 production.

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