of less than 1.2 mm-Hg for each species tested (Table 1).

Oxygen-dissociation curves of blood from these seven species of squirrels differed considerably between species but were almost identical in individuals of the same species. The partial pressure of oxygen required for half-saturation of blood at *p*H 7.40 varied only about 1 mm-Hg for blood from the same species, but ranged from 22 mm-Hg for the prairie dog to 39 mm-Hg for the flying squirrel. In fact, it would be possible to identify any one of these species, apart from the round-tailed ground squirrel and the prairie dog, by determining the position of the dissociation curve. The prairie dog and round-tailed ground squirrel have similar habits and habitats; their curves differ from each other by less than 2 mm-Hg.

The curves shown in Fig. 1 represent the oxygen affinities of hemoglobin within the confines of erythrocytes and in the chemical environment of the circulating blood; they do not indicate the behavior of these hemoglobins in some other physicochemical environment, or any definitive characteristic of the hemoglobin molecule.

It is well established that the hemoglobin molecule of mammalian blood varies with the species and that its relative oxygen affinity is the most characteristic variant. The position of the oxygen-dissociation curve is related to environmental limitations and to the metabolic requirements of the species. The adaptive value of hemoglobin must be considered from both its ability to combine with oxygen in the lungs and its ability to release this oxygen to the functioning cells of the body.

Schmidt-Nielsen and Larimer (*3*) have shown that the dissociation curve of blood of several mammals is related to body size. This correlation is to some extent apparent in studies of the blood of squirrels when the larger and smaller forms are compared. The curve furthest to the right is that for the flying squirrel, while curves for the prairie dog and marmot are far to the left. Squirrels, like many other groups of animals, exhibit great diversity of types, which appears to be correlated with diversity of ecological habitats as well as with habits of the various species; this has been produced by adaptive radiation during the long evolutionary history of this group. Prairie dogs live in deep burrows where oxygen tensions may become low; their oxygen environment may resemble that at high altitude. The marmot is a hibernating animal, and it too appears to have low metabolic requirements. The gray squirrel and flying squirrel are at the other extreme; they live in the "wide-open spaces" and are active. The gray squirrel, however, is diurnal in habit, while the flying squirrel is nocturnal (*4*). The natural history of these animals is described by Walker (*4*).

How and when changes in the respiratory function of the blood originated during evolutionary development of the species are subjects for speculation, but that these changes did occur and that they are advantageous in the struggle of the various species for existence seem evident. This is the picture presented by seven species of squirrels.

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16 February 1965

Control of Enzyme Activity in Growing Bacterial Cells by Concerted Feedback Inhibition

Abstract. *Amino acids of the aspartic acid family are synthesized in bacteria through a multistep branched pathway. In the photosynthetic bacterium* Rhodopseudomonas capsulatus, *the enzyme that catalyzes the first step is specifically inhibited by a combination of two end-product amino acids. Evidence is presented for operation of this kind of "concerted" feedback control as a regulatory device in growing cells.*

The biochemical conversions through which aspartic acid gives rise to L-lysine, L-threonine, L-isoleucine, and L-methionine are believed to be identical in all bacteria (*1*) (Fig. 1). Studies with different bacterial species, however, have revealed the existence of at least three alternative schemes (*2*) of regulation of biosynthesis of these amino acids. One scheme, discovered (*2, 3*) in the photosynthetic bacterium *Rhodopseudomonas capsulatus*, is partly based on control of activity of the first enzyme (β-aspartokinase) by the "concerted" action of two end products, each derived from a separate branch of the pathway. The activity of the cell-free β-aspartokinase is markedly inhibited when L-threonine and L-lysine are present simultaneously; binding of both modifiers to the kinase presumably causes a specific conformational alteration of the protein to a form with greatly diminished catalytic activity. We now present evidence for the operation in vivo of concerted feedback inhibition of β-aspartokinase activity as a regulatory device in *R. capsulatus*.

From the control scheme suggested by experiments (*2*) with cell-free enzyme preparations, excessive accumulation of threonine and lysine in the growing cell should lead to inhibition of activity of β-aspartokinase and homoserine dehydrogenase and, consequently, to a decreased supply of common intermediates required for methionine synthesis. A suboptimum rate of methionine formation should, in turn, lead to depression of the growth rate. This prediction can be tested experimentally by increasing the intracellular concentrations of L-threonine and L-lysine, through addition of the amino acids to the growth medium. Growth experiments (Fig. 2) show the expected effect and also that additional supplementation with methionine causes a reversal of the inhibition due to threonine plus lysine.

When both threonine and lysine are added to the synthetic "basal" medium (containing glutamate as the nitrogen source), growth of *R. capsulatus* is severely inhibited. In the experiments of Fig. 2*A*, supplementary amino acids were added before inoculation of the cultures, and under these circumstances the inhibitory effect of threonine plus lysine is relieved to a significant degree, after a rather extended lag period, when methionine is also present. Addition of lysine to the basal medium results in a slight, but definite, stimulation of growth whereas appreciable inhibition is observed with threonine. The latter amino acid would be expected to interfere with homoserine synthesis through feedback inhibition of homoserine dehydrogenase activity (*2*). The probable reason for the depres-

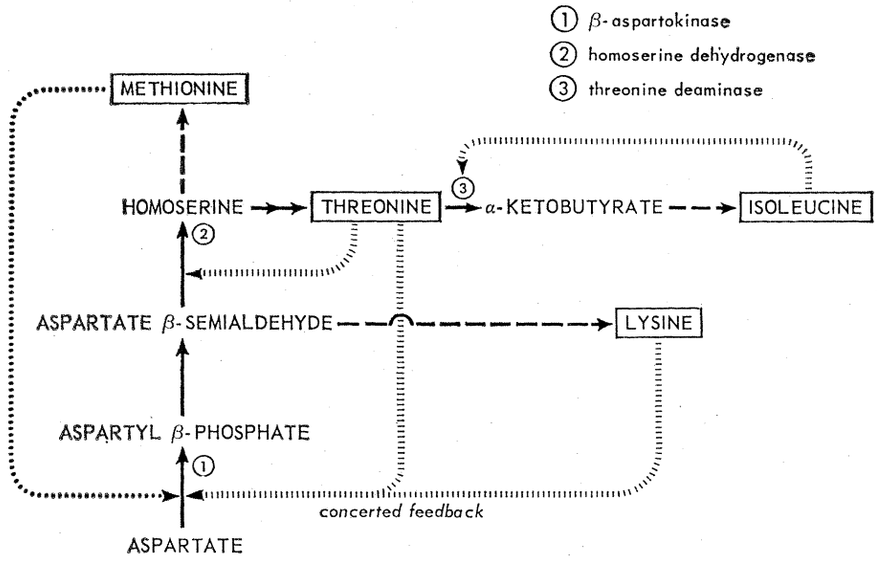

Fig. 1. Scheme for biosynthesis of amino acids of the aspartic family and the control pattern in *Rhodopseudomonas capsulatus*. Feedback inhibition of enzyme activity is indicated by hatched arrows and repression of enzyme synthesis (with glutamate as nitrogen source) by the dotted arrow.

sion in growth rate when only methionine is added to the basal medium is discussed below.

Results substantially similar to those shown in Fig. 2*A* were observed when the nitrogen source for dark aerobic growth was an ammonium salt, except that supplementation with methionine alone caused essentially no inhibition. On the other hand, when *R. capsulatus* is grown photosynthetically in the absence of oxygen, much higher (approximately tenfold) concentrations of added amino acids are required to demonstrate the kinds of effects described. Photosynthetic growth is considerably faster than aerobic growth in the dark, and it is possible that differences in the rates of utilization of intracellular amino acids under the two conditions may account for the different concentration requirements.

The curves of Fig. 2*B* show the effects of adding amino acids to cells just entering the exponential phase of aerobic growth in the dark. In such experiments, cells of comparable physiological status are exposed to the end products, and the reversal by L-methionine of growth inhibition, due to threonine plus lysine, is dramatic.

The possibility that the combination of threonine plus lysine inhibits growth by "multivalent repression" (*4*) of β-aspartokinase synthesis, as well as through concerted feedback inhibition of its activity, was tested by determining enzyme activity in extracts (*5*) prepared from cells grown in the presence and absence of these amino acids. It should be noted that feedback inhibition of β-aspartokinase action by threonine plus lysine is readily reversed by removal of these amino acids (*3*), and all cell-free extracts were therefore dialyzed before estimation of enzyme activity. The specific activities (*5*) of the enzyme in a number of extracts from "basal medium cells" fell in the range 37 to 56, whereas extracts from cells growing slowly in the presence of strongly inhibitory concentrations of threonine plus lysine showed specific activities of 40 to 60. Similar activity was observed in extracts from

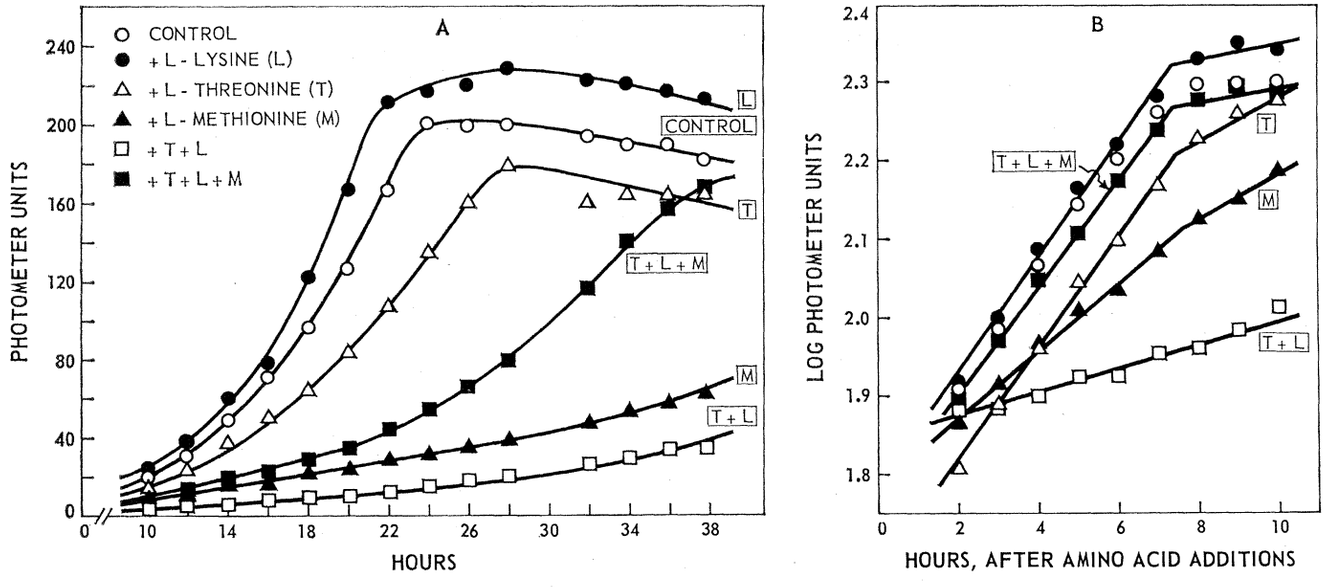

Fig. 2. Effects of added amino acid end products on growth of *Rhodopseudomonas capsulatus*. The basal medium contained glutamate as nitrogen source and other constituents as specified in (*3*), except that the concentration of DL-malate was decreased to 0.2 percent. Other amino acids were added at the following concentrations (millimolar): L-lysine, 1; L-threonine, 0.5; L-methionine, 0.5. The cultures were incubated aerobically (on a rotary shaker) in darkness at 30°C, and growth was estimated by measuring optical density in a Klett-Summerson photometer (filter 66). In *A*, supplemental amino acids were added to the basal medium before inoculation of the cultures; in *B*, the additions were made to cultures just entering the exponential phase of growth.

cells grown in basal medium plus L-threonine or L-lysine. These results clearly indicate that the control exerted by threonine and lysine on aspartyl β-phosphate production in the growing cell occurs through concerted feedback inhibition of enzyme activity and not by a repression mechanism.

In contrast, addition of methionine to the basal medium (glutamate nitrogen source) led to repression of β-aspartokinase formation to the extent that the specific activity was about one-half that of the controls. Repression by methionine has also been noted in *Saccharomyces cerevisiae* (*6*) and may explain, in part, the decrease in growth rate of *R. capsulatus* caused by supplementation with this amino acid only; that is, repression of β-aspartokinase synthesis might be expected to result in deficiency of the common precursors necessary for formation of threonine (and isoleucine) and lysine. The apparent nonparticipation of methionine in feedback control of β-aspartokinase and homoserine dehydrogenase activities implies both that the multifaceted regulatory scheme is such that overproduction of methionine produced biosynthetically cannot occur readily under the range of nutritional conditions usually encountered by *R. capsulatus*, and that a "slow" repression mechanism suffices to ensure modulation of the intracellular methionine pool. Conceivably, under transient conditions of threonine and lysine "excess," continued methionine synthesis may be facilitated by isoenzymes of β-aspartokinase and homoserine dehydrogenase which are inherently insensitive to feedback inhibition, and this remains to be explored.

We conclude that concerted feedback inhibition of β-aspartokinase activity operates as a regulatory device in growing cells of *R. capsulatus* to prevent the overproduction of threonine and lysine. Earlier, we suggested (*2*, *3*) that feedback inhibition of enzyme activity by the concerted action of two or more end products is a phenomenon likely to be of wide occurrence in branched or interconnecting biosynthetic pathways. Concerted feedback inhibition of cell-free β-aspartokinase by L-threonine plus L-lysine has been independently discovered by Paulus and Gray (*7*) during investigation of the *Bacillus polymyxa* enzyme, and the possibility of a similar kind of control of the first enzyme of purine biosynthesis (that is, glutamine phosphoribosylpyrophosphate amidotransferase) is indicated by recent studies with both bacterial (*8*) and pigeon-liver (*9*) enzymes.

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Collagen: Structural Studies Based on the Cleavage of Methionyl Bonds

Abstract. *Eighteen peptides from the α1 fraction and eight from the α2 fraction of rat skin collagen cleaved with cyanogen bromide have been identified by chromatography on carboxymethyl cellulose. The isolated peptides show wide differences in amino acid composition, and their molecular weights vary from several thousand to about 23,000. The data indicate that there are two different chains in the α1 fraction, demonstrating the nonidentity of all three α-chains. The primary structure of each chain appears to be unique throughout its length.*

Physical-chemical studies indicate that the collagen molecule is composed of three chains each with a molecular weight of about 100,000 (*1*). In view of this size, it is not surprising that the separation of peptides in enzymatic digests of unfractionated collagen has not been entirely satisfactory, although useful preliminary information regarding the amino acid sequence in portions of the molecule has been obtained (*2*). The task of structure analysis is further complicated by covalently bonded aggregates in most collagen preparations, and these may contribute additional peptides as a result of interchain cross-links. The chromatographic fractionation of heat-denatured collagen on carboxymethyl cellulose (*3*) has permitted the preparation of single-chain (α) and double-chain (β) components. However, despite the considerable advantage which the use of these components provides, the number of bonds susceptible to the usual proteolytic enzymes is still so large that the satisfactory isolation and analysis of peptides derived from such digests is unlikely (*4*).

The low methionine content of mammalian collagens suggested to us that chemical cleavage at methionyl bonds in collagen chains might provide a relatively small number of protein fragments, amenable to separation and characterization and useful in the study of collagen structure. The ability of cyanogen bromide to cleave methionyl bonds of model peptides in high yields with minimal side reactions (*5*) has been successfully utilized in structural studies of ribonuclease (*6*), sperm whale myoglobulin (*7*), and trypsinogen

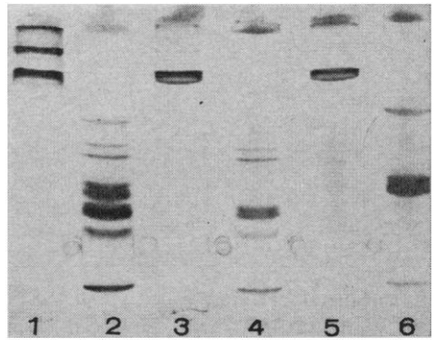

Fig. 1. Acrylamide-gel electrophoresis patterns of acid-extracted rat skin collagen (tubes 1 and 2) and its α1 (tubes 3 and 4) and α2 (tubes 5 and 6) fractions before and after treatment with cyanogen bromide in 0.1*N* HCl. Migration from top to bottom.