SOME QUANTITATIVE RELATIONSHIPS IN BACTERIAL POPULATION CYCLES[1,2]

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Although bacterial population cycles have been rather extensively studied qualitatively, accurate quantitative interpretation of certain aspects of the growth phases is wanting. This is due chiefly to the lack of comparable data on various organisms obtained under controlled, reproducible environmental conditions.

The present investigation was undertaken to define more precisely the reproductive cycles of some of the common saprophytes under controlled, comparable conditions. In particular, the effect of temperature on reproduction was studied. From these growth curves, certain generalized quantitative relationships are derived.

No attempt is made here to review as a whole the literature on growth curves; this has been done adequately by Henrici (1928), Buchanan and Fulmer (1928, 1930), and Rahn (1932). A complete review is also given in the author's original thesis (Jennison, 1932).

METHODS

Broadly, the experiments involved growing several organisms in broth at various temperatures under accurately controlled

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[2] Contribution No. 57 from the Department of Biology and Public Health, Massachusetts Institute of Technology.

conditions, and determining their growth curves over a twenty-four-hour period by plate count.

The species of bacteria used were a secondary consideration except insofar as they fulfilled certain desiderata of technical procedure, namely, ease of handling, absence of spores, and relatively little tendency of the cells to remain attached to each other after reproduction. The following organisms were chosen as representative of four genera of common saprophytes: *Escherichia coli*, an old stock culture and a newly isolated strain; and old stock cultures of *Aerobacter aerogenes*, *Serratia marcescens*, and *Chromobacterium violaceum*. Purity of the cultures was ascertained by the usual methods.

The organisms were "acclimatized" to 22°C. by growing on agar slants, with weekly transplants, for four months before use; they were then grown in broth, with transfers every twelve hours, for a week at this temperature. Growth curves were determined at 22°, 27°, 32°, 37°, and 42°C.—except for the last two species which did not reproduce at 42°—using the 22° cultures for inoculation. (Each organism was also acclimatized (as above) to 27°, 32°, 37°, and, except for the two species mentioned, 42°C. The growth curve of each organism was then determined at the temperature to which it had been acclimatized, by the same procedures as given below. With one exception, the growth of each organism at a given temperature after acclimatization to that temperature was quantitatively the same (within the limits of experimental error) as when acclimatized to 22°. The data for this second series of experiments are not included in this paper, since they were so nearly identical with those reported. The exception mentioned will be discussed later.)

For each organism the growth curves were determined at the four or five temperatures at the same time. Series of culture tubes containing 9 ml. of nutrient broth, which had been brought up to temperature, were inoculated by means of pipettes delivering a known number of drops per milliliter, with 1 to 3 drops of a twelve-hour broth culture of the particular organism, thus keeping the volume of medium approximately constant. As determined by previous experiment, this inoculum gave 1000 to

2000 cells per milliliter, the exact number being found by plate count each time. This quantity is large enough to prevent irregularities in the growth curve, and small enough to give a long period of reproduction. The cultures were immediately incubated at the various temperatures.

At exactly two-hour intervals for twelve hours, and in many cases for twenty-four hours, two (new) tubes were removed at each temperature, stoppered with sterile rubber stoppers, shaken for one minute, and the contents pooled and mixed. Serial dilutions were made in 9 ml. water blanks at room temperature and immediately (within five minutes) plated, 5 plates being made from the proper dilutions. The use of 5 plates for obtaining averages gives more reliable results than does a lesser number (Wilson, 1922; Wilson and Kullmann, 1931). Furthermore, as Wilson and Kullmann have shown statistically, if three or more plates are made in parallel, the elimination of any one plate that is markedly different from its replicates can be made without bias. In our experiments, replicate plates nearly always checked within a few per cent; those few differing by more than 20 per cent (four times the average deviation) from the average were omitted.

Plates were inverted and incubated for forty-eight hours at 37°C. before counting. The dilutions giving 100 to 400 colonies per plate were used for enumeration, which range Wilson (1922) has shown to be optimum for reliable results when averaging three or more plates of the same dilution. Most of our mean counts are averages of five plates.

Conditons of incubation, dilution, etc., were controlled so as to minimize experimental errors. Temperatures of incubation did not vary more than ± 0.15°C. from those specified. The same lot of Difco dehydrated nutrient broth was used throughout, being weighed out rapidly on an analytical balance to the nearest tenth of a milligram to give an 0.8 per cent solution in distilled water, and autoclaved at 15 pounds for twenty minutes. All tubes of broth, and water blanks, contained 9.0 ml. ± 0.1 ml. of liquid when used. The same lot of Difco dehydrated nutrient agar was also used throughout, and the plates poured at 42.0°C.

±1°C. Dilution pipettes (1 ml.) were selected for uniformity of delivery, the average variation being ±1 per cent of volume.

RELIABILITY OF RESULTS

It is difficult to evaluate properly all of the sources of error involved in growth studies and arrive at an exact measure of the precision of results. The *counting error* (i.e., the measure of the reliability of the mean of several replicate plates of the same dilution) is the one most commonly employed by bacteriologists, and can readily be calculated. It may be expressed most simply as the percentage average deviation of a set of plates from the arithmetic mean, although more precise statistical measures are available (Fisher, Thornton, and MacKenzie, 1922; Fisher, 1930; Wilson and Kullmann, 1931). The *counting error* is *not necessarily* a good measure of the *total* error, since it does not take into account errors of dilution. In this study these latter were so much smaller than the counting error, however, that for practical purposes the counting error could be used as a fairly reliable measure of the precision of results.

In the experiments here reported, involving several hundred sets of plate counts, the counting error was consistently low, the average deviation of individual plates of a set from the mean averaging 5 per cent of the mean. This is equivalent to a standard error of the *means* of about ±3 per cent.

In these studies it was also desirable to determine the precision of the bacterial generation times during the logarithmic growth period. This was accomplished in most cases by averaging the generation times calculated for the (usually) two or more two-hour sampling periods during the logarithmic growth phase, and obtaining the average deviation from the resultant mean. (While this method is not statistically precise it is simple, and the results obtained are, within the limits of experimental error, comparable to the values obtained by using the method of least squares.) In those few cases where the phase of logarithmic growth lasted through only about one sampling period (two hours) this method could not be employed. Instead, a formula (Goodwin, 1920) was used which allows computation of the precision of a result

(in this case, generation time) when the precision measures of the components (plate counts at the beginning and end of the sampling period) are known. It was found that the average deviation of the individual generation times from the means was about 7 per cent of the mean. (Standard error of *means* = ±4 per cent.) Similarly, computation of the precision of the temperature quotients, $Q_{10°}$, gave an average deviation of about 10 per cent. (Standard error = ±6 per cent).

THE GROWTH CURVES

In figures 1 to 5 are shown the growth curves at various temperatures for the five organisms, the data for which curves are in tables A to E (appendix). The curves for the different organisms are comparable, since the inoculum was practically the same in each case. Numbers of cells being plotted logarithmically, the slopes of the curves show relative rates of reproduction directly.

The growth curves as a whole are typical of bacterial population cycles, showing a lag period, a phase of maximum (logarithmic) rate of reproduction, and a long period of decreasing rate of growth (Buchanan, 1918). It will be noted (see also tables A to E, appendix) that particularly at the higher temperatures the beginning and end of the logarithmic growth phase is very sharp, which largely obviates the necessity for subjective judgment in determining the length of this phase.

The presence of a lag period at the various temperatures for all organisms except the old strain of *Escherihia coli*, may be explained, except at 22°, as due to the fact that 22° cultures used for inoculation had to become adapted to the higher temperatures before reproducing maximally, since physiologically young cells are more susceptible to environmental changes than old cells (Sherman and Albus, 1923, 1924; Jensen, 1928). It is not clear why the old *Escherichia coli* showed no lag under these conditions. The presence of a lag period at 22°C. is somewhat unexpected, in view of the results of Chesney (1916). He found with the pneumococcus, *Escherichia coli*, *Pseudomonas fluorescens* and *Serratia marcescens* growing at *optimum temperature* that if the

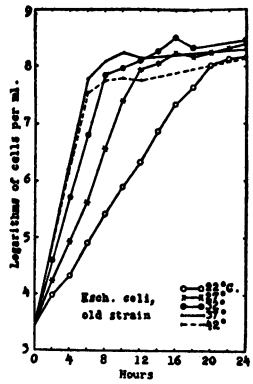

FIG. 1

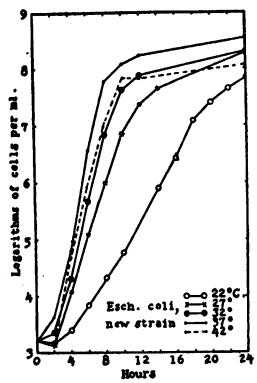

FIG. 2

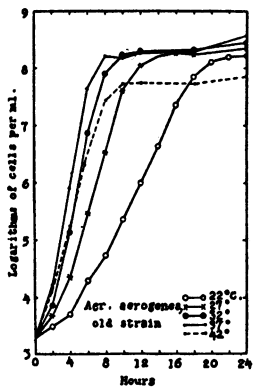

FIG. 3

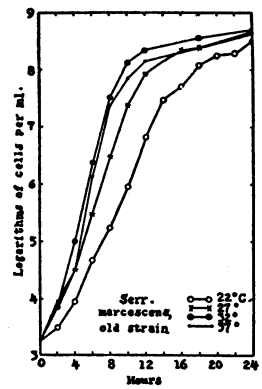

FIG. 4

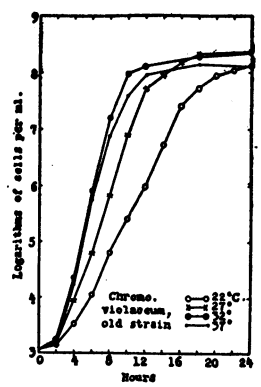

FIG. 5

FIGS. 1 TO 5. GROWTH CURVES OF BACTERIA IN BROTH AT VARIOUS TEMPERATURES, BY PLATE COUNT

culture is reproducing at its most rapid rate (logarithmic growth phase) the bacteria, upon subculture at the same temperature, show no lag period. In our work, the 22°, twelve-hour cultures used for inoculation, while not growing at the optimum temperature, were in the logarithmic growth phase, and it might be expected that upon subculture at 22° the organisms would continue to reproduce maximally. This however, is definitely not the case; the curves for all organisms (with the possible exception of *Escherichia coli*, old strain, which is somewhat irregular during the first four hours at this temperature) show a lag period of from 2 to 4 hours or longer. Duplicate experiments with the five organisms yielded the same results, except that the old strain of *Escherichia coli* showed a definite lag at 22°C. This seems to indicate that the presence of a lag period in subcultures at the same temperature, with bacteria growing at temperatures below their optimum, is dependent, in part at least, upon factors other than the age of the parent culture. Possibly the temperature or the number of cells inoculated is concerned.

Form of the growth curve

There has developed a tendency by some workers to regard bacterial growth in culture as analogous to the autocatalytic phenomena observed in chemistry (Buchanan and Fulmer, 1928, p. 52). To this living phenomenon the name autocatakinetic growth has been applied. A plot of cells against time is represented to be a regular symmetrical sigmoid curve, on which the point of inflection (that point at which the average rate of increase per cell begins to decrease, i.e., the end of the logarithmic phase) is at $b = \frac{a}{2}$, where b is the number of cells at the point of inflection, and a is the maximum bacterial count. While the growth curve of a bacterial culture would be expected to be sigmoid, there is reason to believe that it would not ordinarily be symmetrical about its point of inflection, although there is little experimental data to support adequately either contention. Of two such studies, McKendrick and Pai (1911) found the growth curve of *Escherichia coli* in broth to be reasonably symmetrical;

while Buchanan and Fulmer (1930, p. 63) analyzing the data of Müller on five organisms at various temperatures in a liquid medium, found the growth curves to be exceedingly asymmetrical.

TABLE 1

Point of inflection of growth curves

ORGANISM	TEMPERATURE	"MAXIMUM" NUMBER OF ORGANISMS *a*	NUMBER OF ORGANISMS AT POINT OF INFLECTION *b*	POINT OF INFLECTION IN TERMS OF *a* *a/b*
	°C.	*millions per ml.*	*millions per ml.*	
Esch. coli, old	22°	147.0	20.1	a/7
	27°	246.0	24.4	a/10
	32°	282.0	72.0	a/4
	37°	195.0	60.0	a/3
	42°	136.0	34.3	a/4
Esch. coli, new	22°	68.0	10.2	a/7
	27°	189.0	7.6	a/25
	32°	199.0	7.1	a/28
	37°	350.0	60.0	a/6
	42°	115.0	10.2	a/11
Aer. aerogenes, old	22°	450.0	72.0	a/6
	27°	580.0	42.0	a/14
	32°	670.0	80.0	a/8
	37°	650.0	44.0	a/15
	42°	199.0	27.0	a/7
Serr. marcescens, old	22°	310.0	29.0	a/11
	27°	460.0	23.5	a/20
	32°	490.0	32.0	a/15
	37°	410.0	22.4	a/18
Chromo. violaceum, old	22°	132.0	25.0	a/5
	27°	226.0	50.0	a/4
	32°	216.0	15.7	a/14
	37°	137.0	7.6	a/18

The exact degree of symmetry of our growth curves about the point of inflection, that is, the relative position of this point, could not be determined precisely or directly, without extrapolation from the data, because in no case had the maximum

number of cells been reached. However, by using the number of cells at the end of twenty-four hours as a "maximum" a good approximation was obtained, since such a procedure showed that all the curves were asymmetric in one direction about the point of inflection. Therefore the true figures for total crop would merely show the curves to be more asymmetric in the same direction, and would define more precisely the degree of asymmetry.

In table 1 is given the relative position of the point of inflection of the growth curve of each organism, calculated from the values of $\frac{a}{b}$, where a is the "maximum" number of cells (after twenty-four hours) and b the number of organisms at the point of inflection (end of the logarithmic growth phase). (Data in tables A to E, appendix.) The relative position of each point is given in terms of a, in order to facilitate comparison with the value $a/2$ of a symmetrical curve.

It is apparent that since a symmetrical curve would have its point of inflection at $a/2$, these growth curves are far from symmetrical even when low "maximum" figures are used, the point of inflection in all cases being reached long before it would be for a symmetrical curve. Similar results were obtained in the experiments with acclimatized cultures, all curves being asymmetric. It is impossible to determine directly from our data whether temperature affects the point of inflection, because the true maximum numbers were not reached.

EFFECT OF TEMPERATURE UPON THE MINIMUM GENERATION TIME

The usual numerical measure of the rate of reproduction of bacteria is the generation time (GT), defined as the time (in minutes) necessary for the cells to double in number, assuming that all are dividing. This can be calculated from the well-known formula (Buchanan and Fulmer, 1928, p. 19):

$$GT = \frac{t \log 2}{\log b - \log a}$$

in which t is the time interval in minutes, a is the number of cells at the beginning, i.e., when $t = 0$, and b is the number of cells

after time t. For comparative purposes the minimum generation times (during the logarithmic growth phase) are used.

Generation times were calculated by the above formula from the plate counts made at each two-hour sampling interval (tables A to E, appendix). From these values and an inspection of the growth curves the limits of the phase of logarithmic growth were obtained fairly exactly, since, particularly at higher temperatures, the break at the beginning and end was marked. From the generation times for each two-hour interval during logarithmic reproduction a mean value was obtained. These mean generation times for all organisms are given in table 2. The mean values are calculated only to the nearest minute, since, as we

TABLE 2

Effect of temperature upon the mean generation time during period of logarithmic growth

ORGANISM	MEAN GENERATION TIME IN MINUTES				
	22°C.	27°C.	32°C.	37°C.	42°C.
Esch. coli, old	73	46	33	25	26
Esch. coli, new	65	39	26	20	26
Aer. aerogenes, old	62	33	21	21	29
Serr. marcescens, old	52	37	26	22	
Chromo. violaceum, old	55	34	23	25	

have noted, their standard error is ±4% (about ±1 minute at 37°C.).

The effect of temperature upon the mean generation time is shown graphically for all organisms in figures 6 and 7.

It was noted previously (p. 2) that in a second series of experiments (unreported) the growth curve (and therefore the mean generation time during logarithmic growth) of each organism at a given temperature after acclimatization to that temperature was, with one exception, the same as when acclimatized to 22°. The exception was *Serratia marcescens* at 37°. The mean generation time during logarithmic growth at this temperature, using the 22° culture for inoculation, was 22 minutes (table 2); the culture which had been acclimatized to 37° for

four months showed a mean generation time at 37° of thirty-three minutes. It is not known how long it took the organism to reach this lower rate, but the observation is of interest in that it brings out the possibility that 37° as an "optimum" for *reproduction* of this organism (table 2 and figure 7) may be more apparent than

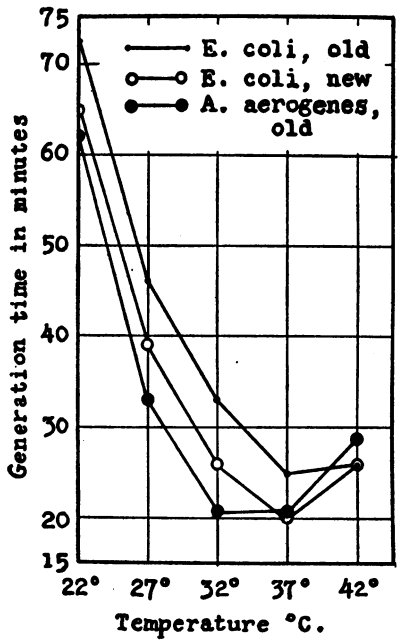

FIG. 6

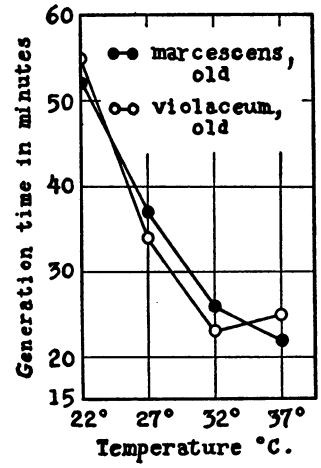

FIG. 7

FIG. 6. EFFECT OF TEMPERATURE UPON THE GENERATION TIME OF BACTERIA DURING THE LOGARITHMIC GROWTH PHASE

FIG. 7. EFFECT OF TEMPERATURE UPON THE GENERATION TIME OF BACTERIA DURING THE LOGARITHMIC GROWTH PHASE

real, depending upon the previous temperature of cultivation of the culture and the phenomenon of "growth momentum."

The faster rate of reproduction at all temperatures up to 42°, of the freshly isolated strain of *Eschichia coli* compared with the old stock culture (figure 6), is worthy of note, and in particular the sharper optimum at 37°. Compared with the two strains of *Escherichia coli*, *Aerobacter aerogenes* seems to have a slightly lower

optimum temperature; its relatively faster rate of reproduction below 37° is also evidence of this. The optimum temperature for *Chromobacterium violaceum* is definitely nearer 32° than 37° (figure 7).

The observed generation times for *Escherichia coli* and *Aerobacter aerogenes* are similar to the values found by other workers (Mason, 1934). The value of twenty-two minutes at 37° for *Serratia marcescens* is considerably smaller than the minimum of thirty-seven minutes recorded for this organism in milk by Heinemann and Glenn (1908). The data for *Chromobacterium violaceum* appear to be new.

THE TEMPERATURE QUOTIENT, $Q_{10°}$, OF THE RATE OF GROWTH

In many chemical reactions a 10°C. rise in temperature increases the rate of reaction two or three times (van't Hoff rule) Since many biological reactions (including reproduction) are governed in part at least by rates of chemical reaction, it is of interest to determine the relationship between the rates of growth of bacteria at different temperatures, even though the significance of the relationship is not well understood.

The ratio between the velocity constants of the rate of growth at two different temperatures may be called the temperature quotient (or coefficient). Customarily, the temperature quotient is recorded for a 10°C. interval as $Q_{10°}$, the formula being

$$Q_{10°} = \frac{k_2}{k_1}$$

in which k_2 is the velocity constant at the higher temperature and k_1 the velocity constant at the lower (Buchanan and Fulmer, 1930, p. 34). The velocity constant k can be calculated from the generation time, as follows (Buchanan and Fulmer, 1928, p. 20):

$$k = \frac{ln\ 2}{GT}$$

It follows from this that

$$Q_{10°} = \frac{GT_1}{GT_2}$$

in which GT_1 and GT_2 are the generation times at the lower and higher temperatures, respectively.

The temperature quotients $Q_{10°}$, for all organisms were calculated by these formulas from the mean generation times during the period of logarithmic growth (table 2) at each temperature. The results are shown in table 3.

As would be expected, the values of $Q_{10°}$ within a particular temperature range vary slightly for different organisms, although the similarities in value are more marked than the differences. It will be noted that $Q_{10°}$ varies inversely with temperature. The temperature quotients are consistently larger in the lower temperatures ranges investigated, so that while with increasing

TABLE 3

Effect of temperature upon the temperature quotient $Q_{10°}$

ORGANISM	$Q_{10°}$ FOR TEMPERATURE INTERVALS		
	22°-32°C.	27°-37°C.	32°-42°C.
Esch. coli, old	2.2	1.8	1.3
Esch. coli, new	2.5	2.0	1.0
Aer. aerogenes, old	2.9	1.6	0.7
Serr. marcescens, old	2.0	1.7	
Chromo. violaceum, old	2.4	1.4	

temperature up to the optimum a concomitant increase occurs in the *absolute* rate of reproduction, it is apparent that the effect of a given temperature increase near the optimum is less, relative to the effect produced by the same increase in the lower ranges. Only within a short range would a 10°C. rise in temperature exactly double the reproductive rate; above this range the effect is less, below it, greater, although the van't Hoff rule may be said to hold roughly.

The order of magnitude of our temperature coefficients is similar to the values observed in the few other cases reported in the same temperature range for bacteria (Barber, 1908; Lane-Claypon, 1909; Chick, 1912) and for yeast (Slator, 1906; Sherwood and Fulmer, 1926).

RELATION BETWEEN THE MAXIMUM RATE OF REPRODUCTION AND LENGTH OF THE LOGARITHMIC PHASE AT THE SAME TEMPERATURE

Inspection of the growth curves (figures 1 to 5) reveals the well-known fact that at lower temperatures the logarithmic phase continues longer, and the rate of reproduction is less, than at higher temperatures. In other words, the duration of the maxi-

TABLE 4

Relation between mean generation time during logarithmic phase and length of logarithmic phase at the same temperature

ORGANISM	TEMPERATURE	MEAN GENERATION TIME (GT)	APPROXIMATE LENGTH OF LOGARITHMIC PHASE (L)	RATIO (GT/L)
	°C.	*minutes*	*hours*	
Esch. coli, old	22°	73	12	6.0
	27°	46	10	4.6
	32°	33	8	4.1
	37°	25	6	4.2
	42°	26	6	4.3
Esch. coli, new	22°	65	14	4.6
	27°	39	8	4.9
	32°	26	4	6.5
	37°	20	6	3.3
	42°	26	4	6.5
Aer. aerogenes, old	22°	62	10	6.2
	27°	33	6	5.5
	32°	21	6	3.5
	37°	21	4	5.2
	42°	29	4	7.2
Serr. marcescens, old	22°	52	10	5.2
	27°	37	6	6.1
	32°	26	4	6.5
	37°	22	4	5.5
Chromo. violaceum, old	22°	55	14	4.0
	27°	34	6	5.5
	32°	23	4	5.7
	37°	25	4	6.2

mum rate of reproduction appears to be inversely proportional to that rate. We have found a roughly quantitative ratio between these two quantities, which is about the same at each temperature over the range of these experiments. This ratio for each organism at each temperature is shown in table 4 (data from tables A to E, appendix, and table 2).

In spite of the fact that the duration of the logarithmic growth phase was not exactly determinable in every case, the ratio of generation time (in minutes) to length of logarithmic phase (in hours) is constant, within relatively narrow limits, for the same organism at different temperatures, approximating 5:1 as an average. There seems to be some indication that the ratio changes above the optimum temperature. It may also be seen from table 4 that for different organisms at the same temperature, the ratio is about the same in each case. The experiments with the cultures acclimatized to each temperature yielded almost identical results.

It is recognized, of course, that both the length of the logarithmic phase and the rate of growth therein are dependent upon the size of the inoculum (McKendrick and Pai, 1911; Graham-Smith, 1920), which fact lends weight to the assumption that the constant ratio found between minimum generation time and duration of that time is of general application.

DISCUSSION

The results of the experiments reported here have been presented for the various genera as a group, in order to bring out the general nature of the relationships. There are, of course, variations between the individual genera, although these differences are less marked than the similarities.

The observations in regard to the presence of a lag period at 22° with all organisms, when the inoculum was from cultures reproducing at a maximum rate at that temperature, needs further investigation, since it has been accepted as a general principle—at least for bacteria at *optimum* temperatures—that if the organisms are in the logarithmic growth phase, a subculture at the same temperature will show no lag (Chesney, 1916).

Buchanan and Fulmer (1928, p. 35), note that it is perhaps necessary also to take into account the effect of differences in numbers seeded in generalizing this principle. Apparently this does not hold in the present case, however, because our inoculum was 1000 to 2000 cells per milliliter, while Penfold (1914) found no lag in subculture at 37°C.—using organisms reproducing at a maximum rate—with about 500 cells per milliliter of *Escherichia coli*, and Chesney (1916) got no lag with 80 cells of *Escherichia coli* under similar conditions. Although the true explanation of our lag is not clear, it may be that bacteria *below* their optimum temperature, when subcultured while reproducing maximally, actually do not continue the maximum rate under the same conditions as do bacteria at optimum temperature.

Our results show definitely that bacterial growth curves, although sigmoid, are not symmetrical about their point of inflection. There seems then to be little justification for trying to compare a growth curve to a (symmetrical) curve of chemical autocatalysis. Furthermore, as stated by Kavanagh and Richards (1934), the demonstration of a good fit of such an autocatalytic curve to a given growth curve is no proof that the growth is autocatalytic.

The finding that the apparent optimum temperature for reproduction of *Serratia marcescens* was dependent upon the previous temperature of cultivation is not in itself new as a general principle; Zikes (1919) for example, showed that the growth-rate temperature-curve for yeast is modified by the temperature at which the strain has previously been cultivated. The observation brings up the question, however, as to how an optimum temperature should be determined—whether the growth-rate temperature-curves should be made from cultures carried at "room temperature" or from organisms first acclimatized to the various temperatures. As Stuart (1925) has shown, from a previously favorable environment (in our case, 22°) an organism may gain momentum which permits it to flourish for a time in unfavorable conditions (37°); several transplants in the new environment are necessary before the effect of growth momentum can be ruled out. In most of the experiments here,

acclimatization had no appreciable effect. In the case of *Serratia marcescens*, however, the decrease of eleven minutes in the generation time at 37°C., after cultivation at this temperature for four months, is significant. Zikes (1919, 1920) found that after cultivation of his yeast at low temperature (8°C.) for some time, growth was more rapid at the lower temperatures than before, and that there was a tendency to develop two optima.

The data presented for the temperature quotients, $Q_{10°}$, of the rate of growth at various temperatures, do not cover a wide enough range to draw broad conclusions as to the operation of the van't Hoff temperature rule in bacterial reproduction. Within the range studied, however, there is a definite decrease in the value of $Q_{10°}$ at higher temperatures with all organisms. This finding is in accord with that of most other workers, that the temperature quotient for the reproduction of microörganisms varies inversely with temperature. While it is apparent that qualitatively the effects of temperature upon bacterial reproduction are in some respects analogous to those exhibited in simple chemical reactions, microörganisms, being complex, may follow a "law" of their own.

SUMMARY

Data are presented on the form of the growth curve, and the effects of temperature upon rate of reproduction, as determined from representatives of four genera of common saprophytic bacteria. The growth curves of the organisms, *Escherichia coli*, *Aerobacter aerogenes*, *Serratia marcescens*, and *Chromobacterium violaceum*, were determined at 22°, 27°, 32°, 37°, and 42°C.—enumeration of cells being by plate count. The following results were obtained.

1. Curves of bacterial population cycles are sigmoid, but are not symmetrical about their point of inflection.

2. The presence or absence of a lag period in subcultures at the same temperature, with bacteria whose optimum is about 37°C., but which are being cultivated at 22°C., seems to be dependent, other conditions being equal, upon factors other than the age of the inoculum.

3. With one exception, the maximum rate of reproduction at a given temperature was the same whether the cultures used for inoculation were first acclimatized to that temperature or to room temperature (22°C.). The exception (*Serratia marcescens* at 37°C.) shows that sometimes the previous temperature of cultivation may influence the optimum temperature, at least for reproduction.

4. The minimum generation time of *Serratia marcescens* may be as low as 22 minutes at 37°C.; of *Chromobacterium violaceum*, 23 minutes at 32°C.

5. The *relative* effect of temperature upon maximum rate of reproduction was progressively less from 22° to 42°C., i.e., Q_{10} varies inversely with temperature.

6. At any temperature up to the optimum, the ratio of minimum generation time (in minutes) to length of logarithmic growth period (in hours) at the same temperature was approximately constant (5:1), both for the same organism at different temperatures and for different organisms at the same temperature.

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APPENDIX

TABLE A

Mean plate counts and generation times of Esch. coli, old culture, in broth

AGE OF CULTURE, HOURS	BACTERIA PER MILLILITER, THOUSANDS					GENERATION TIME, MINUTES				
	22°	27°	32°	37°	42°C.	22°	27°	32°	37°	42°C.
0	2.7	2.7	2.7	2.7	2.7					
2	9.5	17.9	40.0	77.0	74.0	66	44*	31*	31	25*
4	20.8	86.0	500.0	2,350.0	1,760.0	106	53*	31*	24*	26*
6	78.0	420.0	5,870.0	60,000.0	34,300.0	63*	53*	35*	26*	28*
8	251.0	3,500.0	72,000.0	126,000.0	56,000.0	71*	39*	33*	112	169
10	720.0	24,400.0	95,000.0	173,000.0	63,000.0	79*	43*	300	227	706
12	1,980.0	90,000.0	132,000.0	145,000.0	57,000.0	83*	64	324	−19	−19
14	6,900.0	117,000.0	183,000.0			67*	316	324		
16	20,100.0	170,000.0	324,000.0			78*	226	146		
18	43,000.0	152,000.0	207,000.0	165,000.0		110	−19	−20	2000	
20	100,000.0	176,000.0				99	571			
22	129,000.0	204,000.0				333	571			
24	147,000.0	246,000.0	282,000.0	195,000.0	136,000.0	667	444	818	1500	576

* Generation time during logarithmic growth period.

TABLE B

Mean plate counts and generation times of Esch. coli, new culture, in broth

AGE OF CULTURE, HOURS	BACTERIA PER MILLILITER, THOUSANDS					GENERATION TIME, MINUTES				
	22°	27°	32°	37°	42°C.	22°	27°	32°	37°	42°C.
0	1.6	1.6	1.6	1.6	1.6					
2	1.5	1.4	2.4	4.2	1.3	−18	−19	194	85	−19
4	2.5	10.2	19.9	62.0	49.0	160	42*	39	31	23*
6	7.1	128.0	470.0	3,700.0	1,010.0	81	33*	26*	20*	28*
8	21.0	1,020.0	7,100.0	60,000.0	10,200.0	77	40*	31	30	36
10	59.0	7,600.0	43,000.0	121,000.0	67,000.0	81	42*	47	119	44
12		23,500.0	78,000.0	173,000.0	66,000.0		74	141	235	−18
14	800.0	44,000.0				64*	133			
16	2,720.0	59,000.0				68*	286			
18	10,200.0					63*				
20	25,400.0					92				
22	43,800.0					154				
24	68,000.0	189,000.0	199,000.0	350,000.0	115,000.0	190	287	534	713	900

* Generation time during logarithmic growth period.

TABLE C

Mean plate counts and generation times of Aer. aerogenes, old culture, in broth

AGE OF CULTURE, HOURS	BACTERIA PER MILLILITER, THOUSANDS					GENERATION TIME, MINUTES				
	22°	27°	32°	37°	42°C.	22°	27°	32°	37°	42°C.
0	1.9	1.9	1.9	1.9	1.9					
2	3.1	4.7	7.6	16.7	10.3	170	92	60	38	49
4	5.1	22.7	146.0	820.0	170.0	170	53	28	21*	30*
6	19.1	268.0	7,500.0	44,000.0	3,200.0	63*	34*	21*	21*	28*
8	53.0	3,400.0	80,000.0	161,000.0	27,000.0	81*	33*	35	64	39
10	228.0	42,000.0	183,000.0	152,000.0	52,000.0	57*	33*	101	−18	128
12	990.0	113,000.0	193,000.0	180,000.0	55,000.0	57*	85	1714	500	1500
14	4,300.0	164,000.0				57*	226			
16	22,700.0	187,000.0				50*	667			
18	72,000.0	175,000.0	207,000.0	193,000.0	52,000.0	72*	−18	3600	1200	−55
20	127,000.0					148				
22	157,000.0					400				
24	165,000.0	218,000.0	280,000.0	370,000.0	70,000.0	1714	1161	837	387	857

* Generation time during logarithmic growth period.

TABLE D

Mean plate counts and generation times of Serr. marcescens, old culture, in broth

AGE OF CULTURE, HOURS	BACTERIA PER MILLILITER, THOUSANDS				GENERATION TIME, MINUTES			
	22°	27°	32°	37°C.	22°	27°	32°	37°C.
0	1.8	1.8	1.8	1.8				
2	3.1	6.8	7.3	8.7	148	62	59	52
4	8.9	33.0	102.0	31.0	79	53	32	80
6	47.0	312.0	2,390.0	1,390.0	50*	37*	26*	22*
8	176.0	3,000.0	32,000.0	22,400.0	63*	37*	32	30
10	900.0	23,500.0	133,000.0	68,000.0	51*	40	59	75
12	6,500.0	85,000.0	217,000.0	143,000.0	42*	65	169	112
14	29,000.0	143,000.0			56*	160		
16	49,000.0	211,000.0			160	214		
18	122,000.0	240,000.0	360,000.0	235,000.0	92	667	493	507
20	176,000.0				231			
22	190,000.0				1091			
24	310,000.0	460,000.0	490,000.0	410,000.0	169	387	818	450

* Generation time during logarithmic growth period.

TABLE E

Mean plate counts and generation times of Chromo. violaceum, old culture, in broth

AGE OF CULTURE, HOURS	BACTERIA PER MILLILITER, THOUSANDS				GENERATION TIME, MINUTES			
	22°	27°	32°	37°C.	22°	27°	32°	37°C.
0	1.2	1.2	1.2	1.2				
2	1.4	1.5	1.8	1.5	462	293	197	353
4	3.4	9.9	21.7	17.4	92	45	33	34
6	11.4	61.0	780.0	520.0	69	46	23*	25*
8	65.0	690.0	15,700.0	7,600.0	48*	34*	28	31
10	262.0	8,000.0	92,000.0	38,000.0	60*	34*	47	52
12	980.0	50,000.0	126,000.0	88,000.0	63*	46	267	99
14	5,200.0	88,000.0			50*	148		
16	25,000.0	147,000.0			53*	162		
18	51,000.0	202,000.0	184,000.0	137,000.0	118	267	667	571
20	85,000.0				164			
22	111,000.0				316			
24	132,000.0	226,000.0	216,000.0	127,000.0	480	2250	157	−55

* Generation time during logarithmic growth period.