The second effect noted in the treated series was a marked departure from expectation for individual marker genes in approximately 1 percent of the F_2 ears studied. These distorted ratios were of a type which might be expected if gametophyte factor mutation or a chromosomal deficiency were involved. Progeny tests (Aa × aa and reciprocal) should distinguish these two alternatives. If a gametophyte factor were involved the distortion should appear in crosses of the type aa ♀ × Aa ♂ and be absent in the reciprocal. This expectation was not realized. A chromosomal deficiency should be revealed in the Aa × aa cross by a characteristically reduced seed set. A reduced seed set was not observed for any of the progenies tested. The departures from expected equality were similar for both types of back-crosses. In one case, involving the starchy and sugary alleles, the percentages of sugary seeds ranged from 24 to 43 instead of the expected 50 percent. Each of these deviations from expectancy was significant. No satisfactory explanation has been devised for such results but there can be little doubt that some disturbing influence is operative.

Any genetic effects from viral infection are thought to be limited to effects produced prior to or during gamete formation in the treated plants. Recognizable virus symptoms have never been detected in F_1 progeny involving treated gametes. Also we have not succeeded in demonstrating seed transmission of this virus in maize. Inoculation-transfer tests have failed to indicate the presence of virus in normal or chlorophyll-deficient F_2 seedlings.

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6 June 1963

Regulation of Enzyme Activity by Specific Reversal of Feedback Inhibition

Abstract. *Studies with a photosynthetic bacterium have revealed a novel type of regulatory control of enzymes participating in amino acid biosyntheses. Feedback inhibition of enzyme activity can be specifically reversed by ultimate end products of a branched pathway.*

Current understanding of the metabolic regulation of biosynthetic pathways is based on two general mechanisms: the control of enzyme synthesis by repression and induction, and the control of enzyme activity by end-product (feedback) inhibition (*1*). In the latter mechanism, the end product inhibits the activity of the enzyme, which catalyzes the first reaction of a sequence leading specifically to the product in question, thus avoiding oversynthesis.

Inhibition by the end product can in some instances be reversed or counteracted by substrate (*2*), by a heavy-metal cation (*3*), or by relatively high concentrations of certain amino acids which are not direct participants in the biosynthetic sequence in question (*4*). We have uncovered a novel type of reversal, in which the ultimate amino acid end products of a branched biosynthetic pathway reverse feedback inhibition caused by an intermediate or an amino acid derived from an intermediate.

As shown in Fig. 1, the amino acid homoserine is the branch point for the synthesis of threonine and methionine. Threonine has a dual role in that it is an "end product" utilized in protein synthesis and is also an intermediate in the synthesis of isoleucine.

After considering the relationships indicated, it seemed possible that homoserine dehydrogenase, which catalyzes the interconversion of L-aspartic β-semialdehyde and homoserine, might be subject to some sort of regulatory control by the several end products produced from homoserine. Cell-free extracts of the photosynthetic bacterium *Rhodospirillum rubrum*, grown in a synthetic medium with glutamate as nitrogen source (*5*), exhibit high homoserine dehydrogenase activity as measured by reduction of triphosphopyridine nucleotide (NADP) with homoserine as the substrate. As with the enzyme from certain other bacteria (*6, 7*), activity is strongly inhibited by addition of L-threonine; D-threonine does not inhibit. The data of Fig. 2 show that inhibition of the *Rhodospirillum* enzyme by L-threonine is strictly competitive with homoserine; in contrast, noncompetitive kinetics have been reported for the homoserine dehydrogenase of *Escherichia coli* (*7*).

From the standpoint of regulatory control, it is of particular significance that the inhibition of homoserine dehydrogenase activity by L-threonine can be completely overcome by either L-isoleucine or L-methionine, that is, by the ultimate end products of the branched pathway. These effects are illustrated by the data of Fig. 3.

With increasing concentrations of isoleucine or methionine, the inhibition due to threonine is gradually reversed. With isoleucine, a complete reversal is attained when the ratio of isoleucine to threonine is approximately 2; methionine appears to be slightly less effective. A homolog of methionine, L-ethionine, also reverses the threonine inhibition. Inhibition of homoserine dehydrogenase activity by L-threonine and reversal by L-isoleucine or L-methionine have also been observed with preparations purified a hundred fold. Furthermore, essentially similar results were obtained when enzyme activity was assayed by measuring reduction of L-aspartic β-semialdehyde with $NADPH_2$. With regard to specificity,

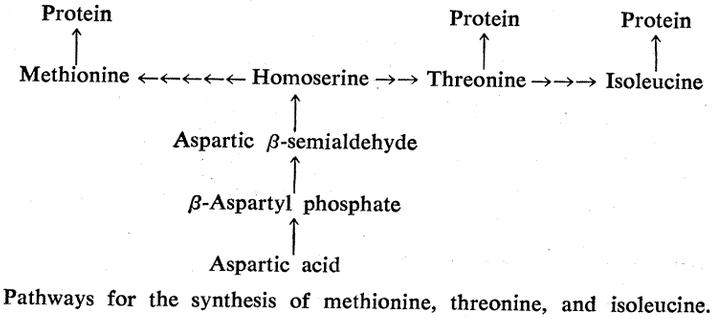

Fig. 1. Pathways for the synthesis of methionine, threonine, and isoleucine.

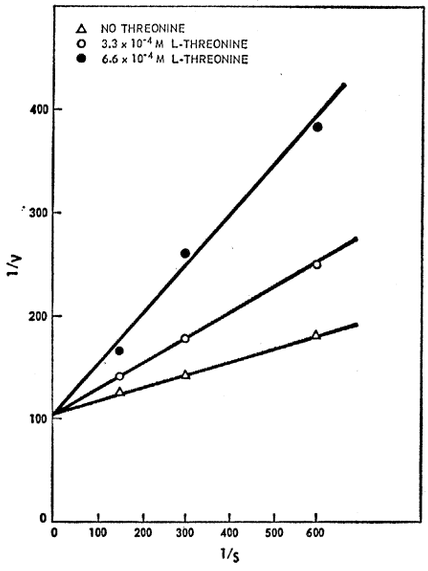

Fig. 2. Competitive inhibition of homoserine dehydrogenase activity by L-threonine; double reciprocal plot of substrate concentration and initial reaction velocity. The reaction mixtures contained, in a final volume of 3 ml: tris(hydroxymethyl)aminomethane, *p*H 8.4, 270 μmole; EDTA, sodium salt, 3 μmole; NADP, 0.4 μmole; L-threonine as indicated; centrifuged sonic extract, equivalent to 1.4 mg of protein. Reduction of NADP at 25°C was measured by following absorbancy at 340 mμ in a cuvette with 1-cm light path. $1/V$, reciprocal of initial reaction velocity, measured as increase in absorbancy per minute (from data obtained during the first 5 minutes after substrate addition). $1/S$, reciprocal of homoserine concentration, in moles per liter.

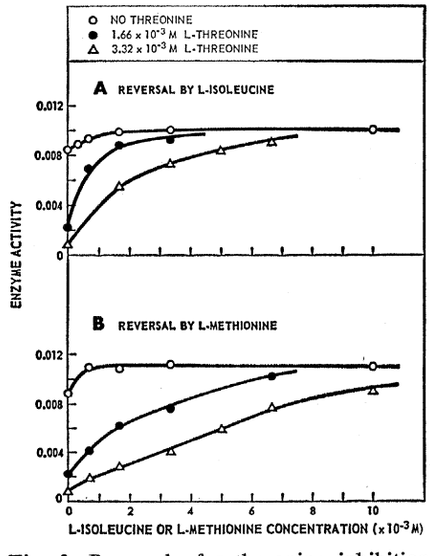

Fig. 3. Reversal of L-threonine inhibition of homoserine dehydrogenase activity by L-isoleucine (*A*) and L-methionine (*B*). The mixtures contained 20 μmole of L-homoserine and the other amino acids as indicated. Other experimental conditions as in Fig. 2. Enzyme activity is expressed as increase in absorbancy (340 mμ) per minute per 2 mg of protein.

it may be noted that the D-isomers of isoleucine and methionine as well as the L-isomers of lysine, leucine, and valine are ineffective in reversing the inhibition caused by L-threonine.

Although the biosynthetic pathways shown in Fig. 1 are based on studies with other organisms, there is no evidence as yet that they differ in *R. rubrum*. In certain bacteria, such as *E. coli*, an important control in isoleucine synthesis is end-product inhibition of threonine deaminase (*8*). In *R. rubrum*, however, we have been unable to demonstrate inhibition of threonine deaminase activity by isoleucine (*9*). Our studies indicate that, in *R. rubrum*, isoleucine exerts control by influencing other reactions in the biosynthetic chain. Preliminary experiments have disclosed that, in addition to its effects on homoserine dehydrogenase, isoleucine also can affect the activity of aspartokinase, the enzyme which phosphorylates aspartic acid (see Fig. 1). L-Threonine strongly inhibits aspartokinase activity and the inhibition is prevented by L-isoleucine, but not by L-methionine.

Evidence indicating a physiological role of these effects was obtained from relevant growth experiments. Growth of *R. rubrum* in the medium mentioned previously is completely inhibited by addition of $3.3 \times 10^{-4}M$ threonine, presumably because of interference with methionine synthesis. Further supplementation with either methionine or homoserine in low concentration ($1.6 \times 10^{-5}M$) does, in fact, abolish the threonine inhibition; similar results have been reported (*10*) with the photosynthetic bacterium *Rhodopseudomonas spheroides*. In the case of *R. rubrum*, reversal of inhibition of growth by threonine is also effected by isoleucine, but considerably higher concentrations are required ($3.3 \times 10^{-4}M$). The foregoing results are evidently consistent with the conclusion that in the intact cell isoleucine can exert regulatory control on methionine synthesis by the mechanism observed in the experiments in vitro, namely, by reversing feedback inhibitions caused by threonine.

The results of this investigation suggest that, in general, metabolic products may exert rapid control of synthesis both by end-product inhibition and by reversal of feedback inhibition established by certain intermediates. Presumably, enzymes subject to controls of the latter kind must be sensitive to critical differences in the relative concentrations of substrate, ultimate end products, and the inhibitory intermediates. A more detailed study of the homoserine dehydrogenase of *R. rubrum* may shed light on the mechanism of such effects, which appear to be examples of "allosteric" interactions (*11; 12*).

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Aberrant Thyroid Tissue in the Mouse

Abstract. *Aberrant thyroid tissue was observed at the base of the heart in seven of 2634 mice of the BALB/c strain. Such tissue was not seen in any of 1033 mice of the Strong A strain. The normal thyroid gland of the BALB/c mouse was observed to be more loose than that of the Strong A mouse and isolated follicles were frequently observed in tissue sections at either pole of the gland which were not attached to the body of the thyroid.*

Aberrant thyroid tissue is known to occur in man and a variety of lower animals. Most aberrant thyroids result from the failure of all or part of the thyroid anlage to descend from the