The Amino Acid Composition of Proteins and Foods

ANALYTICAL METHODS AND RESULTS

Second Edition

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1951

INTRODUCTION TO SECOND EDITION

THE nutritive importance of proteins and the dependence of animals on plants for these substances were first pointed out by G. J. Mulder around 1840 in *The Chemistry of Animal and Vegetable Physiology*. He said, "In both plants and animals a substance is contained, which is produced within the former, and imparted through their food to the latter. It is unquestionably the most important of all known substances in the organic kingdom. Without it no life appears possible on this planet. Through its means the chief phenomena of life are produced."

A few years later Boussingault, writing in the *Économie Rurale* (Paris, 1851) said, "The alimentary virtues of plants reside above all in the nitrogenous substances, and consequently their nutritive potency is proportional to the quantity of nitrogen entering into their composition."

The views of Boussingault were strengthened by Liebig, so that in spite of much evidence against the nutritive equivalence of plant and animal proteins, the view that protein nutrition was essentially nitrogen nutrition remained dominant until 1897 when Rubner recognised that proteins of varying origins were *not* of the same value in nutrition and that therefore there was no one protein minimum but as many minima as there were proteins.

This rather unpromising outlook for research on the biological evaluation of proteins was changed to an hypothesis which could be placed on a rational experimental basis a few years later by Osborne (1907), who concluded his monograph on *The Proteins of the Wheat Kernel* with the following:

"The proportion of lysine [in wheat gluten] is likewise small, especially compared with that obtained from the leguminous seeds. The amount of histidine, however, does not differ very greatly from that of the other seed proteins. What significance these differences have in respect to the nutritive value of these different proteins must be determined by future investigation, for it has only very recently been discovered that such differences exist.

"That a molecule of gliadin can have the same nutritive value as one of casein would seem impossible if one molecule of food protein is transformed into one of tissue protein, for in the former lysine is

wholly lacking. . . . It would seem probable that either the animal requires a variety, so that the relative proportion in which the amino-acids are available for its use shall correspond more nearly to its requirements, or that only a small part of these amino-acids are converted into its tissue proteins and the rest oxidised as such. It is possible that feeding experiments with proteins of known character in respect to the relative proportions of their decomposition products will throw light on these important questions."

About the same time, Willcock and Hopkins (1906) observed that when casein was the sole source of protein for young mice good growth was achieved. However, if the mice were fed a diet containing zein or zein plus tyrosine, they almost immediately lost weight and shortly died. If, however, tryptophan were added to the zein diet, the animals lived for as long as 32 days.

At this time, the general impression arose that proteins of plant origin were inferior to those of animal origin for purposes of nutrition. This idea is still prevalent, for at a conference sponsored by the Children's Fund of Michigan (1944) the following question was asked: "Do you consider vegetable and animal proteins interchangeably adequate in diets for human beings?" The correct answer was given by the speaker as follows: "My view is that an essential amino-acid is essential whether it comes from animal or vegetable protein." This is an example of the widespread impression of a fundamental difference between proteins of plant and animal origin even though Osborne (1908) said: "On chemical grounds there is no more reason for dividing the proteins into two groups of animal and vegetable proteins than there is for making a similar distinction between the carbohydrates."

Shortly thereafter Osborne and Mendel inaugurated their classical studies on the nutritive value of purified proteins and protein-containing foods. These and other investigations formed the basis for the current ideas on the nutritive values of protein foods, which were summarised by Mendel (1923) as follows:

"The proportion of protein in the diet may determine whether larger or smaller absolute amounts of the nitrogenous foodstuffs are consumed; but the actual intake of these is also modified by the character of the non-protein ingredients. The individual instinctively strives to satisfy its calorific needs. A diet rich in fats is consumed in smaller quantity than one poor in fats, consequently the absolute protein intake may vary independently of its concentration

or percentage content in the food. When the absolute intake is small the 'law of minimum' may come into play to limit the efficiency of the whole because of the relative shortage of some essential amino-acid. Thus the protein casein yields relatively little cystine. So long as casein appears in the diet in abundance no deficiency appears; but when the consumption of the proteins is reduced a shortage of the least abundant essential may manifest itself. . . .

"Conversely, when an animal ingests a very large quantity of some protein poor in an essential unit, the absolute amount of the latter thereby available from the great abundance of its precursor may suffice to promote nutritive effects that fail to appear on a lower plane of protein intake."

McCollum, as quoted by Mendel (1923, p. 121), too, remarked that the investigations carried out during the period between 1910 and 1920 on protein foods of plant origin "leave no room for doubt that all the amino-acids necessary for the nutrition of an animal are contained in the proteins found in each of these foods. Certain of these are, however, present in such limited amounts as to restrict the extent to which the remaining ones, which are more abundant, can be utilised. It is for this reason that these proteins are of relatively low biological value unless supplemented by proteins from other sources, the constitution of which is such as to make good their deficiencies. The proteins of the cereal grains are of lower value than those of milk and eggs, or than those of certain mixtures obtained by combining two or more of these seeds."

Until the beginning of the twentieth century, it was generally believed that only intact proteins could be satisfactorily utilised for purposes of nutrition. In 1902, however, Loewi was able to maintain dogs in nitrogen equilibrium on pancreatic autolysates. The following year, Henderson and Dean (1903) attained nitrogen equilibrium in a dog by feeding an H_2SO_4 hydrolysate of meat. Henriques and Hansen (1905) prepared polypetide-free amino-acid solutions by first digesting the protein with trypsin and erepsin and then heating the digest with 20% H_2SO_4. It was found that if the first product gave a pronounced tryptophan reaction it would support nitrogen equilibrium. If, however, a single amino-acid, tryptophan, were lacking, nitrogen equilibrium could not be attained. These experiments formed the basis for the now currently accepted idea that protein nutrition is essentially amino-acid nutrition.

From these investigations, and many others, the concept was

established that the amino-acids can be divided, roughly, into two groups which are called essential and non-essential. Essential amino-acids for growth are those which cannot be synthesised by the animal at a rate sufficient for optimum growth out of the materials ordinarily available in the diet. Our knowledge concerning the essential or non-essential nature of each amino-acid is due to the pioneer investigations of Willcock and Hopkins, Abderhalden, Osborne and Mendel, McCollum, and particularly to the more recent studies of Rose on the growing rat, and of Almquist on the chick.

The term 'indispensable' amino-acid has a significance only when qualified as to species, nutritional state and age of the animal, a fact which is often overlooked. Thus, as Rose (1938) has pointed out, "An indispensable dietary component for maintenance need be synthesised at a rate commensurate with the demands for maintenance only." Rose and Rice (1939) showed that arginine is completely dispensable in the nutrition of the adult dog, while Burroughs, Burroughs and Mitchell (1940) were able to maintain nitrogen equilibrium in the adult rat on amino-acid mixtures devoid of arginine, lysine, histidine, leucine and phenylalanine, provided tyrosine was present in the diet. The evidence presented by Albanese and Frankston (1945) that histidine is required by the adult rat, and that presented by Neuberger and Webster (1945) that lysine also is required, are based entirely upon the refusal of their adult rats to eat amounts of histidine- or lysine-deficient diets sufficient to maintain bodyweight. It is not clear that refusal to eat a given diet by the adult rat is *prima facie* evidence as to its inadequacy. Burroughs, Burroughs and Mitchell (1940) found it necessary to feed their adult rats forcibly on all amino-acid rations since, even on diets containing complete amino-acid mixtures, not enough food was voluntarily consumed to cover calorie requirements.

Determination of the Amino-Acids in Food Proteins

The elucidation of the specific nutritive roles played by certain amino-acids for growth, reproduction, lactation and maintenance indicated the need for information concerning the amino-acid composition of food proteins as they are commonly consumed by man and animals, rather than knowledge of the amino-acid composition of certain purified proteins, which may account for only a small percentage of the total proteins of the food.

ANALYTICAL DIFFICULTIES

It is generally recognised that one of the chief sources of error in protein analysis is the loss by destruction of amino-acids during hydrolysis. The evidence of this loss and methods for its avoidance are discussed in detail in the text and will be mentioned only briefly here. It is obvious that the goal of protein analysts is the development of methods which do not require preliminary hydrolysis. Certain beginnings along this line have been made. Thus, Holiday (1936) and other investigators have been able to estimate the quantities of tyrosine and tryptophan in the intact protein by spectrographic methods. Bates (1937) used a modification of the Voisenet-Rhode *p*-dimethylaminobenzaldehyde method for estimating tryptophan in intact proteins. Mirsky and Anson (1934–35) and others, have utilised the reducing action of the sulphydryl group of cysteine to estimate the quantities of this amino-acid and of cystine in proteins without hydrolysis. A more promising field is the observation of Regnery (1944) that the *Neurospora* mutant "leucineless" developed by Beadle and Tatum (1941) can be used for the determination of leucine in unhydrolysed casein. Extension of this line of investigation is awaited with interest.

The amount of destruction during the conventional acid hydrolysis of a protein is increased by the presence of non-protein substances, especially carbohydrates and lipids. The latter are relatively easy to remove by extraction with the appropriate organic solvents. The former present a more difficult problem (*cf.* Chapter IX).

METHODS OF AMINO-ACID ANALYSIS

For the first 40 years of the present century, knowledge of the amino-acid composition of proteins depended almost entirely on purely chemical procedures. These consisted either in isolating and weighing the individual amino-acids or causing them to react specifically with a compound to form a coloured product. The intensity of the colour of the resulting product was then estimated by comparative colorimetry. In a few instances, the amino-acids were determined by oxidation to more readily measurable compounds. The advantages and disadvantages of the chemical methods are discussed in detail in the text.

Physico-chemical methods of amino-acid analysis by isotope dilution. A compound which has an abnormal isotope content is inseparable from its normal analogue by the usual laboratory procedures. In

the case of a compound containing N^{15} the proportion of this abnormal isotope can be determined by means of the mass spectrograph. The principle of the method of estimating a given amino-acid in a protein depends on adding to a hydrolysate of the protein a definite amount of a pure preparation of the amino-acid containing a known percentage of N^{15} and then to determine the proportion of abnormal to normal N in a pure specimen of the amino-acid isolated from the hydrolysate.

This procedure, which appears to be the most accurate method now available for the determination of amino-acids in protein hydrolysates, is limited only by the availability of the equipment, the isotope-containing amino-acids, and technical ability. It suffers from the same disadvantages of more or less extensive losses of amino-acids during hydrolysis that plague the more widely used procedures.

Biological and microbiological methods of amino-acid analysis. Although biological methods for amino-acid analysis were introduced by Osborne and Mendel in 1914 and are still in use for the estimation of amino-acids in proteins and foods, the results so obtained are seldom considered to be sufficiently reliable, because of the limited number of test subjects employed, to permit a numerical expression of the amino-acid composition of the protein. It should be recalled, however, that the large discrepancy between the "cystine" value of casein as determined by the rat growth method (Sherman and Woods, 1925) and that found by highly accurate chemical procedures, suggested to Jackson and Block (1931; 1932) that methionine may be able to assume all or part of the nutritive functions of cystine. This led to the discovery of the essential nature of methionine. The application of both biological and chemical methods of protein analysis should yield not only more valuable analytical information, but may reveal further details of protein structure.

The determination of an amino-acid based upon its decomposition by a specific enzyme system also is not new. Thus, Jansen (1916) used the formation of ammonia by the successive action of arginase and urease as a specific method for the determination of arginine (*cf.* Chapter I).

Since 1943, however, there have been great developments in the use of biological methods employing micro-organisms and specific enzyme systems for the routine estimation of all the known amino-acids. These methods, furthermore, are fundamentally so similar

in technique that once a procedure is learned for any one amino-acid, it can be applied with equal facility to any other for which suitable conditions have been found. The value of this, especially for routine analysis, is apparent.

However, it should be remembered that the growth of living organisms is subject to many more extraneous influences than are chemical reactions or physical measurements. The results obtained by any microbiological method must be evaluated by one competent in the field of microbiology. The presence or absence of vitamins, of lipoid substances and even of compounds closely related to the amino-acid under examination, may greatly influence the growth of the test organism, and may even determine the "essentiality" of an amino-acid for that organism. Although some of the microbiological methods, such as those for estimating leucine, *iso*leucine and valine, are suitable for application to crude protein-containing foods, other methods must be applied to natural products because of toxic properties encountered at the higher dose levels.

It should also be recalled that, with few exceptions, microbiological methods require partial or complete hydrolysis of the protein and are, therefore, subject to the same hydrolytic losses which have been discussed earlier.[1] This point is often forgotten when the relative merits of microbiological and chemical methods are discussed.

Comparison between chemical and microbiological assay methods for amino-acids. The reception given the microbiological procedures for determining amino-acids has led some workers to claim that the chemical methods are completely antiquated and much inferior to microbiological procedures. On the other hand, others have denied the value of microbiological methods except in certain special cases. It is our opinion that, like so many other instances in the history of physiological chemistry, both procedures will eventually turn out to possess points of superiority, and comparable results obtained by both methods should be more valuable than values obtained by either procedure alone. Furthermore, one cannot justifiably consider determinations for all amino-acids under one heading; either method might be excellent for the determination of one amino-acid but of little value for another.

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[1] Many of the chemical methods give quantitative results with both free and bound amino acids.

In trying to assess the comparative value of chemical and microbiological analysis, one must attempt to weigh the relative importance of absolute accuracy, speed of determination, technical skill required, frequency of analysis, cost of reagents and equipment, and number of proteins to be analysed simultaneously.

The following table reflects our present opinion on the relative suitability of the two types of methods:

Amino-acid	For a few scattered analyses	For many routine analyses
Arginine	Chemical*	Microbiological
Histidine	Chemical	Microbiological
Lysine	Chemical	Microbiological
Tyrosine	Chemical	Chemical
Tryptophan	Chemical	Chemical
Phenylalanine	Chemical	Chemical
Cystine	Chemical	Chemical
Methionine	Chemical	Undecided, probably chemical
Threonine	Chemical	Chemical
Serine	Chemical	Chemical
Leucine	Chemical	Microbiological
*Iso*leucine	Chemical	Microbiological
Valine	Chemical	Microbiological
Glycine	Chemical	Chemical
Alanine	Chemical	Chemical
Aspartic acid	Chemical	Microbiological
Glutamic acid	Chemical	Chemical
Proline	Chemical	Chemical

* Chemical methods include chromatographic procedures.

At the present time it appears to the authors that a combination of chromatographic and chemical methods will yield the most significant results concerning the structure of proteins and peptides. The microbiological procedures will, probably, remain preeminent for routine analyses especially of foods and feeds.

Amino Acid Composition of Food Proteins

The inherent error in calculating protein from nitrogen, especially in certain plants, such as yeasts and soya beans, has long been known. If the plant proteins are isolated in the purified form and then analysed for their constituent amino-acids, and if the results so obtained are used to calculate the amino-acid composition of the tissue from the nitrogen content of the latter, the error may be considerable. But if the tissue is itself analysed for the amino-acids, this error is minimised. Results so obtained show the approximate amino-acid composition of the proteinaceous substances irrespective of the presence of non-protein nitrogen. The majority of the analyses sum-

marised here are intended to be used to indicate the amino-acid composition of foods and not for consideration of protein structure.

Inspection of the data in this monograph indicates that the heterogeneous proteins of actively metabolising living matter, whether from plants or animals, have approximately the same overall pattern of amino-acids. On the other hand, special proteins, such as the keratins, haemoglobins, serum proteins, and hormones, and the storage proteins of seeds, are not as well balanced with respect to their amino-acid distribution. If one risks a tentative generalisation, it may be that the animal tissue proteins contain somewhat more methionine than metabolically comparable plant tissue proteins. However, the report of Mazur and Clarke (1942) that the alga, *Macrocystis*, contains 17% of its total N as methionine N, and that diatoms yield 6.2% of their total N as tryptophan N, is most perplexing. These results, calculated in per cent of protein (N × 6.25), would mean that *Macrocystis* proteins contain 29% of methionine, and the proteins of diatoms contain 7% of tryptophan. The values reported by experienced investigators in this field (Camien *et al.*, 1945; Lugg, 1943, and others) range from 1% to 3% of methionine, and less than 2.5% of tryptophan, in the total proteins of a wide variety of plants.

INTRODUCTION TO FIRST EDITION

IN PREPARING a monograph on the methods and results of amino acid determinations, it is proper to dwell briefly on the object and limitations of such work.

If a study of the structure of a pure homogeneous protein is the point at issue, then determinations of a few amino acids with the highest possible accuracy is of infinitely greater value than rough estimations of the approximate quantity of a large number. Thus, for purely chemical and physico-chemical studies, accurate estimations are prerequisite; while for a broad survey of the field of nutritionally valuable food proteins, the first need is to gain as wide a knowledge as possible of the qualitative and quantitative distribution of the essential amino acids. It has been our experience that a food protein may be a good source of those nutritionally valuable amino acids which are most commonly estimated (i.e., cystine, methionine, arginine, histidine, lysine, tyrosine, and tryptophan) and yet be deficient in one or more of the other essentials for which analytical methods are more difficult and, often, less accurate. If the analytical results of only the first group of amino acids were the sole basis of evaluating the protein, a serious error may have been made in estimating the biological quality of the protein. Thus, the analysis of *all* the amino acids of special nutritional importance, even though some of the results must, at present, be of comparative rather than absolute significance, will yield valuable and pertinent information.

Wherever possible, several procedures have been given for the estimation of each amino acid. If the identical analytical result is obtained by two entirely different procedures, a degree of confidence is imparted to the values which is not given even by closely checking replicate estimations by the same method. It is to be regretted that only a few investigators have used two or more methods for the determination of a single amino acid in the same preparation. It is hoped that the summarizing in one place of the many methods which have been offered for the estimation and determination of amino acids will encourage this type of study.

There is, however, one stumbling block in the accurate determination of amino acids in proteins which must be constantly reempha-

sized. Amino acids methods, with few exceptions, require hydrolysis of the protein before they can be applied. The susceptibility of each and every amino acid to loss during hydrolysis is different and differs not only with the conditions of hydrolysis, but more so with the presence or absence of other substances in the hydrolysis mixture. Thus, various investigators have shown that cystine and especially cysteine may be destroyed during acid hydrolysis when carbohydrates are present, but not in their absence.

Attempts have been made to evaluate hydrolytic losses by adding the amino acid under investigation both before and after hydrolysis. The apparent destruction during hydrolysis can then be determined by the difference in the analytical results obtained. This requires the tacit assumption that the amino acid added to the protein reacts during the hydrolysis in exactly the same way as its analogue in the peptide linkage. A fact which is known to be untrue in the case of certain amino acids. However, this procedure is, it is believed, the best approximation that can be made at the present time whenever hydrolysis is required.

It is interesting to note that in a considerable proportion of the special procedures for protein analysis which have been applied without preliminary hydrolysis, the analytical values obtained to date have been equal or even lower than those following hydrolytic fission.

As in the case of all branches of science, the great majority of methods which have been used are modifications of earlier procedures. It will be the policy of the writers, wherever possible to refer to a method first by the name of the investigator who initially developed or applied it to amino acids; and secondly wherever feasible, by the name of the modification used in the specific investigation. Thus, the Vickery and Leavenworth (1928 B & C) modification of the Kossel-Kutscher (1900) procedure for the separation and isolation of the basic amino acids will be referred to as the Kossel-Vickery method. This system of nomenclature is not uncommon in analytical chemistry and facilitates recalling the general procedure while indicating the particular modification followed.

In the interest of uniformity and to facilitate comparison of data on the same protein by different investigators, the analytical figures given in this monograph have been calculated to 16.0 grams of nitrogen, wherever possible. In certain cases where nitrogen values were not given but the determinations were calculated on the basis

of the moisture, fat, and ash-free preparation the data have been recalculated to 16.0 grams of nitrogen using a generally accepted value for N which is always indicated by placing the N value in parenthesis. In some cases the value (16.0) % of nitrogen is used to indicate that the data are given exactly as in the literature. This notation does not, therefore, always mean that the protein in question was deemed to have 16.0% of nitrogen. It is recognized that this procedure may introduce a certain error, but it is believed more suitable than other methods of presentation.

As all the data in the tables are calculated on the basis of 16 grams of nitrogen, it is only necessary to know the nitrogen content of the protein in order to recalculate the data in the tables to give the approximate amino acid composition of the preparation. If the protein contains 18.6% of nitrogen on a moisture and ash-free basis, then the values in the proper table are multiplied by the factor

$$\frac{18.6}{16.0} \text{ or } 1.16$$

If the nitrogen of the product is only 12.2%, then the amino acid figures are multiplied by the factor

$$\frac{12.2}{16.0} \text{ or } 0.76$$

Although a complete survey of the literature on protein analyses has not 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 unintentionally omitted. 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 that 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 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.

PREFACE TO SECOND EDITION

IN THE Second Edition an attempt has been made to correct the errors and omissions of the earlier edition which were brought to our attention both by private correspondence and in carefully considered reviews, and to include much of the work which has been published since its appearance. However, the most severe criticism concerned the mode of presenting the analytical data. Although we believe that the methods of presentation in the earlier edition (a method which did not originate with us) was the most suitable for the majority of users of this monograph, the fact that values could be interpreted in a way never intended by the authors has caused us to change the form of the tables in this printing. The scheme used in this present edition was arrived at after consulting with a number of eminent protein chemists and nutritionists.

Our object in publishing this monograph is to present to the average reader the widely scattered literature on the methods and results of protein analysis in the most easily useable form. We have no pet ideas to propound and continue to appreciate receiving suggestions for its future improvement.

PREFACE TO FIRST EDITION

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 rôle 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.

W. E. Gaunt has said recently (1942): "Supplies of protein foods for both the human and the stock populations of Great Britain are limited, and this limitation will continue after the War. For the most economical use of these supplies, it is obviously of the utmost importance for us to determine as rapidly as possible the qualitative and quantitative amino acid requirements of farm animals and man." 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

(*cf.* Chapter XI). 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. 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 experiments of our own. Those proteins, for which only a few analytical values of questionable accuracy are available, have been omitted; as have also the majority of those analyses on purified plant proteins which represent only a small proportion of the total proteins of the plant.

Although the number of foodstuffs which have been analyzed, by even reasonably accurate methods, is very limited, it is the purpose of this monograph to summarize the data which have come to the authors' attention, in such a fashion that the analytical values will become more easily accessible and widely available. In order to permit the reader to evaluate the data more accurately, nearly all of the analytical procedures which have been employed by various investigators are presented in detail. Although this may lead to a certain amount of repetition, especially in the first three chapters, it is hoped that the advantages outweigh the disadvantages.

The methods described include procedures for the estimation of

those essential and non-essential amino acids which can be evaluated with some degree of accuracy. The analytical procedures are arranged along the lines which were forged by the methods themselves, for example, the basic amino acids fall into one group: tyrosine, tryptophan and phenylalanine form another; the dicarboxylic amino acids a third; serine and threonine form a fourth; etc.

We are greatly indebted to Dr. W. D. Block, University of Michigan; Dr. M. John Boyd, University of Cincinnati; Dr. W. L. Brown, Georgia Experiment Station; Dr. J. W. Cavett, Charles City, Iowa, Professor A. C. Chibnall, London, England; Dr. M. S. Dunn, University of California at Los Angeles; Prof. Claude Fromageot, Paris, France; Dr. Theodore F. Lavine, Lankenau Hospital; Dr. W. D. McFarlane of McGill University; Dr. W. R. Murrill, University of Michigan: Dr. Ben H. Nicolet, United States Department of Agriculture; Dr. F. E. Reinhart, Franklin Institute; and, Dr. M. X. Sullivan, Georgetown University, for the use of analytical procedures and results prior to publication. We also wish to thank Mr. J. R. Bishop; Drs. A. P. Hellwig, B. F. Buchanan, and M. J. Blish, International Minerals and Chemical Company; Mr. Harry McNeilly, Drs. G. C. Supplee, H. N. Brocklesby, and H. W. Howard, the Borden Company; Dr. Horace Sipple, Evaporated Milk Association; Dr. H. G. Dunham, Difco Laboratories; Dr. B. F. Oser, the Food Research Laboratories; Dr. C. N. Frey, Standard Brands, Inc.; Mr. Arthur Wendel, President, the Wheatena Corporation; and Dr. T. H. Jukes, Lederle Laboratories, for permission to include many analytical data on foods, food products, and special protein hydrolysates.

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, the late Professor Lafayette B. Mendel.

We are especially indebted to Miss Margaret Holtgrave for her meticulous stenographic work and for her preparation of the tables; to Mr. Merrill Webb, and Dr. Herman Gershon for their invaluable aid in the preparation of the bibliography and to Dr. Herbert A. Sober, National Cancer Institute, for preparation of the paper chromatogram charts.