PROTEINS
and
AMINO ACIDS
in
NUTRITION

Edited by

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Stoutness of heart, humility of soul and open-mindedness are the keys to human understanding and happiness; no one endowed with these virtues can be but honest, just and tolerant to his neighbor and himself.

. . . . Melville Sahyun

Acknowledgment

Early in 1945 the late Professor Carl L. A. Schmidt and the editor conferred on the selection of topics for this volume. There was hope at that time that Professor Schmidt could participate in the writing of one chapter. It was soon realized that this was not possible and the editor undertook complete responsibility. However, despite his failing health, Professor Schmidt maintained a deep interest in this undertaking to the very end; in fact I was en route to Berkeley to visit him when I heard of his untimely death.

Dr. Schmidt was not only a scientist and a teacher but a true friend with unselfish interests. His scientific achievements and contributions to the chemistry of proteins and amino acids are too well known to enumerate. To him I owe a deep debt of gratitude.

Herein I also wish to thank Miss Freda Mohrmann for her tireless efforts and valuable assistance in checking all manuscripts for typographical errors and for retyping a large portion of them. I am also indebted to Dr. F. A. Waterman for locating and reproducing copies of pictures of scientists found in this volume.

MELVILLE SAHYUN

Foreword

Man has always been interested in food, of necessity and for enjoyment. Thus the position of nutrition among natural sciences is unique: There is no other subject of greater physiological importance or of greater moment for the welfare of the human race. The knowledge that we have gathered through the efforts of investigators in this field throughout the world should enable us to use our foods intelligently, in health and in disease.

The basic components of the human diet are water, essential mineral salts, vitamins, proteins, fats and carbohydrates. In this volume an attempt is made to point out the important role of protein in nutrition, consequences of protein and amino acid deficiencies, and to a certain extent the existing intimate relationships between proteins and carbohydrates, fats, vitamins and mineral salts, with the obvious purpose of gaining a clearer concept of the fundamentals involved in good animal nutrition. Although in this volume we place greater emphasis on the role of protein than on other essential nutrients, it must not be construed to mean that we can neglect or even minimize the dietary importance of the latter.

Experimental diets were and are used for a definite purpose — to gain knowledge. Pure amino acids of synthetic or natural origin or pure proteins as the sole source of nitrogen in the diet have aided us in determining their biological values and in clarifying our views on the metabolic and catabolic processes of these substances in the animal system. The knowledge we have gained from animal experimentation has led us to institute similar studies in man and in so doing we have enlarged our store of knowledge. We have also learned of hitherto unsuspected differences in the qualitative and quantitative requirements of certain indispensable amino acids and of certain variances in such requirements among different species.

The biological value of a protein is dependent not only on the presence of adequate amounts of indispensable amino acids among its constituents but on the proportions of these components that can be liberated in the digestive tract and absorbed into the circulatory system. Of recent years amino acid mixtures and protein hydrolysates have been prepared for parenteral use in man, and clinical reports have justified their existence. Following this, protein hydrolysates have been produced for oral human consumption. Odor, taste and palatability are fundamental characteristics of good food. By subjecting proteins to the catalytic action of either enzymes or acids the odor, taste and palatability of the resulting product are considerably impaired. If the use of these products is intended to meet dietary protein requirements that the parent protein can accomplish under the same circumstances, then we are needlessly replacing good common foods and burdening the consumer with unnecessary expense. On the other hand, if such protein hydrolysates are used for certain specific therapeutic purposes that the parent protein cannot fulfill, then such preparations have a definite place in therapy. However, they must be subject to regulations governing the use of drugs, and clinical, biological and chemical data must be presented to indicate their safety and chief usefulness.

In considering the economic aspects of foodstuffs we must recognize the necessity of providing adequate food proteins for the greatest number of people without undue cost to consumers. To utilize our present and potential resources with maximum effectiveness, it is mandatory that we evoke the principle of the supplementary relationships of plant and animal proteins. However, under certain impelling circumstances we can justify the use of relatively large quantities of proteins of high biological values.

The intelligent use of foods of different origins can be greatly enhanced by our knowledge of their composition. From a general nutritional standpoint, the breakdown of foodstuffs into their chief constituents is very informative. For this purpose the most comprehensive data available on the composition of natural and processed foods are presented.

MELVILLE SAHYUN

Introduction

HOWARD B. LEWIS

The need for protein in the diet was first pointed out by François Magendie, the great French physiologist, who in his memoir, "Food Substances without Nitrogen" (1816), attempted to see whether animals could live without nitrogenous food (protein). Dogs fed diets of sugar or fat and distilled water became emaciated and died, which led Magendie to conclude that animals cannot live without nitrogenous food.[1] Twenty-three years later (1839), the Dutch chemist, Mulder, suggested the term *protein* as a designation for the universal component of tissues, both plant and animal, "unquestionably the most important of all known substances in the organic kingdom. Without it no life appears possible on our planet. Through its means the chief phenomena of life are produced."[2] Some years later (1899), Verworn[3] wrote: "The proteins stand at the centre of all organic life." Today with the passage of more than a century of intensive research in nutrition, the proteins are still "first" (Greek, πρωτεῖος) in the regulation of vital processes. Despite the discovery of many new factors in cell physiology (*e.g.*, trace elements, enzymes, hormones, vitamins), interest still centers on this group of nitrogenous factors of the diet, the all important proteins. Lafayette B. Mendel who with his teacher, Russell H. Chittenden, and his coworker, Thomas B. Osborne, contributed so importantly to the study of the role of protein in nutrition, writing of Magendie and Mulder in 1923, commented on the "glorification of the albuminous substances — an apotheosis which has persisted in its extreme form almost until the present time."[4] One senses in this that, despite his own research which laid the foundations for the concept of the essential amino acids, Mendel suspected that proteins should or would be displaced as "first" by the newly discovered factors of the diet in the study of which his own work with Osborne pioneered.

The present volume with its varied content should serve to dispel fears that the biological significance of the proteins is solved and no longer an important problem. At no period, in the approximately one hundred years since Mulder, have proteins played so important a role in our thinking. The recent studies of the physical chemistry of the proteins, the use of new physicochemical approaches through the ultracentrifuge, the electrophoresis procedure of Tiselius and the newer studies of x-ray photography of large molecules have served to open up new biological concepts. The more accurate methods of analytical chemistry, particularly the recently introduced microbiological methods of analysis, have made possible an almost complete knowledge of the amino acid composition of a number of proteins, a knowledge which has stimulated anew biological studies of the role of the individual amino acids in nutrition.

The role of the proteins of the plasma in the maintenance of the normal osmotic relationships of the blood, the relation of plasma and tissue proteins, and the origin of the immune proteins of blood plasma have been emphasized.

All well-characterized and highly purified enzymes which have so far been isolated have shown the characteristics of proteins, the so-called enzyme proteins. A large group of hormones have been shown to be proteins, hormone proteins (*e.g.*, insulin and several of the hormones of the anterior pituitary gland); others, notably thyroxine, epinephrine and histamine, are definitely known to be specialized derivatives of amino acids of the protein molecule. Various antibodies including bacteriophage have the properties of proteins. Crystalline proteins have been isolated from plants infected with certain virus diseases, which while apparently lifeless are capable of increasing in quantity in plants into which they are injected and of giving rise to the specific disease. Virus proteins are new in the classification of proteins. It has become evident that the genetic factors in the cell, the genes, are related to the protein portion of the nucleoprotein of the cell or the cell nucleus. All these present new protein problems, which have arisen within the last fifteen years.

The solutions sought for these problems are varied. One of the most important is: By what specific arrangement of amino acids in the protein molecule is the specific biological function achieved? Why should a protein of relatively low molecular weight with an unusually high content of cystine, leucine and tyrosine and completely devoid of methionine and tryptophane exert a regulatory control of the utilization of carbohydrate as does insulin? Why should one group of amino acids as an enzyme protein act as a pepsin on the one hand and as a trypsin on the other? What arrangements of amino acids in the molecule are responsible for the varied phenomena of biological specificity of proteins, so important in immunology and related fields?

The desirable amount of protein in the diet has become of especial interest in the last decade. The early standard of Voit, which was based mainly on surveys of the diet of the German people, seemed to many entirely too high and aroused much controversy. Largely as the result of the experiments of Chittenden, a much lower protein content of the diet was advised and Sherman's recommendation of 1 gram of protein per kilo per day has been accepted generally. Avoidance of high protein diets, particularly in disease, was generally prescribed. In recent years, protein deficiency has been recognized and its role in disease pointed out. Dietary protein in amounts far above those of Sherman's recommendation has been advised, even for patients seriously ill. To one trained in the low protein school of nutrition of the early part of the present century, the recommendation of amounts of protein up to 200 grams daily is amazing. To meet these requirements for higher dietary proteins, particularly for surgical patients, new preparations are available. Increase in the level of protein by the use of hydrolyzed proteins, amino acid mixtures and special proteins has been suggested. These products must be evaluated by careful observations, both experimental and clinical, before they can be accepted without reservation. This is definitely the era of higher dietary protein.

It is hoped that the varied discussions in this volume, presented by workers in their respective fields, may contribute in some part to the solution of some of these problems and, in turn, suggest others. The answers to many of the questions of the biological role of the proteins are not yet available but the discussion should stimulate further investigation. This is the function of the present volume.

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Contents

Chapter 1

Proteins in Nutrition (Historical)*

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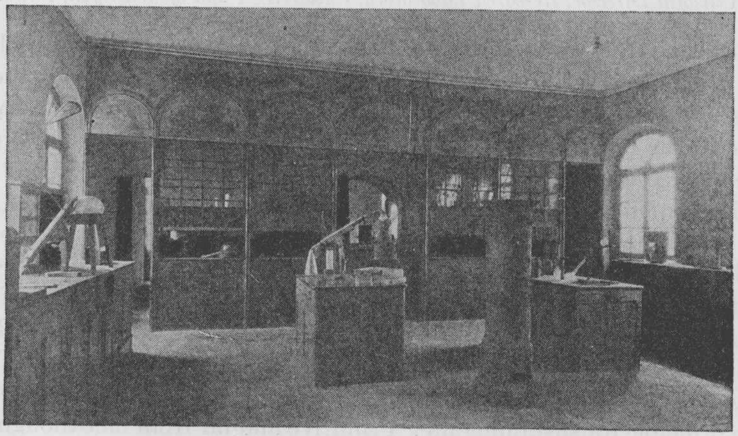
Knowledge of proteins is a relatively recent achievement, though taproots of fact and theory reach back into antiquity. Throughout the ages inquisitive human minds have been intrigued with the nature of food and its use in the body, so it is not surprising that speculative philosophers of Ancient Greece, since they chose omnivorously from the world about them, theorized upon nutriment. The beliefs of Hippocrates (460–377 B.C.) in these matters are summarized in his statement,[1] "There is but one food, but there exist several forms of food." The "Father of Medicine" believed that though variety was evidenced by the many available forms of food, ultimately these were reducible to a single principle. Later the Roman physician Galen (130–200) contributed the theory of the "perfect chyle," believing that during the process of digestion all foods were converted to a single principle for sustaining life. Primitive and far from fact though these theories of the unity of nutriment were, they remained intact for centuries. When the essential nature of protein in nutrition was recognized, protein was viewed as the single food essential for life.

The limited working information of the earliest protein chemists was evolved in the practice of the arts and crafts. Trades dealing with protein materials — baking, cheese manufacture, tanning and the spinning of silk and wool — all contributed information. To Antoine Laurent Lavoisier (1743–1794), Karl Wilhelm Scheele (1742–1786), Daniel Rutherford (1749–1819) and Joseph Priestley (1733–1804) go the honors for the basic discoveries and the techniques which nourished modern chemical theory in its infancy. These men learned to recognize oxygen and nitrogen and to work with the compounds of these two elements and the compounds of carbon. Upon these fundamentals real progress in chemistry began at the end of the eighteenth century and subsequent years saw a rapidly expanding knowledge of biochemistry and physiology and the founding of the science of nutrition in the establishment of protein as the basic nutritional principle.

* A Syllabus (unpublished), prepared by C. M. McCay at Cornell University, Ithaca, New York, for his course in Advanced Animal Nutrition, provided valuable orientation material for this historical review.

In recounting the development of knowledge concerning proteins and their role in nutrition it is difficult to avoid the omission of worthy names, for the field was populous with able minds. We can only hope to include those who for one reason or another spoke earlier or with more eloquence and clarity the truths which were gradually taking form. Those unmentioned worked and reworked the facts and theories of their time, constantly refining them. In the interest of clearer understanding of the thinking of the older workers, frequently we have quoted the exact words which tell of scientific developments in a way which defies improvement.



Liebig's restored laboratory in Giessen, Germany.

With the profound observation of Lavoisier, in 1780, that "life is a chemical function," biochemistry in its modern aspect emerged from its prenatal obscurity to grow and mature in the environment of the new experimental philosophy. The father of modern chemistry spent his few remaining productive years studying respiration and developing many of the exact methods of this science. During the French Revolution, his own "chemical function" was terminated abruptly by the thud of a guillotine.

During the 140 years following the astute observation of Lavoisier, experimental studies in nutrition expanded. These concerned largely the significance of food protein of which a very thorough knowledge was gained before the functions of other nutriment substances were appreciated. Within the history of protein nutrition the whole science passed through infancy and adolescence — the basic facts were verified and the pattern established for the mature growth of recent years. By the end of World War I, nutrition as a distinct science was gaining recognition in all fields of scientific endeavor. In 1922, looking back over the span of years which we are about to consider, the eminent physiologist E. V. McCollum said:[2] "The tree of nutritional knowledge appears, however, to have grown to proportions which reveal the general outlines which it will always present, and further researches by the methods which have hitherto been so productive, can, it seems, only clothe it in attractive foliage and aid it in maturing the rich setting of fruit which has not yet ripened and fallen for the service of man, although a few windfalls which have been tasted reveal the keen enjoyment with which the human race will one day reap the full harvest."

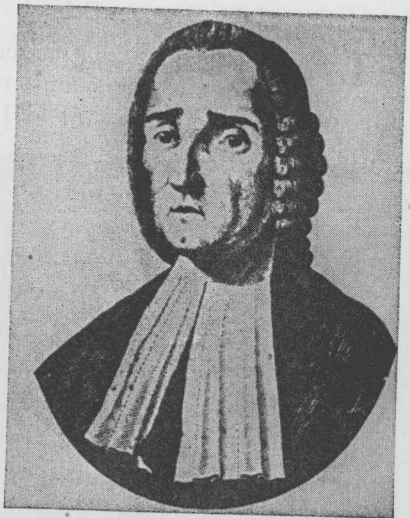
Justus von Liebig: Foundation of the German School

Justus von Liebig (1803–1873) is the inevitable center around whom any discussion of the early history of protein theory must revolve. Liebig was born in Darmstadt and after studying chemistry at Bonn and Erlangen

Justus von Liebig (1803–1873) *and family.*

received his doctorate in 1822. The next two years he spent in Paris and was trained, through his association with Gay-Lussac's laboratory, in the best traditions of the French philosophy. At the age of 21 he returned to Germany to become professor at the University of Giessen. From Giessen for the next 23 years came the students of Liebig and the brilliant writings which continually broadened the understanding and interest of chemists in the physiology of metabolism and nutrition. Liebig's fame is deserved not only for his brilliant original contributions to the literature but also for his ability to gather related facts and popularize them. In these ways he instilled an enthusiasm for the search of knowledge which extended far beyond his own students or the confines of his own country. As translations of his work appeared in English his sphere of influence on scientific thought spread. Publications such as "*Die Thier Chemie*" [3] which appeared first in 1842 and in his later "Familiar Letters on Chemistry" [4] had great influence in establishing centers of biochemical learning in Germany and elsewhere. He became professor at Munich in 1852 where he remained until his death. Liebig's accomplishments are ably summarized by Hubert B. Vickery,[5] his recent historian.

The greatest achievement of Liebig in physiological chemistry was the foundation of the German school. Studies of proteins and their nutritional significance, initiated by the brilliant French workers, were destined to become primarily the province of German research and Liebig was most instrumental in this transition which brought this new blood and intensified interest in the problems.



Iacopo Bartolomeo Beccari (1682–1766)

From the beginning of the protein problem there have been specific points of attack upon which progress in the field has hung. They are embodied in four problems as significant today, in spite of our progress, as they were in the days before Liebig: (1) The variety and distribution of proteins in the biological world; (2) the structure and properties of proteins; (3) the changes in proteins coincident to digestion and assimilation in the body; (4) the functions of proteins in animal bodies.

The Occurrence and Variety of Proteins. Among the very early workers in the isolation of proteins none is more interesting than Iacopo Bartolomeo Beccari (1682–1766), professor at the University of Bologna during most of his life. Among his scientific writings which appear in the "Commentaries of the University of Bologna" is one [6] titled "*de Frumento*" which appeared in 1734, in which Beccari described the preparation of gluten, the protein portion of wheat flour. His method of manufacture is essentially that in use today. The following comments by Beccari, which laid the groundwork concerning vegetable proteins, are translated from the medieval Latin:

"This is a thing of little labor. Flour is taken of the best wheat, ground moderately lest the bran go through the sieve, for it ought to be purified as far as possible in order that all suspicion of mixture should be removed. Then it is mixed with the purest water and agitated. What remains after this process is set free by washing, for water carries off with itself whatever it is able to dissolve. The rest remains untouched.

"Afterward that which the water leaves is taken in the hands and pressed together and is gradually converted into a soft mass and beyond what I could have believed tenacious, a remarkable kind of glue and suitable for many purposes, among which it is worth mentioning that it can no longer

Joseph Gay-Lussac (1778–1850)

be mixed with water. Those other parts which the water carries away with itself for some time float and render the water milky. Afterward they gradually settle to the bottom but do not adhere together; but like a powder return upward at the slightest agitation. Nothing is more nearly related to this than starch or better, it is indeed starch."

Beccari [6] characterized the starchy material of flour, stating that it would ferment to give acid spirits indicating its "vegetable nature." But in contrast the gluten was of "animal nature" for "within a few days it gets sour and rots and very stinkingly putrifies like a dead body." Such was the method of the day for distinguishing proteins from carbohydrates. The belief in the animal nature of gluten was current a century later in Mulder's and Liebig's theory of the identity of animal and plant proteins and the thought that vegetable protein consumed by herbivora becomes directly the flesh and blood of the animal.

By Liebig's time, in fact well before then, the proteins were known to be widely distributed. Nitrogen was found as a constant constituent of all animal tissues by the French chemist Pierre Bertholet (1827–1907). Joseph L. Gay-Lussac (1778–1850), applying his method, concluded that all seeds "contain a principle abounding in azote." The proteins of legume seeds had been studied by Henri Braconnot (1781–1855) and later legumin-like proteins were obtained from a great variety of seeds by extraction with water and precipitation by the addition of acetic acid.

One after another the various protein materials were discovered which later would play such important roles in the examination of nutritive value. Gelatine from bones had been prepared since the days of Robert Boyle (1627–1691) in the seventeenth century. Interest in the food value of gelatine was revived by food scarcity during the French Revolution, at which time Louis Proust (1754–1826) improved the methods of gelatine manufacture. The famous physician and physiologist François Magendie (1783–1855) served as chairman of the French commission for examining the nutritive value of gelatine in 1842. Zein, the protein of corn, was discovered at Harvard University early in the nineteenth century by John Gorham (1783–1829).[7] Casein was well known because of its occurrence in the food trades for centuries.

Early Studies of the Properties and Composition of Proteins. Before the term protein was coined to designate these widely distributed materials, workers designated them variously as albumins or quaternary azotized substances and recognized them as set apart from the hydrates of carbon and fats by their high content of nitrogen. Long before Liebig's birth in Darmstadt, chemistry found, as we have seen, that the albumins would undergo putrefaction spontaneously, in contrast to the fermentation characteristic of carbohydrates. It was also known that upon destructive heat distillation of these substances ammonia, or "alkaline air," was produced. Also, their insoluble salts with heavy metals such as mercury, silver and lead were known. The coagulation of blood serum and egg white was recognized and in a general way the alteration of solubility relations during denaturation had received attention. Hemoglobin had been found to contain iron. Fibrin and the azotized principles of milk and cereals had been examined. It is little wonder that these related materials of various and unique properties captured the attention of many workers.

Basic to the progress of any science is the development of analytical tools by which observations can be gathered. With the albumins, nitrogen analysis was the first key to open new vistas for the old masters. Oxidation of organic material in the presence of cupric oxide, with collection and measurement of the resultant gases, was the most satisfactory of the early methods. It was developed extensively by Gay-Lussac,[8] first while he was professor at the Sorbonne, and later when he was chemist at the *Jardin des Plantes* in Paris. His method was modified by Jean Dumas (1800–1884) and used by Dumas' contemporary, Liebig. Today, the Dumas method still is the classic, having undergone many modifications and adaptation to microprocedure.[9]

Much later, in 1841, F. Varrentrapp and H. Will[10] presented a total