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Endogenous Respiration of *Serratia marcescens* and Oxidation of Added Carbohydrates.* (23338)

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During an investigation of the oxidative patterns of *Serratia marcescens*, cells grown in one of several media consistently yielded an anomalous pattern of substrate oxidation when the data were corrected for endogenous metabolism in the classical manner. Subsequent study of the data indicated that this unusual behavior may be due to suppression of the endogenous metabolism of the organisms. The role of endogenous metabolism during oxidative dissimilation of various substrates has been variously reported. Production of $C^{14}O_2$ from C^{14} "labeled" *Streptomyces coelicolor* was found to be the same during oxidation of glucose and pyruvate as it was in endogenous metabolism(1). Wilner and Clifton(2) reported that the endogenous respiration of a strain of *Bacillus subtilis* was not suppressed during exogenous respiration, and that correction for the former was necessary to obtain satisfactory equations for carbon assimilation. Conversely, endogenous respiration of *Pseudomonas saccharophila* was reported to be inhibited during oxidation of assimilable substrates(3,4), and the oxidation data for various carbohydrates by *Prototheca zopfii* were more amenable to analysis if the endogenous respiration of the organisms was disregarded(5). The present report suggests that the conflicting conclusions of the cited

studies may be a result of different growth conditions, in addition to the differences related to species specificity.

Methods. *S. marcescens*[†] was grown in media containing 5% skim milk solids, 3% Armour's peptone,[‡] and 2% of one of the following carbohydrates. *Neutral glucose*: glucose added to medium prior to sterilization at 121°C for 15 minutes. The pH was 7.0-7.2 without adjustment. *Neutral gluconate*: gluconic acid was dissolved in a few ml water and adjusted to pH 6.0 prior to addition to the medium. Following sterilization the pH dropped to approximately 4.0. Sterile NaOH was added to raise the pH to approximately 6.0. *Acid gluconate*: gluconic acid was prepared as above and added to the medium. However, after sterilization the pH was allowed to remain at pH 4.0. After 16 hours growth on a reciprocal shaker at 30°C, the cells were harvested by centrifugation, washed once with water, and stored at 5°C until used. The Warburg experiments were carried out by conventional manometric technics. Gluconic acid was obtained as glucono-delta lactone from the Eastern Chemical Corporation.

[†] Obtained thru the courtesy of Dr. R. G. Benedict, Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Dept. of Agriculture.

[‡] Mention of commercial products does not imply endorsement by the U. S. Dept. of Agriculture over similar products not mentioned.

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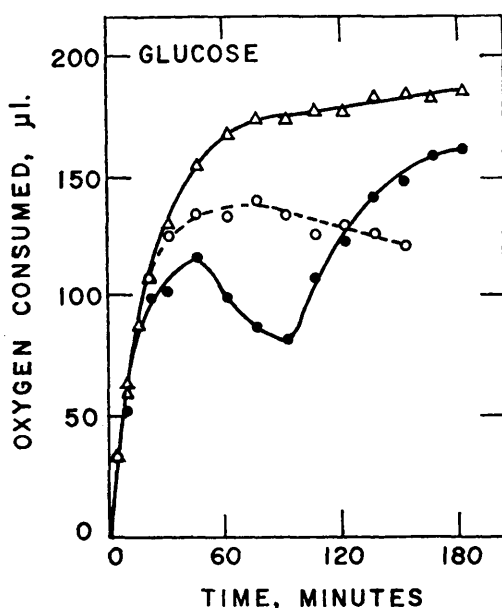


FIG. 1. Glucose oxidation by *S. marcescens* harvested from several media. Each flask contained 0.5 ml 0.05 *M* tris (hydroxymethyl) amino methane buffer, pH 7.2; 100 μ g inorganic phosphorus; 4 mg (dry wt) appropriate cells; 2.5 μ *M* glucose; water to 2.0 ml. Temperature -30°C . Δ — Δ Neutral glucose-grown cells; $\circ$ — $\circ$ Neutral gluconate-grown cells; $\bullet$ — $\bullet$ Acid gluconate-grown cells.

Results. Representative oxygen consumption curves of glucose dissimilation by cells grown on the 3 media were obtained in the classical manner by subtracting endogenous respiration of the cells from the total respiration. Cells harvested from the glucose and neutral-gluconate media oxidized glucose in a manner described by the curves in Fig. 1. The acid gluconate-grown cells, however, produced a minimum in the curve (Fig. 1). This minimum is due to the fact that the rate of endogenous metabolism of the cells was greater than the rate of metabolism in the presence of glucose. Inspection of the endogenous metabolic patterns of the 3 cell preparations showed that respiratory activity of the glucose-grown cells was qualitatively similar to that of the neutral gluconate-grown cells, but that of the acid gluconate-grown cells was considerably higher and varied in rate.

The anomalous pattern of oxidation of the acid gluconate-grown cells in the presence of

substrate could not be exclusively attributed to increased endogenous metabolism. Correction for the high endogenous respiration should yield normal curves as obtained with the other cell preparations. The pattern of respiration was analyzed by comparing rate of oxygen consumption by neutral gluconate- and acid gluconate-grown cells in the presence and absence of glucose (Table I).

Endogenous utilization of oxygen by the neutral gluconate-grown cells proceeded at a low, fairly uniform rate. In the presence of glucose, oxygen consumption was regular, but at an increased rate, then dropped to that of the endogenous cells. However, the acid gluconate-grown cells showed a sharp increase in endogenous rate of oxygen consumption after 30 minutes, which was maintained for approximately an additional 60 minutes before returning to the original lower level. In the presence of glucose, initial oxygen uptake quickly attained a higher rate, which was maintained for approximately 135 minutes before returning to the endogenous rate. The increase in endogenous metabolic rate observed between 30 and 90 minutes was not reflected in the respiratory pattern of the cells in the presence of glucose.

It was also observed that the course of

TABLE I. Course of Oxygen Consumption in Presence and Absence of Glucose by Acid Gluconate- and Neutral Gluconate-Grown *S. marcescens*.

Time, min.	Neutral grown cells		Acid grown cells	
	Endog.	Glucose	Endog.	Glucose
5	0*	29*	5*	34*
10	7	36	16	35
15	8	32	14	47
20	7	28	17	31
30	9	27	36	38
45	16	25	66	81†
60	8	7	70	52
75	10	17	59	47
90	14	8	52	47
105	17	9	33	58
120	0	4	31	45
135	10	6	25	43
150	10	6	22	29
165	—	—	20	31
180	—	—	17	19

* The figures represent μ L oxygen consumed in each time interval.

† This high reading may be due to experimental error since subsequent experiments maintained a constant rate of glucose oxidation.

dissimilation of gluconic acid by the 3 cell preparations was similar to that of glucose. Acid gluconate-grown cells also oxidized 6-phosphogluconate and fructose 1,6-diphosphate in a pattern that varied from that of the other two cell preparations. The observed differences in the response of the cell preparations is not due to the pH of the growth medium alone, since cells grown on acidified (pH 4.0) glucose medium oxidized the various substrates in a manner similar to that of the neutral glucose-grown cells.

Summary. Endogenous oxygen consumption of *Serratia marcescens*, grown on a medium containing glucose at pH 4 and 7 or gluconate at pH 6, continues unchanged during oxidation of glucose and several other substrates. Correcting total oxygen consumption in the presence of a substrate for the endogenous oxygen consumption, affords an accurate representation of the course of oxidation of the substrates. *S. marcescens* grown in glu-

conate-containing medium at pH 4 has a higher endogenous oxygen consumption than cells grown on the other media. At one stage of the endogenous metabolism a sharp increase in rate of oxygen consumption occurs. Such sharp increase in oxygen consumption does not occur during oxidation of glucose and several other substrates. Hence correction of total oxygen consumption in the presence of the substrates for endogenous respiration of the cells may lead to an erroneous interpretation of the data.

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Inhibition of Prothrombin Activation in Sodium Citrate Solutions.* (23339)

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In 25% sodium citrate solution purified prothrombin slowly transforms to thrombin (1). The discovery of this transformation cleared the view that prothrombin, as a single molecular entity, contains all of the structural material required in the thrombin molecule (2). The activation is autocatalytic and is initiated or accelerated by the addition of thrombin. This fundamental consideration alerts us to the possibility that prothrombin activation under physiological conditions may also be autocatalytic, that thrombin may be the only enzyme in prothrombin activation and that other factors are accessory.

The autocatalytic activation of purified prothrombin in sodium citrate solution can be accelerated with an organic compound of

known structure such as 3-chloro-4,4'-diaminodiphenyl sulfone(1). By substituting an amino group for the chlorine atom and thus having 3,4,4'-triaminodiphenyl sulfone the autocatalytic activation is inhibited. In a similar way soybean trypsin inhibitor stops the autocatalytic process(3). We thus have a greatly simplified view of prothrombin activation that may actually be giving us an accurate concept of the minimal chemical requirements for prothrombin activation and its inhibition. The inhibitors 3,4,4'-triaminodiphenyl sulfone and soybean trypsin inhibitor are however not to be anticipated in plasma or serum but apparently no one has inquired whether other inhibitors of prothrombin activation in sodium citrate solution might be found in plasma or serum. With this idea in mind we conducted many experiments and

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