Variation in Sensitivity of *Escherichia coli* to Freezing Damage During the Growth Cycle.* (22523)

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The degree of susceptibility of bacteria to freezing damage has been said to vary during the growth cycle. Fry and Greaves(1) noted better (15%) survival when a 20-hour culture of a paracolon bacillus was dried from the frozen state, than (3%) when a 4½-hour culture was frozen and dried. Similar experiences were recounted at the 1954 London Freeze-Drying Discussion(2).

This variation was examined in the following experiments.

Methods. The general methods employed were similar to those previously described(3). The organism was an old laboratory strain of *Escherichia coli.* Trypticase soy broth (BBL) was used for cultures and dilutions, and trypticase soy agar (BBL) for plate counts. Plate counts were made with tenfold serial dilutions. Freezings and thawings were accomplished with 2.0 ml quantities in 100 x 13 ml pyrex tubes in an alcohol dry ice bath and in a 35° water bath for 10 minutes each. Glycerol was not used in these experiments.

A. *Comparison of sensitivity of a 3-hour and a 24-hour culture of E. coli.* Aliquots of a 3-hour and of a 24-hour culture were subjected to from 1 to 10 cycles of freezing and thawings after which the survivors were determined by the plate counting method (Fig. 1). The cells of the 24-hour culture were more resistant and were destroyed at a slower rate during the repeated freezings and thawings.

B. Changes in sensitivity during growth cycle. Plate counts were made at hourly intervals from a 20-hour 40-ml culture of *E. coli.* Essentially logarithmic growth was observed during the first 5 hours (Table I). Aliquots taken at the same time were counted after 2 cycles of freezing and thawing (Table I). Freezing and thawing was much more destructive for the cells of the logarithmic growth phase, than for the cells of the original inoculum, or those after 5 hours of growth. In this experiment the amount of destruction caused by 2 cycles of freezing and thawing increased from about 75% with the original inoculum, to more than 99.9% after 1 hour, and then decreased after 5 hours.

Discussion. Many of the properties of bacterial populations vary during the growth cycle. This is true for cell morphology and staining(4,5); bacterial metabolism(6,7); susceptibility to injury by heat(8-10); cold (11); and phenol(10); susceptibility to damage by changes in osmotic pressure(12); and by changes in barometric pressure(13); and for susceptibility to injury by ionizing radiations(14).

It is of interest that the changes in susceptibility to freezing damage during the growth cycle are parallel with changes in susceptibility to osmotic pressure(12), and barometric pressure(13), since these pressures have been implicated in the mechanism of freezing damage. However, since the changes

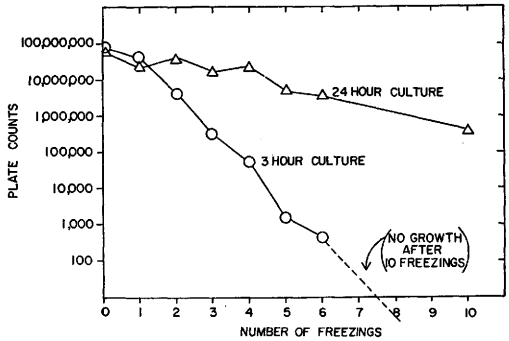

FIG. 1. Plate counts of *E. coli* cultures after successive freezings and thawings.

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TABLE I. Plate Counts of an *Escherichia coli* Culture at Intervals during Growth, Unfrozen and after 2 Cycles of Freezing and Thawing.

Age of culture (hr)	Unfrozen	Frozen twice	Surviving fraction after freezings (%)
0	33,000	9,300	28.2
1	480,000	238	.05
2	2,500,000	1,320	.05
3	32,000,000	4,070	.01
4	183,000,000	102,000	.06
5	600,000,000	44,000,000	7.3
6	620,000,000	162,000,000	26.1
7	950,000,000	73,000,000	7.7
8	760,000,000	72,000,000	9.5
9	650,000,000	102,000,000	15.7

which occur during the growth cycle are poorly understood it does not seem profitable to speculate on these changes in relation to the mechanism of freezing damage.

Since it is apparent that the phase of growth may have a tremendous influence on the susceptibility of bacteria to freezing damage, it is important to consider this factor in the interpretation of freezing damage data.

Summary. The sensitivity of *Escherichia coli* to freezing damage varies during the growth cycle with maximum susceptibility during the period of logarithmic growth.

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Hydrolysis of Amino Acid Amide Derivatives of 2-Aminofluorene, 4-Aminobiphenyl and 4, 4'-Diaminobiphenyl by Leucine Aminopeptidase.* (22524)

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It has been established that carcinogenic amines, such as 2-aminofluorene, 4-aminobiphenyl and 4,4'-diaminobiphenyl (benzidine), combine *in vivo* with tissue proteins or protein precursors(1-3). Although the exact mode of combination with proteins is unknown, Greenstein, *et al.*(4) have suggested that the binding may involve a peptide bond between the amino group of the amine and a carboxyl group of the protein or protein precursor. Moreover, these investigators have demonstrated that peptide derivatives, in which the carcinogenic amine is linked to the carboxyl group of different amino acids, are susceptible to hydrolysis by tissue homogenates. Although the enzyme or group of enzymes responsible for the hydrolytic action has not been identified, it is reasonable to assume on the basis of

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