Endogenous Activity of Bacteria Under Aerobic and Anaerobic Conditions.* (32154)

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The majority of studies on endogenous respiration of bacteria have been conducted under aerobic conditions. In studies in this laboratory on the influence of age of bacteria on their endogenous respiration, comparisons were made of the distribution, with time, of C^{14} between certain C^{14}-labeled, Gram-negative bacteria and the suspending medium under both aerobic and anaerobic conditions. The cells were grown on a synthetic medium with glucose as the bulk source of carbon and energy, small amounts(1) of uracil or of leucine being added to the medium when it was desired to label the RNA with 2-C^{14}-uracil or the protein with U-C^{14}-leucine. To minimize any specific ionic effects the cells were washed and suspended in a buffered salt solution of the same ionic composition as the growth medium but with $(NH_4)_2SO_4$ omitted. $C^{14}O_2$ production served as a measure of aerobic endogenous respiration or of endogenous fermentation (if CO_2 were formed) and also gave some indication regarding the nature of the endogenous substrate(s) when specifically labeled cells were used. C^{14} determinations give no true measure of endogenous metabolism since some of the loss of cellular-C^{14} is by diffusion from living or dead cells. The total decrease in cellular-C^{14} is not a true measure of endogenous metabolism as has been assumed in some studies; it simply is a measure of the amount of carbon lost regardless of cause and is not a measure of endogenous activity alone. The possibility existed that this loss of cellular carbon might be correlated with viability of the cells.

Materials and methods. The general methods were the same as previously described (1,2). *Escherichia coli* K-12, *Serratia marcescens* Z-4 or *Pseudomonas aeruginosa* U-21a were grown for 16 hours on the glucose-ammonium sulfate-buffered salt agar and labeled with C^{14} during growth as described(1) for *E. coli*. Twenty-five ml of the washed cell suspensions containing 120-160 $\times 10^8$ cells per ml of the buffered salt solution were placed in 300 ml Erlenmeyer flasks fitted with an inner tube to contain the CO_2 absorbent (5 N NaOH) as described by Clifton(2). A stopcock was provided for the flasks used in the anaerobic experiments, thus enabling the flasks to be evacuated and filled with N_2 to provide an inert atmosphere. Samples for C^{14}, turbidity and plate count determinations were removed at 24-hr intervals. All tests were conducted at 30°C. Little or no change in pH was noted during the course of the experiments.

TABLE I. Percentage Distribution of C^{14} with Age of Suspensions of Variously Labeled Cells of *Escherichia coli* Under Aerobic and Anaerobic Conditions.

	G*	G_{AN}†	U	U_{AN}	L	L_{AN}
Cells, 24 hr	84	88	81	83	95	92
CO_2	9	3	5	4	0	0
Supernatant	7	9	14	13	5	8
Cells, 48 hr	80	83	69	65	93	88
CO_2	11	8	7	4	0	0
Supernatant	9	9	24	31	7	12
Cells, 72 hr	78	81	56	54	84	86
CO_2	12	10	7	4	0	0
Supernatant	10	9	37	42	16	14

* Original value for G (glucose-labeled) cells was 656 mμc, 1420 mμc for U (uracil-labeled) and 245 mμc for L (leucine-labeled) cells.
† AN indicates aging under anaerobic conditions.

Results. Values for the percentage distribution of C^{14} with time for suspensions of *E. coli*, *S. marcescens* and *P. aeruginosa* are presented in Tables I, II and III, respectively. Similar changes in distribution of C^{14} were noted in experiments of 7 to 10 days' duration although the rate of decrease of cell-C^{14} tended to decrease with time of aging. This did not appear to be directly associated with the numbers of viable cells.

The turbidity readings (Klett-Summerson colorimeter, No. 54 filter) of 1-10 dilutions of the suspensions decreased to about the same extent anaerobically as aerobically

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TABLE II. Percentage Distribution of C^{14} with Age of Suspensions of Variously Labeled Cells of *Serratia marcescens* Under Aerobic and Anaerobic Conditions.

	G*	G_{AN}†	U	U_{AN}	L	L_{AN}
Cells, 24 hr	86	87	75	71	95	92
CO_2	4	1	0	0	1	0
Supernatant	10	12	25	29	4	8
Cells, 48 hr	83	84	64	53	94	85
CO_2	6	3	0	0	2	0
Supernatant	11	13	36	47	4	15
Cells, 72 hr	79	77	55	37	92	82
CO_2	9	3	0	0	2	0
Supernatant	12	20	45	63	6	18

* Original value for G (glucose-labeled) cells was 723 mμc, 814 mμc for U (uracil-labeled) and 505 mμc for L (leucine-labeled) cells.

† AN indicates aging under anaerobic conditions.

TABLE III. Percentage Distribution of C^{14} with Age of Suspensions of Variously Labeled Cells of *Pseudomonas aeruginosa* Under Aerobic and Anaerobic Conditions.

	G*	G_{AN}†	U	U_{AN}	L	L_{AN}
Cells, 24 hr	85	94	83	92	88	93
CO_2	8	1	11	4	7	0
Supernatant	7	5	6	4	5	7
Cells, 48 hr	77	92	68	84	78	87
CO_2	14	1	16	7	13	1
Supernatant	9	7	16	9	9	12
Cells, 72 hr	72	89	64	73	53	82
CO_2	18	3	31‡	11	27‡	1
Supernatant	10	8	5	26	20	17

* Original value for G (glucose-labeled) cells was 920 mμc, 1760 mμc for U (uracil-labeled) and 430 mμc for L (leucine-labeled) cells.

† AN indicates aging under anaerobic conditions.

‡ Lower values for $C^{14}O_2$ at 72 hr noted in other experiments.

for *E. coli* and *S. marcescens* but not for *P. aeruginosa*. Typical decreases in 72 hours were from 115 to 90 for *E. coli*, 140 to 105 for *S. marcescens* and 135 to 63 for *P. aeruginosa* aerobically, to 87 anaerobically.

Typical decreases in plate counts are recorded in Table IV. By the end of 7 days fewer than 1% of the cells of *S. marcescens* or *P. aeruginosa* incubated anaerobically were viable as compared with about 30% aerobically. Approximately 30% of the *E. coli* cells survived for 7 days under either condition. Dawes and Ribbons(3), however, reported a somewhat higher death rate for their strain of *E. coli* anaerobically than aerobically.

Discussion. If one employs $C^{14}O_2$ production from uniformly labeled *E. coli* as an indicator of the amount of energy available from endogenous metabolism, it is apparent (Table I) that much more energy would be available aerobically that anaerobically. However, the decreases in cell-C^{14}, turbidity and viable count were about the same anaerobically as aerobically for uniformly labeled *E. coli*. Also the decreases in cell-C^{14} were about the same for comparative pairs of RNA- or protein-labeled cells although a much higher percentage of the uracil- than of the leucine-label was lost from the cells. These results raise questions regarding the purpose and value of endogenous respiration for *E. coli*.

The loss of C^{14} from uniformly labeled *S. marcescens* (Table II) was about the same under anaerobic as under aerobic conditions. It differed from *E. coli* in that greater decreases in C^{14} content were noted under anaerobic than under aerobic conditions with uracil- or leucine-labeled cells. Neither the uracil nor the leucine (to any appreciable extent) was oxidized by *S. marcescens*. The more marked anaerobic loss of RNA and protein from the cells is in agreement with the more rapid rate of death of this organism under anaerobic conditions. These observations appear to lend support to the concept that endogenous respiration does provide energy of value for survival of the cells.

However, a behavior the reverse of that noted above for *S. marcescens* was observed with variously labeled cells of *P. aeruginosa*. More C^{14} was lost (Table III) from the cells under aerobic than under anaerobic conditions. Cellular-C^{14} values, however, approached each other on longer incubation

TABLE IV. Percentage of Survivors and of Cell-C^{14} After Incubation of Washed Cells at 30°C Under Aerobic and Anaerobic Conditions.

	E. coli		*S. marcescens*		*P. aeruginosa*	
Hr	% Viable cells					
		AN*		AN*		AN*
24	68	64	79	76	100	76
48	57	50	73	33	65	59
72	50	46	72	27	47	18

* AN indicates aging under anaerobic conditions.

(7-10 days). The death rate, as for *S. marcescens,* was higher anaerobically that aerobically. There was, however, a greater decrease in turbidity of the *P. aeruginosa* suspensions aerobically than anaerobically which is consistent with the more marked aerobic loss of C^{14}. These observations, if loss of cellular matter were the major controlling factor, would suggest a higher aerobic death rate for *P. aeruginosa,* but this was not observed. The results with specifically labeled *P. aeruginosa* are in agreement with those of short-term, aerobic experiments by Gronlund and Campbell(4) who reported that some of the RNA and protein labels were lost as $C^{14}O_2$, some as substances which accumulated in the suspending fluid.

Comparison of the results obtained with the different species under the same test conditions makes it apparent that no simple relationship exists between the endogenous metabolism of bacteria and their survival. Dawes and Ribbons(5) concluded that the pattern of endogenous metabolism is determined by the nutritional conditions of the cell and is influenced by test conditions. A similar conclusion with regard to survival was reached by Postgate and Hunter(6). Unfortunately many of the studies have been limited to one organism, generally suspended in a medium of markedly different composition than the growth medium.

Strange, Wade and Dark(7) could find no relationship between the ATP content and viability of *Aerobacter aerogenes* but their results did suggest a possible relationship between the ability of the cell to form ATP and its viability. This was not investigated in the present study but might well serve as a comparative measure of the ability of the cell to provide energy under both aerobic and anaerobic conditions. However, it is possible that no direct relationship exists between the oxidative ability of the cell under starvation conditions and its ability to use ATP. Little is known regarding the control mechanisms. Starving suspensions of *E. coli*(5), for example, oxidize their glycogen reserves at a very rapid rate; as much as 20% of their dry weight was oxidized in 3 hours, although little or no loss of viability occurred in 12 hours.

Summary. The influence of age of cell suspensions on viability and on the distribution of C^{14} between growth-labeled cells of *Escherichia coli, Serratia marcescens* or *Pseudomonas aeruginosa,* CO_2, and the suspension medium was tested under aerobic and anaerobic starvation conditions at 30°C. No direct relationship between the potential amounts of energy available on the basis of $C^{14}O_2$ production or loss of cellular-C^{14} and survival of the cells could be detected. Each species exhibited somewhat different behaviors as regards endogenous metabolism and decreases in cell-C^{14}, in turbidity or in viable counts of the suspensions. Questions are raised regarding the value of endogenous respiration for survival of the cell.

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