

action in the media with the reversing agent could not have occurred. Growth was not restored by placing the seeds in water following the 48-hour treatment with the various mixtures tested.

On the basis of the results obtained, it appears that CDAA inhibits certain sulfhydryl-containing enzymes that are involved in respiration. It further appears that it affects a mechanism even more intimately connected with growth, possibly oxidative phosphorylation.

ERNEST G. JAWORSKI
Monsanto Chemical Company, Organic
Chemicals Division, St. Louis, Missouri

References and Notes

1. P. C. Hamm and A. J. Speziale, "Correlation of structure and activity for a new class of herbicidal chemicals," *Proc. 11th Ann. North Central Weed Control Conf.* (1954), pp. 16-18.
2. J. S. Turner, *New Phytologist* 36, 142 (1937); M. Gibbs, *Nature* 170, 164 (1952).
3. W. W. Umbreit, R. H. Burris, J. F. Stauffer, *Monometric Techniques and Tissue Metabolism* (Burgess, Minneapolis, Minn., 1949), pp. 17-20.

28 October 1955

Evidence for a Negative-Feedback Mechanism in the Biosynthesis of Isoleucine

Recent developments in automation have led to the use in industry of machines capable of performing operations that have been compared with certain types of human activity. In the internally regulated machine, as in the living organism, processes are controlled by one or more feedback loops that prevent any one phase of the process from being carried to a catastrophic extreme. The consequence of such feedback control can be observed at all levels of organization in a living animal—for example, proliferation of cells to form a definite

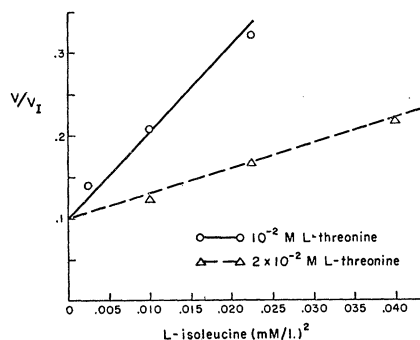


Fig. 1. Competitive inhibition of L-threonine deamination of L-isoleucine. Each point is calculated from an average of duplicate test systems in which keto acid formation from L-threonine was determined. The conditions were the same as those in Table 1 except the substrate and inhibitor concentration.

structure, the maintenance of muscle tone, and such homeostatic mechanisms as temperature regulation and the maintenance of a relatively constant blood sugar level. Because of the complexity of so many biological systems, it is often difficult to postulate a mechanism on the molecular level that would serve in a regulatory function.

Less complex systems for study of internal regulation can be found in the orderly synthesis of protoplasmic components during the growth of bacteria. A simple, though typical, example is the effect of L-isoleucine on the L-threonine requirement of threonineless mutants of *Escherichia coli*. It has been established that a portion of the L-threonine that is supplied in the medium is converted to L-isoleucine (1). In the presence of the latter, this conversion does not occur, and L-isoleucine itself is preferentially utilized (1) with an accompanying sparing effect on L-threonine (2). Exogenous L-isoleucine might effect its own utilization by (i) reversing the equilibrium along the biosynthetic chain or (ii) by specifically inhibiting any of the several enzymatic steps along that chain. However, because of the irreversibility of L-threonine deamination, which is probably the first step in the conversion of L-threonine to L-isoleucine (3), only an inhibition of this step could account for the sparing effect.

Examination of crude extracts of *E. coli* strain K-12 has revealed that L-isoleucine is indeed a strong inhibitor of this reaction (4). The data in Table 1 show the degree of specificity of this reaction. It can be seen that L-isoleucine was about 100 times as inhibitory as the structurally similar amino acid, L-leucine. It has been observed that, of the amino acids tested, only L-isoleucine has a sparing effect on the L-threonine requirement of strain 12B14, a threonineless mutant of *E. coli*.

Preliminary kinetic studies with L-threonine dehydrase activity in crude extracts indicate that the inhibition caused by L-isoleucine is competitive. However, in order for the data to fall in straight lines when they are plotted according to the method of Ebersole *et al.* (5), it is necessary to square the inhibitor concentration (Fig. 1). When the double reciprocal plot of Lineweaver and Burk (6) is employed, it is necessary to square the substrate concentration. This property of the data would be expected if the enzyme combined with 2 molecules of substrate or inhibitor (case II of Lineweaver and Burk). Further experiments are in progress in an effort to decide whether this peculiar kinetic behavior is apparent or real.

It would seem that the interaction between L-isoleucine and L-threonine de-

Table 1. Specificity of inhibition of threonine deamination. In addition to these amino acids at the indicated concentrations, the assay system contained 40 μ moles PO_4 buffer at pH 8.0, 10 μ g crystalline pyridoxal phosphate, 10 μ moles L-threonine and *E. coli* extract with 2 to 3 mg of bacterial protein in a total volume of 1 ml. The reaction mixture was incubated for 20 minutes at 37°C. The extent of deamination was followed by measuring keto acid production by the method of Friedemann and Haugen (7).

Amino acid and concn.	Inhibition (%)
L-Aspartic, $10^{-2}M$	30
L-Alanine, $10^{-2}M$	0
L-Valine, $10^{-2}M$	0
L-Leucine, $10^{-2}M$	55
DL-Homoserine, $10^{-2}M$	0
L-Methionine, $10^{-2}M$	0
L-Isoleucine, $10^{-2}M$	100
L-Isoleucine, $10^{-4}M$	52

hydrase constitutes a negative-feedback loop that could permit the biosynthesis of isoleucine to proceed only when the level of L-isoleucine in the medium or in the metabolic pool has been reduced to a very low level. The biological consequences of this interaction are being studied further in order to decide whether or not the inhibition of L-threonine deamination by L-isoleucine is in fact an important controlling mechanism in biosynthesis.

H. EDWIN UMBARGER
Department of Bacteriology and
Immunology, Harvard Medical School,
Boston, Massachusetts

References and Notes

1. P. H. Abelson, *J. Biol. Chem.* 206, 335 (1954).
2. H. E. Umbarger, *Amino Acid Metabolism* (Johns Hopkins Press, Baltimore, 1955).
3. ———, *J. Bacteriol.*, in press.
4. This work has been supported by grant 4015 of the U.S. Public Health Service and the Eugene Higgins Trust.
5. E. R. Ebersole, C. Guttentag, P. W. Wilson, *Arch. Biochem.* 3, 399 (1944).
6. H. Lineweaver and P. Burk, *J. Am. Chem. Soc.* 56, 658 (1934).
7. T. E. Friedemann and G. E. Haugen, *J. Biol. Chem.* 147, 415 (1943).

21 October 1955

Studies on 4APP: Antineoplastic Action in vitro

In the course of screening experiments for antineoplastic compounds, an isomer of adenine, 4-aminopyrazolo(3,4-d)pyrimidine (4APP) has shown differential cellular damage to several malignant tissues in culture. Antineoplastic activity of this compound has recently been found by Skipper *et al.* (1) on adenocarcinoma 755 in mice. The present report (2) pre-