(*Reprinted from Nature, Vol.* 218, *No.* 5140, *pp.* 464–465, *May* 4, 1968)

Evolutionary Advantage of Control of a Biosynthetic Pathway

BECAUSE uncontrolled production of metabolites is rare in nature it has been concluded that the ability to regulate biosynthetic pathways confers an evolutionary advantage on a species. The advantage has been interpreted as arising from a more efficient use of energy which leads to more rapid growth in a competitive environment. The advantage gained by control of a pathway can thus be estimated by comparing the relative growth rates of two strains which are isogenic, except that one lacks control of the pathway.

We have compared the growth of wild type *Escherichia coli* (*W*) with that of a strain which lacks the ability to control the synthesis of proline (*WP*1) in a mixed culture. Control of this pathway is attributed largely, if not entirely, to end-product inhibition by proline[1,2].

In a mixed culture the ratio of the numbers of the two strains is a function of the initial ratio and of their relative growth rates. This can be expressed as

$$\ln R/R_0 = n\left(\frac{g_W - g_{WP1}}{\bar{g}}\right)$$

where R is the ratio of the amounts of $WP1/W$, g is the generation time, n is the number of generations, and assuming that $\bar{g} = (g_{WP1}g_W)^{1/2}$.

A plot of $\ln R/R_0$ against n yields a straight line, the slope of which can be calculated to give the percentage difference in the generation times of the two strains.

As can be seen from Table 1, there is a consistent decrease in the amount of *WP*1 compared with the amount of *W* with time. The differences in the generation times are strongly dependent on the composition of the growth medium; the richer the medium, the less the growth differential. This is particularly marked when a complete medium is used, but can be seen using glutamic or aspartic acid as well. At a low concentration proline enhances the differential between the two strains, while at higher concentrations it produces a growth pattern approaching that found in minimal medium. These data can be interpreted as indicating that the presence of proline in low concentrations increases the selective advantage of the controlled strain, while at higher concentrations this effect is overcome by the presence of additional nutrient.

Table 1. EFFECT OF THE MEDIUM ON THE RELATIVE GROWTH RATES OF BACTERIA SHOWING CONTROLLED AND UNCONTROLLED SYNTHESIS

Medium	Percentage difference in growth rates	Generations required to change the ratio of *WP*1/*W* 1 : 10
Minimal[1]	4·7	49
Minimal + 1/2 brain-heart infusion	1·8	130
Minimal + glutamic acid 0·1 mg/ml.	3·0	77
Minimal + proline 0·1 mg/ml.	6·7	34
Minimal + proline 0·01 mg/ml.	7·6	30

One ml. of a mixed culture of *W* and *WP*1 which contained approximately equal numbers of the two strains was inoculated into 1 l. of medium. After stationary phase had been reached, 1 ml. of this culture was used to inoculate 1 l. of medium in a flask. Because the dilution 1 : 1,000 is close to 2^{10}, each flask was considered to contain ten generations. Cells of *WP*1 were identified in the mixture by their ability to cross-feed a proline deficient strain. The death rate of the two strains in these conditions was identical; the ratio, *WP*1/*W*, did not change in 4 days at 37° C.

Zamenhof and Eichhorn[3] have shown that derepression of the tryptophan pathway in *Bacillus subtilis* causes a marked decrease in the survival potential of this organism when it is compared with that of the wild type. The change in the ratio for these organisms was 10^5 in twenty-six generations. The effect of the loss of control of the proline pathway leads to much less of a decrease in the survival potential. This can be explained by a difference in the organism or a difference in the generation time, which was 4 h for *B. subtilis*. Another difference is that in the proline pathway the only saving of energy arises from the synthesis of the amino-acid, rather than from the synthesis of a series of enzymes. A pathway subject to end-product inhibition seems to enjoy less of a selective advantage in a competitive environment than one which utilizes a repression mechanism. Loss of control of the pathway would, however, be less harmful to the species.

It is possible that the growth differential measured between regulated and unregulated pathways in an organism can be used to distinguish pathways which are controlled by repression from those controlled by end-product inhibition. This investigation was supported by a U.S. Public Health Service research grant from the National Cancer Institute. One of us (A. B.) is the recipient of a Research Career Development Award.

A. BAICH
M. JOHNSON

Department of Biochemistry and Biophysics,
Oregon State University
Corvallis, Oregon.

Received February 15, 1968.

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Printed in Great Britain by Fisher, Knight & Co., Ltd., St. Albans.