THE METABOLISM OF SPECIES OF STREPTOMYCES

IV. The Effect of Substrate on the Endogenous Respiration of Streptomyces coelicolor[1]

VINCENT W. COCHRANE and MARTIN GIBBS

Department of Biology, Wesleyan University, Middletown, Connecticut, and Department of Biology, Brookhaven National Laboratory, Upton, L. I., New York

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Indirect evidence has been offered (Barker, 1936; Doudoroff, 1940) that with some microorganisms the addition of a readily respirable substrate decreases the rate of endogenous respiration of resting cells. The subject is briefly reviewed by van Niel (1943), who suggests that subtraction of endogenous values may be unrealistic in particular cases.

Streptomyces coelicolor, strain B-3 of Conn (1943), exhibits a high endogenous respiration; the oxygen uptake of freshly harvested washed cells in the absence of substrate may be 15 to 25 per cent of that in the presence of glucose as substrate. With so high an endogenous rate, it becomes important for interpretation of manometric data on substrate utilization to determine whether or not endogenous values should be subtracted. The same problem arises with other actinomycetes and the fungi.

Manometric methods alone cannot give a direct measurement of endogenous respiration in the presence of substrate; hence an experimental design first suggested in principle by Burris (1949) was employed. Cells were grown on glucose uniformly labeled with C^{14}, and the respiration was studied in conventional Warburg apparatus. Endogenously produced CO_2 will thus be labeled; if addition of unlabeled substrate reduces the total activity of the respired CO_2, the extent of such reduction is a measure of the extent of suppression of the endogenous system by substrate. Conversely, if there is no change in total activity of the respired CO_2, one may conclude that the two systems operate independently and that the true measure of oyxgen uptake or CO_2 output caused by added substrate is the total value minus the endogenous value of comparable cells. In the system labeled cells plus unlabeled substrate, it is of course to be expected that specific activity would be affected markedly by the substrate, but only the total activity is relevant to the problem.

EXPERIMENTAL METHODS AND RESULTS

Cells were grown for these experiments in Difco nutrient broth supplemented with phosphate buffer (M/60, pH 7.4), $MgSO_4 \cdot 7H_2O$ (0.001 M), and glucose. The glucose was added aseptically at 16 and again at 60 hours to a concentration in the medium of 0.05 M. At each time period 20 mg of uniformly labeled glucose

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of specific activity 0.53 μc per mg was included. After 72 hours of cultivation on a rotary shaker the cells were harvested and washed twice in saline by centrifugation; an aliquot of a cell suspension containing 6 to 8 mg dry weight was pipetted into each Warburg flask. Respiratory CO_2 was collected at the end of the run by introducing 20 per cent NaOH into a side arm through the venting plug; the last traces of CO_2 were driven over by tipping acid into the main compartment.

After equilibration the contents of the side arm were collected; carrier Na_2CO_3 was added, CO_2 was liberated with acid and passed into $Ba(OH)_2$-$BaCl_2$, and the resultant $BaCO_3$ was collected on a sintered glass disk of known weight and area. Total carbon was determined by back-titration of the $Ba(OH)_2$ prior to collection of the $BaCO_3$. The activity of the $BaCO_3$ was determined in a methane flow proportional counter.

The conditions used and the results of two experiments are displayed in table 1.

TABLE 1

*The effect of substrate on endogenous respiration**

SUBSTRATE	EXPERIMENT 1				EXPERIMENT 2†		
	None		Na-pyruvate		None	Glucose	Na-pyruvate
Time, min	60	120	60	120	60	60	60
CO_2 evolved, μl	47	95	156	—	50	228	233
O_2 consumed, μl	36	68	114	—	35	212	165
$Q_{O_2}^{O_2}$, uncorrected	5.0	4.7	16	—	5.7	35	27
Total activity, mμc	1.58	2.86	1.97	2.86	2.81	2.93	2.77

* Atmosphere O_2; phosphate buffer M/60, pH 5.6; 0.2 ml 20 per cent NaOH added at end of run to side arm, after tipping 0.3 ml 3 N H_2SO_4 into the main compartment; all substrates at 0.033 M; dry weight of cells 7.2 mg in expt. 1, 6.1 mg in expt. 2.

† Average of duplicate flasks.

COMMENT

The data show that concomitant oxidation of glucose or pyruvate has no suppressive effect on endogenous respiration. Consequently, for this organism at least, it is permissible to correct respiratory gas exchange figures by subtracting endogenous values.

At first it might seem necessary to postulate that endogenous carbon dioxide production in *S. coelicolor* proceeds via metabolic pathways completely separate from those involved in substrate oxidation. A more cautious hypothesis is that, whereas reserves and substrate may share a common sequence of terminal reactions, the rate-limiting step for endogenous metabolism is prior to the shared pathway. The "rate-limiting step" in endogenous respiration may, of course, be a spatial limitation reflecting the relative inaccessibility of reserve substances to enzymes. The supposition that endogenous and substrate respiration share a common terminal pathway is introduced for reasons of economy in hypothesis;

a corollary, in view of the data presented, is that the enzymes of the common pathway are present in great excess over the demand put upon them by endogenous metabolism.

SUMMARY

When cells of *Streptomyces coelicolor* are grown in a medium containing glucose uniformly labeled with C^{14}, the addition of a respirable substrate (0.033 M) glucose—or pyruvate—does not appreciably suppress endogenous CO_2 production, as judged by the total radioactivity of respired CO_2. Consequently, in manometric experiments with exogenous substrate of 0.033 M concentration, the subtraction of endogenous values is permissible.

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