

Jervis, H. H. and A. G. DeBusk. NH₂-Terminal
analysis of the conidial proteins of N. crassa.

One of the interests in our laboratory has centered around the initiation of protein synthesis in Neurospora mycelia (Rho and DeBusk 1971 J. Bacteriol. 107: 840, Biochem. Biophys. Res. Comm. 42: 319, and J. Biol. Chem. 246: 6566). The purpose of this study was to analyze the NH₂-terminal amino acids of conidial proteins, thus providing the necessary ground work for elucidation of the protein synthesizing mechanism in this particular stage of fungal growth.

Table 1. The mole percent of DNP-amino acids.

NH ₂ -terminal amino acids	Conidial fractions			Mycelial crude	<u>E. coli</u> B
	Crude	Ribosomal	Soluble		
Alanine	13.6	17.5	12.4	14.6	23.5
Aspartic acid	11.4	4.1	7.0	11.2	1.4
Glutamic acid	11.5	4.0	6.0	10.4	2.2
Glycine	31.4	30.6	33.6	35.1	2.4
Lysine	-	-	3.5	-	-
Phenylalanine	12.6	22.0	14.1	-	-
Serine	6.9	3.0	6.5	6.8	25.4
Threonine	6.4	10.6	8.2	10.9	6.5
Valine	8.0	8.2	8.7	8.5	-
Methionine	-	-	-	-	38.6

sults. The radioactivity appeared at the DNP-phenylalanine spot and at no other. The existence of phenylalanine as a unique NH₂-terminal amino acid in the completed protein of *Neurospora* conidia provides a convenient handle by which this class of molecules may be followed during fungal development.

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The NH₂-terminal analysis employed the method of Sanger (1949 Biochem. J. 45:536), and the DNP-amino acids were separated by two-dimensional paper chromatography. For the first dimension, an ascending one, a solvent of toluene-pyridine-2-chloroethanol-.8 N NH₄OH (5:1:3:3) was used. For the second dimension, a descending one, a 1.5 M phosphate buffer was employed. The presence of all nine of the amino acids at the amino terminal position was confirmed by employing the 1-naphthylisothiocyanate method of Deyl (1970 J. Chromatogr. 48: 231). We believe that this technique will be of particular value in further studies as it is a modification of the Edman method and will allow sequential degradation analysis from the NH₂-terminal to be employed.

Since phenylalanine appeared as a novel NH₂-terminal amino acid in the conidial protein preparations, cells grown on ¹⁴C-L-phenylalanine were also used to confirm these re-