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ABSTRACT

CLIFTON, C. E. (Stanford Univeristy, Stanford, Calif.). Aging of *Escherichia coli*. J. Bacteriol. **92**:905–912. 1966.—The rates of endogenous and exogenous (glucose) respiration decreased much more rapidly than did the viable count during the first 24 hr of aging of washed, C^{14}-labeled cells of *Escherichia coli* K-12 suspended in a basal salt medium devoid of ammonium salts. The rates of decrease of respiration and of death approached each other as the age of the cells increased, but death was not the only factor involved in decreased respiratory activity of the suspensions. The greatest decrease in cellular contents with aging was noted in the ribonucleic acid fraction, of which the ribose appeared to be oxidized, while uracil accumulated in the suspension medium. The viable count and respiratory activities remained higher in glucose-fed than in nonfed suspensions. Proline-labeled cells fed glucose tended to lose more of their proline and to convert more proline into $C^{14}O_2$ than in unfed controls. On the other hand, uracil-labeled cells fed glucose retained more of the uracil than did nonfed cells, but glucose elicited some oxidation of uracil. An exogenous energy source such as glucose aided in the maintenance of a population, but it was not the only factor needed for such maintenance.

A number of investigations on phenomena associated with aging have been reported in recent years (12, 14). Sorokin (14) defined aging as "a process leading to decrease in survival capacity of the individual organism." Since there is no differentiation or natural death in unicellular organisms, the existence of aging in microbial cells has been questioned by some workers. Sorokin, however, presented evidence that aging does occur in microbial cells, and that one of the most salient characteristics of aging at the cellular level is a change in metabolic pattern. Also, it has long been recognized that the age of a culture and the nature of the environment exert marked effects on bacteria. Changes in such aspects as size, shape, metabolic characteristics, staining properties, and nucleoid content have been reported for different species.

Aging, as the term is employed herein, is considered simply as increasing length of time since the cells were harvested from the culture medium and suspended in an environment devoid of at least one extracellular substance essential for growth, e.g., ammonia or an organic substrate. As the chronological age of the cells increases, they undergo change in cellular content and cellular activity, since they are unable to maintain their normal metabolic and regulatory patterns owing to disruption of their enzymatic machinery. In a sense, we are dealing with survival, but survival is of secondary interest. This study deals primarily with changes in C^{14} content and its distribution with time and with endogenous metabolism under conditions of nitrogen or nitrogen and carbon starvation.

MATERIALS AND METHODS

The general methods, particularly for the determination of C^{14} and its distribution in the cells, were the same as previously described (1, 3). The bacteria were grown on a buffered salt solution (3) jelled with agar, and were harvested and tested in the salt solution. Glucose, unless otherwise noted, was employed as the bulk organic substrate in 1% concentration, and $(NH_4)_2SO_4$ (0.1%) was the nitrogen source in the growth medium. An 8-mg amount of proline or leucine or 4 mg of uracil was added per 100 ml of medium when the cells were to be labeled in the protein or ribonucleic acid (RNA) fractions. A 10-μc amount of glucose-*U*-C^{14} for general labeling or 5 μc of proline, leucine-*U*-C^{14}, or uracil-2-C^{14} for specific labeling was added per 100 ml.

Various vessels were employed as containers for the suspensions, but none was entirely satisfactory. Spargers tended to give somewhat uneven flows of air over their surfaces, some of the fine bubbles they produced became airborne, quiescent spaces were established in the vessels, resulting in uneven aeration or mixing, etc. The vessel finally selected for most of the aging experiments in which the cells were not fed was a 300-ml Erlenmeyer flask. This was closed with a rubber stopper through which a 13 × 100 mm test tube was

passed. A hole several millimeters in diameter was blown in this tube just below the bottom of the stopper, and this provided passageway for gaseous exchange. A 2-ml amount of 5 N NaOH was placed in the test tube to act as a CO_2 absorbent, and the small tube was stoppered. A 25-ml amount of the test suspension was placed in each flask, the large stopper was inserted, and the flasks were shaken on a Burril wrist-action shaker. The alkali could be removed daily and replaced without opening the flasks; thus, the chances for contamination of the suspension were reduced. Samples for viable counts and cell C^{14} determinations were obtained from duplicate flasks. All tests were carried out at 30 C in an incubator room.

Various flasks were employed as containers for the suspensions fed during aging. Finally, 250-ml round-bottom flasks (model 9283; Ace Glass Co., Vineland, N.J.) with one side arm and a 14/20 upright joint were selected as most suitable. A vacuum adapter (model 1931; Ace Glass Co.) was passed through the ground-glass joint to serve as an air inlet and outlet. It could be readily removed to obtain a sample of the suspension and could be replaced with ease. Sterile glucose-basal salt solution or the salt solution alone was contained in burettes connected with rubber tubing to a capillary tube passing through the side arm of the flask. The flow of the fluid was controlled by means of a peristaltic pump (model 600-000; Harvard Apparatus Co., Dover, Mass.). The speed of the pump was adjusted to give a flow of about 7 ml/24 hr. The cotton-plugged burette, tubing, and experimental flask were sterilized as a unit in an autoclave. All tests were carried out with aseptic precautions. The suspensions were stirred with 1-inch (2.54-cm), Teflon-coated magnets driven by magnetic stirrers; the flasks were held some distance above the stirrers to reduce heating effects. Cool-Stir stirrers (Technilab Instruments, Los Angeles, Calif.) were used in most of the experiments. Air was blown over the surface of the liquid rather than through it to eliminate foaming effects.

The air was passed through a 500-ml Pyrex gas washing bottle containing 5% NaOH to remove CO_2, then through one with 5% H_2SO_4 to remove ammonia, and finally through distilled water to saturate the air with water vapor. After passing through a filter of sterilized cotton, the air flowed through the experimental flasks and then through 20 ml of 1.0 N NaOH in 130-ml sulfur-absorption tubes (model 39620; Corning Glass Works, Corning, N.Y.). Three-way stopcocks were attached to the bottom of these tubes to permit passage of air through the alkali or drainage of the latter from the tube. The alkali and wash water were collected in 25-ml cylinders, and the volume was adjusted to 25 ml. A test tube only slightly smaller in diameter than the absorption tube was suspended in the latter about 5 cm above the hydroxide solution. Droplets of the alkali condensed on this tube and fell back into the bulk of the absorbent. This procedure reduced spattering of the alkali; any material drying on the tube was readily removed with the wash water. For C^{14} determinations in the Dynacon system 10-ml samples were taken, and, for van Slyke CO_2-*C* determinations, 2-ml samples were used.

RESULTS

The first aging experiments were conducted with cells grown on 1% Casamino Acids(Difco) agar containing 10 μc of algal hydrolysate per 100 ml. A heavy suspension of washed cells, 100 ml, was aerated in a 250-ml wash bottle (Corning 31760, coarse disc; Corning Glass Works), and the CO_2 produced was collected in 1.0 N NaOH. Results of various determinations, made at daily intervals, corrected when necessary for samples removed, are presented in Table 1. The exogenous and endogenous O_2 consumption values were determined in 30-min test periods with samples transferred to Warburg respirometers; 0.1 ml of 0.04 M glucose was added per ml of the suspension for the exogenous tests. Q_{O_2} values are recorded in Table 2.

Results similar to those reported in Table 1 were obtained with glycerol- or glucose-grown cells and with glucose, glycerol, or glutamic acid as the exogenous substrate in Warburg tests. Marked endogenous ammonia production was noted in the suspensions; e.g., in one experiment with glucose-grown cells, 92 μg of NH_3-N was present per ml at the end of 96 hr of incubation; the cellular nitrogen had decreased by 125 μg/ml during that period. Marked decreases in cellular carbohydrate (anthrone) were noted, particularly in the first 1 or 2 hr of aging, at which time ammonia production was not pronounced. Marked

TABLE 1. *Decreases in respiratory activity, viable count, turbidity, and cell N, -C, and -C^{14} noted during the aging of labeled Escherichia coli*[a]

Readings	Original value (0 hr)	Age of suspension			
		24 hr	48 hr	72 hr	96 hr
		%	%	%	%
Endogenous O_2	16 μliters[c]	25[b]	25	23	20
Exogenous O_2, glucose	190 μliters[c]	55	43	26	25
Viable bacteria per ml	35 × 109	93	60	26	17
Klett reading, 1–20	113	87	81	54	46
Cell N per ml	840 μg	89	80	65	52
Cell C per ml	2,970 μg	88	82	70	55
Cell C^{14} per ml	263 mμc	86	76	63	49
$C^{14}O_2$ production, mμc	—	31	20	10	7

[a] Cells were labeled by growth on 1% Casamino Acids agar plus 10 μc of C^{14}-algal protein hydrolysate per 100 ml.

[b] Per cent of original value.

[c] Observed consumption in 30 min by 2 ml of suspension.

TABLE 2. *Comparative Q_{O_2} values for glucose oxidation (calculated from data in Table 1)*

Age of suspension	Q_{O_2} values			
	Per 10^9 viable cells	Per Klett unit	Per μg of cell N	Per $m\mu c$ of cell C^{14}
hr				
0	10.86	3.36	0.22	0.72
24	6.53	2.13	0.14	0.46
48	7.76	1.77	0.12	0.41
72	11.00	1.62	0.09	0.30
96	15.83	1.82	0.11	0.37

increases in absorbancy of the suspension medium at 260 $m\mu$ were noted as the cells aged; this suggested a loss of nucleic acids. With cells uniformly labeled by growth on labeled glucose, the most marked change in amounts of the label was noted in the cellular fractions soluble in hot 5% trichloroacetic acid; the next greatest change was in the insoluble fraction.

To obtain more information regarding the nature of the endogenous substrate(s) and of the substances lost from the cells as they aged, a mutant (K-12A) requiring proline, leucine, and uracil for growth was obtained through the courtesy of Avram Goldstein. This mutant was labeled during growth on the glucose medium in the presence either of uniformly labeled glucose, proline, or leucine, or of uracil-2-C^{14}. Results of subsequent tests showed that strain K-12 could be labeled in the same manner; since distribution of the label was similar to that noted for K-12A, K-12 was employed for these studies. Glucose-labeled cells contained C^{14} in all fractions, uracil-labeled cells contained the C^{14} primarily in the hot trichloroacetic acid-soluble fraction, proline or leucine-labeled cells contained 80% or more of the label in the hot trichloroacetic acid-insoluble fraction, and small amounts of C^{14} from proline- or leucine-labeled cells were present in the ethyl alcohol-soluble fraction and in the pool. The loss of C^{14} from the aging cells and the subsequent distribution of $C^{14}O_2$ in the supernatant fluids are recorded in Fig. 1 from a typical experiment. The total amounts of CO_2-C produced were approximately the same for the three suspensions.

Since turnover of protein or RNA does occur (9), an experiment on aging was conducted with growth amounts of uracil, proline, or leucine present as trapping agents in the suspension medium. No marked difference was noted (Table 3) when uracil-, proline-, or leucine-labeled cells were tested in the presence or absence of the corresponding unlabeled compounds. However, a slight tendency for some trapping of uracil or leucine to occur, but not for proline which is oxidized very slowly by K-12, was evident in the results.

The concept of energy of maintenance requirements has been supported by the results reported by Marr (11) and Mallette (7, 8) and their coworkers from studies on the growth of *E. coli* (11) and on the influence of feeding on growth and viability (7, 8). In the present study, the effects of feeding glucose to *E. coli* on both endogenous and exogenous respiration and on turbidity and viability were determined.

In preliminary experiments, the results from feeding 0.5 μmole or less of glucose per mg (dry weight) of cells per 24 hr were practically the same as those obtained with suspensions diluted with salt solution only. Glucose and fermentation products (evidenced by *p*H decreases) tended to accumulate when more than 10 μmoles was fed per day. The feeding rate employed (ca. 7 ml per 24 hr) provided approximately 1, 2, or 4 μmoles of glucose per mg (dry weight) of cells from 0.01, 0.02, and 0.04 M glucose, respectively. All values reported were calculated from actual flow rates observed. Results of experiments designed to determine the effect of feeding glucose are reported in Tables 4 and 5. The use of labeled cells (Table 4) provided a system for measuring endog-

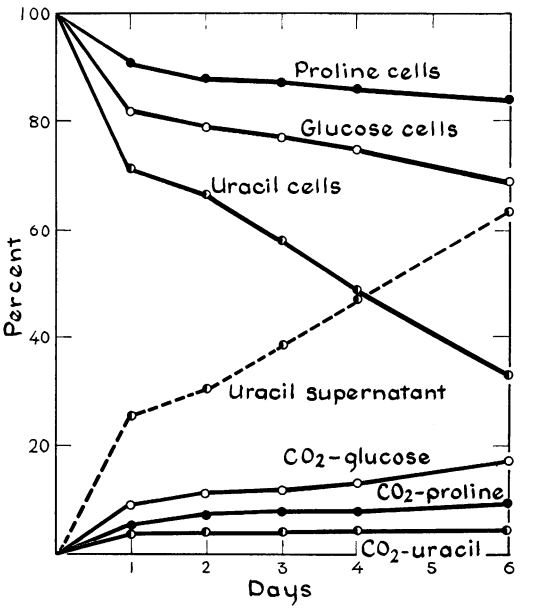

FIG. 1. *Percentage distribution with time of C^{14} from cells of Escherichia coli labeled during growth on glucose in the presence of glucose-U-C^{14}, proline-U-C^{14}, or uracil-2-C^{14}. Initial C^{14} values were 1,110 $m\mu c$ for glucose-, 1,720 $m\mu c$ for proline-, and 2,630 $m\mu c$ for uracil-labeled cells.*

TABLE 3. *Influence of exogenous uracil, proline, or leucine on the loss of C^{14} from glucose-grown labeled Escherichia coli*[a]

Time	Fraction	UU[b]	U	PP	P	LL	L
hr							
24	Cells[c]	640	668	941	962	2046	2100
	CO_2	3	26	28	26	0	0
	Supernatant fluid	240	201	57	63	108	56
48	Cells[c]	396	436	852	839	1875	1970
	CO_2	3	29	43	48	0	0
	Supernatant fluid	504	429	102	117	309	183

[a] Cells were labeled during growth in the presence of uracil-2-C^{14}, proline-*u*-C^{14}, or leucine-*u*-C^{14}. Results are expressed as millimicrocuries.

[b] Double letters, e.g., UU means uracil-grown cells tested in the presence of uracil, etc. Single letters indicate no exogenous uracil, proline, or leucine present during aging.

[c] Original values for cell-C^{14} were 880 mμc for uracil-labeled cells, 1,035 for proline-labeled, and 2,150 for leucine-labeled cells.

TABLE 4. *Influence on CO_2 production of feeding glucose to uniformly labeled (1,820 mμc) Escherichia coli.*

Carbon dioxide produced	Time	Molarity of glucose			
		0	0.01	0.02	0.04
	hr				
$C^{14}O_2$[a]	0 to 24	254	257	259	259
	24 to 48	39	35	37	37
	48 to 72	28	48	29	24
	72 to 96	16	30	75	49
CO_2-C[b]	0 to 24	9.3	3.6[c]	7.1[c]	13.8[c]
	24 to 48	1.9	4.5	9.2	12.4
	48 to 72	1.4	5.2	9.5	11.8
	72 to 96	0.9	4.1	13.0	15.3

[a] $C^{14}O_2$ is expressed in millimicrocuries.

[b] CO_2-C is expressed in milligrams.

[c] Total minus endogenous.

enous respiration in the presence of unlabeled substrate, whereas $C^{14}O_2$ production from labeled substrate (Table 5) gave a true indication of the extent of exogenous respiration when nonlabeled cells were employed.

In the feeding experiments, the Klett-Summerson colorimeter readings (no. 54 filter), corrected for sampling and dilution factors, decreased, but remained higher for suspensions to which glucose had been added. In those suspensions fed glucose, a yellowish-green color developed, and the color increased with age of the suspension and amount of glucose added. This could introduce an error into the turbidity and absorbance readings. The absorbance at 260 mμ of supernatant fluids markedly increased with age of the suspension. Peaks, increasing with age of the suspension, were noted at 260 mμ with supernatant fluids from suspensions fed basal salts or 0.01 M glucose, but the absorbance continued to increase as the wavelength decreased below 260 mμ with samples from systems fed 0.02 or 0.04 M glucose. This indicates the formation or release of other materials absorbing in this range. Ammonia production was pronounced in the control suspension, but little (any ammonia formed probably being assimilated) was present in the suspensions fed glucose.

Results obtained with cells labeled during growth in the presence of proline-*U*-C^{14} or of uracil-2-C^{14} and fed glucose during the aging process are reported in Tables 6 and 7, respectively. The death rates in these experiments were similar to those reported in Table 5. A marked loss of C^{14} from uracil-labeled cells can be noted in Fig. 1 and Table 7. Samples of supernatant fluids from the test reported in Table 7 were subjected to hydrolysis with perchloric acid, and chromatograms were run with either isoproponal–HCl–water (65:17:18) or ethyl acetate–formic acid–water (60:5:35) as the developing solvent. The

TABLE 5. *Percentage distribution of survivors and C^{14} from uniformly labeled glucose fed to aging suspensions of Escherichia coli*

Time	Fraction	Molarity of glucose			
		0	0.01	0.02	0.04
hr		%	%	%	%
48[a]	CO_2	—	69	70	63
	Cells	—	11	12	9
	Supernatant fluid	—	20	18	28
	Survivors	86	110	103	103
96[b]	CO_2	—	72	76	70
	Cells	—	9	10	13
	Supernatant fluid	—	19	14	17
	Survivors	11	85	79	82
168[c]	CO_2	—	73	76	73
	Cells	—	9	9	11
	Supernatant fluid	—	18	15	16
	Survivors	2	34	32	28

[a] Amounts of glucose fed for molarities of 0.01, 0.02, and 0.04 were 370, 750, and 1,270 mμc, respectively.

[b] Amounts of glucose fed from 0 to 96 hr for molarities of 0.01, 0.02, and 0.04 were 720, 1,565, and 2,730 mμc, respectively.

[c] Amounts of glucose fed from 0 to 168 hr for molarities of 0.01, 0.02, and 0.04 were 1,305, 3,135, and 4,725 mμc, respectively.

TABLE 6. *Percentage distribution of C^{14} from proline-labeled cells (1,350 mμc) of Escherichia coli fed glucose (ca. 7 ml per 24 hr)*

Time	Fraction	Molarity of glucose			
		0	0.01	0.02	0.04
hr		%	%	%	%
24	CO_2	5	9	8	6
	Cells	89	86	87	83
	Supernatant fluid	6	5	5	11
48	CO_2	7	12	12	9
	Cells	85	81	81	77
	Supernatant fluid	8	7	7	14
96	CO_2	8	14	15	11
	Cells	82	78	76	71
	Supernatant fluid	10	8	9	18

major amount of C^{14} was detected (4 π Actigraph, Nuclear-Chicago Corp., Des Plaines, Ill.) in the area corresponding to the R_F of uracil, and the intensity increased with age of the suspension. On the basis of R_F values, much of the label appeared to be present as uracil in an unhydrolyzed sample, but this was not studied quantitatively.

TABLE 7. *Percentage distribution of C^{14} from uracil-2-C^{14}-labeled cells (1,000 mμc) of Escherichia coli fed glucose (ca. 7 ml per 24 hr)*

Time	Fraction	Molarity of glucose			
		0	0.1	0.02	0.04
hr		%	%	%	%
24	CO_2	1	8	14	13
	Cells	75	77	77	75
	Supernatant fluid	24	15	9	12
48	CO_2	1	10	18	20
	Cells	50	66	63	66
	Supernatant fluid	49	24	19	14
96	CO_2	1	11	25	25
	Cells	29	56	47	54
	Supernatant fluid	70	33	28	21

DISCUSSION

Many of the enzymatic activities of bacteria decrease with increasing age of the cells. The decreases result from death of the cells, inactivation of enzymes, loss of coenzymes, and other changes. The data in Table 1 show that, in the first 24 hr, the rate of endogenous O_2 consumption by *E. coli* K-12 decreased by 75%, that the exogenous rate with glucose as the substrate decreased by 45%, and that only a 7% decrease occurred in the viable population. (Exogenous refers to the rate of change, or total change, observed after the addition of a substrate, and is a composite value for substrate utilization plus a frequently unknown extent of endogenous respiration.) The marked decrease in endogenous respiration appears to be, to a considerable extent, the result of the utilization of a small amount of a readily oxidized endogenous reserve and of the subsequent slow oxidation of other cellular components, but not necessarily the results solely of enzyme inactivation. Death was not a major factor responsible for the decreases in respiratory activity observed in the first 24 hr.

When the Q_{O_2} values (Table 2) at different times calculated on the basis of cell counts, Klett units, cell N, or cell C^{14}, are compared, the pattern appears to be about the same: fairly large values for freshly harvested cells followed by a decrease of 36 to 40% in the first 24 hr of aging. Thus, the values remained fairly constant with the most striking changes being for Q_{O_2} values calculated on a viable-cell basis. These values, however, give little or no clue as to the fundamental changes occurring in the cells.

Endogenous respiration, as measured either by O_2 consumption over short periods daily or by

$C^{14}O_2$ production from labeled cells over 24-hr periods, decreased slowly with time after 24 hr. The O_2 consumption values are suggestive only because, with such low values, an observed difference of 1 μliter would result in a marked difference on a percentage basis. The percentage decreases in the rate of endogenous $C^{14}O_2$ production, of exogenous O_2 consumption, or in the viable counts were greater than the decreases noted for cell N, C, or C^{14}, and for turbidities with time of aging. The decreases in respiratory activity after 24 to 48 hr appear to parallel the decreases in viable count. The values for C^{14} indicate that about one-half of the initial C^{14} was lost from cells aged 96 hr and that about one-half of this loss was as $C^{14}O_2$, with the remainder as organic matter in the suspension medium.

Changes similar to those in Table 1 were noted for the cells grown with glucose or glycerol as the carbon-energy source, and with glucose, glycerol, or glutamic acid as the substrate in Warburg tests. Cells grown with glucose as the carbon source had a high initial carbohydrate content, but the storage glycogen was utilized as an endogenous substrate in the first 1 or 2 hr of aging. After the depletion of this glycogen reserve, ammonia production became quite evident. The increase of total N content of the supernatant fluids was greater than that of the NH_3-N, and a marked increase in absorbancy at 260 mμ was apparent with time of aging. These results generally agree with those reported by Dawes and Ribbons (4).

To determine the general nature of the endogenous substrates of *E. coli* once the coli-glycogen had been depleted, the distribution with time of C^{14} from uniformly labeled cells or from cells labeled in the protein or RNA fractions was determined. Typical results obtained with suspensions of approximately the same initial turbidity and metabolic activity (CO_2 production) are reported in Fig. 1. These results suggest some utilization of protein as an endogenous substrate, but this utilization was not complete since the proline label did accumulate in the medium. Leucine, however was not oxidized by K-12, but the loss of leucine label from the cells was similar to that noted for proline from proline-labeled cells.

On a percentage basis, it is evident that after 48 hr $C^{14}O_2$ production from glucose-labeled cells continued at a much higher rate than from proline-labeled cells. This suggests that compounds other than protein serve as major substrates for the endogenous respiration of the cells. Since marked increases in absorbancy at 260 mμ appeared together with significant losses of C^{14} from uracil-labeled cells (Fig. 1, Table 3), it seems that, with the ribose moiety being oxidized, RNA might be degraded. This agrees with observations of Dawes and Ribbons (4) and of Wade (15), as well as those of Mandelstam and Halvorson (10), who concluded that degradation of ribosomes supplies much of the free amino acids and almost all of the ribonucleotides passing through the free pool during starvation. Gronlund and Campbell (5) also noted enzymatic degradation of ribosomes during the endogenous respiration of *Pseudomonas aeruginosa*. They concluded that a polynucleotide phosphorylase initiates the ribosome breakdown. Wade (15), too, observed the participation of this type of enzyme during the utilization of RNA by *E. coli*.

The formation of small (but variable) amounts of $C^{14}O_2$ from uracil-labeled cells was noted in all tests and was most pronounced during the first 24 hr of aging. No appreciable amount of an impurity in the uracil could be detected by chromatographic tests, and no O_2 consumption greater than the endogenous was noted in a 2-hr Warburg test period with uracil added to freshly harvested cells. During the Warburg test, no more than 0.3 mμc of $C^{14}O_2$ was produced from the 1,000 mμc of uracil-*2-C*^{14} added to the cell suspension; hence no appreciable amount of an oxidizable impurity was present. This $C^{14}O_2$ production will be considered later.

The test reported in Table 3 was designed to determine whether the presence of unlabeled uracil, proline, or leucine in the suspension medium had any marked effect as a trapping agent on the turnover of these compounds from cells specifically labeled in the RNA or protein fractions. A slightly greater loss of leucine and of uracil than of proline was noted from cells aged 96 hr in the presence of the corresponding compound extracellularly. Accumulation of proline to a lesser extent than that of the other two compounds probably results from some oxidation of liberated proline.

The amounts of endogenous $C^{14}O_2$ produced (Table 4) during the first 48 hr were the same for cells fed glucose as for the suspension diluted only with basal salt solution. Differences, possibly not very significant, can be noted for endogenous $C^{14}O_2$ production after 48 hr. It is apparent from the $C^{14}O_2$ values that endogenous respiration continued in the presence of glucose, although the actual extent of respiration apparently decreased with time. The endogenous values subtracted from those for total CO_2-C gives 0.89, 0.99, and 0.72 mg of CO_2-C per mg of glucose C for suspensions fed 19.5, 39.0, and 73.8 mg of glucose, respectively. It is possible that a slight stimulation of endogenous respiration was induced from feeding glucose to cells aged longer than 48 hr; hence, it is possible that larger corrections for

endogenous respiration might be required. The exogenous values, corrected or uncorrected, remained fairly constant after the first 24 hr.

In other experiments, unlabeled cells were fed uniformly labeled glucose; $C^{14}O_2$ production in these systems gave true values for substrate oxidation. Very little difference (Table 5) was noted in the extent of $C^{14}O_2$ production and the distribution from the glucose-*U*-C^{14} added in different amounts to the suspensions. The percentage values show that feeding the cells maintained the viable populations at higher levels than in starved suspensions and that the fed cells maintained a high level of metabolic activity. Respiration (CO_2-C) of the endogenous control decreased in a manner similar to that in Table 4.

Results of the studies of Marr, Nilson, and Clark (11) and of Mallette (8) indicated that there is a definite energy of maintenance for *E. coli*. In these studies (e.g., Table 1) it was evident that the ability of K-12 to oxidize a substrate such as glucose decreased as the cells aged; this decrease was much less marked when the cells were fed as they aged. Feeding 0.02 to 0.04 M glucose at a rate of approximately 7.0 ml per day appeared to be the optimum for maintenance of the cells. When more glucose was fed, it began to accumulate, and the fermentation products induced decreases in *p*H of the medium. The feeding of 0.02 to 0.04 M glucose corresponds to rates of about 0.01 to 0.02 mg of glucose per mg (dry weight) of cells per hr, a value near that (0.028) reported as a maintenance rate by Marr, Nilson, and Clark (11).

Mallette (8) and McGrew and Mallette (7) reported higher percentages of survivors in their suspensions of *E. coli* fed glucose than we observed. They also observed increases in turbidity after repeated additions of appropriate amounts of glucose, whereas we noted only decreases in turbidity; these decreases were, however, somewhat less in the fed than in the nonfed suspensions. We employed concentrations of bacteria about 10 to 20 times greater than did Mallette, prevented entrance of NH_3 from the air, and vigorously aerated the suspensions. The differences in results, which are of magnitude rather then direction, may arise from strain differences and, to some extent, from these experimental differences. However, it is difficult to understand how turbidity could increase unless actual growth did occur, since we observed such a marked loss of materials, particularly of RNA, from the cells. Our results suggest that energy is required to maintain all the cells over an extended period.

The amounts of C^{14} assimilated in these suspensions of K-12 were lower than those in short-term experiments with glucose-grown cells (2) and tended to diminish with time of aging, as also observed by McGrew and Mallette (7). The lower extent of assimilation probably reflects endogenous oxidation of assimilated carbon. The C^{14} values for the supernatant fluids represent products of incomplete utilization of glucose, since little or no glucose was detected with glucose oxidase.

When proline-labeled cells were fed glucose as the cells aged, the glucose (Table 6) enhanced both the total amount of C^{14} lost from the cells and the portion converted to $C^{14}O_2$, as compared with the unfed control. This may indicate that some energy is utilized in inducing the degradation of proline-containing molecules or their passage across the cell membrane.

Very marked enhancement of $C^{14}O_2$ production is evident from feeding glucose to uracil-labeled cells (Table 7). During the first 24 hr, a small amount (1%) of uracil label is converted to $C^{14}O_2$ from cells receiving no glucose, but none is converted thereafter. Cells fed glucose produced much more $C^{14}O_2$ during the first 24 hr and continued to produce it thereafter, but at much lower rates. About twice as much C^{14} remained in the fed cells as in the unfed cells at the end of 96 hr. This sparing effect of glucose on the loss of the uracil label might be an important factor in the maintenance of cell viability. It is possible that the strains of *E. coli* used by Marr and by Mallette had a lower tendency to lose RNA than does K-12 and, hence, were better able to maintain viability and integrity (turbidity) in the fed suspensions. We have no explantion for the influence of glucose on $C^{14}O_2$ production from uracil-labeled cells other than the possibility that considerable expenditure of energy is involved in the breakdown of uracil to an oxidizable substance.

Gronlund and Campbell (6) reported that the oxidation of endogenous protein or RNA, as measured by $C^{14}O_2$ production during 2 hr from specifically labeled cells of *P. aeruginosa*, was decreased when freshly harvested cells were incubated in the presence of glucose or other exogenous substrates. In our experiments with *E. coli*, the opposite behavior occurred in long-term experiments. Both the nature of the organism and test conditions influence the effect of an exogenous substrate on endogenous respiration.

Sykes (14) discussed the phenomenon of bacterial survival and concluded that death occurs because the cell is unable to follow its proper metabolic cycle or because the cell's enzyme- and protein-synthesizing structure is disrupted. Conversely, the cell survives if it retains its metabolic functions intact or if it is able to bypass any gaps in the cycle. In this study, we noted slowly de-

creasing rates of respiration (both exogenous and endogenous, once excess reserves are oxidized) which were maintained, as were the viable counts, at higher levels when the aging cells were fed glucose. Death was delayed when an extraneous carbon-energy source was available, but eventually one or another of the vital functions of the cells was destroyed with the existing unbalanced conditions. Feeding glucose to suspensions appears to establish different unbalanced conditions than found in unfed control suspension as indicated by the greater loss of the proline label (Table 6), and by the reduced loss of the uracil label, although $C^{14}O_2$ production from uracil was enhanced (Table 7). On the other hand, glucose seemed to provide carbon and energy for some repair, as evidenced by the apparent reincorporation of NH_3 and by greater percentages of survivors in the fed suspensions. The most marked change in composition of the cells occurred in the RNA fraction. This should reduce the ability of the cell to synthesize protein and, hence, to survive. At no time have we noted any substrate-accelerated death of the cells (13), possibly because glucose was not a growth-limiting factor and a balanced salt solution containing Mg^{++} was employed as the suspension medium.

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