"The Significance of Adaptive Enzymatic Patterns in the Study of Terminal Respiration."

R. Y. Stanier

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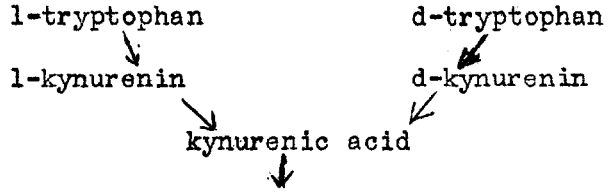
The analysis of adaptive enzymatic patterns, "simultaneous adaptation", can be used to advantage in unravelling microbial metabolic reaction chains. This represents an extension and refinement of the kinetic approach to problems of intermediary metabolism made possible by the substrate-activated nature of many microbial enzymes. The principles of simultaneous adaptation can be stated as follows:

1. If the dissimilation of a given substance A proceeds through a series of intermediates B, C, D, E, F, G. . . . . and, if the individual steps in this chain are under adaptive enzymatic control, adaptation to A will produce cells that are simultaneously adapted to B, C, D, E, F, G.....

2. If adaptation to A fails to adapt the cells to a postulated intermediate X, then X cannot be a member of the reaction chain leading from A.

Although the general validity of these two postulates are self-evident, certain problems arise when they are applied to a given set of experimental data. In the case of postulate 1, simultaneous adaptation to the actual intermediates is a necessary consequence but does not exclude the possibility that the primary substrate may also activate adaptive enzymes not involved in the dissimilation in question. Too little is known about the specificity of activation of adaptive enzymes to assess this possibility accurately, but it should be kept in mind when interpreting cases of simultaneous adaptation. For example, we have recently shown that a bacterium capable of attacking both isomers of tryptophan adaptively shows only partial specificity in its adaptive responses to the isomers. Specific activation of the l-tryptophan-oxidizing system occurs as a result of exposure to the l-isomer, but both enzymatic systems are activated after exposure to the d-isomer. Rigid interpretation according to postulate 1, might imply that the l-isomer is an intermediate in the dissimilation of the d-isomer. However, there is other evidence to indicate that the most probable pathway for the oxidation is:



and hence the adaptation to both isomers after exposure to the d-isomer alone appears to represent a case of non-specific activation. In cases where non-specific activation occurs it is likely to be evident from a consideration of over-all adaptive patterns.

In the case of postulate 2 it has been suggested by several workers that

permeability may constitute a major problem. According to this argument non-adaptation to a postulated intermediate might mean that the intermediate when externally supplied fails to penetrate across the cell membrane. The force of this objection is greatly diminished if it can be shown that adaptation to the substance in question brings about its immediate rapid oxidation.

If this is so the argument for permeability barrier can only be maintained by assuming that adaptation per se represents a change in permeability, and there is already substantial evidence against this belief. A decisive refutation of the permeability argument can be made by the use of dried cell preparations. Our work with such preparations has already shown that pre-establishment of a specific adaptive pattern is a factor of crucial importance in obtaining in vitro activity against presumptive intermediates. An illustration is provided by studies on the oxidation of aromatic compounds by Pseudomonas fluorescens. Recent work has shown that polyhydroxy aromatic compounds are involved in the dissimilation of aromatic acids. Cells grown on mandelic acid or benzoic acid (both of which belong in a common reaction chain) show complete simultaneous adaptation to catechol but not to protocatechuic acid, while cells adapted to parahydroxy benzoic acid show the exact reversal of this pattern. Dried cells grown on mandelate or benzoate show enzymatic activity against catechol but none against protocatechuic acid, while parahydroxy benzoic grown cells are active against protocatechuic but not against catechol. The biochemical implications of this work will also be discussed.

"Bacterial Enzymes as Biochemical Tools."

Fritz Lipmann

Massachusetts General Hospital, Boston

The contribution due to microbial systems in the elaboration of the present metabolic picture will be assessed from the earliest work on fermentation through the development of the vitamin-coenzyme relationship to the present approach to understanding of synthetic mechanisms.

In some detail the steps will be retraced leading from the finding of acetyl phosphate in Lactobacillus delbrückii to the presumed general role of carboxyl activation in biosynthesis. In particular, present results and outstanding problems will be discussed regarding the two-carbon intermediacy in fat and carbohydrate metabolism.

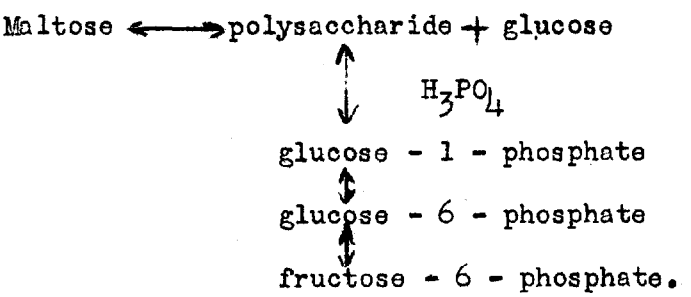
"Enzymes Involved in Bacterial Metabolism of Disaccharides and Polysaccharides."

Michael Doudoroff

University of California, Berkeley

Some microorganisms can utilize certain disaccharides more rapidly or in a different manner than they can use the constituent monosaccharides. In a few cases it has been possible to demonstrate the existence of special enzymes which appear to be responsible for the observed differences in mono- and disaccharide utilization.

Some enzymes catalyze the formation of polysaccharides from disaccharides, such as the production of dextran from sucrose, levan from sucrose and raffinose, and of starch-like polysaccharides from sucrose and maltose respectively. All of the above enzymes may be considered to be transglycosidases since they are capable of exchanging glycosidic linkages. In the case of a mutant of E. coli which is capable of fermenting maltose more rapidly than glucose, it has been shown that this ability may at least in part be explained by the presence of both amylomaltase and phosphorylase. The following reactions have been found to take place with dry cell preparations:



Another type of attack on disaccharides is the direct phosphorolytic cleavage, as found in the phosphorolysis of sucrose by Pseudomonas saccharophila and Pseudomonas putrefaciens. The "sucrose phosphorylase" of Pseudomonas saccharophila has been shown to be also a "transglycosidase", which can use a number of "glucose acceptors" other than phosphate and fructose.

In many cases, the preferential utilization of disaccharides cannot be explained on the basis of knowledge of the enzymes involved.

Thus, the known enzyme mechanisms fail to explain why the entire molecule of maltose or sucrose is utilized rapidly and completely by intact cells of E. coli and of P. saccharophila respectively, while glucose and fructose are not attacked. Other disaccharides, such as melibiose and trehalose, are used rapidly by P. saccharophila, although no phosphorolysis can be detected in vitro or in vivo. Other anomalous cases of rapid disaccharide utilization are found in the metabolism of lactose by certain yeasts and by Lactobacillus bulgaricus.

Several hypotheses for the difference in rates of disaccharide and monosaccharide utilization by intact cells will be discussed.

"Genetic Studies on Lactose Fermentation in Escherichia coli."

Joshua Lederberg

University of Wisconsin, Madison

1. Methods of obtaining mutants.
2. Assay and properties of b-galactosidase, using o-nitrophenyl galactoside.
3. Adaptive properties. (Adaptation to lactose, galactosides). Also, comment on the evocation of the enzyme by lactobionate, which does not seem to react with it.
4. The genetic types of lactose-negative mutants found. Some are specific, others affect maltose, glucose, gluconate-fermenting systems.
5. The effects of the genotype in modifying the adaptive response:

   a) Temperature sensitive allele of Lac_3^- which has a different threshold for the formation of "malto-zymase" and galactosidase. The point that temperature-sensitivity is in the enzyme-forming mechanism, not in the enzyme produced.

   b) The failure of Lac_1^- to respond to lactose, although it does respond to butyl-galactoside, and these cells can ferment lactose. Implications of this separation of the specificity of adaptation and the enzyme produced for mechanism of adaptation, and for Stanier's technique.

6. Patterns of genetic alternative mutations (suppressors) and the uncovering of interesting biochemical problems thereby (e.g. Mal^+Glu^- which Doudoroff will discuss).

ROUND TABLE DISCUSSION ON

ENZYMOLOGY

Society of American Bacteriologists, Cincinnati, Ohio

9:00 a.m. Tuesday, May 17, 1949

The objective of this symposium is, first, to review the status of several pertinent metabolic problems and the enzymatic methods employed in their study, and, secondly, to stimulate discussion of the data, their interpretation, and implication to microbiology and to metabolic patterns in general.

The discussion will be initiated by the workers indicated, assisted by Drs. Kaplan, Spiegelman, Van Niel and Wood; in addition contributions from the floor are solicited.