7. Noll, H. and Braude, A. I., J. Clin. Invest. **40**, 1935 (1961)
8. Nowotny, A., Nature **197**, 721 (1963).
9. Nowotny, A., in "Bacterial Endotoxins," Landy, M. and Braun, W., eds., p. 31. Rutgers Univ. Press, New Brunswick, New Jersey, 1964.
10. Ouchterlony, O., Thesis Karolinska Institute, Stockholm, 1949.
11. Urbaschek, B., 1960, Z. Immunitaetsforsch. **120**, 279 (1960).
12. Urbaschek, B., Verhandl. Deuts. Ges. Inn. Med. **72**, 752 (1966).
13. Urbaschek, B., Ann. N. Y. Acad. Sci. **133**, 709 (1966).
14. Urbaschek, B. and Versteyl, R., Nature **207**, 763 (1965).
15. Urbaschek, B., Versteyl, R. and Götte, D., Naturwissenschaften **53**, 278 (1966).
16. Wharton, D. R. A. and Creech, H. J., J. Immunol. **62**, 135 (1949).

Received Oct. 27, 1967. P.S.E.B.M., 1968, Vol. 127.

Oxidative Assimilation by *Serratia marcescens** (32766)

C. E. CLIFTON
Department of Medical Microbiology, Stanford University School of Medicine, Stanford, California 94305

This study, preliminary to one on endogenous respiration and energy of maintenance, summarizes observations on oxidative assimilation by *Serratia marcescens*. The influence of age of the cells and of cell concentration is considered. Glycerol was employed in most of the tests as the exogenous substrate to avoid any direct polymerization of substrates such as sugar to endogenous reserves.

Materials and Methods. The general methods employed were the same as previously described (1,2). *Serratia marcescens* Z-4 was grown for 16 hours at 30°C nutrient agar (Difco) or on this medium containing 1% glucose. The cells were harvested, washed, and suspended in a basal salt solution (2) to avoid any specific ion effects. In the aging experiments the washed suspensions were shaken in stoppered flasks on a wrist-action shaker at 30°C and samples were removed at daily intervals. Total cell ^{14}C was determined in samples of cells killed with $HgCl_2$ (0.2 ml of 5% $HgCl_2$ per 2 ml of suspension), firmly bound ^{14}C in cells from 2 ml of suspension acidified with 0.2 ml of 10% H_2SO_4. Glycerol was determined colorimetrically (3).

Results. A Qo_2 of 60 was observed following the addition of 8 μmoles (204mμC) of glycerol-U-^{14}C to 6 mg dry weight of freshly harvested cells of *S. marcescens* suspended in 2 ml of basal salt solution. The rate of O_2 consumption (or $^{14}CO_2$ production) decreased abruptly after the time (break-point) that approximately 300 μl or about one half of the theoretical amount (627 μl) of O_2 required for complete combustion had been consumed. The Qo_2 continued to decrease and approached the value (15) observed for the endogenous control. No difference was noted between the behaviors observed with cells grown on nutrient or on glucose agar.

Results of quantitative tests for glycerol in supernatant fluids from nonacidified suspensions indicated that less than 10% of the initial glycerol remained in solution while ^{14}C determinations indicated 22% residual ^{14}C. A portion of this residual ^{14}C probably is in matter relatively resistant to oxidation since the ^{14}C content of the supernatants decreased very slowly after the break-point, a behavior noted with other organisms and substrates (4).

The O_2 consumption values to the break-point, uncorrected for endogenous respiration, suggested about 50% assimilation of the glycerol while the data for total cellular ^{14}C indicated that only about 36% of the initial ^{14}C was present in the cells. One fifth (9 mμc) of the intracellular ^{14}C at the break-point was present in the pool fraction, i.e., material released on acidification of the suspension. The ^{14}C distribution data lead to a more accurate

* This research was supported by Army Research Office Grant HC A-67-G-0014

TABLE I. Influence of Cell Concentration on Oxidative Assimilatory Activity[a] of *Serratia marcescens*.

Dry wt. of cells (mg/ml)	6.0	3.0	1.5
260 μl of O_2 consumed (min)	16	31	62
$^{14}CO_2$ produced (mμC of ^{14}C)	92	92	89
Total cellular ^{14}C	60	47	43
Cellular ^{14}C/mg of cells	10	16	28
Pool ^{14}C/mg of cells	3.6	5.3	8.6

[a] 8 μmoles of glycerol per 2 ml of suspension.

estimate of the extent of assimilation of a substrate than do manometric results since the former are not complicated by endogenous behaviors. In kinetic studies it was observed that the cell ^{14}C content increased to a slight extent for at least 60 min after the break-point and that $^{14}CO_2$ was produced but at a very low rate.

Similar behaviors were noted with glucose-U-^{14}C or glutamate-U-^{14}C as the substrate. Values for the O_2 consumed to the break-point indicated about 45% assimilation of glucose while the total intracellular ^{14}C was only 30% of the initial glucose ^{14}C. The values for glutamate utilization suggested 55% assimilation on the basis of O_2 consumption, 36% for ^{14}C content of the cells.

One might expect that glycerol would be oxidized via pyruvate and the Krebs cycle. It was observed that 61% of the 1-^{14}C, 57% of the 2-^{14}C, and 57% of uniformly labeled glycerol-C atoms were converted to $^{14}CO_2$. About the same extent of assimilation, 20–22%, of the different C atoms was noted. Much greater differences were noted with variously labeled pyruvates. Fifty-five percent of the 1-^{14}C was converted to $^{14}CO_2$ as compared with 36 and 38% from pyruvate-2-^{14}C and pyruvate-3-^{14}C, respectively. About 5% of the 1-^{14}C of pyruvate was assimilated, 16% of the 2- and of the 3-C. The differences between the results obtained with glycerol and pyruvate suggest possible participation of a second pathway of utilization but this possibility was not studied.

Since the concentration of bacteria may influence the extent of oxidation and of assimilation some experiments were conducted to test this possibility. In a typical experiment (Table I) oxidation was halted shortly before the break-point at times when approximately 260 μl of O_2 had been consumed. It is apparent from the results that the time required for an equivalent amount of oxidation, either on the basis of O_2 consumption or $^{14}CO_2$ production, was proportional to the concentration of cells. On the other hand the total amounts of ^{14}C assimilated by the cells in the test periods, or the pool values, were not proportional to the concentration of cells. This lack of proportionality for assimilation by cells of the same age complicates the interpretation of results obtained with cell suspensions of different ages. No satisfactory explanation of the proportionality of respiratory activity, nonproportionaity of C-assimilation can be advanced at this time.

The results of aging experiments with washed cells harvested from nutrient agar or from glucose agar are presented in Table II. The suspensions were made up to the same initial turbidity, shaken at 30°C and samples for the various tests were taken at daily intervals. The amounts of O_2 consumed endogenously in 60 min by 2 ml samples of the suspensions were determined. Eight μmoles of glycerol-U-^{14}C (204 mμC) were then added for the exogenous tests and the times required to consume approximately 300 μl of O_2 were determined. It is apparent that the time required to consume this amount of O_2 increased slowly with age of the cells, approximately doubling by 96 hours and again before 168 hours. The endogenous rate decreased during the first 48 hours and then remained quite constant. The viable count remained fairly high, decreasing in 168 hours to about one third of the initial value. There did not appear to be any direct relationship between the viable count and either the endogenous or exogenous rate of oxidation.

The amounts of $^{14}CO_2$ produced during the exogenous tests were fairly constant but variation was noted as regards the total amount of ^{14}C assimilated by cells of different ages. The same type of behavior was noted on repeating the experiment, the amount of ^{14}C assimilated by glucose agar-grown cells changing from 36 mμC at 0 hour to 76, 68, and 73 mμC at 24, 48, and 72 hours, respectively. Corresponding values for cells from nutrient agar were 39, 50, 48, and 40 mμC. The lesser

TABLE II. Influence on Cellular Activities of Aging of Suspensions of *Serratia marcescens*.

Cell type	Age (hours) 0	24	48	72	96	168
	Endogenous O_2 consumption in 60 min					
G-cells[a]	96	54	39	42	43	42
P-cells	98	45	32	37	39	36
	Time (min) required to consume 300 μl O_2 exogenously[b]					
G-cells	43	53	61	70	78	155
P-cells	33	44	51	63	76	195
	$^{14}CO_2$ produced, mμC/300 μl of O_2 consumed					
G-cells	90	93	96	92	87	100
P-cells	106	103	101	95	99	97
	^{14}C assimilated (mμC)					
G-cells, total	38	71	64	59	54	63
P-cells, total	36	46	34	30	34	46
G-cells, pool	13	15	10	13	8	5
P-cells, pool	8	6	8	—	6	8
	NH_3-N (μg/ml)					
G-cells	26	75	110	155	155	240
P-cells	22	60	90	115	120	220
	Viable cells/ml $\times 10^8$					
G-cells	61	52	48	—	48	18
P-cells	60	56	48	—	45	23

[a] G-cells = glucose agar-, and P-cells = plain agar-grown cells.

[b] 8 μmoles of glycerol/2 ml of suspension.

extent of assimilation by freshly harvested cells may be associated with the higher level of endogenous substrates in these cells. Since concentration of cells also influences the results, as indicated by those reported in Table I, it is difficult to determine the true cause(s) of the variations noted in the results. The variations do not appear to be to any marked extent the result of experimental errors since ^{14}C recoveries generally were very satisfactory (better than 95%).

The results of anthrone determinations indicate that there was a small amount of a readily utilized carbohydrate initially present in the cells and that this was utilized within the first 24 hours. After this time the carbohydrate content of the cells remained fairly constant but did decrease very slowly with time. Nitrogenous compounds appear to be a major endogenous substrate as indicated by the marked increase in NH_3-N with time (Table II). The nature of the endogenous substrate is being studied in more detail.

Behaviors similar to those reported in Table II were observed in other experiments in which glucose or glutamate served as the exogenous substrate.

Summary. The oxidative assimilatory behavior of *S. marcescens* with various substrates is similar to that noted with other bacteria (4), a portion of the substrate being oxidized to CO_2, a portion assimilated by the cells and the remainder converted to extracellular material not utilized readily by the cells. Oxidative activity, but not C-assimilation, was proportional to the concentration of cells over the range studied. No satisfactory explanation for this difference could be advanced. After the first 48 hours of aging of the suspensions the endogenous rate of respiration remained fairly constant for 5 days. However, the rate of exogenous respiration decreased with time but not in proportion to the viable count. Nitrogenous compounds appear to be a major endogenous substrate of *S. marcescens*.

1. Clifton, C. E., J. Bacteriol. **92**, 905 (1966).

2. Clifton, C. E. and Cherry, J., J. Bacteriol. **91**, 546 (1966).

3. Burton, R. M., "Methods in Enzymology," Vol. 3, p. 246. Academic Press, New York, 1957.

4. Clifton, C. E., Ann. N. Y. Read. Science **102**, 655 (1963).

Received Oct. 30, 1967. P.S.E.B.M., 1968, Vol. 127.