Oxidative Assimilation and Endogenous Respiration of *Rhodotorula graminis** (33697)

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Most studies on oxidative assimilation and endogenous respiration of yeast have been conducted with fermentative species, particularly *Saccharomyces cerevisiae*. In studies preliminary to more detailed ones on endogenous respiration the oxidative characteristics of a nonfermentative yeast, *Rhodotorula graminis*, were studied.

Materials and Methods. The general methods were the same as previously described (1, 2). A strain of *Rhodotorula graminis* CBS 3043 from the collection of Dr. Herman Phaff was grown on a basal salt medium (2) with the phosphate concentrations adjusted to yield a final pH of 5.5. One % glucose was employed as the C-source, 0.1% $(NH_4)_2SO_4$ as the N-source. In some experiments the cells were uniformly labeled during growth in the presence of 15 μCi of glucose-U-^{14}C/100 ml of the agar medium. Cells from 16-hr cultures were harvested, washed twice and suspended in the basal salt solution of pH 5.5 unless otherwise noted. The cell concentration was adjusted to approximately 5 mg of dry weight/ml of suspension.

Results. The results of a typical experiment on oxidative assimilation, during the utilization of 8 μmoles of glucose containing 72 mμCi of glucose-U-^{14}C, and on endogenous respiration by *R. graminis* are reported in Fig. 1. The amount of O_2 consumed during the first 20 min following the addition of the glucose was only about 1.5 times the endogenous value. This ratio fell to 1.3 for 60 min. A tenfold increase in glucose concentration had no appreciable effect on the rate of O_2 uptake except that the maximum rate was maintained over a proportionally longer period of time. The rates and extents of O_2 consumption were practically the same over a pH range of 5.5–8.1.

A decrease (break) in the rate of O_2 consumption in the exogenous system is apparent before 30 min in Fig. 1. The O_2 consumed during the first 30 min corresponded to 16% of the theoretical value for complete combustion, no correction being made for concomitant endogenous respiration. Oxidation had proceeded to 11% of completion as calculated indirectly on the basis of $^{14}CO_2$ production with an RQ of approximately 1.0 in the presence of glucose. This extent of oxidation corresponds to an O_2 uptake of 118 μl for the oxidation of glucose. The difference, 54 μl, between the total O_2 uptake of 172 μl and that calculated for the oxidation of the glucose is about 45% of the endogenous value of 119 μl for 30 min and represents an apparent inhibition of endogenous respiration of about 55%. Since respiration continued to a reduced extent for some time following the addition of sulfuric acid (final concentration ca. 2% and some time is required to remove the KOH from the Warburg vessels the $^{14}CO_2$ values, and hence the calculated O_2 uptake, may be somewhat high. Therefore the calculated extent of inhibition of endogenous respiration in the presence of glucose would be high. Somewhat lower values were noted in experiments of longer duration with 20 μmoles of glucose rather than 8. The longer experimental periods reduced the errors of $^{14}CO_2$ adsorption and residual production in the Warburg vessels. Results of other tests with cells uniformly labeled during growth indicated that endogenous $^{14}CO_2$ production was inhibited 10–20% following the addition of glucose to endogenously respiring cells. Results of this more direct method for the measurement of endogenous respiration probably represent more accurately the influence

* Supported by Army Research Office Grant HCA-67-G-0014. The technical assistance of Mrs. Rogers Johnson is gratefully acknowledged.

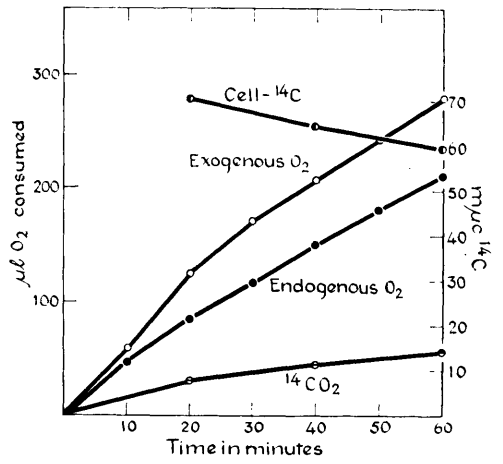

FIG. 1. Oxygen consumption (endogenous and exogenous), ^{14}C-assimilation and $^{14}CO_2$ production from 8 μmoles (72 mμCi of ^{14}C) of uniformly labeled glucose by *R. graminis*.

of glucose on the endogenous respiration of *R. graminis*.

In other experiments the amounts of total- and of firmly bound-^{14}C (i.e., ^{14}C not released on acidification) in the cells were determined. It was noted in preliminary experiments that acidification tended to cause some of the cells to clump and to stick to the walls of Warburg flasks. Because of this behavior a suspension of cells was shaken in a large flask in the Warburg bath. Glucose (8 μmoles in 0.2 ml/2 ml of suspension) was added to the suspension and 2-ml samples were removed at appropriate times. These samples were added to 0.2 ml of 20% H_2SO_4 or 5% $HgCl_2$ in the centrifuge combustion tubes employed with the Dynacon system. The O_2 consumption was determined concurrently in the Gilson respirometer and $^{14}CO_2$ production from Dynacon measurements on the KOH from the center well of Warburg flasks.

The results of tests for total- and firmly bound-^{14}C are presented in Table I. It is apparent, as in Fig. 1, that most of the $^{14}CO_2$ production occurred during the first 30 min. By the end of this time 16% of the glucose-^{14}C had been converted to $^{14}CO_2$. At the end of 30 min ca. 78% of the initial glucose-^{14}C was within the cells and most of this label was firmly bound, i.e., not released on acidification. The nature of the storage product(s) has not been determined but it is apparent from results presented in Fig. 1 and Table II that the assimilatory product is not oxidized rapidly. It may be that the initial bursts of respiratory activity noted represent, in part at least, expenditure of energy for transport of glucose into the cell and its conversion to cellular reserves.

The results presented in Table I show that 19% of the label present in the cells at the end of 30 min was in pool material, extracted on acidification of the suspension. The size of the pool increased to some extent with time after 30 min. No explanation for this increase in size of the pool, or shift in the distribution of cell-^{14}C, can be advanced. The same behavior was noted in other experiments.

In some experiments the cells were fractionated (3) to determine the distribution of the label. In the experiment reported below

TABLE I. Distribution with Time of 73 mμCi (8 μmoles) of Glucose-U-^{14}C in a Washed Suspension of *R. graminis*.

Min:	(mμCi)					
	30		60		90	
	—[a]	—[b]	—[a]	—[b]	—[a]	—[b]
$^{14}CO_2$[c]	11.3		16.1		18.4	
Cells	45.9	56.4	40.0	52.7	33.6	50.9
Supernatant	16.5	4.4	18.3	3.8	22.0	3.3

[a] Cell suspensions acidified.
[b] Cells killed with $HgCl_2$.
[c] $^{14}CO_2$ determined following acidification of suspensions in Warburg flasks.

TABLE II. Endogenous O_2 Consumption and NH_3–N or $^{14}CO_2$ Production by Suspensions of Labeled *R. graminis*.*

Time (min)	O_2 (μl/ml)	NH_3–N (μg/ml)	$^{14}CO_2$ (mμCi/3 ml)	% Cell-^{14}C→$^{14}CO_2$
0–60	167	7	6.5	2.5
60–120	114	9	3.4	1.2
120–180	77	23	2.3	0.9
180–240		27	1.8	0.7
240–300			1.2	0.4
300–360			0.2	0.1

* Five mg dry weight of cells and 90 mμCi ^{14}C per ml.

the cells were treated with $HgCl_2$ to halt respiration before the break in rate of O_2 uptake was noted and with 20 rather than 8 μmoles of glucose-U-^{14}C as substrate. At this time (40 min) 44% of the initial label was intracellular, 41% was in the suspension medium and 15% had been released as $^{14}CO_2$. The cells were removed by centrifugation and then extracted in turn with cold 5% trichloroacetic acid, hot 75% ethanol and hot 5% trichloroacetic acid. ^{14}C-determinations indicated that 45% of the label present in the cells at harvest was in the pool fraction (low mol. wt. compounds soluble in cold trichloroacetic acid). Six % of the intracellular label was present in both the alcohol and the hot trichloroacetic acid soluble fractions and the remainder, 43%, was in the insoluble residue. The nature of the assimilatory products has not been determined. These results indicate, as reported (4) for various bacteria, that the label is widely distributed in the cell after short periods of oxidative assimilation although much of it may be concentrated in some of a few primary assimilation products. These results indicate the complexity of oxidative assimilation as it occurs under conditions of N-starvation.

The rate of endogenous O_2 consumption by freshly harvested cells of *R. graminis* was high but decreased quite rapidly with time of shaking. The same behavior was noted for $^{14}CO_2$ production by uniformly labeled cells tested over a longer period of time. About 6% of the initial cellular-^{14}C was released as $^{14}CO_2$ during a 6-hr test period.

The RQ of freshly harvested, endogenously respiring cells ranged from 0.83 to 0.86 and tended to decrease as the age of the suspension increased. The RQ in the presence of free glucose (initial value of 8 μmoles/2.2 ml) in the medium was around 1.0, but this value decreased rapidly after the break in rate of O_2 consumption was noted. Nitrogenous "matter" appeared to be, in part at least, a substrate for endogenous respiration as indicated by marked NH_3 production (Table II). The cells contained a pool of amino acids (materials extracted with hot water, which reacted with 2,4,6-trinitrobenzene sulfonic acid) and the size of this pool decreased on shaking for 2 and 5 hr. Very little change in the carbohydrate (anthrone method) content of the hot-water soluble material was noted during the same time periods.

An attempt was made to determine the chemical fraction(s) supplying the substrate for endogenous respiration. Samples of a suspension of labeled cells were fractionated (3) and the ^{14}C-content of each fraction was determined at the beginning and at the end of a 4-hr period of endogenous respiration. The cell-^{14}C content (120 mμCi/ml) decreased by 9% during the test period. The most marked numerical decrease was noted in the fraction insoluble in hot 5% trichloroacetic acid but this fraction constituted about 80% of the total cell-^{14}C content. No appreciable change on a percentage basis was noted in the distribution of the label in the various fractions. This is in agreement with the general observations reported (4) for various bacteria.

Summary. Results of the ^{14}C-distribution studies suggest that equilibria (steady state conditions) are maintained between the vari-

ous constituents of the cells, at least during the early stages of endogenous respiration and before death becomes a major factor. The loss of nitrogen from the cells as NH_3 would result in some shift in internal conditions. The results indicate that the oxidative assimilatory and endogenous respiratory activities of the oxidative yeast *R. graminis* are similar to those reported for fermentative yeasts and for bacteria. The results of the RQ studies and those on the amino acid pool and on NH_3 production indicate that amino acids are a major endogenous substrate for this organism. Carbohydrate, which is an endogenous substrate for various species of yeast, does not appear to act as such to a marked extent for *R. graminis*.

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Received Sept. 4, 1968. P.S.E.B.M., 1969, Vol. 130.

The Effect of Interferon on Focus Formation and Yield of Murine Sarcoma Virus *in Vitro* (33698)

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The available evidence supports the view that interferon plays a significant role in host-resistance to primary viral infections (1). Moreover, various oncogenic viruses recently were shown to be susceptible to the inhibitory action of interferon (2–6). With the demonstration of a viral agent inducing sarcomas in mice (7, 8) and the development of an *in vitro* assay system for this virus (9), it has become possible to test the susceptibility of this murine sarcoma virus (MSV) to the inhibitory action of interferon. The data presented below indicate that mouse serum interferon induced by Newcastle disease virus *in vivo* does interfere with focus formation and yield of MSV *in vitro*.

Materials and Methods. A cell-free pool of murine sarcoma virus (MSV) derived from sarcomas in Balb/c mice was kindly provided by J. Moloney, NCI, NIH, and was used as test material in all these experiments. The titer of this virus was $10^{7.6}$ pfu/ml. There was a direct relationship between dilutions of this virus and the focus count. The Herts strain of Newcastle disease virus (NDV) was obtained through the courtesy of S. Baron, NIAID, NIH. It was propagated in embryonated chicken eggs and had a titer of $10^{9.3}$ pfu/ml. Vesicular stomatitis virus (VSV) was obtained from Y. Hirshaut, NCI, NIH; it was propagated in mouse embryo fibroblast cultures and had a titer of $10^{7.0}$ pfu/ml. Concentrated plasma-derived Rauscher leukemia virus (RLV) was obtained from Mason Research Institute and demonstrated a titer of between 10^6 and 10^7 $TCID_{50}$/ml by means of an interference assay using MSV as challenge virus (10).

Mouse embryo fibroblast (MEF) cell cultures and chick embryo fibroblast (CEF) cell cultures were prepared from 9–14-day-old embryos by trypsinization. Medium for these cultures consisted of Eagle's minimum essential medium (MEM) supplemented with glutamine and with 10% (for growth) or 2% (for maintenance) heat-inactivated fetal calf serum (Hyland). The JLS-V9 cells, a cell line derived from mouse bone marrow (11), were grown in McCoy's 5A medium supple-

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