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AMINO ACID HANDBOOK

METHODS AND RESULTS OF PROTEIN ANALYSIS

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Objectives

We hope to do two things in this monograph. First, to describe in detail tried and proven examples of the three most widely used methods of amino acid analysis, *i.e.*, by microorganisms, by column chromatography, and by paper chromatography. It is hoped that sufficiently detailed directions will be given for each of these methods so that the analyst will be able to determine all of the amino acids which commonly occur in proteins without the need of recourse to the original literature. In contrast to our previous monographs on amino acids (Block and Bolling, 1945, 1951) no attempt is made to describe the many modifications proposed by various investigators although some comments are made concerning difficulties and possible pitfalls which may be encountered during the analysis.

The second part of the monograph is devoted to a tabulation of the amino acid composition of proteins, biologically active polypeptides and foods. The majority of the values for the amino acids in proteins and foods have been calculated as grams of amino acid per 16.0 grams of nitrogen in order to facilitate comparison between the results of different analysts on various preparations and to permit more ready use of the data for nutritional calculations.

ABBREVIATIONS

Mtd.	Method
N	Nitrogen
S	Sulfur
M.R.	Molar Ratio
ARG	Arginine
HIS	Histidine
LYS	Lysine
TYR	Tyrosine
TRY	Tryptophan
PHE	Phenylalanine
CYS	Cystine
MET	Methionine
SER	Serine
THR	Threonine
LEU	Leucine
ISO	Isoleucine
VAL	Valine
GLU	Glutamic acid
ASP	Aspartic acid
GLY	Glycine
ALA	Alanine
PRO	Proline
HYD	Hydroxyproline

Preface

"THE CURRENT trend of the investigation of the chemistry of nutrition is emphasizing the significance of the amino acids as the fundamental factors in all problems in which hitherto the role of proteins has been involved. . . . Obviously the relative values of the different proteins in nutrition are based upon their content of these special amino acids which cannot be synthesized in the animal body and which are indispensable. . . ." These views, set forth by T. B. Osborne and L. B. Mendel in 1914, cover the accepted facts on protein nutrition today. The extension of the pioneer experiments of Willcock and Hopkins, Osborne and Mendel, and others were brought to a successful conclusion by the well-known results of one of Professor Mendel's distinguished students, Professor W. C. Rose of Illinois.

With the proof of the essential nature for animal nutrition of methionine, histidine, lysine, tryptophan, phenylalanine, threonine, leucine, isoleucine, and valine, and the special importance of cystine, arginine, tyrosine, and glycine as shown by W. C. Rose, H. J. Almquist, R. W. Jackson, H. H. Mitchell, and others, the nutritive evaluation of protein foods, based on their comparative amino acid composition became a possibility. A reasonably accurate knowledge of the amino acid composition of a protein permits an approximation of its nutritive value and, more important, allows the choosing of different proteins so that they become mutually supplementary. The formulation of diets based on amino acid composition results in a great saving of time and cost over the long and tedious trial-and-error type of animal test employed heretofore.

If the amino acid requirements of an animal are known, even approximately, the proper quantities and combinations of food proteins can be chosen, provided that their essential amino acid composition has been estimated by methods of comparable accuracy. Evidence is beginning to accumulate concerning the special importance of certain amino acids in pre- and post-operative treatment, in wound healing, in blood regeneration, in learning processes, and even in the etiology of mental disease. Again, a knowledge of the amino acids present in available proteins may permit the use of relatively inexpensive sources rather than the more costly purified amino acids. Amino acid analysis has revealed rich sources of specific amino acids

in protein products which were heretofore unsuspected. Protein analysis is also valuable in revealing new uses of the protein-containing by-products of industry, and, in quickly revealing specific deficiencies in natural and prepared foodstuffs.

The degree of experimental accuracy in the amino acid analysis of the naturally occurring heterogeneous proteins, which may vary within wide limits because of preparative difficulties as well as changes in the amino acid composition of the tissue or organism itself, does not need to be as high as that required in a study of the molecular structure of those rare proteins which have been shown to be homogeneous substances (*cf.*, Colvin, 1954). Although in all analytical work, one must strive for the determination of absolute values, nevertheless, much valuable information of a permanent nature can be achieved by comparative amino acid analyses, especially in relation to protein nutrition.

This monograph gives many of the analytical values from the literature as well as some hitherto unpublished results of our own. Those proteins for which only a few analytical values are available have been omitted.

Many of the thoughts and ideas expressed in this monograph are the result of the kind suggestions and criticisms of various scientists, including Drs. Max Bergmann,* R. Keith Cannan, George R. Cowgill, Israel S. Kleiner, Icie Macy-Hoobler, William A. Perlzweig,* Carl L. A. Schmidt,* M. X. Sullivan, Donald D. Van Slyke, Hubert B. Vickery, and above all, Professor Lafayette B. Mendel.*

Although a survey of the literature on protein analyses has been attempted, the authors realize that many valuable contributions, especially those in foreign journals and in periodicals not devoted primarily to physiological chemistry, have been omitted unintentionally. It is also probable that, due to the many calculations and transpositions of data from the literature, which were necessary in the preparation of this monograph, numerical errors have been made. The authors welcome the correction of any erroneous statements or calculations and hope that readers will be so kind as to call their attention to important papers on this subject which have been omitted.

We wish to acknowledge our indebtedness to the writers and publishers of the various scientific books and journals from which the greatest part of the descriptions of experimental details, the analytical

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*Deceased.

results, and almost all the figures and diagrams were taken. Although specific credit is given in the text or bibliography, it is hoped that any omissions will be brought to the authors' attention for rectification.

R. J. B.

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PART B

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AMINO ACID HANDBOOK

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