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General Discussion

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Patricia H. Clarke, F.R.S. (*Department of Biochemistry, University College London, U.K.*). Several speakers have discussed phenomena associated with growth of microorganisms on surfaces. In the natural environment much of the surface area available for microbial growth consists of plant structures. Perhaps Professor Veldkamp might have comments on microbial associations with plant surfaces.

H. Veldkamp. The epidermis of plant leaves is covered by a waxy coating, the cuticle. Microbial degradation of this layer occurs by means of exoenzymes and the development of a microbial flora on the surface is strongly dependent on the wettability of the cuticle. Once a flora of microbes (moulds, yeasts, bacteria) has developed which erodes the leaf surface, the underlying tissue releases organic compounds and inorganic ions. Often only a small part of this is utilized by the microflora on the leaf surface because large quantities of material may be washed off by rain. Therefore the activities of the surface-eroding flora may have a considerable influence on the entire ecosystem of a natural vegetation.

J. R. Quayle, F.R.S. (*Department of Microbiology, University of Sheffield, U.K.*). This question is probably being addressed in the first place to Professor Harder and Professor Kuenen. Many catabolic and biosynthetic enzyme systems have now been shown to be derepressed at low growth rates under substrate limitation. This means that the organism is triggered to respond immediately to transient appearance of substrate. By contrast, other enzyme systems need the presence of an inducer and hence the organism cannot respond as quickly to the appearance of substrate as it could if the enzyme system were merely repressible. What then are the particular advantages of the inducible system over the repressible system?

W. Harder (*Department of Microbiology, University of Groningen, The Netherlands*). Unfortunately, very little is known about the significance of the two strategies in microbial competition in Nature. Therefore we can only speculate about the possible advantages of inducible enzyme systems over repressible systems. I should like to suggest that the first strategy may be of advantage to organisms thriving in environments in which the turnover rate of substrate(s) is relatively high and in which the nature of the growth substrate changes in time. The alternative strategy is probably advantageous to organisms that occur in environments in which the turnover rates of various compounds is low and in which the composition of the available nutrient mixture is fairly constant in time. We know very little about the last group of organisms and it is therefore entirely possible that our current ideas about the relative incidence of the two strategies is not correct.

J. G. Kuenen (*Laboratory of Microbiology, Delft University of Technology, The Netherlands*). Many different strategies may have developed during evolution to cope with the fluctuations and diversity of nutrients in the environment. Indeed the reaction of *Ps. oxalaticus* to low supply of oxalate seems to anticipate a possibility for autotrophic growth. If it has competitive value for the organism to react this way this trait may survive. On the other hand it may well be that the organism is never exposed for a prolonged time to extreme slow growth under oxalate limitation. In the latter case the derepression of RuBP carboxylase may just be an incidental property of the system for which selection has taken place on entirely different grounds.

Thiobacillus A2, a very versatile, facultatively chemolithotrophic *Thiobacillus* when grown on acetate, did not show derepression of RuBPcase even at not very low growth rates. In fact the same is true for *Ps. oxalaticus* growing on acetate at very low dilution rates. It is interesting that *Thiobacillus* A2 always maintains low levels of inducible enzymes, allowing low but substantial respiratory capacities for a large number of substrates (10–80 μl O_2 mg^{-1} d.m. h^{-1}). This permits the organism to respond extremely rapidly to changes in the quality and quantity of available substrates. This makes up, to some extent, for its relatively slow response compared with non-versatile (specialist) organisms. The latter bacteria always maintain very high capacities for their specialist substrates, even under starvation conditions. In this background, derepression rather than induction seems to be a compromise between constitutive and inducible enzymes, since in the absence of catabolite repression such enzymes 'act' as constitutive.

B. S. Hartley, F.R.S. (*Department of Biochemistry, Imperial College, London, U.K.*). One can guess the mechanism for the important physiological response that Professor Quayle mentions. Repressors have poor promoters and translation signals, so in conditions of starvation they will compete poorly for the scarce components essential for protein synthesis. Paradoxically this will *increase* the levels of the enzymes that they control, which have better promoters and translation signals. Hence the cell becomes less specialized but fitter to react rapidly to growth on a variety of new foodstuffs that might adventitiously turn up: an important selective advantage.

N. G. Carr (*Department of Biochemistry, University of Liverpool, U.K.*). The adoption of induction in addition to derepression of enzyme synthesis as a strategy by bacteria undergoing nutritional limitation may, to an extent, be understood from the natural history of the organism's ecological niche. Nutritional limitation often leads to a general derepression of enzymes that are required for all, or most, nutritional régimes. Considerable selective advantage would be gained by acquisition of inductive control over the formation of an enzyme that is only required for a particular nutrient that may be only intermittently available, thus preventing the synthesis of protein that may not always be necessary. There would be particular selective pressure favouring inductive control over an enzyme that represents a relatively large, say 1–2 %, proportion of the total cell protein when fully expressed.

Patricia H. Clarke. Catabolic enzymes are mostly inducible. In many cases it has been observed that the substrate affinities are fairly low with K_m values of the order of 1 μM. This relatively low substrate affinity is of no disadvantage under fully induced conditions when large amounts of catabolic enzymes are synthesized. On the other hand, the recorded $K_{inducer}$ values are often one or two orders of magnitude lower. The organism is thus able to respond fairly quickly to a sudden appearance of a metabolizable substrate even at low concentrations. Also, at very low growth rates in the absence of alternative carbon sources, the influence of catabolite repression will be negligible and the enzyme system will be poised to respond to the inducer.