THE FIXATION OF CARBON DIOXIDE BY GROWING AND NONGROWING YEAST

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Although studies with labeled carbon have revealed that CO_2 is an active participant in the complex pattern of intermediate metabolism (Werkman and Wood, 1942; Buchanan and Hastings, 1946; Wood, 1946), little information is available concerning the dynamics of CO_2 metabolism in the living organism. The available evidence indicates that the degree to which CO_2 is incorporated into the cellular components of an organism depends, among other things, upon whether the organism is resting (nongrowing) or in an active state of protein synthesis (growing). Thus, experiments with rats (Bloom *et al.*, 1947; Schubert and Armstrong, 1949) and mice (Brues and Naranjo, 1949; Skipper *et al.*, 1949) have shown that growing cells tend to fix and retain CO_2 carbon to a greater extent than do nongrowing cells. Microorganisms are particularly suitable for a study of this nature since such cells can be grown from almost zero mass to "adult size" under well-defined conditions. In the present investigation, a comparison is made between the rate of uptake of labeled CO_2 by yeast cells undergoing rapid proliferation in an atmosphere containing labeled CO_2 and nongrowing cells under the same conditions. Data are also presented that describe the manner in which changes in the concentration of the environmental CO_2 influence the fixation of CO_2 by growing yeast cells.

METHODS

General. The yeast used in these experiments was *Saccharomyces cerevisiae* (Red Star strain) maintained on glucose agar slants. Inocula for the experiments to be described were taken from the growth of a 48-hour slant suspended in 5 ml of sterile saline solution.

The synthetic medium of Olson and Johnson (1949) was modified by replacing asparagine with DL-alanine. In experiments with "nongrowing"[2] yeast, DL-alanine and $(NH_4)_2HPO_4$ were omitted from the medium and replaced with an equivalent amount of K_2HPO_4. The latter modification did not seriously impair viability but did inhibit multiplication.

Samples of the CO_2 in the system were trapped in a solution of 2 N NaOH contained in a 15-ml centrifuge tube (*h*, figure 1*B*). The $BaCO_3$ precipitate produced by adding a few drops of saturated $BaCl_2$ solution was successively washed with CO_2-free water and methanol by repeated centrifugation. The precipitate

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[2] The term "nongrowing" as used in this paper is a relative one. Actually the mass of the yeast cells did increase to some extent (see figure 2).

was transferred to an aluminum planchet, dried under an infrared lamp, and counted with a thin mica-window Geiger tube.

Yeast suspensions were centrifuged and successively washed with 0.1 N H_2SO_4, water, methanol, and ether. The residue was dried at 37 C and finally over P_2O_5 to constant weight. (It is evident that this residue consists mainly of the protein and nucleoprotein components of the cell.) The dry powder was counted after being compressed into an aluminum planchet by means of a modified Parr pellet press. The activity of the yeast was calculated as $BaCO_3$ by using a conversion factor obtained by comparing the counts of several combusted samples of yeast (Van Slyke and Folch, 1940), collected as $BaCO_3$ with samples prepared as described. This data also permitted the calculation of the dry weight of the yeast as carbon (Calvin *et al.*, 1949).

All samples of $BaCO_3$ and yeast were of infinite thickness, and hence the observed counts, after background correction, were proportional to the specific activity (SA). Sufficient counts were made to establish an accuracy of ± 2 per cent (Calvin *et al.*, 1949).

Experiments in a small-volume system. The first experiments were performed with an apparatus of relatively small capacity. One-liter Erlenmeyer flasks were each fitted with rubber stoppers containing a gas inlet and outlet tube and two test tubes for isolating components of the medium during the sterilization procedure (figure 1*A*). In a typical experiment, 100 ml of the basal medium were introduced into the main compartment of the flask, $NaHCO_3$ solution into one test tube, and HCl into the other. Whenever necessary, requisite amounts of NaCl were introduced into the medium in order to maintain ionic balance. The flask was then plugged with cotton and autoclaved for 10 minutes at 15 pounds pressure. After the solution had cooled, 1 ml of a solution containing $NaHC^{14}O_3$ was added to the bicarbonate tube and 1 ml of yeast suspension to the medium. The sterile stopper assembly was inserted into the flask and tightly wired in place. A stream of O_2 was passed through the flask for 2 minutes, after which time the flask was sealed by connecting inlet to outlet with a sterile rubber tube. The contents of the test tubes were then "tipped" into the main compartment. After incubation in a water bath at 30 C with constant shaking for the required period, CO_2 was released by the addition of 25 ml 30 per cent trichloroacetic acid and collected in alkali. Samples of yeast and CO_2 were prepared for counting in the manner already described. The initial SA of the CO_2 in the system was obtained by preparing a flask in the same manner but neither inoculating nor incubating it.

Experiments in a large-volume system. As will be subsequently pointed out, the preliminary small-volume experiments revealed that the output of CO_2 that accompanied yeast catabolism caused an appreciable diminution in the SA of the CO_2 in the system. This in turn affected the SA of the yeast in such a manner as to render the data difficult to evaluate. This objection was largely eliminated by constructing a closed system (figure 1*B*) of such magnitude that the CO_2 output of the yeast had a negligible effect on the total CO_2 concentration and its SA.

In experiments with growing yeast, a 3-liter Erlenmeyer flask, *j* (figure 1*B*), containing 1 liter of sterile medium was inoculated with 10 ml yeast suspension and attached to the remainder of the system. The entire volume of the system

was thoroughly flushed with O_2, and the desired amount of isotopic CO_2 was introduced through side arm *g* (figure 1*B*). The culture was gassed and agitated during the incubation period by the action of pump *f* (figure 1*B*). The original CO_2 concentration, as measured with a Haldane gas analyzer, was high enough (10.66 per cent) so that the CO_2 output during the ensuing experimental period had a negligible effect on the subsequent SA of the CO_2 in the system (see figure 2). Aliquots of the yeast suspension, removed at intervals with a syringe attached to *i* (figure 1*B*), were treated as described, and simultaneous samples of gas were taken for SA measurement. The experiment was continued until the yeast cells failed to increase in mass.

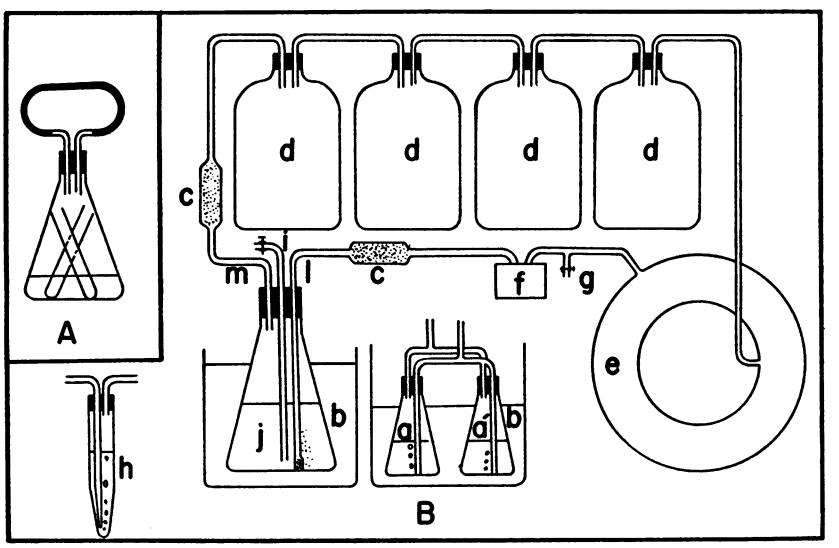

Figure 1. Apparatus for exposure of yeast to $C^{14}O_2$. *A:* Flask used in small-volume experiments. *B:* Apparatus for large-volume experiments—*a-a'*, 1-liter flasks; *b*, 30 C water bath; *c*, gas filters; *d*, 12-gallon carboys; *e*, butyl rubber inner tube; *f*, gas pump; *g*, gas-sampling side arm; *h*, alkaline trap for CO_2; *i*, culture-sampling connection; *j*, 3-liter flask.

In experiments with "nongrowing" yeast, cells that had been centrifuged from a liter of nonradioactive culture after maximum growth had been attained (60 hours) were washed twice with sterile saline and suspended in a liter of nitrogen-free medium. The flask was attached to the apparatus and allowed to incubate at 30 C for 238 hours with periodic sampling of gas and yeast. The initial CO_2 concentration in this case was 10.09 per cent. The continued viability of the cells was evidenced by a slow continuous rise in the CO_2 content of the system. This was confirmed by periodic staining of a drop of the suspension with methylene blue. Approximately 75 per cent of the yeast cells were alive (resisted staining) by the completion of the experiment.

Effect of CO_2 concentration on $C^{14}O_2$ uptake by growing yeast. Each of two 500-ml flasks, *a* and *a'* (figure 1*B*), containing 100 ml of sterile medium were inoculated with 1 ml yeast suspension, placed in the bath at 30 C, and connected at

l and *m* (figure 1*B*). Growth was allowed to proceed for 18 hours. The gas was sampled for CO_2 concentration, using the Haldane gas analyzer, and SA at the beginning and end of each exposure. Yeast samples were prepared in the usual manner. Successive pairs of flasks were inserted, and the process was repeated with different initial concentrations of CO_2. At lower levels the CO_2 output of the yeast served to raise the CO_2 concentration, but in order to obtain higher concentrations additional nonlabeled CO_2 was introduced between exposures.

RESULTS AND DISCUSSION

Small-volume experiments. In table 1 are shown the data of a typical experiment conducted in the system of limited volume and planned to ascertain the rate of uptake of CO_2 by growing yeast. It is quite apparent that the CO_2 concentration,

TABLE 1

Rate of uptake of CO_2 by growing yeast in small-volume system

(To 100 ml basal medium described in text were added 6 mM $NaHCO_3$, 5 mM HCl, 1 ml $NaHC^{14}O_3$ solution [chemically negligible], and 1 ml yeast inoculum containing 2.8 mg [dry weight] equivalent to 0.11 mM carbon. Final volume was made to 130 ml. Incubation was conducted at 30 C with shaking)

FLASK NUMBER	INCUBATION PERIOD	YEAST CARBON	FINAL SA CO_2 (AS $BaCO_3$)	FINAL CO_2 CONCEN.*	SA YEAST (AS $BaCO_3$)
	hours	*mM*	*cts/min*	*mM*	*cts/min*
1	0	0.11	4,045†	6.0†	—
2	6	0.68	3,152	7.7	178
3	12	2.27	2,168	11.2	169
4	24	4.33	1,558	15.6	135
5	48	8.02	1,119	21.7	123
6	60	8.89	1,039	23.4	135
7	72	8.95	919	26.4	124

* CO_2 concentration (final) $= \frac{\text{SA } CO_2 \text{ (initial)}}{\text{SA } CO_2 \text{ (final)}} \times CO_2$ concentration (initial).

† Initial values used in equation in footnote above.

as evidenced by its decrease in SA, had undergone a substantial increase as the period of incubation was prolonged. Although the yeast carbon appears to exhibit a downward trend in SA, the effect of the process of growth on these values is obviously masked by the concomitant change in the SA of the environmental CO_2 within a single incubation period.

In table 2 are given the data of an experiment designed to determine the difference in the fixation of CO_2 between growing and "nongrowing" yeast. Here again the large quantity of CO_2 produced by both the growing and "nongrowing" yeast markedly reduced the SA of the CO_2 in the system. With this effect in mind, it is still significant to note, however, that the SA of the growing yeast was almost 10 times as great as that of the nitrogen-deprived yeast. This was true in spite of the fact that the catabolic activity of both cultures was essentially the same, as evidenced by the production of CO_2.

Large-volume experiments. By the use of the large-volume system, difficulties due to the production of CO_2 by the metabolizing yeast were largely eliminated, so that it now became possible to relate the SA of the yeast carbon directly to the SA of the CO_2 in the system. The data relative to the rate of fixation of $C^{14}O_2$ by growing and "nongrowing" yeast are summarized in figure 2. The slight increase in the mass of the "nongrowing" yeast may possibly be explained as a transition from high-nitrogen to low-nitrogen yeast as discussed by Ehrensvärd (1948). In confirmation of the results obtained in the preliminary small-volume experiments, the rate of incorporation of labeled carbon differed markedly between the two cultures. The carbon of the rapidly proliferating yeast rapidly attained a SA that remained relatively constant at a value that indicated that about 4.8 per cent of the yeast carbon was derived from the "atmospheric" CO_2.[3] This result seems consistent with the observation by Ehrensvärd (1948) that

TABLE 2

Uptake of CO_2 by growing and "nongrowing" yeast in small-volume experiment

(Cultures were incubated at 30 C for 60 hours with shaking)

CULTURE	ADDITIONS TO BASAL MEDIUM			SA CO_2 (AS $BaCO_3$)		CO_2 PRODUCED*	SA YEAST (AS $BaCO_3$)
	$NaHCO_3$	HCl	NaCl	Initial	Final		
	mM	*mM*	*mM*	*cts/min*	*cts/min*	*mM*	*cts/min*
Growing†	3.0	2.5	15.0	12,900	1,649	20.5	167
	6.0	5.0	12.5	6,450	1,527	19.3	119
Nongrowing‡	3.0	2.5	15.0	12,900	1,710	19.6	20
	6.0	5.0	12.5	6,450	1,536	19.2	15

* See first footnote to table 1.

† To 100 ml basal medium described in text were added $NaHCO_3$, HCl, and NaCl in the proportions shown in table, 1 ml $NaHC^{14}O_3$ solution (chemically negligible), and 1 ml yeast suspension. Final volume was made to 130 ml.

‡ Each culture was grown in the medium above omitting $NaHC^{14}O_3$, centrifuged, washed, and suspended in the nitrogen-free medium with isotope.

CO_2 is readily fixed into the amino acids of protein derived from growing cultures of *Torulopsis utilis*. Using $C^{13}O_2$ he found that about 3.5 per cent of the protein carbon originated from the inflowing labeled CO_2.

In contrast to the growing cells, the slowly proliferating but metabolically active cells assimilated CO_2 to a considerably less extent. At the end of 10 days, only 1 per cent of the yeast carbon apparently had its origin from the air. Whether the amount of yeast carbon derived from the air by "nongrowing" cells can ever reach the level attained by growing cells cannot be safely predicted on the basis of these data. The two SA curves of figure 2 suggest that this will not happen in any period of time that will allow "nongrowing" cells to remain viable. The re-

[3] Per cent of yeast carbon fixed from CO_2 =

$$\frac{\text{SA yeast (as } BaCO_3)}{\text{SA } CO_2 \text{ (as } BaCO_3)} \times 100.$$

sults do indicate that growing yeast rapidly accumulates a large store of fixed carbon that does not again turn over in the metabolic pool. Extension of the SA curves indicates that more than three-quarters of the original fixed carbon in that fraction of the yeast cell studied remains in the cell after 10 days of fairly active catabolism.

It is of interest to compare the results obtained here with studies of phosphorus turnover in growing and nongrowing cells reported by others. The turnover of labeled phosphorus has been found to be much greater in the desoxynucleic acids of regenerating rat liver (Brues *et al.*, 1944) and the nucleo-

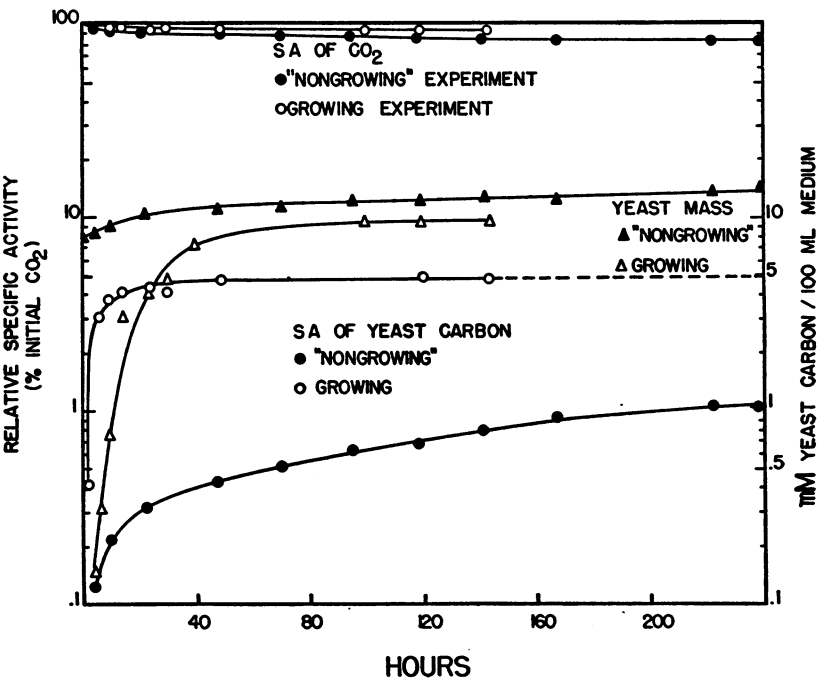

Figure 2. Rate of uptake of fixed carbon by growing and "nongrowing" yeast. Since the relative SA of the CO_2 remained close to 100 throughout the experimental period, the relative SA of the yeast carbon closely approximates the percentage of carbon derived from CO_2 (see footnote 3).

protein fraction of growing yeast cells (Spiegelman and Kamen, 1946) than in the corresponding nongrowing cells. These experiments supplement the considerable body of evidence that indicates that the phosphorus-containing proteins, nucleoproteins, by virtue of their ability to donate energy, may be the controlling factors in protein synthesis (Spiegelman and Kamen, 1946, 1947). The fact that the energy required for the assimilation of CO_2 may likewise be linked to phosphorylation (Lipmann and Kaplan, 1949) could offer an explanation for the rapid uptake of CO_2 under those conditions that favor protein synthesis.

Effect of CO_2 concentration on CO_2 uptake by growing yeast. As previously pointed out, changes in CO_2 concentration appeared to influence the fixation of

CO_2 by growing yeast in the small-volume experiments. In order to study more precisely the nature of this effect, use was again made of the large-volume system to minimize the influence of metabolic CO_2. It can be seen from figure 3 that at lower levels of CO_2 this attempt was not entirely successful. At higher levels of CO_2 concentration, however, the effect of metabolic CO_2 on the fixed carbon value could be considered negligible. In spite of the wider variations in fixed carbon values encountered at lower concentrations of CO_2, the smooth curve, which could be drawn, revealed that the amount of yeast carbon coming from

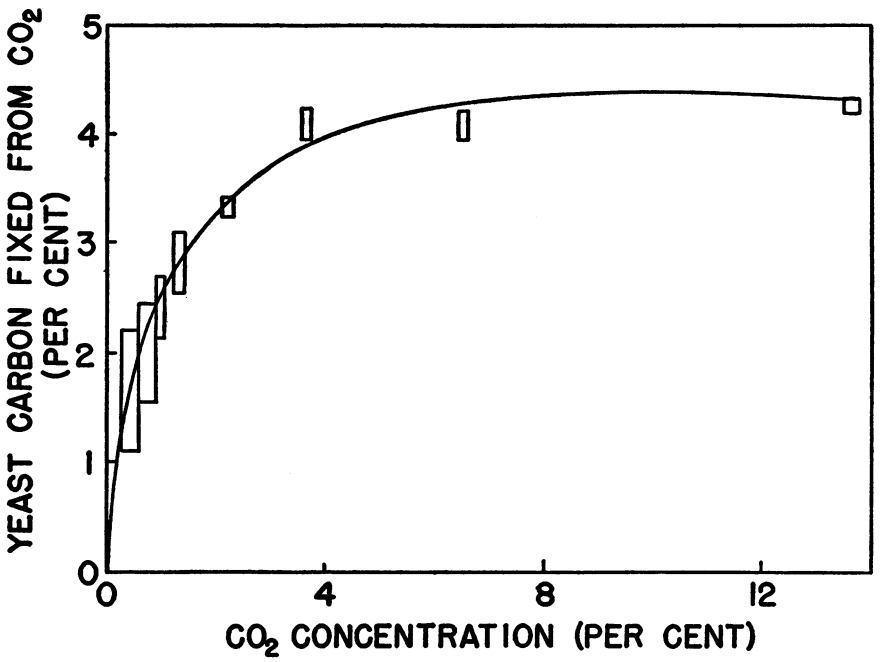

Figure 3. Effect of CO_2 concentration on uptake of fixed carbon in growing yeast. The sides of each rectangle indicate the magnitude of the changes produced by metabolic CO_2 during each exposure period: horizontal side, the change in CO_2 concentration due to the CO_2 output by the yeast; vertical side, the range of fixed carbon values (see footnote 3) based on the initial and final SA of the CO_2. The SA of the yeast carbon used in calculating fixed carbon values is the average of duplicate samples. The curve shown is the best one that could be drawn through the mid-point of each rectangle.

CO_2 increased with the CO_2 concentration up to 4 to 5 per cent, then becoming essentially constant. This maximum value is in substantial agreement with that obtained from the data in figure 2. It is interesting to note that Elsden (1938) had previously reported that succinic acid production by *Escherichia coli* increased with CO_2 concentration up to 5 per cent, but above this level there was no further increase. The curve shown in figure 3 is qualitatively similar to that reported for dark fixation in *Scenedesmus* (Benson and Calvin, 1948). Whether such a curve for yeast would have the same metabolic implications as discussed by Benson and Calvin (1948) cannot be answered from the present data.

SUMMARY

Yeasts were cultured in closed systems containing isotopic CO_2 in order to determine the percentage of total carbon (in that fraction that had been acid-washed and ether-extracted) originating from CO_2.

Growing yeast cells rapidly fixed CO_2, obtaining as much as 5 per cent of their total carbon from the air. After an exposure of 10 days under the same CO_2 tension, "nongrowing" cells contained only 1 per cent fixed carbon.

At low concentrations of CO_2, the uptake of this carbon by growing yeast is proportional to the CO_2 concentration. When the CO_2 concentration exceeds 5 per cent, the percentage of the total carbon represented by fixed carbon becomes essentially constant.

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