PHENOTYPIC, GENOTYPIC, AND CHEMICAL CHANGES IN STARVING POPULATIONS OF *AEROBACTER AEROGENES*

ARTHUR P. HARRISON, JR. AND FELIX R. LAWRENCE

Department of Biology, Vanderbilt University, Nashville, Tennessee

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ABSTRACT

HARRISON, ARTHUR P., JR. (Vanderbilt University, Nashville, Tenn.) AND FELIX R. LAWRENCE. Phenotypic, genotypic, and chemical changes in starving populations of *Aerobacter aerogenes*. J. Bacteriol. **85**:742-750. 1963.—Cells harvested from postlogarithmic (maximal stationary phase) *Aerobacter aerogenes* cultures and starved in dilute sodium phosphate at 40 C remained viable for many hours. On the other hand, most cells from logarithmic-phase cultures succumbed, although a relatively small number remained viable. This viable segment of the original population thus responded like cells from postlog-phase cultures and, in fact, had properties in common with them. The residual segment was comprised of cells of two kinds. The first were mutants; when cultivated, harvested during log-phase growth, and again starved, they were resistant. The second were wild-type; they responded exactly as before. During starvation, the mutant is at an advantage because it has the ability to convert from susceptible log-phase physiology to resistant postlog-phase physiology more rapidly than can wild-type. The mutant differs from wild-type in yet other ways. It is smaller in size, slower in growth rate, lower in ribonucleic acid (RNA)-deoxyribonucleic acid ratio, greater in light-scattering ability, and, during the first 4 hr of starvation, it loses a higher proportion of its RNA. Selection of mutants of low growth rate between periods of active clonal growth indicates that evolutionary advantage may not necessarily be with the fast-growing members of the clone.

When *Aerobacter aerogenes* from a logarithmic-phase culture is starved in 0.03 M sodium phosphate buffer (pH 7.1) at growth temperature (40 C), there results a decline in viable cells concomitant with a loss of cell substance in the absence of gross cell lysis. The decline is less at high than at low initial cell densities because cell effluent accumulating from large cell mass may serve as nutriment for survivors (Harrison, 1960). Thus, the survival curves may have "tails" or, at intermediate initial cell densities, after an initial decline may even manifest a subsequent rise in viable cell numbers (Fig. 4*a*); in either case, a small residual population may persist for relatively long periods. The purpose of the present research has been to determine whether this residual population comprises solely of chance survivors no different from the original population except perhaps in physiological state, or whether the residual population represents a starvation-resistant segment (mutants) of the original population. A sensitive test to detect clonal differences in physiology within an aging population has been developed. Starvation-resistant mutants have been isolated, characterized, and compared with wild-type cells.

MATERIALS AND METHODS

The test organism was a nonmotile, nonencapsulated strain of *A. aerogenes*. Stock cultures were maintained on slants of the following composition (w/v): $Na_2HPO_4 \cdot 7H_2O$, 0.60%; $NaH_2PO_4 \cdot H_2O$, 0.15%; KCl, 0.05%; $MgCl_2 \cdot 6H_2O$, 0.02%; Na_2SO_4, 0.03%; NH_4Cl, 0.10%; glucose, 0.15%; and agar, 1.6%. For plating, the following medium was used: Trypticase (BBL), 0.5%; glucose, 0.5%; K_2HPO_4, 0.20%; and agar, 1.6%.

Distinctive colonial mutants, identical in their response to starvation (Fig. 1) and mixed together in the test suspensions, permitted identification of segments within the total population. The S colonies were smooth and opaque, whereas the T colonies were translucent. The S and T cells grew equally well on the Trypticase agar in mixtures on plates, and were easily differentiated. In fact, owing to their opacity, S colonies in minority could be detected within areas of confluent T growth; thus, an enumera-

tion of S colonies in ratio with T colonies as great as one to several thousand was possible. Both types bred true, and spontaneous mutation to the contrasting colonial type was rare and did not complicate interpretation of the experiments.

An experiment was carried out as follows. Cells (S or T) from a stock slant were inoculated into 5 ml of synthetic liquid medium and were incubated at 40 C overnight without aeration. Then, 0.2 ml served as inoculum for 10 ml of medium in a nephelometer cuvette (17 by 150 mm), with a tube for aeration inserted through the aluminum cap. The liquid medium contained the same ingredients as the stock agar, but lacked agar. Growth conditions were varied to obtain cell crops of different physiological states, and will be described below. Growth was followed by means of nephelometry by use of a Coleman model 9 Nephocolorimeter. (Turbidities are expressed in nephelos units based on Coleman standards.) The 10-ml culture was decanted into a 50-ml centrifuge tube, 10 ml of buffer (0.60% $Na_2HPO_4 \cdot 7H_2O$ and 0.15% $NaH_2PO_4 \cdot H_2O$; pH 7.1) at 40 C were added, and the cells were washed by means of three alternate centrifugations (5,000 $\times$ *g* for 5 min) and resuspensions in 20 ml of buffer. The resulting concentration of washed cells was approximately 2×10^8/ml. Samples (used full strength, diluted with buffer, or mixed together in different ratios with samples of washed cells of contrasting colonial morphology from a second culture, as the particular experiment demanded) were used to prepare test suspensions in 5-ml volumes in Pyrex test tubes (15 by 150 mm) fitted loosely with glass caps. Immediately, the test suspensions were placed in a water bath at 40 C. This instant corresponds to zero time on the abscissas of the figures herein, and occurred within 30 min after harvesting. The starving suspensions were not forcibly aerated. Viable-cell counts were made by spreading an appropriately diluted 0.1-ml sample over nutrient agar plates. The initial count was calculated from a plating of the original washed-cell suspension. All plates were incubated at 37 C for 18 to 24 hr. The plating diluent was the same composition as the plating medium, but lacked agar. The buffer was prepared in large quantity in a polyethylene carboy, and the desired volume, as needed, was decanted into an Erlenmeyer flask, then autoclaved and cooled just prior to use.

To obtain sufficient cells for chemical analyses, as well as for total cell volume and dry-weight determinations, cultivation was in 120 ml of medium in bottles with sintered-glass spargers for aeration. Washing was carried out in a manner equivalent to that employed with the cuvette cultures. For cell volume and weight determinations, the final washing was with water. Nucleic acid was extracted by means of a 30-min treatment with 5% trichloroacetic acid at 100 C, and the protein precipitate, after washing once with buffer, was dissolved with 1 N NaOH at 100 C. Ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and protein were determined by means of the Bial, Dische, and biuret reactions as described by Schneider (1957) and Layne (1957). As standards, yeast nucleic acid, salmon-sperm DNA, and crystalline bovine serum albumin (from California Corp. for Biochemical Research) were employed. Total cell volume was determined by centrifugation of washed cells for 1 hr at 2,000 $\times$ *g* in a Shevky-Stafford and McNaught graduated centrifuge tube. Dry weight was ascertained by transferring the pellet to a small test tube or crucible and heating at 100 C to constant weight. For potassium and magnesium analyses, the cells were washed with demineralized water in lieu of the sodium phosphate buffer. They were placed in a platinum crucible, air-dried at 100 C to constant weight, ashed at 900 C for 2 hr, and finally taken up in 5 ml of 0.04 N HCl. This solution was diluted, compared with standard KCl solutions by means of flame photometry, and subjected to the Titan-yellow colorimetric test for magnesium (Granick, 1950).

Log-phase cells were obtained from cultures growing at the maximal rate (generation time of approximately 28 min) at 40 C in liquid medium containing 0.10% NH_4Cl and 0.15% glucose. The cells were harvested when the culture attained a concentration of approximately 4×10^8 cells/ml, requiring about 3.5 hr of incubation. Postlogarithmic (maximal stationary phase) cells were obtained from cultures limited by a glucose concentration of 0.05%. The cells were harvested after the growth rate had become negligible (some time after exhaustion of glucose, usually after 12 hr of incubation) and the cell concentration was approximately 4×10^8/ml.

In the figures which follow, S cells will be represented by circles and T cells by triangles.

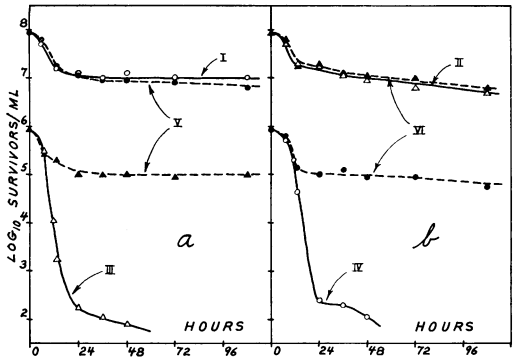

FIG. 1. *Viability of Aerobacter aerogenes cells at 40 C in 0.03 M sodium phosphate (pH 7.1).* I *and* IV: *Log-phase S cells alone.* II *and* III: *Log-phase T cells alone.* V: *Log-phase S cells (in majority) with log-phase T cells (in minority).* VI: *Log-phase T cells (in majority) with log-phase S cells (in minority).*

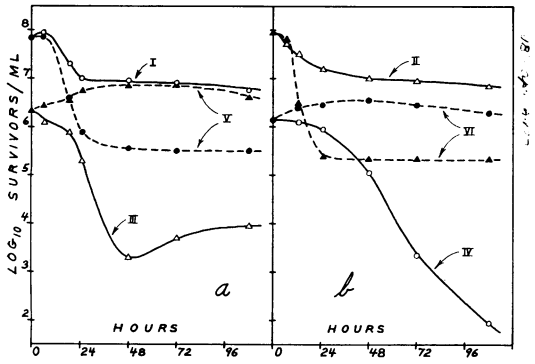

FIG. 2. *Viability of log- and postlog-phase Aerobacter aerogenes cells at 40 C in 0.03 M sodium phosphate (pH 7.1).* I: *Log-phase S cells alone.* II: *Log-phase T cells alone.* III: *Postlog-phase T cells alone.* IV: *Postlog-phase S cells alone.* V: *Log-phase S cells (initially in majority) with postlog-phase T cells.* VI: *Log-phase T cells (initially in majority) with postlog-phase S cells.*

Continuous curves represent S or T cells alone, whereas broken curves represent S and T cells mixed.

RESULTS AND DISCUSSION

Analysis of survival curves. Figure 1 summarizes a typical experiment with log cells. By comparing suspensions I and IV or suspensions II and III, the effect of initial cell concentration upon decline will be noted. This effect was also demonstrated in mixture, where the minority segment declined along with the total population (suspensions V and VI).

Figure 2 illustrates that postlog (maximal stationary phase) cells suffer less from starvation (compare suspensions III and IV with their counterparts, Fig. 1) and, when mixed with log-phase cells, grow at the expense of the latter (suspensions V and VI). The inhibition of log-phase cells is not due to an elaboration of a toxic product by postlog-phase cells, but simply to a more successful competition for cell effluent by them. Thus, buffer filtered (Millipore HA filter) from postlog-phase cell suspensions is not harmful but, on the contrary, somewhat beneficial to log-phase cells. Postlog-phase cells from cultures limited by NH_4Cl rather than by glucose also inhibit log-phase cells.

An earlier example of cell populations interacting through the agency of cell effluent was brought to our attention by W. D. McElroy. Loss of synthetic ability in *Achromobacter fischeri* was found to be inversely proportional to the density of the suspension tested (Miller, Farghaly, and McElroy, 1949). Moreover, when wild-type *A. fischeri* was mixed with auxotrophic mutants in minimal medium in which the metabolic activity of the wild-type strain was low due to suboptimal growth temperature, they observed a selective advantage favoring the auxotrophs and concluded that this may be "due to a greater loss of essential nutrients from the wild-type cells." Another example of an inverse relation between initial cell density and subsequent decline in viability was described by Major, McDougal, and Harrison (1955) with *Escherichia coli* frozen in water at −22 C.

Analyzing starvation decline of clonal admixtures aids in detecting similarity or dissimilarity in physiological state. Log-phase cells from two cultures of the same growth rate decline in concert (suspension V, Fig. 1), whereas log-phase cells from two cultures differing slightly in growth rate (altered by incubation temperature or nutriment concentration) manifest decline imbalance. The most extreme decline imbalance is that which results upon admixture of log-phase cells (rapid growth rate) with postlog-phase cells (negligible growth rate; suspension V, Fig. 2).

Figure 3 summarizes an experiment in which this technique was employed to determine the physiological state of cells on various portions

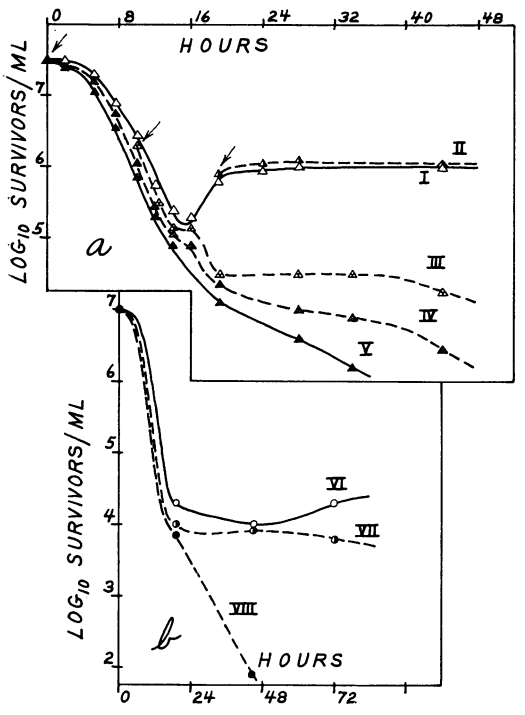

FIG. 3. *Analysis of decline of log-phase Aerobacter aerogenes cells at 40 C in 0.03 M sodium phosphate (pH 7.1).* I: *Log-phase T cells alone (control).* II: *Log-phase T cells to which postlog-phase S cells (10^8/ml) were added after 19 hr (at arrow).* III: *Same, but with the postlog-phase cells added after 10 hr.* IV: *Postlog-phase cells added at outset.* V: *Log-phase T cells alone (as in* I*) but in presence of 16 μg/ml of chloramphenicol.* VI: *Log-phase S cells alone (control).* VII: *Log-phase S cells to which unwashed 48-hr T cells (from* I*) were added at outset.* VIII: *Log-phase S cells to which washed 48-hr T cells (from* I*) were added at outset. (Note: The broken curves represent only one segment of a mixed suspension; the viability of the other segment is not pertinent and, to avoid congestion, has not been plotted. The other segment, of course, responds as indicated in Fig. 2.)*

of the decline curve. (At intermediate initial cell densities, between 5×10^6 and 5×10^7 cells/ml, the starvation curves are the most complex, showing a minimum and a subsequent increase in viable count.) In Fig. 3*a*, postlog-phase cells have been added at various times during starvation of the log-phase cells. For clarity, the curves to the left of the minimum have been spread apart; actually, they superimpose upon the control curve. Also, for clarity, the postlog-phase cell segment has not been plotted. This and replicate experiments demonstrate that the addition of postlog-phase cells to the left and to within several hours of the minimum will prevent regrowth of the log-phase cells almost to the same extent as exposing them to chloramphenicol (16 μg/ml). However, the addition of postlog-phase cells to the residual population has no effect whatsoever. This finding suggests that the cells to the right of the minimum represent those log-phase cells that have converted to postlog-phase cell physiology or a state closely akin thereto. This view was substantiated when the residual population was used to challenge freshly harvested log-phase cells. In Fig. 3*b* (curve VIII), it will be noted that the residual population from Fig. 3*a* when washed and added to log-phase cells now acts just as postlog-phase cells do, causing inhibition of the log-phase cells. However, unless the residual cells are first washed, sufficient effluent will be added to them to more than meet the needs of all cells and, therefore, inhibition will not occur (curve VII, Fig. 3*b*). This supports the view that postlog-phase cells exert their inhibitory effect not by the secretion of some toxic material but by competitive adsorption.

Isolation of "starvation-resistant" mutants. To decide whether there resides in the residual population cells genotypically different from those in the original suspension, the experiment summarized in Fig. 4 was undertaken. Here, individual clones (colonies) were selected at various points on the decline curve (Fig. 4*a*). Stocks were prepared from these isolates. Each stock, in turn, was challenged with log-phase cells just as in Fig. 1*a*. Decline ratios were calculated (Fig. 4*b* and 4*c*). If the test clone and the indicator clone (S-type cells) decline in concert, the ratio will be unity; if decline imbalance results, ratios higher than unity will occur. The results are plotted as histograms in Fig. 5. The isolates from the initial population (zero time) spread about a decline ratio of 0.5 to 4.0. Upon aging of the suspension, however, cells able to bring about very high decline ratios (from 16 to 2,008) accumulated. The decline ratio obtained predicts the showing the test organism will make when starved alone. Thus, all isolates giving a ratio between 0.5 and 4.0 decline exactly like wild-type cells (curve IV, Fig. 1*b*), whereas those isolates giving the high ratios are resistant to

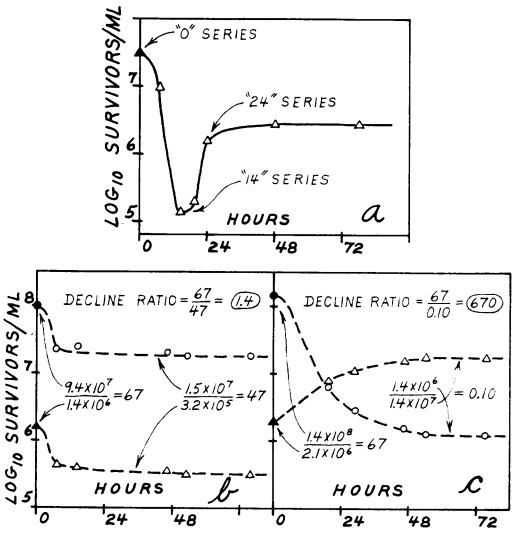

FIG. 4. *Isolation of clones during starvation of log-phase T cells, and determination of their response (expressed as a decline ratio) when mixed with log-phase S cells and again starved (a). (b) Response of a typical wild-type T clone; (c) response of a typical starvation-resistant T clone.*

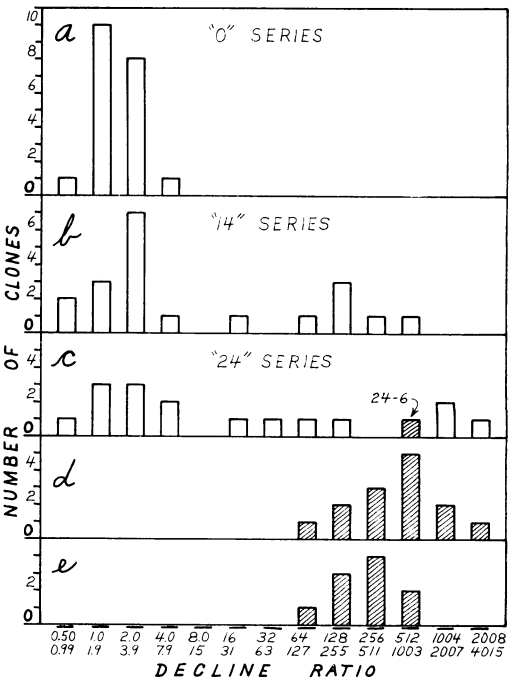

FIG. 5. *Decline ratios obtained with the clones isolated in Fig. 4: (a) 20 clones selected at zero time ("0" series, Fig. 4a); (b) 20 clones selected after starvation for 14 hr ("14" series, Fig. 4a); (c) 17 clones selected after starvation for 24 hr ("24" series, Fig. 4a); (d) 14 clones obtained from clone 24-6; and (e) 10 clones obtained from 24-6 after 21 additional generations.*

starvation, persisting in the buffer to the same degree as postlog-phase cells (curve IV, Fig. 2b). Moreover, these resistant cells breed true. This is demonstrated with mutant 24-6 (Fig. 5d and 5e).

Approximately half the survivors are starvation-resistant mutants and half are wild-type. The latter may be envisioned as cells which, by chance, converted to postlog-phase physiology before losing sufficient effluent to succumb. One wild-type survivor was leucine-deficient. All other survivors, mutant and wild-type, were unchanged in nutrition.

Conversion of mutant and wild-type from log- to postlog-phase physiology during starvation. The previous findings lead to the view that the starvation mutants owe their resistance to a more rapid conversion from log- to postlog-phase physiology. Thus, they do not remain long enough in the susceptible log-phase state to succumb. To verify this view, we made use of the well-known difference in resistance between log- and postlog-phase cells to deleterious circumstances such as heat. In these experiments, a fraction from the starving suspension at 40 C was transferred to 5 ml of buffer at 53 C, and the sensitivity to heat ascertained by customary plate counts. The volume of sample

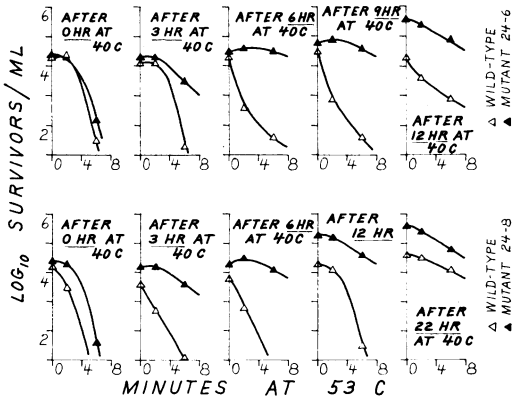

FIG. 6. *Viability at 53 C after various intervals of starvation at 40 C in 0.03 M sodium phosphate. (Comparison of the conversion from heat sensitivity to heat resistance by wild-type and mutant cells.)*

transferred was increased from 0.05 to 0.5 ml during the test period to compensate for starvation decline in the suspensions at 40 C. Figure 6 summarizes two such experiments. (Similar results were obtained with samples added to 0.2 mg/ml of lead nitrate at 40 C and, although not so clear-cut, with ultraviolet irradiation at 20 C.) Of three mutants examined, all converted to heat resistance more rapidly than did wild-type cells. The time required for conversion was the same, whether mutant and wild-type were tested separately or were mixed as a single suspension. Apparently, at this low cell density, neither affects the conversion of the other. The acquired resistance was of the same magnitude as the resistance of cells harvested from a postlog-phase culture.

Mutant and wild-type cells from postlog-phase cultures starved in mixture manifested no decline imbalance. Apparently, with postlog-phase cells, there is little interaction between the cells. This is borne out by the fact that viability of cells from postlog-phase cultures is influenced relatively little by initial cell density (Harrison, 1960).

The following explanation of the population changes in a starving log-phase *A. aerogenes* suspension (Fig. 4*a*) is in agreement with all the experimental data. The log-phase cells lose cell substance. As a result of this (or owing to a dearth of a particular nutrient) they are stimulated to make physiological adjustments. Thus, the declining economy of the population is acted upon by two factors: the accumulating cell effluent and the changing physiology of individual cells. A cell, once adjusted to the new circumstances, becomes a more successful scavenger and, as a result, grows at the expense of those cells not yet so adjusted (because it utilizes effluent that otherwise would be recovered by them). Therefore, the survival curve (Fig. 4*a*) is a composite of two curves. One represents decline of the susceptible log-phase segment; the other represents growth of a segment which has adjusted to use effluent as food. This growth, at first cryptic but then detectable as the declining population falls in viable numbers below the growing population, is itself a composite. One segment is composed of mutants which, although present initially in low proportion, have increased to above this proportion, since rapid adjustment has allowed them to begin scavenging effluent early. The other segment is composed of wild-type chance survivors that have adjusted late, but are now also successfully scavenging effluent. The wild-type, once adjusted, is at no disad-

TABLE 1. *Comparison of mutant and wild-type log-phase cells of Aerobacter aerogenes*

Trait	Mutant (24-20)	Wild-type (T cells)	Probability of difference being due to chance alone[a]
Growth rate, divisions per hr[b]	1.9	2.5	<0.001
Nephelos equivalent per g of dry cells[c]	2.8×10^6	2.2×10^6	<0.001
No. of cells per g of dry cells[d]	2.6×10^{12}	1.7×10^{12}	<0.001
Potassium, g per g of dry cells[e]	5.2×10^{-3}	5.1×10^{-3}	—[f]
Magnesium, g per g of dry cells[e]	2.2×10^{-3}	2.2×10^{-3}	—[f]
Fraction of chemical weight that is DNA[g]	0.033	0.025	<0.001
Fraction of chemical weight that is RNA[g]	0.24	0.27	0.05–0.1
Fraction of chemical weight that is protein[g]	0.73	0.71	>0.1

[a] From "Student's" t-test.

[b] Means from 16 pairs of results (16 experiments). Calculated from the slope of the growth curve (nephelometric) during cultivation.

[c] Means from ten pairs of results (ten experiments). Obtained by multiplying the nephelos value (Coleman units) at time of harvest by ml of culture harvested and dividing this figure by the dry weight of these harvested cells.

[d] Means from ten pairs of results (ten experiments). Cell number determined by plate count.

[e] Means from five pairs of results (five experiments). The harvested cells were washed with water rather than the customary phosphate buffer.

[f] Not determined.

[g] Means from nine pairs of results (nine experiments). By chemical weight is meant the sum of the three components: DNA, RNA, and protein.

vantage; on the contrary, it may be expected to have a slight advantage owing to its higher growth rate (Table 1).

The presence of wild-type cells in the residual population may be accounted for in another way.

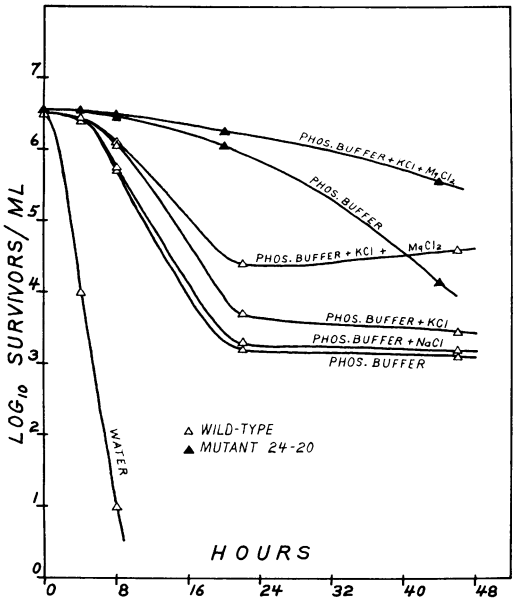

FIG. 7. *Viability of log-phase Aerobacter aerogenes at 40 C in various menstrua: demineralized water; 0.03 M sodium phosphate (pH 7.1) alone or with 0.01 M NaCl, 0.01 M KCl, or 0.001 M $MgCl_2$.*

This requires the assumption that the mutant secretes some substance which alters or protects wild-type. However, the more simple explanation already presented seems sufficient. [Eddy and Hinshelwood (1953) suggested that populations may make temporary nonheritable adjustments to adverse circumstances.]

Effect of K^+ and Mg^{++} ions on survival and postlog-phase conversion. At an initial concentration of 10^6 to 10^7 cells/ml, survival of mutant and wild-type log-phase cells can be increased by incorporating 10^{-2} M KCl and 10^{-4} to 10^{-3} M $MgCl_2$ into the sodium phosphate menstruum, (Fig. 7). In some experiments, replacing the $MgCl_2$ with $CaCl_2$ brought about an equivalent improvement, but this effect was not always reproducible and may have been due to traces of contaminating Mg^{++}. The beneficial effect of these salts is due to their cations; 10^{-2} M NaCl does not improve survival. With demineralized water as menstruum, wild-type log-phase suspensions may become sterile within a few hours. However, at initial concentrations of 10^8 to 10^9 cells/ml, survival in water is about the same as in sodium phosphate.

The presence of K^+ and Mg^{++} ions in the phosphate does not alter the rates of conversion from heat sensitivity to heat resistance already described (Fig. 6). Furthermore, the potassium and magnesium content and retention in mutant

TABLE 2. *Decrease of Aerobacter aerogenes log-phase cell contents as a result of 4 hr of starvation at 40 C in sodium phosphate (pH 7.1)*[a]

Effect	Mutant (24-20)	Wild-type (T cells)	Probability of difference being due to chance alone[b]
Decrease in dry-cell weight,[c] %	19	13	—[d]
Decrease in magnesium[c], %	32	37	—[d]
Decrease in potassium[c], %	91	93	—[d]
Decrease in RNA[e], %	45	31	0.01–0.02
Decrease in DNA[f], %	12	11	—[d]
Decrease in protein[f], %	14	9.4	>0.1
Change in viability[g], %	+3.8	−37	0.01–0.02

[a] Cell concentration during starvation: approx 5×10^6/ml.

[b] From "Student's" t-test.

[c] Means from two pairs of results (two experiments).

[d] Not determined.

[e] Means from 14 pairs of results (14 experiments).

[f] Means from six pairs of results (six experiments). The DNA decrease in the replicate experiments varied greatly, between 0 and 30%, for both mutant and wild-type. The protein decrease in five experiments was uniformly greater in the mutant, but one unusual experiment yielded an opposite result; hence the high probability value.

[g] Means from 12 pairs of results (12 experiments). Calculated from plate counts. The mutant mean represents a slight (negligible) increase in viability.

and wild-type log-phase cells are not appreciably different (Tables 1 and 2).

Analyses on mutant and wild-type cells. To compare mutant with wild-type for a particular trait, one culture of each was prepared and tested simultaneously as a single experiment. The experiment, therefore, yielded two results, and replicate experiments yielded a set of paired results. (This method is preferable to sequential tests where a series is carried out first with mutant, then with wild-type, or vice versa.) The degree of significance was determined by means of "Student's" t-test, which analyzes the difference between the two results from each experiment. Comparisons between mutant 24-20 and wild-type are presented in Table 1. (Replicate mutants were similar to 24-20.)

Observe that the mutant is smaller and slower-growing (Table 1). (Enrichment of the synthetic medium with 0.1% yeast extract increased the growth rate of mutant and wild-type in the same proportion, to 2.7 and 3.6 divisions/hr, respectively.) Also, the mutant has greater light-scattering properties; thus, nephelometric comparisons between the two correlate more closely with cell number than with mass, although nephelometric measurements on either alone correlate with mass, as is customary (Monod, 1942). Both have the same water content (81 ± 2%). The slower-growing mutant has the lower RNA-DNA ratio. These ratios are 7.3 and 11. This is as expected from the work of Schaechter, Maaløe, and Kjeldgaard (1958), where the dependency of the growth rate on the RNA-DNA ratio was demonstrated.

Postlog-phase cells of mutant and wild-type contain the same percentage of water as do log-phase cells and have the same light-scattering properties, but are only about one-half the mass of log cells. The DNA, RNA, and protein fractions for the mutant are 0.052, 0.19, and 0.77; for wild-type, 0.041, 0.24, and 0.72. Thus, the RNA-DNA ratios are 3.7 and 5.9. The low RNA-DNA ratio of postlog-phase cells (compared with log-phase cells) was described by Lockhart and Powelson (1954).

Chemical changes during starvation. In addition to the greater rate of conversion to heat resistance already described (Fig. 6), a greater loss of RNA (and possibly protein), and a lesser loss of viability, was manifested by the mutant when starved for 4 hr (Table 2). There appears to be little difference in loss of DNA, potassium, and magnesium. The difference in loss of dry weight is undoubtedly due to the unequal loss of RNA and protein, which are the main cell constituents other than water. Apparently, there is very little lipid or carbohydrate (other than pentose) in *A. aerogenes* cultivated in simple synthetic media; the sum of RNA, DNA, and protein (chemical weight) represents well over 90% of the cell dry weight (*see also* Strange, Dark, and Ness, 1961). The greater RNA loss by the mutant during the first 4 hr of starvation is noteworthy. It is during this interval that the mutant converts from log- to postlog-phase physiology (Fig. 6). It is interesting, further, that a suspension of the mutant can lose approximately 20% of its dry weight with no apparent loss in the number of viable cells. On the other hand, this same loss in mass by a suspension of wild-type cells (as a result of starvation for 19 hr) occurs concomitantly with a loss of viability of well over 99% (Table 3). When the cells were starved

TABLE 3. *Decrease of Aerobacter aerogenes log-phase cell contents as a result of 19 hr of starvation at 40 C in sodium phosphate (pH 7.1)*[a]

Effect	Mutant (24-20)	Wild-type (T cells)	Probability of difference being due to chance alone[b]
Decrease in dry-cell weight[c], %	17	18	—[d]
Decrease in RNA[e], %	70	73	≫0.1
Decrease in DNA[e], %	37	48	≫0.1
Decrease in protein[e], %	36	40	≫0.1
Decrease in viability[e], %	70	>99	0.01–0.02

[a] Cell concentration during starvation: approx 5×10^6/ml.
[b] From "Student's" t-test.
[c] Means from two pairs of results (two experiments).
[d] Not determined.
[e] Means from six pairs of results (six experiments).

for 19 hr, no significant difference between mutant and wild-type losses of RNA, DNA, and protein was revealed (Table 3).

Some evolutionary considerations. During periods of active proliferation, the fastest-growing cells of a clone will be at an advantage. In nature, however, a clone does not grow unrestricted for long; the cells will succumb to predator, toxic materials, or starvation. Starvation can occur from depletion of nutriment either as a result of growth or as a result of dilution, as after rain. Our research has shown that, when *A. aerogenes* is shifted rapidly from growth to starvation conditions, a selection of resistant mutants will occur, and that these mutants are characterized by a slow growth rate. In nature, such a selection of slow-growing cells may thus offset selection of fast-growing cells during periods of active proliferation. Accordingly, just as in the fable of the tortoise and the hare, the race may go to the persistent rather than to the swift.

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