# SYMPOSIUM ON MULTIPLE FORMS OF ENZYMES AND CONTROL MECHANISMS[1]

## II. Enzyme Multiplicity and Function in the Regulation of Divergent Metabolic Pathways

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### Introduction

The past two decades have seen tremendous advances in our knowledge concerning the biochemical mechanisms of synthesis of numerous cellular constituents. When considered together, one cannot help but marvel at the overwhelming complexity of cellular metabolism with its maze of overlapping and interlocking metabolic pathways leading to diverse end products. Even more impressive, perhaps, is the phenomenal capacity of the living organism to regulate its metabolism so as to direct an orderly flow of precursor compounds along the multitude of biosynthetic pathways to provide adequate supplies of each critical end metabolite. The effectiveness of this control is clearly illustrated by the simple experiments of Roberts et al. (13), showing that the biosynthesis of specific amino acids from isotopically labeled glucose is selectively discontinued when the specific amino acid in question is supplied exogeneously to the growth medium. In addition to showing that the organism has the capacity to discontinue the synthesis of those compounds adequately supplied, these experiments demonstrate that the ultimate end metabolites themselves serve as regulatory stimuli.

During the past several years, the researches of many laboratories have been directed to a study of the mechanism of end-product regulation of metabolism. (For excellent reviews of this work the reader is referred to various articles in the following publications: D. M. Bonner [ed.], *Control Mechanisms*, The Ronald Press Co., New York, 1961; Cold Spring Harbor Symp. Quant. Biol., vol. 26, 1961.) These studies led to the demonstration that the end product of a biosynthetic pathway may exert two types of restraint on those reactions leading to its synthesis, namely, "repression" and "feedback inhibition," or as it is now sometimes called "retroinhibition." Repression refers to the situation in which accumulation of the end product leads to a cessation, i.e., repression, of the synthesis of various enzymes involved in its formation (4), whereas feedback inhibition refers to the situation in which accumulation of the end metabolite causes a specific inhibition of the activity of preformed enzymes catalyzing,

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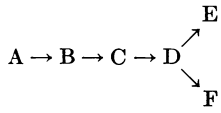
usually, an early step in the metabolic sequence leading to its formation (20). It is a consequence of either type of regulation that excessive accumulation of a particular end product will result in decreased activity of the over-all metabolic pathway leading to its synthesis. The simple elegance of these control mechanisms in the economy of the cell is at once apparent, since they allow dynamic and selective restraints of a metabolic pathway when the end product of that pathway is present in adequate amounts to satisfy the cellular requirements. Thus, the precursor compounds and the energy normally needed for the synthesis of an abundant end product can be diverted along other metabolic pathways.

Although, in principle, end-product regulation can provide the specificity and automatism essential to effective metabolic control, there are some unique situations in which such regulation would appear to present difficulties. For example, consider the situation illustrated below, in which a given sequence of reactions, A to D, represents a metabolic pathway common to the biosynthesis of two ultimate end products, E and F.




It is evident that in this situation control of reactions A through D by excessive accumulation of either end product, E or F, could cause a serious problem. If accumulation of E results in inhibition or repression of any enzyme in the sequence A through D, this could lead to curtailment of the production of D and hence to a deficiency of F.

## Enzyme Multiplicity and Function in the Regulation of Aspartate Metabolism

### *Aspartokinases of Escherichia coli*

In an effort to determine the role and consequences of end-product regulation in the metabolism of such divergent pathways, studies were carried out at the Institut Pasteur on the metabolic control mechanisms of aspartate metabolism in *E. coli*. The intermediary metabolism of aspartate presents a particularly interesting situation since, as is shown in Fig. 1, aspartate is a common precursor of three different

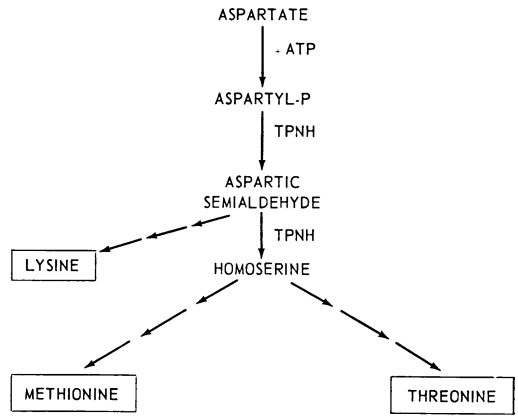



FIG. 1. *Aspartate metabolism in Escherichia coli.*

amino acids: threonine (5, 10, 2, 1), lysine (1), and methionine (1, 8).

It is obvious from examination of Fig. 1 that a serious difficulty may arise if enzymes catalyzing the early steps in this metabolic pathway are susceptible to repression or feedback inhibition by any of the ultimate amino acid end products. With such control, an excessive accumulation of any one of the three ultimate end product amino acids could reduce the production of aspartylphosphate or aspartic semialdehyde and, hence, could result in a deficiency in synthesis of the other two amino acids.

This unique situation was examined in greater detail by analysis of the end-product control of the enzyme aspartokinase, which catalyzes the first step in the metabolism of aspartate, i.e., the reaction between aspartate and adenosine triphosphate (ATP) to form aspartyl-P (17, 18).

*Feedback inhibition.* To determine whether aspartokinase is susceptible to feedback control, the ability of various amino acids to inhibit this enzyme in crude sonic extracts of *E. coli* was determined. As shown by the data of Table 1, significant inhibition was observed by the amino acids L-lysine, L-threonine, and DL-homoserine, whereas methionine was without effect. Although the degree of inhibition varies somewhat as a function of the growth conditions and extraction procedure, crude sonic extracts are usually maximally inhibited 30 to 50% by either lysine or threonine and 10 to 15% by DL-homoserine. The significance of these results in the cellular regulation of aspartokinase ac-

tivity is suggested by the fact that only those amino acids derived from aspartyl-P are capable of inhibiting this enzyme; all other amino acids that are normal constituents of proteins are without effect, as are also the D isomers of lysine and threonine and the lysine homologue, L-ornithine. The concentrations of L-lysine, L-threonine, and DL-homoserine required for one-half maximal inhibition under standard assay conditions are about 3 to 5 $\times 10^{-4}$ M. At a concentration of $10^{-2}$ M, used in the experiments summarized in Table 1, maximal inhibition by each of the individual amino acids is achieved. It is therefore of special significance that the simultaneous addition of two amino acids results in an inhibition of aspartokinase activity that is roughly equal to the sum of that observed for each independently. This result suggested the possibility that crude extracts contain three different aspartokinases, one of which is selectively inhibited by lysine, one by threonine, and one by homoserine. This interpretation was substantiated by the partial separation of two distinct aspartokinases upon fractionation of the crude cell-free extracts with ammonium sulfate. As is illustrated in Table 2, a protein fraction precipitating between 0 and 37% saturated ammonium sulfate contains aspartokinase that is inhibited 88% by lysine but is resistant to inhibition by threonine. In contrast, the fraction precipitating between 37 and 50% saturated ammonium sulfate is inhibited 73% by threonine and hardly at all by lysine. The existence of a third enzyme sensitive to homoserine is suggested by other experiments showing that the ability of homoserine to inhibit aspartokinase activity varies independently with respect to inhibition by lysine and threonine.

The lysine-sensitive and threonine-sensitive aspartokinases are further differentiated on the basis of their stability to heat and to inactivation by auto-oxidation. Exposure to air at 0 C in the absence of added sulfhydryl compounds results in a rapid loss of activity of the threonine-sensitive enzyme, but only a gradual loss of the lysine-sensitive enzyme activity. The rate of inactivation is greatly accelerated by heat. Thus, heating at 45 C for 10 min results in complete destruction of the threonine-sensitive enzyme but only 16% inactivation of the lysine-sensitive activity (17). It was further noted that some

TABLE 1. *Inhibition by various amino acids of aspartokinase in extracts of Escherichia coli K12 HfrC**

| Amino acid | Inhibition of aspartokinase activity | |
|---|---|---|
| | Expt 1 | Expt 2 |
| | % | % |
| L-Methionine | 0.0 | 0.0 |
| L-Lysine | 36.0 | 28.7 |
| L-Threonine | 39.5 | 36.5 |
| DL-Homoserine | 7.3 | 12.2 |
| L-Lysine + L-threonine | 85.0 | 76.4 |
| L-Lysine + DL-homoserine | 43.0 | 42.0 |
| L-Threonine + DL-homoserine | 46.0 | 43.0 |
| L-Threonine + DL-homoserine + L-lysine | 86.0 | 82.5 |
| L-Lysine + L-methionine | 36.5 | — |
| L-Threonine + L-methionine | 38.0 | — |
| L-Lysine + L-methionine + L-threonine + DL-homoserine | 83.0 | — |

*Each reaction mixture contained ATP, 10.4 mM; tris HCl buffer (pH 8.1), 94 mM; $MgSO_4$, 1.6 mM; $\beta$-mercaptoethanol, 10 mM; L-aspartate, 10 mM; $NH_2OH$, 800 mM; KCl, 800 mM; 0.9 mg of crude sonic extract of *E. coli* HfrC; and the other amino acids as indicated, 10 mM, except DL-homoserine which was 20 mM; total volume, 1.0 ml. Incubation was at 26 C for 30 min. The reaction was stopped by the addition of 1.0 ml of Lipmann's $FeCl_3$ reagent, and, after centrifugation, the amount of asparthydroxamate present was measured at 540 m$\mu$ in a Beckman DU spectrophotometer. Aspartokinase activity is defined as the optical density at 540 m$\mu$ $\times$ 1,000.

TABLE 2. *Separation of lysine-sensitive and threonine-sensitive aspartokinases from extracts of Escherichia coli K12 HFrC*

| Protein fraction | Protein | Total aspartokinase* | Inhibition by | |
|---|---|---|---|---|
| | | | L-Lysine | L-Threonine |
| | *mg* | *units* | % | % |
| Sonic extract | 1,225 | 208,000 | 33.3 | 50.2 |
| Dialyzed $NH_4SO_4$ precipitate (0–37%) | 395 | 46,000 | 84.7 | 2.7 |
| $NH_4SO_4$ precipitate (37–50%) | 285 | 110,000 | 3.6 | 73.0 |

*Aspartokinase activity of each fraction was measured in the presence and absence of 10 mM L-lysine and L-threonine. Otherwise, conditions were as described in Table 1.

TABLE 3. *Repression of aspartokinase by growth of Escherichia coli K12 HfrC on L-threonine and L-lysine*

| Supplements to growth medium | Aspartokinase* (units/mg) | | Feedback inhibition by | |
|---|---|---|---|---|
| | Total | Decrease | L-Lysine | L-Threonine |
| | | | % | % |
| Minimal medium..... | 184 | — | 28.5 | 46.0 |
| L-Threonine (10 mM).... | 164 | 20 | 30.0 | 43.0 |
| L-Lysine (10 mM)..... | 137 | 47 | 0 | 74.0 |
| L-Lysine (10 mM) + L-threonine (10 mM).... | 70 | 114 | 0 | 64.0 |

* Measured as in Table 1.

degree of protection against heat denaturation of the two enzymes is exerted by their respective end-product inhibitors (17).

Kinetic analysis of the inhibition by lysine and threonine of the individual lysine-sensitive and threonine-sensitive aspartokinases, respectively, shows that inhibition by L-threonine is competitive, whereas inhibition by L-lysine is noncompetitive (17). Although noncompetitive in nature, the inhibition by lysine is reversible.

*Repression.* To ascertain whether the various aspartokinases are subject to repression, a study was made to determine whether normal cellular concentrations of the various enzymes are influenced by the presence of high concentrations of threonine, lysine, methionine, or homoserine during growth (17, 18). Typical results showing the influence of threonine and lysine are summarized in Table 3. As can be seen from the data, the crude sonic extract obtained from bacteria grown on a minimal medium contains 184 units of aspartokinase activity per mg of protein; this activity was inhibited 28.5% by the addition of L-lysine to the enzyme assay system and 46% by L-threonine. When the organism is grown on a medium supplemented with $10^{-2}$ M L-threonine, a small reduction in total aspartokinase activity occurs, and the susceptibility to inhibition by threonine relative to inhibition by lysine is slightly reduced. This suggests that barely detectable repression of the threonine-sensitive enzyme may have occurred as a result of growth in the presence of added threonine. On the other hand, sonic extracts of *E. coli* grown on a minimal medium containing $10^{-2}$ M L-lysine contain much less total aspartokinase activity, and that which is present is no longer sensitive to inhibition by L-lysine. This result indicates that growth in the presence of lysine causes complete repression of the lysine-sensitive aspartokinase. In similar experiments, it was established that growth on homoserine or methionine does not influence the composition of the aspartokinase activity of sonic extracts.

It is of further interest that cell-free extracts of *E. coli*, grown in the presence of both threonine and lysine, contain considerably less total aspartokinase than would be expected from the sum of the repressions observed when the organism is grown in the presence of each amino acid alone. This result appears particularly significant in light of the recent discovery by Freundlich et al. (6) of the phenomenon of "multivalent repression," in valine, leucine, isoleucine, and pantothenate metabolism. In this situation, repression requires the concerted action of several end products of divergent metabolic pathways. The possibility therefore remains that one or both of the lysine-sensitive and threonine-sensitive aspartokinases may be controlled by multivalent repression. [Since this manuscript was prepared, the author has been made aware of an unpublished report by Freundlich (6*a*), demonstrating that the threonine-sensitive aspartokinase and homoserine dehydrogenase of *E. coli* and *Salmonella typhimurium* are subject to multivalent repression by threonine and isoleucine.] The fact that complete repression of the lysine-sensitive enzyme occurs when the organism is grown in the presence of lysine alone does not eliminate the possibility that repression of this enzyme requires the simultaneous presence of threonine and lysine, since endogenously produced threonine may be present in a sufficient concentration to cause repression of the lysine-sensitive enzyme in the presence of added lysine.

To summarize the above observations, it is obvious that *E. coli* produces three distinct aspartokinases that are subject to differential regulation by feedback inhibition or repression. Two of these enzymes are readily separated from each other by simple ammonium sulfate fractionation. Of these two aspartokinases, one is specifically and noncompetitively inhibited by L-lysine, and its formation is completely repressed when the bacterium is grown in the

presence of L-lysine. The second aspartokinase is specifically and competitively inhibited by L-threonine, but its formation is only slightly repressed when the organism is grown in the presence of added threonine; greater repression of this enzyme occurs when the growth medium is supplemented with both lysine and threonine. A relatively smaller amount of a third aspartokinase, which is specifically inhibited by DL-homoserine, is present also, but this enzyme has not received much attention as yet.

Aside from differences in relative heat stability, the three aspartokinases are recognizable in crude extracts only by their differential inhibition and repression by L-lysine, L-threonine, and DL-homoserine. They catalyze identical reactions between ATP and aspartate to form aspartyl-P, and cannot be differentiated on the basis of their responses to changes in concentrations of aspartate, ATP, $Mg^{++}$, or hydrogen ion.

### *Regulation of Later Steps in Aspartate Metabolism of E. coli*

In view of the fact that aspartyl-P formation represents the first step in a divergent metabolic pathway leading ultimately to the production of lysine and threonine, the synthesis of two distinct aspartokinases under differential control by lysine and threonine is readily understandable, since this permits independent regulation of the pathways leading to the biosynthesis of lysine and of threonine. A dilemma is thus avoided which otherwise would result if a single aspartokinase, susceptible to end-product regulation by these different amino acids, existed. In the latter instance, overproduction of one amino acid could result in a reduction of aspartokinase activity to a level below that necessary to provide adequate synthesis of the other amino acid.

A question which follows logically from this interpretation is whether the biosynthesis of lysine and threonine involves two parallel biosynthetic pathways in which all of the common enzymatic steps are spatially separated within the cell, or, alternatively, whether the separate aspartokinases supply a common pool of aspartyl-P from which both amino acids are ultimately derived. This question is resolved by the recent experiments of Gilvarg (7) and of Patte et al. (12) which contraindicate the existence of parallel pathways. Gilvarg (7) has isolated an auxotropic mutant of *E. coli* (M-145) with multiple nutritional requirements for threonine, methionine, and the immediate lysine precursor, diaminopimelate. The demonstration that the complex nutritional requirement of this mutant is due to impairment of a single genetic locus concerned with the biosynthesis of aspartic semialdehyde dehydrogenase precludes the existence of multiple enzymes catalyzing the conversion of aspartyl-P to aspartic semialdehyde (see Fig. 1). Other studies by Patte et al. (12) have failed to demonstrate the existence of more than one homoserine dehydrogenase catalyzing the further conversion of aspartic semialdehyde to homoserine.

The absence of multiple enzymes catalyzing other steps in the aspartate pathway indicates that the various aspartokinases present in *E. coli* must supply a common pool of aspartyl-P from which the three amino acids, lysine, threonine, and methionine, are ultimately derived. The presence of separate aspartokinases independently controlled by repression and feedback inhibition is understandable, since this permits end-metabolite control of the first step in the biosynthetic pathway, yet restricts this control in such a way that an overproduction of one amino acid can never cause a reduction in the size of the aspartyl-P pool to a level below that necessary for the biosynthesis of the other amino acids derived from aspartyl-P. It is evident that this explanation is untenable unless secondary controls are present to direct the flow of a diminished aspartyl-P pool toward the synthesis of the less abundant amino acids. The explanation is therefore strengthened by recent investigations in several laboratories showing the existence of multiple secondary control sites in the metabolism of aspartate. The results of these studies are summarized in Fig. 2. Of special significance is the fact that specific end-product regulation occurs for enzymes 5, 10, and 12 (Fig. 2) which catalyze the first steps in the diverging pathways leading to lysine, threonine, and methionine, respectively. Thus, as was shown by Yugari and Gilvarg (26), enzyme 5, catalyzing the condensation of aspartic semialdehyde with pyruvate to form dihydropicolinic acid (7*a*), is under selective feedback inhibition by lysine. Similarly, Wormser and Pardee (25) have presented evidence for feedback inhibition by threonine of enzyme 10,

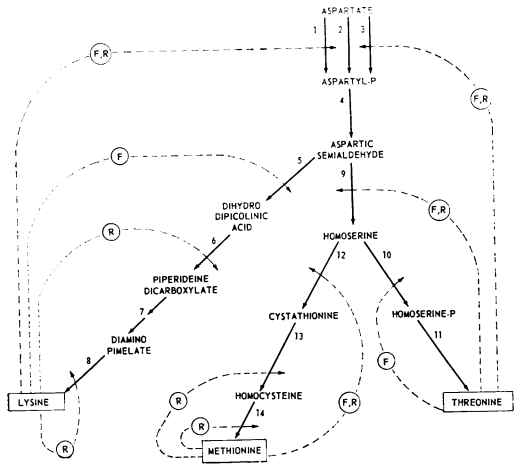



FIG. 2. *End-product regulation of aspartate metabolism. F = feedback inhibition; R = repression.*

the homoserine kinase that catalyzes the first branch step in the biosynthesis of threonine. Finally, Rowbury and Woods (14, 15) have reported feedback inhibition and repression of enzyme 12 which catalyzes the first branch reaction leading to methionine, namely, the reaction of homoserine with cysteine to form cystathionine.

It is apparent that differential control of the branching reactions by their ultimate end metabolites provides an effective means of directing the flow of aspartyl-P to the more essential amino acids. Consider, for example, the situation in which the organism is presented with an excess of lysine. The immediate effect is a reduction in the synthesis of aspartyl-P by inhibition and repression of the lysine-sensitive aspartokinase (Fig. 2, enzyme 1). The reduced supply of aspartyl-P still produced by action of the threonine-and homoserine-sensitive aspartokinases (Fig. 2, enzymes 2 and 3) is subsequently prevented from going to lysine by inhibition of enzyme 5 at the branch point of the lysine pathway; the aspartyl-P is thereby diverted preferentially into the threonine and methionine pathways. In an analogous manner, excesses of either threonine or methionine could direct the flow of aspartyl-P toward the less abundant amino acids.

In addition to these critical secondary controls at the branch points of the diverging pathways, Fig. 2 shows that the branches leading to lysine and methionine are under further end-product regulation. Thus, the reductase (enzyme 6) catalyzing the reduction of dihydropicolinic acid to piperideine dicarboxylate is repressed by lysine, and the diaminopimelate decarboxylase (enzyme 8) is repressed by lysine (11). [J. Boezi, G. N. Cohen, and T. C. Patte (*unpublished data*) have obtained evidence that the reduction step involved in the conversion of aspartic semialdehyde to lysine is under control by repression. Recent studies by Gilvarg (7*a*) indicate that this reduction step involves the reaction: dihydrodipicolinic acid + reduced triphosphopyridine nucleotide + $H^+ \rightarrow$ $\Delta'$-piperideine-2,6-dicarboxylate + triphosphopyridine nucleotide$^+$.] Repression by methionine also has been observed for enzymes 13 (14) and 14 (4, 24) of the methionine pathway. The efficacy of these multiple control sites in the smooth regulation of aspartate metabolism is weakened by the apparent lack of a specific control of aspartokinase by methionine. Perhaps it is the function of the homoserine-sensitive aspartokinase to maintain an adequate supply of aspartyl-P for methionine biosynthesis in the presence of simultaneous excesses of lysine and threonine. However, a much more alarming deficiency, in an otherwise sensible regulatory mechanism, is the discovery by Patte et al. (12) that *E. coli* contains but a single homoserine dehydrogenase (enzyme 9) that is under strong specific end-product control by threonine. This makes little sense because homoserine dehydrogenase acts at a step preceding the divergence of the pathways leading to threonine and methionine. As a consequence of this situation, it might be expected that an excess of threonine could retard the synthesis of homoserine to a rate inadequate to provide for the synthesis of methionine. That this eventuality is, in fact, realized is supported by the observation that supplementation of the growth medium with high concentrations of threonine causes an appreciable retardation of growth, and that this can be overcome by the simultaneous addition of methionine (Cohen, *personal communication*). In the absence of added methionine, the lag in growth rate is eventually overcome by induction of an enzyme system that catalyzes the decomposition of this excess threonine, thereby relieving that end-product control. Although these observations point up an obvious flaw in the basic control mechanism, they are a

further testimony of the remarkable adaptability of the organism to meet complications arising in the regulation of its metabolism.

### *Control of Aspartate Metabolism in Other Organisms*

The scheme presented in Fig. 2 summarizes the present knowledge concerning the metabolism of aspartate in one strain of *E. coli* (K12 HfrC). It is not to be inferred that all sites of end-product control have been determined or that identical patterns of regulation will be found in other organisms. Limited examination of other strains of *E. coli* reveals quantitative differences in the concentrations of the three different aspartokinases. For example, in *E. coli* strain W the total aspartokinase level is higher than in the K12 strain, and the relative amounts of the threonine-sensitive and homoserine-sensitive enzymes are considerably lower (18). More marked differences are noted in two other microorganisms. Recent studies by Robichon-Szulmajster and Corrivaux (13*a*) with *Saccharomyces cerevisiae* reveal qualitative as well as quantitative differences in the control of aspartokinase activity. Moreover, the susceptibility of the aspartokinase activity of this organism to feedback inhibition is greatly influenced by the cultural conditions. As shown by the data of Table 4, when *S. cerevisiae* is grown on a minimal medium without amino acid supplements, the aspartokinase activity of cell-free extracts is inhibited 85 to 100% by threonine and 30 to 40% by homoserine. Little or no inhibition is observed with either methionine or lysine. If the growth medium is supplemented with 20 mM L-threonine or L-lysine, there is an appreciable reduction (84 and 70%, respectively) in the total concentration of aspartokinase; however, that which is formed shows the same response to feedback inhibitors as does the enzyme produced on minimal medium. When the medium is supplemented with 5 mM homoserine, there is a 55% repression of aspartokinase synthesis, and the enzyme formed is relatively more sensitive to inhibition by homoserine. However, the most surprising results are obtained when the growth medium is supplemented with methionine or its homologue, ethionine. These substances not only cause repression (23%) of aspartokinase synthesis but they impart a decided sensitivity to inhibition of the aspartokinase by methionine (21%) and ethionine (28%).

The pathway of lysine biosynthesis in *S. cerevisiae* (3, 8*b*, 8*c*) and in other yeasts (19) is not the same as that found in *E. coli*. In *S. cerevisiae*, lysine is not derived from aspartate but is formed by a mechanism in which $\alpha$-aminoadipic acid and saccharopine are intermediates (3). It is, therefore, not surprising that lysine fails to inhibit the aspartokinase activity of extracts from the yeast grown on minimal medium. On the other hand, for the same reason it is difficult to reconcile the marked repressibility of the aspartokinase by lysine, and, even more so, the appearance of an enzyme susceptible to feedback inhibition by lysine when the organism is grown in the presence of methionine. Nevertheless, it seems probable that these curious results only expose deficiencies in our knowledge of the amino acid metabolism of yeast and

TABLE 4. *Repression and feedback inhibition of aspartokinases in Saccharomyces cerevisiae**

| Addition to growth medium | Aspartokinase activity (total units) | Repression | Feedback inhibition of aspartokinase activity by 10-mM concn of | | | |
|---|---|---|---|---|---|---|
| | | | L-Threonine | L-Homoserine | L-Methionine | L-Lysine |
| | | % | % | % | % | % |
| None | 93.6 | 0 | 85–100 | 30–40 | 0 ± 10 | 0–6 |
| Threonine, 20 mM | 14.7 | 84 | 85–100 | 15 | 0 | 0 |
| Lysine, 20 mM | 28.3 | 70 | 100 | 7 | 0 | 0 |
| Homoserine, 5 mM | 41.8 | 55 | 100 | 70 | 0 | 0 |
| Methionine, 20 mM | 74.0 | 21 | 90–100 | 20 | 21 | 28 |
| Ethionine, 20 mM | 71.6 | 23 | 100 | 38 | 14 | 16 |

* Taken from Robichon-Szulmajster (*unpublished data*).

emphasize the need for more detailed studies in this area.

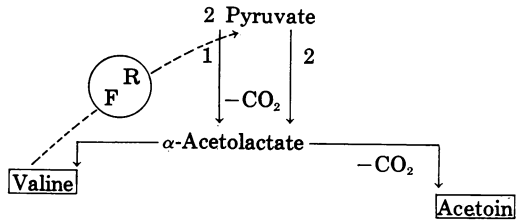
Other studies by Karassevitch and Robichon-Szulmajster (8*a*) have shown that homoserine dehydrogenase synthesis in *S. cerevisiae* is specifically repressed by methionine and that this enzyme is susceptible to feedback inhibition by lysine, methionine, and threonine. Since inhibition by the latter two amino acids is not additive, it is concluded that a single homoserine dehydrogenase with multiple inhibition sites is formed. This is in contrast to the situation in *E. coli* where threonine alone controls the synthesis and activity of homoserine dehydrogenase (Fig. 2). The sensitivity of yeast homoserine dehydrogenase to inhibition by lysine presents the same anomaly discussed above in connection with the observed inhibition of yeast aspartokinase by lysine.

The lack of universality in the control mechanism of different organisms is again illustrated by the studies of Nara et al. (9) on the aspartate metabolism of *Micrococcus glutamicus*. These investigators were unable to detect any kind of end-product regulation of aspartokinase in this organism. On the other hand, synthesis of homoserine dehydrogenase is strongly repressed by methionine, whereas its activity is specifically inhibited by L-threonine.

## Enzyme Multiplicity in the Control of Common Steps in Synthetic and Degradative Processes

### *α-Acetolactate Formation*

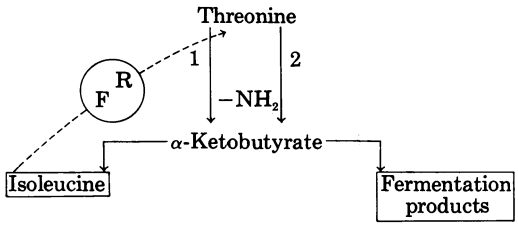
The demonstration that the multiple aspartokinases of *E. coli* are under specific end-product control suggests the possibility that the multiplicity of enzymes frequently observed to catalyze the same biochemical reaction in a single organism may indicate situations in which the product of the catalyzed reaction is a common precursor in divergent metabolic pathways. This possibility was already suggested by the earlier studies of Umbarger and Brown (22), showing the existence of two enzymes in *Aerobacter aerogenes* that catalyze the condensation and decarboxylation of pyruvate to form α-acetolactate. This reaction may be regarded as the first step in the biosynthesis of valine, and is involved also in the formation of acetoin during the dissimilation of glucose at acid pH. α-Acetolactate is thus a common intermediate in two diverging pathways. The situation is analogous to that encountered in aspartate metabolism except that the pathway leading to valine may be regarded as biosynthetic in function, whereas the formation of acetoin is an end product of glucose degradation at acid pH and is, therefore, associated with the energy metabolism of the organism. It is evident that end-product regulation of the biosynthetic pathway is desirable to avoid overproduction of the amino acid valine; however, such regulation of acetoin synthesis is undesirable, since the energy metabolism of the organism may depend upon unhindered accumulation of acetoin.




It is obvious that a single enzyme catalyzing α-acetolactate formation could not be subject to regulation by valine without disturbing the energy metabolism of the organism, especially when it is grown at acid pH. Again, this dilemma is solved by the formation of two distinct enzymes. One enzyme is synthesized at neutral to alkaline pH and is under specific regulation by valine (22). This enzyme has a relatively high pH optimum and is presumably concerned with the biosynthesis of valine. The other enzyme is elaborated only at acid pH when acetoin becomes a significant end product of glucose metabolism; it is completely refractory to end-product regulation and, presumably, is concerned primarily with glucose metabolism.

### *Threonine Deaminase*

A comparable situation is encountered in the metabolism of threonine by *E. coli* when the organism is grown anaerobically on mixtures of amino acids in the absence of fermentable sugar. Under these conditions, the deamination of threonine to form α-ketobutyrate is a key enzymatic step in the biosynthesis of isoleucine, on the one hand, and in the energy metabolism of the organism, on the other.




Studies by Umbarger and Brown (23) reveal that, as in the above instance with pyruvate metabolism, *E. coli* avoids the conflict between end-product regulation and energy metabolism by synthesizing two different threonine deaminases, one of which is under selective control by isoleucine, whereas the other is formed only under anaerobic conditions, in the absence of fermentable sugar, and is not subject to end-metabolite regulation. The significance of these observations in explaining the multiplicity of enzymes catalyzing identical reactions was recognized by Umbarger (21), who enunciated the principle that "whenever a chemical reaction in a cell is catalyzed by an enzyme whose formation and activity are under the rigid control of repression and end-product inhibition, a second enzyme will be needed by the cell if there is some other essential role for that reaction to play in the cell."

## ENZYME MULTIPLICITY IN AROMATIC AMINO ACID METABOLISM

One final example illustrating the validity of this generalization comes from the recent studies on the enzyme catalyzing the condensation of phosphoenolpyruvate and D-erythrose-4-phosphate to form 3-deoxy-D-*arabino*heptulosinic acid-7-phosphate. As is shown in Fig. 3, this reaction is the first step in the biosynthesis of the two aromatic amino acids, tyrosine and phenylalanine. The studies of Smith et al. (16) show that *E. coli* contains two different enzymes catalyzing the condensation reaction. One of these enzymes is under specific control by repression and feedback inhibition by tyrosine, whereas the other is under specific control by phenylalanine. The two enzymes are separable by ammonium sulfate fractionation, are inhibited noncompetitively by their specific end product amino acids, and are further differentiated by their susceptibility to heat inactivation. This situation is, therefore, comparable in nearly every respect to that encountered with

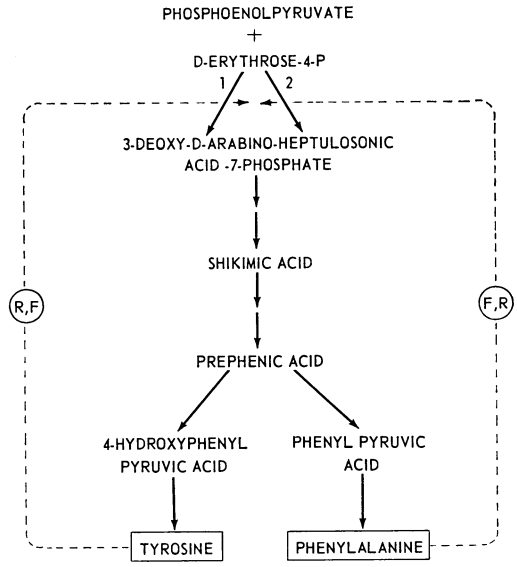



FIG. 3. *End-product regulation of aromatic amino acid metabolism. F = feedback inhibition; R = repression.*

the aspartokinases of *E. coli*. Although it would be dangerous to conclude on the basis of these few examples that, in general, multiple enzymes are elaborated to facilitate end-product regulation of initial steps in divergent metabolic pathways, it is, nevertheless, probable that these are not isolated examples. Certainly it might prove fruitful to investigate the existence of multiple enzymes catalyzing early steps in other divergent pathways, and conversely to investigate the repression and feedback inhibition by reasonable end products of isozymes already discovered.

## MULTIVALENT REPRESSION

The foregoing discussion has been concerned with a review of the evidence showing that organisms sometimes synthesize multiple enzymes to avoid complications which otherwise can arise in the end-product regulation of the first step in divergent metabolic pathways. It is therefore of special interest to consider the mechanism of end-product regulation in the unique situation where a single enzyme catalyzes an early step in two independent, parallel pathways, one of which is also a divergent pathway. Such a unique situation exists in the biosynthesis of the branched-chain amino acids, leucine, valine,

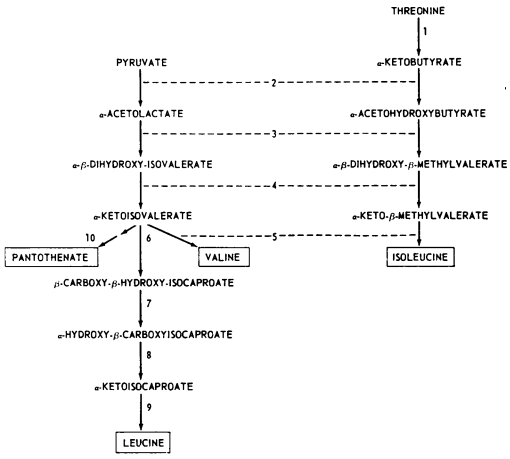



FIG. 4. *Metabolism of branched-chain amino acids.*

and isoleucine. Figure 4 summarizes the sequences of reactions involved. It is seen that in one pathway, shown on the left, pyruvate is condensed to form α-acetolactate which is converted to α-β-dihydroxyisovalerate; this in turn is converted to α-ketoisovalerate which is a common precursor in the biosynthesis of valine, leucine, and pantathenate. The right hand pathway involves a homologous series of reactions leading to the synthesis of isoleucine. Of special importance for the present discussion is the absence of multiple enzymes catalyzing any of the various steps; moreover, enzymes 2, 3, 4, and 5 catalyzing the consecutive steps in the valine pathway are responsible also for catalysis of corresponding steps in the isoleucine pathway. Here, then, the dilemma considered earlier for divergent pathways is intensified by the fact that single enzymes have dual roles in two independent pathways. Freundlich, Burns, and Umbarger (6) have recently investigated end-product regulation of this unique situation in *E. coli* and *S. typhimurium*. Their studies led to the discovery that no repression of the common enzymes in these pathways occurs unless each of the ultimate end products, valine, isoleucine, leucine, and pantothenate, are simultaneously present in excessive concentrations. (The role of pantothenate in multivalent repression, not discussed in reference 6, was reported by H. E. Umbarger at the meeting of the American Chemical Society, Cincinnati, Ohio, January 1963.) Thus, by imposing the obligatory requirement for a concerted action of all ultimate end metabolites to produce repression, the organism has found a way, other than by evoking enzyme multiplicity, to control metabolic pathways in which several end products are derived from common precursors. This kind of concerted control has been referred to as multivalent repression (6).

It must not be assumed that multivalent repression and enzyme multiplicity represent mutually exclusive mechanisms for the control of divergent pathways. One recalls the data of Table 3 showing that the depression of aspartokinase activity in cell-free extracts of *E. coli*, grown in the presence of excesses of both threonine and lysine, is considerably greater than that expected from the sum of the repressions observed when the organism is grown in the presence of each amino acid alone. These results suggest that multivalent repression may supplement the elaboration of multiple aspartokinases as an auxiliary mechanism for controlling the first step of aspartate metabolism (6*a*).

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