COMMUNICATIONS TO THE EDITOR

p-HYDROXYPHENYLPYRUVIC ACID FUNCTION IN *NEUROSPOSA CRASSA*[1]

Sir:

Investigation of a mutant strain of *Neurospora crassa* (T-145)[2] requiring tyrosine for growth (2×10^{-4} molar for maximum growth equal to wild type) revealed that the addition of amino acids at relatively low concentrations resulted in unusual growth inhibitions in the presence of tyrosine. These amino acids proved to be competitive with tyrosine over a wide concentration range.

Studies of individual L-isomers of the inhibitory amino acids revealed the inhibition indices to fall in the range of 5–250. The D-isomers inhibited in a competitive manner also, but had a higher index. While phenylalanine was inhibitory (inhibition index = 8) it was found that the related compounds, phenylpyruvic acid and phenyllactic acid, showed no inhibition. Of 23 L-isomers of amino acids tested for competitive inhibition with L-tyrosine, eighteen showed complete inhibition of growth within the relative substrate-inhibitor concentrations indicated above while five caused no inhibition at any concentration. The inhibitory amino acids are: alanine, α-aminobutyric acid, aspartic acid, citrulline, cysteine, glycine, glutamic acid, histidine, isoleucine, leucine, methionine, norleucine, norvaline, phenylalanine, serine, threonine, tryptophan and valine; non-inhibitory amino acids are arginine, hydroxyproline, lysine, ornithine and proline.

Examination of the two groups of amino acids suggested at once that a major system such as transamination might be involved. This led to the testing of the keto analog of tyrosine (*p*-hydroxyphenylpyruvic acid).[3] These studies revealed that *p*-hydroxyphenylpyruvic acid completely satisfies the growth requirements of the mutant. Even more significant is the fact that it completely reverses the inhibition of the above amino acids in the presence of tyrosine. Relief of the inhibition occurred over a wide range of concentration (up to 100 times the concentration required for complete inhibition) of the inhibiting amino acid. The α-keto analog of tyrosine shows activity in both cases mentioned above within the same concentration range in which tyrosine is active alone.

A suggested hypothesis that would explain all the data is one in which the inhibitory amino acids block the conversion of tyrosine to its keto analog which is normally utilized by the organism for some essential function. The exact nature of the genetic block is not clear but the evidence is strong in favor of the conclusion that the keto acid serves some essential function other than the formation of tyrosine.

BIOCHEMICAL GENETICS LABORATORIES
DEPARTMENT OF ZOOLOGY
UNIVERSITY OF TEXAS
AUSTIN, TEXAS
A. GIB DEBUSK
ROBERT P. WAGNER

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(2) Obtained by A. Gib DeBusk, J. M. Weaver and R. A. McRorie in conjunction with the Genetics Group, University of Texas, by means of ultraviolet irradiation.

(3) We are indebted to Dr. Alton Meister of the Department of Health, Education and Welfare, Public Health Service, National Institute of Health, Bethesda 14, Maryland, for a sample of this compound for preliminary studies with this mutant.

CONFIGURATION OF DIHYDROSPHINGOSINE

Sir:

In a recent communication[1] we presented evidence for the *erythro* configuration of the natural dihydrosphingosine. We have now completed the resolution of the synthetic *erythro*-1,3-dihydroxy-2-aminoöctadecane, and one of the optical isomers is identical with the natural base, as shown in the table:

		Triacetyl m.p. °C.	Specific rotation
Natural dihydrosphingosine		100–102	$[\alpha]^{30}D$ +18° (chf.)
Synthetic enantiomorph	*erythro*	98–100	$[\alpha]^{22}D$ +19.2 (chf.)
	threo	46	.....

On the basis of these data and in conjunction with a previous report on the configuration of the amino carbon of dihydrosphingosine[2] it is now conclusively established that the natural dihydrosphingosine is *erythro*-D-1,3-dihydroxy-2-aminoöctadecane.

Experimental.—A warm solution of *erythro*-1,3-dihydroxy-2-aminoöctadecane (1.5 g.) in 50 cc. of ethanol was added to a warm solution of 740 mg. of finely powdered L-glutamic acid in 95 cc. of 50% ethanol. The slightly turbid solution was evaporated *in vacuo* to dryness. Vigorous foaming at the end of the distillation was overcome by adding two or three 50-cc. portions of ethanol. The dry salt was dissolved in 250 cc. of 90% ethanol (90 cc. ethanol–10 cc. distilled water) and left overnight at 20–22°; 750 mg. of a crystalline salt was filtered off. From the mother liquor 300 mg. more was separated after 24 hours. The combined solids were recrystallized once from 150 cc. and twice from 125 cc. portions of 96% ethanol and finally from 100 cc. of 90% ethanol. The pure salt melted incompletely at 136–140° and decomposed at 165–170°. The salt was decomposed with sodium carbonate and extracted with ether. The free base was converted into the triacetyl derivative which after two crystallizations from ethanol melted at 98–100°; $[\alpha]^{22}D$ −19.35 (0.066 g. in 10 cc. of chloroform).

The first two mother liquors from the above salt were concentrated. The residual salt was dis-

(1) H. E. Carter, D. Shapiro, J. B. Harrison, THIS JOURNAL, **75**, 1007 (1953).

(2) H. E. Carter and C. G. Humiston, *J. Biol. Chem.*, **191**, 727 (1950).