AGEING WITHOUT REPRODUCTION AND THE VIABILITY OF YOUNG BACTERIAL CELLS AT LOW TEMPERATURES

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Since it was first shown that the actively-reproducing, young cells of the coliform and proteus groups of bacteria are killed by a number of mild inimical factors which are relatively or entirely without effect on mature cells, an extensive literature has developed on the physiological differences between young and old bacterial cells. This subject has been admirably summarized and discussed in a recent review by Winslow and Walker (1939).

It is known that such treatments as a sudden drop in temperature from 37° to 2°C., and the transfer to weak saline solutions containing only 2 to 5 per cent sodium chloride, which are without measurable effect upon mature cells, are lethal to a high percentage of young cells; likewise, exposures to temperatures only slightly above the maximum for growth (50° to 53°C.) kill a much larger percentage of the young cells than of the mature ones; and exposures to germicidal agents such as phenol also show the greater sensitivity of the young cells (Sherman and Albus, 1923). More striking is the fact that sudden environmental changes even within the natural range of growth of the organism may kill a large proportion of the young cells (Sherman and Cameron, 1934). For example, when a culture of *Escherichia coli* growing at 45°C. is suddenly chilled to 10°C., more than 90 per cent of the young cells may be killed; however, the change must be a sudden one, apparently a "shock" effect, as the gradual cooling of an actively growing culture (at 45°C.) to 10°C., over a period of 30 minutes, has no apparent effect. Of interest also is the fact that during the "lag" period mature bacterial cells, which are unaffected by such mild environmental changes, become sensitive just before active reproduction begins, indicating a definite

physiological rejuvenescence in which the old cells acquire the properties of young ones before cell division starts (Sherman and Albus, 1924); and that earlier in the lag phase other differences may be detected, such as in heat tolerance (Elliker and Frazier, 1938) and in fermentative abilities (Hegarty, 1939).

Aside from the subject of the present work, are the equally interesting morphological changes in bacterial cells during the various phases of the culture cycle (Clarke and Ruehl, 1919; Henrici, 1921–22) which have been especially exploited by Henrici in a series of valuable investigations and the whole topic reviewed in a monograph (Henrici, 1928). Likewise aside from the immediate concern of this paper are the many fundamental and valuable contributions to the physiology and biochemistry of young bacterial cells; these, however, are summarized by Winslow and Walker (1939).

II

Some simple points of considerable theoretical interest, and perhaps of practical importance, arise in connection with this general problem. For example, the question of whether or not young bacterial cells age, thus acquiring the properties of mature cells, when held under conditions which preclude growth. One would naturally expect that they would, at least under some conditions, but so far as we are aware the question does not appear to have been answered. If ageing in bacteria is independent of growth, it would be of interest to know something concerning the extent to which the two processes can be divorced. As ageing, like other biological phenomena, is undoubtedly dependent upon a complex of chemical reactions, it is logical to expect that it should be subject to rather rigid limitations.

Related to the foregoing problem and perhaps dependent upon it, is the question of the relative viability of young and mature bacterial cells in the absence of growth and distinctly lethal factors, as at temperatures below the minimum for reproduction, or in the dry state. The practical implications of this question are rather great; in the preservation of bacterial cultures, and probably in a number of other connections. Off hand, it might be

expected that young cells, being more delicate, would die faster than mature cells under such conditions; but, if ageing can take place in the absence of growth, just the opposite might be the logical expectation. At any rate, it is a simple question of theoretical and physiologic interest which should be answered.

III

We approached this problem by holding young cells of *Escherichia coli* at 1°C. A 4-hour culture at 37°C. was gradually cooled (about 15 minutes) in a water bath to 1°C., at which temperature it was maintained in an incubator placed in a refrigerator room of lower temperature. Periodically, a portion of the culture was removed, warmed gradually to 37°C., and subjected to cold shock by instantaneous chilling to 1°C.; counts being made, before and after the cold shock, by the plate culture method. At each period, also, a portion of the warmed sample was incubated at 37°C. and plate counts made at hourly intervals for at least three hours. Thus, the criteria of youth in the cells were sensitivity to cold shock and the ability to initiate reproduction without lag. As a control, mature cells (24-hour culture in 1 per cent peptone) were held at 1°C. and periodically subjected to the same treatments, in this case the old cells being transferred to fresh 1 per cent peptone to determine lag.

TABLE 1

Young cells of Escherichia coli held at 1°C.

DAYS HELD	PER CENT KILLED BY COLD SHOCK	PER CENT INCREASE DURING 1ST HOUR	PHYSIOLOGICAL CONDITION
0	75		Young
2	70	200	Young
4	60	500	Young
7	70	110	Young
14	95	180	Young
29	65	160	Young
36	65	140	Young

Note: These results, as well as those in tables 2, 3 and 4, have been substantiated by two additional experiments.

The results of this experiment are condensed in table 1. Young

cells remained young over the 36-day period; they continued to be sensitive to cold shock, and retained the ability to reproduce without lag.

It is unnecessary to tabulate the data on the mature cells. As would be expected, they retained the properties of mature cells, under the conditions of this experiment.

Simultaneously with the experiments on *Escherichia coli*, similar tests were conducted with *Streptococcus lactis*—but with quite different results. In this case, the organism was grown in a broth containing 1 per cent tryptone, 0.5 per cent yeast extract, 0.2 per cent K_2HPO_4, and 0.05 per cent glucose. As young cells of *Streptococcus lactis* are not measurably sensitive to cold shock,

TABLE 2

Young cells of Streptococcus lactis held at 1°C.

DAYS HELD	PER CENT INCREASE DURING 1ST HOUR	PHYSIOLOGICAL CONDITION OF CELLS
2	140	Young
4	140	Young
7	25	Mostly mature
14	15	Mostly mature
21	0	Mature
36	0	Mature
42	0	Mature

the criterion of youth in the cells was the ability to initiate growth without lag.

Table 2 shows that at 1°C. the young cells of *Streptococcus lactis* remained young for less than one week and then progressively aged, losing entirely the ability to grow without lag. Although the young cells of *Streptococcus lactis* showed ageing at 1°C., the mature cells gave no indication of becoming young—the only probable result under the conditions of this test. Rejuvenescence without growth, if possible, is quite a different problem demanding appropriate technique.

The empirical conclusion to be drawn from these experiments is that at 1°C. young cells of *Escherichia coli* do not age, whereas the young cells of *Streptococcus lactis* do; that the process of age-

ing has not been divorced from growth in the case of the coliform organism, but that the young cells of *Streptococcus lactis* may become mature in the absence of reproduction. However, it scarcely seems logical to conclude that these two bacteria are fundamentally different in such an important respect. It should be remembered that the minimum temperature for growth of *Streptococcus lactis* is usually below 5°C., that of *Escherichia coli* about 8°C., and that some disparity in their minimum temperatures for ageing is quite possible.

Additional experiments on this phase of the problem are obviously indicated: Tests with young cells of *Escherichia coli* at higher temperatures, but below the minimum for growth, in an effort to obtain ageing without reproduction in this species; experiments with streptococci which have higher minimum temperatures for growth than does *Streptococcus lactis*, in order to see if ageing can also be inhibited at low temperatures in the young cells of this genus. Studies should also be made of young bacterial cells under other conditions where growth is inhibited; as dried, frozen, and in non-nutrient liquids. The possibility of rejuvenescence in the absence of reproduction likewise deserves investigation. It is hoped that further work along these lines may be initiated in the near future.

IV

Work on the other objective of this study, to determine the relative death rates of young and mature bacterial cells at temperatures below the minimum for growth, was encompassed in the same series of experiments which have already been described. Young and mature cells (4-hour and 24-hour cultures at 37°C.) of *Escherichia coli* and *Streptococcus lactis* were held at 1°C., plate counts and tests for the physiologic condition of the cells being made intermittently.

In table 3 it is shown that the young cells of *Escherichia coli* die much more rapidly than do the mature cells, when held at 1°C. As has been shown, the young cells at this temperature remain young, and the mature cells show no indication of becoming young. In view of the greater general sensitivity of young cells,

this result is probably in line with logical expectations. In contrast, the behavior of young cells of *Streptococcus lactis* (table 4) is exceedingly interesting.

TABLE 3

Death of young and mature cells of Escherichia coli at 1°C.

DAYS HELD	VIABLE BACTERIA PER ML.	
	Young cells	Mature cells
0	8,600,000	
2	1,470,000	650,000,000
4	490,000	460,000,000
7	125,000	440,000,000
14	4,400	192,000,000
29	400	95,000,000
36	72	43,000,000
42		39,000,000
51		16,400,000
62		10,400,000

TABLE 4

Death of young and mature cells of Streptococcus lactis at 1°C.

DAYS HELD	VIABLE BACTERIA PER ML.	
	Young cells*	Mature cells
0	28,000,000	
2	16,000,000	350,000,000
7	18,700,000	167,000,000
14	12,200,000	38,000,000
21	11,500,000	10,100,000
36	2,800,000	<10,000
42	2,650,000	<1
51	2,060,000	<1
62	620,000	

* See table 2.

The greater viability of "young" cells of *Streptococcus lactis* loses its mystery and becomes quite logical in view of the results reported in the preceding section. As already reported, at 1°C. the young cells of this organism matured in about one week. It is therefore our belief that the differences in the relative viabil-

ities of young and old cells of *Escherichia coli* and *Streptococcus lactis* are to be explained on the basis of the ageing, without reproduction, of the latter organism at the temperature used in these experiments; but further investigations should be made before a definite conclusion is drawn on this point.

V

Aside from the points of theoretical interest, the results reported, if subsequently verified, have some obviously important implications of a practical nature; as in the viability of stored bacterial cells and cultures. For example, diverse results have been reported on the longevity of cultures of the same organism when stored at temperatures below the minimum for growth. In some such cases it is entirely possible that each of the conflicting investigators was entirely correct in his conclusions, under the conditions of his own experiment. The results obtained would obviously be very markedly affected according to whether or not the culture had passed its logarithmic phase of growth before storage, and, further, if the nature of the organism or the storage temperature used were such as to permit ageing in the absence of growth.

Further discussion of the biological implications of the results obtained in this work should await additional and more incisive experiments.

SUMMARY

Young cells of *Escherichia coli* held at 1°C. remained physiologically young throughout the 36-day experimental period.

Young cells of *Streptococcus lactis*, on the other hand, progressively aged at 1°C., having the properties of mature cells after about one week.

The death rate of young cells of *Escherichia coli* at 1°C. was faster than that of mature cells.

In contrast, young cells of *Streptococcus lactis* at 1°C., possibly because of their ability to age at this temperature, had greater viability than did mature cells.

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