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PROTEINS and AMINO ACIDS in NUTRITION

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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

CARL LOUIS AUGUST SCHMIDT

Born on March 7, 1885, in Brown County, South Dakota. From 1924 until he passed away on February 23, 1946, in Berkeley, he was Professor and Chairman of the Department of Biochemistry at the University of California, Berkeley, California.

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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Chapter 1

Proteins in Nutrition (Historical)*

ELIOT F. BEACH, PH.D

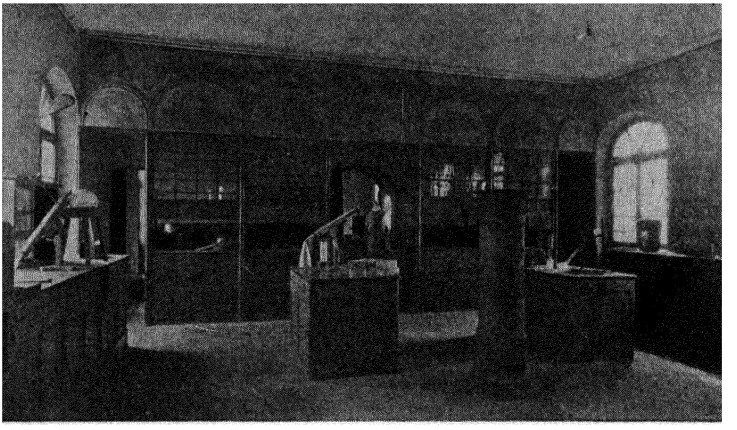
Assistant Director, Research Laboratory, Children's Fund of Michigan, Detroit

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.

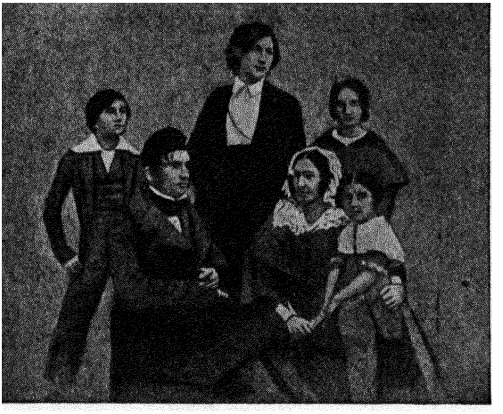
With the profound observation of Lavoisier, in 1780, that "life is a chemical function," biochemistry in its modern aspect emerged from its prenatal obscurity to grow and mature in the environment of the new experimental philosophy. The father of modern chemistry spent his few remaining productive years 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

nitrogen method based on the liberation of ammonia by heating protein with alkali, followed by gravimetric estimation of the ammonia as its chloroplatinate. Although the slow, tedious procedure had certain fundamental inaccuracies, the method had certain technical advantages over that of Dumas when applied to metabolic observations and it was used in many early studies of that type. Remembering the slow and difficult procedures available to chemists early in the nineteenth century, one is doubly impressed with the inestimable service rendered on behalf of our knowledge of proteins by the Danish chemist, J. Kjeldahl (1849–1900),[11] of Carlsberg, when in 1883 he presented his method for catalyzed digestion of nitrogenous materials in sulfuric acid to produce ammonia quantitatively.

François Magendie (1783–1855)

Before Liebig became prominent, amino acids had been discovered. Although workers of that era were far from the modern concept of amino acid structure, their observations sowed the seed which would later produce the theory of the amino acid building blocks, or "*bausteine*," of the protein molecule. In 1810 the British physician, William Wollaston (1766–1828),[12] discovered cystine in certain urinary calculi and named the substance cystic oxide. William Prout (1785–1850) did elementary analyses of the substance but many years were to pass before sulfur would be found to be one of its component elements and would be detected as one of the products of protein disintegration.

In France, Proust,[13] working with the flavoring matter of cheeses, in 1819 isolated from cheese a white compound which he called casein oxide. A year later, Braconnot,[14] director of the Horticultural Gardens in Nancy, obtained the same compound from sulfuric acid digest of muscle fiber and wool and named it leucine. This was the first use of the modern type of

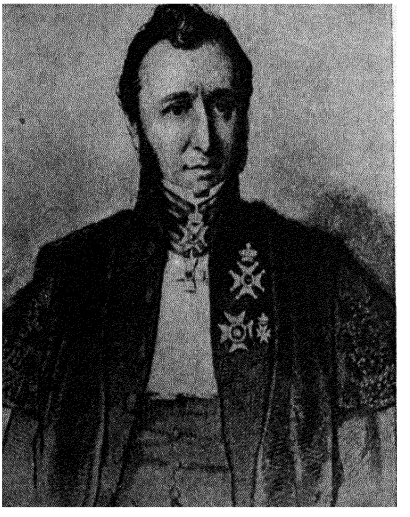
acid hydrolysis for isolation of amino acids and the first demonstration that protein hydrolysis yielded simpler crystalline compounds. Also in 1820 in the same work, Braconnot described the isolation of glycine from the acid hydrolysate of fish glue. Because of its characteristic sweet taste the product was called "sugar of gelatin."

The discovery of tyrosine was a contribution of Liebig.[15] He reported in a brief paper in 1846 the separation of this compound from casein after fusion with caustic potash, dissolving in water and neutralization with acetic acid. A year later he obtained the same compound [16] from fibrin and serum albumin. The product was finally isolated by acid hydrolysis, using the earlier technique of Braconnot. Liebig and his students also applied oxidizing agents such as manganese dioxide and chromic acid during acid hydrolysis of proteins, thus obtaining and identifying a series of acids and aldehydes. The idea of studying the degradation products of protein, which was to play such an important role in the next generation, stems from Liebig's imaginative genius.

Of all Liebig's contemporaries, the Dutch chemist, Gerard J. Mulder (1802–1880), provided the greatest immediate stimulus to Liebig's work. The theories of Mulder had great influence in unifying thought concerning the "quaternary azotized substances." In his paper of 1838 in the *Bulletin des Sciences Physiques et Naturelles en Neederlande* and reprinted a year later in the *Journal für praktische Chemie*,[17] under the title "*Ueber die Zusammensetzung einiger thierischen Substanzen*," Mulder first coined the term "protein" as a class name for the "quaternary azotized substances." The word, derived from the Greek, *proteios*, meaning "primus," or "primary," shows the conviction with which Mulder viewed protein as fundamental to life. Though our horizons have broadened since, many biochemists of today believe that the elusive process of life itself resides principally in protein. Certainly, modern research on the nucleoproteins has produced strength for the belief that protein constitutes the true lifestuff of protoplasm.

Mulder made analyses of blood fibrin, egg albumin, wheat gluten and other proteins for the elements nitrogen, carbon, hydrogen, oxygen, sulfur and phosphorus, and deducted from these values the best empirical formula for protein on the basis of atomic proportions. He concluded that all protein materials are identical in their relative proportions of hydrogen, oxygen, carbon and nitrogen and that these together constitute the nucleus occurring in all protein materials of the plant and animal kingdoms. Protein bodies, he found, differed only in regard to the quantity of phosphorus and sulfur attached to their common nucleus, the combination being represented symbolically as "Pr + SP." Mulder did not claim for his empirical formula of the protein nucleus any merit beyond that of being the best approximation possible with the current knowledge — the best that can be said for any theory.

Mulder was concerned with a problem in protein nutrition which was destined to pique the minds of many successors. It was easy to understand how carnivorous animals living upon flesh could obtain flesh for body construction, but the herbivora likewise grow and increase in flesh without consuming meat in their food. On the basis of his analyses and his belief that protein substances in plants are identical with those in animals, he



Gerard J. Mulder (1802–1880)

reasoned that plant proteins must be as effective as flesh in replenishing the blood and flesh. This became a central and guiding concept of proteins in nutrition, for Liebig and for workers in many years to follow. In Mulder's famous "*Ueber die Zusammensetzung einiger thierischen Substanzen*" [17] we see the framing of a great theory:

"*Es scheint also, dass die Thiere ihre wesentlichsten näheren Bestandtheile unmittelbar aus dem Pflanzenreiche ziehen. Es ist möglich, dass der Pflanzeneiweissstoff Schwefel und Phosphor in einem andern Verhältnisse als der thierische Eiweissstoff, der Faserstoff u.s.w. enthält; aber der quaternäre organische Körper ist das Protein selbst.*

"*Die pflanzenfressenden Thiere sind also, aus diesem Gesichtspuncte betrachtet, von den fleischfressenden nicht verschieden. Beide werden durch das Protein genährt, durch denselben organischen Körper, der eine Hauptrolle in ihrer Oekonomie spielt.*"

Liebig accepted from Mulder the theory of the unity of proteins, and embellished it with his clever imagination and keen reasoning. The brilliant theory contained in his "Animal Chemistry" [3] held sway for many years in animal physiology.

"When animal albumin, fibrine, and caseine are dissolved in a moder-

ately strong solution of caustic potash, and the solution is exposed for some time to a high temperature, these substances are decomposed. The addition of acetic acid to the solution causes, in all three, the separation of a gelatinous, translucent precipitate, which has exactly the same characters and composition, from whichever of the three substances above mentioned it has been obtained.

"Mulder, to whom we owe the discovery of this compound, found, by exact and careful analysis, that it contains the same organic elements and exactly in the same proportion, as the animal matters from which it is prepared; insomuch, that if we deduct from the analysis of albumin, fibrine, and caseine, the ashes they yield, when incinerated, as well as the sulphur and phosphorus they contain, and then calculate the remainder for 100 parts, we obtain the same result as in the analysis of the precipitate above described, prepared by potash, which is free from inorganic matter.

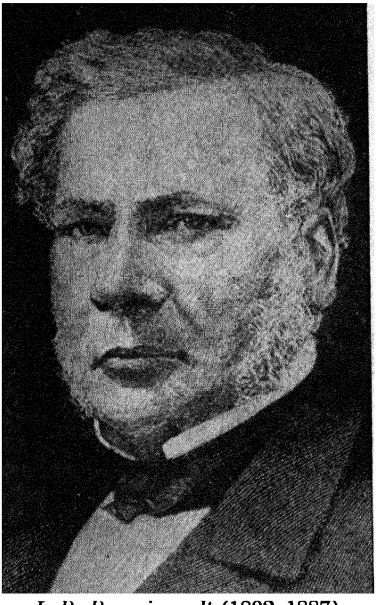
"Viewed in this light, the chief constituents of the blood and the caseine of milk may be regarded as compounds of phosphates and other salts, and of sulphur and phosphorus, with a compound of carbon, nitrogen, hydrogen, and oxygen, in which the relative proportion of these elements is variable; and this compound may be considered as the commencement and starting-point of all other animal tissues, because these are all produced from the blood.

"These considerations induced Mulder to give to this product of the decomposition of albumen, etc., by potash, the name of proteine, from πρωτεύω 'I take the first rank.' The blood, or the constituents of the blood, are consequently compounds of this proteine with variable proportions of inorganic substances."

However, a few years later Liebig's analyses of the protein material prepared according to Mulder's method yielded evidence that proteins vary in elementary composition particularly with reference to the behavior of the element sulfur. This indication of diversity in protein composition led to a bitter argument with Mulder and eventually displaced Mulder's theory. Though Liebig only recognized the existence of four different sorts of protein his work marked the beginning of the present day vision of the individuality in the composition of each specific protein in nature.

Metabolism of Proteins. After nitrogen was found to be a component of excreta, the fate of nitrogenous foods during metabolism also received attention. Lavoisier early had established the viewpoint that atmospheric nitrogen did not serve as nutriment for the body, nor were nitrogenous end-products of metabolism exhaled in respiration. This premise was not accepted by all scientists and was difficult to prove experimentally. Chemists like Sir Humphry Davy (1778–1829) took a position opposite to Lavoisier's and it was over 120 years before the polemics on this matter were silenced by the modern painstaking respiration experiments of August Krogh (1874–),[18] in Copenhagen.

The first nitrogen balance studies with animals were designed to test the point of possible fixation of atmospheric nitrogen by animal bodies. Credit for the conception of such studies, which would serve in later years in building the theories of protein in nutrition, is due J. B. Boussingault (1802–1887), a French contemporary of Liebig. Boussingault, primarily an



J. B. Boussingault (1802–1887)

agricultural chemist, was born in Paris. At the age of 20 he went to South America as representative of a mining concern, travelled in Peru and Bolivia and knew the hero Simon Bolivar. His wife's estate in Bechelbrom, Alsace, was the experimental farm on which he carried out much of his famous work on rotation of crops, animal feeding and the fertilizers.

The nitrogen balance studies of Boussingault [19] appeared in 1839 and are the first on record. One subject was a lactating cow whose food, urine, feces, and milk were collected and analyzed for nitrogen, carbon and hydrogen. A second experiment [20] was performed with a horse as the subject. One cannot fail to be impressed with the amount of work involved in these studies. The only method then available, that of Dumas, presents real difficulties when applied to analysis of foods and excretory products. The significant conclusion which Boussingault made from his experiments is that animals, unlike leguminous plants, do not fix atmospheric nitrogen. The nitrogen content of the excreta of animals does not exceed the nitrogen content of their food. Inasmuch as his analyses of excreta and milk did not account for all of the nitrogen consumed by the animals, he postulated that nitrogenous waste products may have been lost through the expired air.

Boussingault's concept of the nitrogen balance is best illustrated by excerpts from his treatise on "Origin of Animal Principles," in his book "Rural Economy":[21] "It is now generally admitted that the food of animals must necessarily contain azote; and this circumstance has led to the inference, that the herbivorous tribes obtain from their food the azote which enters into the constitution of their bodies.

"In a general way, the individual consuming a certain portion of food every day, nevertheless does not increase in his average weight. This is what occurs with animals upon the quantity of food which is known to be sufficient for their keep; and it has been found that the human subject, living very regularly, returns at a certain hour, or at certain hours of the day, to a certain mean weight. Grooms, farm servants, etc., are perfectly well aware of the fact, that with a certain allowance of hay and corn, a horse will be kept in the condition necessary to do the work required of him without either gaining or losing in flesh.

"Under such circumstances, the whole of the elementary matter contained in the food consumed ought to be found in the dejections, the excretions and the product of the act of respiration. And assuming that this is so, it might then be maintained that none of the elements is assimilated, assimilation being taken in the sense of an addition of principles introduced with the food to the principles already present in the body. Yet is there unquestionably assimilation in the sense that the alimentary matters of the food become fixed in the system, having there undergone modification or change; and that they replace, or come instead of other elements of the same kind, which are daily thrown off by the vital acts of the economy.

"During the nutrition of a young animal, and also in the process of fattening an adult, things go on differently, here there is unquestionably definitive fixation of a portion of the matter contained in the food; there is no longer balance between waste and the supply; an animal then increases in weight notably and rapidly.

"Looking at the question of feeding in the most general way, then, I admit that an adult animal, upon the daily allowance, voids a quantity of matter in its various excretions precisely equal to the quantity which it receives in its food: all the elements, the same in nature and in quantity, which are contained in the food, are also contained in the excrements, vapors, and gases, which pass off from the living body; carbon and azote, hydrogen and oxygen, phosphorus, sulfur and chlorine, calcium, magnesium, sodium, potassium and iron, as they are all encountered in the food so are they all encountered in the body, and also in the excretions of an animal; and it seems certain that no one of these primary or simple substances can be wanting in the nutriment without the body very speedily feeling the ill effects of its absence. . . .

"In what has just been said, I take it for granted that animals do not absorb or assimilate any of the azote which forms so large a constituent of

the air they breathe; and I am warranted in this by the researches of every physiologist of any name or distinction."

Through Liebig's experiments and teachings on respiration and nitrogen excretion the field of intermediary metabolism was materially developed. He classified protein-containing foods as "plastic foods," in contrast to fat and carbohydrate materials which he called "respiratory foods." To the plastic foods he attributed the support of the body flesh and blood and its renewal, while the respiratory foods were visualized as the source of animal heat through oxidation: [3]

"According to what has been laid down in the preceding pages, the substances of which the food of man is composed may be divided into two classes: into *nitrogenised* and *non-nitrogenised*. The former are capable of conversion into blood; the latter incapable of this transformation.

"Out of those substances which are adapted to the formation of blood are formed all the organized tissues. The other class of substances, in the normal state of health, serve to support the process of respiration. The former may be called the *plastic elements of nutrition;* the latter, *elements of respiration.*

"Among the former we reckon vegetable fibrine, vegetable albumen, vegetable caseine, animal flesh, and animal blood.

"Among the elements of respiration in our food are fat, starch, gum, cane sugar, grape sugar, sugar of milk, pectine, bassorine, wine, beer, spirits."

His discussion of the utilization of the constituents of milk by the young animal is a good example of the application of these theories:

"The carbon and hydrogen of butter, and the carbon of the sugar of milk, no part of either of which can yield blood, fibrine, or albumen, are destined for the support of the respiratory process, at an age when a greater resistance is opposed to the metamorphosis of existing organisms; or, in other words, to the production of compounds, which in the adult state are produced in quantity amply sufficient for the purpose of respiration.

"The young animal receives the constituents of its blood in the caseine of the milk. A metamorphosis of existing organs goes on, for bile and urine are secreted; the matter of the metamorphosed parts is given off in the form of urine, of carbonic acid, and of water; but the butter and sugar of milk also disappear; they cannot be detected in the faeces.

"The butter and sugar of milk are given out in the form of carbonic acid and water, and their conversion into oxidised products furnishes the clearest proof that far more oxygen is absorbed than is required to convert the carbon and hydrogen of the metamorphosed tissues into carbonic acid and water."

But he differentiates protein from other food principles by stating that the plastic element resists oxidation to such a degree that it is not used until the respiratory elements (fat and carbohydrate) are all utilized. This was essential in his theory: [3]

"If the albumen of the blood, which is derived from the plastic portion of the food, possessed in a higher degree the power of supporting respiration, it would be utterly unfit for the process of nutrition. Were albumen as such, destructible or liable to be altered, in the circulation, by the inhaled oxygen, the relatively small quantity of it, daily supplied to the blood by the digestive organs, would quickly disappear; and the slightest disturbance of the digestive function would of necessity put an end to life.

"As long as the blood contains, besides albumen, other substances, which surpass it in attraction for oxygen, so long will the oxygen be unable to exert a destructive action on this, the chief constituent of the blood; and the significance of the non-nitrogenous part of the food is thus made clear.

"Starch, sugar, and fat, serve to protect the organised tissues, and, in consequence of the combination of their elements with oxygen, to keep up the temperature of the body.

"The sulphurised and nitrogenous constituents of food determine the continuance of the manifestations of force; the non-nitrogenous serve to produce heat. The former are the builders of organs and organised structures, and the producers of force; the latter support the respiratory process; they are materials for respiration.

"The necessity for the simultaneous presence of both, of the plastic and respiratory materials, and for their due admixture, is now obvious. The sum of both, daily required by the body, depends on the amount of oxygen taken up; their relative proportions depends on the causes of loss of heat and expenditure of force."

One of the most unique theories of Liebig was that the metabolism of the plastic, or protein, elements formed the basis of animal motion and muscle force. He reasoned that, inasmuch as the muscles' predominant constituent is protein, protein must be the product metabolized during muscle contraction. His conclusion was confirmed by erroneous and uncontrolled observations of urinary nitrogen excretion during exercise. As a new chemistry evolved in the next generation, the downfall of his theory caused Liebig great disappointment: [3]

"But however closely the conditions of the production of heat and of force may seem to be connected together, with reference to mechanical effects, yet the disengagement of heat can in no way be considered as in itself the only cause of these effects.

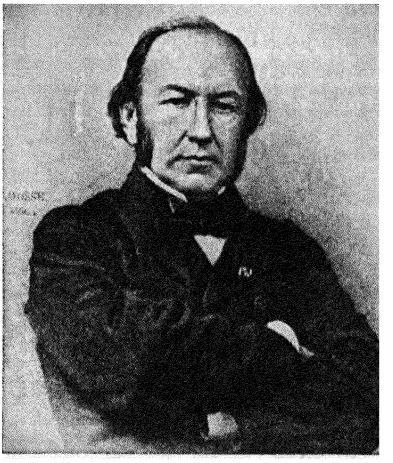
"All experience proves, that there is, in the organism, only one source of mechanical power; and this source is the conversion of living parts into lifeless, amorphous compounds. . . .

"The amount of azotised food necessary to restore the equilibrium between waste and supply is directly proportional to the amount of tissues metamorphosed.

"The amount of living matter, which in the body loses the condition of

life, is, in equal temperatures, directly proportional to the mechanical effects produced in a given time.

"The amount of tissue metamorphosed in a given time may be measured by the quantity of nitrogen in the urine."



Claude Bernard (1813–1878)

Function of Protein as Food. Interest in protein as a nutriment increased with the development of protein chemistry. Of the older masters in this field none is more prominent than Magendie, the teacher of Claude Bernard (1813–1878), who is often designated as the father of experimental medicine. Magendie became interested in the then current use of special dietary treatment for the cure and prevention of urinary calculi. Such diets were particularly poor in nitrogenous substances and this led him to study the effects of nitrogen-free foods upon dogs.[22] Experimental animals were fed single, protein-free substances such as sugar, olive oil, gum or butter as their only food. All of the dogs developed severe debility and died in a month or less (see page 16). He found this could be prevented by feeding protein-containing food substances. Thus, Magendie proved that to maintain life animals require nitrogenous material in their diets in organic form. During the experiments he recorded also the fact that the dogs on certain diets developed peculiar lesions around the eyes. Presumably this is the first record of the occurrence of the ophthalmia symptoms of vitamin A deficiency which were to be rediscovered a century later during studies of the vitamins.

Boussingault, the great "farmer of Bechelbrom," working with animal feeding and recognizing the importance of nitrogen in the economy of plants, followed Magendie's teaching regarding the essential nature of nitrogenous nourishment for animals. So impressed was he with this idea that he analyzed foods and animal feeds for nitrogen, inferring from the

MEMOIRE

Sur les propriétés nutritives des substances qui ne contiennent pas d'azote.

Lu à l'Académie des Sciences le 19 août 1816.

Par M. F. Magendie.

On n'a que des connaissances très-superficielles sur le mouvement moléculaire qui constitue la nutrition des animaux; on sait d'une manière générale que ce mou-

vement existe; les excrétions habituelles, la nécessité des alimens, plusieurs maladies, quelques expériences directes en sont des preuves incontestables. On sait encore que ce mouvement est plus rapide chez les enfans et dans la jeunesse que dans un âge plus avancé. On a aussi reconnu qu'il présente des modifications remarquables dans les différentes classes d'animaux; mais c'est à-peu-près là que se bornent les notions positives touchant le mouvement nutritif, son mode particulier, les variations qu'il doit subir dans chaque organe, les lois qui le régissent sont à-peu-près entièrement inconnus.

* * * * *

J'ai pensé qu'on pourrait acquérir quelques notions exactes sur ce sujet en soumettant des animaux, pendant le temps nécessaire, à une nourriture dont la composition chimique serait rigoureusement déterminée.

Les chiens étaient très-propres à ce genre d'expériences; ils se nourrissent, comme l'homme, également bien de substances végétales et animales.

Chacun sait qu'un chien vit très-bien en ne mangeant que du pain; mais, en le nourrissant ainsi, on n'en peut rien conclure relativement à la production de l'azote dans l'économie animale; car le gluten que contient le pain est une substance très-abondante en azote. Pour obtenir un résultat satisfaisant, il fallait nourrir un de ces animaux avec une substance réputée nutritive, mais qui ne contînt pas d'azote.

A cet effet, j'ai mis un petit chien âgé de trois ans, gras et bien portant, à l'usage du sucre blanc et pur pour tout aliment, et de l'eau distillée pour boisson : il avait de l'un et de l'autre à discrétion.

Les sept ou huit premiers jours, il parut se trouver assez bien de ce genre de vie; il était gai, dispos, mangeait avec avidité et buvait comme de coutume. Il commença à maigrir dans la seconde semaine, quoique son appétit fût toujours fort bon, et qu'il mangeât jusqu'à six ou huit onces de sucre en vingt-quatre heures. Ses excré-

tions alvines n'étaient ni fréquentes ni copieuses; en revanche, celle de l'urine était assez abondante.

La maigreur augmenta dans la troisième semaine, les forces diminuèrent, l'animal perdit la gaîté, l'appétit ne fut pas aussi vif. A cette même époque, il se développa, d'abord sur un œil et ensuite sur l'autre, une petite ulcération au centre de la cornée transparente; elle augmenta assez rapidement, et, au bout de quelques jours, elle

ANNALES

DE

CHIMIE ET DE PHYSIQUE,

Par MM. Arago, Berthollet, Biot, Bouillon-Lagrange, Chaptal, Chevreul, d'Arcet, Deyeux, Dulong, Gay-Lussac, Hassenfratz, Laugier, Monge, Prieur, Séguin, Thenard *et* Vauquelin,

Rédigées par MM. GAY-LUSSAC et ARAGO.

TOME TROISIÈME

A PARIS,

Chez CROCHARD, Libraire, rue de l'Ecole-de-Médecine, n° 3, près celle de la Harpe.

1816.

avait plus d'une ligne de diamètre; sa profondeur s'accrut dans la même proportion; bientôt la cornée fut entièrement perforée, et les humeurs de l'œil s'écoulèrent au-dehors. Ce singulier phénomène fut accompagné d'une sécrétion abondante des glandes propres aux paupières.

Cependant l'amaigrissement allait toujours croissant; les forces se perdirent; et quoique l'animal mangeât, par jour, de trois à quatre onces de sucre, la faiblesse devint telle, qu'il ne pouvait ni mâcher ni avaler; à plus forte raison, tout autre mouvement était-il impossible. Il expira le trente-deuxième jour de l'expérience. J'ouvris son cadavre avec toutes les précautions convenables; j'y reconnus une absence totale de graisse; les muscles étaient réduits de plus des cinq sixièmes de leur volume ordinaire; l'estomac et les intestins étaient aussi très-diminués de volume et fortement contractés.

La vésicule du fiel et la vessie urinaire étaient distendues par les fluides qui leur sont propres. Je priai M. Chevreul de vouloir bien les examiner; il leur trouva presque tous les caractères qui appartiennent à l'urine et à la bile des animaux herbivores, c'est-à-dire que l'urine, au lieu d'être acide comme elle l'est chez les carnivores, était sensiblement alcaline, n'offrait aucune trace d'acide

relative nitrogen contents their nutritive value. His paper [23] on the subject, in 1836, is summarized in his "Rural Economy" [21] and illustrates the way in which recognition of the special nutritive virtues of protein dawned in the minds of men:

"The identity, in point of composition and properties, which appears to obtain between certain substances derived from either kingdom of nature, naturally led to the conclusion that animals do not form or originate the substances which enter into their organization, but that they find these ready formed in their food, merely appropriate them; whence we must conclude, that herbivorous animals assimilate several of the proximate principles of plants immediately, causing them to undergo but slight modifications, and that the elements of the animal tissues and fluids pre-exist in vegetables. . . .

"It is by no means easy to ascertain precisely the amount of the azotized constituents, gluten, and albumin, contained in plants; to do so requires both time and pains. But let it be once admitted that the nutritive properties of forage increase in the precise ratio of these matters, this is clearly as much as to say that the value is in proportion to the quantity of azote contained in the food, and that it becomes a matter of the highest moment to have at hand a ready mode of determining the point. I believe it infinitely better to get at the quantity of azote immediately, which is easily done, than by any round-about and laborious process to ascertain the amount of albumen and gluten; the quantity of azote ascertained, it is most easy to deduce the quantity of albumen and gluten — in other words, of *flesh* — contained in each particular species of food examined, for, as in a general rule, vegetable food does not contain any other azotized principle. It is true, indeed, that all the azotized principles of vegetable origin cannot be considered as nutritious; some of them, on the contrary, are virulent poisons or active medicines, according to the dose in which they are administered. But these poisonous substances are not met with in appreciable quantity in the plants which are commonly grown for the food either of man or beast. Still, all the truly nutritious articles of food contain an azotized principle. The experiments of M. Magendie have shown, that substances which contain no azote, such as sugar, starch, oil, will not support life; and, on the other hand, it is ascertained that the quality of alimentary matter, flour for example, increases with the amount of gluten which it contains. It is because the seeds of the leguminous vegetables are richer in azotized principles — that is, in *flesh* — that they are also more highly nutritious than the seeds of the cereals.

"These several considerations, therefore, induce me to conclude, that *the nutritious principles of plants and their products reside in their azotized principles, and consequently that their nutritious powers are in proportion to the quantity of azote they contain.* From what procedes, however, it is obvious that I am far from regarding azotized principles alone as sufficient

for the nutrition of animals; but it is a fact, that every highly azotized vegetable nutritive substance is generally accompanied by the other organic and inorganic substances which concur in nutrition."

Prout did much for the advancement of chemistry and nutrition early in the nineteenth century. After his medical training in Edinburgh, he settled in London. While practicing his profession he maintained an active interest in chemistry, discovering hydrochloric acid in gastric juice, showing that atomic weights are all multiples of that of hydrogen, and observing the excretion of uric acid as the principle nitrogenous component of reptile and bird urine. His outstanding contribution to knowledge of proteins in nutrition was his recognition of the three proximate, or staminal, principles in foodstuffs: the "saccharina, oleosa, and albumosa," or the carbohydrate, fat, and protein of today. This work [24] appears in his dissertation "Chemistry, Meteorology and the Function of Digestion, Considered with Reference to Natural Theology" published by the Royal Society in the series of Bridgewater treatises endowed fo the purpose of demonstrating "The Power, Wisdom, and Goodness, of God, as Manifested in the Creation."

In stating the staminal principles of food Prout helped focus attention upon their dietetic significance. He was impressed particularly with the nutritive potential of milk, arguing teleologically, as we are prone to do today, that here if ever is the perfect nutriment, elaborated as it is by nature for the nutrition of the growing young. He believed in the providential symmetry of its contents of fats, proteins and carbohydrates and looked upon milk as the prototype of all foods:

"The composition of the substances by which animals are usually nourished, favours the mixture of the primary staminal elements forming the basis of their food; since most of these substances are compounds, of at least two, of the staminal elements. Thus most of the gramineous and herbaceous matters contain the saccharine and the albuminous elements; while every part of an animal contains at least albumen and oil. Perhaps therefore, it is impossible to name a substance constituting the food of the more perfect animals, which is not essentially a natural compound of at least two if not of all the three proximate elements which afford nourishment to organized bodies. But in the artificial diet of man, we see this great process of mixture more strongly exemplified. He, dissatisfied with the spontaneous productions of nature, culls from every source; and by the force of his reason, or rather of his instinct, forms in every possible manner, and under every disguise, the same great alimentary compound. This after all his cooking and his art, how much soever he may be disinclined to disbelieve it, is the sole object of his labour; and the more nearly his results approach to this object, the more nearly do they approach perfection. Even in the utmost refinements of his luxury and in his choicest delicacies, the same great principle is attended to; and his sugar and flour, his eggs and butter, in all their various forms and combinations, are nothing more or less, than disguised imita-

tions of the great alimentary prototype *milk*, as furnished to him by nature."

Liebig visualized blood rather than milk as the prototype of all food. He believed that food protein entered the system and whether of vegetable or animal origin could be incorporated without alteration into the blood and used for formation of the tissues bathed by the blood: [3]

"The nutritive process in the carnivora is seen in its simplest form. This class of animals lives on the blood and flesh of the graminivora; but this blood and flesh is, in all its properties, identical with their own. Neither chemical nor physiological differences can be discovered.

"The nutriment of carnivorous animals is derived originally from blood; in their stomach it becomes dissolved, and capable of reaching all other parts of the body; in its passage it is again converted into blood, and from this blood are reproduced all those parts of their organisation which have undergone change or metamorphosis. . . .

"The process of nutrition in graminivorous animals appears at first sight altogether different. Their digestive organs are less simple, and their food consists of vegetables, the great mass of which contains but little nitrogen. . . .

"Vegetables produce in their organism the blood of all animals, for the carnivora, in consuming the blood and flesh of the graminivora, consume, strictly speaking, only the vegetable principles which have served for the nutrition of the latter. Vegetable fibrine and albumen take the same form in the stomach of the graminivorous animal as animal fibrine and albumen do in that of the carnivorous animal.

"From what has been said, it follows that the development of the animal organism and its growth are dependent on the reception of certain principles identical with the chief constituents of blood.

"In this sense we may say that the animal organism gives to blood only its form; that it is incapable of creating blood out of other substances which do not already contain the chief constituents of that fluid. We cannot, indeed, maintain that the animal organism has no power to form other compounds, for we know that it is capable of producing an extensive series of compounds, differing in composition from the chief constituents of blood; but these last, which form the starting point of the series, it cannot produce.

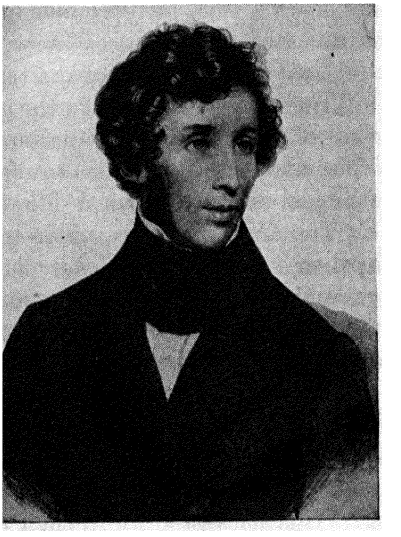
"The animal organism is a higher kind of vegetable, the development of which begins with those substances, with the production of which the life of an ordinary vegetable ends. As soon as the latter has borne seed, it dies, or a period of its life comes to a termination."

Carl Voit and His Contemporaries

After Liebig's major pronouncements, protein for years was to monopolize the center of the stage in nutrition studies. In naming protein as the one "plastic" or structural food, thereby relegating all other principles only

to the support of respiration, he had done little to change the Hippocratian doctrine of the single alimentary principle. Protein assumed the focal point of the still unitarian theory of nutrition.

Carl Voit (1831–1908)



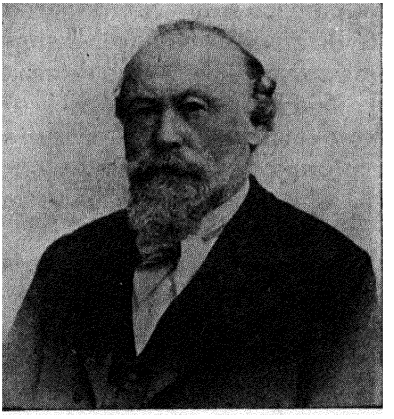
Frederick Wöhler (1800–1882)

During the last half of the nineteenth century new methods and the perfection of old ones brought about continual changes in beliefs regarding protein in nutrition. Throughout this period Carl Voit (1831–1908), a great research physiologist and teacher, was prominent in contributing to this progress. He criticized Liebig for overemphasis of protein, presenting his view of fat, carbohydrate, and protein as the alimentary trinity. Even so, Voit never completely divorced himself from the habits of the past and as a consequence he overestimated the protein requirement of man.

Voit was born in Bavaria, and educated at Wurzburg and Munich, where he graduated in medicine. In his thesis, entitled "The Circulation of Nitrogen in the Animal Organism," he dedicated himself to his life work. He studied for a year in Gottingen under Frederick Wöhler (1800–1882) and then returned to Munich in 1863 to become a professor. One of Voit's great contributions was his extensive observations with perfected nitrogen balance techniques which he applied to men and dogs under various conditions of diet. Also, with a great expenditure of time he succeeded in developing a respiration calorimeter for the study of energy exchange in his subjects. Perhaps even more important was the training and inspiration he gave to a distinguished group of students who share with him the honor of establishing a great new body of facts and theories in nutrition.

Eduard Pflüger (1829–1910), one of Voit's most distinguished contemporaries, succeeded Hermann Ludwig Ferdinand von Helmholz (1821–1894)

as professor of physiology at Bonn and worked broadly in the field of intermediary metabolism. He was a great worker and volatile in scientific argument, a characteristic which led him into bitter controversies with Voit and other noted physiologists of their day. Throughout Pflüger's extensive published works, particularly *Pflüger's Archives*, his greatest monument, long and impassive arguments are found. Especially pertinent to the subject of proteins were two disputes with Voit which raged for years. One related to the formation of fat from protein, which Voit contended was possible and Pflüger said did not occur. The other was based on Pflüger's contention that ingested protein could not be metabolized until it became a firmly bound and integral part of the body's cellular structure. In contrast,



Eduard Pflüger (1829–1910)

Voit theorized that protein ingested is normally taken up in the form of circulating or storage protein (roughly, the protein of plasma and that loosely bound to body cells) and metabolized directly when needed.

How impressive the facts these men established were in their lasting value! How unimpressive the theories over which they contended! Voit proved the breadth of his vision when he said, "The value of a theory is not measured by its length of life but rather by the work which it stimulates."

Nitrogen Balance Studies. No single accomplishment in this period produced more fundamental progress in understanding protein nutrition than the development of the techniques of nitrogen balance study, for this procedure revealed the significance of nitrogenous foods in the body economy. The technique conceived by Boussingault many years earlier was applied in refined form by F. Bidder and C. Schmidt, at Dorpot. Their results [25] appeared in 1852 in the classical publication "*Die Verdauungssäfte und der Stoffwechsel*" and demonstrated that in adult cats food nitrogen ingested could be accurately accounted for in the urine and feces excreted. In the hands of Voit and his students nitrogen balance studies became the power-

ful instrument in metabolism work. After 1860 numerous papers by Voit and coworkers appeared, particularly in the *Zeitschrift für Biologie*, of which Voit was editor.

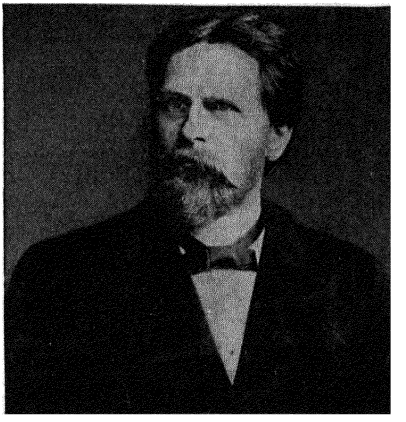
Voit studied the metabolism of nitrogen in dogs during starvation by determining urea excretion.[26] At first, during the withholding of food if ample fat stores existed in the body, there was a relatively low excretion of nitrogen, for fat was supplying a part of the internal energy needs of the subject. Later, after depletion of stored fat, nitrogen excretion increased, for body protein then was being used to provide for the portion of total metabolism which body fat formerly had served. Voit found also that exercise during starvation produced increased excretion of nitrogen, since the demands of activity augmented energy metabolism, which was being supported solely by body protein. However, dogs receiving ample fat and carbohydrates to cover their caloric needs for exercise did not show an increased excretion of nitrogen. Thus, Voit demonstrated the fallacy of Liebig's dictum that protein is the fuel of muscle contraction.

These conclusions were confirmed and extended by M. Pettenkofer and Carl Voit [27] with human subjects. They used the respiration calorimeter for measuring the respiratory quotient and demonstrated that, during fasting, body fat was the main source of energy. When food containing sugar and starch was given, energy was derived mainly from combustion of carbohydrates. Pettenkofer and Voit concluded that fat and carbohydrate are the principal sources of energy in muscle contraction, though they recognized the importance of protein in maintaining the structure of the muscular machine.

The death blow to Liebig's theory that protein was the source of muscle energy was dealt by A. Fick and J. Wislecenus, at Wurzburg. In 1865 they reported observations [28] on their own urinary nitrogen excretion while climbing the Faulhorn, an Alpine peak 2000 meters in altitude. During the ascent they consumed a virtually nitrogen-free diet rich in calories and their analyses showed that the protein metabolized during the experiment yielded far less energy than was necessary to lift their bodies and packs 2000 meters, therefore fat or carbohydrate must have been the principal fuel.

In 1867 Voit [29] reported the nitrogen balances of dogs receiving diets of meat only. He observed that nitrogen equilibrium could be attained at varying levels of protein intake. However, following a shift from one level of protein intake to another there was invariably a time lag before the attainment of equilibrium at the new level. A dog accustomed to a relatively low protein intake would, when given a higher protein intake, show a positive nitrogen balance for a few days, indicating protein storage, before equilibrium at this higher level of intake was finally established. Likewise, a shift from high to lower, though adequate, levels of protein intake resulted in protein loss for a few days. This and many other experiments of similar

character led Voit to his theory of two types of body protein. He visualized "organ protein" as the stable form of protein forming the essential structures of the body and not readily available for metabolic needs, in contrast to the "circulating" or "storage" type comprising the plasma proteins and that loosely bound in cells of certain organs, which may be readily mobilized for metabolism in periods of need. In this regard, Voit made the interesting observation that in starvation organs such as muscle and liver lose much of their substance, while brain and heart change little; an indication that the latter do not contain much storage-type protein.

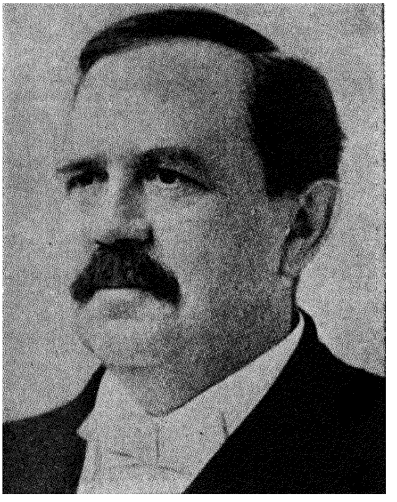


Max Rubner (1854–1932)

The determination of fecal nitrogen was an integral part of the refined nitrogen balance technique. Max Rubner (1854–1932), a student of Voit, devised a method for testing availability of protein from various food sources to the body by determining the difference between food nitrogen and fecal nitrogen. He found [30] that low-residue foods such as eggs, meat and cheese resulted in a fecal loss of only 2.5 to 3 per cent of the nitrogen consumed. From vegetables containing much roughage, such as carrots or coarse black bread, as much as 40 per cent of the original nitrogen was found in the feces. Thus, Rubner indicated the differences in nutritive efficiency of the proteins of different foodstuffs, on a basis principally related to factors involved in their digestion.

Felix Hirschfeld, in Berlin, 1890, also reported [31] that heavy exercise will not produce greater nitrogen excretion than the resting state provided the increased caloric needs are amply covered by non-nitrogenous foods. In stressing the importance of meeting caloric needs, Hirschfeld emphasized an important point which frequently had led to erroneous conclusions concerning protein requirement; earlier workers had not grasped the significance of energy balance as well as nitrogen balance in determining the overall picture of metabolism.

Pflüger, in 1891, published his classic "*Die Quelle der Muskelkraft.*" [32] Constantly at odds with the Voit school, he could be depended upon to take a rather different view of protein metabolism. Admitting that carbohydrate and fat might serve as the source of a part of the muscle energy, yet he believed that muscles could not be exerted without some loss of nitrogen. Pflüger showed that pure flesh alone would support dogs in a lean state and that protein must be capable of yielding energy for muscle activity for the



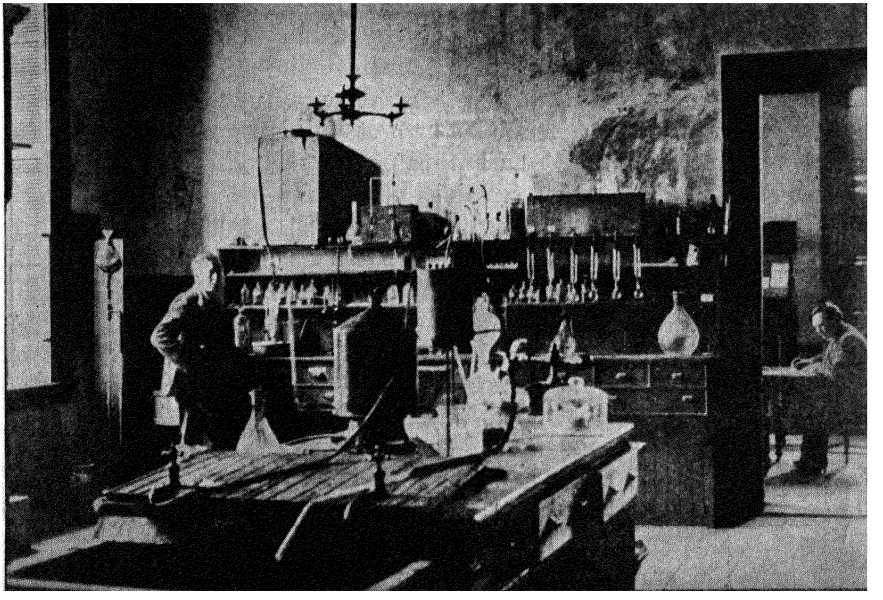
W. O. Atwater (1844–1907)

carbohydrate and fat contents of the meat diet he gave experimental dogs was so low as to be capable of furnishing scarcely that energy needed for the heart beat alone. Leaning heavily upon the old dicta of Liebig he retained the belief in protein as the key material of life, stating in his paper that the nutritive needs of any organism are determined by its amount of "flesh" (or protein) for stored fat and carbohydrate have no life. In protecting the cardinal position of protein he said, "*Die stickstoffreien Bestandtheile des Thierleibes sind todter Stoff. Nur das Eiweiss ist lebendig und vollzieht alle eigentliche Arbeit des Lebens.*"

Three years later, Nathan Zuntz (1847–1920),[33] at Berlin, found that when dogs were returned to normal dietaries after a period of low nitrogen feeding they tended to store a very substantial portion of their daily protein allowance and that they would do this despite heavy exercise. His experiments showed that the dogs' expenditure of calories in work during the protein storage was so high that only 4.4 per cent of the calories could be accounted for as metabolized protein, therefore, fat and carbohydrate were the predominant sources of muscular energy.

Hundreds of such nitrogen metabolism experiments were performed from

1860 to 1900. W. O. Atwater (1844–1907), a former student of Voit, compiled the then extant data on balance experiments. This work, done in conjunction with C. F. Langworthy, was prepared at Wesleyan University in Middletown, Connecticut, for the United States Department of Agriculture under the title "A Digest of Metabolism Experiments." [34]



Atwater's Laboratory

Levels of Dietary Protein. Logically, the observations of nitrogen balance produced the question of the most desirable level of protein intake. The problem was complicated by the fact that nitrogen equilibrium could be attained at a variety of levels of protein intake, provided the total needs of the body for calories were met. For this reason an experiment leading to an unequivocal answer to the problem was virtually impossible.

Carl Voit was first to face this problem. After extensive nitrogen balance studies of dogs, and dietary surveys of moderately active men he was convinced that 118 grams of protein daily was the desirable allowance. This level, known as the Voit standard, was set forth in 1881 in his classic "*Physiologie dęs Stoffwechsels.*" [35] The standard became a storm center of controversy which lasted well into the twentieth century. Although attacked by many opponents it was vigorously defended by Voit and his students, who held tenaciously to the 118 gram standard in the face of contrary evidence, some of it their own. In 1889 Voit published observations on a vegetarian diet in "*Ueber die Kost eines Vegetariers.*" [36] His subject, a Munich paperhanger, had lived in reasonably good health and vigor for

three years on a diet of fruit, oil, and graham or pumpernickel bread. His daily protein consumption was only 54 grams, a level well below half of the recommended Voit standard. Voit tested this type of diet briefly on other subjects, including one of his laboratory assistants. He actually concluded that it was conceivable to maintain health at protein intake levels somewhat below his original standard. Although he was aware that both in Europe and the Orient, large groups subsisted on low-protein diets, Voit warned constantly of the dangers of low-protein intake to health and vigor and of the dyspepsia and intestinal catarrh produced by the excessively high intake of starchy foods in vegetarian diets.

Voit's position in recommending a high-protein intake was strengthened by the work in Berlin of Immanuel Munk. Munk demonstrated [37] that dogs consuming a diet rich in meat remained healthy for long periods, but when the protein allowance was reduced to a point still capable of maintaining nitrogen equilibrium the dogs lost strength and declined, with severe intestinal digestive symptoms. Many years later it became evident that the detrimental effect of these low-protein diets resided in their deficiency of water-soluble vitamins. At the time, however, the experiment was interpretable only as clear evidence in favor of liberal amounts of protein in the diet. Munk concluded that it was unsafe to allow the daily dietary intake of protein to fall below 100 grams for the average man.

Further support for Voit's standard came from Atwater,[38] Voit's former student, in the United States. In 1894 he published surveys of the dietary habits of industrial workers in New England which demonstrated that it was common practice to consume as much or more protein than the 118 grams per day. Four years later, A. P. Bryant,[39] also working for the United States Department of Agriculture, demonstrated in his surveys that the average dietary chosen in the United States yielded 100 to 106 grams of protein, although some indigent families managed to secure only as little as 70 grams per person daily.

On the other hand, Voit's standard had many opponents before the turn of the century. Hirschfeld was among the first of the outstanding physiological chemists to demonstrate that substantially less protein in the diet is consistent with health. In 1887 he published his "*Untersuchungen über den Eiweissbedarf des Menschen*" [40] in which he demonstrated the adequacy of 40 to 45 grams of nitrogenous matter (protein plus non-protein nitrogenous material) daily for equilibrium in men under severe exercise. In 1889 Hirschfeld sought to overthrow the Voit school in his "*Betrachtungen über die Voit'sche Lehre von dem Eiweissbedarf des Menschen.*" [41] He reviewed many experiments which appeared to make Voit's position in regard to dietary protein allowance untenable.

Many others, including Muneo Kumagawa, a Japanese student,[42] and G. Klemperer,[43] both in Berlin, demonstrated in careful experiments that

low-protein allowance was consistent with health, strength and nitrogen equilibrium.

Protein and Specific Dynamic Action. Discovery of the specific dynamic action of protein foods is generally attributed to F. Bidder and C. Schmidt, in their experiments on cats. However, the fact that ingestion of food has a stimulating effect on metabolism has been general knowledge since the days when the famous Sanctorius chair balance was used in the observation that food ingestion increased the rate of insensible perspiration loss. Bidder and Schmidt were the first to link clearly with the protein element the stimulus of food to metabolism. In "*Die Verdauungssäfte und der Stoffwechsel,*" in 1852, they reported [25] that following meat consumption cats showed a twofold oxygen consumption and carbon dioxide output over that of the post-absorptive condition.

A clearer understanding of specific dynamic action resulted from the work of V. Mering and N. Zuntz,[44] in 1877. They demonstrated that all substances, whether digestible or not, when ingested, produce an increase in oxygen consumption and concluded that a portion of the specific dynamic effect of food is attributable merely to the increased glandular and smooth muscle activity induced by the presence of a mass in the gastrointestinal tract. This, however, would account for only a trifle of the specific dynamic effect of protein foods. The workers further demonstrated that lactic acid, glycerine, or glucose injected into animals would not stimulate metabolic rate appreciably, but that peptone produced by the tryptic digestion of protein possessed a marked stimulatory power. They concluded that the split-products resulting from digestion of food protein account for the principal specific dynamic effect of proteins.

Quite logically this remarkable stimulatory effect was called into theories regarding the position of protein as a nutriment. Pflüger, who had always favored protein as a food material specially suited for the maintenance of vigor and muscular strength, took the specific dynamic action of protein as a matter of special significance. In 1899 he reported [45] work on cats and dogs which seemed to demonstrate that increased protein in the diet resulted in a higher metabolic rate and increased strength, while decreased protein allowance produced the opposite effect even though the protein was replaced by an isodynamic allowance of fat and carbohydrate.

Nutritive Values of Proteins. Among physiologists throughout the last half of the nineteenth century the orthodox view of protein was not essentially changed from that held by Mulder and Liebig. The unitarian theory prevailed that protein from whatever source was one and the same substance with all other proteins. There was little basis for believing that proteins differed nutritionally. Rubner had indicated that protein from vegetable sources was less readily available to the system than meat and egg

protein, but this could be explained by the differences in the digestibility of foods of varying fiber content.

As early as 1863 a British worker, William S. Savory, was experimenting with the relative nutritive merits of various types of food. His results [46] are of little practical significance today in comparison to his purpose and method of approach to the problem. Working at St. Bartholomew's Hospital in London, he furnished one of the first records of using the rat in nutrition experiments. With his nitrogen balance studies, he measured changes in body weight and concluded that a non-nitrogenous diet will fail to support life in the rat. Of the use of rats in such experiments Savory said rats were chosen as subjects "because they are omnivorous, and will readily feed on almost any kind of diet. Moreover, from their size, they are very convenient to manage." Destiny held something special in store for the lowly rat in the next century when it played a major role in revealing the nutritive value of proteins.

Gelatin was the only protein substance recognized in the nineteenth century as being inadequate for nutrition, and in this respect differing from other proteins. This characteristic of gelatin, however, did not disturb greatly the unitarian view of protein held by the physiologists, for after all, they reasoned, it was not a natural protein but was, rather, an albuminoid substance derived by artificial means and, therefore, not to be classed as true protein material.

Voit, in his "*Ueber die Bedeutung des Leimes bei der Ernährung,*" [47] which appeared in 1872, demonstrated that gelatin as the sole source of nitrogen in the diet would not maintain nitrogen equilibrium in dogs. He succeeded in showing that gelatin is somewhat more efficient than an equivalent amount of fat or carbohydrate in minimizing nitrogen loss from the body when other sources of protein were absent from the diet. This placed gelatin in an anomalous position as a nutriment. In attempting to classify gelatin in accordance with Liebig's old system Voit was at a loss for it seemed to be something more than a "respiratory food" in its ability to spare circulating protein and minimize the loss of organ proteins, but, on the other hand, gelatin was something less than a "plastic food" for it would not serve as a material for construction of cellular material. Several years later, Örum, at Copenhagen, substantiated Voit's observations on the failure of gelatin to support nutrition in his "*Versuche über den Nährwerth des Leimes.*" [48]

At this time Otto Nasse, in Halle, made the interesting discovery that the aromatic amino acid tyrosine is the basis for the well-known red color reaction of proteins with Millon's reagent.[49] Gelatin, which did not give the red color reaction, therefore could not contain tyrosine within its structure. In 1894 Munk,[50] in a discussion of the nutritive properties of gelatin and its failure to serve as the sole source of protein, listed various ways in which gelatin differs from other proteins. Among them he included the

failure of gelatin to give the Millon reaction and the fact that it lacks tyrosine. These were the first scientific writings to hint that amino acid constitution might be the basis for differences in the nutritive value of proteins.

In nitrogen balance experiments workers like Erwin Voit (brother of Carl Voit) and Alexander Korkunoff [51] were attempting to determine the minimum amount of protein nitrogen necessary for maintenance of equilibrium. Various and conflicting results were obtained. At the end of the nineteenth century, bits of evidence were beginning to convince one thoughtful man that in some way proteins from different sources might differ in their nutritive ability to sustain animals. This man was Rubner. In 1897, in Leyden's "*Handbuch der Ernährungstherapie*" [52] he states: "*Das suchen nach einem Eiweissminimum wird überhaupt wie von einem Erfolg begleitet sein, weil es eben nicht ein, sondern viele Eiweissminima, mit welchen die Ernährungslehre rechenen muss, gibt. Ein Eiweissminimum lässt sich nur feststellen, wenn mann ganz genau bestimmt, mit welchem Nahrungsmittel es erreicht werden soll.*" This statement that there are as many proteins as there are protein-containing foods foreshadowed the discovery of varying biological values among proteins.

Protein Analysis. While the more physiologically-minded workers were attacking, through animal and human experimentation, the problem of the use of protein within the organism, those more chemically inclined were contributing perhaps even more fundamental observations. Chemical studies were gradually uncovering a new facet to the protein problem, one which would be the key to a bright new world in nutrition. One after another of the amino acids, the simpler crystalline "bausteine" of the complex protein molecule, were being discovered. The history of their discovery during the nineteenth and early twentieth centuries is well told by Vickery and Carl L. A. Schmidt [53] and by Melville Sahyun.[54] It was in the analysis of proteins for these components that men learned of the great diversity of proteins which actually exists in nature.

The ingenious chemical work of Emil Fischer (1852–1919), in Berlin, was perhaps the greatest single contribution ever made to the understanding of protein chemistry. Fischer [55] devised the method of separating the aliphatic amino acids through the fractional distillation of their ethyl esters. This method of systematic analysis led to the discovery of new amino acids, as well as to the recognition that proteins differ in their amino acid constitution.

At the turn of the century Albrecht Kossel (1853–1927) and F. Kutscher (1866–1927), at Marburg, presented their classic method for the analysis of protein substances for the three basic amino acids, histidine, arginine, and lysine, known as the hexone bases. In 1900 the method [56] was applied to gluten, thymus histone, the spermatozoa proteins of various fish, to gliadin of wheat, zein from maize, and to gelatin. These analyses revealed great

diversity in the composition of the proteins. They found that gliadin entirely lacked lysine. On the other hand, the protamine of salmon sperm consisted principally of arginine, with only a small proportion of other amino acids present. Here was evidence, indeed, of the intricate construction of the world of proteins.

Modern Concepts of Protein

By 1900 enough was known of the physiology and chemistry of proteins to indicate that rarely before had chemists and physiologists a more obvious common meeting ground than in the solving of the many problems relating to the function of protein and its meaning to life. The relative merits of high-protein and low-protein diets continued to be argued throughout the early 1900's, with equally distinguished authorities supporting both sides of the argument. Not until new approaches produced a deeper understanding of proteins in nutrition did the storm of conflict subside.

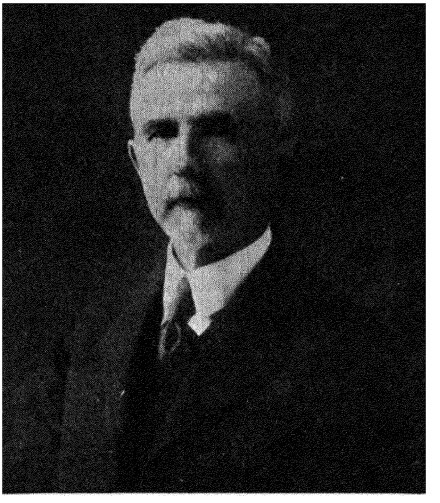
Protein Requirements. At the turn of the century, V. Sivén, a Finn, at Stockholm, produced evidence [57] that protein intake drastically reduced below the Voit standard was not detrimental. Sivén, a rather small man weighing only 59 kilograms, found that his body could maintain nitrogen equilibrium with a total nitrogen intake of only 4.52 grams daily, roughly 28 grams of protein, provided he consumed with it an adequate quantity of non-protein calories.

Contrariwise, accumulating evidence was showing that people performing heavy muscular work tend to consume large quantities of protein. For many years it had been common practice in training athletes to supply liberal amounts of protein foods. A study by C. D. Woods and E. R. Mansfield,[58] in 1904, of food consumed by Maine lumbermen revealed surprisingly high intakes of both calories and protein. In their periods of heaviest work the men consumed 247 grams of protein and 8200 calories per day. Similarly, Lavonius, at Helsingfors, reported [59] studies of the food habits of two athletes who daily consumed 180 to 220 grams of protein. The view of dietary protein as fundamental to muscle strength and endurance had persisted for generations following the dictum of Liebig.

In 1904, at Yale University, Russel Henry Chittenden (1856–1943), one of the most confirmed exponents of moderation in dietary habits, published his "Physiological Economy in Nutrition." [60] The book contained an extensive record of nitrogen balance experiments conducted with members of the University faculty, college athletes, and a group of U. S. Army Hospital corpsmen in training in New Haven. The data demonstrated that maintenance of nitrogen equilibrium, health, and endurance, as well as increased physical strength from training, could be attained with a diet containing one-third to one-half the amount of protein recommended in the Voit standard.

Chittenden's experiments stimulated the members of the opposing school

of thought to renewed efforts. Francis G. Benedict (1870–), one of the most active American students of metabolism and for many years associated with Atwater, presented a strong argument [61] favoring the high-protein dietaries which had been used by many investigators. "Dietary studies all over the world," said Benedict, "show that in communities where productive power, enterprise and civilization are at their highest, man has instinctively and independently selected liberal rather than small quantities of protein."



Russel Henry Chittenden (1856–1943)

In 1907 Chittenden added more evidence favoring moderate feeding of protein in "The Nutrition of Man." [62] Chittenden's new experiments demonstrated that dogs could survive for months in excellent health, even gaining in weight and storing body protein, on diets providing very low intakes of nitrogen (about 0.23 gram of nitrogen per kilogram of body weight per day). The data contrasted with the results of Munk [37] and Theodor Rosenheim,[63] who some years earlier had failed to maintain dogs on diets containing much more protein. Chittenden attributed their failure to lack of proper cleanliness and hygiene in the care of their animals.

At that time, prior to the recognition of specific vitamins, workers had no way of knowing that dietary factors other than protein were involved in the contradictory results. Being more of a skeptic than many of his contemporaries regarding the wisdom of man's instinctive judgment in food selection Chittenden said: "The results of these experiments with dogs, which we have recorded in such detail, are in perfect harmony with the conclusions arrived at by our experiments and observations with man, and serve to strengthen the opinion, so many times expressed, that the dietary habits of mankind and the dietary standards based thereon are not always

in accord with the true physiological requirements of the body. If these views are correct, and the facts presented seemingly indicate that they are, it is time for enlightened people to give heed to such suggestions, that their lives may be ordered more nearly in accord with the best interests of the body. Physiological economy in nutrition is not a myth, but a reality full of promise for the welfare of the individual and of the community in general. Ignorance on dietary matters should give place to an intelligent comprehension of the body's needs, and an adequate understanding of how best to meet the legitimate demands of the system for nourishment under given conditions of life."

One of the most gifted critics of Chittenden's low-protein dietary was Major D. McCay, professor of physiology at the Medical College of Calcutta. His valuable but amusing document, "The Protein Element in Nutrition," [64] sought to disprove Chittenden's stand by relating the physical development and character of various Indian races and tribes to the protein contents of their respective dietaries: "We believe that diet, and particularly the level of nitrogenous metabolism attained, has an immense influence on the formation of those desirable characteristics so well exemplified in the races whence is drawn our best fighting material. Further, although we have made little or no attempt to advance arguments for or against vegetarianism, it will be found that those races, whether of the hills or the plains, who are distinguished above all others for their martial qualities, are never vegetarians, but, on the contrary, usually large meat-eaters. The Sikh lives on wheat, other cereals, milk, and meat, particularly pork, of which he is specially fond. The Rajput, Pathan, Baluchi, etc., are all large meat-consumers, while the more or less purely vegetarian races, such as the Brahmins, are gradually being eliminated as recruiting sources for the Indian Army. We shall study the influence of the protein element of the dietaries of these races presently, simply pointing out now that the elimination of the several causes advanced by Kellogg, effected by comparison of tribes or races living under identical conditions as regards sexual excesses, climate, immature age of marriage, etc., brings out in its proper aspect the determining influence of diet on the character formation of a people. All the evidence we have been able to collect from observations on the different tribes and races of India points to a high level of protein interchange in the body accompanied by a high development of physique and manly qualities; whilst under the opposite conditions, poor physique, and a cringing, effeminate disposition is all that can be expected."

Mikkel Hindhede (1862–), at Copenhagen, became an enthusiastic supporter of the low-protein regimen. His able work yielded evidence of nitrogen equilibrium in man at extremely low levels of protein consumption. A 70-kilogram subject could be adequately maintained with as little as 40 grams of protein daily, mainly in the form of potatoes.[65–68] Emil Abderhalden (1877–), at Halle, also observed the remarkable nutritive

quality of the potato, showing that 4.5 grams of nitrogen from this source daily was consistent with nitrogen equilibrium.[69]

By the end of World War I the theories of proteins in nutrition were well advanced and the contribution of the vitamins to health clearly understood. Everyone had obtained practical experience in the consumption of low-protein dietaries. The controversy itself had been the greatest stimulus to its solution. From the new facts and experiences emerged a sounder and more mature evaluation of the subject. Benedict, who had supported the high-protein dietary originally, carried out a study of the nitrogen metabolism of a group of college students[70] and concluded that restriction of calorie and nitrogen intakes leads to lower levels of metabolism which are adequate and safe. Nine to ten grams of nitrogen daily in the diet appeared ample. "Protein curtailment is an assured and physiologically sound practice," he said.

Protein Split-Products and Their Nutritive Value. The fundamental work on the amino acid composition of proteins continued to gain momentum as the twentieth century opened. The work of Emil Fischer, of Kossel, and of their followers continued to reveal implications relating to all phases of protein theory, including nutrition. The multiplicity of proteins in nature, the specific, component amino acids in them, the theory of the peptide linkage — all were becoming a part of biochemical understanding.

In 1901, Kossel, using his own work and that of Fischer and of other leaders, presented a summary of the current knowledge regarding the structure of proteins and amino acids. The old theory formulated by Mulder of the "ideal" or "original" protein, which in its day had served well, was finally laid to rest. Breadth of vision had displaced it:[71] "*Jedenfalls ergiebt sich aus allen Betrachtungen, dass die Eiweisskörper eine Gruppe sehr verschiedenartiger Verbindungen bilden. Gewöhnlich hat man sich das Eiweiss als einen Körper von bestimmten feststehenden Eigenschaften gedacht und hat sich wohl einen Idealeiweisskörper construirt, ähnlich wie Goethe eine Urpflanze oder Idealpflanze erdachte. Die jenigen Eiweisssubstanzen, welche diesem Ideal nicht entsprachen, hat man als mit Defecten behaftet in eine niedere Gruppe, die der Albuminoïde, zusammengefasst. Diese Betrachtungsweise kann nicht aufrecht erhalten werden. Der heutigen Naturforschung liegt das Bedürfniss zu Grunde das organische Product als Glied einer sich entwickelnden Reihe aufzufassen, ein Bedürfniss, welches in den phylogenetischen und ontogenetischen Forschungsrichtungen seinen deutlichsten Ausdruck findet. So nehmen wir auch das complicirte Eiweissmolekül nicht ein für allemal als gegeben an, sonder wir suchen ein System von Eiweisskörpern zu finden welches, von den einfachsten Gliedern zu den complicirtesten fortschreitend, uns das innerste Wesen dieser vielgestaltigen Körper enthullt.*"

Shortly thereafter, Sir Frederick Gowland Hopkins (1861–) and Sydney Cole, at Cambridge University, isolated the amino acid tryptophane,[72] which was destined to play a prominent part in nutrition research. They

were interested in the substance responsible for the trytophane color reaction, and the pink color reaction of proteins with a mixture of glyoxylic acid in glacial acetic acid, known as the Adamkiewicz test, was a subject of their research. These workers found that by precipitation with mercuric sulfate and sulfuric acid a crystalline substance responding strongly with both color reactions could be isolated from casein digested with the pancreatic enzyme. This crystalline chromogen was found to be a derivative of indole and its elementary composition determined. The substance was called tryptophane.

Thomas B. Osborne (1859–1929)

Pursuant to this work, Thomas B. Osborne (1859–1929) and Isaac F. Harris, at the Connecticut Agricultural Station in New Haven, applied the tryptophane color reaction to 35 protein substances prepared in their laboratory.[73] Certain proteins such as the alcohol-soluble protein of the oat kernel and vicilin from peas gave no color reaction, presumably because the amino acid tryptophane was absent. Osborne and Harris arranged their list of 35 proteins in the order of intensity of the tryptophane color reactions and demonstrated the diversity of proteins with respect to tryptophane content.

The amino acid concept led to new concepts of the chemistry of digestion and new understanding of the mode by which proteins from food become available to the organism. At Heidelberg, Otto Cohneim [74] discovered that a digestive ferment capable of causing the destruction of peptones and

peptides exists in the intestinal mucosa. Proteins and peptones which originally gave the characteristic biuret reaction no longer did so after digestion by this new enzyme from the intestinal wall, which Cohneim named erepsin. In 1906, he demonstrated [75] that free amino acids are formed in ereptic digestion. The view was revolutionary. Hitherto the concept had been that in the intestine the blood proteins were synthesized and absorbed. Now it became apparent that intestinal digestion produced complete disruption of the protein material.

Almost simultaneous with Cohneim's observation, Kutscher and J. Seemann, at Marburg, presented studies [76] of the contents of the intestines of dogs with intestinal fistulas. They demonstrated that free tyrosine, leucine, lysine, and arginine could be isolated directly by precipitation and crystallization from the intestinal chyme. It was evident that protein of the diet was not absorbed directly, intact, but assimilation occurred primarily by absorption of the simpler, split-products, such as amino acids and possibly the very simple peptides. Thus, the animal organism received for its use, not the original dietary protein, but the units with which the dietary protein was constructed.

Loewi, also at Marburg, was first to demonstrate that protein digestion products are adequate for maintaining nitrogen equilibrium in dogs.[77] He used autodigested pancreas, so prepared as to be free of any peptides giving the biuret reaction, as the sole dietary source of nitrogen. With this diet the animals succeeded in building up body protein. The observation was amply confirmed by Abderhalden and his coworkers,[78–81] in Berlin.

Abderhalden and Franz Samuely [82] conducted another study which illustrates the changing viewpoint of that time. A horse was depleted of blood through routine phlebotomy and concurrently was fed gliadin of wheat as its protein food. The horse continued to synthesize blood proteins characteristically poor in glutamic acid, though the dietary protein was rich in glutamic acid. This demonstrated two facts: (1) That dietary protein is altered before it appears in the blood; and (2) that specific body proteins cannot be influenced in amino acid composition by the feeding of dietary proteins of different composition.

Indispensable Amino Acids. One link was missing in the proof that a mixture containing only amino acids would support life in animals when used as the sole dietary source of nitrogen. No one had been able to demonstrate that the enzymatic digests contained only free amino acids with no admixture of simple abiuret peptides. Only the use of an acid hydrolysate of protein completely split into its component amino acids would serve to demonstrate the synthesis of body protein from these ultimate "*bausteine.*"

Such an experiment was carried out by Yandell Henderson,[83] a former student of Carl Voit, and Arthur L. Dean, at Yale University. The acid hydrolysate of protein prepared by these workers was fed to dogs. The amino acid mixture was readily absorbed and rapidly converted to urea, a

finding consistent with Abderhalden's observation that isolated amino acids fed to dogs are excreted largely as urea. Such a mixture exerted a nitrogen-sparing effect in the animals but was totally incapable of maintaining nitrogen equilibrium or promoting the synthesis of new flesh. Essentially the same findings were published by Abderhalden and Peter Rona [78] a year later. At the same time failure of acid hydrolysates of protein to replace dietary protein was being observed, Hopkins and Cole were isolating the amino acid tryptophane at Cambridge. Soon it would become evident that the failure of acid hydrolysates in nutrition was owing to the destruction of the essential tryptophane.

One of the finest contributions to knowledge of the nutritive value of proteins was made at Cambridge by Edith G. Willcock and Hopkins with their demonstration that tryptophane is an indispensable amino acid.[84] These workers found that when fed as the only dietary protein, the alcohol-soluble protein (zein) of corn failed to maintain mice. The diet so composed was poorly consumed and within a brief period the animals receiving it lost weight and died. Inasmuch as zein gave no color test characteristic of tryptophane, Willcock and Hopkins knew tryptophane was absent. They added tryptophane to the zein diet and observed marked beneficial effects upon the mice. The animals ate much more food and remained healthy for longer periods. In similar experiments the amino acid tyrosine had no protective action.

Willcock and Hopkins were aware that zein contained little if any lysine in its structure for they wrote significantly: "The addition of the missing tryptophane group to zein has, it is also clear, no power to convert such loss [of body weight] into equilibrium or gain; a fact possibly due to other deficiencies in the zein molecule, such as the absence of lysine or the lack of some other amino-acid not yet observed." They opened a new door for biochemistry with the statement: "We are no longer bound to Liebig's view, or to any modification of it which implies that the whole of the proteid consumed is utilized as intact proteid, nor are we even compelled to assume that the whole of what is broken down in the gut is resynthesized before utilization. Proteid products may function in other ways than in the repair of tissues or in supplying energy. It is highly probable that the organism uses them, in part, for more specific and more immediate needs. The discovery of substances absolutely essential to life, highly specific [referring to hormones] and elaborated in special organs, suggests that some part, at least, of the protein products set free in the gut may be used directly by these organs as precursors of such specific substances."

A year prior to Willcock and Hopkins' report, M. Kauffmann published from Berlin a significant report [85] on the dietary shortcomings of gelatin. He demonstrated that gelatin, which fed alone is incapable of supporting animals, is much improved in this regard if tyrosine and tryptophane, both absent from gelatin, are added to it. However, such mixtures were still in-

adequate to maintain nitrogen equilibrium in his experimental dogs. By adding cystine, which also is virtually absent from gelatin, in addition to tyrosine and tryptophane Kauffmann succeeded in supporting nitrogen equilibrium in his dogs. The results suggested possible essential nutritional roles for all three of these amino acids.

The observations of Willcock and Hopkins were followed by the discovery of other essential amino acids and resulted in a remarkable clarification of the position of proteins in nutrition. The field was virtually dominated by

Lafayette B. Mendel (1872–1935)

Osborne and by Lafayette B. Mendel (1872–1935), at Yale University. Working together they became the most fertile combination of minds ever directed toward studies of nutrition. Using albino rats as their test subjects, they studied the nutritive value of gliadin, the predominant protein of wheat and rye.[86] Their interest focused on gliadin because the protein contains little lysine. They discovered that adult animals could be adequately maintained on a diet containing gliadin as the only protein, but that the same diet failed to support growth in young rats. Addition of lysine made gliadin adequate in supporting growth. Therefore, they concluded that lysine is essential for growth but not for maintenance.

Two years later the same workers extended the work with zein which had been initiated by Willcock and Hopkins.[87] They demonstrated that zein could be made adequate for maintenance of animals by the addition of tryptophane and become adequate for supporting growth upon the further addition of lysine. Furthermore, they demonstrated that the milk protein lactalbumin when fed as a supplement to zein was effective in correcting the nutritional defects of the incomplete protein. Presumably, the beneficial

effect of lactalbumin, with zein, was the result of its liberal contents of tryptophane and lysine. This was one of many demonstrations of the ability of one protein to compensate for the nutritional defects of another: "The lack of tryptophane can also be made good, as might be expected, by supplementing the zein ration with proteins which do contain this lacking amino-acid. Thus even gliadin stopped the decline. The relative efficiency of different proteins in preventing the failure with zein apparently depends to a dominant degree, in so far as maintenance is concerned, on their comparative yield of tryptophane.

"Where growth is involved in addition to maintenance, the lysine factor, as well as others, not yet more accurately ascertained, must also be taken into account. Here, then, is evidence of the relative economy of different proteins in maintenance, based upon the content of one or more of the amino-acids essential for the proper functioning of the organism (in so-called maintenance) or for new tissue construction (in growth). Obviously the relative values of the different proteins in nutrition are based upon their content of those special amino-acids which cannot be synthesized in the animal body and which are indispensable for certain distinct, as yet not clearly defined processes which we express as maintenance or repair."

To Osborne and Mendel we also owe the clear formulation of the theory of specific amino acids as limiting factors in the nutritive value of proteins and as determinants of the "protein minimum" necessary for optimal growth. For any protein which is capable of supporting growth in young animals there is a specific minimal quantity which must be fed daily to produce optimal growth. For instance, they found casein would produce maximal growth in albino rats if incorporated to the extent of 18 per cent of the diet. Lower concentrations of lactalbumin in the diet would produce maximal growth, thus lactalbumin demonstrated higher efficiency. In 1915, Osborne and Mendel fed to rats diets containing a lower concentration of casein, which resulted in suboptimal growth of their animals, and then produced normal growth rates by adding to the diet the sulfur-containing amino acid cystine. They concluded that cystine is the "first limiting factor" of the nutritive value of casein and postulated its indispensable function in nutrition.[88]

In 1917, a student of Mendel, Howard B. Lewis, at the University of Illinois, substantiated the belief that a specific body need for cystine exists.[89] In dogs showing extremely negative nitrogen balances when ingesting suboptimal intakes of protein provided by beef heart, Lewis demonstrated that small additional amounts of cystine in the diet almost abolished the nitrogen loss. In his experiment the effect was specific for cystine, because other amino acids such as tyrosine and glycine were ineffectual. Harold Ackroyd and Hopkins [90] presented evidence of the essential nature of either or both of the basic amino acids arginine and histidine, in 1916.

The discovery of indispensable amino acids so clarified the problem of

proteins in nutrition [91–94] that the theory envisioned by Mendel [1] has to the present day remained essentially unaltered except in specific detail:

"Today we are concerned with the question whether this or that protein, whatever its biological origin, will yield the characteristic desired amino-acids, such as tyrosine and tryptophane, leucine and lysine, glycocoll and cystine, histidine and arginine. Our attention is fixed on the building-stones or units out of which the great protein structures are put together. Instead of referring to the proteins in terms of their physical properties or empirical composition — their content of carbon, hydrogen, oxygen, nitrogen or sulphur — at least so far as the problems of nutrition are involved, the time has arrived for estimating their behavior in the organism on the basis of the quota of each of about eighteen well-defined amino-acids which the individual representatives of this group of foodstuffs can yield. Many, if not all, of these amino-acids are essential for the construction of tissue and the regeneration of cellular losses. In proportion as any specific protein can furnish these constructive units it may satisfy the nutritive needs of the body. The efficiency of the individual protein in this respect must depend on the minimum of any indispensable amino acid that it will yield; for it is now known that some of them cannot be synthesized anew by the animal organism. If, for example, a protein or mixture of proteins comparatively deficient in their yield of the sulphur-containing amino-acid, cystine, be furnished alone to supply the body's nitrogenous requirements the production of new, cystine-yielding molecules of protein will be limited by the amount which is available in the diet. An excess need not be wasted, for it can be burned up like sugar or fat to provide energy; but new construction or growth is limited by the minimum of the essential unit."

Vitamin Chemistry. Our present concepts of proteins in nutrition were well-founded before biochemists had obtained any clear understanding of the vitamins. Much of the evidence concerning protein had been gathered from brief experiments on nitrogen balance in animals, conducted sufficiently long to observe the relatively rapid changes in protein economy, but not over a span of time in which avitaminoses would cloud the results. Difficulty in maintaining animals on restricted synthetic dietaries was encountered generally in any extended feeding experiment, though the diets contained all of the then-recognized food principles. Coincidentally, knowledge of deficiency diseases was being accumulated by the medical profession and biochemists were becoming aware of certain accessory factors, occurring in small amounts in natural substances, which are essential in a healthful diet.

Long-term feeding experiments with restricted dietaries were doomed to failure until a method was devised for satisfying the requirements for vitamins. Most fortunate in devising rations suitable for long-term feeding studies were Osborne and Mendel. Their use of so-called "protein-free milk" as a ration component was the key to success in their early work.[95]

This substance was obtained by removing casein from milk and subsequently, the heat-coagulable proteins, then concentrating the resultant liquid. The use of "protein-free milk" was not as coincidental as it might seem, for its development stemmed from Osborne and Mendel's recognition of the ability of milk to protect health. The superior value of protein-free milk in maintaining animals apparently was not owing to its mineral content, for milk ash would not produce the same effect when incorporated in a synthetic diet of protein, fat, and carbohydrate. Somewhat later the value of the Osborne and Mendel supplement was understood to reside in its content of the water-soluble vitamin.[96]

The use of protein-free milk in synthetic dietaries for the study of amino acid and protein requirement was severely criticized, not without reason, by the University of Wisconsin workers, particularly by McCollum [2] and his associates. They reasoned that protein-free milk contributes a small but significant amount of nitrogen in unknown form, perhaps in sufficient amount to offset the deficiencies of the protein under examination and that this would obscure results obtained with it. It was many years before the general conclusions of Osborne and Mendel concerning proteins and amino acids could be adequately tested and substantiated through the use of more highly purified vitamin preparations.

Hopkins was one of the early leaders in nutrition to recognize the need of vitamins for the maintenance of experimental animals. In experiments on the growth promotion value of small additions of milk to restricted dietaries, he substantiated the belief of Osborne and Mendel that milk contains health-protective factors.[97]

Butter had been used in experimental dietaries for some years without encountering vitamin A deficiency. McCollum and Marguerite Davis at the University of Wisconsin were the first to publish observations on the ability of a fat-like substance in butter to promote growth and health.[98] Out of such observations grew the knowledge of the fat-soluble vitamin A.

To a great extent the studies on protein values in nutrition stimulated the discovery of vitamins. In turn, recognition of the need for vitamins enriched the field of protein studies, both in its future progress and in explaining many of the controversial observations of the past.

Biological Value of Proteins. The concept of biological value evolved with the many-sided attack on the inner secrets of the protein molecule. Karl Thomas (1883–), at Berlin, a student of Rubner, was the first to apply the term *biologische Wertigkeit* with reference to protein and devised the first method of expressing the value quantitatively.[99, 100] He observed from nitrogen balance studies of himself that a bread diet furnishing 13.1 grams of nitrogen was just adequate for nitrogen equilibrium. In contrast, 5.5 grams of nitrogen in the form of potatoes would serve equally well. He ascribed this difference among proteins from different sources in ability to satisfy requirements, to their relative values in satisfying nitrogen needs of

the organism: "*Dieser Unterschied beruht auf einer verschiedenen Verwertbarkeit der Stickstoffsubstanz im Organismum. . . . Die Stickstoffsubstanz des Weissmehls, der Kartoffel, der Milch muss in verschiedenem Grade für den Eiweissbedarf des Körpers herangezogen werden.*"

For quantitating biological value, Thomas determined the minimal total nitrogen excretion when a calorically-adequate, protein-free diet was consumed. This minimal figure is the quantity of body proteins used to cover metabolic needs for nitrogen. Thomas reasoned that the ideal dietary protein would be one so perfectly constituted with respect to amino acid that it could produce nitrogen equilibrium when fed in the diet at a level equivalent to the minimal metabolic nitrogen excretion. The expression of biological value used by Thomas was the number of parts of nitrogen in any protein food which would spare 100 parts of the body nitrogen. Although Thomas controlled the calorie factor in his experiments on biological values, there were many other variables over which no control was attempted. Time wrought changes in the method of estimating biological value and many of the early observations were vitiated. Nevertheless, the central idea of Karl Thomas has remained.

The question of the biological value of proteins was studied extensively in the United States, particularly by two schools of workers. The University of Wisconsin group, E. B. Hart (1874–), E. V. McCollum, Harry Steenbock,[101] and associates, conducted animal experimentation with a variety of species, including rats, swine [102] and cattle,[103] testing mainly the biological values of naturally occurring food materials.[104–107] The general approach through studies with natural foodstuffs led to development not only in the field of proteins in nutrition but also in knowledge of mineral and vitamin requirements. The Connecticut group, comprising T. B. Osborne of the Connecticut Agricultural Experiment Station and Lafayette B. Mendel of Yale, worked principally with rats and the biological value of isolated proteins. Both schools contributed heavily to the furtherance of the understanding of proteins in nutrition and fortunately their diverse viewpoints and approaches led to great progress in the science.

Osborne and Mendel had demonstrated that gliadin supports maintenance but not growth in white rats. Obviously, the amino acid requirements for these two processes differ. It became evident that the biological value of protein based upon a criterion of maintenance [108] of the adult body might be different from that based upon growth.[109] Even broader criteria of biological value were introduced by McCollum and the Wisconsin workers who enumerated reproduction, fertility, lactation and longevity as functions presenting specific demands for nutriments, stating that there is no assurance that a food supply inducing the most rapid growth is consistent with highest fertility of the mature animal or that either of these is consistent with greatest longevity or success in lactation. Reasoning in this way, a multiplicity of biological values became evident possibilities.

Osborne, Mendel, and Edna Ferry expressed biological value using growth as a criterion.[110] They determined in young albino rats the maximum amount of weight gain per gram of protein fed. The superiority of lactalbumin over casein for growth purposes was apparent from the fact that over an 11-week period of testing, rats gained 2.06 grams of body tissue per gram of lactalbumin fed, as in comparison with 1.76 grams per gram of casein in the diet.

Interest in the performance of dairy cattle led to the use of lactation as an index of the biological value of dietary protein. Hart and G. C. Humphrey, at the University of Wisconsin, carried out extensive and painstaking studies of the influence of various cattle feeds and of milk itself in supporting the production of milk proteins.[111–113] Milk protein in the diet of cows was found to be the most efficient for formation of protein in the milk secreted. Similarly, in studies of human subjects the proteins of fresh cow's milk were demonstrated by B. Raymond Hoobler (1872–1943) [114] to be more efficient than others such as the vegetable and cereal proteins in the production of human milk proteins and in the protection of maternal tissues against excessive nitrogen loss during milk production.

The period of 1911 to 1920 was prolific in the advancement of the biological method of testing foodstuffs, and its application both to isolated proteins and to natural food materials. From the use of this new tool with many species, information was obtained not only about protein but also concerning the vitamins and mineral elements in nutrition. Leaders in the work were Osborne and Mendel in Connecticut, Hart and McCollum of Wisconsin and the many inspired students who stemmed from their teaching.

It was the work of these men and their contemporaries which in little more than a century produced the information establishing the modern understanding of proteins in nutrition. In tracing the history of proteins it is evident that the history, as of any science, is a review of growth during successive generations, of the development of those enduring facts which constitute the science itself. Only upon the basis of these facts is one able to evaluate the true significance of recent work in all its complexity. What one accepts as almost instinctual in his modern thinking was new and intricate a few years ago. Just as surely, today's experiments and complex theories will be polished by the work of many until the contribution of a whole generation melts into a few more simple facts upon which future building can take place.

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Chapter 2

The Biological Utilization of Proteins and Protein Requirements

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The content of foods in the various nutrients is important information in their nutritive evaluation. In fact, the application of nutritional principles to the practical problems of feeding people rests largely upon tables of food composition and yet these tables contain many elements of uncertainty that have been recently discussed by Elvehjem.[32] The incomplete availability of food nutrients in the body demands particular emphasis because the nutritive value of a food depends upon what the body can extract from it in digestion and, of the fraction digested, upon what it can assimilate in metabolism. Attempts have been made to take such factors into account by subjecting foods to chemical reagents and enzymes in the laboratory with the object of separating available from unavailable substances. A notable attempt of this kind was published by Horwitt, Cowgill and Mendel,[60, 61] while the work of McCance and Lawrence [91] on the carbohydrates of foods, and the scheme of analysis of animal feeds proposed by Crampton [27] are worthy of note. But these laboratory methods of simulating some of the conditions of animal digestion have not commended themselves to nutritionists in general, at least in this country, although this circumstance does not by any means reflect upon the accuracy of the methods proposed or detract from the importance of the underlying problem.

Elvehjem has expressed the hope that "Eventually it should be possible to determine the nutritional value of any diet through the use of figures for dietary requirements, tables of food composition and a calculating machine." But such a hope neglects the difference, however small, that always exists between the total content of nutrients in a food material and the available content. One need only mention the presence in foods of carbohydrates entirely resistant to animal enzymes, that themselves contribute little to the energy value of foods and by their presence in plant cell walls may seriously impede the digestion of cell contents; the interaction of inorganic ions in the intestinal tract with the production of highly insoluble salts; and the existence in foods of substances that exert powerful anti-enzyme activity to realize the futility of ever expressing the complete nutritive value of foods by even the most complete laboratory analysis.

The animal organism itself must be the court of last resort in assessing the nutritive value of food materials.

In particular, the protein value of a food obviously cannot be assessed even approximately by a Kjeldahl determination, or by a true protein determination; an analysis for amino acids by modern methods may give an excellent nutritive evaluation of the absorbed fraction of a food protein,[115] or it may give a very inadequate one. In any case it throws little if any light on protein digestibility. The first step in any study of the biological utilization of dietary protein is concerned with digestive utilization, and such studies necessarily relate to the fate of dietary nitrogen within the digestive tract. It is problematical whether any better method than this of studying protein digestion will be developed.

The Digestive Utilization of Dietary Protein

The enzymes in the alimentary tract are capable of hydrolyzing proteins completely to their constituent amino acids and prosthetic groups. The fact that this so rarely occurs in actual nutrition, except for some of the animal proteins, has presented a problem for many investigations.

Mendel and Fine published a series of reports [97] concerned with the digestibility of vegetable proteins *per se*, when removed from the structural pattern in which they have been deposited. *In situ* they are fairly indigestible because cellulosic and hemicellulosic membranes impose an indigestible barrier between digestive proteases and their substrates. Also, the large mass of bulky material in many vegetable products may disturb the time relations of digestion by stimulating intestinal motility unduly. The experiments of Mendel and Fine, performed upon dogs and in a few cases upon human subjects, showed that the proteins of wheat, barley and corn are as digestible *per se* as those of meat, *i.e.*, 93 to 96 per cent. However, proteins of the soybean, navy bean and garden pea proved to be resistant to proteolysis in their own rights, giving digestion coefficients of 80 to 85 per cent. The proteins of cottonseed are even more refractive to digestion, yielding coefficients of only 67 to 75 per cent.

In animal feeds, the content of fibrous material is an important factor in determining the digestibility of the organic matter, the correlation coefficients for different animals ranging from -0.75 to -0.91. The author elsewhere [110] has reviewed this phase of the subject.

The resistance of legume proteins to digestion in the animal seems to be explained, wholly or in part, by their association with anti-enzymes. Aqueous extracts of soybeans [20, 44] and navy beans [20] contain a trypsin-inhibiting substance, which seems to account for the low digestibility of the protein of raw soybeans.[45] The properties of the inhibitor have been studied by Klose, Hill and Fevold [73] and it has been recently isolated by Kunitz.[75] It is a protein of the globulin type. On heating, the anti-enzyme is destroyed and a more complete digestibility of the protein ensues.[124, 173]

Melnick [96] has observed a marked difference in the susceptibility of the proteins of raw and processed soybeans to enzymic digestion. During *in vitro* digestion, methionine is released much earlier from the heated than from the unheated protein, even though eventually the same amount of methionine is released in both cases. Methionine limits the nutritive quality of soybean proteins. Hence, if the rate of absorption of methionine may further limit the utilization of the other amino acids of soybean proteins, then this finding may have a bearing on the low biological value of the proteins in the raw bean.

One naturally associates the relatively low digestibility of cottonseed proteins with the presence of gossypol, the toxic principle in the cottonseed. This idea receives some support from the observations of Jones and Waterman [68] that the *in vitro* digestion of cottonseed globulin and of casein by pepsin and trypsin was markedly depressed in the presence of 1 per cent of gossypol. Gallup and Reder,[40] however, were unable to confirm this effect of gossypol in digestion experiments on mature rats, although the ratio of gossypol to protein in their diets was less than is commonly found in cottonseed. Be that as it may, the removal of gossypol from cottonseed by solvent extraction is associated with an increase in the digestibility of its protein (unpublished data). On the other hand, the application of moist heat to cottonseed depresses the digestibility of the protein,[39] possibly by favoring a combination of gossypol and protein that is resistant to enzyme attack.*

The digestibility of food proteins by human subjects has been reported in scattered publications, mostly in the older literature. The only attempt in this country to compile and collate these results, of which the author is aware, was made by Atwater in 1899.[7] From this study Atwater developed a table of average digestibility of the main organic nutrients in different classes of foods:

Coefficients of Availability of Nutrients of Different Groups of Food Materials and of Total Nutrients of Mixed Diet

	Protein (%)	*Fat* (%)	*Carbohydrate* (%)
Animal foods	97	95	98
Cereals	85	90	98
Legumes, dried	78	90	97
Sugars and starches			98
Vegetables	83	90	95
Fruits	85	90	90
Vegetable food	84	90	97
Total food	92	95	97

It will be noted, according to Atwater, that food proteins are the most variable in their digestibility, which means that the digestion of proteins in

* This idea is contained in a private communication from Dr. Frank E. Caruth.

the alimentary canal is the most readily impaired by extraneous factors, such as those discussed above.

Another product of Atwater's studies was the computation of a table of *available* nutrients in human foods,[8] by which is meant the digestible nutrients. For undivulged reasons, his publications with Bryant on the chemical composition of American food materials [6] neglected entirely the availability of the contained nutrients. The shortcomings of the ordinary proximate analysis of foods in defining their nutritive value may be accentuated by an extreme case. This method of analysis cannot distinguish between dried whole milk and a properly devised mixture of feathers, mineral oil, inulin and barium sulfate.

The coefficients of digestibility thus far considered relate to "apparent," not "true," digestibility, because they place the metabolic products in the feces in the same class with the undigested food protein. This "apparent" digestibility is of practical significance because the so-called metabolic nitrogen of the feces is the cost of digestion in terms of nitrogen and should be charged against the main nitrogen-containing nutrient, protein. However, for many reasons and on some occasions it is important to know the actual digestibility of the dietary protein. In measuring the true digestibility of protein (nitrogen) it is necessary to deduct from the fecal nitrogen the nitrogen of body origin (unabsorbed intestinal juices, mucosal detritus, mucus, etc.).

It seems reasonable to expect that the nitrogen in the feces would be reduced to that of body origin by removing all protein from the diet. The residual metabolic nitrogen has originated largely from digestive juices secreted into the gastrointestinal tract. The amount of these juices secreted will bear some relation to the amount of digestive work to be done, *i.e.*, the mass and character of the food ingested. The degree to which these secretions will be reabsorbed will depend upon the rate of peristalsis, which in turn is influenced by the mass and character of material in the intestines. Considerations such as these induced Mendel and Fine [98] to propose a method of measuring the metabolic nitrogen in the feces:

(1) Determine the volume and nitrogen content of feces resulting from the food under investigation.

(2) Determine the fecal nitrogen resulting from a nitrogen-free diet to which has been added an amount of indigestible non-nitrogenous matter that will yield approximately the same volume of feces as was obtained in 1.

(3) Subtract the fecal nitrogen in 2 from that in 1. The excess of nitrogen over 2 is presumably due to undigested or unabsorbed nitrogenous matter of the food under study.

Mitchell and Hamilton [123] have applied this method to the problem of the true digestibility of oat hulls and alfalfa meal by swine.

The author has discussed elsewhere [103] this method of measuring the metabolic nitrogen in the feces and has shown from experimental evidence

then available in the literature that for animals of several species the following general principles seem to hold:

"(1) The most important factor determining the amount of metabolic nitrogen in the feces is the amount of dry matter consumed.

"(2) Another factor of great importance is the concentration of indigestible non-nitrogenous material in the ration. The greater the concentration of such 'roughage,' the greater the fecal excretion of metabolic nitrogen per unit of dry matter consumed.

"(3) The presence of protein in the ration or of other materials known to stimulate the digestive glands, exerts no appreciable influence on the excretion of metabolic nitrogen in the feces.

"(4) The size of an animal is not an important factor in determining the amount of metabolic nitrogen in the feces per 100 grams of dry matter consumed, provided the animal is not consuming abnormally large or abnormally small amounts of food relative to its body weight.

"(5) Animals varying in size from the rat to man excrete approximately 0.2 gram of metabolic nitrogen in the feces per 100 grams of dry matter consumed on rations containing minimal amounts of roughage. This figure may be greatly exceeded on rations containing high concentrations of indigestible carbohydrates."

Later work in the author's laboratory indicates that the metabolic fecal nitrogen per 100 grams of dry food consumed approximates 0.1 gram for rats, pigs and human beings on low-roughage diets.

Schneider [148] has discussed in some detail the relationships between food intake, body weight and metabolic nitrogen in the feces. It was later shown from the same laboratory [107] that the substitution of fat for starch in the diet of the rat does not affect the excretion of metabolic nitrogen in the feces, even though the total amount of dry fecal material produced is considerably increased thereby. An alcohol supplement to the diet does not increase the excretion of metabolic products in the feces, but probably does increase the digestibility of the basal diet.[108] Finally Schneider [149] showed that the metabolic products in the feces of the rat and the pig consist of two fractions, one related to a constant type of metabolic wastage, possibly a phase of the endogenous catabolism of nitrogen, and the other related to digestive work as represented by the amount of dry food consumed. In the human subject, the first fraction is inconsiderable if it exists at all.

This relationship between dry matter consumed and metabolic nitrogen in the feces would lead one to expect a positive correlation between the nitrogen (or protein) content of a food expressed on the moisture-free basis and its apparent digestibility, since the protein in a low-protein food would be charged with metabolic fecal nitrogen induced by a high proportion of non-nitrogenous material. As early as 1870, Stohmann [158] suspected such a relationship and proposed a formula for the estimation of the amount of digested protein in sheep rations from a somewhat complicated function

of the protein as related to the non-protein material fed. The author [103] has demonstrated that, for cattle feeds, a high degree of correlation actually exists between protein content on the moisture-free basis and the apparent digestibility of protein. From a later and more complete compilation of data on the digestibility of cattle feeds,[130] the author has developed the following prediction equation for roughages containing more than 5 per cent of protein (N × 6.25) on the dry basis:

$$D = 42.636\ (P - 5)^{0.21476}$$

in which D is the percentage apparent digestibility of protein and P is the protein content of the feed on the dry basis. It would be interesting to investigate this relationship in human nutrition if sufficient data were available for study.

In many recent studies in the author's laboratory, the relationship for the young growing rat of the metabolic nitrogen of the feces to the dry matter consumed has averaged 1.164 mg per gm of dry food consumed in 156 trials; the standard deviation is 0.1822 mg of nitrogen and the coefficient of variation is 15.7 per cent. The fact that these measurements of the metabolic nitrogen of the feces were made with diets containing about 4 per cent of whole egg protein, instead of with a diet practically free of nitrogen, may account for the fact that the above average is considerably less than the one previously cited [103] of 1.9 mg. For two adult human subjects on a low-nitrogen diet, reported by Bricker, Mitchell and Kinsman,[21] the metabolic nitrogen in the feces amounted to 0.91 and 1.14 mg per gm of dry matter consumed. It seems evident that other factors than the amount of dry matter consumed determine the excretion of metabolic nitrogen in the feces. Hence, in measuring the true digestibility of protein by the method proposed by Mendel and Fine, or any equivalent method, it seems important to plan the nitrogen-free diet to simulate the protein-containing diet as closely as possible in all respects except the protein component.

Among the factors that may modify the ratio of metabolic fecal nitrogen to dry matter consumed, for the laboratory rat at least, is the amount of food consumed. Schneider [148] has discussed this point and has shown that, due to the presence in the feces of a quota of body nitrogen related to body weight rather than to food consumed, an intake of food less than the amount required to maintain body weight is associated with a high ratio.

In his study on the nitrogen metabolism of steers on rations containing alfalfa as the sole source of nitrogen, Titus [168] concluded that the metabolic nitrogen of the feces varied directly with the moisture content. The conclusion is based upon the fact that the expected linear relationship between the nitrogen content of the food and that of the feces revealed deviations from expectation when the feces contained high concentrations of water. A less credible deduction from the data of the experiment is that the intercept on the fecal nitrogen axis of the line describing the relationship between

nitrogen intake and fecal nitrogen is not a good estimate of the metabolic nitrogen in the feces. Hence, "the amount of nitrogen in the feces of a steer consuming a nitrogen-free ration may not safely be taken as a measure of the amount of metabolic nitrogen resulting from the ingestion of an equal weight of alfalfa, or other feeding stuffs." This deduction, so contrary to current opinion, is based upon the fact that the intercept above referred to is appreciably less than an estimate derived from an equation by which metabolic fecal nitrogen is related to the amount of dry matter digested and the weight of water in the feces. The validity of the latter relationship is not established, nor has the difference between the two estimates been shown to be statistically real.

Bosshardt and Barnes [18] defend the same conclusion that the fecal nitrogen on a nitrogen-free diet is not a good measure of metabolic fecal nitrogen; their data were obtained with growing mice. Using essentially the same mathematical methods as Titus in analyzing their data, they assume, unlike Titus, that the intercept on the fecal nitrogen axis of the line describing the relation between nitrogen intake and fecal nitrogen is the "true" measure of metabolic nitrogen. From the fact that this intercept is statistically different from a value secured with a nitrogen-free diet, they assume that the latter is in error as a criterion of the metabolic fecal nitrogen on protein-containing diets. However, in analyzing the data plotted in a second figure another statistically aberrant value obtained with a diet containing nitrogen is discarded in plotting the curve, apparently as possessing no significance whatever.

Neither the data of Titus, nor those of Bosshardt and Barnes, seem adequate to disprove the proposition that the nitrogen content of feces secured on a no-protein diet, or one containing only a small amount of a completely digestible protein, measures satisfactorily for the same animal the metabolic fecal nitrogen secreted on a protein-containing diet equal in amount and composition except for the protein component.

In order to present a general picture of the differences in "true" digestibility among different food materials, the values appearing in Table 2–1 have been assembled from the literature, mainly from the author's laboratory. The values relate to the young growing rat and to food materials that have been largely defatted if they contained originally considerable proportions of fat, and then dehydrated at low temperatures and ground finely. If they are in error with reference to practical methods of feeding protein foods, they over-estimate the truth.

The digestion of protein in the alimentary tract may not yield a residue, for one reason or another resistant to proteolytic action, that contains the essential amino acids in the same relative proportions as in the original material.[67] Hence, the digestible portion may possess a different nutritional value than the amino acid content of the original protein would lead one to expect. Also, the possibility of amino-acid synthesis within the intestinal

Table 2–1. The Digestibility by Growing Rats of the Nitrogen in Various Food Materials, Corrected for Metabolic Nitrogen in the Feces

Food Material	*True Digestibility* (%)	*Food Material*	*True Digestibility* (%)
	Animal Foods		
Muscle meats	99	Milk, fresh raw	95
Liver	97	Milk, dried skim	93
Kidney	99	Casein	99
Salmon	99	Lactalbumin	98
Egg, whole	100	Cheese, Swiss	98
Egg, white	100	Cheese, Limburger	96
Egg, yolk	93	Tankage	83
	Cereals and Breads		
Wheat, whole	91	Corn, flaked and toasted	80
Wheat, germ	95	Rice, whole	96
White flour	100	Rice, polished	97
White bread, 70 per cent extr.	91	Rice, bran	83
White bread, 80 per cent extr.	92	Oats, rolled	93
White bread, 85 per cent extr.	89	Barley	90
White bread, 100 per cent extr.	85	Millet	85
Corn, whole	94	Hegari	87
Corn, germ	85	Kaoliang	90
	Legumes		
Peanuts, roasted	96	Navy beans, cooked	85
Peas, cooked	90	Mung bean	86
Soybean flour	96	Lentil	83
Soybean curd	96		
	Other Vegetables		
Potato	89	Alfalfa leaf	79
Cabbage	65	Lady's finger	70
Sweet potato	57		
	Tree Nuts		
Almond	94	Filbert	91
Brazil nut	96	Pecan	71
Cashew nut	96	English walnut	84
Coconut	86		
	Miscellaneous		
Cottonseed flour	90	Yeast, brewers'	93
Cocoa	38	Sunflower seed flour	94
Pumpkin seed	92	Watermelon seed	92
Sesame seed	92		

tract by bacteria would lead to the same result. Martin [90] has presented some evidence that such syntheses may occur, but Williams and Watson [176] were unable to find any clear indication that arginine synthesis occurs in the gastrointestinal tract of rats subsisting upon an arginine-free diet. This problem should be pursued further. Certainly in the ruminant, in which food stagnation and bacterial proliferation occur in the proximal, as well as in the distal, portions of the alimentary tract, massive amino-acid synthesis occurs from simple forms of nitrogen, and the cellular protein thus produced is passed on to the true stomach and the intestines of the animal, where effective utilization is made of it.[46, 47, 84] It is difficult to see how animals with a simple stomach, and consequently with bacterial proliferation practically restricted to the colon and cecum, can benefit from the synthesis of amino acids unless such acids are destined for other purposes than the construction of protein molecules within the bacterial cells.

Metabolic Utilization of Dietary Protein

The utilization of proteins in metabolism, as in digestion, can be studied most effectively by the nitrogen balance technic. The balance of nitrogen in the body, commonly obtained by deducting from the intake of nitrogen that excreted in urine and feces, measures the nitrogen added to the body stores. In the growing animal, it measures the growth accretion in terms of nitrogen or protein. In mature animals it measures the growth of those tissues that continue to grow throughout life. The neglect of the dermal excretion of nitrogen in computing the nitrogen balance is probably not an error of consequence except with human subjects under conditions inducing considerable sweating. If the balance is negative, the body is losing nitrogen at the indicated rate.

The nitrogen balance does not, however, measure the amount of dietary nitrogen used to replace losses incurred in the endogenous catabolism. The reality of this catabolism is universally admitted; because of this continuous tissue erosion the body needs a continuous supply of protein merely to maintain the *status quo*. The magnitude and the constancy of the endogenous catabolism of nitrogen is, unfortunately, a matter of controversy. Folin [38] was the first to distinguish, from his studies on the effect of the plane of protein nutrition on the composition of the urine, between a constant catabolism of nitrogen, related to body size, and a variable catabolism of nitrogen, dependent upon the level of protein consumed. The interpretation of these experimental facts by Folin, but not their validity, has been seriously challenged by the work of Schoenheimer and his associates involving the use of hydrogen and nitrogen isotopes.[150] These classical investigations demonstrated clearly a state of dynamic, not static, equilibrium between the tissues and the amino acids of the blood plasma and intercellular fluids. It has been concluded by some, including Schoenheimer himself, that the distinction between the two types of nitrogen catabolism made by Folin has

been entirely swept away. The author has discussed this apparent conflict of evidence elsewhere.[111] Certainly there is nothing in Schoenheimer's work that denies the existence of a constant type of catabolism of nitrogen-containing compounds in the tissues. In fact, his beautiful analysis of the creatine-creatinine reaction illustrates the type of reaction of which Folin's endogenous catabolism is composed: its rate is not affected readily by the variable presence in the tissues of the creatine precursors; the reactions in which creatine is involved are irreversible, including transmethylation from choline to creatine as Simmonds and du Vigneaud [153] have recently shown. The term "endogenous catabolism" is still applicable, since its independence of the protein intake implies its restriction to functional tissue constituents, though not necessarily protein in nature. Also, there is still a variable metabolism of nitrogenous substances, the rate of which is determined by the magnitude of the supply of dietary amino acids, regardless of whether this metabolism involves dietary amino acids only, or whether tissue constituents also participate in it. While the term "exogenous" in its original meaning is not descriptive of this phase of nitrogen metabolism it may still be applicable in the sense that the amount of nitrogen undergoing transformation, either anabolic or catabolic in type, is determined by the exogenous supply of nitrogen.

In measuring the extent of the metabolic utilization of protein, it is essential to credit the truly absorbed nitrogen with the nitrogen used to replace endogenous losses as well as the nitrogen added to the body stores. Attempts to measure the utilization of protein in metabolism by the percentage of the nitrogen intake (or of the absorbed nitrogen) represented by the nitrogen balance are incomplete and inadequate because one important function of dietary protein in the body is entirely neglected. The dietary nitrogen used to replace endogenous losses is estimated by measuring the magnitude of these losses in a separate experimental period in which the animal is fed a nitrogen-free diet, or one extremely low in nitrogen. This is the method introduced by Folin,[38] and later used by Thomas [167] in his estimations of the "biological value" of food proteins in adult human nutrition.

If the food protein under test is fed to an animal in period 1 and a nitrogen-free diet, similar to the test diet in all essential respects but protein, is fed in period 2, the urinary nitrogen in period 2, after proper adjustment of the animal to diet is assured by the attainment of a constant day-to-day output of the nitrogen in the urine, is assumed to represent the endogenous nitrogen of Folin. Deducting this amount of nitrogen from the urinary nitrogen excreted in period 1 (after proper consideration of any difference in body weight that may have developed between periods 1 and 2) should give the dietary nitrogen wasted.

Obviously, this procedure assumes, first, that the level of urinary output of nitrogen on a nitrogen free diet, attained after a short period (varying

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with different species of animals [156]) during which the more labile stores of nitrogenous compounds are catabolized, measures the amount of nitrogen that must be replaced from the diet to maintain the integrity of the tissues. It assumes, also, that the endogenous nitrogen catabolism during specific nitrogen inanition remains constant during periods of protein feeding, *i.e.*, that the constant type of endogenous catabolism is independent of the "exogenous metabolism" of nitrogen in the original Folin sense.

The assumption that the endogenous loss of nitrogen determines the size of the maintenance requirement of protein seems as well justified as the common assumption that the basal metabolism of energy determines the basal requirement of energy. However, long-continued subsistence upon a nitrogen-free diet, as in the experiments of Smith [154] and of Deuel and associates,[29] will result in much lower levels of urinary nitrogen, just like protracted fasting will induce subnormal basal metabolic rates. But neither these low levels of urinary nitrogen nor the low metabolic rates thus induced have any relation to normal nutrition and normal maintenance requirements of protein and energy. The experiments of McCollum and Steenbock [92] on the pig, and of Mitchell [101] and of Mitchell and Carman [120] on the rat, prove that it is possible to maintain nitrogen equilibrium in animals on amounts of dietary nitrogen no greater than the losses of nitrogen in urine and feces on a nitrogen-free diet, using such proteins as lactalbumin and the proteins of whole milk and whole egg, and even the proteins of corn and oats.

That the endogenous urinary nitrogen observed after a period of protein-free feeding adequate to induce a levelling-off of the urinary nitrogen is unaffected in magnitude by subsequent feeding of dietary protein is a proposition difficult to prove though readily conceivable.* The independence of the output of creatinine and the protein intake constitutes supporting evidence. The failure of Burroughs, Burroughs and Mitchell [22] to lower consistently the endogenous output of urinary nitrogen of mature rats by the feeding of individual amino acids, mixtures of amino acids or proteins of high nutritive quality is also supporting evidence.

It should be noted that the proposition under discussion implies not only a constant endogenous erosion of the tissues, but also a complete utilization of tissue protein in replacing endogenous losses of nitrogen on a protein-free diet. The latter corollary would be proven invalid if it could be shown

* Interesting circumstantial evidence of the continuity of the endogenous catabolism of nitrogen is afforded by the experiments of Ackerson and Blish [1] on molting and non-molting hens. The average endogenous nitrogen (urine N + fecal N on N-free diet) of 69 non-molting hens was 143 mg per kg body weight per day. That of 37 molting hens was 217 mg per kg per day. The biological value of corn protein was determined on 19 non-molting hens and on 6 molting hens, assuming in the latter case the continuity of the accelerated endogenous catabolism indicated above throughout the period of corn feeding. The biological values of corn protein were 68 for the non-molting hens and 67 for the molting hens.

that any source of dietary nitrogen could depress the endogenous urinary nitrogen of Folin. Brief reports of work carried out by Swanson and her associates [157, 177] indicate that mature rats may excrete more nitrogen in the urine on a low-nitrogen diet than on a diet containing 3.5 per cent of protein from whole egg, egg white or egg yolk. On the other hand, diets containing an equivalent amount of protein from pork or rat muscle, casein, or gelatin induced the usual increase in urinary nitrogen output over the endogenous level. Furthermore, a daily dose of 30 mg of *dl*-methionine was as effective as 400 mg of nitrogen derived from egg protein. It is to be noted that the proteins exhibiting this unusual effect are relatively high in methionine plus cystine, whole egg containing 6.5, egg white 6.7 and egg yolk 4.9 per cent per 16 gm of nitrogen; [17] on the other hand, the proteins not exhibiting the anomalous effect are all nutritively deficient in the sulfur-containing amino acids, casein containing 3.8 per cent, gelatin 0.5 per cent and muscle (beef) 4.6 per cent. It should also be noted that the diets used in these experiments are relatively high in fat, containing 20 per cent. Allison and coworkers [5] have noted the same situation in dogs rendered hypoproteinemic by plasmapheresis and low-nitrogen feeding. Such dogs excrete less nitrogen on a diet containing egg white than on a no-protein diet. The experimental diets contained 22 per cent of fat.*

Swanson interprets her data to mean that "egg proteins supply nitrogenous metabolites that are more efficiently utilized than those arising in the catabolism of body tissues coincident with existence on a protein-free diet." Allison concludes less specifically: "The data can be interpreted to mean that egg white spares body nitrogen." However, in both investigations the condition of severe protein deficiency coupled with a high-fat intake are conducive to an impairment in liver function that may well involve the metabolism of protein. Li and Freeman [81] showed that "exogenous fat impairs liver function in the protein-deficient dog." The effect of methionine in depressing the endogenous nitrogen catabolism of the protein-deficient animal [99, 157] may be a sequel to its beneficial effect on liver function.[100]

In early work in the author's laboratory the urinary nitrogen output of rats on a low protein, high fat diet occasionally was observed to exceed the output on a no-protein diet. In the paper first describing the nitrogen-balance method of measuring the biological value of proteins by growing rats,[101] this situation was observed with lactalbumin, containing 6.8 per cent of sulfur-containing amino acids,[17] but not with milk proteins containing only 4.4 per cent. In their study of the effect of individual amino acids on the endogenous urinary nitrogen of mature rats, Burroughs *et al.*,[22] using diets containing 15 per cent fat, noted six instances out of 40 in which a significant depression occurred. These all related to abnormally high levels

* This work has since been reported in detail by M. K. Brush, W. J. Willman, and P. P. Swanson, in *J. Nutrition*, **33**, 389 (1947).

of endogenous urinary nitrogen and four of them involved methionine or cystine. The protracted experiments on low-nitrogen nourishment reported by Smith [154] and by Deuel and others [29] were carried out with diets containing little or no fat; otherwise they may have terminated disastrously.

In using the endogenous output of nitrogen in the urine to measure the amount of dietary nitrogen required to maintain the integrity of the tissues, in the course of a determination of the biological value of protein, the purpose is not to reduce the urinary nitrogen to the lowest possible level. Such a level can be obtained only after severe protein depletion and obviously bears no relation to the nitrogen requirement of maintenance under conditions of adequate nutrition. In an analogous sense, the basal requirement of energy is related to the metabolic rate immediately subsequent to the completion of absorption from the alimentary canal, not to the much lower value obtained after protracted fasting. The analogy is strengthened by the establishment of a relationship between the endogenous nitrogen of the urine, obtained after a short period of protein depletion, and the basal metabolism, obtained after a short period of fasting, by Smuts,[156] confirming earlier work by Terroine and his associates. This relationship, to be discussed in more detail later, is such that, for species of animals differing widely in size, 2 mg of endogenous nitrogen are excreted per calorie of basal heat. In the determination of biological values of proteins in the author's laboratory, it is now customary, in view of the Terroine-Smuts findings, to adjust the estimations of endogenous urinary nitrogen in periods where body weight changes have developed, in proportion to the three-fourths power of the body weight, rather than to the body weight itself. The essence of this procedure has been substantiated by Olson and Palmer.[137]

The determination of the biological value of food proteins with growing rats in the author's laboratory, involves the following steps:

(1) The determination of the absorbed nitrogen after due allowance is made for the metabolic nitrogen in the feces as discussed in the preceding section. Feces markers (Fe_2O_3 and Cr_2O_3) are used to distinguish the feces of different experimental periods.

(2) The urinary nitrogen is determined for each test protein in collection periods extending over 7 days and preceded by preliminary periods of at least 4 days but preferably 7 days.

(3) The endogenous nitrogen in the urine * is determined in a 7 day period on a diet containing approximately 4 per cent of protein from whole egg, preceded by a 7 day preliminary period on the same diet. The low-egg nitrogen diet and all test diets are equalized in fat (and fiber) content, the fat content being no more than 12 per cent. The work of Mitchell

* In 156 recent determinations of the endogenous urinary nitrogen of young growing rats, the average value was 0.605 mg per $W_{gm}^{0.75}$, with a coefficient of variation of 15.3 per cent.

and Carman,[120] Burroughs *et al.*[22] and of Treichler [169] demonstrate to our satisfaction that under the conditions imposed, egg protein does not depress the output of endogenous urinary nitrogen nor of creatinine.

The significance and accuracy of biological values of food proteins as determined in the author's laboratory have been assessed by Mitchell, Burroughs and Beadles,[116] the average standard deviation for the same protein being 3.7 percentage units. For a group of 10 rats, the standard error of the mean biological value would thus equal $3.7 \div \sqrt{10} = 1.2$. There is a tendency, not always evident,[105] for biological values determined immediately subsequent to a period of low-nitrogen feeding to be higher than otherwise, but this tendency can be minimized or removed, by increasing the length of the pre-feeding period, following the standardizing period, to 7 days or more. There is also a tendency, as Murlin and associates [133] have recently shown with human subjects, and our own data on rats reveal, for the endogenous urinary nitrogen determined subsequent to a period of feeding a protein of high biological value to be higher than that determined after feeding a protein of low value. Probably this tendency also may be minimized by increasing the prefeeding period to the standardizing period. However, for reasons given above, the author definitely cannot subscribe to Murlin's recommendation that endogenous nitrogen excretion in the urine be determined only after a series of experimental periods during which a large deficit of body nitrogen has been accumulated, some 60 gm for the human subject.

In Table 2–2 will be found a summary of biological values of food proteins secured upon growing rats, as compared with a chemical score proposed by Mitchell and Block.[115] The biological values have been selected from the literature and from unpublished data in the author's laboratory on the basis of comparability of experimental conditions (young rats, level of dietary protein 9 to 12 per cent). The chemical score is 100 minus the maximum percentage deficit of the given protein in an essential amino acid compared with the proteins of whole egg taken as a standard, due consideration being given to the relationship in metabolism between cystine and methionine. The two methods of measuring the nutritive quality of proteins are highly correlated ($r = +0.86$). The regression equation of biological value (y) on chemical score (x) is:

$$y = 38.6 + 0.634x$$

For proteins containing some of all of the amino acids essential for growth the biological value will vary between 39 and 100. For incomplete proteins, the value will vary between 0 and 39.

The values in the table reveal the general superiority of animal over plant proteins, although the distinction is not by any means universal. Meat proteins, for example, depending upon their connective tissue content, may vary in biological value from 80 down to 60.[114] On the other hand, the

Table 2–2. The Biological Value of Food Proteins for Growing Rats and a Chemical Score Based on Content of Essential Amino Acids

Food Material	*Biological Value* (%)	*Chemical Score* * (%)	*Food Material*	*Biological Value* (%)	*Chemical Score* * (%)
		Animal Foods			
Beef muscle	76	71	Red salmon	72	
Beef heart	74	65	Milk, raw, liquid	90	68
Beef liver	77	70	Milk, dried skim	84	
Beef kidney	77	65	Milk, evaporated	88	
Veal, fibrous	62		Lactalbumin	84	66
Pork tenderloin	79		Casein	73	58
Pork, ham	74		Cheese, Swiss	73	
Egg, whole	94	100	Cheese, Limburger	69	
Egg, white	83	78†	Tankage	48	
Egg, yolk	96				
		Cereals and Breads			
Wheat, whole	67	37	Wheat bran	74	
White flour	52	28	Wheat, puffed	64	
White bread, no milk	45		Corn, whole	60	28
White bread, 2 per cent milk solids	48		Corn, germ	78	39
White bread, 6 per cent milk solids	50		Rice, white	75	44
Wheat bread, 70 per cent extraction	51		Oats, rolled	66	46
Wheat bread, 80 per cent extraction	54		Barley	64	
Wheat bread, 85 per cent extraction	53		Millet	56	
Wheat bread, 100 per cent extraction	56		Kaoliang	56	
Wheat germ	75	38	Hegari	53	
			Rye	58	

Table 2–2. The Biological Value of Food Proteins for Growing Rats and a Chemical Score Based on Content of Essential Amino Acids (*continued*)

Food Material	*Biological Value* (%)	*Chemical Score* * (%)	*Food Material*	*Biological Value* (%)	*Chemical Score* * (%)
		Legumes			
Navy beans, cooked	38		Soybeans, raw	59	49
Mung beans	58		Soybean flour	75	
Peas, raw	48		Soybean curd	65	
Peanut, roasted	56	24			
		Other Vegetables			
Potato	67		Sweet potato	72	
Cabbage	76		Alfalfa leaf	61	60
		Tree Nuts			
Brazil nut	54		Pecan	60	
Cashew	72		English walnut	56	
Almond	51		Coconut	71	
Filbert	50				
		Miscellaneous Foods			
Cottonseed flour	62	37	Yeast, brewer's	63	45
Sunflower seed flour	65	53	Cocoa	37	
Linseed meal	78	35	Pumpkin seed	63	
Sesame seed	71	39	Watermelon seed	73	

* 100 minus the maximum percentage deficit in an essential amino acid as compared with the proteins of whole egg.[115]

† Later analyses by Block (private communication) give a lysine content of 6.5 gm per 16 gm of N rather than 5.0 gm as previously reported.[117]

biological values of the proteins of corn germ, wheat germ, wheat bran, white rice, certain grades of soybean flour,[124] the cashew nut, and a number of other plant foods fall within the range of values for animal proteins.

Mitra and Mittra [129] have presented evidence to the effect that the proteins of cow's milk are superior in biological value to those of the goat and the buffalo. Deuel and coworkers [28] have presented further evidence of the superior quality of fish proteins, as compared with casein. Mushroom protein is low in nitrogen (11.8 per cent) and is only about 32 per cent as good for the growth of albino rats as is casein or the proteins of soybean meal.[37]

Increasing the extraction of flour in wheat milling increases the protein content and the biological value of the protein, but decreases protein digestibility.[55] The net result is an increase of about 10 per cent in net protein value of the bread.* The addition of 6 per cent of milk solids to white bread raises the biological value of the protein by 5 percentage units, depresses the digestibility by 2 units, but increases the net protein value by some 25 per cent because of the concomitant increase in protein content.[54]

The digestibility and biological value of the proteins of many Indian foods for rats have been reported by Basu and associates [12–15] and by Swaminathan.[163] It is unfortunate that in much of this work mature rats, or nearly mature rats, were used, although the level of dietary protein was 10 per cent or more. Unless the rats have been previously depleted in protein, such levels of dietary protein may exceed the ability of the rat to store protein, in which case spurious biological values will be obtained.

The method of measuring the nutritive value of proteins by the nitrogen balance technic has been applied to other animals than the rat. The author has discussed elsewhere [17, 109] the species differences in protein utilization as revealed by the scanty information available. The agreement in this respect between the rat and the chicken is not good, possibly because of the comparatively greater requirement of glycine and arginine by the chick than by the rat. Except for the oil meals, the reported differences between growing rats and growing pigs are not greater than have been reported from different laboratories for the same species. The disagreement for the oil meals is probably a result, not of different degrees of utilization of protein by the two species, but rather of different processing methods used in preparing the meals.[124]

The utilization of dietary proteins by ruminants is modified by the synthetic activities of the paunch microorganisms,[46, 47] so that no agreement with single-stomached animals would be expected. From the fact that the biological value of the nitrogen of rations containing 10 to 12 per cent of conventional protein (N × 6.25) generally varies only within a few per cent from 60, it is an attractive theory, which needs more direct confirma-

* The net protein value of food is its protein (N × 6.25) content on the dry basis times the net utilization expressed as a decimal ([104, 119]). The net utilization is the digestibility of the N times the biological value divided by 100.

tion, however, that a considerable proportion of the protein ultimately utilized by the ruminant is microorganismal protein, regardless of the nature of the nitrogenous compounds contained in the ration as consumed.[64] Further confirmation of this idea is afforded by the experiments of Louw and Van Der Wath [85] on meat meal and corn; for both of these feeds, the biological value of the protein for sheep was 67. Swanson and Sherman [164] have discussed the interpretation of nitrogen balance data obtained with growing dairy heifers and have shown, in harmony with the theory above proposed, that the biological values of the proteins of feeds differing as widely in amino-acid content as corn and milk are approximately the same for the ruminant animal.

While nitrogen balance methods have been used in studying the protein requirements of children, only two instances have been found in the literature in which an attempt was made to determine the biological value of dietary protein. One was reported by Wagner,[171] cited by Sumner and Murlin.[160] In this work, a group of children from 10 to 12 years convalescing from hilus gland tuberculosis exhibited biological values of 60 for milk protein and 71 for egg protein. The other was reported by Edelstein and Langstein,[31] in which a value of 73 was found for cow's milk, 88 for woman's milk, 87 for lactalbumin and 73 for casein. That the child is inefficient in his utilization of nitrogen, as he is in his utilization of calcium, is indicated by the nitrogen balance studies of Petrunkina.[145] Although not designed to measure protein utilization, balances were obtained at different levels of intake of nitrogen from a mixed diet. From the data tabulated, it may be computed that each increase of 100 mg in nitrogen intake is, on the average, associated with an increase in nitrogen retention of about 42 mg.

The Biological Value of Proteins for Maintenance. That the metabolic utilization of proteins is different during growth than during adult life was shown with rats by Osborne and Mendel [140] in 1916. Although their work did not involve the nitrogen balance method, they were able to show clearly in carefully controlled experiments that, for the growing rat, casein is distinctly superior to edestin, while for the mature rat the reverse is true. In their later work on the nutritive value of the proteins of the wheat kernel and its milling products,[141] they found that the wide difference in the nutritive value for growth existing between the proteins of whole wheat and those of wheat endosperm largely disappeared when the comparison is made with adult rats. Sumner [159] has reviewed the literature on the effect of age on the biological value of proteins in the rat, and has contributed data of her own indicating a somewhat poorer utilization of the absorbed nitrogen from milk and from egg by mature as compared with young rats. The work of Burroughs, Burroughs and Mitchell,[23] establishing a qualitative difference in the amino acid requirements of growing and of mature rats, revealed a rational basis for differences in protein utilization.

It should be emphasized that determination of the biological value of

protein in the mature rat, as in the young rat, must be made at levels of intake insufficient to cover the requirements of the animal as measured by its ability to retain dietary nitrogen; otherwise spurious values will be obtained. For the mature rat, this generally means that the nitrogen balance should be negative to be sure of satisfying this requirement. If the rat has been seriously depleted of protein, nitrogen intakes inducing positive balances of nitrogen may presumably be permitted for a time at least.

A number of experimental methods have been employed in studies of protein utilization in adult men and women: the method of Thomas [167] involving the computation of biological values in the original meaning of the term; the method of Murlin and associates,[161] in which the Thomas method is modified by use of an egg-protein diet as a standard rather than a nitrogen-free diet; and the method of Sherman [152] in which different sources of protein are compared on the basis of the balances of nitrogen secured on comparable, and low, intakes of nitrogen. None of these methods is ideal in experimentation with adult human subjects, either because of the difficulty in tolerating the experimental diets, the purely comparative nature of the results secured, or the impossibility of computing a protein requirement for nitrogen equilibrium. Recently, Bricker, Mitchell and Kinsman [21] adapted to adult human experimentation a method that had been applied to adult dogs by Melnick and Cowgill [93] and Allison and Anderson,[4] based upon the rectilinear relationship between the nitrogen balance of a mature animal and the intake of nitrogen, or of absorbed nitrogen.

Block and Mitchell [17] have recently summarized available information on the metabolic utilization of dietary protein by adult human subjects. The result is definitely discouraging because of the lack of agreement among the different investigators and even among different reports from the same laboratory. As an example, the proteins of milk are given a biological value of 100 by Thomas,[167] 74 by Bricker *et al.*,[21] 62 by Sumner and Murlin,[160] 51 by Martin and Robison [89] and by Lintzel,[82] and 43 by Lintzel and Bertram.[83] In Murlin's laboratory, the biological value of the protein of whole egg by adult human subjects has been rated at 65,[160] 92,[134] and 102.[132]

Apparently, experimental procedures employed in the determination of the biological values of food proteins in adult human nutrition have not been sufficiently well standardized to permit profitable comparison among values obtained in different laboratories. In attempting to decide whether any one set of values may be more reliable than another, Block and Mitchell [17] employed as a yardstick the chemical scores, such as those listed in Table 2–2, based upon the relative contents in the essential amino acids between a given protein mixture and the proteins of whole egg taken as a standard. It will be remembered that these chemical scores were found to be highly correlated with the biological values of proteins for growing rats.

Some justification for this attempt was offered in the fact that egg proteins were shown to be superior to milk proteins for adult humans by Sumner and Murlin [160] and Sumner, Pierce and Murlin,[161] and, for children, by Wagner.[171]

The failure of most of the data on human adult subjects summarized by Block and Mitchell [17] to reveal any correlation with the chemical scores may be due to imperfection of technic, quite understandable in a field of research beset with so many difficulties. The results reported by Martin and Robison,[89] by Cheng, Li and Lan [26] and by Bricker, Mitchell and Kinsman [21] do display some degree of correlation, whatever other faults they may possess.

The author offers the following estimates of the biological value of a few food proteins for human adults, based upon what seems to be known and upon what may be surmised from the amino acid composition:

Whole egg	78	*Whole wheat*	55
Milk	74	*Corn meal*	43
Meat	72	*Peanut flour*	42
Soy flour	65	*White flour*	41
Rolled oats	60		

The Net Utilization of Dietary Proteins

The net utilization of proteins takes into account the utilization in digestion and in metabolism, *i.e.*, the product of the digestion coefficient and of the biological value, divided by 100. Such figures are summarized in Table 2–3. The table also includes estimates of net utilization by another method, based not upon nitrogen balance data, but upon growth and food consumption data. The ratio of gain in weight of growing rats to protein consumed, the "protein efficiency ratio," was first proposed by Osborne, Mendel and Ferry,[143] and in modified form has been used more extensively than any other method of protein quality appraisal. The method has been criticized by the author elsewhere,[112] but with all its faults it seems to be a good method, particularly for proteins of better nutritive quality, since the protein efficiency ratios, when carefully selected for comparability, are highly correlated both with chemical scores based upon contents of essential amino acids ($r = +0.89$) and with net utilization values by the nitrogen balance method ($r = +0.84$), as Block and Mitchell [17] have shown.

In the selection of protein efficiency ratios for inclusion in Table 2–3, only those ratios were taken that relate to diets containing 10 ± 2 per cent of conventional protein ($N \times 6.25$), and to feeding periods of 4 to 8 weeks. The ratio is known to vary with the length of the feeding period,[142] with the sex of the rat,[58] and with the proportion of protein in the diet.[143] From the regression equation of protein efficiency ratio (y) on chemical score (x), *i.e.*, $y = 0.56 + 0.0321x$, it appears that the ratio will assume a maximum value of 3.77. Barnes and associates [10] have reported a value of 3.8 for the

Table 2–3. The Net Utilization of Food Proteins for Growing Rats and Their Over-all Nutritive Value as Measured by the Protein Efficiency Ratio

Food Material	*Net Utilization* * (%)	*Protein Efficiency Ratio* †	*Food Material*	*Net Utilization* * (%)	*Protein Efficiency Ratio* †
		Animal Foods			
Beef muscle	76	3.2	Oysters		1.3 ‡
Beef heart	74	3.1	Clam		2.1
Beef liver	75	2.7	Shrimp		2.2
Beef kidney	76	2.9	Eggs, whole	94	3.8
Sheep muscle		3.1	Eggs, white	83	2.6
Veal, fibrous	62		Eggs, yolk	89	
Pork tenderloin	79	3.3	Milk, raw, liquid	86	
Pork, ham	71	2.7	Milk, dried skim	80	2.9
Beef brain		3.0	Lactalbumin	83	2.9
Pork brain		2.9	Casein	68	2.2
Beef blood serum		2.1	Cheese, Swiss	72	
Red salmon	71		Cheese, Limburger	66	
			Tankage	40	1.3
		Cereals and Breads			
Wheat, whole	61	1.5	Corn germ	66	2.6
White flour	52	1.0	Corn gluten		0.7
White bread, no milk	42	1.1	Corn flakes, toasted	42	0.8
White bread, 2 per cent milk solids	43		Rice, white	70	1.9
White bread, 6 per cent milk solids	44		Rice, puffed		0.6
Wheat bread, 70 per cent extraction	47		Oats, rolled	61	2.2
Wheat bread, 80 per cent extraction	49		Barley	58	1.8
Wheat bread, 85 per cent extraction	47		Millet	51	1.2
Wheat bread, 100 per cent extraction	48		Buckwheat		2.5
Wheat germ	71	2.9	Kaoliang	50	1.0
Wheat bran	73	2.0	Hegari	46	0.6
Wheat, puffed	57	0.7	Rye bread		1.7
Corn, whole	49	1.2			

Table 2–3. The Net Utilization of Food Proteins for Growing Rats and Their Over-all Nutritive Value as Measured by the Protein Efficiency Ratio (*continued*)

Food Material	*Net Utilization* * (%)	*Protein Efficiency Ratio* †	*Food Material*	*Net Utilization* * (%)	*Protein Efficiency Ratio* †
		Legumes			
Navy beans, cooked	32	1.2	Green peas		1.9
Mung beans	50		Green peas, cooked		0.4
Cow pea		1.1	Lima beans, raw		0.2
Peas, raw	44	1.1	Lima beans, cooked		1.2
Peas, baked		0.8	Soybeans, raw	50	0.5
Peanut flour		1.9	Soybean flour	72	2.3
Peanuts, roasted	54		Soybean curd	62	
		Other Vegetables			
Potato	60		Cabbage	41	0.9
Potato, tuberin	67	2.0	Alfalfa leaf	49	2.4
Sweet potato	41	1.5			
		Tree Nuts			
Brazil nut	52		Pecan	43	
Cashew	69		English walnut	47	
Almond	48		Coconut	61	
Filbert	46				
		Miscellaneous Foods			
Cottonseed flour	56	2.0	Yeast, brewer's	56	0.9
Sunflower seed flour	61		Yeast, torula		0.8
Linseed meal	72	1.9	Pumpkin seed	60	
Sesame seed	65		Watermelon seed	67	
Cocoa	14				

* True digestibility times biological value divided by 100.

† Gain in body weight in grams per gram of protein consumed.

‡ This value for oysters [66] is greatly at variance with that reported by Lanham and Lemon [76] who report a value of 2.5 for oyster protein, higher than that of any other sea food tested.

proteins of whole egg. The fact that y assumes a value of 0.56 when $x = 0$, is due to the fact that low-quality proteins tend to give too high a ratio. It would be interesting to know whether the pancreas protein preparation of White and Sayers,[175] apparently possessing unusual growth-promoting properties for rats,[69] would give a protein efficiency ratio as high as 3.8 when measured under the conditions specified above.

Hegsted, Hay and Stare,[50] using methods of feeding that can lay no claim to accuracy, nevertheless present interesting evidence concerning the relative growth-promoting qualities of human blood proteins. At a 20 per cent level of dietary proteins, fibrin promoted as good growth as milk proteins, blood albumin supported no growth at all, while γ-globulin was intermediate in value. Mitchell and Block [115] have computed the following chemical scores for these proteins: milk 68, fibrin 63, γ-globulin 27, and serum albumin 20.

In order to limit the protein efficiency ratio to metabolic utilization, Hoagland and others [57] have recently suggested the introduction of a digestibility test in conjunction with the growth test, and the computation of a ratio of body weight gain per gram of digestible nitrogen consumed. Boshardt and coworkers [19] have proposed many more modifications and refinements of the current procedure in computing the protein efficiency ratio. Other methods of evaluating the nutritive quality of proteins have been proposed involving the regeneration of plasma proteins [24, 95, 174] or the regeneration of liver protein.[48] In poultry nutrition, there seems to be a place for methods of measuring the protein quality of supplements added to a standard practical basal diet [53] and for various crude chemical procedures.[34, 35]

The Supplementary Relations Among Proteins

Individual protein foods are rarely consumed alone. Rations and diets, containing many foods in each, are devised in practical nutrition in order (1) properly to cover the requirements of the body for all nutrients, and (2) to satisfy the appetite which is, in many animals at least, closely meshed with the body's requirements of nutrients. In this process of combining foods into diets, proteins may lose their individuality with reference to metabolic utilization: the amino acids of other foods may supplement the amino acids of a given food and *vice versa*, so that the metabolic utilization of the combined proteins exceeds the mean utilization of the individual proteins. Whether such supplementation will or will not occur, will depend upon the essential amino acids limiting the metabolic utilization of the two proteins. If the limiting essential amino acids are the same for both proteins no supplementation will occur; if they are different some supplementation will always occur.

The amino acid deficiencies of food proteins in so far as they have been determined by biological assay, mainly upon growing rats, are indicated

in Table 2–4. Included in the table, also, are the limiting amino acids indicated by the contents of essential amino acids determined analytically, according to the method proposed by Mitchell and Block.[115]

The agreement between chemical and biological evidence on the amino acid limiting the metabolic utilization of proteins is generally good: 16 cases of agreement against 6 disagreements, one of which was secured with chicks (sunflower seed). The disagreements for blood plasma albumin, soybeans, peas, alfalfa, and rye may well be the result of a slight error in amino acid assay, emphasizing a second amino acid deficiency at the expense of a first.* In these cases, the amino acid analyses[17, 115] indicate, in the order of foods above given, the following limiting amino acids rather than the ones named in Table 2–4: tryptophane, methionine, methionine and isoleucine, and methionine. Obviously indications based on amino-acid assay are less reliable than the results of bio-assay, provided the latter is sufficiently well controlled.

It will be noted from the table that animal foods are generally deficient in the sulfur-bearing amino acids or in isoleucine, cereal proteins in lysine, leguminous seeds in cystine and methionine, other oil-bearing seeds in lysine, and leaf proteins apparently also in the sulfur acids. These facts explain the general existence of supplementary relations between the proteins of animal foods and those of the cereal grains and breads, as revealed by the experiments of Mitchell and Carman,[120] Hoagland and Snider [59] and Hoagland, Ellis, Hankins and Snider.[57] In fact, it is possible to combine animal (tankage) and cereal (corn) proteins to give a mixture with a biological value exceeding that of either one of the component foods.[126]

One of the most remarkable instances of amino-acid supplementation is that reported by Rapp, Skinner and McHargue,[147] showing that the addition of lysine to tobacco seed protein raised the biological value from 51 to 79. Mitchell [102] and Block and Mitchell [17] have reported other instances of the same kind.

By combining foods into diets, if this is done with discrimination, the individuality of the component food proteins as regards metabolic utilization may be lost. At a 10 per cent level of dietary protein the biological

* In a private communication, Dr. R. J. Block makes the following comment on these discrepancies: ". . . in view of the complex relationship between cystine, methionine, choline and fat, I think the chemical data on soybeans, peas and alfalfa should be considered confirmatory to the biological findings.

"We have checked the chemical analyses on serum albumin and find a value of 1.7 per cent for isoleucine rather than 2 as previously given. This coupled with the fact that the tryptophane requirements are modified by the amounts of niacin and possibly pyridoxine in the diet, may put serum albumin in the class of chemical confirmation with biological data.

"This leaves only rye showing a discrepancy. It may well be that the reported lysine figure, which was carried out by the microbiological method was high or that the sample fed may have suffered some heat inactivation or both."

Table 2–4. Amino Acids Limiting the Nutritive Value of Food Proteins

Food Protein	*Limiting amino acid*		*Chemical Evidence* [b]	*Biological Evidence* [c]	*Reference* [d]
	Amino acid	*Deficit (%)* [a]			
Animal Foods					
Beef muscle	Cystine and methionine	29	+	+	128
Horse muscle	Cystine and methionine	35	+		
Chicken muscle	Cystine and methionine	31	+		
Crustacean muscle	Cystine and methionine	28	+		
Heart	Isoleucine	35	+		
Kidney	Cystine and methionine	35	+		
Liver	Isoleucine	30	+		
Brain	Isoleucine	36	+		
Blood plasma	Isoleucine	62	+	+	51
Blood albumin	Isoleucine	75	−	+	50
Blood fibrin	Isoleucine	37	+		
Blood γ-globulin	Methionine	73	+		
Blood hemoglobin	Isoleucine	79–99	+	+	3
Globin	Isoleucine			+	30, 138
Cow's milk	Cystine and methionine	32	+	+	113
Casein	Cystine and methionine	42	+	+	70
Lactalbumin	Methionine	34	+		
β-Lactoglobulin	Histidine	29	+		
Human milk	Methionine	46	+		
Human colostrum	Cystine and methionine	34	+		
Egg albumin	Threonine	22[h]	+		
Gelatin	Tryptophane	100	+		
Cereals					
Whole wheat	Lysine	63	+	+	128
Wheat germ	Isoleucine	62	+		
White flour	Lysine [e]	72	+	+	57
Gliadin	Lysine	86	+	+	94
Whole corn	Lysine	72	+	+	128
Corn germ	Methionine	61	+		
Corn gluten	Lysine	89	+		
Zein	Lysine	100	+	+	139
Oats, rolled	Lysine	54	+	+	128
Rice, white	Lysine	56	+	+	71
Rye	Lysine	42	−	+	65
Hegari	Lysine			+	155
Kaoliang	Threonine			+	88
Legumes					
Navy bean, Phaseolus vulgaris	Cystine and methionine [g]			+	62
Phaseolin	Cystine and methionine			+	62
Lima bean, Phaseolus lunatus	Cystine and methionine			+	36
Adsuki bean, Phaseolus angularis	Cystine and methionine			+	63

Table 2–4. Amino Acids Limiting the Nutritive Value of Food Proteins
(*continued*)

Food Protein	*Limiting amino acid*		*Chemical Evidence* [b]	*Biological Evidence* [c]	*Reference* [d]
	Amino Acid	*Deficit (%)* [a]			
Legumes (*continued*)					
Soybeans	Cystine and methionine	40	−	+	128
Peanut	Methionine	76	+		
Arachin	Methionine	85	+	+	9
Pea, field	Cystine and methionine	66	−	+	178
Pea, garden	Cystine and methionine	66	−	+	16
Other Vegetables					
Potatoes	Cystine and methionine			+	16
Leafy vegetables	Methionine	44	+		
Beet tops	Methionine	51	+		
Alfalfa leaves	Cystine and methionine	40	−	+	48
Miscellaneous					
Cottonseed	Lysine	63	+		
Cottonseed flour, autoclaved	Lysine			+	136
Flaxseed	Lysine	65	+		
Sesame seed	Lysine	61	+	+	41
Sunflower	Lysine	47	+	−	42
Edestin	Lysine	69	+	+	162
Tobacco seed oil meal	Lysine			+	147
Yeast, brewers'	Cystine and methionine	55	+	+	72
Yeast, torula	Cystine and methionine			+	72

[a] Percentage deficit in the indicated amino acid as compared with the proteins of whole egg.[115]

[b] Plus sign indicates existence of chemical (structural) evidence that amino acid named is the limiting one.[115] Minus sign indicates that evidence concerning limiting amino acid named is based on biological evidence only.

[c] Plus sign indicates existence of biological evidence that amino acid named is the limiting one. Minus sign indicates that evidence concerning limiting amino acid named is based on chemical evidence only.[115]

[d] Reference numbers for biological evidence. Many other references might, of course, be cited. Those cited were selected on the basis of priority or soundness. The evidence of a few cannot be vouched for by the author of this review.

[e] The deficiency of white flour proteins in lysine for the growing rat has been shown by Bricker *et al.*[21] to apply to the adult human also.

[f] The finding of Kik [71] that rice proteins are supplemented effectively by more than one amino acid is such an anomalous result as to require confirmation by some more exact method than a simple paired-feeding test.

[g] It is interesting to note that Pittman [146] has confirmed on adult human subjects the cystine deficiency of the proteins of the navy bean.

[h] Later analyses by Block (private communication) give a lysine content of 6.5 gm per 16 gm of N rather than 5.0 gm as previously reported.[117]

values of the mixed proteins of human diets for the growing rat have been found to range from 83 to 89 for typical Chinese omnivorous diets [172] and from 78 to 81 for diets served to Royal Air Force airmen's messes.[86] In these diet tests, all meals are mixed together in preparing the experimental diets for the rats, a procedure that implies supplementary relations between the food proteins of adjacent, or even of terminal, meals. This assumption is worthy of specific investigation in order to discover to what extent the proteins of single meals need mutual supplementation. This problem is but one phase of the more general one concerned with the need (or advantage) of balancing each meal as well as the day's (or the week's) diet.

However, in thus combining foods into diets, the characteristic digestibilities of the individual proteins may not be lost. In a sense, it is more important, especially in animal feeding, to avoid foods containing poorly digestible proteins than to avoid ones with proteins possessing low biological values. The latter can be corrected by proper supplementation, but the former cannot. Wheat-germ protein is lower in biological value than corn-germ protein (see Table 2–2), but higher in digestibility (Table 2–1); in diets it may thus prove to be a more desirable protein. The heating of cereal proteins in the preparation of breakfast foods may be a matter of no consequence in practical nutrition if only the biological value is impaired, because when consumed with the usual proportion of milk proteins this impairment is entirely corrected.[17] However, if the digestibility of the proteins is impaired, as in the flaking and toasting of corn (see Table 2–1), there is no known method of food combination that will remedy the situation. In this same connection, it is interesting to note that Evans [33] has reported a correlation of + 0.925 between the digestible organic sulfur of a series of animal feeds for poultry and the gain in weight of chicks per unit of supplementary protein consumed in rations in which protein is the only limiting factor.

Protein Requirements

The most practical phase of protein nutrition relates to the amounts of protein of different varieties and combinations required by animals and by man for various purposes. Many methods have been used in arriving at the desired result. Perhaps the most popular method is that involving the adjustment of the protein intake to the lowest level compatible with nitrogen equilibrium, or with maxiumm growth, or milk production, or egg production. This method yields information of practical value, but it is quite empirical in that it contributes little to the science of protein nutrition aside from the result secured. In particular, it sheds no light upon the relation of body loss of nitrogen to the dietary nitrogen required to replace it, or of the nitrogen stored in the body to the dietary nitrogen required to produce it. This section will be concerned largely with information of the latter type and largely with the human subject. Similar studies for cattle,[106]

swine,[121] and poultry [117, 118] have been published from the author's laboratory.

Protein Requirements for Maintenance. The protein required for maintenance is used in replacing the endogenous losses of nitrogen in the urine. The relationship of these losses to the basal metabolism in adult man was shown by Palmer, Means and Gamble [144] in defining a close relationship between the creatinine output in the urine, a characteristic feature of the minimum endogenous catabolism of nitrogen, and the basal expenditure of energy. This relationship was later studied in children by Talbot and his associates,[165] who showed that, for children as well as adults, the basal metabolism can be satisfactorily estimated from the output of creatinine in the urine. A natural sequel of this work was the broad generalization of Terroine and Sorg-Matter,[166] confirmed by Smuts,[156] that for many different species of animals, the endogenous nitrogen losses (in the adult, at least) bear a very constant relationship to the basal metabolism of energy. According to Smuts, this relationship is such that 2 mg of endogenous nitrogen are lost in the urine per calorie of basal heat. The ratio, however, may be disturbed in the rat by age [169] and by factors that affect differently the two components of the ratio.[170]

These investigations established the fact that the maintenance requirement of protein depends, other things than body size being equal, not upon body weight, but upon basal metabolism, and upon body surface to which basal metabolism is related. The recent failure of Murlin and his colleagues [133] to confirm this relationship in a group of 5 adult human subjects varying in weight from 52 to 70 kg, may be due to those individual variations in physiological functioning that make it difficult even to establish the surface area law with reference to basal metabolism in subjects not differing greatly in size, as Murlin [131] has himself pointed out. The observations of Hegsted *et al.*[52] are in harmony with the above proposition.

Another factor than body size that determines the maintenance requirement of protein is the amount of food energy consumed. Neumann [135] has clearly shown that the greater the energy intake the smaller the amount of a given protein supply required for nitrogen equilibrium. This is an expression of the sparing effect of carbohydrates and fats upon the exogenous protein catabolism. It would seem that the most significant amount of dietary protein of a given description required for maintenance would be that amount required on a maintenance energy diet. Muscular activity *per se* does not increase protein requirements if the energy requirements are satisfied.[25, 127]

The early work on the protein requirements of maintenance in man has been compiled by Sherman.[151] In utilizing the data from 109 experimental periods belonging to 25 investigations on 29 men and 8 women subjects, Sherman proceeds on the following assumptions: "Probably the best indication of the normal protein or nitrogen requirement is to be obtained by

averaging the observed nitrogen output in all available experiments in which the intake appears to have been barely sufficient or not quite sufficient to result in equilibrium of intake and output." The average result thus obtained from data not particularly well adapted to the purpose was 44.4 gm of conventional protein per 70 kg-person. No sex difference was revealed and 90 per cent of the values fell within the range 29 to 56 gm of protein per 70 kg of body weight. The source of the protein was quite varied and the average value pertains to no particular protein or protein mixture.

Leitch and Duckworth [79] reported a somewhat similar compilation of data on the protein requirements of maintenance in man, but they did not exclude the results of obviously adequate intakes of nitrogen as high as 16 gm daily. Using a mathematical method of deriving a protein intake for which the chances of negative and positive balances are equal, they arrive at figures of 48.2 gm and 52.4 gm per day, depending upon whether all of the data are included or only those data for which the nitrogen intake was no more than 10 to 11 gm daily. These values relate to no particular dietary protein or protein mixture, or to no particular body weight, the authors evidently proceeding on the baroque assumption that body size is a matter of indifference in determining protein requirements.

Some of the more recent attempts to determine the amount of protein required by adult man for nitrogen equilibrium are summarized in Table 2–5. The experiments of Martin and Robison and of Lauter and Jenke were carried out by the method of Thomas.[167] The experiments of Murlin and associates have not been interpreted by them in terms of protein requirements. The figures given in the table were computed by the author in accordance with the Thomas method, assuming the endogenous N in the urine to average 1.142 gm per m^2 (Table 5 of reference [133]), the metabolic N in the feces to average 1.08 gm per day (Table 2 of reference [133]), or 0.624 gm per m^2, and the biological values of the proteins studied to be those cited in Table 1 of reference [132].

The requirements of protein reported by Bricker, Mitchell and Kinsman, and by Hegsted and others, were obtained by a modification of a method developed by Melnick and Cowgill [93] and previously discussed (page 64). It offers great promise, since in 2 or 3 experimental periods on the same protein source, it permits an estimation of the biological value, of the nitrogen balance on a nitrogen-free regime, and of the amount of nitrogen required for equilibrium.

Except for the unaccountably high values for protein requirements obtained by Martin and Robison, and the somewhat low values of the Murlin group, the indicated requirements are reasonably harmonious and may be summarized approximately as follows in grams of conventional protein (N × 6.25) per 70 kg-man of 1.8 m^2 body surface: milk 24, soy flour 25, meat 26, potato 30, white flour 40, mixed balanced diet 27.5, and a balanced all-vegetable diet 32.

Table 2–5. Estimates of the Protein Requirements of Adult Man for the Maintenance of Nitrogen Equilibrium

Authority	*Protein Source*	*Protein Requirement*		
		N per Basal Cal (mg)	*N per m^2 (gm)*	*N × 6.25 per 70 kg* [a] *(gm)*
Martin and Robison [89]	Milk			44.9
	Whole wheat bread			66.8
Lauter and Jenke [78]	Beef			26.3
	Potato			29.6
	Wheat flour			38.4
Bricker, Mitchell and Kinsman [21]	Milk	2.76	2.17	24.4
	White flour	4.76	3.74	42.1
	Soy flour	2.88	2.26	25.4
	Soy-white flour [b]	3.38	2.65	29.8
	Mixed diet [c]	3.12	2.45	27.6
	All-vegetable diet [d]		2.88	32.4
Hegsted *et al.*[59]	Vegetable protein $\frac{2}{3}$, meat protein $\frac{1}{3}$ [e]		2.41	27.1
	Whole egg		1.77	19.9
Murlin *et al.*[132]	Beef steak		1.94	1.92
	Haddock		1.92	21.6
	Corn germ		1.84	20.7
	Cottonseed flour		2.04	23.0
	Yeast		2.13	24.0

[a] With a surface area of 1.8 m^2.

[b] Containing 36 per cent soy flour protein and 64 per cent white flour protein.

[c] Mixture of foods modified from list 1 proposed by the Food and Nutrition Board of the National Research Council (Circular 115, 1943). The diet contains about 47 per cent of animal protein.

[d] Distribution of nitrogen: 50 per cent from white flour, 12 per cent from other cereal products, 13 per cent from potatoes, 17 per cent from other vegetables, and 8 per cent from fruits.

[e] The amount of each food in the all-vegetable diet described above was decreased by one-third and sufficient meat added to supply approximately the amount of nitrogen thus removed.

The observations of Kon and Klein [74] on the value of whole potato in human nutrition relate to two human subjects, a man and a woman, who lived over a period of 157 days in apparent nitrogen equilibrium and in good health on a diet in which the nitrogen was supplied practically solely from the potato. The daily intake of conventional protein, computed to 70 kg of body weight, averaged 40 gm for the man and 27 gm for the woman. Kon believes these observations afford substantial confirmation of the claims of Hindhede [56] on the dietetic virtues of the potato and the magnitude of the protein minimum. Incidentally, these values are not greatly different from that for the potato given in the preceding paragraph.

Basu and Basak [11] have reported daily protein requirements for men of standard body weight ranging from 38 to 60 gm for various Indian all-vegetable diets.

Such figures for the protein requirements of adult men and women relate to the condition of nitrogen equilibrium, determined by the equality of the intake of nitrogen from the diet and the output in urine and feces. No account is taken of the loss of nitrogen through the skin, which always occurs and may attain considerable proportions under conditions of activity, emotion and environment favoring sweat secretion. They also take no account of the growth of tissues, such as the epidermis and epidermal structures, that occurs throughout life. Bricker, Mitchell and Kinsman [21] have attempted to assess these factors on the basis of available information, admittedly scanty, and have revised their values upwards to account for them. The revised values, for a 70 kg-man (1.8 m^2 surface area), range from 42 gm of conventional protein per day for milk to 74 gm per day for white flour. These figures do not cover sweat gland activity, which may or may not increase protein requirements depending upon whether or not sweat nitrogen represents solely protein metabolites whose excretion is merely diverted from kidney to sweat glands. Sweat is known to contain amino-acid nitrogen,[49] suggesting an increased wastage of the products of protein digestion.

Protein Requirements for Maintenance and Growth. In the growing child, the requirement for dietary protein includes not only the replacement of the inevitable endogenous losses of nitrogen but also the additional protoplasmic material contained in the growth increment. Lauter [77] reports evidence obtained with girls 11 to 15 years old indicating that the losses of endogenous nitrogen in the urine per kg of body weight are within the normal range for adults, which would mean a decreased loss per m^2 of body surface. However, the question at issue requires further evidence in view of data of contrary significance that may be cited for chickens [2] and laboratory rats.[122]

The growth requirement of protein should bear some relation to the amount of protein retained daily in the body above that used to replace tissue losses. This is commonly estimated by the nitrogen balance, but the nitrogen balance in a short experimental period may be greatly distorted as a measure of true growth by the prior plane of protein nutrition, the condition of the protein stores, and the many variables, intrinsic and extrinsic, that modify the disposal of dietary protein in the body. One might expect that the daily protein acquisition during growth would be small, since the average daily increase in weight by children after the first few months of life does not exceed 20 gm or so, and is of the order of 4 to 10 gm for the period from 2 to 12 years of age.[125] If these increments in weight contain 20 per cent of protein the daily protein accretion would amount to 1 to 4 gm. If the biological value of dietary protein is taken as 50, the digestible pro-

tein required to cover this amount of protein growth would range from 2 to 8 gm daily. This agrees with figures cited by Macy (page 140 of reference [87]) for children from 4 to 12 years of age, obtained in nitrogen balance studies covering considerable periods of time.

This small increment in body protein, together with a maintenance requirement of 12 to 15 gm per day, if the ratio of endogenous loss of N to body weight is approximately that of the adult, seems to require protein intakes of 2 to 3.5 gm per kg of weight [80, 87] and to be associated with extreme wastages of dietary protein that belie the high biological values of dietary protein for children reported by Edelstein and Langstein.[31]

A complete picture of the nitrogen economy of the growing child cannot be drawn at the present writing.

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Chapter 3

Caloric, Vitamin and Mineral Requirements with Particular Reference to Protein Nutrition

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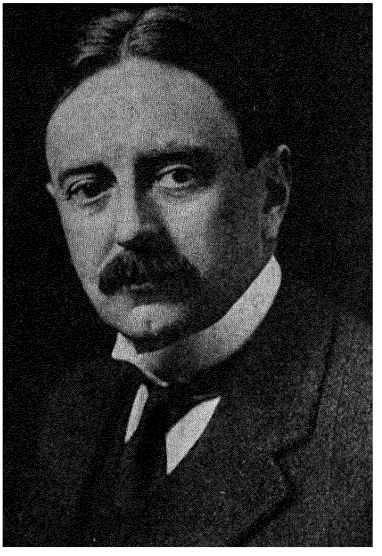

Graham Lusk

Born in Bridgeport, Connecticut, on February 15, 1866. After receiving his Ph.B. degree in 1887 from Columbia University, he went to Munich where he obtained his Ph.D. degree in 1891. While in Germany he came under the influence of Carl von Voit and acquired a profound knowledge of the physiology of nutrition. On his return to America he went first to Yale, then to New York University, and finally to Cornell Medical College where he occupied the chair of physiology until his death on July 18, 1932.

Lusk's major interests were animal calorimetry, metabolism of amino acids, fat and carbohydrate, and the specific dynamic action of foods. One of his contributions was his text, "The Elements of the Science of Nutrition."

Introduction

The normal requirements of the body for protein are conditioned by factors other than the nature of the protein itself. The level of protein intake necessary to establish nitrogen equilibrium is related to the nature of the foodstuffs which are being oxidized concomitantly as well as to the actual quantity of such calories which are available. Several of the intermediary transformations of the amino acids are controlled by enzyme systems of which some of the vitamins may be component parts. This means a less efficient protein utilization may result when there is a deficiency of certain of the vitamins.

The Food and Nutrition Board of the National Research Council in its recent formulation of the recommended daily allowances of calories, pro-

tein, minerals and vitamins has attempted to set standards for good nutrition. The original values which were also approved by the National Nutrition Conference for Defense in 1941 were slightly modified in 1942.[233] Since that time it has become apparent that the allowance for the B vitamins was overgenerous and a 1945 report has included a downward revision of the levels of the B vitamins at caloric intakes above 3000 calories.[234] According to Jeans,[149] this last compilation of suggested levels of intake represents the best group judgment which can be obtained and is the majority opinion of a large number of qualified individuals. Although these tables giving the recommended daily allowances are exceedingly valuable for group calculations, it should be realized that normal individual requirements may vary markedly with variations in the composition of the diet. With increasing proportions of carbohydrate, a large content of the B vitamins is indicated; on the other hand, nitrogen equilibrium may readily be obtained at a considerably lower figure on a high carbohydrate diet than on a predominantly fat diet. However, the proposed levels are not merely the minimal quantity necessary to prevent actual deficiencies, but they were designed to allow a fairly liberal margin to insure good nutrition and to provide for protection of all body tissues. These recommendations are meant merely as an aid in the formulation of good nutrition; in this respect they have served as a valuable yardstick.

In the present summary, the salient factors influencing the requirements for calories, minerals, vitamins and proteins will be considered in turn. The relation of these dietary requirements to that of protein will be viewed as well as the various alterations in protein metabolism which may arise as a result of one or more deficiencies in some of the essential components of the diet.

Caloric Requirements

Total calories. Although it is a fairly simple procedure to obtain reliable experimental evidence as to the basal level of heat production, it is much more difficult to predict accurate figures for total metabolism. Such variables as the amount of activity together with the efficiency of such work, the specific dynamic activity of the foodstuffs, the effect of environmental temperature and the nutritional state must be taken into account in each individual case along with the basal metabolism. The latest recommendations of the National Research Council Committee on Foods for total caloric allowances are summarized in Table 3–1.

Age is an important factor in determining the caloric requirement. The total recommended allowances are highest for girls in the 13–15 year age group and for boys between 16 and 20 years. The suggested values for the 24-hour caloric requirement of these adolescent years exceed those assigned for the adults of the same sex except for that adult group which is characterized as the "very active group." There is ample experimental justification for the high caloric demands of growing children.

Table 3–1. Recommended Caloric Allowances
(Food and Nutrition Board, National Research Council)[234]

Children			*Adults*		
Age in Years	*Calories/24 Hours*		*Activity*	*Calories/24 Hours*	
	Boys	*Girls*		*Men 70-kg*	*Women 56-kg*
Under 1	100/kg	100/kg	Sedentary	2,500	2,100
1–3	1,200	1,200	Moderately active	3,000	2,500
4–6	1,600	1,600	Very active	4,500	3,000
7–9	2,000	2,000	Pregnancy *	——	2,500
10–12	2,500	2,500	Lactation	——	3,000
13–15	3,200	2,800			
16–20	3,800	2,400			

* Latter half.

Sex is also a vital consideration in establishing the total caloric needs. The allowances suggested for women vary from 84 per cent of that of man where sedentary occupations are involved to only 67 per cent in cases where very active individuals are concerned. The variations in the first example are largely to be ascribed to average differences in weight between the sexes, since they are based on the requirements of a 70-kg man and a 56-kg woman. Although basal requirements are proportional to the surface area, this average weight for women is only 80 per cent of that of men. The smaller caloric allowance for the woman doing active work, as contrasted with men in the same category, is obviously a reflection of the lower work capacity of women under such conditions. The caloric needs are not increased during the first half of pregnancy and they are only moderately augmented during the last half of this period. It is surprising that a considerably greater rise in caloric needs occurs during lactation than during pregnancy and in this case the food requirement equals that of a woman engaged in active physical work.

The most obvious factor which alters the requirement of energy in the normal individual is the amount of activity. The values which are postulated are only rough approximations. They must obviously vary with the severity of the task and with the length of time that it is continued over the 24-hour period. The energy expenditures in a number of simple tasks based on the results of Atwater *et al.*,[24] of Benedict and collaborators [42–44] and of Becker *et al.*[39] are summarized by MacLeod [193] and included in Table 3–2.

Of all forms of work, that of lumbering is probably the most strenuous. On the basis of dietary studies, Woods and Mansfield [312] calculated the average caloric production of fifty Maine lumbermen at 8000 calories per

Table 3–2. The Effect of Several Types of Activity on Total Heat Production [193]

Activity	Calories per Hour	
	Total	Per kg
Sleeping	65	0.93
Awake, lying still	76	1.10
Sitting, quietly	100	1.43
Sitting, reading	105	1.50
Standing, relaxed	105	1.50
Standing, at attention	115	1.63
Walking		
Slowly (2.6 m/hr)	200	2.86
Moderately fast (3.75 m/hr)	300	4.28
Very fast (5.3 m/hr)	650	9.28
Running (5.3 m/hr)	570	8.14
Walking down stairs	364	5.20
Walking up stairs	1100	15.8
Light exercise		
Sewing	111	1.59
Knitting	116	1.66
Singing	122	1.74
Typewriting	140	2.00
Ironing	144	2.06
Dishwashing	144	2.06
Sweeping	169	2.41
Shoemaking	180	2.57
Moderate exercise		
Carpentry	240	3.43
Metal working	240	3.43
Industrial painting	240	3.43
Heavy exercise		
Stoneworking	400	5.71
Sawing wood	480	6.86

day. This would seem excessive except for the demonstration of the high heat production of sawing wood and because of the higher general level of metabolism of individuals having such an occupation. On the basis of metabolism experiments, Atwater and Benedict [24] have found that a 7-day bicycle rider riding for a total of 16 hours during the day had an energy expenditure of approximately 9300 calories per day. This is about the same caloric expenditure as calculated by Lusk [187] for the long distance runner. Since it was known that energy requirements for a 70-kilogram man to run 5.3 miles in an hour was 480 calories, it was suggested by Lusk that the total energy expenditure for the 80-mile run from Milwaukee to Chicago in a 15-hour period required a total of approximately 8900 calories, 1680 calories

of which were required for the basal metabolism. Liljestrand and Stenström [178] report the total energy expenditure of swimming at the rate of 2 miles per hour as approximately 624 calories per hour. In the swimming of the English Channel which required 15 hours, it seems probable that approximately 10,000 calories would be expended and this does not take into consideration the increased loss of energy due to the greater conduction in the cold water. In climbing mountains it has been estimated that the hourly caloric requirement may be as high as 767 calories.[187]

Although the average energy expenditure of the American farmer based on the food consumption was reported between 3500 and 4000 calories in 1903 by Atwater,[23] it must be considerably lower than this figure at the present time with the greater use of labor-saving equipment. The high energy production of Hungarian peasants during the harvest [107] which was estimated at 6000 calories per day, must be a reflection of the lack of machinery and the predominant use of manual labor. The soldier under usual conditions expends approximately 4000 calories per day. This value does not exceed that which has been proposed by the National Research Council Committee on Foods for active individuals. Although a daily consumption as high as 8000 calories per day has been reported for American cavalrymen previous to World War I, it was shown that these figures were erroneous inasmuch as they were based on food offered rather than on food actually consumed. Lusk calculated the caloric requirement of the 70-kilogram soldier marching a total 30 miles per day carrying a 20-kilogram pack as about 4000 calories. Murlin and Hildebrandt [218] have estimated the average caloric requirement of the American soldier during World War I as approximately 4000 calories. Cathcart [72] reported on the basis of his own experimental work during World War I the caloric requirements of many occupations of the British soldier (Table 3–3).

Cathcart estimates the average daily expenditure of energy for the recruit as about 3500 calories. Heat production for people who are engaged in so-called sedentary occupations may be greater than is realized if the work is continued over a long period of time. Lusk,[187] in making an analysis of the energy expenditure of a pianist,[223] calculated that calories were increased only from 53 to 95 when the music of Mendelssohn was performed and to 128 when the *Appassionata* of Beethoven was played. The metabolism reached three times the basal level when the very rapid *Tarantella* of Liszt was being performed. In the experiments of Loewy and Schroetter [180] the powerful effect required in the playing of the music of Liszt is also emphasized where there is an increase of 270 per cent over the basal level. Reading aloud produced an increase of 64 per cent whereas the rise in metabolism was about doubled (129 per cent) with cello playing as compared with the violin. Even the work of dancing may require an extreme expenditure of energy. A group of Swedish investigators [126] showed that the waltz required 280 calories per hour, the fox trot 335, the polka 529,

Table 3–3. The Energy Requirement of the British Soldier in Various Occupations as Determined by Cathcart [72]

Activity	*Calories per Hour*
Lying, post absorptive	67
Standing, at ease	76
Lying, after meals	75
Sitting, lectures	86
Standing, attention	90
Using Lewis gun	94
Kit inspection	130
Anti-gas drill	145
Musketry	178
Guard and sentry drill	181
Bayonet practice	215
Company drill	228
Fatigues	241
Marching, drill order	319
Field work	330
Entrenching	331
Marching, battle order	379
Assault	383
Marching, full equipment	413
Route marching	457

and the mazurka the maximum expenditure of 761 calories per hour, which is equivalent to the amount of work required in climbing mountains.

Although it would appear that we may have a maximum rate of energy expenditure as high as 1100 calories per hour for stair climbing, the total maximum heat production over 24 hours is somewhere in the neighborhood of 9000 to 10,000 calories for such widely varying tests as bicycle riding, swimming, lumbering and such feats of work. However, after considering the most reliable results on the expenditure of energy by farmers and by soldiers, it would appear that the value of 4500 calories given as the suggested requirement for an individual doing a very active work should usually be considered as sufficient.

Basal Requirements. The variation in the daily requirement as postulated by the Food and Nutrition Committee of the National Research Council, is to a great extent dependent on variations in basal metabolism. Thus, the energy production during periods of rest is considerably higher in men than in women. Moreover, not only sex but also age is an important factor because the highest heat production per unit of surface area occurs during early childhood. The values for an average normal individual at various age periods is given in Table 3–4 which summarizes the results of Boothby, Berkson and Dunn.[55] These data are based on the average of a large number of individuals in each group.

Table 3-4. Standard Values for Basal Metabolism in Calories per Square Meter per Hour [55]

Age on Last Birthday	Calories		Age on Last Birthday	Calories	
	Male	Female		Male	Female
6	53.0	50.6	19	42.3	36.7
7	52.4	49.1	20–21	41.4	36.2
8	51.8	47.0	22–23	40.8	36.2
9	50.5	45.9	24–27	40.2	35.7
10	48.5	45.9	28–29	39.8	35.7
11	47.2	45.3	30–34	39.4	35.7
12	46.8	44.3	35–39	38.7	35.7
13	46.4	41.4	40–44	37.4	34.9
14	46.4	41.4	45–49	37.4	34.9
15	46.4	40.1	50–54	36.7	34.0
16	45.7	38.8	55–59	36.1	33.2
17	44.8	37.8	60–64	35.5	32.6
18	43.2	36.7	65–69	34.8	32.3

It is interesting that there seems to be some evidence that the level of basal metabolism is also to some extent affected by the nature of the occupation. Takahira and associates [287] showed that the basal metabolism of sedentary workers was as much as 7 per cent below that of individuals engaged in more active occupations. Thus, whereas the merchant had an average basal metabolism of 36.5 calories per square meter per hour, the policeman had a basal level of 38.9 calories per square meter per hour. The differences were more evident in the female subjects, teachers giving an average caloric production of 31.5 and laborers 35.1 calories per square meter per hour.

However, from a practical standpoint, it should be realized that basal metabolism is determined not only by age, by sex and by occupation but the actual level is a function of the amount of active metabolizing tissue that the individual possesses. In other words, it is dependent on the body weight of the individual. Thus, the very large individual is constantly producing calories at a higher level than the small individual. Although it has been found that the actual basal metabolism is proportional to body weight, the value which gives the most consistent comparison with persons of widely varying size and with different species of animals is based on surface area. Surface area is a function of two-thirds of the body weight according to the formula of Meeh, or 0.425 power of the body weight according to the formula of DuBois and DuBois.[96]

Vitamin Requirements

Although it is not known how many of the vitamins are required by man, the Committee on Food and Nutrition of the National Research Council

considered that there was sufficient evidence to assign minimum daily requirements for a number of the better known vitamins.[234] It was recognized at the outset that there are in general two different classes of substances, *i.e.*, those which had to do with the maintenance of the structural tissues of the body and secondly, those which are concerned with the oxidative processes of the body. In the first group are vitamin A (concerned with the maintenance of the epidermis and the various epidermal appendages), vitamin C (necessary for the production of cartilage and of all collagenous tissue) and vitamin D (required for the maintenance of bone structure because of its effect on calcium and phosphorus metabolism). In the second category are those members of the B complex, such as thiamine, which are concerned with certain intermediary changes in carbohydrate metabolism. Equally as important as thiamine is riboflavin which is the prosthetic group of the "yellow enzyme" and which is also a requirement for carbohydrate breakdown. Another important member of the B complex is niacin which is a component of the so-called coenzymes I and II. These are also important in catalyzing certain reactions involving intermediary changes in carbohydrate metabolism. Pyridoxine seems to be to some extent related to intermediary protein metabolism, but insufficient is known about this vitamin to give a postulation as to the amount required. There is some evidence also that man requires pantothenic acid, choline, biotin, inositol, *p*-aminobenzoic acid, and possibly also folic acid, but there is no indication of the quantity required. The latest recommendation for the requirement of the various vitamins where the Food and Nutrition Board of the National Research Council believed adequate information was available is given in Table 3–5.

There are several variations in vitamin requirements which appear on inspection of Table 3–5. In the first place, the requirement for those vitamins concerned with structural tissues such as vitamin A and D and ascorbic acid is not influenced by the amount of activity, although the need is increased in pregnancy and lactation. On the other hand, with those vitamins of the B complex which are parts of different coenzyme systems, their need is directly proportional to the amount of activity in which the person engages. Another fact which is evident from this table is that the highest requirement during life occurs from the 16th to the 20th year in the case of the boys, and from the 13th to 15th year in the case of the girls, which parallels the maximum caloric requirement. There is also an indication that the male in general requires a greater amount of the various vitamins than the female, although this is not the case with vitamin A or with vitamin D. Another surprising fact is that the requirements for the various vitamins are increased during lactation to a higher value than occurs at any other time during the life cycle of the female. This presumably is because of the large excretion of the various vitamins in the milk and the dependence of such excretion on a sufficient intake of the diet.

Table 3-5. The Recommended Daily Allowances for Various Vitamins [234]
(Committee on Food and Nutrition, National Research Council)

Age or Activity	Vitamin A (I. U.)		Ascorbic Acid (mg)		Thiamine (mg)		Ribo-flavin (mg)		Niacin (mg)	
	M	F	M	F	M	F	M	F	M	F
Children										
Under 1 year	1500	1500	30	30	0.4	0.4	0.6	0.6	4	4
1–3	2000	2000	35	35	0.6	0.6	0.9	0.9	6	6
4–6	2500	2500	50	50	0.8	0.8	1.2	1.2	8	8
7–9	3500	3500	60	60	1.0	1.0	1.5	1.5	10	10
10–12	4500	4500	75	75	1.2	1.2	1.8	1.8	12	12
13–15	5000	5000	90	80	1.6	1.4	2.4	2.0	16	14
16–20	6000	5000	100	80	2.0	1.2	3.0	1.8	20	12
Adult										
Sedentary	5000	5000	75	70	1.5	1.2	2.2	1.8	15	12
Moderately active	5000	5000	75	70	1.8	1.5	2.7	2.2	18	15
Very active	5000	5000	75	70	2.3	1.8	3.3	2.7	23	18
Pregnancy (latter half)	——	6000	—	100	—	1.8	—	2.5	—	18
Lactation	——	8000	—	150	—	2.3	—	3.0	—	23

Vitamin D is recommended for children under one year and for women during pregnancy and lactation in amounts of 400–800 I.U. daily. It is also stated that it is undoubtedly necessary for older children and adults. When not available from sunshine, it should be provided probably up to the minimum amounts recommended for infants (400–800 I. U. daily).

Mineral Requirements

The requirements for an adequate amount of mineral in the diet both quantitatively and qualitatively have been recognized for many years. Since the symptoms of the deficiency are not often clear-cut and are much less spectacular than those occasioned by lack of the vitamins in the diet, there is little positive information on the absolute quantities of the various minerals which are required. Not only are the minerals such as calcium, potassium, sodium, magnesium and iron needed along with chloride, phosphate and sulfate, but there is a series of minerals which are necessary in extremely small amounts. Hence, the latter are referred to as the "trace elements." Some of the deficiency symptoms occasioned by the absence of the trace elements are more readily recognizable than those brought about by deficiencies in the better known mineral constituents. In the group of trace elements, probably the most important is copper but zinc, iodine, cobalt, manganese and many others also play an important role. One of the things which complicates the picture as far as the requirement for inorganic constituents is concerned is the fact that some of these are supplied by other foodstuffs than the ash. Thus, the protein ordinarily contains an adequate

amount of sulfur. Proteins such as casein, ovovitellin and nucleoproteins also supply definite quantities of bound phosphate. Another source of the phosphate is in those phospholipids such as lecithin and cephalin which are present in our every day foods. Iron and copper are also obtained as components of protein molecules although it must be admitted that the iron present in the hemoglobin molecules is not as well utilized as inorganic iron salts. The recommended daily allowance for calcium and iron as suggested by the Committee on Food and Nutrition of the National Research Council [234] is given in Table 3–6.

Table 3–6. The Recommended Daily Allowances for Calcium and Iron [234]
(Committee on Food and Nutrition, National Research Council)

Age or Activity	*Calcium (gm)*		*Iron (mg)*
	Male	*Female*	*Male and Female*
Children			
Under 1 year	1.0	1.0	6
1–3	1.0	1.0	7
4–6	1.0	1.0	8
7–9	1.0	1.0	10
10–12	1.2	1.2	12
13–15	1.4	1.3	15
16–20	1.4	1.0	15
Adults (All degrees of activity)	0.8	0.8	12
Pregnancy		1.5	15
Lactation		2.0	15

Iodine requirements are 2 to 4 micrograms daily per kilogram of body weight. This is readily met by the use of iodized salt. The use of iodine is of especial importance in adolescence and pregnancy.

Copper is needed by infants and children to the extent of 0.05 mg per kilogram body weight. Adults need 1 to 2 mg per day. It is necessary in about one-tenth the amount that iron is needed.

The requirements for calcium and iron are similar to those of vitamins A and D as well as ascorbic acid since all are concerned with structural development. The increased need for calcium during pregnancy and particularly during lactation reflects the increased requirements in the first place for development of the bony structure of the fetus and in the second place it is an indication of the large calcium loss which normally occurs in milk. The need for iron, however, is little changed in pregnancy and in lactation.

Another factor which complicates an exact statement of requirement is that the mineral requirements are bound up with those of the vitamins and also particularly with the activity of certain of the glands of internal secretion. The metabolism of calcium is not only related to the amount of vita-

min D available, but it is also markedly influenced by the hypo- or hyperactivity of the parathyroid glands. The metabolism of iodine, on the other hand, is intimately bound up with that of the thyroid gland. The most distressing symptoms which follow the removal of the adrenal cortex, namely the dehydration symptoms, result from the absence of the adrenal cortical hormone which results in a loss of control of the excretion of the sodium ion. By an increased administration of sodium chloride under such conditions, many of the immediate distressing conditions associated with adrenalectomy can be avoided.

Another fact which is of importance in determining the amount of a special mineral which is necessary, is the form in which it is fed. Insoluble compounds, which will not dissolve in the hydrochloric acid of the stomach cannot be absorbed. Inorganic iron is better utilized than iron which is combined with heme in the hemoglobin molecules. Not only must one consider the form in which the substance is at the time it is ingested but the other foods eaten along with the mineral-containing foods are also of importance. The concomitant ingestion of oxalate-containing foods along with calcium prevents the absorption of calcium. Moreover, on high fat diets there may be a tendency for insoluble calcium and magnesium stearates to be excreted in the stool. On the other hand certain amino acids increase the solubility of the calcium and magnesium salts.[169, 170] This fact also is in agreement with the report of McCance, Widdowson, and Lehmann,[188] that protein facilitates the absorption of calcium and magnesium. It also probably explains why vitamin D may have a favorable action on increasing the retention of calcium on a carbohydrate diet but not on a protein diet.[56]

Protein Requirements

There is a wide divergence of opinion as to the optimum standards of protein in the diet. On the one hand, we have the generous estimates of Carl Voit made some years ago in Germany of 118 grams daily [303] as well as those of Atwater in this country [22] where 125 grams was the suggested level per day. However, both of these values have been arrived at as a result of dietary surveys of actual protein intakes of representative groups of the two countries. Although the rat when given an opportunity will actually pick out the various foods in the proportion which will give satisfactory growth,[237] there is no proof that man will choose a perfect diet which contains the optimum proportions and amounts of the various foodstuffs even if he has an entirely free choice in the matter. There is therefore considerable doubt as to the significance of dietary surveys in deciding on the correct protein standards.

On the other hand, the aforementioned values are in sharp contrast with those of Chittenden who worked at Yale University.[73] This investigator advocated a protein intake of 45 grams per day and based his estimate on

the results obtained over a period of nine months on himself and on several colleagues who were able to maintain nitrogen equilibrium on this low intake of protein. Mendel, who participated in this study, was able to maintain nitrogen equilibrium over a period of about seven and one-half months on a nitrogen content of 6.53 grams per day which is equivalent to a metabolism of 40.8 grams of protein or about one-third of the Voit standard. Another one of the subjects, Underhill, had an average nitrogen excretion over a six month period of 7.81 grams daily while during the last two months it amounted only to 6.68 grams per day. During the whole period, Underhill kept a constant body weight which was considered a fairly good indication that a condition of nitrogen equilibrium obtained. Chittenden in his own case was able to maintain nitrogen equilibrium with an even smaller intake of protein. At the start of the experiment on October 13th, Chittenden weighed 57.5 kilograms which was exactly his weight on the termination of the test on June 28th of the following year. His average daily excretion of nitrogen for the entire period of nearly nine months was only 5.70 grams whereas during the last period of 77 days this investigator maintained a nitrogen balance of 5.40 grams per day. In the conclusions of his interesting book entitled "Physiological Economy in Nutrition," [73] this author states:

"Confining our conclusions to general statements, it may be said that our results, obtained with a great diversity of subjects, justified the conviction that the minimal proteid requirement of the healthy man under ordinary conditions of life is far below the generally accepted dietary standards, and far below the amount called for by acquired taste of the generality of mankind. Expressed in different language, the amount of proteid or albuminous food needed daily for the actual physiological wants of the body is not more than one-half that ordinarily consumed by the average man. Body weight (when once adjusted to the new level), health, strength, mental and physical vigor, and endurance can be maintained with at least one-half of the proteid food ordinarily consumed; a kind of physiological economy which, if once entered upon intelligently, entails no hardship, but brings with it an actual betterment of the physical condition of the body. It holds out the promise of greater physical strength, increased endurance, greater freedom from fatigue, and a condition of well being that is full of suggestion for the benefit of health."

In a later publication of Chittenden [74] this author indicates that his data "are seemingly harmonious in indicating that the physiological needs of the body are fully met by a metabolism of protein matter equal to an exchange of 0.10 to 0.12 gram of nitrogen per kg of body weight per day, provided a sufficient amount of non-nitrogenous food is taken to meet the energy requirements of the body." This level of nitrogen would correspond with 44 to 53 grams of protein a day for a man of average weight, that is 70 kilograms or 154 pounds. The Swedish investigator, Sivén,[269] also

indicates that nitrogen equilibrium can be established at a level lower than that ordinarily found in the urine during the periods of fasting. The latter investigator was able to establish nitrogen equilibrium after three days when the food nitrogen contained 6.6 grams, although his experiments were of a short duration compared with those of Chittenden.

Although the experiments of Chittenden were sufficiently prolonged to indicate that it is possible for the human being to live over long periods of time on a relatively low protein intake provided there is a sufficient source of calories from non-protein foods, there is no proof that such diets are optimal. It is true that Chittenden lived to a ripe old age and that during the period of nine months when he was on the very low protein diet he reported his health was exceptionally good. He indicated that rheumatism of the knee joint disappeared and such minor ailments as "sick headaches" and "bilious attacks" no longer occurred periodically as they had before. He states further: "There was a greater appreciation of such food as was eaten: a keener appetite and more of an acute taste seemed to be developed, with a more thorough liking for simple foods." Chittenden felt that the ingestion of the large quantity of protein ordinarily taken in the diet is a form of self indulgence.

In the recommended daily allowances as published by the Committee on Food and Nutrition of the National Research Council [234] considerably higher levels for protein intake are suggested. These are summarized in Table 3–7.

Table 3–7. The Recommended Daily Protein Allowances for Children and Adults [234] (Committee on Foods and Nutrition, National Research Council)

Protein Allowances (gm)					
Children			*Adults*		
Age (years)	*Sex*		*Activity*	*Sex*	
	Boys	*Girls*		*Men*	*Women*
Under 1	3–4/kg	3–4/kg	Sedentary	70	60
1–3	40	40	Active	70	60
4–6	50	50	Very active	70	60
7–9	60	60	Pregnancy		85
10–12	70	70	Lactation		100
13–15	85	80			
16–20	100	75			

Age and sex are primary considerations in establishing the level of recommended protein intake. There is a graded increase in suggested requirements for the boys and girls which are identical up to the 13- to

15-year age group. The requirement reaches a maximum value of 100 grams per day for the rapidly growing boys in the 16 to 20 year group but drops back to 70 grams for the girls in the same age group. In the case of adults, the values of 70 grams for men and 60 grams for women indicate that there is a continued sex differentiation in the requirement for protein. It is interesting that no difference in protein requirements are postulated for the man engaged in active occupations as contrasted with the individual in sedentary work. This is in line with the discovery in 1866 by Pettenkofer and Voit [228] that work was done not at the expense of protein but rather at the expense of the energy obtained from the breakdown of carbohydrates and fats. Although there may be a slightly increased nitrogen excretion following very severe exercise due to the wear and tear of the tissues, the recommendations for the protein requirement by the National Research Council are believed to be sufficiently liberal to take care of any such contingencies. In fact, in a recent study of the protein requirement of 26 adults ranging in age from 19 to 50 years, it was found that a mixed diet containing both animal and vegetable proteins sufficed for nitrogen equilibrium when the protein consumption was under 35 grams daily.[140] The authors conclude "that the National Research Council's daily allowance of 70 grams of protein for an adult weighing 70 kg is most generous and could, if necessary, be reduced to 50 grams and still provide approximately 30 per cent margin above requirement."

Considerations in Establishing Nitrogen Equilibrium Level. The factors concerned with the level at which nitrogen equilibrium can be established are as follows:

Effect of the Biological Value of the Protein. It is likely that the more similar the makeup of the dietary protein is to the average composition of the essential amino acids in body tissues, the lower the level at which nitrogen equilibrium can be established. Proteins to be of high biological value must contain not only all of the essential amino acids but they must be present in the proportion which they are needed for the building up of body tissue.

The brilliant work of Rose and his collaborators on amino acids is summarized in a review by Rose.[241] Of the ten essential amino acids, at least four of them can be utilized by the rat for growth when given in the form of the unnatural isomer. These are tryptophane,[45, 47, 302] methionine,[148] phenylalanine [245] and histidine,[80] although d^+ histidine appears to be less efficiently utilized than the natural l^- isomer.* In distinction to the interchangeability of d^+ and l^- tryptophane for the growth of rats, it has been reported that mice exhibit poor growth [7] or fail to grow [159] when the

* In accordance with the terminology suggested by Dunn,[97] all naturally occurring amino acids are considered to be members of the *l* series while the unnatural ones are those of the *d* series. Their actual effect on the plane of polarized light is indicated by + or − which follows the letter designation of the series.

unnatural isomer is used. Mice are able to utilize both d^+ and l^- histidine equally effectively for growth [289] although, using dose as a criterion, d^+ histidine was found to be poorly utilized in the guinea pig,[101] rabbit [3] and dog.[5] Occasionally the d form of an amino acid may actually be toxic as has been demonstrated by Artom and Fishman [21] for d^+ serine.

There are less satisfactory methods for comparing the effectiveness of the various amino acids in man. The best procedure for the human subject is the ability of the protein or amino acid mixture to maintain nitrogen equilibrium. It has been known for a number of years that it is possible to obtain nitrogen equilibrium and even growth when amino acids rather than native proteins are administered orally. Abderhalden and Rona [1] were the first to demonstrate that nitrogen equilibrium could be brought about in the dog when a pancreatic digest of casein was administered over a period of 16 days in an amount containing two grams of nitrogen. It is interesting also that they were unable to obtain a positive nitrogen balance when casein hydrolyzed by acid was administered. Henriques [142] later reported that protein digests would afford a satisfactory nitrogen equilibrium as long as they gave a test for tryptophane, but in the absence of this one amino acid, nitrogen equilibrium could not be attained. More recently, it has been affirmed by Elman [102] that when hydrochloric acid digests of casein are deficient in tryptophane, it is impossible to obtain a nitrogen balance without fortifying the mixture with tryptophane. On the other hand, a prolonged positive nitrogen balance can be demonstrated when an enzymatic casein digest is fed to weanling rats. These animals grew as satisfactorily and attained maturity as quickly when fed on such a fresh pancreatic digest of casein as on the unhydrolyzed casein.[214] The casein digest was nutritionally equal to or in several cases superior to that of unhydrolyzed protein when growth rates were followed at several levels between 5 and 20 per cent of the diet. Moreover, the effectiveness for regeneration of serum protein in hypoproteinemic dogs was similar with the unhydrolyzed and hydrolyzed casein. By nitrogen balance studies, Rose and his associates [242] have been able to establish nitrogen equilibrium in man when 95 per cent of the nitrogen was taken in the form of the ten amino acids previously proved as essential for the rat. Surprisingly enough it was later found that histidine was not required for the maintenance of nitrogen balance in the adult [45, 46, 242, 243] and arginine also apparently could be synthesized rapidly enough so that it was not required in the human subject. Although methionine was necessary for obtaining nitrogen equilibrium, cystine was not required when sufficient methionine was present.[13] It is now generally considered that there are only eight essential amino acids in the case of man which are the same as required for growth in the rat with the exception of histidine and arginine.

Seuffert and Marks [260] were unable to establish nitrogen equilibrium in a

dog where the diet contained only asparagin, leucine, tyrosine, tryptophane, glutamic acid and alanine as the nitrogen components.

Proteins in which any one of these indispensable amino acids is deficient will have a low biological value. If the particular amino acid is completely absent, as in the case of tryptophane in the protein, gelatin, then the biological value of the protein is zero. A number of cereal proteins are low in lysine and in some cases also in tryptophane. These are gliadin (obtained from wheat and corn), hordein (obtained from barley), and zein (obtained from corn). Rats will not grow when gliadin is given as the sole protein while death actually ensues when zein lacking in tryptophane is the only protein in the diet. It was believed by Mendel [202] that tryptophane was a requirement for maintenance while lysine was necessary for growth.

However, it is possible for such proteins of low biological value to contribute an important part to the nutritive value of the diet. On hydrolysis, the amino acids liberated from such deficient proteins may help to make the total amino acid mixtures of the several proteins ingested a very satisfactory one for maintenance or for promoting growth. Such facts were demonstrated in the beautiful pioneer work of Mendel.[201] From a practical standpoint in human nutrition, these effects of deficient proteins are not of great importance. Such proteins are seldom found alone, but they usually occur in a mixture with several other proteins which more or less complement each other. Therefore, when the sources of proteins in the diet are fairly well diversified, there is little danger of the protein being inadequate from the qualitative standpoint.

Apparently the human organism cannot use the unnatural forms of the amino acids very effectively. Thus, when d^+ tryptophane was ingested, an aberrant intermediate compound was excreted in the urine. This substance was not present when the natural, l^-, form was taken or when ordinary protein meals were consumed.[10] It was recently shown that the human subject can utilize acetyl d^+ tryptophane as readily as l^- tryptophane when administered orally.[15] Only the natural l^- phenylalanine [7] and l^- tyrosine [17a] can be metabolized by man while the d^+ isomers largely escape oxidation.

Albanese could detect no differences in the utilization of the methionine enantiomorphs by the human subject [7] and nitrogen equilibrium could be established in the complete absence of cystine. In a later study, it was reported that d^+ cystine as well as l^- cystine can be utilized by man.[8] Likewise, the d^- form as well as the natural l^+ form of arginine was found to be utilized by the human subject; [17] in addition, it was shown that both isomers were acted on by the arginase in human liver although rat arginase was ineffective on d^- arginine.

On the other hand, Albanese *et al.*[14] have found that d^+ histidine, the unnatural form, is poorly utilized by man. From 82 to 97 per cent of the

d^+ histidine, ingested as the racemate, was excreted in the urine and identified as the *d*-form. Murlin *et al.*[217] as a result of extensive experiments on man have come to the conclusion that the use of *dl*-amino acids, even those of the essential group of amino acids, is nutritionally uneconomical.

The normal human subject excretes about 3 mg of tryptophane per kg per day.[11] As small an amount as 6–9 mg of this amino acid per kg daily was found to be sufficient to establish nitrogen equilibrium on a tryptophane-deficient diet.[243] The normal urinary excretion of arginine by male adults has been found to vary between 50 to 150 mg daily.[12]

There are a number of ways in which the nutritive value of a protein may be determined. In addition to the classical methods which involve the assessment of their effect on growth, there are several other more refined technics. One of these methods is that of Osborne, Mendel and Ferry [224] in which the nutritive value is considered to be a function of the ratio, x, of the gain in weight to the protein intake. This method is somewhat unsatisfactory, according to Boas Fixsen,[53] because protein before being used for growth must first be used for maintenance. Then this value x (the ratio of gain of weight to amount of protein consumed) will increase until an optimum is reached and when additional protein is consumed, this foodstuff will be used for energy and the value x will decline. McCollum and Shukers [189] modified this general procedure by determining the actual amount of protein which is retained when a given amount of protein is administered, this foodstuff being fed at an arbitrary level.

Another procedure which has been employed for the determination of biological activity of protein is that of McCollum, Simmonds and Parsons[190] where the relative nutritive value of the protein is compared by testing its effect on growth, fertility, lactation, infant mortality, and longevity. The different proteins are fed in comparable basal diets in equal proportions. Boas Fixsen [53] has criticized this method of procedure by suggesting that some proteins give better results for one function and others for another function. She also states that it is probably unjustifiable to assume that all dietary essentials are as yet recognized and that the absence of these from diets over a long period of time may be the cause of the unsatisfactory response. In addition she emphasized that since no records are kept of the food intake in this particular method, it is possible that the differences indicated for different proteins are related to variations in the amount of protein ingested.

Another method which has been quite widely employed is that of Mitchell and Beadles [209] which involves the paired feeding method. When comparable amounts of the proteins under investigation are ingested, their nutritive value is assessed by the amount of the resulting growth. Boas Fixsen [53] has questioned this method by stating that when an animal is not allowed to satisfy its appetite entirely (as occurs with a high quality protein), it is questionable whether such results are strictly comparable

with those of animals which eat all they want (as occurs with the animals receiving the more deficient proteins).

The classical procedure which has served most widely to evaluate the biological values of the different proteins is the technic first outlined by Mitchell.[205, 206] This method is based on the ability of the protein under consideration to replace the body protein which is being metabolized. The greater the amount of the digested protein which is retained, the higher its biological value. The retained protein is considered to be the difference between the protein absorbed and that lost as urinary nitrogen. The latter value is corrected for metabolic nitrogen which is the amount of nitrogen excreted on a protein-free diet.

Mitchell and Block [210] have recently proposed a new procedure, the calculation of which is based on the chemical composition of the respective proteins. (For details, see Chapter 2 by Mitchell.) However, there are certain imperfections in this procedure which may in part be traceable to inaccuracies in the data. In the case of the animal tissues such as muscle, liver, kidney, and heart, the proteins have a higher ranking on the chemical scale than on the biological one. Since the proteins from these tissues possess considerable amounts of non-protein nitrogenous substances which may have little value in relation to the animal functions in the tissues and since the biological value is determined on the basis of the total nitrogen present, it seems probable that the value for the true protein may be somewhat higher than is indicated by the usual method for determination of biological value. On the other hand, the values obtained on wheat germ and corn germ protein are considerably lower on the basis of chemical composition than were found by their biological performance. The greatest discrepancy was detected with certain cereal proteins which uniformly possess a considerably higher value when estimated by the chemical methods than was noted by the biological assay technic. It is believed in a number of cases that heat has a destructive effect on the proteins as far as growth is concerned which is not reflected in the chemical composition.

It is uncertain how much importance should be attached to strepogenin in relation to the biological value of protein. This substance, so named by Sprince and Woolley [282] for a growth factor necessary for hemolytic streptococci, has been shown by the latter author [315] to be important in the growth of mice. Somewhat slower growth obtained in these animals on a diet devoid of strepogenin than on one containing this factor although ultimately the same eventual body weights were attained in both groups. Since the active compound is apparently a peptide, the activity would be absent in amino acid mixtures or in acid hydrolysates but would probably be present in enzyme digests. Crystalline trypsinogen and purified casein are excellent sources of this factor while some proteins are largely devoid of it.[282] These data suggest that the method of evaluation of biological value of protein merely on the basis of amino acid composition may need

revision. Woolley [316a] has recently shown that synthetic seryl- glycyl- glutamic acid possesses considerable strepogenin activity although it had a lower order of effectiveness than the product prepared from natural sources. Scott *et al.* [255a] believe that Factor S and strepogenin are identical.

The heating of proteins may increase their biological value, as in the case of soybean protein, by destroying a proteolytic-inhibiting substance in the raw bean which slows down the rate of digestion.[134] On the other hand the application of heat is known to exert a depressing effect on the nutritive value of corn and oat protein. In the latter case, the biological value is greatly lowered by the gun explosion procedure but not by the usual methods of domestic cooking.[216] Stewart, Hensley and Peters [284] have also noted that processing exerts a deleterious effect on proteins from oats or mixed cereals.

Mitchell and Block,[210] using a method suggested by Murlin *et al.*,[219] have reported the biological value of processed cereal proteins (oats-corn-rye mixture) as only 86.6 per cent of that of the unprocessed material. These authors suggest the following possible causes for the lowering in nutritive value where actual destruction of the amino acids does not occur: (1) A decreased digestibility of the proteins, (2) a decreased digestibility which involves the disproportionate loss in the feces of certain amino acids as has been reported for arachin,[151] or (3) the application of heat to a protein may bring about certain combinations between terminal groupings which become resistant to proteolytic action and result in the formation of atypical peptides. These may be absorbed as such and excreted in the urine without further breakdown as has been suggested by two groups of workers.[181, 235]

Relative Importance of Carbohydrate and Fat. Carbohydrate has long been known to exert an important "protein-sparing" action. Without carbohydrate or fat in the diet, Voit [305] found that nitrogen equilibrium could be reached in dogs only when five times the quantity of protein destroyed in the fasting animal was fed. When fat was given with the protein, the amount of protein required to establish equilibrium was considerably lowered, being 1.5 to 2 times the fasting value.[304]

However, when carbohydrate is the predominant food, nitrogen equilibrium can be established at a level considerably below that of fasting. As noted earlier, Sivén [269] was able to obtain nitrogen equilibrium at a level of 6.26 grams daily, while the value obtained by Chittenden [73] over a two and one-half month period was 5.40 grams daily. Lusk,[186] in experiments which he carried out on himself while he was working in the laboratory of Carl Voit, found that the sudden withdrawal of carbohydrate from the diet and its replacement by fat resulted in an increase in protein breakdown as reflected by the change of urinary nitrogen from an average of 11.44 grams to 17.18 grams per day.

The sparing effect of carbohydrate on protein metabolism is also beau-

tifully demonstrated by an experiment of Kayser cited by Sherman.[262] The subject was on a diet of approximately 2600 calories and the total nitrogen of the food was kept at approximately 21 grams daily. During the first four days of the period when carbohydrate calories were replaced by fat, a negative nitrogen balance occurred which reached the maximum value of 4.98 grams on the 3rd day of the experiment. Within two days after replacing the fat by an isocaloric carbohydrate diet the positive nitrogen balance had again been established. The experiments of Cathcart [71] carried out on himself and of Landergren [167] are similarly conclusive.

The most pronounced action of carbohydrate in sparing protein occurs when this foodstuff is given to an individual who is on protein-free diet. Under such conditions the protein metabolism is reduced to the so-called "wear and tear" quota (also called the "nitrogen minimum"). The minimum values for total nitrogen for 24 hours under such conditions which have been found are 1.75 grams in the experiments of Deuel *et al.*[95] and 1.58 grams in the report of Smith [271] which are equivalent to 24.1 and 24.2 mg per kg respectively. The values obtained on man are lower on the kilogram basis than any result which has been reported on pigs, cattle, dogs, or rabbits, with the exception of one value on sheep of the large number cited by Mitchell and Hamilton [212] where an equally low level was found.[251] In cases of specific nitrogen hunger, carbohydrate alone would appear to exert a sparing action on the protein metabolism. In the experiments of Voit on a dog [305] no change in the level of fasting nitrogen was observed by giving 100, 200, or 300 grams of fat per day. According to Voit, the ingested fat was merely oxidized in place of the animal's own body fat. Bartmann [36] found that when fat was fed, up to 150 per cent of caloric requirement, the protein metabolism was lowered to a maximum of only 7 per cent. In the experiment carried on by Landergren [167] which has been referred to earlier, after the urinary nitrogen had been lowered by the ingestion of a protein-low carbohydrate diet from a level of 12.76 grams per day to 3.76 grams, the carbohydrate was replaced by fat. The urinary nitrogen promptly rose within 3 days to a level of 9.64 grams. It is therefore evident that carbohydrate and not fat has the ability to spare protein in conditions of nitrogen starvation. These experiments also demonstrate that carbohydrate possesses a sparing action at several different levels of protein metabolism.

The reasons why carbohydrate should exert this effect are not entirely clear. Kocher [158] believes that the glucose offers intermediate compounds which can be transformed to amino acids in the presence of ammonia and thus serve as some of the source of protein building stones. Cathcart, on the other hand,[71] has suggested that the ketolytic effect of carbohydrate and the protein-sparing action are intimately related, since he believes that ketosis is associated with an increase in protein catabolism. A more

logical explanation is suggested by Landergren.[167] This investigator is of the opinion that in the balance of carbohydrate in the diet, increased amounts of protein are broken down in order to serve as a source of blood sugar. It is well known that fat cannot provide a source of carbohydrate in the animal body.[94] This hypothesis of Landergren seems to be supported by the evidence that the administration of carbohydrate to a phlorhizinized dog results in a marked diminution in the level of excreted nitrogen.[93, 240] The results of Nash [221] in which it is shown that the injection of insulin into phlorhizinized dogs also results in a decreased protein metabolism would seem to offer further circumstantial evidence for the action of carbohydrate on protein metabolism. In any event, although we cannot as yet give an answer to the reason for the sparing action of carbohydrate, the results are certainly sufficiently conclusive to leave no doubt that this foodstuff possesses such an activity.

Effect of Caloric Level. Although fat, when fed to the extent of 150 per cent of the caloric requirement, has practically no sparing effect on protein metabolism,[36] carbohydrate is able to exert its sparing action when it contributes considerably less than 100 per cent of the total caloric intake. In the experiment of Chittenden [73] where the nitrogen metabolism was maintained at a level under 6 grams over a period of 9 months, the average caloric intake was only somewhere in the neighborhood of 1600 calories. Voit and Korkunoff [304] were able to establish nitrogen equilibrium in the dog while it was losing 28 grams of carbon daily, so it is possible to prevent protein loss while there is at the same time a loss of fat or carbohydrate. In the experiments of Deuel *et al.*[95] the low levels of nitrogen excretion were obtained on a diet furnishing only 1800 calories. Over the 71 days of the test, the subject lost about 11 kilograms of body weight while at the same time minimum nitrogen levels were being found in the urine. On the day on which the minimum nitrogen figure was reached, there was an intake of only 1525 calories of which carbohydrates furnished 1156, fat 366 and protein 3. However, in some recent experiments of the author and others [150] where a protein-low diet consisting of approximately 600 calories in the form of carbohydrate and about 5 grams of protein were taken daily, the values for the urinary nitrogen were not reduced to the same extent as they normally are over a 10-day period on a protein-free diet in which ample carbohydrates are available. Just where the critical caloric level lies between 1500 and 600 calories in order that protein metabolism be spared to a minimum is a moot question. In subjects who were fed on a diet containing approximately 12 grams of protein daily with a total caloric value of 2000 largely in the form of carbohydrates, it was found over a 10-day period that urinary nitrogen was in most cases reduced to values under 3 grams daily.[150] It is therefore evident that in order to obtain a sparing action on the protein metabolism either when protein is given simultaneously or in the case of practically protein-free

diets, caloric levels do not necessarily need to equal values as high as 2500 calories per day, but the sparing action may be noted when the caloric intake is only 1500 calories daily. However, it would appear that when the caloric level is reduced to as low as 600 calories daily, a maximum sparing action on the protein does not occur even when the ingested calories are largely in the form of carbohydrates.

Intravenous vs. *Oral Administration.* The employment of protein digests for intravenous injection or other forms of parenteral therapy have become quite common of late. It is impossible to administer native proteins repeatedly by pathways other than by mouth without causing a severe anaphylactic reaction and without a large proportion of the material being lost in the urine. However, if hydrolyzed protein or purified amino acids are employed, a satisfactory utilization of the material can be obtained when such solutions are given by vein. It is logical to expect the amino acid mixtures to serve as satisfactorily as sources of protein when introduced by the parenteral route as by mouth. Inasmuch as it is believed that the normal course of protein utilization involves a primary breakdown of the protein in the gastrointestinal tract followed by an absorption of the mixed amino acids so formed and a distribution of this amino acid mixture by the blood to the tissues for building new proteins and for conversion to other compounds, there is no reason why the direct introduction of the amino acid into the blood stream should not give identical results as when oral administration is employed. Shohl, Butler, Blackfan and MacLachlan [266] were able to obtain nitrogen equilibrium in infants following the administration of a casein hydrolysate obtained by enzyme action when this material was administered orally or following the parenteral administration provided that glucose and salt solutions were given simultaneously. In later work (Shohl and Blackfan [265]), it was also found that nitrogen equilibrium can be established in normal infants when a mixture of the crystalline amino acids is given intravenously. These authors showed that when 6.05 grams of nitrogen per kilogram per day was administered, an adequate retention of the amino acids took place. Madden and coworkers [195] have also been able to demonstrate a positive nitrogen balance in dogs which had hypoproteinemia when these were injected with mixtures of the ten essential amino acids.

The difficulties in the intravenous technique have made it impossible to obtain studies on nitrogen balance over long periods of time. However, in a recent report of Kade, Houston, Krauel and Sahyun,[153] it has been demonstrated that dogs can be kept in nitrogen equilibrium over a period of approximately a month when no nitrogen is given except in the form of a casein digest fortified with tryptophane. During the first three weeks the amino acid digest was administered by mouth along with sucrose, dextrine, and various other supplements to the diet, and it was demonstrated that a positive result obtained throughout when not

only the urinary nitrogen but also the fecal nitrogen was taken into consideration. Following this period of three weeks was a period of one week during which the amino acid mixture was given intravenously and the other components were administered by mouth. This was followed by another period of 7 days in which the amino acid digest and other components were again administered by mouth. During the whole period a positive nitrogen balance obtained which was equally satisfactory during the interval of intravenous administration as during the fore and after periods when the amino acids were administered orally. These results were not open to the criticism that additional protein was given by mouth simultaneously with the intravenous administration and they were also sufficiently long in duration to indicate that the nitrogen equilibrium was a real one. It is well known that after periods of fairly high nitrogen intake, a positive balance may occur for 4 or 5 days thereafter if the protein intake is suddenly lowered quite irrespective of the nature of the protein itself. Therefore, periods of longer than 1 or 2 days are necessary to establish the efficiency of a substance for maintaining nitrogen equilibrium.

It should be emphasized, however, that nitrogen equilibrium can be obtained only if the protein digest contains all of the essential amino acids. The results of Cannon and collaborators [41a, 65, 112a] with hypoproteinemic rats indicate that when tryptophane or leucine is absent from such mixtures they are entirely unsatisfactory for establishing nitrogen equilibrium. Digests which have been prepared by acid hydrolysis are inadequate because tryptophane is destroyed; their potency can be restored when they are fortified with tryptophane. When protein is hydrolyzed in an alkaline medium, as with sodium, potassium or barium hydroxide, tryptophane is not destroyed although cystine is lost and a certain amount of ammonia is set free. However, a considerable degree of racemization of the amino acid occurs which results in a decreased utilization of the hydrolysate. Sahyun [248] has suggested that hydrolysates be employed which are mixtures of those prepared by acid and alkaline hydrolysis; these would contain an adequate amount of tryptophane and be satisfactory for intravenous therapy.

Another procedure for the preparation of the protein hydrolysates involves the use of enzymes; in this case also a mixture of enzymes is necessary if anything approaching a complete hydrolysis is to be obtained.[63] This process has the disadvantage of being very slow and requiring special equipment for large scale production. It is impossible to remove the enzymes from the final hydrolysis. There is, moreover, some danger of immunological activities if the proteins are not broken down at least to the stage of polypeptides. It is not known how effective such mixtures can be as a source of tissue protein if the breakdown to the amino acids is not complete. In any case, the most satisfactory protein digest for intravenous administration would seem to be those which are digests of

several proteins so chosen that the resulting amino acids approach an optimum mixture. Cox and Mueller [81] have recently compared the efficiency of nitrogen retention when enzymatic hydrolysates and mixtures of amino acids are given intravenously. It was found that at minimal levels just sufficient to obtain nitrogen equilibrium the amino acid mixture was more effective than the enzymatic digest. At higher levels the digest appeared to give a greater nitrogen retention. More amino acids were lost in the urine after the amino acid mixture was injected while more peptide nitrogen was excreted after the digest was administered. Recent reviews on intravenous therapy with amino acids are those of Martin and Thompson [198] and of Elman.[103]

A retention of nitrogen for growth necessitates the simultaneous presence of all the natural amino acids. If a protein is given which is deficient in one of the amino acids and the missing amino acid is given later, nitrogen equilibrium cannot be obtained nor can growth occur. It was demonstrated by Berg and Rose [48] that when rats are fed on a tryptophane-free ration, better growth occurs if the tryptophane is administered at frequent intervals. Elman [102] found that when an acid hydrolysate of casein (deficient in tryptophane) was given intravenously to dogs, a positive nitrogen balance occurred only if tryptophane was added to the hydrolysate. When tryptophane was given separately several hours after the administration of the hydrolysate, a negative nitrogen balance obtained. It has recently been demonstrated by Geiger [115] that when rats are given a diet deficient in methionine during the day and methionine at night growth does not occur. Similar failures in growth were noted when tryptophane was given separately to rats on a tryptophane-free diet or when lysine was fed during the night to rats on a lysine-free diet during the day. However, Geiger found that when the lysine-free diet and the one containing lysine were fed simultaneously in separate containers, the rat soon learned to ingest sufficient of the missing amino acid to bring about normal growth.[115] Apparently, there is no mechanism for the body to store any excess of the essential amino acids, so that they may be used later when incomplete mixtures of the other building stones of proteins are available.

Relationship between Protein Metabolism and Vitamins.* Many interrelations in the metabolism of protein and the vitamins have been recently disclosed. In the absence of certain specific amino acids, vitamin deficiencies may arise which are amenable to treatment with additional protein. On the other hand, in hypovitaminosis of specific vitamins, an upset may occur in the normal metabolism of certain amino acids and abnormal intermediary products may result, which products may in some cases be excreted in the urine. Of most profound interest has been the

*The interrelationship of the protein and the vitamins has been reviewed by Mitchell.[207]

recent demonstration that the clinical symptoms of vitamin deficiencies may be exaggerated when increased quantities of proteins or amino acids are given in those instances where the missing vitamin is concerned with some phase of protein metabolism. There has been increasing evidence that an important function of some of the vitamins may be as components of enzyme systems which are related to the catalysis of certain intermediary reactions of amino acids or carbohydrates.

The earlier results were based on the relationship of the protein metabolism to the vitamins of the B complex without attempting to identify which vitamin was related to one specific effect. Thus, Hartwell [137] and Reader and Drummond [232] found that when the protein content of the diet was increased without a concomitant rise in the B complex, poor growth of the rats obtained. Moreover, the development of a B-avitaminosis in rats was found to be accelerated by a high protein diet.[290] Finally, when rats are free to choose their diet, they consume more protein when B vitamins are available.[236]

Relation of Riboflavin Requirement to Protein Metabolism. The earlier reports with riboflavin indicated that this vitamin exerts an influence on protein metabolism. Kleiber and Jukes [157] found that the retention of protein by chicks was decreased when the diet was deficient in riboflavin while somewhat similar findings have been reported on rats.[286] Conversely the urinary excretion of riboflavin by dogs and rats has been shown to bear an inverse relation to the level of protein intake.[239, 249] These data are all in agreement in indicating that riboflavin has a profound influence on protein metabolism. The recent work has attempted to determine the role which riboflavin plays in the different enzymatic changes involved in the intermediary metabolism of protein.

Riboflavin is the key constituent of flavoproteins which comprise a number of enzyme systems related to the yellow enzyme. Riboflavin was first recognized as a compound of biological importance when it was found to be an integral part of the yellow enzyme.[306, 307] We now consider it to act as the prosthetic group in this and other enzyme systems. There are a number of enzyme systems containing the flavin group which are now known to be required to bring about specific changes of amino acids or other protein derivatives.

One such important enzyme is *d*-amino acid oxidase which catalyzes the oxidation of *d*-amino acids. Although the *d*-series of amino acids is not the normal one and such amino acids are generally considered as foreign substances to the tissues, definite quantities of *d*-amino acid oxidase are normally present in the animal body. Decreased quantities of this enzyme have been demonstrated in riboflavin deficiency. Thus, Axelrod, Sober and Elvehjem [27] have shown a greatly lowered content of *d*-amino acid oxidase in the liver of rats and a somewhat lowered content in the kidney during ariboflavinosis. Whether the lowering in *d*-amino acid oxidase

activity is the result of a decrease in the specific protein of the enzyme or of the flavin-containing prosthetic group is not thoroughly proved by this study. However, it has been demonstrated also by other workers [222] that a decrease in flavin-adenine dinucleotide occurs in riboflavin deficiency. It would appear that this decreased activity might logically be ascribed to the lowered content of riboflavin. Further support to this supposition is afforded by the results of Rossiter [247] who demonstrated that the oxygen consumption of rat liver preparations from rats on flavin-deficient diets in a medium containing *dl*-alanine was markedly lower than that of similar preparations from rats on the same basal diet to which riboflavin had been added. Moreover, the addition of flavin-adenine dinucleotide brought the oxygen consumption of the livers from the deficient animals to the same level as that of the normal animals where the dinucleotide had been added to the diet. This would seem to indicate that the active flavoprotein can be rapidly rebuilt when necessary flavin-adenine dinucleotide is available. The total riboflavin content of the tissues is considerably greater than that present as *d*-amino acid oxidase since a number of other enzyme systems are known in which riboflavin is present. Since Axelrod *et al.*[27] demonstrated that the total riboflavin content of the tissues was also reduced in riboflavin deficiency as well as *d*-amino acid oxidase, it might be expected that an upset in other enzyme systems might occur in ariboflavinosis.

Such investigations have been made on another riboflavin enzyme system, namely xanthine-oxidase. This enzyme is responsible for the oxidation of hypoxanthine to xanthine as well as the oxidation of the latter compound to uric acid. It was proved by the work of Ball [30, 31] and Corran and coworkers [78, 79] that xanthine-oxidase is a flavoprotein in which the prosthetic group consists, at least in part, of riboflavin-adenine dinucleotide. Xanthine-oxidase has been recognized since its isolation and characterization by Burian.[60] It is therefore obvious that riboflavin indirectly plays a commanding role in the metabolism of the purines.

That alterations in the metabolism of the purines may be expected in riboflavin deficiency is indicated by the experiments of Axelrod and Elvehjem.[25] These workers found that the xanthine-oxidase content in the livers of rats was greatly diminished in riboflavin deficiency. When riboflavin was fed to animals having such a deficiency, the xanthine-oxidase activity was restored to normal provided that the food intake was restricted to prevent any considerable growth. However, where *ad libitum* feeding was employed, the riboflavin requirement for other growth processes took priority over the requirement for xanthine-oxidase so the restoration of the enzyme was less complete. Moreover, no decrease in uricase activity results in riboflavin deficiency.

There are many other catalytically active flavoproteins than *d*-amino acid oxidase and xanthine-oxidase but these are not primarily concerned

with protein metabolism. Among these are liver aldehyde oxidase,[118] cytochrome reductase,[132, 133] succinic dehydrogenase,[26] diamine oxidase,[317] a glucose-oxidizing enzyme,[112, 215] a fumaric acid reductase [116] as well as the system which oxidizes codehydrogenases I and II, which is generally known as the "old" yellow enzyme. Presumably, the activities of all these enzyme systems are related to the riboflavin content of the diet.

The fact that the epitheliodystrophic cataract produced in tryptophane deficiency closely resembles that resulting from ariboflavinosis, might suggest that tryptophane metabolism is related to riboflavin and leads Albanese and Buschke [9] to state: "Although the changes in tryptophane deficiency are not identical with those seen in other conditions (including ariboflavinosis) causing epitheliodystrophic cataracts, the similarity of the pathological picture is striking and suggests that some common metabolic path is interrupted in these disturbances."

Relation of Pyridoxine to Amount and Quality of the Protein Ingested. Although pyridoxine was the first compound isolated and identified as the anti-acrodynia factor, it has been proved recently that other related compounds possess equal or in some instances greater activity in promoting reactions ascribed to vitamin B_6. In order to account for the discrepancies in the apparent pyridoxine content when determined by the yeast method and the *Streptococcus lactis R.* assay,[279] it was suggested that an unknown active product, "pseudopyridoxine," was also present which could stimulate the growth of the *Streptococci* but not the yeast. Snell [276] believed that the substances in question might be the aldehyde or amine of the alcohol, pyridoxine. When these substances were prepared,[136] it was found that both the aldehyde (pyridoxal) and the amine (pyridoxamine) were five thousand to nine thousand times as active as was pyridoxine with *Streptococci lactis R.* This would seem to indicate that the "pseudopyridoxine" is, in reality, pyridoxal or pyridoxamine.

McHenry and Gavin [191] were the first to suggest that pyridoxine was especially needed for the satisfactory metabolism of protein. On a diet composed primarily of protein and in which carbohydrate and fat were excluded, the total fat content of the carcass of rats was increased appreciably above the control level only when pyridoxine was included. It was suggested, therefore, that pyridoxine is required specifically for the change of protein to fat. However, this viewpoint has been criticized by Mitchell [208] who points out that fat deposition is a normal concomitant of growth. If a diet causes growth, there will be a simultaneous fat deposition. A close correlation between these phenomena has been reported by MacKay *et al.*[192] It is suggested that fat deposition from protein cannot be considered a specific function of pyridoxine but is similar to what would be exhibited when growth was resumed after any essential dietary component such as sodium chloride was added to a previously deficient diet. There are several amino acids in which the normal metabolism re-

quires the presence of an adequate amount of pyridoxine. One of the most important of these is tryptophane. Lepkovsky and Nielsen [172] first reported the presence of a green pigment-producing compound in the urine of pyridoxine-deficient rats. This same compound, later identified as xanthurenic acid,[173] was found to be excreted by dogs,[111] pigs,[309] mice [204] but not by chickens [173] when a similar dietary deficiency obtains. In the case of dogs, xanthurenic acid was excreted only after tryptophane was given. It appeared after the dogs had been on the pyridoxine-free diet for only 30 days and continued throughout extended periods up to 407 days.[28] In the pyridoxine-deficient swine, xanthurenic acid excretion was increased after tryptophane.[69, 70]

The interrelation between xanthurenic acid and tryptophane was first suggested by Musajo,[220] who found that on high protein diets, rats and rabbits excrete both kynurenic and xanthurenic acids. When a tryptophane-deficient diet was fed to rats suffering from a pyridoxine deficiency, xanthurenic acid disappeared [173] from the urine while it reappeared when tryptophane was again added to the diet. That tryptophane was the only amino acid involved in xanthurenic acid synthesis was indicated by the fact that it disappeared from the urine within 6 to 12 hours when the casein in the diet was replaced by a tryptophane-free protein as acid-hydrolyzed casein, zein or gelatin.[173] In a later study, Ried, Lepkovsky, Bonner and Tatum [238] reported that of the compounds related to tryptophane, only l^- tryptophane and kynurenine were precursors of xanthurenic acid in the vitamin B_6-deficient rat. Compounds which may be used in place of tryptophane for growth in the normal rat, such as d^+ tryptophane,[45, 302] indole-3-pyruvic acid,[49, 147] indole-3-lactic acid [38] and abrine,[62, 119, 120] are not convertible to xanthurenic acid in the pyridoxine-deficient animals. This indicates that their metabolism must follow a different pathway. Negative results were also obtained with indole-acetic acid, indole plus serine and kynurenic acid. On the other hand, when pure xanthurenic acid was fed, it was excreted quantitatively in unchanged form in the absence of pyridoxine while in the normal rat it disappeared completely and none appeared in the urine after its oral administration. There would appear to be a species difference between the rat and the dog since the normal dog is unable to utilize xanthurenic acid. However, with adequate pyridoxine, tryptophane is changed to kynurenine and then to kynurenic acid which is excreted by the dog. No xanthurenic acid is formed under these conditions.[28]

The relation between tryptophane metabolism and pyridoxine becomes apparent when pyridoxine analogs are fed to the rat. When desoxypyridoxine (2, 4-dimethyl-3-hydroxy-5-hydroxymethylpyridine) was administered along with tryptophane [229a], more xanthurenic acid was excreted than in control animals; the effect was exaggerated in pyridoxine-deficient animals. A methoxypyridoxine (2-methyl-4-methoxymethyl-3-hydroxy-5-

hydroxymethylpyridine) showed some B_6 activity on pyridoxine-deficient rats although it acted to some extent as a competitor when fed to normal rats.

The importance of vitamin B_6 in the metabolism of tryptophane has been indicated by an entirely new method of approach. The addition of *l*– tryptophane to the diet of pyridoxine-deficient mice lessened their survival time [204] as well as increased their xanthurenic acid excretion. From 10 to 24 per cent of the tryptophane was accounted for as this urinary chromogen. The feeding of increased amounts of casein also contributed to the ill health of the mice. It would appear that the increased protein metabolism causes a more rapid depletion in the tissue stores of pyridoxine. Schweigert *et al.*[255] have demonstrated that the body stores of pyridoxine particularly in young mice are much lower after a pyridoxine-free diet if the diet contains 50 per cent of protein than when it contains 10 per cent. In some cases fatty livers also developed. That species differences exist is also indicated by the failure to observe similar differences in the pyridoxine content of rats fed on different protein levels. That the metabolism of amino acids other than tryptophane is related to pyridoxine is also postulated because of the fact that the feeding of casein contributed to the ill health of the mice to a greater extent than its equivalent tryptophane content.[204] Axelrod, Morgan and Lepkovsky [28] report that the ingestion of tryptophane by dogs which had severe vitamin B_6 deficiency produced nausea, anorexia and sometimes collapse, although normal animals or those moderately deficient failed to exhibit any of these symptoms after tryptophane was given. The development of the dermatitis in rats on a pyridoxine-deficient diet has been shown to occur more readily when protein makes up 30 per cent of the diet than when fed at lower levels.[77] This shows that the intermediary protein reactions have a priority on the pyridoxine supplies before it can be utilized for the reactions concerned with the metabolism of the epidermis. These experiments are also in line with the results of Richter and Hawkes [237] who found that rats lost their appetite for protein when the B-complex vitamins were removed from the foods available. Although thiamine restored the appetite for carbohydrate, riboflavin and niacin were only partially successful in reviving the appetite for protein. The complete desire for protein did not return until pyridoxine was again available.

More recently, Sarma, Snell and Elvehjem [250] have found that the addition of *dl*-tryptophane or indole to a diet containing suboptimal quantities of pyridoxine or pyridoxal causes a growth retardation. This is interpreted to mean that when a vitamin is required for the intermediary metabolism of an amino acid, that function has the priority and the vitamin cannot also serve for other reactions required for growth.

Since the feeding of *dl*-methionine also brought about a growth retardation in pyridoxine-deficient rats,[250] it was concluded that this vitamin

may be concerned with the intermediary metabolism of this sulfur-containing amino acid. However, no growth-depressing effect was noted for *l*-cystine or *dl*-alanine so this function of pyridoxine is not a generalized one. Moreover, the addition of tyrosine and histidine did not decrease the survival time of mice on a pyridoxine-deficient diet [204] so the metabolism of these amino acids must be considered to be independent of vitamin B_6. The fact that oleic acid exerts a depressing action on the growth of rats on a suboptimal pyridoxine intake [250] further strengthens the hypothesis that this type of test is of value in establishing normal interrelations of vitamins and intermediary metabolism. The relationship of pyridoxine to the metabolism of the unsaturated fatty acids has been largely accepted since the work of Birch.[52]

Pyridoxine also seems to be concerned in some cases with bacterial decarboxylation of tyrosine although this amino acid exerted no deleterious effect on growth on a pyridoxine-free diet. Bellamy and Gunsalus [40] have demonstrated that pyridoxine accelerates the decarboxylation rate of tyrosine by cell suspensions of *Streptococcus faecalis* although fairly high concentrations of pyridoxine were required. Gunsalus and Bellamy [129] later found that pyridoxal was the more active compound. This was the same substance earlier identified as "pseudopyridoxine" which had been obtained by autoclaving pure pyridoxine with cystine [275] or by treatment with peroxide.[67]

It was later reported [130] that the coenzyme of the *l*-tyrosine decarboxylase is actually pyridoxal phosphate. Subsequently this coenzyme was demonstrated by Umbreit *et al.*[293, 294] and by Baddiley and Gale [29] to be identical with the codecarboxylase isolated from yeast earlier by Gale and Epps.[114] Apparently pyridoxal phosphate (prepared either enzymatically by the action of adenosine triphosphate on pyridoxal or chemically by the reaction of pyridoxal and phosphoryl chloride) is the same as the natural codecarboxylase which occurs in plant and animal tissues, yeast and bacteria. The synthetic product which has been highly purified as the barium salt [131] has been shown to contain one atom of phosphorus per molecule of pyridoxal.

The role of pyridoxal phosphate as a codecarboxylase appears to be a general one as it functions not only in the decarboxylation of tyrosine [29, 40, 130] but also in that of lysine,[113] arginine,[29, 294] ornithine [29] and as the codecarboxylase for dopa carboxylase which acts on 3,4-dihydroxyphenylalanine (dopa).[123] Moreover, the codecarboxylase activity of mammalian tissue has been shown to be proportional to the pyridoxine level of the diet.[41] However, it could not be demonstrated as a component of the *l*-histidine decarboxylation system [104] or in a codecarboxylase preparation from *Cl. welchii* which was active on an l^+ glutamic acid.[288] Martin [197] has observed that the addition of 5 per cent of tyrosine to the diet caused less toxicity in the absence of pyridoxine than when vitamin B_6

was present. He ascribes this to the decreased decarboxylation of this aromatic amino acid which prevents the accumulation of the toxic amine.

Another function which has recently been ascribed to vitamin B_6 is its role as a coenzyme in transamination.[276] A possible clue to the mechanism is given by the *in vitro* demonstration that the heating of pyridoxal and glutamic acid together results in the production of pyridoxamine and ketoglutaric acid.[277, 278] The reaction is a reversible one. The biological conversion of pyridoxamine into pyridoxal can be brought about by *Streptococcus faecalis* in the presence of pyruvate.[41]

Other recent investigators have questioned the cotransaminase activity of pyridoxal. Although Kritzmann [165] stated that a coenzyme was required for aspartic acid transaminase, Cohen [76] was unable to demonstrate any such requirement. Likewise, Leloir and Green [171] found no evidence of dissociable prosthetic groups or members of B complex in two highly purified transaminase preparations, although Green *et al.*[123] later presented evidence that a coenzyme was present in two purified transaminases from pig heart which had properties similar to pyridoxal phosphate. Moreover, Lichstein and Cohen [176] reported no reduction in transaminase activity of a suspension of *Streptococcus faecalis* grown in a pyridoxine-poor medium although a decrease in the rate of decarboxylation of tyrosine was noted. Possibly these results only indicate the lower requirement of the transaminase enzyme for pyridoxal than of the decarboxylase or that the transaminase has priority in the competition for the small amounts of pyridoxine which were known to be present in the medium. It should be remembered that Schlenk and Snell [252] did find a decreased rate of transamination between oxalacetic and glutamic acids in tissues of rats on a pyridoxine-deficient diet. Moreover, the reaction could be accelerated by the addition of pyridoxal and adenosine triphosphate. Lichstein, Gunsalus and Umbreit [177] have been able to demonstrate in highly purified cell-free enzymes from *Streptococcus faecalis* that pyridoxal phosphate functions as a coenzyme in the glutamate-aspartate transaminase. This was proved by the demonstration of the activating effect of pyridoxal phosphate on the transaminase apoenzyme grown on a pyridoxal-deficient medium as well as by resolving the cell-free enzymes grown with pyridoxal and restoring the activity of the apoenzyme with pyridoxal phosphate. Finally, Ames *et al.* [20a] have recently reported that the transaminase activity of heart and kidney tissues of rats on a vitamin B_6-deficient diet was only 40 per cent of the normal value while succinoxidase which does not contain pyridoxine was present at a level which was 80 to 90 per cent of normal. While pyridoxine or pyridoxamine either with or without adenosine triphosphate was ineffective in reactivating such tissues, the addition of pyridoxal phosphate or pyridoxamine phosphate at the same low level did produce a reactivation of transaminase activity. That this was a specific effect is indicated by the fact that the addition of these compounds

was without effect on the activity of succinoxidase. The higher blood levels of urea and N.P.N. obtained in dogs and rats on high protein diets lacking in pyridoxine have been ascribed to an impairment in transaminase activity.[139]

Still an additional function of pyridoxine (pyridoxal phosphate) appears to be in the synthesis of tryptophane. Umbreit *et al.*[295] have found that a mixture of serine and indole are changed to tryptophane by an enzyme prepared from the mycelium of *Neurospora sitophila* provided pyridoxal phosphate is present as a coenzyme. According to Schweigert [254a] *Lactobacillus arabinosus* requires vitamin B_6 for the metabolic conversion of indole or anthranilic acid to tryptophane. As in most other reactions mediated by vitamin B_6, pyridoxal or pyridoxamine are more active than pyridoxine.

Relation of Niacin Requirement to Amount and Quality of the Protein Ingested. The requirement for niacin is closely related to the type and amount of the protein in the diet. The urinary excretion of total nicotinic acid (trigonelline and acid-hydrolyzable derivatives) by dogs has been shown to be inversely proportional to the level of protein fed.[249] Wintrobe, Stein, Follis and Humphreys [310] failed to produce niacin deficiency in swine when the diet contained 26 per cent of casein, but such a deficiency rapidly developed when the protein level was reduced to 10 per cent. That either protein or niacin could act independently to clear up the deficiency symptoms is indicated by the fact that the addition of niacin to the protein-low diet or the increase of the protein level by augmenting the vitamin-free casein in the diet were both equally effective. Not only was the deficiency indicated by the poor physical condition of the animals and by their failure to grow but it was confirmed by the low level of excreted nicotinuric acid, trigonelline and related compounds which are the end products of niacin metabolism. The beneficial effect of tryptophane in correcting niacin deficiency of the pig [183a] and of the chick [57a, 57b] has also been reported.

The quality of the protein would appear to be equally important in determining the niacin requirement. The white rat, which ordinarily is not susceptible to niacin deficiency as it can synthesize this vitamin [89] as well as coenzymes I and II,[90] had a greatly reduced growth level when corn meal or corn grits were added to a protein-low diet.[162] Such a depression in growth did not occur when the corn was added to a basal diet containing 15 to 20 per cent of casein and it could be cleared up by the addition of niacin to the diet. It was later demonstrated by Krehl, Teply, Sarma and Elvehjem [164] that the addition of as little as 0.05 per cent of *l*− tryptophane to such growth-depressing diets removed their inhibitory effect. It was found that 50 mg of tryptophane under these conditions was nutritionally equivalent to 1.0–1.5 mg of niacin.[161] The growth-inhibitory effects of low-tryptophane, low-niacin diets could be produced on non-

corn diets when the sugar was sucrose. These in turn would respond to added tryptophane or niacin or to a carbohydrate which will produce a favorable intestinal flora for synthesis of added niacin.[161]

Further evidence of the role of tryptophane may be adduced from a recent report of Krehl, Sarma and Elvehjem [160] where it was shown that the deleterious action of corn grits in a synthetic diet can be prevented by fibrin, egg albumin or soybean globulin which are practically niacin-free but which are excellent sources of tryptophane. All of these data suggest that the beneficial effect of protein in niacin deficiency is related to its tryptophane content. Such beneficial effects do not occur when the proteins of corn are the chief source of protein since these are very low in tryptophane. However, recently Krehl *et al.*[159a] have interpreted the growth depression of zein and other proteins containing a low content of tryptophane when fed in conjunction with a low-niacin diet as due to the imbalance of the amino acid mixture. The excess of glycine has some deleterious action although such an effect was not noted when the carbohydrate was dextrine instead of sucrose.

The most convincing proof that nicotinic acid is derived from tryptophane is the demonstration that the urinary niacin is increased when tryptophane is administered to rats as well as to men.[227a, 248a, 267] Rosen, Huff and Perlzweig [246] have reported that the other niacin end products in the urine (nicotinuric acid and trigonelline) are also excreted in decreased amounts when a trytophane-free protein as gelatin is used as compared with the excretion on a basal diet where casein is employed. Equally striking was the finding that the administration of tryptophane orally or subcutaneously is followed by a marked increase in the urine derivatives of niacin. The fact that this effect can be produced when the tryptophane is introduced parenterally as well as after oral administration precludes intestinal synthesis as the explanation for the action of tryptophane. In a later communication, Singal *et al.* [267a] report that rats receiving tryptophane excrete in the urine not only increased amounts of nicotinic acid and a methylated derivative of nicotinic acid but also an unidentified product which is converted to niacin by acid but not by alkaline hydrolysis. This latter product was found to be excreted in the largest percentage on the highest intake of tryptophane. It presumably represents an intermediate product in the tryptophane niacin transformation. Spector and Mitchell [280] believe the physiological effects of niacin and tryptophane are not related to the synthetic capacities in the gastrointestinal tract but possibly to some unknown function in which these two compounds participate interchangeably.

The depressing effect of corn grits also was found in the dog which is an animal normally requiring niacin. A threefold increase in the niacin requirement followed the inclusion of the corn grits in the diet.[163]

The recent work of Woolley [313] suggests another possible explanation

for the deleterious activity of corn grits on growth and the compensatory effect of other proteins, tryptophane or niacin. This investigator demonstrated that the feeding to mice of 3-acetyl-pyridine, an analog of niacin, resulted in the death of the animals with pellagra-like symptoms. The toxic action of the 3-acetyl-pyridine could be completely negated by the addition of niacin or by the inclusion of extra *dl*-tryptophane in the diet in amounts as small as 0.1 per cent.[314] It is suggested that the harmful effect of the corn may be due to the presence of a structural analog of nicotinic acid. Woolley [316] has recently separated a "pellagragenic" agent from corn by chloroform extraction of a sodium hydroxide extract. This substance markedly reduced growth rate, produced a mild diarrhea and caused a considerable reddening of the skin and tongue in mice, all of which are classical symptoms of pellagra. The condition could be prevented when niacinamid was added to the ration. Whether the active compound is an analog of pyridine is not as yet known. Krehl *et al.*[159a] have recently shown that another analog of niacin, pyridine-3-sulfonic acid, causes growth inhibition in the rat fed on a low protein level. This effect could be counteracted by a high protein diet or by niacin. Such compounds as 3-CN-pyridine, indole, indole-3-acetic acid and anthranilic acid were shown to be ineffective in replacing tryptophane or niacin but likewise they did not compete with these substances.

It would thus appear that not only a dietary deficiency of niacin or its precursor, tryptophane, may be the sole cause of pellagra but the presence of a pellagragenic agent may also play a role. The association of pellagra with diets high in corn can then be ascribed to the low niacin content of the corn, the low concentration of the niacin precursor, tryptophane, in the corn proteins and lastly to the toxic effect of possible pellagragenic agents which act antagonistically to niacin.

If tryptophane is the mother substance of niacin as would appear probable, there is no indication by what pathway such a transformation may occur. Since the indole ring may be changed to the quinoline ring in many animals, it is possible that the kynurenic acid or xanthurenic acid so formed might be broken down to a pyridine derivative which could be changed to nicotinic acid.

Guggenheim [127] has suggested an entirely different biosynthesis of nicotinic acid from arginine which is as follows:

$$\textit{Ornithine} \xrightarrow{-NH_2} \textit{Proline} \xrightarrow{+H_2} \Delta\textit{-aminovaleric acid}$$

$$\xrightarrow{-H_2} \Delta\textit{-aminodehydrovaleric acid} \underset{-H_2O}{\overset{+CH_2O}{\rightleftharpoons}} \textit{Guvacin} \xrightarrow{-H_2O} \textit{Nicotinic acid}$$

Huff and Perlzweig [146] obtained some evidence supporting this hypothesis by demonstrating that the stimulatory effect of Δ-aminovaleric acid on the synthesis of niacin, as determined by the rate of urinary excretion, could be produced by the administration of glycine and ammonium lactate

and these results tended to indicate that the pathway through Δ-aminovaleric acid is not the only possible one.

*Relation of Choline to Protein Metabolism.** Choline is one of the vitamins generally classed as a member of the B complex. Moreover, because it functions not only as a vitamin but also as a component of the structural tissues, it is now generally referred to as a "vitagen." Choline has been known for a long time as a component of the phosphatide, lecithin, which is widely distributed in the animal and plant kingdom. In the animal, lecithin is believed to be present in every cell; it is concentrated in the liver and especially in the nervous tissue. Lecithin is present in considerable quantities in crude soya oil which is the chief commercial source of this phosphatide. Because of its emulsifying properties, it finds wide application in the food industry.

The discovery of the relationship of choline to protein metabolism resulted from the fact that both of these substances possess a lipotrophic action, *i.e.*, are able to prevent the accumulation of fat in the liver or to cause the disappearance of such liver fat if it has already been laid down. Best and associates [50] had first demonstrated that on a low protein diet where high levels of certain fats were present, fatty livers quickly developed in rats. Lecithin in small amount could prevent this condition; likewise, choline was very effective in so doing but the glycerol, fatty acids and phosphoric acid in the lecithin molecule were all completely inactive.[51] Several years later, Tucker and Eckstein [291, 292] found that methionine was equally effective as a lipotrophic agent although its demethylated homolog, homocystine, was lacking in the lipotrophic action.[268] This behavior of methionine was later demonstrated by du Vigneaud *et al.*[298, 300] to be related to its ability to serve as a transmethylating agent whereby it yields the methyl groups which enable the readily synthesizable aminoethyl alcohol to be changed to choline. It was found that on a choline-free diet 89 per cent of the methyl groups in the choline isolated from the tissues of rats fed deuterio-methionine could be demonstrated to have originated from this amino acid.

That choline may also serve as a transmethylating agent has also been convincingly proved. Although homocystine cannot sustain the growth of rats on a choline-free diet,[297, 301] it was found to be entirely effective in place of methionine when choline was present.[57, 244, 308] The work of du Vigneaud and collaborators [299] proved that under such conditions, the methyl groups of choline were transferred to homocystine with the resultant synthesis of methionine. Thus, the requirement for choline must be increased when the protein intake is greatly reduced. On a high intake of protein (particularly of proteins high in methionine), choline can be to a considerable extent synthesized *de novo*. Conversely, in the absence of

* An excellent summary of the importance of choline as a dietary factor is given by Lucas and Best.[183]

methionine, adequate nutrition obtains with homocystine if the supply of choline is plentiful.

Relation of Other B-Complex Vitamins to Protein Metabolism. Although the study of the physiological action of thiamine has been chiefly concerned with its activity as cocarboxylase, this vitamin is important in the breakdown of amino acids as well as sugar. Thus, it has been known for some time that there is a marked accumulation of pyruvic acid in the blood in thiamine deficiency [182, 229] and that definite amounts of this keto acid are excreted in the urine in B_1 avitaminosis. The amount of the keto acid so eliminated is markedly increased after the administration of carbohydrate.[135, 263] However, since pyruvic acid is the primary product obtained following oxidative deamination of l^+ alanine, it is evident that a failure to dispose of this intermediate in a satisfactory way may embarrass the orderly mechanism of alanine degradation and may prevent the body from obtaining the principal amount of energy by its complete oxidation.

The suggestion that pyruvic acid is one of the intermediates produced from cysteine by liver slices and especially by chloroform-treated liver extract indicates another possible source of the ketoacid from amino acids.[273] However, this result is contrary to the *in vivo* tests of Butts *et al.*[61] with cystine (which is presumably readily convertible to cysteine) where it was conclusively demonstrated that this amino acid cannot serve as a source of glycogen although the urinary sulfate excretion indicated that the bulk of ingested cystine was absorbed and metabolized. Were pyruvic acid a normal intermediate of cystine breakdown, glycogen formation should be demonstrated after cystine feeding.[261]

The role of thiamine in increasing the oxidation and utilization of α-ketoglutarate in avitaminotic rats [33] should not be looked on exclusively as an effect on carbohydrate metabolism, important as ketoglutaric acid is in the Kreb's cycle. Since ketoglutaric acid is the product formed on oxidative deamination of glutamic acid, one can hardly question the importance of the α-ketoglutarate oxidase as this one amino acid is concerned.

The decarboxylation of α-ketoglutaric acid to succinic semialdehyde, of pyruvic acid with the formation of acetoin, and of α-ketobutyric acid with the formation of propionin are all examples of reactions which are catalyzed by diphosphothiamine metalloproteins.[124]

Another member of the B complex, *p*-aminobenzoic acid, has been shown to enhance the oxidation of tyrosine by tyrosinase [200] while it accelerates the oxidation of another aromatic compound, namely *p*-cresol.[311] There seems to be some evidence that folic acid or folic acid-like compounds are in some way related to the metabolism of tyrosine. Swendseid *et al.*[286a] found that the metabolism of this amino acid was altered in pernicious anemia. In later work a lower rate of oxidation of tyrosine was reported for liver homogenates prepared from rats where a pteroylglutamic acid (PGA) deficiency had been induced by the feeding of sulfasuxidine.[240a]

This relationship was further indicated by the fact that the oxidation of tyrosine was largely restored when PGA was added to the liver preparations.

The first indication that pantothenic acid may be related to protein metabolism is seen by the finding of Nelson *et al.*[221a] that the urinary excretion of this vitamin paralleled the level of dietary protein. That this was not a result of increased intestinal synthesis is indicated by the fact that fecal pantothenic acid was independent of the level of protein in the diet. It is believed by these authors that protein may spare the pantothenic acid.

Relation of Ascorbic Acid to Protein Metabolism. Although it has been recognized for some time that ascorbic acid and its oxidation product, dehydroascorbic acid, have important functions as an oxidation-reduction system, their role in protein metabolism has only recently begun to be recognized. Ascorbic acid is necessary for collagen formation and many of the more frank symptoms of scurvy are related to the failure of this protein to be formed.

The relation of faulty wound healing to the level of ascorbic acid available in the tissues is undoubtedly traced to the requirement of vitamin C for collagen formation. Lanman and Ingalls [168] first reported the poor repair of tissue and the improper collagen formation in experimental wounds in scorbutic guinea pigs. A similar slow healing was reported in man but only after the subject had been on an ascorbic acid-free diet for six months and the blood ascorbic acid level had been zero for several months.[185] An improvement was noted in this case when ascorbic acid was given intravenously without any other change in the dietary regime.

When the vitamin C deficiency is less acute, there is some evidence that wound healing is less satisfactory than when adequate stores are available. In carefully controlled studies on guinea pigs, Bartlett *et al.*[34] found that normal guinea pigs were able to bring about a mobilization of ascorbic acid in the scar tissue of a wound while this was not possible for animals on the vitamin C-deficient diet. The postoperative levels of vitamin C in the scar tissues of the guinea pigs on the normal and deficient diets were 6.53 and 0.29 mg per cent respectively; the preoperative levels in the same tissues were 1.57 and 0.25 mg per cent respectively. A further proof of the importance of ascorbic acid in wound healing was the demonstration that the average pressures to cause skin separation in vitamin C-saturated and deficient animals was 140 and 70 mm of mercury respectively while the pressures required to cause complete ruptures of the wound in the two groups were 258 and 127 mm mercury respectively. Lund [184] concluded after a study of recoveries from operations in 43 patients that "when nonradical operations were performed, more complications and deaths occurred in the patients with low reserves (of ascorbic acid) than in those with high reserves." Additional evidence of the therapeutic use of vitamin C is also given by Crandon, Lund and Dill.[82]

Hartzell, Winfield and Irvin [138] found that a low plasma vitamin C was

present in 19 of 20 cases of postoperative wound disruption. On the other hand, Carney [66] was unable to find a correlation between serum ascorbic acid level and the rate of wound healing on soldiers on the Italian front. However, the bulk of evidence seems to support the thesis that a relationship between vitamin C stores and the healing of wounds does exist.

That ascorbic acid might have a generalized effect on protein metabolism is indicated by the suggestion that it may be concerned with the production of urea in the liver,[199] a phenomenon which is supported by the demonstration that it exerts a stimulatory effect on the activity of arginase.[152]

There is considerable evidence that ascorbic acid has a specific role in the breakdown of histidine and the aromatic amino acids. The reaction between ascorbic acid and histidine *in vitro* has been the basis of a specific test for this amino acid.[4, 100] Apparently the reaction involves a cleavage of the iminazole ring [125] since the ammonia which originates in this reaction is too great to be accounted solely from the amino group in the side chain. Such an *in vitro* analogy would fit in with the postulated cleavage which Edlbacher [98, 99] believes is brought about by histidase. However, Greenblatt and Pecker [125] were unable to demonstrate any increased destruction of histidine in *in vivo* tests on man, rabbits and guinea pigs where large doses of ascorbic acid were injected intravenously or intraperitoneally although it is possible that the conditions may have been somewhat artificial in the latter tests.

It is believed that ascorbic acid may be concerned with decarboxylation of amino acids in general [4, 144] including histidine. However, here again it has been impossible to demonstrate that the administration of large amounts of ascorbic acid increases the activity of the gastric glands due to the production of added histamine.[125]

The most striking alterations in protein metabolism on ascorbic acid deficiency have been noted with phenylalanine and tyrosine. This is exhibited either by the appearance of *p*-hydroxyphenyllactic and *p*-hydroxyphenylpyruvic acid or homogentisic acid in the urine. These compounds undoubtedly originate from phenylalanine or tyrosine. Levine *et al.*[175] found that premature infants on high protein diets excreted considerable amounts of *p*-hydroxyphenyllactic and *p*-hydroxyphenylpyruvic acids and that this could be abolished by the administration of ascorbic acid.[174] On the other hand, full term infants failed to excrete these abnormal end products in high protein diets although the excretion could be provoked by the feeding of phenylalanine or tyrosine in doses of one gram per kilogram of body weight. In this case also the abnormality responded to ascorbic acid treatment. It is possible that the appearance of *p*-hydroxyphenylpyruvic and *p*-hydroxyphenyllactic acids may constitute one of the first objective symptoms of ascorbic acid deficiency.

The feeding of tyrosine in amounts in excess of 0.5 gram to normal guinea pigs was found to cause the excretion of homogentisic, *p*-hydroxyphenyl-

pyruvic and *p*-hydroxyphenyllactic acids, but these metabolites disappeared when ascorbic acid was also given.[259] Phenylpyruvic acid was excreted when fed in excess while it appeared from the urine with ascorbic acid administration.[257] On the other hand, the metabolism of *p*-hydroxyphenylpyruvic acid was found to proceed independently of ascorbic acid.[257] In a later paper, these authors have found that the requirement for ascorbic acid is specific for *l*⁻ phenylalanine, *l*⁻ tyrosine and phenylpyruvic acid while a large series of closely related intermediates were as satisfactorily metabolized in the absence as in the presence of vitamin C.[35] The adrenalin content of the adrenals of ascorbic guinea pigs has recently been shown to be considerably above normal.[32]

On a constant intake of *l*⁻ tyrosine, the excretion of homogentisic acid was found to be inversely proportional to the quantity of ascorbic acid given.[258] It has recently been shown that ascorbic acid is without effect on human alcaptonuria.[256] The far-reaching effects of the deficiency of ascorbic acid are shown by the fact that liver slices from scorbutic guinea pigs are unable to oxidize *l*-tyrosine although this ability could be restored by the *in vivo* or *in vitro* addition of vitamin C.[166] Quastel and Wheatley [230] have reported a similar stimulatory effect of ascorbic acid on the oxidation of butyric and crotonic acids by liver slices from scorbutic guinea pigs. Darby and coworkers [91] have noted, however, that the liver slices from normal and scorbutic guinea pigs were equally efficient in producing a hydroxyphenyl compound from phenylalanine, in metabolizing tyrosine as measured by the disappearance of hydroxyl groups and in conjugating phenol.

The earlier suggestion that thiamine-deficient rats excreted phenylpyruvic acid after large doses of phenylalanine [75] must be modified as a result of the investigations of Kaser and Darby [154] who found no difference in the ability of the normal and the thiamine-deficient rats to metabolize phenylalanine. However, the suggestion that the synthesis of ascorbic acid is dependent on the presence of thiamine [121, 285] would seem to argue for the possibility of such a relationship.

The ascorbic acid-dehydroascorbic acid system has been shown to be effective in the reduction of methemoglobin. Vestling [296] demonstrated the quantitative reduction of this pigment to hemoglobin *in vitro* at a pH of 7.0 by the ascorbic acid system. That such a reaction can also be catalyzed *in vivo* is indicated by the report of Deeny, Murdock and Rogan [92] who have treated a case of familial idiopathic methemoglobinemia successfully with ascorbic acid. The oxygen capacity of the blood was increased from 13.2 to 22 volumes per cent by the administration of 2 grams daily of ascorbic acid over an eight-day period.

Relation of α-Tocopherol (Vitamin E) to Metabolism of Creatine. The tocopherols have several important functions in addition to their activity as the antisterility vitamin. One of the most important of these functions is as an antioxidant. The superior keeping qualities of vegetable oils as

contrasted with animal fats is largely attributed to the much higher concentration of the tocopherols which they possess.

It is surprising that α-tocopherol in addition seems to have some activity in relation to the metabolism of creatine. When rabbits or guinea pigs are placed on a vitamin E-free diet, they develop a muscular dystrophy along with a concomitant creatinuria. This condition is usually referred to as "nutritional muscular dystrophy." Although rats ordinarily are not susceptible to this deficiency, Evans and Burr [106] demonstrated a paralysis in suckling rats prior to weaning when the mother was on a vitamin E-free diet. Goettsch and Pappenheimer [117] reported profound changes in the muscles of rabbits and guinea pigs maintained on a scorbutic diet which was also deficient in vitamin E. That vitamin E is related to muscular dystrophy is also indicated from the fact that sheep and goats,[197] ducks,[227] mice,[226] and hamsters [145] develop muscular dystrophy on vitamin E-free diets. Dogs with chronic biliary fistulae also develop muscular disorders which have been ascribed to the failure in absorption of vitamin E.[59]

Synthetic α-tocopherol or natural vitamin E cures the disease in rabbits and in guinea pigs [105, 144, 264] simultaneously with the lowering or abolition of creatinuria.[116, 213] Clinical improvement was practically always followed by a parallel decrease in creatinuria. It would seem improbable that the action of tocopherol is a direct one on creatine; it would rather appear that its action is indirect, which enables the normal metabolism of creatine to take place. Tocopherol therapy on cases of muscular dystrophy in man have given somewhat disappointing results although Milhorat and Bartels [203] have recently reported a marked improvement when inositol and tocopherol were given simultaneously or after the parenteral administration of a water-soluble conjugation product prepared by reacting inositol and α-tocopherol.

Effect of Vitamin K on Synthesis of Prothrombin. Although the mechanism of action of vitamin K is as yet not entirely clear, its connection with protein metabolism is established by its requirement in the synthesis of the serum protein, prothrombin. After the experimental demonstration that a bleeding tendency developed in chickens on a diet limited in fats,[83] Dam,[84, 85] as well as Almquist and Stokstad,[19, 20] furnished convincing evidence for the existence of a vitamin-like organic factor which is required by the chick for maintaining a normal clotting power of the blood. Since this factor was concerned with blood clotting, Dam [85] called it vitamin K (Koagulations Vitamin). Schönheyder [253] later demonstrated that the delayed clotting time in vitamin K-deficiency was associated with a lowered prothrombin content of the blood. The other components necessary for blood clotting were shown to be present in normal amounts; the relation of the prothrombin level to vitamin K was proved by the demonstration that the level of this constituent could be restored to normal by the administration of vitamin K.

The prothrombin deficiency may occur or be produced in many species other than the chick. Thus, it has been experimentally produced in the duck, goose and pigeon.[88, 254] In the rat [122] and the dog,[270] a hypoprothrombinemia exists when the bile is excluded from the intestine by biliary fistulae. When sufficient vitamin K is present, this hypoprothrombinemia can be cured when bile or the bile salts are administered. In cases of obstructive jaundice in man, the attendant hypoprothrombinemia may be counteracted by a similar treatment.[84, 274] The hemorrhagic tendency frequently noted in the new born is also due to a deficiency in blood prothrombin; [141] here the cause is not the failure to absorb the vitamin K but rather the inadequacy of its synthesis in the intestinal tract as long as this remains sterile. An important part of the vitamin K necessary is normally synthesized by bacterial action in the gut.

Although there are several reports which indicate the inability to produce a hyperprothrombin level by excessive doses of vitamin K,[6, 18, 87] Field and Link [109] have demonstrated that a hyperprothrombinemia persisting for several days can be brought about in the dog, rabbit and rat after single large oral doses of 2-methyl-1, 4-naphthoquinone (the compound with most vitamin K activity). It has been further stated that such methyl xanthines as theophylline, theobromine, and caffeine induce a state of hyperprothrombinemia in the dog, rabbit and rat which may be maintained by a single dose as long as 5 days or by repeated small doses up to a month.[108] In the so-called sweet clover disease of cattle, a hypoprothrombinemia occurs with a bleeding tendency and this is usually fatal. The causative agent for the sweet clover disease has been identified by Link and his associates [64] as 3,3′ methylene-*bis*-(4-hydroxycoumarin) or dicumarol. The synthetic product was shown to have identical physiological effect with the natural product [283] and it was found that the toxic action could be counteracted by an excess of vitamin K.[225] Dicumarol has served as a valuable anticoagulant in medicine but it is only effective when administered to the live animal. This makes it seem probable that it functions by suspending the production of prothrombin in the liver. Dicumarol appears to be broken down to salicylic acid in the animal body. Although salicylic acid has little antivitamin K activity in the rabbit [179] or dog, it has been reported to lower the prothrombin level of normal human blood.[231]

The action of vitamin K in causing a synthesis of prothrombin is not a direct one but one which is mediated through the action of the liver. When the liver is injured in cats and dogs by administration of chloroform or carbon tetrachloride [54, 58] a lowered blood prothrombin results which cannot be relieved by the administration of vitamin K. This is taken as presumptive evidence of the role of the liver in prothrombin synthesis. Future research will undoubtedly reveal how vitamin K functions in producing the prothrombin molecule.

Conclusions

It would appear that one can no longer consider that the sole function of protein is the building of body tissue. Although it is true that a constant replacement of the tissue proteins broken down by the normal catabolic processes must be made, certain amino acids must continually be available in the tissues for such lightly specialized functions as transmethylation and transamination. Inasmuch as choline can be synthesized in the animal body if transferable methyl groups are available, it is obvious that the choline requirement will be dependent on the protein intake since the amino acid, methionine, is the compound which will furnish the necessary methyl groups. The interdependence of proteins and vitamins is further illustrated by the synthesis of niacin from tryptophane. Vitamin deficiencies may be to a considerable extent related to the protein level in the diet.

Conversely, the satisfactory utilization of protein as well as carbohydrate requires an adequate vitamin intake. The failure of certain intermediary reactions of the amino acids to occur in specific vitamin deficiencies has been noted in a number of instances and unusual end products are excreted. Since the vitamins, particularly those of the B complex, serve as components of widely varying enzyme systems, such irregularities in the metabolism of the amino acids must be related to an interference in some essential enzyme reaction.

Not only must protein metabolism be considered as intimately bound up with the vitamins but the composition of the other foodstuffs also is of importance. The level at which nitrogen equilibrium can be established is a function of the level of caloric intake. Moreover, carbohydrate plays a much more important role in this respect than does fat. Although no important relation of the minerals to protein requirement has as yet been demonstrated, this foodstuff in the future probably will be shown to be important in this respect. Certainly, future research will disclose a greater complexity in the relations of the metabolism of the various foodstuffs.

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Chapter 4

Economic Aspects of Food Proteins

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Very few foods ordinarily represented in any significant proportions in human dietaries completely lack protein. Sugars and syrups and various fats and oils are the outstanding examples of foods substantially represented in human dietaries which do not contribute food protein. Since food proteins are not produced, distributed, or consumed apart from other constituents in foods, it is apparent that consideration of the economic aspects of food proteins commands consideration of those *foods which contain protein.* In short, the economic aspects of food proteins are inseparably linked with the economic status of most of the world's basic food supplies.

It would be a serious mistake to assume that because sugar, fats and oils contained no protein these items were without influence upon the food protein economy in this and various other countries. The presence of these non-protein food materials, in proportions which furnish approximately a third of the caloric value of our national dietary, means that foods supplying the remaining two-thirds of the calories must carry the *entire load of most* other dietary essentials than calories if such are to be adequately provided. High levels of consumption of sugar, fats and oils can create a nutritional demand for food protein of relatively high biological value especially in the dietaries of children. This phenomenon is particularly in evidence in diets wherein the quality and quantity of food protein are marginal without the inclusion of high proportions of sugars and fats.

One of the established dietary patterns in this country, which is frequently singled out for special comment, consists largely of whole ground cornmeal, fat-back or sow-belly, and molasses. Does anyone have doubts as to what effect the fat-back or sow-belly and molasses in this diet have on levels and adequacy of food protein ingestion? This dietary pattern seemingly is firmly entrenched. Perhaps we all prefer the diet we have *unless* one we like better is available to us. But if we look at this diet from the point of view of those who consume it, they seemingly have made a blind discovery of a principle in the physiology of nutrition; namely, how to reduce dietary bulk and increase satiety value — sugar and fat together will accomplish such effect. The consumers of this diet have apparently solved one problem of immediate concern to them and run afoul of long-term

consequences resulting from that solution. Sugars and fats are proper adjuncts to human dietaries so long as their consumption is restricted to quantities which do not prevent achievement of adequacy in the diet as a whole.

The subject matter of this chapter, in general, is concerned with the following: (1) A survey of the primary sources of food protein; (2) A summary of the efficiencies of farm animals as converters of feed protein to food protein; (3) Economy in food protein purchases; (4) Trends in consumption of various protein-containing foods in continental United States; (5) Total food protein supplies, continental United States; (6) The relationship of population density to levels and kinds of food protein consumed; and (7) The food protein problem of the poor.

Primary Sources of Food Proteins

Since all plant and animal tissues contain protein, it is clear that all foods comprised in whole or in part of such materials will provide protein in a measure dependent *both* upon the level of the protein in the food itself *and* upon the quantity of the food ingested. The plant kingdom alone possesses the ability of building proteins from constituents of the air and soil, using radiant energy of the sun in the process. By contrast, the animal body cannot build protein from any such simple nutrients but, nevertheless, continually uses protein in metabolism so that man, as well as other animals, is dependent upon plants for food protein supplies. Animal products, to whatsoever degree they are used as sources of food protein do so, in the last analysis, as a result of the conversion of plant proteins into such proteins as are represented in milk, eggs, and animal flesh. Plants, then, are the primary sources of all food proteins and of all animal-feed proteins. Of course no thoughtful biologist would ever contend that an intrinsic appraisal of plant proteins rests merely on their value as nutrients in foods and animal feeds. Plant proteins are essential to the life processes by which all nutriment for man and animals is provided. The aggregate proteins inherent in edible parts of plants rarely, if ever, are completely lacking in any one of the amino acids currently classed as indispensable components of the diet of the human species.[1]

Bacterial Synthesis of Protein. Certain nitrogenous substances other than protein can, to some extent at least, replace formed protein or amino acids in animal feeds. Several recent publications [2–6] leave no doubt about this matter. Urea and other simple nitrogenous compounds, especially when fed to ruminants, have been found to exert a dietary protein-sparing effect. This is generally credited to the activity of microorganisms which are able to utilize the nitrogen of these simpler nitrogenous compounds in the synthesis of their body proteins. The bacterial proteins thus formed add subsequently to the food protein supply of the animal. This phenomenon has been demonstrated most clearly in the digestive tracts of cattle, sheep, and other ruminants [6, 7] where the ingested feed stagnates and un-

dergoes extensive fermentation in the rumen, recticulum, and omasum — the so-called first, second, and third stomachs of the animal. However, this synthesis of protein by bacterial action in the digestive tract offers no exception to the general thesis that plants, and not animals, are the world's primary sources of food proteins. Bacteria are prevailingly considered to belong to the plant kingdom and it is they that build this protein in the digestive tract. It might be added that, morphologically, the digestive tract of any animal is but an invagination or infolding of the external surface of the body so that the contents of the digestive tract are still strictly outside the animal body proper.

The bacterial synthesis of proteins in the digestive tract is not limited to ruminants,[2] but because of the extensive bacterial activity in the esophageal pouches of such animals, and thus the greater opportunity for subsequent digestion and absorption, this phenomenon would seem quite certain to be of much greater importance in ruminants than in non-ruminants. In the practical feeding of animals this process has not been exploited to any important degree by the feeding of such substances as urea or ammonium salts. Proteins derived directly or indirectly from the higher plants remain the chief source of protein in animal nutrition.

Yeast Synthesis of Protein. Yeast plants can also synthesize protein from such starting materials as urea and ammonium salts. This knowledge is widely applied in the commercial manufacture of yeasts and, in the hands of German chemists, reached a rather high stage of development during World War I, as an outgrowth of the high cost and scarcity of grains in their country.[8] The production of protein from non-protein materials and the conversion of grain proteins through the activities and propagation of yeasts provide protein generally reported [9, 10] to be of relatively high nutritive value. Most of the yeast protein at present represented in human dietaries is largely incidental to the use of yeast as leavening agents in flour-converted products.

Dehydrated yeasts, containing 45 to 50 per cent of protein, have been tried, on more or less experimental bases, in human dietaries [11, 12] and have been used in emergency types of rations on occasion. Yeast proteins have also been shown to possess high nutritive value as protein concentrates in livestock feeds.[13, 14] For the present, yeast proteins, other than such quantities as are included by reason of the use of live yeast as leavening agent, have not been accorded acceptance to any significant extent in human dietaries. The dehydrated products are, by comparison with other available protein concentrates suitable for livestock feeding, relatively expensive. For these reasons yeast proteins can perhaps be regarded principally as potential reserves of food and feed protein which can be pressed into service if, or when, man sees fit to add them to his diet or eventually finds it economically expedient to use them in animal feeds.

Higher Plant Syntheses of Protein. In the higher (green) plants photosynthesis is, practically speaking, a necessary part of the production of

plant protein. Plants or leaves kept in darkness can synthesize protein but only if some available form of carbohydrate is provided, so in the last analysis the activities of the chloroplasts are an essential feature in the plant synthesis of protein.[15–17] The details of the process by which the higher plants build amino acids and proteins is not known.

The nitrogenous reserve substances in grains and seeds are largely proteins,[18] while those in underground storage organs such as fleshy roots and tubers often include relatively high proportions (50 per cent or more) of non-protein nitrogen [18, 19] including amino acids and amides such as asparagine and glutamine.[20, 21] In general the non-protein nitrogen compounds in tissues of the higher plants include various amino acids, amines, amides, purine bases, and alkaloids, the distribution of which is variable among species and organs. The extent to which various non-protein nitrogenous compounds, other than amino acids, in plant tissues can replace dietary protein is, in most instances, not known although the indications are that the amides in potatoes may be quite effective in this respect.[19, 22]

Practically the whole of the reserve proteins of plants consists of simple proteins and includes globulins, glutelins, prolamins, and albumins. In general the reserve proteins of plants contain from 2 to 3 per cent more nitrogen than the average for animal proteins. Of the twenty-odd plant proteins isolated by Osborne,[23] the percentage of nitrogen therein ranged from 15.9 to 19.0. This is the explanation for the smaller factors used for the conversion of percentages of nitrogen to corresponding percentages of crude protein in the case of most plant proteins as compared with animal proteins.[18, 21] The factors recently suggested by Jones [21] for use in converting percentages of nitrogen in various items of food (or animal feeds) into terms of protein are shown in Table 4–1.

Except for the family *Leguminosae*, it is generally accepted by plant physiologists that the higher plants, including those which commonly provide food proteins in human dietaries, absorb practically all of their nitrogen supplies in combined form (such as nitrates) from the soil. The *Leguminosae* also derive nitrogen by the same means but, in addition, have a second and more abundant source of nitrogen at their service as a result of their symbiotic relationship with the tubercle bacterium, *Bacillus radicicola*.[24–26] Early in the growth of leguminous plants their roots are infected with the tubercle bacteria from the soil. These bacteria take nourishment from the plant and later provide the plant with large amounts of fixed nitrogen derived from elemental nitrogen of the atmosphere. As a result of this extra source of nitrogen all tissues of leguminous plants are comparatively rich in nitrogen. The seeds of leguminous plants such as soybeans, peas, lentils, and various species of kidney beans are well known to be richer in protein than the seeds of many other plant families. For the same reason legume-forage for animals provides a richer source of protein than the grasses (*Graminaceae*) and various other forage crops.

Table 4–1. Factors Suggested for Use in Converting Percentages of Nitrogen in Various Substances into Percentages of Protein [21]

Substance	*Factor Suggested*
Cereal Grains	
Wheat, endosperm	5.70
Wheat, embryo	5.80
Wheat, bran	6.31
Wheat, whole kernel	5.83
Rye	5.83
Barley	5.83
Oats	5.83
Rice	5.95
Corn (maize)	6.25
Oilseeds and Nuts	
Hempseed	5.30
Cottonseed	5.30
Sunflower seed	5.30
Flaxseed	5.30
Squash seed	5.30
Pumpkin seed	5.30
Sesame seed	5.30
Cantaloupe seed	5.30
Almonds	5.18
Coconut	5.30
Brazil nut	5.46
Hazelnut	5.30
Walnut	5.30
Peanut	5.46
Soybean	5.71
Butternut	5.30
Castor bean	5.30
Substances of Animal Origin	
Milk	6.38
Eggs	6.25
Meats	6.25
Gelatin	5.55
Leguminous Seeds	
Navy bean	6.25
Lima bean	6.25
Mung bean	6.25
Velvetbean	6.25
Adzuki bean	6.25
Jack bean	6.25

The proteins in leaf and stem tissues and in the berries, pomes, drupes, fleshy achenes, etc., commonly called fruits, have not been intensively studied. In general the nitrogenous materials contained in leaves are to be considered as rather transient materials awaiting transportation to the stem, roots, or seeds of the plant. The fleshy leaves of such plants as the onion and cabbage, however, are exceptions to this generalization since these represent permanent storage organs comparable to fleshy roots. As Pirie [27] has pointed out, leaf proteins represent virtually untapped resources of proteins that could be partially extracted and used as human food if, or when, there is real need for this. At present the total proteins ($N \times 6.25$) of plant materials, other than those in seeds and storage organs of plants, probably do not contribute more than 5 per cent of the crude protein in the average dietary of the United States. In view of the fact that asparagine (semi-amide of aspartic acid) probably represents both a nitrogenous end-product of protein catabolism in plants as well as a nitrogenous product for the synthesis of plant protein,[15–17] it is very likely that the nitrogenous matter of leaves and germinating seeds and grains will be found generally to contain very appreciable proportions of nitrogen in this form. It is generally supposed that glutamine, which is also widely distributed in plants, may have functions similar to those of asparagine.[15–17]

Most protein-containing foods representing tissues of the higher plants are associated with moderate to rather high proportions of carbohydrates as well as with more or less indigestible fiber (cellulose and similar roughage types of materials). A few plant proteins such as those in common oil-bearing seeds, edible nuts, the coconut, embryo portions of grains, the avocado, etc., none of which provides more than rather insignificant quantities of protein in most human dietaries, are associated with substantial proportions of fats or oils. By contrast, food proteins of animal origin are predominantly associated with fats and carry little carbohydrate and no roughage materials. Milk proteins, of course, are associated with fat and carbohydrate (lactose) in more or less similar proportions, and liver usually contains appreciable amounts of carbohydrate also.

Protein-Sparing Effect of Carbohydrates. Both fats and carbohydrates can spare protein by serving as sources of food energy and thus relieve the necessity for the use of protein as fuel. In addition to this, carbohydrates have long been known to exert a *specific* protein-sparing effect by reason, presumably, of reactions between certain intermediary products of carbohydrate metabolism and the end-products of protein metabolism. When dietary supplies of protein are minimal or restricted, carbohydrates exert a marked protein-sparing effect provided they are the major source of food calories and total energy requirements are at least covered. This attribute of carbohydrates may well be a highly important factor of economy particularly in the nutrition of people in certain densely populated countries and/or of people in low-income groups where the dietary conditions just mentioned

very frequently prevail. In Figure 4–1, reprinted here from a publication of Bennett,[28] are shown the relative positions of potatoes, wheat, and other cereal grain products as provisioners of calories in the average national

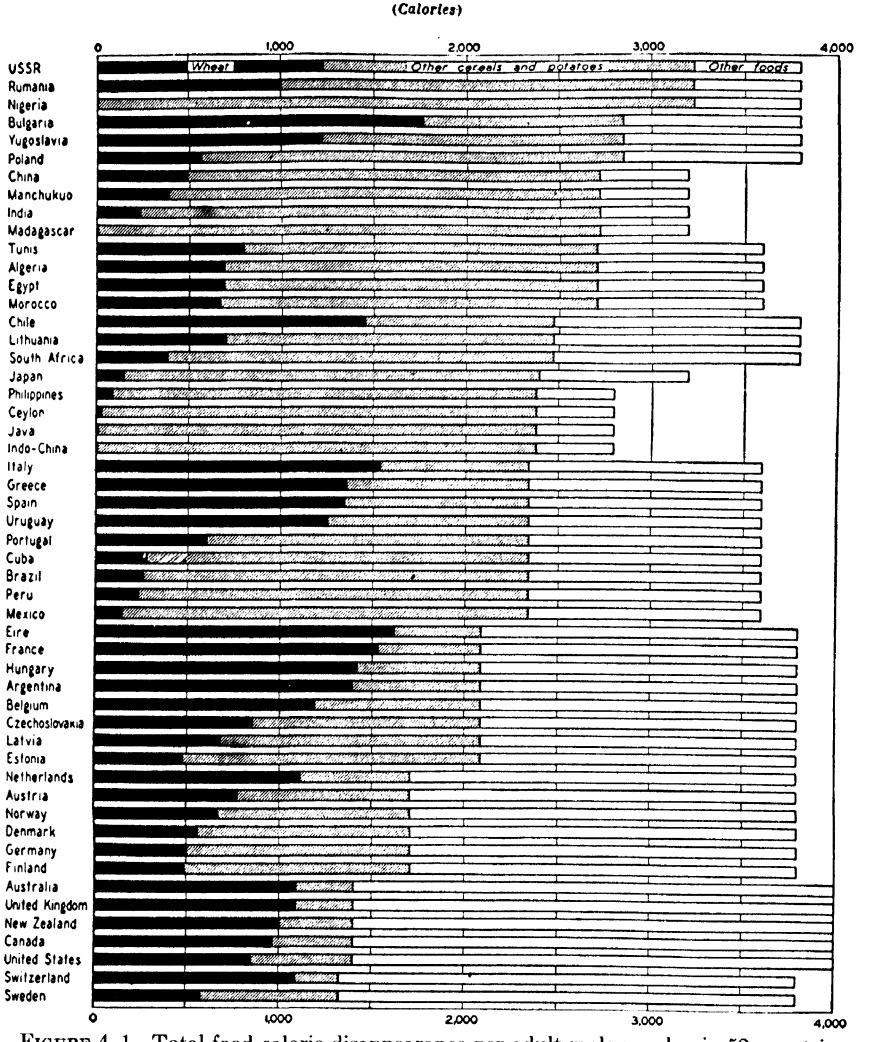

FIGURE 4–1. Total food-calorie disappearance per adult male per day in 52 countries (1933–38), contributed respectively by wheat, other cereals and potatoes, and other foods.[28] (*Courtesy Food Research Institute, Stanford University, California*)

dietaries, computed on the basis of calories per adult male per day, for 52 countries. As will be clearly evident later on in this chapter, the average national dietaries associated with these higher proportions of food calories provided in the form of carbohydrates (mostly starches) are also those usually most restricted in food protein. It is thus apparent that carbohy-

drates can function and probably do function specifically to effect economy in the utilization of food protein.

Effect of Roughage Materials. In large degree the presence of roughage in plant tissues accounts for the lower digestibility of plant proteins as compared with proteins of animal origin. The experiments of Snyder [29] conducted in 1899 and 1900 on the digestibilities of bread made with whole wheat and white flours demonstrated this phenomenon very clearly and these results have been confirmed many times since on humans and on other species of animals. It is now general knowledge that with increase in the crude fiber content of diets the digestibility of dietary protein (and of other nutrients also) is correspondingly decreased. This observation has been taken into account in the Mitchell method [30] for determining the biological values of food proteins since the level of roughage influences the quantity of nitrogen absorbed. Nevertheless, from a practical standpoint some sacrifice in the net utilization of dietary protein has to be accepted in the feeding of all animals nourished either on mixed or vegetarian food supplies as a natural consequence of the roughage present in many foods of plant origin.

According to investigations of Cowgill and Anderson,[31] a satisfactory laxation rate in human adults on ordinary types of mixed diets is usually accomplished by a daily ingestion of 90 to 100 mg of crude fiber per kilogram of body weight, or by a total daily ingestion of 6 to 7 grams of crude fiber for a man of 70 kilograms. Naturally some considerable variations were observed among individuals. It is also to be noted that the fiber in foods is not all of one type nor are all indigestible to the same degree. The kind, the quantity, the degree of digestibility, and the state of subdivision of roughage materials in diets are each an important factor in determining the relative acceptability or non-acceptability of diets as well as in affecting the net utilization of food nutrients. The calorie, protein, vitamin, and mineral values jointly considered by no means determine the practicability of human dietaries. The general character, palatability, digestibility, assimilability, texture, and state of concentration of foods which go to make up a total diet are of great importance in both human *and* animal feeding. This subject has been accorded more consideration usually in animal than in human feeding.

Snyder,[29, 32] Lawes and Gilbert,[33] Martin and Robison,[34] and Macrae *et al.*[35] all found that unreasonably high proportions of crude grain fiber in human diets are accompanied by adverse physiological responses including discomfort and difficulty in consuming such diets. It has also been shown [32, 35] that in the case of whole grain products finer grinding increases the level of physiological tolerance for the crude fiber therein. However, our common fruits and garden vegetables also contain considerable fiber so that any rational program which purports to alter the consumption of foods of higher or lower than usual fiber content commands considered and prac-

tical judgment of the fiber content and the bulk of the diet *as a whole*. It should be obvious that for individuals whose total food requirements are relatively small (sedentary types of individuals), as compared with those of laborers or otherwise active persons whose food ingests are large, whole grain types of flour or bread might be acceptable and advantageous and vice versa.

The trend toward reduction of the bulk and roughage in human dietaries is very obvious to any and all who follow dietary trends. It was unmistakably evident at least as far back in time as ancient Egyptian civilization and the trend is continuing and expanding. Sugimoto [36] reports that about 200 years ago in Tokugawa's Era, the Japanese began to eat polished rice and since then its use has gradually become universal in Japan. The same thing is happening in China and India and elsewhere. The steady tendency to reduce dietary bulk and food roughage is clearly evident in this country as we have turned more and more to concentrated forms of foods (sugar, fat and oils, meats, etc.) with the major part of our dietary roughage and bulk derived from fibrous fruits and vegetables.

The diets of people in the low-income groups are likely to be bulky and high in roughage. The fruit and vegetable (salads and the like) eaters are predominantly among those whose diets are decidedly satisfactory in satiety value and not overloaded with indigestible residues derived from other foods. The desire of the low-income groups as regards less bulky and less roughage types of diets will very likely have to be satisfied before substantial quantities of fruits and vegetables will be acceptable to them.

Sugimoto [36] reports a very pertinent experiment on the subject of various degrees of rice milling in relation to Japanese dietaries. Adult Japanese subjects given an allotment of about 650 calories per day in the form of bean paste, soybean curd, and small amounts of other vegetable products were allowed in successive periods such quantities of polished rice, half-polished rice, 70 per cent polished rice, and unpolished rice respectively as were desired as sources of food energy. All subjects greatly reduced their calorie intake during periods when the unpolished rice was provided, consuming much less rice in this form than would meet energy requirements. The experiment was repeated with the same results. The investigator concludes: "It appears that in the unpolished rice period not only is the caloric value insufficient for the requirements of the body, but the absolute output of food in the feces is greater than in any of the other periods when rice of other grades of polishing is eaten; the nitrogen balance under these conditions is almost always negative." The practical implications of such experiments as these cannot be ignored, for the masses of undernourished people will give little heed to the "natural food" recommendation if their diets as a whole do not provide physiological satisfaction.

There can be no doubt but that the bulkiness and the high proportion of cellulose and similar materials in diets comprised solely or in high degree of

unrefined plant materials — whole grain meals, fruits, and vegetables — is a major disadvantage in the use of such diets. The importance of roughage content and satiety value of diets especially as they relate to low income groups and working men was clearly recognized by scientists at least as far back as 1858.[33] Knowledge of the importance of *enough but not too much* roughage is fully appreciated and applied in the feeding of livestock, even in the case of dairy cows which have a relatively enormous tolerance for roughage compared with man. The fallacy and folly of disregarding this factor in human nutrition is abundantly apparent, and no universal prescription can be given for the quantity of roughage human dietaries should provide.

Plant Protein Contributions to Human Dietaries. Plant products, directly consumed, provide for more protein in human dietaries than animal products. The cereal grains as a group, and directly consumed, provide far more protein than any other major class of foods. Rice, strange as it may seem, probably provides more food protein in the world's dietaries than any other single food item. It is very obvious why plant proteins occupy this position in the world's food protein economy — they are the primary sources of food protein and, therefore, the least expensive. The economic position of various grains relative to one another has been comprehensively reviewed by Jasny [37] and by Wickizer and Bennett.[38] The great majority of the world's population can afford neither to produce nor to buy more than very limited quantities of protein-containing foods of animal origin. The relatively high cost of animal proteins or of animal products is inherent in the inefficiencies of animals as converters of feed protein and other feed nutrients (all of which, in the last analysis, are derived from plants) into animal proteins and other formed nutrients in animal products. It has been estimated by Leitch and Godden [39] that, considering the total livestock population of the United Kingdom for the years shortly prior to World War II, the efficiency of conversion of feed protein to food protein was 11 per cent. As will be apparent later, this low efficiency of protein conversion by livestock is accompanied by certain other attributes which in some considerable measure offset the high costs entailed in the conversion. Nevertheless, the low efficiency of livestock as converters of plant to animal forms of protein is an enormous factor in the differences which prevail in cost between plant and animal proteins, or more practically, between foods which represent important sources of plant and animal proteins respectively.

Rice provides the inhabitants of the densely populated areas of Monsoon, Asia with a large and decidedly major proportion of their total protein as well as calories.[38, 40] The proteins of rice and maize supply the average native of Java and Madura with approximately 75 per cent of his total dietary protein.[41] The protein in the average national diet of China is in large degree provided by cereal grains, among them rice, wheat, millet, maize, oats, kaoliang, and barley. Scientists reporting from China,[42]

India,[43–46] and Japan [47] on the protein quality of rice actually used in those countries leave little doubt but that the protein in this cereal leads practically all other common cereals in excellence of its protein. In many regions of China the cereal component of the diet consists of mixed cereals. Adolph [48] states that in a given community the proportions of various cereals in the mixture come close to being that which would provide the maximum in nutritive quality of protein. This practical and applied knowledge of nutrition has been forged from the depths of long race experience.

In recent years plant proteins have provided about 40 per cent of the total protein in the average diet of the people of the United States. Among various nations of the world plant proteins, consumed directly as food, contribute to average national dietaries almost any proportion between 40 and 90 per cent of the total dietary protein.

The protein in plant tissues is always comprised of several different proteins, never is it confined seemingly to a single protein. Although an individual or isolated protein obtained from various agricultural food crops may completely lack one or more of the indispensable amino acids (zein of maize, for example, lacks certain indispensable amino acids), it has never been demonstrated that the total protein of maize or of any important food crop is completely lacking in any one of the indispensable amino acids. The protein of gelatin, a derived protein product, is incomplete, and the nutritive qualities of many pure proteins of animal origin have never been assessed. Nevertheless, it is clearly obvious that no distinction can be made between the total protein in foods of plant and animal origin respectively on the basis of the completeness or incompleteness of their total proteins.

Almost any ordinary protein-containing foodstuff comprised of plant tissues, if consumed in quantities which would provide adequately for calorie needs, would at the same time adequately cover maintenance needs for food protein and allow a considerable margin of excess.[1] Because of various supplementary relationships among plant proteins, many combinations of protein-containing plant foods would provide more liberal supplies of food protein than many plant proteins consumed as the sole source of protein. It should not be assumed, however, that the proteins of all combinations of protein-containing foods are supplementary. Neither can it be assumed that the proteins in two or more protein-containing foods of plant origin, because they are supplementary in some one set of proportions, are necessarily supplementary in any and all proportions. Of course human dietaries rarely contain only one source of food protein regardless of whether the diet is of vegetarian or near-vegetarian type, a mixed type, or is composed entirely of animal products. As long as the food protein from single or mixed food sources is complete; *i.e.*, devoid of no indispensable amino acid, such protein could, if eaten in the proper quantity, supply *all* food protein requirements. The quantities of food protein required for a given

physiological function (maintenance, growth, etc.) would, however, vary inversely with the biological value of the protein. In the case of many food plant materials, however, the level of protein in the product, for example in spinach, would be limiting. In other words, one could not possibly eat enough of some food plant materials to provide the necessary food protein even for maintenance.

A diet of common food items then would generally have to be low in calorie value or very high in fats, sugar, or purified starch before an adult would be in danger of short supplies of protein for purposes of body maintenance. Presumably man does, in some regions of the world, consume diets with high proportions of foods very low in protein. One such diet, we may assume, would be provided where calorie needs are in large measure supplied by cassava (also called manioca or mandioca), the material from which our tapioca and arrowroot starch are obtained. The fleshy roots of the cassava, usually of the bitter species (Manihot utilissima), after processing to remove the hydrocyanic acid, are widely used in Brazil and a few other tropical regions where it is grown as a cheap source of food energy. The cassava root is very low in protein, much lower than our white potato. In the laboratory, of course, one could arrange almost any number of diets with the protein component inadequate for maintenance, but in practical human dietaries such diets appear to be very uncommon. Neither man nor animals will long consume diets highly deficient in protein except under the most stringent circumstances. Even if the protein ingest is rather restricted the body possesses a capacity for increasing its efficiency in the utilization of protein.

Nutritional surveys accompanied by investigations for recognizable signs of protein deficiency states [49] among various groups of people in this and other countries have been singularly in agreement in bringing to light little evidence of specific protein undernutrition [50, 53] among adults when the calorie values of the diets were adequate. Thus the saying of Bayliss, "Take care of the calories and protein will take care of itself" is in large measure the case for the average adult at least.

Infants, children, and pregnant and lactating women have additional food protein requirements over and above those required for maintenance by reason of having to build new protein. Terroine [1] considers that even these extra food protein needs could be adequately met by almost any common protein-containing food provided such food is eaten in quantities which will cover energy requirements. We do not have experimental evidence to prove or disprove this supposition. Terroine's argument is that the increased need for calories would automatically increase the level of food protein intake sufficiently and that most of our deductions about food protein requirements for growth, etc., are based on short-lived, very rapidly growing animals (such as rats and farm animals) which must build protein at much greater rate relative to body size than the human child. However,

for various reasons and in spite of the strength of the arguments presented the writer does not consider a strictly vegetarian diet practical for all persons beyond weaning age.

The thesis of Terroine rests on the condition that the calories of the diet are to be derived from common protein-containing foods. In practice children's diets as well as those of pregnant and lactating women, in this, and various other countries, include substantial proportions of calorie value from sugars and visible fats which do not provide food protein. Secondly, it is generally recognized that among peoples on near-vegetarian diets, such as the majority of the Chinese and Asiatic Indians consume, there is difficulty in the rearing of children. Adolph,[48] who has spent years in the Far East, considers that food protein is the number one qualitative need for the improvement of diets in that region. We note also that such protein deficiency states as have been noted in nutritional surveys made in this country show higher incidence among growing children than among adults.[52] It would appear, therefore, that infants, children, and pregnant and lactating women are the real benefactors of foods of high biological value. Because of the calcium and riboflavin values associated with milk proteins, it would be logical and at the same time the most economical solution to stress the use of milk as the preferred animal product for providing the extra protein needs for children and for pregnant and lactating women.

The author is not advocating for anyone an abstemious use of animal proteins but merely pointing out the economic factors involved in the use of plant and animal proteins for purposes of supplying the needs for food protein. Probably most of us would be no happier swelling the ranks of herbivore than of carnivore. It will be left to the reader to justify luxus consumption of high quality, expensive food proteins if he feels impelled to, but it is not in the nature of things to justify it or the basis of nutritional need or economy in the utilization of food protein.

Animals as Feed Protein Converters

The exploitation of plants as primary sources of animal-feed proteins and other feed nutrients has made possible in some countries, in general in those less populated and/or more advanced in industrialization, a program of very intensive animal husbandry. We do not have accurate data on either the world's total human or animal population, but various recent estimates would suggest a figure of about 2.2 billions for the former and normally on the order of 1.75 billions for the latter (exclusive of poultry but including livestock such as oxen and water-buffaloes, etc., kept primarily as beasts of burden). Even admitting the roughness of these estimates, it is, nevertheless, a fact that much of the world's food protein economy does, and must of necessity, rest on primary rather than secondary sources of food protein.

The livestock population, exclusive of horses and mules, in the United States as of January 1, 1942 was estimated [54] as follows: 75,162 thousand

head of cattle (including 26,398 thousand head of dairy cattle and dairy heifers 2 years old or over), 56,735 thousand head of stock sheep and feeder lambs, 60,377 thousand head of hogs, 474,910 thousand chickens, and 7623 thousand turkeys. The figures for poultry would not include those raised and sold within the year. Based on estimates of the Bureau of the Census,[55] the total human population of the United States as of January 1, 1942 was 133,953 thousand. It is obvious, therefore, that such livestock numbers in relation to numbers of people represent a very substantial reserve of food (and food protein) which would cushion the impact of temporary food shortage. Various other countries possess similar food reserves. Any country with large livestock numbers can, through liquidation of a suitable proportion of these and by falling back upon increased use of primary foods which otherwise would have been consumed by the livestock, withstand dislocation of the food economy that otherwise would be attended with disaster. In short, intensive livestock husbandry signifies wealth in terms of land capable of producing food or in terms of goods exchangeable for feedstuffs.

Grazing animals (cattle and sheep) can, of course, be fed entirely on range and pasture grass. Such feeding practice is, however, not at all usual since in the case of dairy cows the milk yield would be low and in the case of meat-producing animals the low weight of the animal and inferior quality of the meat would operate against maximum success of the enterprise. The usual procedure is to feed dairy cattle grain and other feed concentrates in addition to pasture grass, silage, hay, etc. Range and grass-fed beef cattle are commonly fattened in dry lot with grains for some months before marketing. In general, hogs and poultry are provided with a larger share of their feed in the form of grain than other types of livestock; dairy cattle come next, and beef cattle and sheep follow in order. It must not be forgotten, however, that livestock as a whole do derive a large share of their feed requirements from materials unsuitable for human consumption.

Our total livestock population, exclusive of horses and mules, consumed during the year beginning October 1, 1941, about 85 million tons of grain and about 1.8 million tons of dry weight equivalent of skim milk, buttermilk and whey, including milk fed to veal calves.[54] These products comprise the major share of the materials suitable for direct human consumption which were consumed by livestock for purposes primarily of increasing food supplies in the form of animal products. Materials suitable for direct consumption by man are reputed to comprise about one-third of the digestible nutrients consumed by livestock in this country. The remainder of nutrients is obtained from roughage materials including pasture and range grass, hay, stover, silage and beet pulp, and feed concentrates including oil-seed cakes and meal, millfeeds, brewer's and distiller's grains, gluten feed and meal, tankage, fish-meal, etc. Of course where the density of population requires diets of lower proportions of animal products, some of these

products raised for animal feeds (for example, timothy, alfalfa, and clover hays) and some of the materials representing by-products of other industries would not be produced. In such areas the program of agricultural production would be altered in major ways, along with the material standard of living.

Both livestock and cultivated plants do provide other materials than food, including food protein, but these are not of concern here. In return for the feed protein (in the last analysis derived from plants) we obtain food protein of enhanced biological value in products widely valued by the majority of people as highly palatable foods — milk, cheese, eggs, and meats. Most persons accept these foods as providing important dietetic values and satisfactions, some two million or so persons in this country rejecting consumption of flesh foods. These values, like material values of everything else, are not obtained without cost in terms of labor and materials which in turn is to be paid for by the consumers of these products. World food economy does not make it possible for us to obtain something for nothing. You and I and everybody else are tradesmen, exchanging some form of goods or services for other goods and services.

Animals even under the best feeding practices are not efficient converters of primary food protein. Depending upon species of animal, product produced, level of production, feeding practice in relation to productive capacity, length of the feeding period of the animal considered, and so forth, the efficiency of conversion of feed protein to food protein can probably be said to range between about 8 and 45 per cent, excluding breeding stock animals. The higher percentage applies to the milk cow considered *only* during a relatively short period of maximum milk production and not of the cow during an entire life span or any substantial part of that time. The overall estimate of 11 per cent efficiency of protein conversion for all livestock of the United Kingdom was mentioned earlier. As overall estimates, various authors suggest on the order of 18 per cent for the dairy cow (complete life span including calf and cow beef production) as well as for poultry and egg production, and roughly 10 per cent or less for meat production other than the types just mentioned.[56, 57] Regardless of what particular figure one chooses or considers fair or representative of the situation, the fact remains that for a given production of food protein, livestock must consume manyfold such quantities of protein in the form of feed.

In order to present a brief picture of the relative efficiencies of farm animals as converters of feed protein to food protein, the author has selected from a large volume of literature on this general subject what seemed best adapted to yielding a concise resumé of the available information in simple, direct, and readily comprehensible terms. Various reviews of this subject have recently been reported by Leitch and Godden,[39] Jennings,[58] and Maynard.[59] The reviews just cited are based upon original data and/or earlier reports of many contributors, and through these reviews the reader

Table 4–2. Efficiency of Production of Food Protein and Food Energy from Feed Protein and Feed Energy Respectively by Various Species of Farm Animals *

	Efficiency	
	Energy (%)	*Protein* (%)
Milk †		
Winter feeding:		
7.2 gals. daily	40.9	47.0
4.8 gals. daily	35.1	43.5
2.4 gals. daily	24.6	35.1
Summer feeding: grazing only		
4.8 gals. daily	29.5	25.7
Complete year:		
720 gals. milk / 1 calf: birth value — Spring calving	19.5	23.6
720 gals. milk / 1 calf: birth value — Autumn calving	17.1	20.0
Whole life:		
2160 gals. milk / 3 calves: birth value / Cow beef — Average of spring and autumn calving	13.9	18.1
Eggs		
Medium light bird: average weight 4 lb		
1 egg daily	19.6	43.6
200 eggs yearly	13.3	29.8
120 eggs yearly	9.0	20.2
Medium heavy bird: average weight 5.5 lb		
1 egg daily	17.7	39.7
200 eggs yearly	11.8	26.4
120 eggs yearly	7.9	17.7
Table Poultry		
Spring chicken: 3.75 lb at 15 weeks	5.8	26.3
Fat cockerels: 4.75 lb at 18 weeks	8.2	21.8
Beef		
Baby beef: birth to 7 cwt. live weight	8.6	15.5
Baby beef: birth to 9 cwt. live weight	11.1	11.1
Fat bullock: birth to 10.7 cwt. live weight (grass fattened)	8.2	8.9
Fat bullock: birth to 12.5 cwt. live weight (stall fattened)	7.9	8.7
Fattening period only: winter feeding		
9 cwt. to 10.7 cwt. live weight	16.0	11.9
10.7 cwt. to 12.5 cwt. live weight	16.3	9.0
Fattening period only: grass fattened.		
8.5 cwt. to 10.7 cwt. live weight	15.7	5.1
Lamb		
Feeder lamb: fattening period		
70 to 100 lb in 12 weeks	17.2	13.2
Bacon and Pork		
Bacon:		
40 lb to 220 lb live weight: restricted feeding	28.2	15.2
40 lb to 220 lb live weight: unrestricted feeding	22.3	13.3
Pork:		
40 lb to 160 lb live weight: restricted feeding	27.4	19.0
40 lb to 160 lb live weight: unrestricted feeding	22.1	15.3

* Data in above table are taken from publication of Leitch and Godden.[39]

† Volumes of milk originally reported in imperial gallons are here computed to U. S. gallons.

who so desires will have no difficulty in tracing most of the major reports available on the subject.

The author is presenting here in Table 4–2 a brief summary of the data reported by Leitch and Godden and will confine the discussion to comments in explanation of the data therein represented with only brief reference to such other pertinent information as seems desirable to a comprehensive view of the situation. As a matter of general interest, the data in Table 4–2 include also the figures from Leitch and Godden on the efficiency of energy conversion by various farm animals.

Dairy Cows as Converters of Feed Protein to Food Protein. The mature dairy cow has three basic needs for protein; namely, for body maintenance, for production of new tissue associated with calving, and for production of milk. The dairy cow can thus be evaluated as a converter of feed protein to food protein on various bases. Considering the protein-cost of three calvings, milk production (2,160 gal total), and meat production as cow beef, the average dairy cow (approximately 1,100-lb body weight) has an overall efficiency for protein conversion of about 18 per cent.

If the protein-cost of calving and protein-cost of cow beef production are eliminated from consideration, the average dairy cow will be rated a much more efficient converter of protein. In other words, milk protein production is a much more efficient process than production of flesh protein, and production of protein represented in that stored in the calf is more efficiently produced than cow beef. For a given size cow, the efficiency of milk protein production will of course vary with the yield of milk. All dairymen recognize that high milk production is important to the highest success of their enterprise, and the most favorable breeding and feeding practices are arranged accordingly.

The greatest interest in the dairy cow is of course milk production, which is the prime reason for breeding and raising dairy cows. Since milk proteins together with other nutrients of milk combine to provide the most valuable food of all animal products, the greatest importance attaches to the efficiency of dairy cows as milk producers with veal and cow beef production largely assessed as by-products, these latter not without real value in their own right, naturally. As a converter of feed protein to food protein considered apart from meat production, then, a well-fed, high milk producing strain of dairy cow would rank above all other farm animals. The average dairy cow, as usually fed, would very probably derive about one-third of her total nourishment for milk production from grain and the rest from roughage materials and feed concentrates largely unsuitable for direct human consumption. Halnan [60, 61] assesses the efficiency of the dairy cow producing 720 gallons of milk (roughly 6,200 lb) a year at 35.8 per cent; that is, considering only the protein-cost of producing the milk and maintaining the cow for the year. This figure is in excellent agreement with that reported by Leitch and Godden.

From the standpoint of land-use and number of farmers who include it among their farm-operations, milk production is of great significance in our total agricultural economy. There are on United States farms at present over 25 million head of dairy cows, and milk is produced on about 70 per cent of all farms in the United States.[55] The average cow is said to remain in the milking herd about 5 years [62] so that each year something over 5 million dairy heifers under one year of age and around the same number between one and two years of age are in process of being raised as replacements for the milking-herd. The first calving of dairy heifers is usually at 2 to 2½ years, depending upon rate of growth and maturation of the animal.

The average milk production per cow on farms in the United States, excluding that sucked by calves, is estimated [55] to be around 4,600 pounds per year. A national average such as this represents of course includes milk production by cows that were bred as beef cattle with milk production merely an incidental by-product, some cows that certainly must not have been well-fed, and cows with inherently widely different milk-producing capacities. It can be concluded beyond doubt that the dairy cow, if properly fed and managed, possesses the highest degree of efficiency of all livestock in the conversion of protein in feedstuffs into protein (milk) for human dietaries.

Poultry as Converters of Feed Protein to Food Protein. Chickens as they appear in human dietaries can for the most part be placed in three general categories depending on weights and ages; namely, broilers, fryers, and roasters. According to Chatfield and Adams,[63] the live weights of broiler-class chickens range from 1½ to 2½ pounds, age 8–12 weeks; the live weights of fryers from 2½ to 3½ pounds, age 14–20 weeks; and roasters include live weights beyond 3½ pounds and are usually from 5- to 9-month old birds. The edible portion of the flesh of birds in each category, including as edible portion the flesh, fat, skin, and giblets, contains very nearly the same proportions of protein, roughly 20 per cent, except in case of very fat birds.

According to poultry nutritionists,[64, 65] the protein in the feed of chickens should be around 18 to 20 per cent of the weight of the feed for best practical results, with some small variations on one side or the other of this range recommended at different stages of growth. In general, successful poultry producers appear not to vary the protein content of the rations much outside of this range of 18 to 20 per cent, nor do poultry nutritionists in general recommend much deviation from this range.

Ewing [65] reports that a broiler type of chicken, 12 weeks of age and weighing on the average 2.57 pounds (live weight) would consume on the average 3.64 pounds of feed per pound of meat produced. Assuming then that the feed contained 18 to 20 per cent of protein and that the meat produced contained roughly 20 per cent of protein, the efficiency of conversion of feed protein to meat protein would be on the order of 27 to 30

per cent. In similar studies, 17-week-old fryers averaging 3.52 pounds live weight consumed 4.80 pounds of total feed for the production of one pound of meat, thus effecting an efficiency of feed protein to meat protein conversion of around 21 to 23 per cent. Similarly 20-week-old chickens averaging 4.17 pounds live weight consumed an average of 5.28 pounds of total feed per pound of meat produced, the efficiency of feed-protein conversion in this case amounting then to 19 to 21 per cent.

In comparison with the above data, Halnan [60] of the Animal Nutrition Institute of Cambridge, England, has assessed the efficiency of the hen as a converter of feed proteins to meat proteins at 18.0 per cent and Leitch and Godden (see Table 4–2) for spring chickens and fat cockerels at 21.8 and 26.3 per cent respectively.

The lower efficiency of conversion with increasing weights of the birds is in large measure due to corresponding increases in the protein-maintenance requirements; in other words, *increment* in protein must be added on to a protein base maintenance which steadily increases with the size of the birds.

Considering egg production, Ewing [65] reports that in a well-managed, full-fed flock around 24 grams of feed protein are required by hens producing eggs at the rate of 220 eggs per year for production of a standard sized egg weighing 50 grams and containing 6.7 grams of protein, such representing a high rate of egg production. The efficiency of conversion of feed protein to egg protein is, therefore, around 28 per cent, whereas Halnan [60] of England reports an efficiency for the hen producing 140 eggs per year the figure 31.6 per cent. Leitch and Godden (see Table 4–2) report for the hen producing 120 and 200 eggs per year figures of 20.2 and 29.8 per cent respectively. These percentages for efficiency of protein conversion in egg production refer only to total protein-costs entailed during the egg producing period; the cost of producing chickens for meat is here considered separately as a distinct enterprise.

It is clear, therefore, that chickens, as producers of eggs, provide a higher quality of food protein and produce it more efficiently than chickens raised for meat production. Chickens and other forms of poultry, when fed according to the feeding practices recommended for maximum yields of edible products and for maximum profit to producers, are potential competitors of man for the foods consumed in substantial degree.

Turkeys and chickens of all types produced in the year beginning October 1, 1941 are estimated to have been provided with 338 thousand tons of dry weight equivalent of skim milk, buttermilk, and whey, of which somewhat less than two-thirds was fed to hens and pullets, and with 20,869 thousand tons of grain including corn, oats, barley, sorghums, wheat, and others.[54] Dry skim milk is considered a highly desirable feed concentrate in chicken feeds because of the riboflavin and pantothenic acid content of milk as well as the excellent quality of the protein provided.[64]

Beef Cattle as Converters of Feed Protein to Food Protein. In average per capita consumption in the United States, the use of beef exceeds that of any other type of meat except pork. By comparison, the consumption of lamb and mutton is only about 10 per cent of that of veal and beef combined.

Beef cattle are of course especially bred to obtain stock that has large and efficient flesh-producing capacity. Although other types of beef including culled stock from dairy herds and cow beef add considerably to the total beef consumption by way of canned and other processed products and less expensive types of beef as well, the discussion here will deal principally with the efficiency of beef-bred cattle in converting digestible crude protein of feedstuffs into edible meat proteins.

Haecker [66] has estimated that around 48 to 52 per cent of total protein in veal calves (100- to 250-pound live weight) would be represented in the edible parts of the animal; of the total protein in yearling beef calves (500- to 600-pound live weight) roughly 55 to 65 per cent is available in the edible parts, and for steers of 1,200-pound live weight on the order of 62 per cent of the total protein is represented in edible parts of the animal. This does not mean that the rest of the feed-converted protein is of no use; some is represented in hides used as leather, and meat scraps are used in livestock feeds, etc., but it does mean that much of the conversion of feed protein by beef cattle is directed into other channels than foods for human dietaries. Haecker's data on the efficiency of the beef steer in converting feed protein to beef protein are for calves reared from an initial live weight of about 100 pounds to successive 100-pound live weight increments up to and including beef steers of 1,413 pounds live weight. The efficiency of feed protein to food protein conversion of course varies with the size of the animal produced, varying throughout the entire range of relatively short-term intensive feeding between about 11 and 14.5 per cent. It is to be noted that less intensive feeding, and storing the animals over longer periods, would result in significantly lower efficiencies in protein conversion.

Halnan [60] reports an efficiency for conversion for feed protein into edible meat proteins in the form of baby beef at 7.8 per cent, and for larger beef cattle and grass-fed beef efficiencies of conversion of 5.9 per cent (Norfolk beef) and of 5.4 per cent (grass-fed beef). The data of Leitch and Godden are shown in Table 4–2.

As significantly offsetting this relatively low feed protein to food protein conversion of beef animals, the reader's attention is called to the fact that such animals normally derive a large share of their protein from pasture or range grass and other types of roughage materials. Most of the grain and other feed concentrates fed to beef cattle is provided during the fattening period just prior to marketing. In the United States, the total grain consumption by beef cattle during the year beginning October 1, 1941 was estimated at 7,888 tons,[54] a relatively small proportion of the total tonnage of all feed consumed by these animals.

Hogs as Converters of Feed Protein to Food Protein. The hog is a heavy fat-producer as well as the largest producer of meat of all livestock species raised in this country. Halnan [60, 61] reports an efficiency of the hog as a producer of food protein from feed protein of 21.2 per cent. The data of Leitch and Godden (see Table 4–2) indicate the relative efficiency of the hog raised to 160-lb and 200-lb live weights respectively as converters of feed protein to pork protein. The fat hog, of course, since emphasis in feeding is on bacon and fat production with feeding arranged accordingly, is the less efficient type in protein conversion.

Hogs are heavy consumers of primary foods as well as secondary foods which are suitable for direct consumption by man. In this category, it is estimated that for the year beginning October 1, 1941, hogs in this country consumed 38,527 thousand tons of grain, 30,509 thousand tons of which was corn, or roughly about 40 per cent of the total corn *produced* in our country that year. Hogs also were fed during the same year 904 thousand tons of dried milk products expressed in terms of dried equivalent of skim milk, buttermilk, and whey fed on farms.[54] From a nutritional standpoint considering the relative merits of various animal products, the use of any milk suitable for human consumption that is fed to hogs (or poultry) is an unfortunate situation and a misuse of a highly nutritious food. In spite of the protein in grains, milk products and various other feed concentrates fed to hogs produced in this country, Jennings [58] states that hog-feeding practices, considered on a national scale, would be improved by feeding substantially more feed protein concentrates than have been provided in recent years.

Of the total 1.8 million tons of dry weight equivalent of skim milk, buttermilk, and whey fed to livestock on farms, approximately 90 per cent of this was fed to hogs and poultry.

Sheep as Converters of Feed Protein to Food Protein. Sheep and lambs, to greater extent than any other species of livestock, derive nourishment from grazing on pasture and range grass. Only a relatively minor part of their nourishment is derived from grains and other concentrated feeds. The efficiency of lambs as feed- to food-protein converters has not been directly assessed as far as this author is aware, except during the fattening periods when concentrated feeds are fed (see Table 4–2). On any other basis, estimates of protein derived by means of grazing would be essential if practical conditions of feeding were to be the basis considered.

In view of the circumstances that sheep and lambs are wool-producers in addition to the fact that a very large proportion of their feed is pasture or range grass, these animals would seem to be entitled to some substantial priorities as meat-producing animals above those of the hog especially in regions where grazing land was plentiful and grain crops relatively scarce. Such, in general, is the case in various parts of the world.

In summary then, it may be concluded that the order of efficiency of

various farm animals as converters of feed protein to food protein is as follows: the dairy cow as producer of milk protein, the hen as producer of egg protein, poultry as producer of meat protein, and other meat-producing animals following (pork, small beef animals, lambs, and large beef animals). This is the same order as has been reported for relative efficiency in food protein production when total nutrient consumption is taken as the basis of consideration.[58] The livestock first to be thrown into competition with man for primary foods would be intensively fed hogs and poultry. The type of livestock which ranks far ahead of others as provisioner of food for man is without question the dairy herd.

Economy in Food Protein Purchases

The purchase of a given quantity of plant protein in the form of common staple food items usually entails less cost to the consumer than the purchase of an equal quantity of animal protein in such forms as milk, cheese, eggs, or meat. Many factors operate to bring about these differences in costs to consumers. These factors are associated with costs of production and distribution. In Table 4–3 are shown the comparative costs of 50 grams of

Table 4–3. Retail Costs (August 15, 1939) of Various Major Food Items in Quantities which Carry 50 Grams of Proteins *

Item	*Cost per Unit (cents)*	*Cost per 50 gm Protein (cents)*
Grain Products		
Bread, white	7.8/lb	12.0
Bread, whole wheat	8.8/lb	10.8
Cornmeal	4.0/lb	4.8
Flour, white	35.8/10 lb	3.7
Oats, rolled	7.1/lb	5.5
Vegetables		
Beans, navy	5.8/lb	2.9
Potatoes	34.4/15 lb	14.9
Dairy products		
Cheese	24.7/lb	11.4
Milk, evaporated	6.7/14½ oz	11.6
Milk (grocery)	11.0/qt	16.1
Milk, (delivered)	12.0/qt	17.6
Meat, fish, poultry		
Beef, round	36.4/lb	23.3
Chicken, roasting	29.2/lb	26.2
Ham, whole	27.4/lb	20.5
Lamb, leg	27.6/lb	20.4
Salmon, Red, canned	23.1/lb	12.6
Eggs	32.0/doz	22.6

* Obtained from data on "Retail Food Prices by Cities," published January 15, 1946 by U. S. Department of Labor, Bureau of Labor Statistics.

food protein in the form of various widely used food items included in our average national dietary. These data are based upon retail food prices in large cities as of August 15, 1939, this date being selected because it represents a time when food prices were not subject to price controls. These prices, therefore, reflect rather a normal situation as regards relative cost in the production and distribution of the food items listed. The retail prices of quantities of various common protein-containing foods which would provide 50 grams of food protein were computed rather than for those of other quantities merely because the 50-gram level, even if supplied by a single item of almost any one of our common protein-containing foods, would provide adult maintenance needs for food protein.

Production Costs. As has been seen in the previous section, several-fold more food plant material must be consumed by animals in order that the animal may produce the same quantity of food whether measured in terms of dry weight, calories, or food protein. In the production of meats, as contrasted with milk or eggs, a very considerable share of the product produced is inedible — hides, feathers, meat scrap, inedible offal, and the like. All animals obviously are more efficient as converters of feed to total body protein than they are as converters of feed to *edible* protein. Some of the cost of producing the inedible forms of protein is absorbed in hides used as leather, meat scrap used in animal feeds, and so on. Nevertheless, it is clear that animal proteins must always cost more than a like quantity of protein in the food from which they are produced.

Whatever is used in the way of capital goods (the tractor, the plow, packing plants and equipment, mills and their equipment, the baker's ovens and tools, and so on) in the production of food products must also be purchased or use of these paid for, since back of these stand costs in terms of labor and materials. Any materials added to a food product, such as shortening, sugar, and yeast added to flour in bread-making; containers for food, cartons, cans, bottles; and anything added by way of labor have all to be added into the cost of the final product. There is nothing more mysterious involved here than in the case of, let us say, a physician who has a capital investment in education for his profession, and such goods as an x-ray machine, a stethoscope and such, and who must pay office rent and labor costs of his assistants to which he adds his services, in return for *all of which* he hopes to derive something more than a bare-subsistence living. If he is successful in his management, offers needed and acceptable services, and is efficient, he will probably make a profit which will give him added purchasing power for things he wants but does not himself produce. Some type of goods or services is all anyone has to offer in exchange for other types of goods and/or services. Somebody pays for everything that does not represent a free gift of nature (such as the oxygen we breathe).

All processing of foods such as pasteurizing of milk, making of bread or

cheese, curing of meats, milling of flour, rolling of oats are examples of services which add to the cost of production of food products.

Distribution Costs. No product, food or non-food is of the least service unless it is distributed. Distribution involves cost in the way of transportation from place of production to place of consumption, handling and storage along the line, setting up of retail stores, or delivery to us from creameries, butcher-shops, bakeries, and groceries. By the same token, the physician must distribute his services by office appointments or house-calls. These are all services the consumers of the goods or service have included for him in the price of his purchases.

Food Protein Purchases in Relation to Price of Foods. A relatively large share of human energy is directly or indirectly associated with the production and distribution of foods. The food economy of the world, a nation, or a family comprises a large share of the total economy of such groups. This is presumably the basis for the saying "if you would gain a general idea of the prosperity of a people, you have only to eat with them." By and large this is true. Food purchases do in large measure reflect the purchasing power and the general standard of living of people.

The effects of the level of family income upon the daily per capita consumption of total food proteins and on the types of protein-containing foods consumed is well illustrated in Table 4–4. These data represent results of several thousands of family food records. In the circular referred to in the footnote to this table many other aspects of the data obtained in the survey are discussed in detail, as are also the methods of assembly and treatment of the data. Only that portion concerned with summarization of the immediate topic under discussion is presented here. The term *consumption* as applied to this set of data does not exclude table or kitchen waste; that is, the quantities of food and/or nutrients indicated were taken out of retail channels of supply and in an economic sense were "apparently consumed." These data are assembled here merely as an illustration of more or less the common trends in various factors of food economy among families or households with moderate incomes. Further sets of data collected would, of course, show various modifications, but the general trends would be much the same among families of moderate income, and most readers will probably find these trends quite in accord with their observations or what they may generally have assumed to be the case.

The reader will note from the data presented in Table 4–4 that: (1) as household income increases, the per capita expenditures for food steadily increase; but the *percentage* of income spent for food does not materially increase; (2) higher household income is associated with smaller number of persons comprising the household; (3) as household income increases the per capita consumption of food protein correspondingly increases as does consumption of all of the more expensive forms of protein-containing foods;

Table 4–4. Estimated per Capita Daily Supply of Protein and Trends in Consumption of Major Protein-Containing Foods in Relation to Weekly Income, Weekly Expenditure for Food, per Cent Income Spent for Food, Household Size, and Food Energy Value — Employed White Wage Earners and Clerical Workers in Cities *
(Survey Conducted December 1934–February 1937)

Weekly Expenditure for Food per Capita	*Range in Average Weekly Household Income for Preceding 3 Months*		*Range in per Cent of Income Spent for Food*	*Range in Average Household Size*	*Total Number Weekly Food Records*	*Per Capita Protein per Day*	*Range in Average Apparent per Capita Consumption in 3 Months*				*Food Energy Value per Capita per Day*
	Total	*Per Capita*					*Eggs*	*Total Fluid Milk Equivalent*	*Meat, Fish and Poultry*	*Grain Products, Flour Equivalent*	
			(%)			(gm)	(doz)	(qt)	(lb)	(lb)	(calories)
$1.00–$1.24	$18.85–$21.14	$3.39– $3.62	29.8–31.0	5.56–5.84	17	45–55	3.0– 4.8	11–17	11.2–14.0	38.1–48.6	1950–2110
$1.25–$1.87	$22.00–$34.60	$4.36– $6.69	23.0–36.2	4.09–6.39	358	56–68	2.3– 6.3	20–41	14.1–26.1	29.5–44.9	1860–2470
$1.88–$2.49	$24.74–$37.22	$5.89– $8.43	25.3–37.4	3.42–5.37	706	70–86	3.7–12.8	27–48	20.3–37.5	29.2–50.6	2210–2980
$2.50–$3.12	$27.85–$39.38	$7.12–$10.97	25.2–38.3	2.74–4.23	672	80–101	4.3–11.2	32–70	25.4–52.9	32.1–51.6	2510–3370
$3.13–$3.74	$27.40–$39.27	$8.15–$15.32	22.6–38.5	2.33–3.46	327	93–110	6.0–10.4	25–59	34.5–48.9	39.0–53.8	2900–3570
$3.75–$4.37	$27.05–$39.95	$9.68–$17.15	23.3–41.0	1.96–3.08	116	107–123	8.0–10.9	38–58	42.0–67.6	44.1–53.4	3290–3870
$4.38 and over	$30.74–$37.82	$10.39–$20.44	26.3–42.6	1.75–2.96	55	111–144	9.3–13.3	49–73	52.2–75.2	43.4–64.7	3530–4870

* Data included in this table are a re-arrangement of statistical survey data taken from U. S. Dept. Agriculture Circular No. 507, "Diets of Families of Employed Wage Earners and Clerical Workers in Cities." The data in this original publication are given in terms of averages for various seasons included in the period of study; the number of families included in each seasonal record not being given, it was impossible to compute the above data in terms of weighted means but the trends in consumption in relation to economic status are fully apparent from the ranges in averages taken directly from the original data.

and (4) it would appear that the groups representing those households with the two highest levels of income either indulged in a luxus consumption of food protein and food calories or wasted considerable quantities of their food purchases. The data in this table were collected during 1934 to 1937 when average income and food prices were both lower than at present. Among families with substantially lower purchasing powers than this group, the percentage of income spent for food would be greater. *Most* families in the United States spend between 25 and 40 per cent of their income on food purchases.

Nutritional Economy in Food Protein Purchases. The nutritional function of food protein is to supply the body's *specific* needs for protein. Some margin of excess over specific needs is quite generally recommended by nutritionists.

A luxus ingestion of food protein, that is ingestion of quantities beyond what could be considered a reasonable excess over needs (30 to 50 per cent is the order of excess usually recommended) entails use of the overload as a source of food energy. The use of food protein as a source of fuel is very inefficient since the waste products of protein catabolism (largely urea) carry with them about 1.25 calories per gram of protein ingested. Aside from the nutritional uselessness and the physiological waste involved, any significant luxus ingestion of food protein practically always means that the diet carries a fairly high proportion of relatively expensive types of protein-containing foods.

In Table 4–5 the author has assembled data, based on the earlier scientific reports as indicated, showing: (1) the quantities of protein derived from single items of common food materials which have been found adequate for the maintenance of nitrogen equilibrium in adults (computed for a 70-kg adult); (2) the quantities of protein derived from mixtures of various food materials, which it might be reasonable to use in practical dietaries, required for the same purpose; and (3) the average calorie values for the various protein-containing foods or food mixtures which carry the requisite food protein to maintain nitrogen equilibrium in a 70-kg adult. It is to be understood that the data computed here by the author are in some greater or lesser degree an approximation since exact nitrogen equilibrium was not attained in every case; the protein derived from the principal foods involved and listed in column one varied from 90 to 100 per cent of the total protein intake, and in computation of calories, *average* fuel values for the foods concerned were used. In many cases, however, the original publications carried all of the data as shown except for the column of figures pertaining to calorie values. Published data omitted from the discussion here included those on (1) food combinations consisting primarily neither of single items nor of items approaching practical dietaries, and (2) on food mixtures wherein the author was uncertain of the proportion of nitrogen

Table 4–5. Food Protein Requirements for Adult Maintenance in Terms of Single Food Items and Mixed Dietaries in Relation to Calories Associated Therewith

Source of Food Protein *	*Total N from Food Listed* (%)	*Computed Quantities for 70-kg Adult*			*Authors on Whose Work Computations Were Based*
		Equilibrium Requirement		*Fuel Value of Food Coincident with N-equilibrium Requirement* (*calories*)	
		Total N @ Day (*gm*)	*Crude Protein* † *@ Day* (*gm*)		
1. Beans (*Phaseolus vulgaris*):					
cooked	90–94	5.6; 6.3	35; 39.5	560; 630	Pittman [70]
baked	90–94	6.3; 6.4	39; 40	620; 640	Pittman [70]
2. Beef, lean round	97/98	5.6–5.7	35	300	Rose *et al.*[67]
3. Bread:					
White	100	9.3; 10.3	53; 59	1650; 1850	Abderhalden *et al.*[68]
White (5% total N from apple & butter)	95	5.3	30	920	Sherman [69]
4. Cornmeal (3% total N from fruit)	97	5.4; 5.6	34; 35	1380	Sherman *et al.*[72]
5. "Flour":					
White (made into biscuits)	91–95	7.2	41	1350	Bricker *et al.*[71]
Whole wheat (baked in loaves, raised with baking powder)	99	11.2–14.5	65–85	1800–2300	Martin *et al.*[34]
6. Milk, pasteurized	97/98	5.3; 5.4	34	670	Rose *et al.*[67]
Milk, pasteurized	91–95	4.2	27	530	Bricker *et al.*[71]
7. Oatmeal (5% total N from apple and cornstarch)	95	5.5; 5.8	32; 34	920	Sherman *et al.*[73]
8. Potatoes, white	99.9	6.8	42	1780	Rose *et al.*[22]
9. Soybean curd	98	5.6	32	350	Rose *et al.*[67]
10. Soy flour	91–95	4.4	25	170	Bricker *et al.*[71]
11. Soy and white flour (N 36% from soy flour)	91–95	5.1	29	670	Bricker *et al.*[71]

Table 4–5. Food Protein Requirements for Adult Maintenance in Terms of Single Food Items and Mixed Dietaries in Relation to Calories Associated Therewith (*continued*)

Mixed Dietaries ‡

Diets Comprised of Plant Products					
1. White bread 50, vegetables 30, misc. cereal products 12, fruits 8	100	5.1	30	1230	Hegsted *et al.*[74]
2. White bread 66.6, vegetables 19.9, misc. cereal products 8, fruit 5.5	100	5.0	30.5	1140	Hegsted *et al.*[74]
3. White bread 33.3, soy flour biscuits 33.3, vegetables 19.9, misc. cereal products 8, fruit 5.5	100	4.9	29	840	Hegsted *et al.*[74]
4. White bread 33.3, wheat germ biscuits 33.3, vegetables 19.9, misc. cereal products 8, fruit 5.5	100	5.0	29	920	Hegsted *et al.*[74]
Diets Comprised of Plant and Animal Products					
1. Lean beef 33.3, white bread 33.3, vegetables 19.9, misc. cereal products 8, fruits 5.5	100	5.0	31	890	Hegsted *et al.*[74]
2. Beef 19.90, eggs 8.61, milk 23.02, oatmeal 6.43, soy-wheat biscuits 23.50, vegetables 15.95, fruit 2.59	91–95	4.7	30	680	Bricker *et al.*[71]

* All food items listed in this column were fed in one or another of usual forms, cooked if generally so served, fruits raw or cooked.

† Nitrogen factors used: for wheat products 5.7; for milk 6.38; for all other food materials 6.25.

‡ The figures immediately following each food item in extreme left hand column below refer to percentages of nitrogen derived from the respective items.

contributed by the separate items. Thus the primary interest here was in single food items and/or practical dietaries.

It will be noted from the data presented in Table 4–5 that almost any one of our common protein-containing foods alone can cover adult maintenance needs for food protein when it is consumed in quantities that would provide 50 grams or less of food protein per day. Such quantities of these foods as would provide 50 grams of protein, moreover, would, in most instances, not provide more than around 40 per cent of the gross calorie needs of a moderately active 70-kg adult. Thus it has been proved, as nearly as the science of nutrition and physiology *can prove anything,* that if the calorie requirements are adequately covered by common, staple protein-containing foods the protein needs for maintenance *at least* are also adequately covered and a liberal margin of excess food protein is simultaneously provided. This is a thesis propounded years ago by Sir William Maddock Bayliss, eminent English physiologist, who in conjunction with Starling was discoverer of the first known hormone, secretin, and who is widely known for his "Principles of General Physiology" published in 1914. This same thesis has been put forth and emphasized anew by Terroine.[1]

Although the data in Table 4–5 show some variations among results of different investigators, the only marked exception to these generalizations is whole wheat bread. The failure of whole wheat bread to provide adequate protein with smaller ingest of protein than was the case is due undoubtedly, not to the quality of protein represented therein, but to low digestibility of the protein. Probably the net calorie value of the bread was substantially lower than computed here also. Both these divergences from the general trend are matters associated, we may assume, with the relatively high fiber content of whole wheat bread.

It is also of interest to note that soybean products and other dried legume types of food material, which represent very inexpensive types of food protein, are adequate for supplying maintenance needs for food protein in quantities not significantly different at all from the quantities required in the form of animal products (milk or meat). It is very possible that the results obtained in terms of additional weight increments in young rats when legume protein, as the sole source of food protein, is supplemented with methionine [75] are not applicable, or not of significance, to the human species. By reason of the relatively large amount of hair growth and use of sulfur-containing amino acids in this process young rats, as compared with humans, may require relatively more methionine.

There is no scientific evidence known to the writer which supports *physiological need* for food proteins of especially high biological value for purposes of maintenance of nitrogen equilibrium in normal adults. The normal adult (exclusive of pregnant and lactating women) who consumes food protein of highest biological value does so for reasons entirely aside from physiological need. The usual recommendation of food protein levels of intake

which will provide the caloric equivalent of 10 to 15 per cent of the total calories stands firm for the adult who has only the needs for maintenance to be satisfied. Even with a diet in which sugars and fat comprise one-third of the total dietary calories, adult maintenance needs for protein can still be satisfied and a margin of excess food protein be provided to the extent usually of 40 to 50 per cent by consumption of common, staple protein-containing foods (such as those listed in the first section of Table 4–5) to supply the remainder of the needs for calories. Actually *the ingestion* of fats and sugars together must add up to something less than 30 to 35 per cent of the total calories in the average diet of the United States by reason of the relatively high waste involved in fats "apparently consumed." According to the most comprehensive assessment ever made of nutrient waste in this country, Pearl [76] estimated in 1917–1919 that about 25 per cent of the fats "apparently consumed" were in fact not actually ingested but wasted. By contrast, the same authority estimated that about 5 per cent of the protein disappearing from retail food channels was lost in the form of edible food wastage.

There is no sound scientific basis for debate on whether or not consumers of large quantities of meat (and, therefore, of meat proteins) are in any way nutritionally better off than persons who consume moderate amounts of food protein of such quality and quantity as will adequately supply specific protein needs and provide some reasonable margin of excess as a factor of safety. Chittenden's earlier stand on this has not been proved untenable by subsequent research. All reference to such "evidence" as the strength and endurance of the Eskimo and other arguments of similar patterns do not take stock of even the major portents of the situation. In the case of the Eskimos, for example, Stefansson,[77] a scientist who has lived many years among these people, reports that, even before associations with white men, this race of men was relatively short-lived and that, in general, Eskimos mature and age quite young, a fact which he suggests *may* be due to a "speeded-up" metabolism. All such statements as that of Rubner who claimed that high protein diets are "a right of civilized man" are a matter of personal philosophy and, therefore, not in need of further consideration here.

The food protein requirements for maintenance plus such physiological processes as entail production of new protein (growth, pregnancy, and lactation) are not accurately known. It is, nevertheless, obvious from data presented in the latter part of Table 4–5 that when as much as one-third of the total food protein is provided by various mixtures of food plant materials or by mixtures of food plant materials and animal products, the maintenance needs for protein are about the same as when milk or meat proteins alone supply the total protein. The proportion of about two parts of protein derived from staple grain-products to one part of milk or meat protein is also a ratio in which supplementary relationships of the protein from these

two classes of foods is *usually* much in evidence. When economy in utilization of food protein or of money available for food purchases is of practical moment, the proportion of two-thirds to one-third of plant to animal protein respectively would seem to be adequate for meeting the food protein needs for growth, pregnancy, and lactation, provided calorie needs were also met.

Failure to take advantage of the maximum supplementary relationships of protein in different foods means either that (1) the people subsist on protein supplies which are overloaded with plant proteins, or (2) the people consume luxus quantities of animal protein. Mixed dietaries, largely restricted to one source of food protein (*e.g.* cornmeal along with fat-back and molasses), or which carry excessive amounts of animal proteins, *both* represent lack of rational utilization of food protein.

Trends in Food Protein Consumption in the United States

All of the data presented in this section refer to average per capita consumption of food protein or of protein-containing foods, as fits the situation, on the basis of apparent consumption or of quantities available for consumption. The foods involved entered into regular channels of trade for purchase by consumers. No allowances were made for losses along the way nor for kitchen and table waste incurred after purchases were made. Most of the data presented in this section were obtained directly from materials collected and published by the Bureau of Agricultural Economics, United States Department of Agriculture, Washington, D. C.

Since trends in consumption of various foods by a population may vary from year to year by reason of more or less transient influences, a comprehensive view of the trends can be obtained only by examination of data on consumption levels over a period of years. The data here presented cover for the most part the period from 1909 to 1944 inclusive, that for the year 1944 being a tentative estimate.

Per Capita Consumption of Food Protein. In Table 4–6 are presented the total quantities of food protein available from all foods for consumption per capita per day within continental United States for the years 1909 to 1944 inclusive.[78] These data presumably are less accurate for the years preceding about 1924 than in later years since the quantities of several important protein-containing foods are not listed in current publications of the United States Department of Agriculture for years prior to about 1924. Fairly complete records of grains and meats available for consumption have been in evidence for over 40 years.

It will be obvious from the data in Table 4–6 that the people of continental United States have for the past 35 years and more had abundant supplies of food protein, and except for the years commonly referred to as the period of economic depression, the per capita supplies available have amounted to 90 grams or more per day. Probably all but a relatively small

Table 4–6. Food Protein Available for Consumption per Capita per Day, Continental United States, Calendar Years 1909–1944 *

Year	*Protein (gm)*	*Year*	*Protein (gm)*	*Year*	*Protein (gm)*
1909	101			1935–39 average	89
1910	99	1925	93	1940	93
1911	99	1926	93	1941 †	93
1912	100	1927	92	1942 †	96
1913	96	1928	93	1943 †	98
1914	97	1929	92	1944 †	100
1915	95	1930	91		
1916	95	1931	90		
1917	98	1932	87		
1918	96	1933	86		
1919	96	1934	89		
1920	93	1935	85		
1921	91	1936	90		
1922	93	1937	89		
1923	94	1938	91		
1924	94	1939	91		

* Quantities of nutrients computed by Bureau of Human Nutrition and Home Economics on the basis of estimates of apparent consumption (retail basis), including estimates of foods supplied by farm and city gardens, prepared by Bureau of Agricultural Economics. No deductions have been made in the nutrient estimates for the loss or waste of food in the home or for destruction or loss of nutrients during the preparation of food.

† Civilian only.

proportion of these supplies available for consumption actually reached United States' kitchens. When the dietary allowances for food protein ingestion recommended by the Food and Nutrition Board of the National Research Council [79] are weighted according to the composition of the population (age, sex, physiological status) as of recent years, the per capita food protein allowance amounts to around 65 or 66 grams per day. These recommended food protein allowances are, moreover, decidedly liberal especially when so large a proportion of the food protein is derived from proteins of high biological value as is the case in the average United States diet. It would appear that per capita food protein supplies of United States represent *about 100 per cent overage* beyond per capita supplies which would provide about 30 to 50 per cent margin of excess over food protein needs. By no stretch of the imagination can it be contended that the people of the United States face a problem of overall or general food protein shortage. The United States' supplies of food protein are, and have, for years, been abundant, and a large proportion of the supplies are proteins of highest biological value.

Statistics on *average* per capita consumption, of course, indicate absolutely nothing about the distribution of food protein among individuals. For all practical purposes, the normal adult who is adequately provided with food calories, not overwhelmingly derived from fats and sugars, and who has no physiological need for food protein beyond that needed for maintenance can be summarily dismissed from concern in the matter of food protein supply. Children, and pregnant and lactating women who have food protein requirements beyond those required for maintenance could have these extra needs provided by ingestion of the quart of fresh, pasteurized milk (or its equivalent in other forms of milk) generally recommended for them along with enough other foods commonly represented in an average mixed diet to complete their requirements for food calories. This solution of the problem would also provide them with their extra needs for calcium and riboflavin at the same time. Adding small proportions of milk to various food mixes or incorporating milk in relatively expensive foods is neither an economical nor an effective means of solving this problem of better distribution of milk proteins (and other nutrients in milk), since the problem is primarily associated with persons in the lower income groups who are not in position to benefit substantially by such a program. Moreover, this would not guide the right quantities to the right people, but would in large degree represent poor economy in the utilization of milk protein since much of that so distributed would be consumed by persons not in need of more. We are, then, far ahead on food protein production in relation to our progress in food protein distribution.

Some not inconsiderable members of the population have special needs for food protein by reason of various diseased and idiocratic conditions. This problem cannot be discussed in broad perspective as it relates to food

protein economy since consideration of individual cases is of the utmost importance and must of necessity be left to physicians and the dietitians and others who work with them.

Per Capita Trends in Consumption of Plant and Animal Proteins. During the period 1935–39, the food protein consumption in continental United States was represented to the extent of about 44 per cent by plant protein and about 56 per cent by animal protein.[78, 80] Most of this protein was provided by three major classes of foods: namely, grain products, 28 per cent; meats, poultry, game and fish, 27 per cent; and dairy products, excluding butter which provides very little, 21 per cent. Minor deviations from this pattern have occurred since, but in view of various arbitrary controls and restrictions placed on different food items these are of uncertain significance in assessing trends under normal conditions. Protein-containing foods of plant origin also provided about 62 per cent and 6 per cent respectively of the total carbohydrate and fat content of our national dietary during this period as contrasted with about 40 per cent of the fat and 6 per cent of the carbohydrate contributed by animal products exclusive of fat cuts of meat.

Per Capita Trends in Consumption of Grain Products. A 35-year trend in per capita consumption of grain products is shown in Table 4–7. As regards wheat flour, the data presented indicate more or less stability in level of consumption over the years since about 1932, whereas for many years prior there had been gradual and steady decline in per capita consumption of wheat products. Wheat flour is, except for 2 to 3 per cent of the total, consumed in the form of white flour-converted products. The per capita consumption of maize or corn, the second-ranking species of grain represented in the national dietary, is perhaps still slowly declining. Maize is widely known to be an important dietary constituent largely in the southern states.

Wheat is variable in protein content but probably most of that produced would carry on the average between 12 and 14 per cent of crude protein ($N \times 5.7$). The emergency flour of 80 per cent extraction probably averaged around 11.5 per cent of protein. White patent flour would normally average around 10.8 per cent and bread flours around 11.5 per cent of protein. The protein in millfeeds ordinarily makes up most of the difference between levels of protein in wheat and various types of flour. Millfeeds are fed largely (to the extent of about 70 per cent of the total millfeed produced) to dairy herds and poultry. The quantity of millfeed fed to livestock for the year beginning October 1, 1941 in the United States has been estimated at about 4.75 million tons.[58] Gluten feed and meal largely from wet-milling of maize contribute only about 20 per cent as much millfeed as wheat and are disposed of in much the same way as wheat millfeeds.

From the standpoint of food protein economy it is doubtful whether this diversion of wheat and corn offal, derived from milling processes, is of any

Table 4–7. Trends in Apparent per Capita Consumption of Major Grain and Grain Products 1909–1944, Continental United States *
(Civilian Consumption Since 1941)

Year	Wheat		Rye Flour	Rice (Milled Products)	Corn		Oatmeal
	Flour (White, Whole Wheat, Semolina)	Cereal			Meal	Cereal	
	(lb)	(lb)	(lb)	(lb)	(lb)	(lb)	(lb)
1909	209.4	3.0	—	—	66.2	1.3	3.2
1910	211.9	3.0	5.0	—	62.8	1.3	3.3
1911	206.8	3.0	5.0	—	60.8	1.3	3.2
1912	212.5	3.0	5.0	—	58.2	1.3	3.3
1913	206.6	3.0	5.1	—	55.6	1.3	3.1
1914	207.2	3.1	5.1	—	52.7	1.3	3.4
1915	200.0	3.1	4.6	—	52.0	1.4	3.6
1916	205.1	3.1	5.3	—	53.6	1.4	3.6
1917	198.4	3.2	6.9	—	54.2	1.4	4.6
1918	164.3	3.2	7.7	5.6	56.0	1.5	5.2
1919	196.8	3.2	6.9	3.4	35.4	1.5	5.5
1920	186.5	3.3	4.5	5.2	35.0	1.5	5.7
1921	177.7	3.4	3.7	4.5	34.3	1.6	5.8
1922	181.4	3.5	3.7	5.3	36.3	1.6	5.8
1923	176.3	3.5	3.7	5.2	35.7	1.7	5.4

Table 4-7. Trends in Apparent per Capita Consumption of Major Grain and Grain Products 1909–1944, Continental United States * (Civilian Consumption Since 1941) (*continued*)

1924	175.1	3.6	3.6	5.4	32.3	1.8	5.6
1925	176.9	3.6	3.6	5.2	29.4	1.8	4.5
1926	177.7	3.6	3.6	5.6	29.1	2.1	5.2
1927	173.5	3.6	3.5	6.2	28.7	2.4	6.5
1928	177.3	3.5	3.5	5.8	29.7	2.8	5.8
1929	172.6	3.5	3.4	5.8	30.5	3.1	5.2
1930	168.9	3.5	3.2	5.3	28.0	3.0	6.0
1931	159.9	3.5	3.5	5.7	26.4	3.0	6.3
1932	157.4	3.5	3.0	5.3	26.3	2.4	5.7
1933	152.6	3.5	2.6	6.0	25.4	1.9	4.9
1934	153.3	3.5	2.5	4.7	25.0	1.6	4.5
1935	150.3	3.5	2.0	5.6	24.5	1.4	4.0
1936	157.5	3.5	2.3	5.2	24.4	1.6	3.9
1937	152.8	3.6	2.3	5.9	23.5	1.8	3.8
1938	153.4	3.7	2.2	6.2	23.2	1.9	3.8
1939	151.4	3.9	2.3	5.7	23.7	1.9	3.8
1940	147.4	3.8	2.4	6.2	23.7	1.9	3.9
1941	152.2	3.7	2.3	6.2	22.5	2.3	4.0
1942	155.7	3.8	2.7	5.9	21.8	2.5	4.8
1943	161.3	3.7	3.3	6.3	22.6	2.6	4.0
1944 †	(161.0)	(3.7)	(2.8)	(6.7)	(22.6)	(2.5)	(3.2)

* Data presented in this table for years prior to 1929 taken from Agricultural Statistics 1944; data subsequent to 1928 taken from Agricultural Statistics 1945.

† Data for this year preliminary.

importance so far as human dietaries in this country are concerned. The presence of the branny portions of wheat significantly reduces the human digestibility of the protein in whole wheat; ruminants can make much more efficient use of the protein in millfeed products than can humans. The consumption of higher levels of vegetables and fruits, actual or recommended, has in large measure disposed of the necessity for consideration of wheat fiber as a source of roughage materials which perhaps might have served more useful purpose in days when fruit and vegetable consumption was much lower. Added to this are the facts that (1) much of the protein in millfeeds is used in the production of milk and egg proteins, the highest in biological value we have and the most efficiently produced, and (2) the enrichment of white flour and bread has restored to flour the mineral and vitamin values considered to be of practical importance in food selection.

The United States is a wheat surplus producing country. Although some considerable amounts of wheat and flour are imported to United States from Canada, we are a net-exporting country for wheat. Except for alterations associated with need for wheat and flour in Europe as a result of World Wars I and II, the trend in wheat and flour exportation of the United States has declined rapidly since the turn of this century. For further information on this subject the reader is referred to a publication by Shollenberger,[81] various other earlier and later publications of the Bureau of Agricultural Economics, and the series of "Wheat Studies" published by the Food Research Institute, Stanford University, California.

Per Capita Trends in Consumption of Meats, Poultry, and Eggs. Trends in per capita consumption of eggs and flesh foods, except for fish, are presented in Table 4–8. The United States, in years prior to World War I, was a rather substantial net-exporter of meats. Due to subsequent increases in the United States population and domestic demand, not matched by corresponding increase in meat production, expansion of livestock production in the Southern Hemisphere and various trade relationships which will not be further discussed here, we reached the stage in the five-year period 1935–39 where total meat production and total meat consumption were not greatly different.[82] During these years meat exports were offset, or more than offset, by meat imports.

The trend in per capita consumption of meats in this country has, in general, been gradually downward for some years prior to World War II. During World War II various factors, economic and otherwise, were injected which interfered with normal relationships between supply and demand. The downward trend in per capita consumption of meats has been largely effected by a downward per capita consumption in beef.

The severe droughts of 1934 and 1936 resulted in the feeding, to all types of livestock, of only about 75 per cent of the usual quantities of grain during the years 1934–37.[58] This was attended with a large reduction in hog production. During the years 1935–39, United States was a larger net importer

of beef, veal, and cattle than in the years immediately preceding. The combined result of these circumstances included reductions in per capita consumption and export of pork during most of this period, 1935–39, and a reduction in per capita consumption of meats. The production and consumption of lamb and mutton, not nearly as important a factor in our animal protein economy as hogs or beef, have very nearly balanced over the past 35 years. United States' imports and exports of lamb and mutton have been negligible in most years.[82]

The per capita consumption of chickens on a dressed weight basis has not changed greatly over a considerable number of years except during World War II when poultry, in some degree, offset the lower consumption of pork or beef. The per capita consumption of eggs has, except for the depression years, shown a slightly increasing trend or perhaps a trend toward being stabilized at a figure not far from one egg daily. Obviously many persons in this country do not consume an egg every day or nearly every day — eggs are not inexpensive foods nor are they inexpensive sources of food protein. There was a significant decline in per capita consumption of eggs during the depression years of the 1930's.

Per Capita Consumption of Dairy Products. National trends in per capita consumption of various kinds of dairy products are shown in Table 4–9. Except for cheese and butter, data are largely nonextant prior to 1920. Part of the explanation of this is given in the footnote to the table and part of the explanation is due to the fact that some items are relatively new on the scene or have become items of substantial economic importance only recently.

The commerical production of ice cream is said to have begun about 1850 and underwent rapid expansion after the introduction of the ice-cream cone at the World's Fair in 1904.[83] Ice cream is more or less of a luxury item for many families. This is reflected by a marked downward trend in per capita consumption of ice cream during the years of general economic depression and rapid recovery shortly thereafter. A part of the protein in ice cream is supplied as dry and condensed milks.

The per capita consumption of cheese was relatively stable between 1910 and 1934 and thereafter has tended toward substantial increase as, also, have the quality and variety of product.

Evaporated milk has shown rapid increase in per capita consumption over a period of at least 25 years while condensed milk has rapidly declined. Much of the evaporated milk supply is used as a relatively inexpensive form of milk for infants and children or entire families. Dairy products are, in some degree, competitive with one another. An example of this can be noted in the data presented in Table 4–9. During the depression years of the 1930's the per capita consumption of evaporated milk, one of the less expensive forms of dairy products, steadily increased while the per capita consumption of ice cream, butter, and fluid milk and cream declined.

Table 4–8. Trends in Apparent per Capita Consumption of Meats, Poultry and Eggs, 1909–1944, Continental United States *
(Civilian Consumption Since 1941)

Year	*Meats (Dressed Weight)*					*Poultry (Dressed Weight)*		
	Beef (*lb*)	*Veal* (*lb*)	*Lamb and Mutton* (*lb*)	*Pork (Excluding lard)* (*lb*)	*All Meats* (*lb*)	*Chickens* (*lb*)	*Turkeys* (*lb*)	*Eggs* (*number*)
1909	74.2	7.3	6.7	67.0	155.2	19.6	—	293
1910	70.4	7.2	6.5	62.3	146.4	20.6	—	306
1911	68.5	7.1	7.3	69.1	152.0	20.8	—	329
1912	64.5	6.9	7.7	66.7	145.8	19.9	—	311
1913	63.3	6.3	7.2	66.9	143.7	19.4	—	303
1914	62.0	5.8	7.1	65.1	140.0	19.3	—	295
1915	56.4	5.9	6.1	66.5	134.9	19.2	—	313
1916	58.9	6.4	5.9	69.0	140.2	18.4	—	299
1917	64.7	7.2	4.5	58.9	135.3	17.7	—	281
1918	68.5	7.3	4.8	61.1	141.7	17.8	—	284
1919	61.5	7.8	5.7	63.9	138.9	19.0	—	303
1920	59.1	8.0	5.4	63.6	136.1	18.3	—	299
1921	55.5	7.6	6.1	64.8	134.0	17.8	—	299
1922	59.1	7.8	5.1	65.8	137.8	18.9	—	316
1923	59.6	8.2	5.3	74.2	147.3	19.4	—	327

Table 4-8. Trends in Apparent per Capita Consumption of Meats, Poultry and Eggs, 1909–1944, Continental United States *
(Civilian Consumption Since 1941) (*continued*)

1924	59.5	8.6	5.2	74.0	147.3	19.2	—	324
1925	59.4	8.6	5.2	66.8	140.0	19.8	—	318
1926	60.3	8.2	5.4	64.1	138.0	19.7	—	339
1927	54.5	7.3	5.3	67.7	134.8	21.0	—	342
1928	48.7	6.5	5.5	70.9	131.6	20.2	—	338
1929	49.7	6.3	5.6	69.7	131.3	19.8	1.7	334
1930	48.7	6.4	6.6	66.6	128.3	21.5	1.8	329
1931	48.3	6.6	7.1	68.0	130.0	19.4	1.7	331
1932	46.4	6.6	7.0	70.3	130.3	19.7	2.1	311
1933	51.2	7.1	6.7	69.6	134.6	20.3	2.4	295
1934	64.9	9.7	6.4	65.0	146.0	18.8	2.2	287
1935	53.0	8.0	6.8	48.1	115.9	18.1	2.1	278
1936	57.8	8.3	6.6	54.8	127.5	18.1	2.7	287
1937	54.8	8.6	6.6	55.4	125.4	18.0	2.7	306
1938	54.0	7.6	6.9	57.8	126.3	16.8	2.7	308
1939	54.4	7.5	6.6	64.3	132.8	18.6	3.0	311
1940	54.7	7.3	6.6	72.4	141.0	18.0	3.6	316
1941	60.5	7.6	6.8	66.5	141.4	19.4	3.6	311
1942	61.2	8.0	7.2	61.5	137.9	21.5	3.7	311
1943	49.6	7.9	6.4	72.4	136.3	28.0	3.4	345
1944 †	(55.1)	(11.2)	(6.6)	(76.7)	(149.6)	(23.6)	(3.3)	(351)

* Data presented in this table for years prior to 1929 taken from Agricultural Statistics 1944; data subsequent to 1928 taken from Agricultural Statistics 1945.

† Data for this year preliminary.

Table 4–9. Trends in Apparent per Capita Consumption of Major Dairy Products 1909–1944, Continental United States *
(Civilian Consumption Since 1941)

Year	All Milk for Human Consumption	Fluid Milk and Cream (Milk Equiv.)	Condensed and Evaporated Milks (Canned Weights)	Dried Whole Milk	Non-Fat † Dry Milk Solids for Human Consumption	Cheese (Excludes Pot, Cottage, Bakers', Full-skim American)	Ice Cream	Butter		
								Actual Weight	Milk Equivalent	
									Butter × 21	Percentage of Total for Human Consumption
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
	(lb)	(lb)	(lb)	(lb)	(lb)	(lb)	(lb)	(lb)	(lb)	(%)
1909	—	—	5.47	—	—	3.90	—	17.8	373.8	—
1910	—	—	—	—	—	4.39	—	18.4	386.4	—
1911	—	—	—	—	—	4.14	—	18.7	392.7	—
1912	—	—	—	—	—	4.01	—	16.6	348.6	—
1913	—	—	—	—	—	4.42	—	16.5	346.5	—
1914	—	—	9.03	—	—	4.46	—	17.0	357.0	—
1915	—	—	—	—	—	4.34	—	17.2	361.2	—
1916	—	—	—	—	—	4.07	—	17.3	363.3	—
1917	—	—	—	—	—	4.28	—	15.8	331.8	—
1918	—	—	—	—	—	3.88	—	13.8	289.8	—
1919	—	—	9.38	—	—	4.21	—	15.3	321.3	—
1920	—	—	8.61	0.08	—	4.17	—	14.8	310.8	—
1921	—	—	9.87	—	—	4.16	—	16.2	340.2	—
1922	—	—	10.90	0.02	—	4.30	—	17.0	357.0	—
1923	—	—	11.49	0.05	—	4.39	—	17.8	373.8	—

Table 4-9. Trends in Apparent per Capita Consumption of Major Dairy Products 1909–1944, Continental United States *
(Civilian Consumption Since 1941) (*continued*)

1924	796.3	352.7	11.8	0.05	—	4.5	8.0	18.0	378.0	47.5
1925	801.6	353.5	11.7	0.07	—	4.6	9.4	18.0	378.0	47.2
1926	817.8	354.4	11.8	0.10	—	4.7	9.2	18.5	388.5	47.5
1927	812.5	353.3	11.6	0.10	—	4.4	9.4	18.1	380.1	46.8
1928	804.9	353.9	12.2	0.07	—	4.4	9.4	17.5	367.5	45.7
1929	811.7	356.3	13.6	0.11	—	4.6	10.0	17.4	365.4	45.0
1930	814.8	350.9	13.5	0.10	—	4.6	9.1	17.2	361.2	44.3
1931	835.1	348.4	13.3	0.06	—	4.4	7.8	18.0	378.0	45.3
1932	829.6	350.3	13.9	0.08	—	4.3	5.8	18.1	380.1	45.8
1933	812.3	348.8	13.7	0.09	—	4.5	5.5	17.8	373.8	46.0
1934	812.7	333.4	14.9	0.12	—	4.8	6.6	18.2	382.2	47.0
1935	799.0	335.4	16.1	0.15	1.6	5.2	7.3	17.1	359.1	44.9
1936	791.7	340.6	15.8	0.15	1.7	5.3	8.9	16.4	344.4	43.5
1937	796.5	342.3	16.6	0.11	1.9	5.5	10.2	16.3	342.3	43.0
1938	794.7	338.3	17.2	0.12	2.1	5.8	10.1	16.4	344.4	43.3
1939	824.0	344.0	17.7	0.13	2.2	5.9	10.9	17.3	363.3	44.1
1940	820.5	343.1	19.2	0.15	2.2	6.0	11.3	16.9	354.9	43.3
1941	806.9	350.6	18.2	0.17	2.4	6.0	13.5	15.9	333.9	41.4
1942	839.0	371.8	18.3	0.19	2.3	6.3	15.9	15.6	327.6	39.0
1943	761.2	402.5	18.6	0.38	2.0	5.0	12.1	11.7	245.7	32.3
1944 ‡	(787.6)	(422.6)	(16.1)	(0.32)	(1.6)	(5.0)	—	(12.0)	(252.0)	(32.0)

* Data on per capita consumption of most dairy products listed in this table have been limited to the years 1924–1944 inclusive, prior to which dates statistics on some products could be presented only as interpolated data. Figures in columns (2) through (9) for the years 1924–1928 inclusive were taken from Agricultural Statistics 1944; data subsequent to 1928 were taken from Agricultural Statistics 1945. Figures in columns (10) and (11) are values computed from these data by the author.

† Data not available prior to 1935.

‡ Data for this year preliminary.

Whether the increase in per capita consumption of fluid milk and cream from 1942 on represents a trend in that direction or merely reflects a temporary shift in various factors of our overall food economy remains to be seen.

Butter is not at all an important source of food protein. It carries usually only about 0.6 per cent of milk protein. The milk equivalent (fat basis) of one pound of butter is usually reckoned at 21 pounds of whole fluid milk. From the standpoint of nutritive value, butter is not considered to rank any higher than margarine fortified with vitamin A.[84] Table, salad, and cooking fats, including fat cuts of meat, butter, and margarine, together provide only about 2 per cent of the per capita food protein supplies of the United States.

In recent years the protein in skim milk and buttermilk, by-products of cream separation and butter-making, represented over half of the total milk protein produced in this country, or on the order of 55 billion pounds (fluid milk) per year. This would be enough to supply one-third of our population with at least a pint of milk (or about 17.5 grams of milk protein) per day. Somewhere on the order of 17 per cent of these defatted milk products was, in 1937, used in the manufacture of dried skim milk, casein, cottage cheese, etc.,[83] and about 83 per cent was fed to livestock on farms. In recent years the manufacture of butter has normally accounted for approximately 80 per cent (on the basis of milk used in production) of the total manufactured dairy products.

It is unfortunate that, from the standpoint of nutritive value, the nonfat by-product of butter and cream should be of greater value than the milk-fat while the milk-fat, sold as cream and butter with the by-products used as livestock feed materials, should be of greater value to the producer. To deplore this situation is one thing, to resolve it in the best interests of nutrition is quite another. It is, of course, the consumer's food dollars which have provided the support for the present disposal program of milk fat and defatted milk products. That is what supports all food production enterprises. In a thorough-going system of free enterprise, it is the consumers, sometimes with and sometimes without the stimulus of a competitive product, who can bring about shifts in food production programs. The immediate competitive product of butter is fortified margarine, the less expensive product of the two. The chief product in competition with defatted milk products would appear to be defatted soybean meal, at least in the line of animal feeds. Some nutritionists seem to overlook the fact that a market or demand for a given food product is essential to continuing production of that food product. So whether a decline in consumers' purchase of butter and converted, defatted milk products would result in more whole milk especially for the children and pregnant and lactating women in low-income families who are in need of more milk, depends upon the demand (and purchasing power) for milk by the families involved. Food protein

adequate apparently to solve most, if not all, of the food protein problem in this country is already being produced in the form of milk. The problem then is reduced to how to get milk effectively distributed to those who *need* the available supply.

Per Capita Consumption of Fish and Fish Products. The nutritive attributes of fish have long been recognized and firmly established as empirical knowledge of the human race. Most fishes are enormously prolific and thus stock the sea with vast food resources; but as the volume of fishing and efficiency of capture increase, it becomes increasingly important to ensure stability of the stock.

The commercial fisheries of the United States are located off the Alaskan Coast, off our Atlantic, Pacific, and Gulf coastal states, in the Great Lakes, and many other smaller lakes, in our coastal rivers, and in the Mississippi River and its tributaries. Our annual production of landed fishery products, in recent years totalling from 4 to 5 billion pounds, includes some substantial portions of fish not used for food, mussel shells, and various other inedible products. In addition to around 1.5 billion pounds of the edible portion of fishery products used for human food, the fisheries of United States and Alaska supply us annually with large quantities of vitamin and other fish oils; fish meals made from entire fish, fish-residue, defatted residues, bone and scraps; and shells, some of which are ground and used in poultry feeds. Our country also imports and exports various fishery products.

The fish meals and fish-residue meals, constituting by-products of the fisheries industry, usually contain around 65 per cent of proteins that provide excellent returns when added to various poultry and livestock feeds. Fishery products, therefore, provide high-quality proteins used both directly and indirectly as sources of protein in our national dietary.

Surveys on production of fishery products in the continental United States and Alaska together with data on foreign trade and disposition of these products have made it possible to obtain information on the apparent consumption of fishery products used for food in United States. The data shown in Table 4–10 for the 10-year period prior to World War II are based upon output of manufactured products and fresh fish marketed, imports, exports, shipments to and from territories with deductions for imports processed in United States as manufactured products, and changes in cold storage holdings.[85] Undoubtedly these data reflect some duplication of a few items reported at more than one stage of processing. Nevertheless, these data do reflect the general trend of the position of fishery products in our national dietary.

Due to contingencies of war, it was not possible to conduct the usual annual statistical surveys on apparent consumption of edible fishery products during 1941 to 1945. However, since data on over 70 per cent of the catch continued to be collected by the Division of Commercial Fisheries and the Alaskan Division of the Fish and Wildlife Service and various United States

Table 4–10. Apparent Consumption of Edible Fishery Products, Continental United States, 1930–1940.

Year	*Total Consumption* (*thousand lb*)	*Per Capita Consumption* (*lb*)
1930	1,575,206	12.8
1931	1,567,812	12.6
1932	1,402,129	11.2
1933	1,486,266	11.8
1934	1,768,061	14.0
1939 *	1,934,955	14.8
1940 *	1,866,242	14.1

* Preliminary.

fishery agencies, reasonably good *estimates* of the total production were possible for these years.[86, 87] Except for the reduced catch in 1942 occasioned by military requisitioning of some of the larger vessels formerly used by fishermen, the annual productions of edible fish during 1941 to 1945 were as great or greater than the 1935–39 average.

According to several sources, there would appear to be a slow and gradual trend toward higher per capita consumption of fishery products in this country; but in view of simultaneous improvements in the methods of collecting data and complexities involved in disposal of fishery products, the true situation may only become a certainty after a period of some years.

A very extensive and detailed survey of fresh and processed fishery products representing apparent human consumption in the continental United States for the year 1931 was made by the United States Bureau of Fisheries (now merged with the Fish and Wildlife Service).[88] Since no large or important changes in the general trend of our national consumption pattern have been evident subsequently, the findings of this survey pertinent to the subject-matter under discussion are here set forth in Table 4–11.

Since table waste of 10 per cent would seem maximal in consumption of such products, it appears safe to conclude that fishery products contribute annually around 2 pounds of protein per capita to our national dietary.

Meats, fish, and poultry are often grouped together in presentation of statistical data on food production and consumption and in various types of evaluations of human dietaries. At our present national rates of consumption of items within this basic food group, fish products contribute 10 per cent of the total protein provided by this entire group, which is considerably in excess of the protein contributions of either lamb and mutton or veal.

The per capita rate of consumption of fishery products is far from uniform over different areas of United States, the largest per capita consump-

Table 4–11. Apparent Consumption and Protein Contributions of Fishery Products, per Capita Basis, Calendar Year 1931 *

Fishery Product	*Product Weight* (*lb*)	*Protein Content of Product* (%)	*Protein Contribution* (*lb*)
Fresh fish	7.134 †	17	1.213
Canned fish	3.166	20	0.633
Salted fish	0.972	26	0.253
Smoked fish	0.288	18	0.052
Dried fish	0.045	38	0.017
Fresh clams and oysters	0.645	7	0.045
Canned clams and oysters	0.202	9	0.018
Fresh crabs and lobsters	0.175	16	0.028
Processed crabs and lobsters	0.171	20	0.034
Fresh and processed shrimp	0.256	16	0.041
Other shellfish	0.096	16	0.015
Total	13.150		2.349

* Evaluation of the statistical survey in terms shown in this table was supplied by Dr. Hugo Nilson, Chemist, Fishery Technological Laboratory, Fish and Wildlife Service.

† Includes an estimated 2 pounds of edible portion of fish caught for home consumption.

tions being in coastal regions near large fisheries and lowest in those communities far inland which are also removed from large inland waterways.

The biological or nutritional quality of the proteins contained in various species and types of fish compares very favorably with that of muscle meats of beef, veal, pork, lamb and mutton. A larger proportion of fishery products used in mixed dietaries instead of more expensive meats would be a decided economic boon to families with moderate or moderately low income without entailing any appreciable sacrifice of protein quality or quantity.

Compared with many foreign countries, the per capita consumption of fishery products in continental United States is relatively low. Japan ranks first in annual per capita consumption of fishery products, the normal rate for that country being 50 to 60 pounds per capita per year. Next in order of descending rates of consumption among countries recently reporting are Sweden, Norway, Denmark, Portugal, England and Wales, Canada, and the Netherlands, followed by various other countries of continental Europe whose per capita rates of consumption of fishery products are similar to that in our own country.[88] Clearly, the annual per capita rates of consumption of fishery products in various countries vary roughly with the annual per capita rates of production of fishery products and in general also with the relative proportions of their populations in close proximity to fish-producing areas.

Sixty per cent or more by weight of the fishery products consumed as human food in continental United States reach consumers in the form of fresh and frozen products.[88] Market surveys conducted by the Fish and Wildlife Service show that a relatively few varieties of fresh- and salt-water fishery products account for 70 to 90 per cent of the total consumption of this group of foods. This continues to be the case despite the fact that there are no less than 175 varieties of edible fish and shellfish represented in continental United States supplies. Some edible varieties included in the catch, of course, are available only in limited quantities, but supplies of many others, currently little used for human food are, or could be made, available for the increased enjoyment and to the economic benefit of United States consumers if used in place of more expensive types of flesh foods.

Salmon, in fresh, frozen, canned, smoked, and dried forms, lead all other varieties of fish represented in our national dietary. Following this the group consisting of cod, haddock, hake, pollock, and cusk ranks second in consumption, then sardines, and fourth, oysters. Eight additional species or groups can be listed as contributing most of the remainder of our national consumption of fishery products — in descending order of consumption these are sea herring (excluding sardines), mackerel, halibut, clams, crabs, tuna and tuna-like fishes (including Pacific yellowtail), and shrimp.[88] This customary restricted selection of fishery products to relatively few, popular, higher-priced varieties probably cannot be obviated until consumers in general gain further appreciation of the enjoyment and value inherent in consumption of a wider variety of fishery products.

For the years immediately ahead, it would appear that our national per capita consumption of fishery products would continue in general at much the same rate as currently. This, of course, would make for some expansion in the production of fishery products as the population density in this country continues to increase. There are reputed to be potentialities for expansion in production of fishery products suitable for human food within continental United States and Alaska to match the highest per capita rate of consumption recorded for any foreign country; namely, 50 to 60 pounds of edible products per capita annually. There can be no gainsaying that fishery products constitute a potential and substantial reserve of protein-rich food products which can be made available to United States consumers if or when the need or popular demand becomes evident.

Total Food Protein Supplies, Continental United States

The total production or consumption of various foods and/or food nutrients, apart from consideration of the numbers of persons who consume these, may mean much or little depending upon the type of information desired. If one is interested in such matters as average productivity of land devoted to food production, land utilization, and intensity of food

crop cultivation, then overall food production is a part of the data that would be useful. Some persons are interested as a matter of curiosity or of gaining some idea of how great an enterprise food production really is.

National consumption of foods involves consideration, not only of foods produced within the area of a nation, but also of food import and export. The chief food exports of the United States normally have been, over recent years, wheat and pork products, corn, and concentrated milks. Our chief food imports normally have been sugar, tropical fruits, fats and oils, and various dietary accessories such as coffee, cacao, tea, and spices. Although a very large amount of our food moves within the borders of our country, very little enters into trade with other countries of North America or in intercontinental trade with Europe, Asia, or Africa.

It comes as something of a surprise to many people to learn that so little of the world's food production moves in intercontinental trade — only about 6 per cent of total production. Of the six continents, Europe is the only one which imports more food than she exports — her imports of food total about 25 per cent as much as she produces.[89, 90] The bulk (about 90 per cent) of the world's intercontinental food shipments consist of grains and seeds and their products, and about two-thirds of this is wheat and corn. Asia, the largest food-producing continent, exports only about 2 per cent of her production, and Oceania, a relatively small producer in terms of world food supplies, exports about 60 per cent of her production.[90]

One can get an approximation of total food protein available for consumption for various years and of *the trend* in volume of food protein supplies available for consumption over the years by multiplying the per capita consumption figures for total protein as presented in Table 4–6 by the respective population numbers estimated for those years. This procedure is simply a reversal of the final process involved in the computation by which the data in Table 4–6 were derived. As the reader will have noted from this table, the food protein available for consumption per capita in continental United States for the year 1909 through 1944 has not altered substantially. Over these years the population has very substantially increased and this has been met in two ways; namely, by increased food production and by decreased exportation of foods.

Various statistical surveys have been made of the total food protein supplies of continental United States and the contributions of various food items or food groups to this supply. One such survey was published by Pearl [76, 91] covering the seven-year period 1911–12 to 1917–18 inclusive. This survey was the result of studies of national food supplies by the United States Food Administration of which Mr. Herbert Hoover was Chief. The survey (by years) included national food production, net imports and exports of foods, food carry-overs, gross consumption in tonnage of various foods and food nutrients (allowing for inedible refuse), and consumption of nutrients per adult-man equivalent per day (allowing

for inedible refuse and estimated cooking and table wastage). In the year 1916–17 the gross consumption of food protein by the population of the United States was estimated at 3,714,893 metric tons, or roughly 8.2 billion pounds. The population of the United States was then about 103 millions.

According to estimates of the Bureau of the Census,[55] the population of the United States had by 1945 reached 138,955,000, and our food protein available for consumption, assuming a trend much as it has been in recent years, would have mounted to around 5 million metric tons or on the order of 11 billion pounds. A recent survey of the food protein production of United States in terms of four major food groups: namely, meats, dairy products, poultry and eggs, and beans and peas, was published in 1942 by the Committee on Milk, Meat and Legumes, Food and Nutrition Board of the National Research Council.[92] This survey, like the one of Pearl, was occasioned by a national emergency but was much less comprehensive and given in much less detail.

The average adult in this country consumes in one year around 0.75 short tons (1,500 pounds) of food per year, or about one-third this quantity of food on a dry-weight basis. On the as-purchased weight basis this food contains about 1.5 per cent of protein, on a dry-weight basis about 4.5 per cent of protein. This food, of course, includes sugars and fats which contain no protein, and the computation includes no correction for wastage. These figures provide merely rough estimates of the quantity of food and food protein which an average adult withdraws from the national supplies annually.

The actual cropland harvested in the United States in 1939 was estimated to be approximately 321,242 thousand acres, or roughly not quite 2.5 acres per capita. Some of this, of course, yielded crops not directly suitable for human consumption, and by more intensive cultivation some of it could be made more productive. The best farm land is already under cultivation or is allotted to farm buildings, timber, and such. We could also search out some further marginal land and by expending more labor and materials make it productive in greater or less degree. We could drain some swamps probably. By such means as these we could add some acreage to our cropland, but all these measures offer much less in the way of increased food production and exact a great deal more labor and expense than many assume. Our capacity to increase food production is not inexhaustible.

Considering a world population of about 2,200 millions, the earth's surface would allow for each person about 60 acres, of land surface about 15 acres, and then, after elimination of land in deserts, mountains, and other unproductive areas, there would remain, according to economists, the equivalent of about 1.0 to 1.5 acres of reasonably productive land per person.[90, 93] If the one acre was intensively cultivated, let us say in cereals, it

would provide the food equivalent in calories for an average adult man each year, provided there were no crop failures and the land was well cared for. In densely populated countries there is not even one acre of cropland available per person.[40] Some of the population would require less than one adult man. Of course the land would not be entirely planted in grains, but it approaches that *closely* where pressures of population force men to produce the maximum food calories that the land is capable of producing in types of food that can be carried over from one season to another.

So, because of our extensive grazing lands and relatively large acreage (per capita) in cropland, it becomes intelligible how we, in the United States, manage to consume a relatively rich diet including a high proportion of animal products which are inefficiently produced by animal conversion of feedstuffs. There is no magic in food production, and no amazing scientific advances have enabled us to make two blades of grass grow where other men can make but one grow. Farm machinery made it possible to cultivate land with less man-power, but it did not make the land produce more except where factors such as deeper plowing were effective. Some of that man-power released has gone into producing materials for the farmer and some of it is engaged in the production of a myriad of other things associated with our industrialized life. The result — a relatively high standard of material living and, in line with the main thesis of this discourse, a high level and high quality of protein in our national diet.

Population in Relation to Food Protein Supplies

Civilization is credited with having its beginning when man took to a settled abode with plant cultivation and animal husbandry supplanting his more precarious methods of searching for food. By this stroke man made an enormous stride forward in the achievement of more abundant food supplies. Simultaneously he also set the stage for rapid increase in the numbers of his kind. Insecurity of his food supply was lessened but by no means banished.

Crop failures, through droughts, floods, and pests, destruction of his animals and other food reverses through war and raids, and various other conditions which dislocated factors of production and distribution of food supplies have all added a share to work havoc with man's objective to achieve security in food supplies. Famine and starvation have dotted the whole history of civilized man. Despite these hazards, set-backs, and disasters man has clung to food plant cultivation and animal husbandry as the most practical means of obtaining food supplies.

With but brief respites, civilized man has always had a struggle to keep food supplies ahead of the numbers of people to be fed. Most biologists have noted that other forms of life (plant and animal) also tend to populate up to the limit of available food supplies; this is perhaps a universal "law of life." We in this country are not likely to be so keenly aware of this trend

of balance between population and food supply because we *still* live in a relatively sparsely populated country where new agricultural lands have rather recently been settled. By contrast, a country like China is densely populated and, according to Buck,[40] farmers, having settled most of the country centuries ago, have brought under cultivation not only the good land, but a great deal of sub-marginal land. The rural population of China is 85 to 90 per cent of the total population. A farm household in China averaging 6.2 persons lives on a farm averaging about 4.18 acres; in United States the average size of farm families is about 4.2 and the average size of farms about 157 acres. Adolph [48] generalizes on the overall food situation in China very simply as follows: "Our experience in the Far East leads us to believe that in normal times the question of sufficient calories usually takes care of itself. That is to say, in a closed area or in a large agricultural country undisturbed by much industry when the population pressure is in equilibrium with the food supply, it would appear that people live or die, depending upon whether the supply of calories is sufficient or not."

Wars always make people very much aware of food supplies; post-war periods always find people in more or less social upheaval and with a desire to see some measure of social gains instituted. There is widespread hope that man may somehow manage to provide himself generally with a higher standard of living including a richer and more varied diet with relief from famines and other less severe food shortages. Some are sure this can be done if man will but put his effort into doing it. Others see some very definite limitations considering that the area of arable land is limited and that population continues to increase. Clearly the matter is beset with some imponderable factors.

There are no new continents awaiting man's discovery. The land area of the world is finite and a large proportion of it is incapable of producing food or feed crops. Productive land presupposes good soil, but to this must be added an adequate supply of sunlight, favorable temperature suited to a growing season, adequate and reliable rainfall (or possibilities for irrigation), and suitable topography.

As regards population pressures, Malthus wrote in 1798 in his "Essay on the Principle of Population" that "the power of population is indefinitely greater than the power of the earth to produce subsistence for man." About 135 years later, Pearl [91] in 1924 writes on the same general topic as follows: "The important consideration is that population, so far as we know, always has grown and certainly is now growing at a rate which, if continued, will sometime completely populate the habitable portions of the earth with a density which will be the maximum consistent with existence of human beings." "Nothing," Pearl continues, "which has happened since 1798 has in the least degree mitigated or softened or altered in any true sense the relentless insistence of Malthus' logic. On the contrary, the developments of the last century have made it far plainer even than it was to so clear-

visioned a major prophet as he was that the population of the world cannot go on increasing at anything like the rate of growth that has prevailed in the past more than a short time longer."

Land devoted to raising such high energy yielding crops as potatoes, rice, or corn produces relatively large amounts of food per acre of crop land. Such crops as these supplemented with small amounts of other foods mostly of plant origin are capable of sustaining greater numbers of people than most other uses to which the land could be put. These three foods are the major items in the diets in various regions of the world where food supplies are highly restricted. Other cereals and root crops, with variations from place to place would, in general, follow the three foods specifically named as major items in areas where populations are dense relative to available crop land. The potato and grain diet was the basis of the German war- and pre-war program of self-sufficiency in the way of food supplies for themselves and the countries they occupied for a time. At the other extreme, land devoted to producing animal products for human consumption would provide only a relatively small return in food. So a diet which provides liberal amounts of milk, eggs, meats, fruits, and leafy vegetables, in addition to potatoes and grain products, automatically demands more land per capita for those who consume such varied diets than is required for production of food for dietaries approaching the strictly vegetarian type.

Against the forces of population pressure and limitations of arable land areas man possesses a degree of inventive and scientific genius. This human attribute has in the past aided and presumably in the future will continue to lend aid in increasing food production. The addition of two new food crops, natives of the New World — maize and potatoes — made important contributions to human dietaries. Hybrid maize is a recent development which added an average of about 15 per cent to potential yields of maize. The Haber process for fixation of nitrogen paved the way for wider use of chemical fertilizers and various other means have been exploited in greater or less degree to increase food supplies. But these lines of progress have not been anything like the spurt made in potential food production which arrived with opening of the New World and its large areas of land suitable for growing food crops. As East [93] has pointed out, "Mechanical invention simply made it possible for a given unit of man power to cultivate more land and to distribute its products more rapidly and equitably." Man's inventive genius thus far has not actually been a large factor in the race between food production and population increase. Aside from spurious and local examples of "surplus foods" which were after all merely a matter of poor distribution, the world has never had a real food surplus.

The settlement of new continents has always had a favorable effect temporarily on man's food supplies and for clearly obvious reasons. Countries of Western Europe are practically the only countries that have an excess of food imports over food exports and this is managed by export of

other types of commodities in exchange for food. This type of exchange is possible only because these are highly industrialized countries and because other countries can still produce more food than they need and are willing to exchange some of it for various non-food materials and industrial products.

There are no huge stock-piles of food in the world and never have been. In two-thirds of the world the people live very strictly on a hand to mouth food economy. If a crop failure materializes as it does in some of these areas almost every year, people face starvation. In this country the carry-over of food from one crop season to another is estimated at about 6 per cent of annual consumption.[90] However, those countries, including our own, which have a large livestock population have therein a temporary or short-term buffer against food shortage. If crops fail, more or less of the livestock population, as fits the given circumstances, is liquidated, which makes this reserve source of food available. Having done this, further adjustment can be made through consumption of a more vegetarian type of diet if necessary. Consumption of food plant materials directly represents more economical use of the available short supply of food crops than the conversion of plant foods into animal products. Many countries do not have large livestock populations and many being over-populated and hard-pressed normally to balance food crops and population numbers are shortly faced with critical food shortages when a major crop fails.

The human body is highly adaptable to a wide variety of dietary patterns and if occasion demands, it can make some highly effective physiological adjustments. Nevertheless, nobody doubts that there are physiological minima in the way of nutrient supplies which will sustain life and which will promote normal life processes even though the science of nutrition cannot now precisely define all of these minima. The human body cannot go on building up nutritive-debt so there cannot be overage of population in relation to food supply. For the support of health, vigor, and stamina man requires some margin of food nutrients over and above minimum requirements. By and large the quantitive consumption of food by various peoples does not vary greatly except as climate and body size decree, but the type of food consumed and its nutritive qualities otherwise do vary greatly. The most immediate need for food is caloric value but calories alone will not long suffice. Because of the higher nutritive requirements of children and for women during child-bearing and lactating periods, this group is especially hard hit by inadequacies in the food supply. In countries where the food supplies are highly restricted, mothers tend to nurse their children for considerable periods, but this merely shifts the problem from child to mother. There is usually, in these same countries, a high death rate among children which is in part due to nutritive qualities of the food supply, but very commonly involves a high proportion of preventable and untreated disease also.

Except for a very small amount of food to be found growing wild, food is not free; its procurement entails cost to the consumer. The farmer and livestock breeder can no more produce free goods than the scientist can invest in education and donate his labor and investment without recompense in some kind. Economic processes the world over operate on the basis of exchange of various types of goods and services for other types of goods and services. Division of labor and specialization of professions command such exchange. If there is any more practical way than this to manage procurement of the variety of goods and services needed or desired, man has never found it.

There can no longer be any argument on whether or not the so-called "Law of Malthus" is really operative. It is operating in full view in countries such as China and India. Whether or not greater food production could halt it is like asking whether a pail can be filled with water if it has a hole in the bottom which continues to grow larger and larger. The level of water is bound to sink unless your ability to carry water keeps up with the outflow and, figuratively speaking, the level for the world as a whole is already low. Huntington [94] sums up the situation like this; "The upshot of the whole matter seems to be that one of two things must happen — either the standards of living of many countries — yes, of the majority of the peoples of the world, must decline because of increasing density of population or the birth rate must be lowered until the growth of population ceases to outstrip the growth of man's ability to produce food and the raw materials which are needed to maintain a high standard of living."

The world's food protein supplies are, of course, one of the factors in the larger problem of overall world food supplies. The fact that the highest quality proteins are also the most expensive to produce adds further to the economic aspects of the problem. World food surveys, even of major food items, have been few and it is obvious that many difficulties stand in the way of obtaining such data. But having briefly considered the world food problem, it might be of some interest to see the problem further unfolded as it relates to food protein supplies in such degree as is possible at present.

In Table 4–12 are shown the per capita, pre-war protein supplies of 70 countries together with the proportions of the supplies estimated to have been derived from animal and plant products respectively. These data were released recently by the Food and Agriculture Organization of the United Nations,[95] and represent presumably the largest scale attempt ever made at surveying world protein supplies. In the release it is stated that these figures must necessarily be regarded as provisional and incomplete. Nevertheless, they provide a comprehensive view of the pre-war food situation. In most cases, the data refer to the period 1935–38 or 1935–39; but as regards this and other details, the reader should consult the original reference. In order that the reader may be better equipped to evaluate the data

readily in terms of population numbers involved, the writer has included in the table a column giving the estimated population of each country. The population figures were taken from the last issue of the "Encyclopædia Britannica," and for some countries of course the population data are much more accurate than in others and this should be kept in mind. The writer has also converted the contributions of protein supplied in the form of animal and plant proteins into percentages of the total per capita supplies from original data expressed in terms of grams. The term "protein supplies" as it applies to data in Table 4–12 refers to *supplies available* in the respective countries listed, which countries probably represent about 90 per cent of the world's total population. It is perfectly clear that the major part of the world's food protein supplies are derived from foods of plant origin.

Nutritionists often recommend that from ⅓ to ½ of the total dietary protein should be derived from high quality animal proteins in the form of milk, cheese, eggs, and meats (including fish and poultry) at least for children and for women during periods of child-bearing and lactation.[79] A quick glance at the figures in Table 4–12 will make it evident that even if the world's entire food protein supplies were pooled and distributed according to recommended allowances (assuming for the sake of argument that such a plan were possible) among the world's population such a goal would not come anywhere near fulfillment. Other important features of the data shown in this table will not require further comment here. It is all too clearly obvious that world food protein supplies are not adequate to provide a high standard of diet as regards protein quality. Under present conditions at least a large population of livestock devoted to food production is absolutely prohibitive in many of the more densely populated countries. Fresh fluid milk is one of the most perishable, easily contaminated and least conveniently transportable foods. Except in processed form (evaporated, condensed, or dried), therefore, milk is usually consumed in the country of production and in large measure near centers of production. Dairy products in other forms, butter, cheese, etc., move into more expanded markets. The greatest development in production of dairy products has occurred in the western hemisphere (Canada, United States, Argentina, and a few other countries), Continental Europe (especially in the Scandinavian countries and central and western Europe), the British Isles, Australia, and New Zealand. In general those countries which consume rather liberal quantities of milk and other dairy products, usually consume rather liberal amounts of meat also. Much of the European livestock is in part supported by imported feeds and feed protein-concentrates. By these means, industrialized European countries can achieve a much richer and more varied diet, including relatively high protein quality, than if they operated on a program of self-sufficiency in food supplies.

In very few countries is the human population known to be stationary or

declining; in most cases it is increasing, although in many it would appear to have passed the peaks of *increasing rates* of growth. There is continual population pressure on food protein supplies (and food supply generally) in this country as well as in almost all other countries. Population pressure is a relative term and varies in degree from country to country.

The population of the United States has grown from around 4 millions in 1790 to around 63 millions in 1890, and to around 131.5 millions in 1940. According to inter-censal estimates of the Bureau of Census issued September 15, 1946, our total population on July 1, 1946 had reached 140,840 thousands. These inter-censal estimates which take into account death rates, birth rates, and the like, must also be corrected and adjusted later on in light of data from the last census and those to be obtained at the next census period. Obviously in a new country the size of ours there is in its early history a great excess of land per capita. As the land becomes settled and the country becomes more and more populous, the per capita area of land is reduced and must perforce be more intensively utilized. Our relatively large area of land per capita, plus certain natural resources plus our industrial development are all major factors in supporting our so-called high standard of living. From where we are now, any substantial increment of population places more pressure on the various factors of our economy, and our ability to maintain a high standard of living will be put to test. Human effort and intelligence are large factors in utilizing natural resources to gain and maintain a high standard of living, and as our population increases future generations will have an increasingly larger problem — these will have larger national and world problems than our generation.

In 1924 Pearl [91] ventured a prediction or description of the trend in rate of population growth for our country based upon mathematical considerations and extrapolation of data which were found to describe empirically the course of our population changes in the past. Pearl showed that from 1790 to 1920 the population growth in the United States followed very closely a certain empirical equation which by ordinary means of curve fitting would assume the form of a logarithmic parabola. It is merely an empirical expression without encumbrance as regards any theory of population growth. In extrapolating his curve, Pearl predicted the course of population on the basis of constancy or essential constancy of such factors as land area, standard of living, methods of agriculture, etc. Of course, if any and all sorts of shifts without number were to be entertained in predictions, nothing but a fantasy could result. Thus he described and extrapolated his findings on the number of people who would be living within the present area of the United States on the basis of past trends, assuming no intervention of cataclysmic alterations of circumstances or fundamentally new forces that would throw us off the general course followed earlier.

If or when, owing to discoveries or new circumstances, a new cultural stage was entered upon, a new cycle of population growth might also be

Table 4–12. Apparent per Capita Consumption of Protein and Proportions of Plant and Animal Proteins Respectively Provided by Prewar Food Supplies of 70 Countries *

Country	Estimated Population †	Protein per Day		
		Total Protein (gm)	Animal ‡ Protein (%)	Plant ‡ Protein (%)
North America				
United States	131,669,275	88	57	43
Canada	11,506,655	87	54	46
British Isles				
Eire	3,020,000	92	45	55
United Kingdom	46,154,200	80	54	46
Scandinavia				
Denmark	3,844,312	76	58	42
Norway	2,930,000	83	49	51
Sweden	6,284,722	88	61	39
Iceland	120,264	101	62	38
Finland	3,850,000	80	46	54
Central & Western Europe				
Switzerland	4,220,000	89	54	46
France	41,980,000	87	44	56
Germany	79,612,442	77	44	56
Netherlands	8,840,000	78	47	53
Austria	7,009,014	79	46	54
Belgium	8,310,000	77	42	58
Czechoslovakia	10,500,000	72	35	65
Southeast Asia Mainland				
Burma	16,119,000	73	44	56
Malaya	3,500,000	57	25	75
Siam	14,464,489	58	36	64
Indochina	21,652,000	55	31	69
Major S. E. Asia Islands				
Java and Madura	41,901,542	43	9	91
Philippines	16,000,303	55	45	55
Middle East				
Turkey	16,200,694	101	26	74
Palestine	1,569,000	74	22	78
Syria & Lebanon	3,630,000	77	34	66
Egypt	15,920,703	69	12	88
Iran	15,000,000	66	26	74
Iraq	3,560,456	61	26	74
Trans-Jordan	300,000	64	23	77
North Africa				
French Morocco	6,500,000	75	35	65
Tunisia	2,700,000	69	36	64
Algeria	7,490,000	63	30	70

Table 4–12. Apparent per Capita Consumption of Protein and Proportions of Plant and Animal Proteins Respectively Provided by Prewar Food Supplies of 70 Countries * (*continued*)

Southern Europe				
Spain	26,222,000	85	24	76
Italy	43,950,000	81	23	77
Greece	7,336,000	65	22	78
Portugal	7,702,000	74	31	69
Cyprus	383,967	85	16	84
Eastern & S. E. Europe				
Yugoslavia	13,934,038	87	21	79
Rumania	19,933,802	87	22	78
Bulgaria	6,370,000	90	20	80
Hungary	10,817,286	89	27	73
Poland	35,200,000	72	26	74
USSR	170,467,186	88	19	81
Oceania				
New Zealand	1,624,714	96	64	36
Australia	7,068,689	90	66	34
Eastern Asia				
Manchuria	43,233,954	88	6	94
Japan	72,875,800	67	18	82
China	420,803,578	68	7	93
Formosa	5,609,000	52	27	73
Korea	22,633,853	70	21	79
Indian Peninsula				
Ceylon	6,061,000	51	22	78
India	365,900,000	56	16	84
West Africa				
French West Africa	14,944,830	68	24	76
East Africa				
Kenya-Uganda	7,325,551	55	22	78
Madagascar	3,669,328	50	26	74
South Africa				
Union of South Africa	9,589,898	75	31	69
Central America				
Honduras	57,061	49	20	80
Costa Rica	656,129	49	43	57
El Salvador	1,787,930	52	27	73
Mexico	19,473,741	59	34	66
Caribbean				
Cuba	4,227,587	68	43	57
Puerto Rico	1,869,255	55	31	69
Dominican Republic	1,654,993	54	26	74
South America				
Argentina	13,129,723	111	57	43
Uruguay	2,122,628	102	62	38
Paraguay	1,104,773	99	61	39
Brazil	41,356,605	73	36	64
Chile	4,679,494	70	34	66
Peru	7,023,111	58	14	86
Colombia	8,986,106	62	47	53

* Basic data on protein supplies were taken from "World Food Survey" of the Food and Agriculture Organization of the United Nations, released July, 1946, Washington, D. C.

† All figures in this column are those given in the "Encyclopædia Britannica" (1943 copyright).

‡ The original data on plant and animal protein respectively were given in grams, the percentages shown here were computed by the author.

begun. In such event a new curve, starting from the base line of the already attained population of the old epoch, would, according to Pearl, need to be constructed. The newly constructed curve would arise from a new base but retain the essential features of the old curve in describing the trend of population growth.[96] Pearl's curve describing the probable future trend of population growth in the United States, based on past records and conditions, is shown in Figure 4–2.

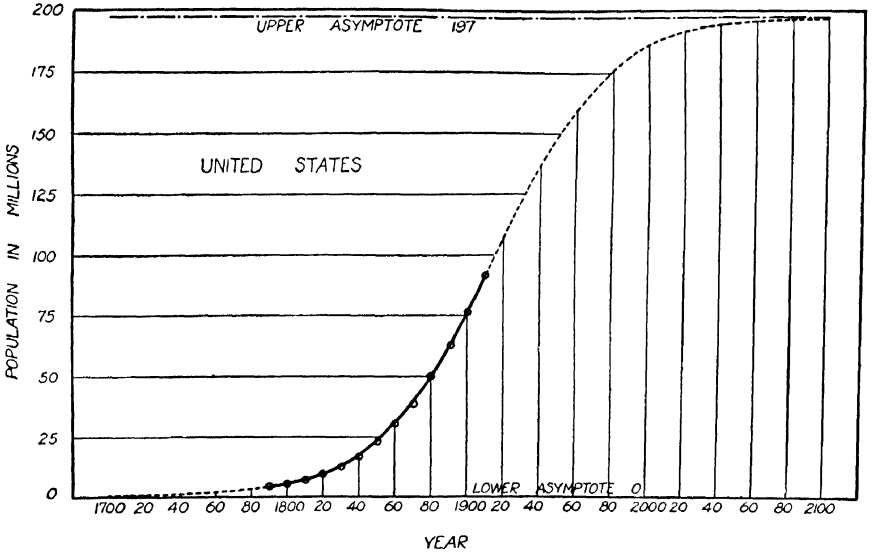

FIGURE 4–2. The trend in population growth of the United States based upon census data from 1790 to 1910 as predicted by Pearl.[91] (*Courtesy Williams and Wilkins Co., Baltimore, Maryland*)

Pearl makes it clear that his predictions shown on the curve as a dotted line to the right of the solid black line (describing past trends in population numbers) are subject to a probable error and that as new data are accumulated on the subject the predictions should be corrected in light thereof. Nevertheless, the reader will note that the "proof of the pudding" has worked out very well for the years since Pearl presented his chart. Now of course a lot of cataclysmic alterations and fundamentally new forces *could* come into this picture. We could lower our standard of living very appreciably, someone might find a way to make 3 or 5 or 10 blades of grass grow where one now grows, a horde of insects might completely overrun the country, and so on — those would be fundamentally new forces if one wishes to dream. But headed as we were and are, our population numbers appear to be speeding along approximately as Pearl predicted for the years immediately ahead. This chart is presented here merely for imparting some idea of what may be ahead of us in the way of problems of food protein production and food supplies in general as our population increases. It is clear

that if we maintain our present status, we must intensify our efforts and do so still further if we are to have further quantities of goods to share and trade.

World surveys of detailed food consumption patterns are not available. However, a special Joint Committee set up by the Combined Food Board [80] has published rather detailed data on the nutrients available from various major food groups for civilian consumption per capita per day for the years 1935–39 (average), 1943, and 1944, for the United States and Canada, and for the years 1934–38 (average), 1943, and 1944 for the United Kingdom. The data therein relating to average available supplies of food protein for one or the other of these two intervals 1934–38 or 1935–39 and for the years 1943 and 1944 respectively are shown in Table 4–13.

So far as the writer can decipher from the total data released by this special Joint Committee, the oft-repeated statement that "people of the United States are the best-fed people in the world" would seem to have very little basis in fact. Probably with regard to palatability, the peoples in any one of these three countries would prefer the type of their own country's pre-war diet to that of either of the other two. We are probably as well-fed as the people of any country of similar size. However, our national dietary appears to be less rich and varied than that of certain smaller countries, such, for example, as that of New Zealand. The differences in nutritive quality of pre-war United States and Canadian per capita average food supplies for the years 1935–39 were of no practical nutritional significance and neither the United States nor Canadian per capita supplies for this period showed important differences from those of the United Kingdom, 1934–38. Although some shifting in the items available as sources of specific nutrients had necessarily to be made during 1943 and 1944, this situation did not seemingly entail consumption of significantly less nutritious foods. However, in the United Kingdom the food supplies during 1943 and 1944 were less varied, and according to most accounts were considered less palatable. Fruits and various vegetables, other than potatoes, were quite restricted. The food-rationing system of that country forced many of the populace to alter their accustomed dietary habits rather markedly, but this is not of importance to the present thesis.

It is clear from the data in Table 4–13 that in spite of having far less cultivated land area per capita than United States or Canada, the food supplies of the United Kingdom have not, normally, represented any inferiority as regards nutritive quality. In years shortly preceding World War II, Great Britain produced only about 35 to 40 per cent of her food. The peoples of Great Britain produced during those years the whole of their milk, nearly all of their potatoes, a large proportion of their eggs and poultry, 50 per cent of their meat, but only 25 per cent of their wheat and 10 per cent of their butter, but are reported [97] to have attained from 65 per cent to 75 per cent self-sufficiency in the way of food supplies during the last war.

Table 4–13. Food Protein Available for Civilian Consumption per Capita per Day, United States, Canada, and the United Kingdom

Source of Food Protein	*1935–39 Average*		*1934–38 Average*	*1943*			*1944*		
	United States (*gm*)	*Canada* (*gm*)	*United Kingdom* (*gm*)	*United States* (*gm*)	*Canada* (*gm*)	*United Kingdom* (*gm*)	*United States* (*gm*)	*Canada* (*gm*)	*United Kingdom* (*gm*)
Dairy products	19.5	20.3	13.8	22.7	23.9	18.1	22.7	24.5	17.4
Meats	20.3 *	19.7	18.4	22.5 *	25.0	14.8	23.5 *	25.7	14.9
Poultry, game, and fish	6.1	6.3	6.9	6.8	7.8	4.9	6.4	6.9	5.2
Eggs	4.9	4.3	3.5	5.6	5.0	3.0	5.6	5.2	3.4
Fats and oils	0.2	0.3	0.2	1.3	0.2	0.1	0.1	0.2	0.1
Potatoes	3.1	4.3	3.6	3.1	4.5	5.5	3.1	4.2	5.7
Dry beans, peas, soybeans, and nuts	4.2	3.6	2.7	5.9	3.3	2.4	6.2	4.6	3.1
Tomatoes and citrus fruit	0.7	0.5	0.4	0.8	0.7	0.2	0.8	0.8	0.2
Other fruits	0.9	0.5	0.5	0.7	0.4	0.3	0.8	0.5	0.3
Leafy, green, and yellow vegetables	1.6	0.8	1.4	1.9	0.8	2.0	2.0	1.0	2.0
Other vegetables	1.3	0.5	0.5	1.4	0.4	0.7	1.4	0.5	0.7
Grain products	25.8	28.9	28.1	26.5	31.5	35.7	26.8	31.3	33.9
Beverages	0.2	0.2	0.3	0.2	0.2	0.6	0.2	0.2	0.5
Total, all sources	88.8	90.2	80.3	99.4	103.7	88.3	99.6	105.6	87.4

* Includes fat pork cuts.

Normally the meat-producing animals in Great Britain are the objects of more or less intensive feeding in large measure on imported grains and feeds. Denmark specialized in dairy products on much the same basis. So while Great Britain has normally been far from self-supporting in the way of food supplies she has been economically self-supporting by reason of her industrial development at home and in her colonies, by which means she has obtained exchange values with which to purchase food from less populated food surplus-producing regions of the world. The purchase of butter from Denmark would, of course, be a case of purchasing from less populated regions once removed. In varying degrees many countries of Western Europe obtain their food supplies and support a relatively rich and varied diet for their people by such procedures. Economically there are two factors necessary for the operation of such programs: (1) an *excess* of food production in some countries, and (2) a *relatively* high degree of industrialization in others. These facts are presented merely to illustrate that population, in and of itself, may be no cause for restricted dietaries, but it is obvious that "Malthus' law" is only suspended in its application by reason of the fact that all countries are not as over-populated as, for example, China. Perhaps they never will be, for no one can truly predict how future generations may manage their population problems, and no one can truly say that some new force may not intercede to prevent universal over-population. On the other hand, it cannot be said that Malthus was entirely mistaken nor that we lack indications of the trend in human population of which he clearly spoke.

The Food Protein Problem of the Poor

Exclusive of situations wherein relief feeding is temporarily extant, the dietary patterns of civilized men everywhere represent, of necessity, adjustment between the economic status of the individual or family and the prevailing conditions of the food economy of the given area. Only *within* the circumscribed limits of these two conditions can an individual or family normally exercise freedom of choice in dietary pattern. If one or the other of these two major conditions of existence is depressed in substantial degree, dietary habits will *and must* change. Nutrition education, if intelligently applied, would enable a person to make rational food choices within the limitations set by prevailing economic factors, but it could not do more for anyone. The food protein problem of the poor is an integral part of the food problem of this group in general, and it cannot be separated from the larger problem to any important degree.

The statement that poverty is the chief cause of malnutrition and undernutrition is an irrefutable fact but the word "poverty" has become hackneyed by overusage in attempts to stir human emotions with sentiments of pity and compassion for others. We should have pity and compassion for others, but unless that is followed up by a practical and a constructive

program of assistance, such emotions merely constitute indulgence in cheap sentiment.

Poverty implies lack or insufficiency of goods or services to permit an exchange for such total goods and services as one needs to live decently. Poverty is a relative term, of course, and the popular concept of a decent living varies in different places and moves upward with the progress of mankind in achievement of higher average standards of living. Any plan for *basic relief* of poverty should encompass all degrees of poverty or it will, sooner or later, amount only to a vicious circle of favoritism.

Some of the chronically poor are so because they are victims of incurable disease or are beset with serious physical or mental handicaps. Those incapable of securing a decent livelihood by reason of such circumstances probably represent a rather small proportion of the chronically poor. The only means of relief for such cases is through direct aid supported by members of society in more fortunate circumstances.

Some numbers among the chronically poor are not inclined to exert themselves by reason of having lost or never acquired an interest in struggling for a decent living and others are merely underprivileged people who could and would make a sincere effort to better their economic condition if they knew how to cope with situations directed to such an end. Ignorance or non-acceptance of the concept of one's responsibility to be productive and useful or ignorance of the ways and means of making a decent living are obviously predisposing causes of chronic poverty. These are causes of poverty which society has a very clear and definite responsibility to remove from its midst. Very few of the chronically poor are helpless and very few have been substantially helped by pity. The situation calls for more constructive assistance than transient forms of charity and pity and public benevolence.

Chronic poverty thrives on ignorance the world over and any program for relief of the causes or conditions of poverty which does not have education of the people involved as the basic objective would seem to this writer to side-track the prime issue. Certainly a program which puts a premium on indolence or ignorance is to be condemned. Relief feeding, then, becomes a sedative to be used accordingly and education directed toward helping people with a sub-average standard of living to increase their possibilities for usefulness to society and to themselves becomes the basic and long-term objective.

Education will not make all men opulent in material goods, but few persons with a reasonable amount of education and training are among the group classed as being in a state of chronic poverty. We, in this country, crossed the bridge of compulsory education long ago; we just have not seen it through to an *effective* and practical solution for some, and these we left behind as non-participants in our national and community activities by means of which most of us make our living. When folk have useful goods

or services *to offer* and show a willingness to offer these, there will be far less of poverty, far less malnutrition, and far less undernutrition than now exists. It is certainly a part of the responsibility of every intelligent and educated man and woman in our country to see that any and all who are capable of being trained and educated so that they can contribute to society and receive in return their share of the world's goods and services are provided that opportunity. Perfect justice in human relations may be a long way off but the just deserts of the chronically poor as members of the human family need not be as remote as they have been in any country. Is there any more effective or more fundamental kind of help men can give their fellowmen than an equal opportunity with themselves to gain a proper share of the world's goods and services or as near that as inherent capacities permit? Is there any more fundamental and effective way of reducing poverty without risk of harming many whom we had hoped to help?

Carefully considered and circumspect improvements in the nutritive qualities of staple low-cost foods and the development of further low-cost food items of high nutritive value and wide acceptability could expedite the process of obtaining more adequate diets for those with low incomes. However, the inadequate diets of the poor are not of a uniform pattern and the poor as well as other economic groups have various food preferences. So it is not to be expected that one or even a few low-cost food items can be ushered in hastily to solve the entire nutritional problem of the poor. Whether or not improvements in nutritive qualities of existing staple articles of food or development of inexpensive new foods would be in the best interests of the poor requires consideration of the cost to the consumers, the degree of acceptability of the items and the probable level of consumption of the products proposed. Any food purporting to be a boon to the poor must be within economic reach of those with low incomes; it should enjoy ready acceptance by significant numbers of the poor, and it should supply needed nutrients and be consumed in effective quantities by the poor.

In some parts of the world, poverty and the malnutrition and undernutrition which accompany that state are the direct result of over-population together with under-education. In various countries of the Orient, over-population is a prime factor in the relatively low economic status of the majority of the people. There illiteracy and general lack of education among the masses have an almost incredibly high incidence.[40] One way or another an over-population problem which has reached the stage of more people than the land and degree of industrialization of the country together can support would seem to call for intelligent cooperation on the part of a large segment of the people. Basic education widely disseminated among the people would certainly be a great boon in working out the wide-scale solution required.

As the reader will have noted from data presented in Table 4–4, most of the problems of the low income groups, including procurement of an ade-

quate diet and increased purchasing power for other things, are resolved rather automatically with increase in family purchasing power. As people can be taught to be more productive and useful in the society in which they live, they will enhance their powers to gain such basic economic security as human society can provide its members. Ignorance or indolence (both remedial by proper education) almost doom one to the maximal risk of living in poverty; knowledge, training, and a willingness to be productive and useful are the best tools we have to avoid the risk of having to live in poverty.

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Chapter 5

The Nutritive Aspects of Meat and Meat Products

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The terms meat and meat products are used to include the skeletal muscle tissues of beef, pork, veal, lamb, mutton, poultry and game; and other organs which may be utilized for food, such as the tongue, liver, kidney, heart, pancreas, brain, thymus and spleen. The importance of meat in the diet is evident from the production and consumption statistics.

Production and Consumption

The average annual production of meat in the United States for 1939 to 1941 was about twenty-one and one-half billion pounds. For the year 1945 it was about twenty-seven billion pounds.

From 1909 to 1945 the average annual per capita consumption (estimated on retail weights) of meat and fish in the United States ranged from 128 to 164 pounds. The average for 1935–39 was 137 pounds and for 1940–44 was 152 pounds.[153] Beef and pork constitute the major portion of meat consumed in the United States (Table 5–1). The quantity of meat consumed varies in different sections of the country and with the level of economic status.[131–133, 149]

Table 5–1. Production and Civilian Consumption of Meat in the U. S.

	Per Capita Civilian Consumption in Pounds		*Production in Millions of Pounds*	
	*1946**	*Average 1939–41*	*1946*	*Average 1939–41*
Beef	60.3	56.5	9,200	7,428.3
Pork	68.5	67.7	10,300	9,355.0
Lamb and Mutton	6.1	6.7	980	891.3
Veal	9.3	7.5	1,400	999.3
Poultry	27.0	22.1	3,830	2,962.0
Total	171.2	160.5	25,710	21,635.9

* Preliminary estimate.

Table 5–2 gives the average per capita consumption of meat (excluding poultry and game) in various countries.[69, 151]

Table 5–2. Estimated Average Annual per Capita Consumption of Meat Exclusive of Poultry and Game, 1930–1934

Country	*Total Meat (lb)*	*Country*	*Total Meat (lb)*
Argentina	266.4	Norway	73.0
New Zealand	228.9	Italy	35.5
Australia	201.7	Austria †	110.2
Canada	144.2	Finland †	86.0
United Kingdom	140.5	Hungary †	75.0
Denmark	137.6	Czechoslovakia †	61.7
United States	136.5	Poland †	46.3
Germany	112.6	Greece †	46.3
Netherlands	100.6	Bulgaria †	44.1
France	95.9	Rumania †	37.5
Belgium	86.4	U.S.S.R. †	37.5
Sweden *	79.4	Yugoslavia †	35.3

* For 1932–1933.
† During pre-war period.

Grades of Meat

Federal grades of carcass beef and wholesale cuts have been established as follows: *prime, choice, good, commercial, utility, cutter and canner.*[152] Only the first five grades are sold in retail markets. Quality is determined largely by the degree of fatness or finish, method of feeding, and by the quality of the animal.[6] *Prime*, the highest grade of beef, is obtained from high quality animals fed to a high degree of finish. It is available only in limited quantities mostly for exclusive hotels, restaurants and clubs. *Choice* is the highest grade regularly available. *Good* is the best grade that is available in volume for purchasers of high grade beef. *Commercial*, constituting a large percentage of production, is of moderately high quality. *Utility* is the lowest grade usually sold in retail stores where consumers purchase relatively low-priced meats. Cutter and canner grades are sold as prepared meats. There are five grades of veal: *choice, good, commercial, utility*, and *cull;* six of lamb: *prime, choice, good, commercial, utility*, and *cull.*

Since the higher quality of meats contains larger amounts of fat, they are lower in percentage of protein. There is no relation between the content of connective tissue of the lean of the rib or round and the market grade.[109] Retail prices of beef cuts are not determined by their nutritive value but by other considerations such as relative supply, flavor, tenderness, grain, color, general appearance and convenience of cooking.[46]

Structure and Composition of Meat

The lean of meat consists of two types of muscle tissue, the voluntary (striated) and involuntary (smooth or non-striated). The major portion is striated. Non-striated muscles occur in the heart, along the arteries in the walls of the alimentary canal, and in other organs. The striated muscle consists of bundles of muscle fibers held together by connective tissue.[112, 147] The fibers are composed of many fibrils arranged parallel to each other and to the fiber axis. The fibrils are about 1 μ and the fibers 10 to 100 μ in diameter. The fibrils are imbedded in the sarcous medium. A framework of connective tissues is distributed throughout the system of fibers. The connective tissues containing collagen, elastin and vascular material are called extracellular protein, and the protein of the fibrils and sarcoplasm is called intracellular.[3] In addition to the muscle tissues, meat contains varying amounts of adipose tissue, the proteins of which are chiefly collagen and elastin.

Muscle proteins may be classified into two groups, the plasma and stroma. The plasma proteins are a complex mixture, but consist chiefly of the globulin myosin which in the form of a concentrated gel makes up the fibrils; and the globulin X and albumin-like myogen, which form the chief constituents of the sarcoplasm. The following percentages are reported for rabbit muscle: myosin 57, globulin X 18, myogen 9, and stroma 16.[3]

About two per cent of the fresh weight of muscle tissues consist of extractives of which about 0.7 per cent is organic and 1.3 per cent inorganic. The nitrogenous extractives contain free amino acids, creatine, methyl guanidine, carnosine, inosine, carnitine, sarcosine, etc. Muscle tissues also contain small quantities of glycogen, glucose and lactic acid.

Collagen and elastin in varying proportions are the chief constituents of connective tissues. Both substances are insoluble in cold water but may be hydrolyzed by enzymes. When boiled with water, collagen is converted into soluble gelatin.

Tenderness of meat varies with the kind, age, part of the carcass, and degree of ripening; and is to some extent related to the amount and distribution of connective tissue.[109] There is some difference in the collagen and elastin content of different cuts of meat, but no relation between the grade of meat and the connective tissue content of the lean in round and rib cuts.[109] The connective tissues contain much larger amounts of collagen than elastin (Table 5–3).

Proximate Composition of Meats

The chief constituents of meat are moisture, protein, fat and minerals. Small quantities of carbohydrates, including glucose and glycogen, are present; the latter may be stored in the liver in amounts of a few per cent.[139] Meats contain small quantities of nitrogenous extractives which are included in the protein analysis (N $\times$ 6.25).

Table 5–3. Collagen and Elastin Content of Lean Meat in Per Cent of Total N.
(After Mitchell *et al.* [109])

	Collagen	*Elastin*
Round steak, outer	10.8	0.30
Round steak, inner or top	10.0	0.10
Porter house steak	10.9	0.08
Sirloin steak	8.0	0.08
Ribs 10th to 11th eye	8.2	0.03
Tenderloin	8.9	0.05
Chuck ribs 2nd, 3rd	14.6	0.30
Navel	15.8	1.02
Fore shank	23.0	0.09

The composition of meat is affected greatly by the proportions of fatty and muscular tissues present. These proportions vary with the type of animal, the stage of growth and degree of fatness. Formulae for calculating the proportions of the three kinds of tissues and estimating the composition of carcasses and cuts of meat have been developed.[17, 18, 47, 48] Typical analyses of trimmed hams from 175-lb hogs and 250-lb hogs in per cent are respectively fat 29.12 and 38.82, protein 15.38 and 13.29, moisture 54.81 and 47.80, ash 0.81 and 0.64. Thus there is a considerable range in the proximate composition of the different grades and cuts of meat.[2, 23, 46, 57, 118, 139, 147, 148, 154] The amount of protein in the edible portion of meats will usually range from 15 to 20 per cent. In Table 5–4 is given the average composition of typical meat cuts and meat products.

Amino Acid Composition of Meat

Precise data regarding the amino acid composition of meats and other protein containing foodstuffs are not available. Accurate and convenient methods of analysis have not been devised for all of the amino acids. Losses during hydrolysis and errors in the methods of determination of the amino acids are difficult to avoid.[7, 8, 141] Recently microbiological methods have been developed for the quantitative determination of amino acids and increased efforts have been devoted to the determination of the amino acid composition of foods.[4, 31, 40, 42, 54, 66, 78, 79, 85, 121, 123, 126, 128, 135]

A compilation of those values considered most reliable for meats is given in Table 5–5. These values will be revised in the future as more reliable methods of analysis are developed and larger numbers of samples are analyzed. Amino acid values for whole egg and milk are included in the table for comparison since both contain complete proteins of high biological value.

The analyses show that meat contains all of the indispensable amino acids in liberal quantities and that the proteins of meat are biologically

Table 5–4. Composition of Typical Meat Cuts and Meat Products (Edible Portion) [23, 154]

Type of Meat	Per Cent			Mg per 100 Gm			Calories per Lb	Per Cent Ash
	Moisture	Protein	Fat	Calcium	Phosphorus	Iron		
Beef chuck	65	18.6	16	11	200	2.8	988	0.88
Beef hamburger	55	16.0	28	9	172	2.4	1431	
Beef loin	57	16.9	25	10	182	2.5	1327	0.84
Beef rib	59	17.4	23	10	188	2.6	1255	0.83
Beef round	67	19.3	13	11	208	2.9	879	0.95
Beef rump	53	15.5	31	9	167	2.3	1545	0.77
Pork Boston butt	60	16.6	23	10	179	2.5	1237	0.80
Pork ham fresh	53	15.2	31	9	164	2.3	1540	0.80
Pork ham smoked	42	16.9	35	10	182	2.5	1740	5.00
Pork loin	58	16.4	25	10	177	2.5	1318	0.90
Pork picnic	52	14.8	32	9	160	2.2	1572	0.80
Pork spare rib	53	14.6	32	8	157	2.2	1567	0.80
Pork sausage	43	11.0	44	6	116	1.6	2021	2.10
Veal loin	69	19.2	11	11	207	2.9	797	1.00
Veal round	70	19.5	9	11	210	2.9	720	1.00
Veal leg	68	19.1	12	11	206	2.9	843	1.00
Lamb leg	64	18.0	17	10	194	2.7	1042	0.90
Lamb shoulder	58	15.6	26	9	168	2.3	1314	0.80
Lamb chop	64	18.0	17	10	194	2.7	1042	0.80
Chicken	66	20.0	13	16	218	1.9	880	0.80
Turkey	58	20.0	20	23	320	3.8	1188	0.80
Beef tongue	68	16.4	15	30	119	6.9	916	0.86
Beef liver	70	20.0	5	8	373	12.1	594	1.40
Beef heart	75	6.5	7	10	236	6.2	572	1.10
Brains	78	10.6	9		345		580	1.40
Sweet breads	54	11.8	33				1560	1.11
Frankfurters	64	15.2	14	9	164	2.3	912	3.10
Bologna	63	14.8	16	9	160	2.2	984	3.00

complete. The values given here are averages for different cuts. The lean tissues of different parts of the same animal and of beef, pork, veal and lamb are very similar in amino acid composition.[42, 84, 86, 121, 126] Compared with egg proteins, meat proteins are higher in histidine and lysine and lower in leucine, isoleucine, valine and methionine. Meat proteins contain larger amounts of arginine, histidine, lysine and methionine and smaller amounts of leucine, isoleucine and valine than milk proteins.

Several studies have been made on the effect of cooking meat on the stability of the amino acids. From 86 to 100 per cent retentions of valine, leucine and isoleucine were found in veal, lamb and pork. Less than two

Table 5–5. Amino Acid Content of Edible Portion of Meat in Per Cent of the Protein (N × 6.25) [7, 40–42, 84, 86, 121, 123, 126]

	Whole Egg Block [105]	*Whole Milk* [66]	*Beef*	*Pork*	*Lamb*	*Veal*	*Liver Beef Pork Lamb*	*Beef Heart*	*Tongue Beef and Pork*	*Kidney Beef Pork Lamb*	*Beef Brain*	*Beef Thymus*	*Beef Spleen*	*Chicken Muscle*
Arginine	6.4	3.5	6.4	6.4	6.2		6.0	6.5	6.5	6.2	6.0	6.6	6.1	7.1
Histidine	2.1	2.4	3.9	3.8	3.2	3.5	2.8	2.6	2.7	2.6	2.7	1.8	2.6	2.3
Lysine	7.2	8.1	8.9	8.7	8.8		7.3	8.3	8.6	7.1	6.7	8.3	7.6	8.4
Leucine	9.2	11.8	7.6	7.2	8.1	7.6	8.4	7.7	7.2	7.9	7.7	5.9	7.8	
Isoleucine	8.0	6.5	5.7	5.7	5.4	5.6	5.4	5.4	5.6	5.2	5.0	3.8	4.3	
Valine	7.3	6.2	5.3	5.5	5.4	5.3	6.5	5.5	5.5	6.0	5.5	4.3	6.0	
Methionine	4.1	2.2	2.5	2.4	2.4		2.2	2.3	2.4	2.1	2.1	1.4	1.9	3.2
Cystine	2.4		1.4				1.3	1.2		1.5				1.3
Threonine	4.9	4.8	4.5	4.5	4.8	3.9	4.4	4.6	4.6	4.5	4.7	3.6	4.2	4.7
Tryptophane	1.5	1.4	1.4	1.4	1.4	1.1	1.6	1.3	1.2	1.4	1.4	0.7	1.2	1.2
Phenylalanine	6.3	4.6	4.2	4.2	4.3	3.8	5.4	4.6	4.4	4.8	5.4	2.9	4.4	4.6
Glutamic Acid			14.2	11.8	11.9									16.5
Tyrosine	4.5	5.5	3.4				4.6	4.4		4.8				4.3
Glycine	2.2		5.0				8.5							
Alanine			4.0											
Serine			5.4				7.3	5.9		6.1				4.7
Aspartic Acid			6.0				6.9	6.9						
Proline			6.0											

per cent of these amino acids were found in the drippings.[126] Some values for the amino acid composition of fresh and cooked cuts of beef, pork and lamb are given in Table 5–6.

Table 5–6. Average Amino Acid Content of Fresh and Cooked Cuts of Meat in Per Cent of Protein (N × 6.25) [42]

	Fresh Beef		*Cooked Beef*		*Fresh Pork* [c]	*Fresh Lamb* [d]	*Cooked Pork* [c]	*Cooked Lamb* [d]
	Choice [b]	*Utility* [b]	*Choice* [b]	*Utility* [b]				
Arginine	6.7	6.9	6.4	6.5	7.3	7.5	6.5	7.0
Histidine	3.2	3.1	2.7	2.8	3.7	3.0	3.2	3.0
Lysine	8.5	8.5	8.0	8.3	8.0	7.8	7.9	7.8
Leucine	8.4	8.9	7.9	8.4				
Isoleucine	5.3	5.4	5.1	5.0	5.0	4.8	5.1	5.1
Valine			5.5	5.6	5.2	5.2	5.0	5.2
Methionine	2.4	2.1	2.4	2.3	2.2	1.8	2.0	2.2
Cystine	1.4	1.4	1.3	1.3				
Threonine	3.9	4.0	4.2	4.1	4.2	4.1	4.3	4.1
Tryptophane [a]	0.8	0.8	0.8	0.9				
Phenylalanine	4.1	4.0	4.1	4.0				
Glutamic Acid	14.3	14.1	13.9	13.7	11.8	11.9	12.5	12.8

[a] Values probably low.

[b] Average values for chuck, flank, neck, plate, rib and rump.

[c] Average values for rib chop, loin chop, blade steak, spare ribs, loin roast and shoulder roast.

[d] Average values for rib chop, loin chop, shoulder chop, leg roast, rolled breast and rolled loin.

When beef is fed to rats at a low protein level (8 per cent) increased growth is obtained by additions of cystine to the ration, indicating that at this low level beef is deficient in cystine or methionine or both.[110] Mitchell and Block [105] compared the amino acid analyses of various protein foods with whole egg and calculated the percentage deviations for each of the essential amino acids. The percentage of the limiting essential amino acid was compared with the results of biological tests. In most cases there is fairly good agreement with the biological tests. In beef muscle and kidney the limiting essential amino acid appears to be cystine plus methionine, in beef liver and beef heart isoleucine, and in gelatin tryptophane. Amino acid analyses indicate a higher relative value than the biological tests for animal tissues. This may be due to the fact that animal tissues contain some non-amino acid nitrogen which is included in the protein analysis. Based on the true protein, the relative values for meat products are probably higher than indicated by the biological tests.

Since collagen and elastin (Table 5–7) are extremely deficient in several of the essential amino acids, variations in the amounts of these constituents

Table 5-7. Approximate Amino Acid Content of Gelatin and Elastin (N × 6.25)

Amino Acid	*Collagen Gelatin* (%)	*Elastin* (%)
Arginine	8.8	0.9
Histidine	1.0	0.0
Lysine	4.5	
Tyrosine	0.3	1.5
Tryptophane	0.1	0.0
Phenylalanine	2.1	3.1
Cystine	0.1	0.2
Methionine	1.0	0.4
Threonine	1.5	2.5
Leucine	3.7	
Isoleucine	1.7	
Valine	2.1	
Glycine	23.6	27.5
Alanine	9.2	0.0
Serine	3.3	
Glutamic acid	10.3	2.5
Aspartic acid	5.9	0.0
Hydroxyproline	13.0	1.9
Proline	15.3	14.2

in different cuts of meat will affect the amino acid composition. Mitchell *et al.*[109] found 23 per cent of the total nitrogen as collagen in the fore shank, 15.8 per cent in the navel, and 14.6 in the chuck ribs of beef, but the other cuts ranged from 8 to 10.9 per cent. Significant differences were not found in the amino acid composition of six cuts of choice and utility beef.[42] The methods for estimation of the amino acids may not be sufficiently accurate to determine the small differences existing in these cuts.

Nutritive Value of Meat Proteins

Biological Value. Mitchell *et al.* [100, 102, 103, 106-108] have determined the biological value of the protein in meats and meat products. The term "biological value" is used to express the percentage of the absorbed nitrogen that is retained by the animal for growth and maintenance. Since this value will be lower at higher levels of intake, relatively low levels are fed (8 to 10 per cent). The biological value of different proteins is dependent on the percentage deficits of their limiting essential amino acid.[105]

Digestibility is not considered in determining biological value. Since the digestibility as well as the biological value is a function of the nutritive value, the net protein value is a better measure of the nutritive value of the protein than the biological value.[51] As Hegsted *et al.*[51] have pointed out, "Most textbooks of nutrition present extensive tables of biologic values

without presenting the corresponding digestibility. Both values are equally important in determining the nutritional value of the proteins to the individual. Perhaps the best method of comparing the relative nutritional values is to compute the product of the digestibility and biologic value. This figure, called the 'net protein value,' should represent the per cent of the particular protein actually available to the animal for purposes other than energy."

Hoagland and Snider [58–62, 64] have studied the nutritive value of the protein of different meats and organs. Biological values were based on the gain in weight per gram of protein consumed. Hegsted and Worcester [52] have shown that "Proteins are classified relative to each other in the same manner, and with equal accuracy, by either gain in weight alone or protein efficiency (gain per gram of protein eaten)." Beef, pork, veal and heart, liver and kidney from cattle, swine and sheep are all of high nutritive value and have about the same value for maintenance and growth.[60] Veal, beef and hog brains, beef and hog tongues and spleens have about the same nutritive value as the lean meat and about the same as dried whole milk. The proteins of sweetbreads, tripe, beef cheek meat, beef lips and casein are lower in value.[59] It may be noted from Table 5–5 that the methionine content of sweetbreads (thymus) is considerably lower than that of the lean meats, kidney, heart and liver. Due to the high content of connective tissue the proteins of tripe, beef cheek meat and beef lips are also probably lower in methionine and tryptophane content. The value of the protein in all cuts of pork meat is high and about equal.[65, 104] The connective tissue content (collagen and elastin) of pork meat cuts shows little variation, from 8.6 to 12.3 per cent.[104]

Digestibility. Compared with most vegetable proteins, animal proteins are considerably higher in digestibility. Meat proteins are almost completely digestible, ranging from 98 to 100 per cent.[105]

Table 5–8. Biological Value, Digestibility and Net Protein Value of Meats

	Biological Value	*Digestibility*	*Net Protein Value*
Beef muscle	76–81	100	76–81
Pork ham	74	100	74
Veal	62–84	100	62–84
Beef liver	77	97	75
Beef kidney	77	99	76
Beef heart	74	100	74

Supplementary Effects. Biological and net protein values are measures of the value of the protein when used as the sole source of protein in the

diet. Such values do not take into account the supplementary values of proteins when fed in a mixed protein diet.

Although the biological values of meat proteins are not as high as egg proteins, they are particularly well suited to supplement the proteins derived from cereals and other vegetable proteins. As Mitchell [102] has stated, "Among the animal foods it is evident that meats and meat products are pre-eminent as sources of protein. Although the biological values of animal tissue proteins (nitrogen) are appreciably lower than those of eggs or of milk, the higher content of protein in animal tissues, either on the fresh or the dry basis, offsets or more than offsets their greater losses in metabolism." Mitchell and Carman [108] conclude that "The marked supplementary relations between meat proteins and cereal proteins established in this and other similar investigations are to be explained not only by the different character of meat and cereal proteins, indicating different types of amino acid deficiencies, but also by the fact that the supplementary fraction in each case is large, *i.e.*, 25 to 50 per cent of the absorbed protein." Hoagland and Snider [65] found the biological value of pork much higher than that of white bread containing 4 per cent and whole wheat bread containing 3 per cent of skim milk solids. Mixtures containing equal quantities of nitrogen from bread and pork had biological values equal to pork. Mixtures containing 1 part of nitrogen from pork and 2 parts from bread had values somewhat lower than pork but much higher than bread alone. The high lysine content of meat makes it particularly well suited to supplement the lysine deficient cereals (oats, wheat, corn, and rice). McCollum *et al.*[88] found that the proteins of muscle, kidney, and liver consistently gave higher biological values when combined with cereals and legumes than did milk proteins.

Minerals in Meat

The three mineral elements most likely to be deficient in the average diet are calcium, iron and phosphorus. The amounts of these elements in the edible portion of different kinds of meat are shown in Table 5–4. Winton and Winton [147] give detailed ash analyses of some of the early work of Katz on meat from different animals and of Lawes and Gilbert on the whole animal. They have also compiled analyses from much of the more recent literature on the minor mineral elements in meat.

Iron. Meats are among the best food sources of iron.[35, 39] Beef, pork, veal, lamb, and poultry contain from 2.2 to 3.8 milligrams per 100 grams of edible portion. Liver with about 12 milligrams per 100 grams is one of the richest food sources of iron. Tongue contains 6.9 milligrams per 100 grams, and heart 6.2 (Table 5–4). The iron in meat is well utilized.[97]

Phosphorus. Meats, containing from 160 to 200 milligrams per 100 grams, are a fair source of phosphorus. Liver, heart and brains contain about twice as much phosphorus as the lean meats. The phosphorus in

different cuts of beef, beef heart, and beef liver is well utilized by the human.[80, 82, 116]

Magnesium. Lean meat contains about 27 milligrams and liver about 20 milligrams of magnesium per 100 grams of fresh weight.[118, 142] Kruse *et al.* have studied the symptoms resulting from magnesium deficiency in animals.[77]

Calcium. Meats are not considered a good source of calcium since they contain only from 8 to 30 milligrams per 100 grams of fresh meat.

Copper. The copper content of the tissues and organs have been studied by a number of workers.[55, 71, 75, 83, 89] The following are typical values in milligrams per kilogram of fresh weight: beef 0.8 to 1.2, pork 3.1, lamb 4.2, veal 2.5, beef liver 21.5, hog liver 6.5, beef kidneys 1.1. Copper is an important supplement to iron in hemoglobin synthesis in the rat.[50]

Manganese. Meat contains the following amounts of manganese in milligrams per kilogram of dry tissue: beef liver 15, beef T-bone steak 0.65, pancreas 3.3, veal chops 1.3, pork chops 1.6, lamb chops 1.2, and calf brains 2.3.[89, 115, 117, 127] Manganese may be an important element in nutrition.[113]

Zinc. The zinc content of lean meat ranges from 26 to 50 milligrams per kilogram and of liver from 80 to 120 milligrams.[38, 117, 136] Stirn *et al.*[134] have shown that zinc is indispensable in the nutrition of the rat.

Aluminum. The edible portion of beef contains 5, mutton 4.3, pork 4.4, calf liver 17.3, and pig liver 17.7 milligrams per kilogram of aluminum on the fresh weight basis.[81, 140]

Meat is an important dietary source of iron, phosphorus and a number of the minor mineral elements. Liver and other organ meats are especially rich in iron and the minor elements.

Vitamin Content of Meat

In contrast to the very uniform amino acid composition of lean meat in different parts of the same animal, in different animals of the same species and in the different species, the vitamin content is more variable. This is especially striking in pork where large variations occur in the thiamine content.

The contents of the more important vitamins in meat — thiamine, riboflavin and niacin — for which the human requirements are better known are given in Table 5–9.

Since the amount of these water-soluble vitamins is greater in the lean than in the fatty tissues, there will be some variations depending upon the grade of meat. The values in the table are representative average values.

Thiamine. The lean tissues of pork are very high in thiamine.[142] Seven samples of pork hams ranged from 0.91 to 1.90 milligrams and nine samples of pork loin from 1.1 to 2.6 milligrams per 100 grams of fresh weight. The thiamine intake of swine affects very greatly the thiamine content of

Table 5–9. The Vitamin Content of Meat in 100 Gm Edible Portion [142, 154]

Meat Item	*Thiamine (mg)*	*Riboflavin (mg)*	*Niacin (mg)*
Beef chuck	0.12	0.15	5.0
Beef dried	0.11	0.22	3.7
Beef hamburger	0.10	0.13	4.3
Beef rib roast	0.11	0.14	4.7
Beef roast canned	0.02	0.24	4.5
Beef round	0.12	0.15	5.2
Beef rump	0.10	0.12	4.2
Pork Boston butt	1.05	0.21	4.5
Pork ham fresh	0.96	0.19	4.1
Pork ham smoked	0.78	0.19	3.8
Pork loin	1.04	0.20	4.4
Pork picnic	0.94	0.18	4.0
Pork spare rib	0.92	0.18	3.9
Pork sausage	0.22	0.15	2.3
Veal loin	0.18	0.27	6.3
Veal round	0.18	0.28	6.4
Veal leg	0.17	0.27	6.3
Veal stew meat	0.17	0.26	6.0
Lamb leg	0.21	0.26	5.9
Lamb shoulder	0.18	0.23	5.2
Lamb sirloin	0.21	0.26	5.9
Chicken	0.11	0.18	8.6
Turkey	0.12	0.19	7.9
Tongue	0.22	0.27	5.0
Liver	0.27	2.80	16.1
Heart	0.54	0.90	6.8
Kidney	0.27	2.05	10.0
Brains	0.25	0.26	6.0
Pancreas	0.32	0.53	5.0
Bologna	0.31	0.30	3.0
Frankfurters	0.19	0.23	2.4

pork.[53, 98] An increase of 100 per cent in the thiamine content of pork was obtained when the intake was increased from 1,318 to 3,447 micrograms of thiamine per pound of feed. A further increase from 3,447 to 5,761 micrograms resulted in an increase of only 15 to 20 per cent in the pork. When the thiamine content of the ration is 5,800 micrograms per pound, the saturation point of the muscles appears to be attained. At the lower level of intake, the shoulder, center loin and ham end contained 0.79, 0.95 and 1.03 and at the higher level 1.73, 2.31 and 2.39 milligrams per 100 grams of fresh weight respectively.[98] Beef is much lower in thiamine than pork, while lamb and veal contain slightly more than beef. Beef, pork, veal, and

lamb liver are slightly higher than veal tissues. Beef, pork heart and pork kidneys contain about half as much as pork muscle, and are excellent sources of thiamine.

The studies of Stiebling and Phipard [133] show the large contribution that meat, fish and poultry make to the total thiamine content of the diet. Families in the East, North, and Central States, spending from $1.88 to $2.49 per capita weekly for food, spent 24.2 per cent for meat, poultry and fish and obtained 30 per cent of the thiamine of the diet from this food group. The thiamine content of the diet rose as the level of expenditures for food increased. Most groups failed to reach a dietary level of 1.5 milligrams of thiamine until weekly expenditures reached $2.50 to $3.12 per person.

Riboflavin. Beef, pork, lamb, and veal liver, and kidneys are excellent sources of riboflavin. The liver of all four species contains about 3.0, kidney 2.0, heart 0.9 and brain 0.26 milligrams per 100 grams of fresh weight. The lean meats have about one-tenth as much riboflavin as liver.[29, 30, 63, 96, 142] From the studies of Stiebling and Phipard [133] showing that 25 per cent of the total amount spent for food by wage earners and clerical workers in cities was for meat and from their analyses of meat Waisman and Elvehjem [142] have estimated that meat and meat products furnish nearly 30 per cent of the riboflavin requirements. The more money spent for food, the richer the diets are in riboflavin. When per capita weekly expenditures were $1.88 to $2.49, the diet contained an average of about 2 milligrams of riboflavin.

Niacin. Meats are among the richest food sources of niacin. The lean tissues of beef, pork, lamb, and veal, and the tongue, heart, brains, and pancreas are similar in niacin content (5 to 6 milligrams per 100 grams of fresh weight). The liver and kidney contain from two to three times as much. The daily human requirement of niacin is supplied by 100 grams of fried liver, or 200 grams of beef or veal.[142] From the food consumption data of Stiebling and Phipard,[133] Waisman and Elvehjem [142] have estimated that two-thirds of the requirement was supplied by the meat consumed in the diet of wage earners and clerical workers in the cities of the North Atlantic States. Thus, meat may readily furnish the major amount of the niacin requirement.

Vitamin A. Lean meats contain only small amounts of vitamin A, but liver is a very rich source.[142] The amount of the vitamin will vary, depending upon the feed. Typical values in international units per 100 grams fresh weight are: beef liver 15,000, lamb liver 25,000, pork liver 8,000, veal liver 10,000, and kidney 800.

Vitamin D. The lean meats contain about 10 international units of vitamin D, beef and pork liver 45, calf liver 10, and lamb liver 20.[142]

Ascorbic Acid. Only the liver, kidney, sweetbreads, and brain are good sources of ascorbic acid. Lean meats contain only very small amounts. Meats are considered as only a fair source of vitamin C.[142]

Pantothenic Acid. The following average values in milligrams per 100 grams fresh weight have been reported for the pantothenic acid content of meats: beef 0.60, lamb 1.0, pork ham 1.25, veal 1.45, beef brain 1.63, heart 1.9, kidney 3.4, tongue 1.1, liver 5.5, and poultry 0.9.[142]

Although Spies *et al.*[130] conclude that pantothenic acid is necessary in human nutrition, little is known regarding the human requirements. From studies with dogs, Elvehjem [34] suggests that the human requirement may be close to 5 milligrams per day. On this basis, 100 grams of liver would furnish the daily requirement.

Pyridoxine. Average values for the pyridoxine content of meat in milligrams per 100 grams of fresh weight are as follows: beef 0.4, lamb 0.3, pork ham 0.6, veal 0.4, beef liver 0.7, lamb, pork, and veal liver and kidney and heart 0.3.[142] Pyridoxine is essential for the rat, pig, chick and dog.[34] The significance of pyridoxine in human nutrition is not known, although Spies *et al.*[129] noted additional improvement by administration of pyridoxine after giving niacin, riboflavin and thiamine.

Choline. Beef, pork, veal and lamb contain from 70 to 144 milligrams of choline per 100 grams of fresh weight. The kidney, heart and liver are somewhat higher.[91] Meats are considered a good source of choline.

Biotin. Schweigert *et al.*[124] found kidney and liver an excellent source of biotin. Heart, pancreas and chicken are also good sources. The following values were obtained in milligrams per gram of dry tissue: pork kidney 6.23, beef kidney 4.05, lamb liver 4.38, beef liver 3.53, veal liver 2.82, and pork liver 2.84. The significance of biotin in human nutrition is not known.

Inositol. Williams *et al.*[146] have shown that heart, kidney, brain and spleen are especially rich in inositol. The possible significance of inositol in human nutrition is not known.

Folic Acid. Schweigert *et al.*[125] determined the folic acid content of several cuts of meat. The folic acid content of liver and kidney is higher than that of muscle tissue. Cooperman *et al.*[27] have shown that a folic acid deficiency in the monkey induces a deficiency in the antianemia factor, retards growth and causes suboptimal hemoglobin levels. Higgins[56] reported a marked antianemic effect when 80 micrograms of folic acid daily was given to rats in which anemia was induced experimentally. Kornberg *et al.* found that folic acid had a preventative and corrective action on hemorrhagic anemia.

In addition to the contribution that meat makes to the vitamin content of the diet, it supplies unknown factors. As Elvehjem [34] has stated, "Aside from the gustatory significance of properly prepared natural foods, the greatest value obtained from their consumption is that they supply the unknown factors along with the known."

Effect of Cooking and Processing on Nutritive Value

Effect on Vitamins. A number of studies have been made on the retention of thiamine, riboflavin, and niacin in meat during cooking.[11, 28, 72, 90, 92, 93]

Vitamin retention in meat alone is higher in roasting and broiling than in braising, and lower in stewing. The total retention of thiamine in meat and drippings after braising and roasting was 50 to 62 per cent, after broiling 70 to 80 per cent, and after stewing 51 per cent. Riboflavin and niacin retentions in the meat alone were 52 to 85 per cent. Total retentions in the meat and drippings were 90 to 101 per cent for riboflavin, 90 to 100 per cent for niacin, and 62 to 70 per cent for thiamine.[90, 92] In variety meats, 90 per cent of the riboflavin and niacin is retained in the meat and drippings.[93] In well-done beef, 62 to 72 per cent of thiamine, 73 to 87 per cent of riboflavin, 65 to 91 per cent of niacin, and 69 to 86 per cent of pantothenic acid are retained.[28] In rare beef, 71 to 80 per cent of thiamine, 75 to 98 per cent of riboflavin, 66 to 83 per cent of niacin, and 85 to 95 per cent of pantothenic acid are retained.[28] From 87 to 114 per cent of choline and 42 per cent of pyridoxine are retained in cooked meats.[91] Cheldelin *et al.*[24] found very small losses in riboflavin, pantothenic acid and inositol, with beef 60 per cent loss in biotin and with lamb no loss. Losses in folic acid were high, but it may be that the loss was not actual but due to a binding of the folic acid during cooking. Schweigert *et al.*[124, 125] found a retention of 65 per cent folic acid during cooking and 80 per cent of biotin.

Schweigert *et al.*[122] found 80 per cent of the thiamine, 97 per cent of the riboflavin, and 100 per cent of the niacin of the fresh ham retained in the cured hams.

The retentions of vitamins in pork and beef during canning are similar to those of household cooking. Rice and Robinson [119] report the following percentage retentions: thiamine 60 to 70, niacin 90 to 100, riboflavin 90 to 100, and pantothenic acid 70 to 80. Commercially canned cured pork (12-oz cans) retains 67 per cent thiamine, 90 per cent riboflavin, 94 per cent niacin, and 76 per cent pantothenic acid. Thiamine retention in 6-pound cans is 40 to 50 per cent. Practically no losses occurred during storage in niacin, riboflavin and pantothenic acid for a period of 219 days. Above 120° F, slight losses of riboflavin and pantothenic acid occur. Thiamine retention was 52 per cent after 293 days' storage.

Greenwood *et al.* [43] found that the rate of thiamine destruction is doubled for a 10° rise in temperature as contrasted with a tenfold increase in the rate of destruction of heat-resistant bacteria. Thus, under conditions which permit uniformly rapid heating, higher temperatures, with the shorter times needed for processing, are more favorable to thiamine retention. No significant losses in riboflavin, niacin or pantothenic acid were found after storage for one year at 45°, 70° and 98° F. Thiamine retention was 89 to 100 per cent at 45° F, 59 to 76 per cent at 70° F, but only 12 to 20 per cent at 98° F.[36]

Rice and Robinson [119] found the following percentage retentions of vitamins in the dehydration of meat: beef 76 thiamine, 105 riboflavin, 92 niacin, and 68 pantothenic acid; pork 63 thiamine, 104 riboflavin, 92 niacin, and 73 pantothenic acid.

Whitmore *et al.*[144] determined the vitamin content of commercial samples of dehydrated meat before and after storage at intervals up to 42 weeks. No significant loss occurred in riboflavin or niacin even at the highest temperature 120° F. No loss in thiamine occurred at 40° F, but at 70° F there was a definite loss after 30 days and a large loss after 42 weeks. At 100 and 120° F, thiamine loss occurred at one week and was almost complete after 10 weeks' storage.

Effect on Proteins. Morgan and Kern [111] found that the cooking of meat lowered the biological value of beef. On the other hand, Rice and Robinson [120] report that the nutritive value of proteins in meat is not significantly impaired during dehydration or commercial canning, except when very low protein diets are considered. Hoagland and Snider [65] obtained slightly lower biological values in pork with the more severe cooking conditions, particularly with longer time of cooking. Amino acid retention studies in the cooking of meat, as mentioned previously, show good retention. Wilder and Kraybill [145] found no loss in lysine content of fresh uncured pork luncheon meat when it was canned by commercial process. By chemical analysis, the cured canned meat showed a slight loss of lysine during the canning process. But rat feeding tests did not detect impairment of the lysine content.

It seems that with good cooking practice there is probably no serious impairment of the nutritive value of the proteins in meat. Very severe processing may result in greater injury. Lowered biological value, due to cooking and processing, may have little significance when the proteins are consumed in mixed diets. More work is needed to evaluate properly the effect of processing on the nutritive value of meat proteins.

Fats in Meat

Meats make an important contribution to the total fat of the average diet. The average fat content of the various cuts of meat ranges from about 10 to 35 per cent. The amount varies with the kind of meat, the type of cut, and the degree of finish of the animal.

The fatty acids of beef, pork and lamb fats consist chiefly of palmitic, stearic, oleic, and linoleic acids. They also contain smaller amounts of arachidonic and linolenic acids.

Burr and Burr [15, 16] showed that, when rats were fed on diets extremely low in fat, growth was greatly retarded and a diseased condition developed which could be cured by administration of unsaturated fatty acids or linoleic acid. Subsequent studies show that linoleic acid gives good growth response, but has little effect on curing the skin disease. Linoleic and arachidonic acids seem to have similar effects in promoting growth and correcting the diseased skin condition.[14] The requirement of the rat has been estimated at one per cent of linoleic acid in the diet. A similar level has been proposed for the human.[13] Although the requirement of linoleic acid by

man has not been determined by direct experiment, there is extensive evidence of its beneficial effect in curing or alleviating skin diseases.[49]

The linoleic acid content of lard is about 8 to 12 per cent, and of beef, fat, and mutton tallow about 3 per cent. These fats are prepared from adipose tissues and are storage fats. In addition to the storage fat contained in the adipose tissue of meat cuts, the lean tissues and organs contain phospholipids, which are good sources of unsaturated fatty acids.

Meat in Special Diets

Meat has an important place in special diets where high quality animal protein is desired. The high content of protein, the excellent palatability and wide variety of methods of preparation make it especially useful in these diets. High protein diets are recommended for restriction of weight during pregnancy,[1] for patients before and after operation,[21, 33] for patients with severe burns,[25, 67] for hemoglobin building following blood donations,[94] for blood formation,[45, 114] for wound healing,[138] and for convalescents.[150]

Greater susceptibility to rheumatic fever of children was found associated with deficient intake of protein.[26] Boyd [10] stresses the need of meat in the diet of growing children not only for its protein content but also for iron and niacin. The importance of adequate protein in the diet for preservation of the antibody mechanism in disease resistance has been emphasized by Cannon.[20] Burke *et al.*[12] and Ebbs *et al.*[32] found a definite relationship between the adequacy of the diet of the mother during pregnancy and the condition of the children at birth. McLester [95] stresses the importance of a liberal protein diet in treatment of obesity. Meats, and especially liver, have been found of value in the diet for pernicious anemia.[99]

Some Fallacies Regarding Meat

Some false conceptions regarding meat are frequently held by large numbers of people. Scientific evidence is available to discredit these misconceptions.

Digestibility. There is a popular impression that there are wide differences in digestibility of white and red meats, of pork and beef, and of tough and tender cuts. Grindley *et al.*[44] in extensive experiments found the average digestibility of the protein and fat in veal, mutton, pork and beef cooked in various ways to be 98 per cent. No difference was found with the different kinds of meat, methods of cooking or relative fatness of the meat. Many more recent studies show that meat is practically completely digestible.[65, 105]

Diets in Disease. The older conception that high protein foods, such as meat, should be avoided in cases of arteriosclerosis, hypertension, cardiac disease, nephritis and rheumatism is now known to be erroneous.[5,19,37,73,74,143] Thomas [137] comments, "The Greenland Eskimo, on a carnivorous diet, exhibits no increased tendency to vascular or renal disease." In discussing

the experiment in which Stefannson lived on nothing but meat and fish (including fat) for a year, McClellan [87] states, "From the tests and observations made, no evidence of kidney irritation or decrease of kidney function was noted, functional tests were above the average at the end of the year."

Nutrients Contributed by Meat in the Diet

The importance of meat in the diet in the United States can be seen from Table 5–10, which shows the amount of the important nutrients furnished by meat, fish and poultry.[70, 153] Howe [70] has shown, on the basis of food prescribed for the Armed Forces in 1941, that meat furnished the following percentages of nutrients in the diets: calories 21.8, protein 42, fat 41, phosphorus 29.9, iron 33.8, vitamin A 14.6, thiamine 52.8, riboflavin 30, and niacin 65.3.

Table 5–10. Percentages of Total Nutrients Contributed by Meat, Poultry, Game and Fish in the Food Supply of United States [70, 153]

	Calories	*Protein*	*Fat*	*Calcium*	*Phosphorus*	*Iron*	*Vitamin A*	*Thiamine*	*Riboflavin*	*Niacin*	*Ascorbic Acid*
Civilian											
1917–18	10.7	26.8	22.2	2.2		27.0	8.3	30.2	19.2	44.8	2.0
1925–29	10.4	26.8	21.5	2.2		26.7	7.5	32.9	18.4	47.2	0.9
1935–39	10.4	27.0	20.5	2.1		24.8	6.4	30.0	17.3	48.1	0.9
1944–45	11.5	28.0	22.0	1.9		23.4	7.5	25.4	15.9	40.5	1.4
Army *	21.8	42.0	41.2	3.4	29.9	33.8	14.6	52.8	30.0	65.3	1.8

* Prescribed for May–October 1941.

In the civilian diet in this country, meat contributes the following percentages of the total nutrients of the diet: niacin 41, protein 28, thiamine 25, iron 23, fat 22, riboflavin 16, and calories 12. No other food group supplies an equivalent amount of all of these nutrients. Meat is also an important source of other vitamins and of non-identified nutrients.

In addition, the savory and satiety values of meat make it one of the most valuable foods in our diet. Carter, Howe and Mason [22] state, "The palatability, variety, ease with which the flavor may be modified, facility of preparation, concentration of proteins, and digestibility are factors which have made meat the most important protein of the adult human dietary." McLester [95] states, "Meats have the greatest satiety value of all foods — they 'stick to the ribs' longest — it gives a greater degree of satisfaction and sense of well-being over a longer period of time than any other food."

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Chapter 6

The Amino Acid Requirements of Avian Species

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Introduction

For some time it has been possible to maintain and grow rats on highly purified diets. On the other hand, the more complicated nutritive requirements of the chick could not be met except by the use of some natural feedstuffs. The chick is comparatively sensitive to the lack of a number of dietary factors, including vitamin K, choline, manganese, potassium, magnesium, certain carbohydrates, and the more recently recognized members of the vitamin B complex. The provision of these factors in substantially protein-free forms has been a necessary prerequisite to experiments on the amino acid requirements of chicks. Several reviews have appeared dealing with the amino acid requirements of the fowl.[2–4, 8, 53] It is the purpose of the present review to summarize the earlier work and to add newer information. Since it is impossible to cite here the specific references to the now large volume of literature on amino acid studies with other species, the reader must be referred to other chapters of these volumes, and to other recent and general reviews.[6, 21]

Individual Amino Acids

Arginine. The necessity of dietary arginine for the rapid growth of the chick was noted with diets containing whole casein.[22] This work showed that the ability of the chick to synthesize arginine was probably less than that of the young rat. With diets very low in arginine, the extreme inability of the chick to synthesize arginine was demonstrated.[57] Rapid loss of weight was completely reversed by added arginine, but not by ornithine.[57] Later, it was shown that a diet partially deficient in arginine could be supplemented equally well by arginine or citrulline,[55] indicating that the chick either uses the latter directly in place of arginine or converts it to arginine. The impossibility of the Krebs-Henseleit cycle for the regeneration of arginine from ornithine in chicks became obvious.[2, 53, 55, 57] It was pointed out that this result was in harmony with the relatively low excretion of urea, and the known absence of arginase in the chick, except for small amounts in the kidney.[2, 53, 57] This organ is probably the site of

the production of ornithine for the detoxication of benzoic acid (as ornithuric acid) and the simultaneous increase of urea output.[33]

Studies with isotopically-labelled arginine, comparable to the work done with rats, have not been reported for the chick. In pigeons, arginine labelled with N^{15} in the amidine group is retained to a much greater extent than in rats. Synthesis of arginine from labelled precursors, as in the rat, does not seem to take place in the pigeon.[24]

A dietary deficiency of arginine will limit not only growth but also creatine formation in the chick.[18, 46] The feeding of creatine, creatinine, guanidoacetic acid or arginine improved both the growth rate and the tissue creatine content.[18, 46] These results showed a "sparing action" of the first three compounds on arginine, creatine being most effective.[18, 46] By the more accurate procedure available for creatine, it was shown that the liver and kidney contained only small amounts in comparison to breast and leg muscle.[18]

Similar growth and diet studies have not been made with the pigeon. However, it has been shown that pigeon kidney slices are comparatively inefficient in the synthesis of guanidoacetic acid from glycine and arginine.[27] Furthermore, isotopically-labelled arginine is employed for creatine formation distinctly less efficiently in the pigeon than in the rat.[24] While it is impossible to draw parallel comparisons between the chick and the pigeon from the work reported, the impression is strong that the metabolism of arginine differs appreciably in these avian species.

Histidine. Histidine has been established as an indispensable amino acid for the growth of the chick.[57] On histidine-deficient diets chicks do not lose weight nearly so seriously as might be expected,[8, 57] and this observation may indicate a very low requirement for maintenance or a limited synthesis.

Lysine. By using various combinations of proteins such as zein, edestin, and casein, single deficiencies of lysine were produced in chick diets. The calculated levels of lysine, as well as the levels produced by added crystalline lysine, pointed to an adequate intake at 0.9 per cent lysine in the diet.[4, 17]

Sesame meal, which is deficient only in lysine for the chick,[39] was used to supply all the protein in diets for chicks and turkey poults.[43] The quantitative lysine requirement of the chick was confirmed. The corresponding requirement of the poult, 1.3 per cent of the diet, was found to be proportional to the higher protein requirement, which is approximately 24–25 per cent of the diet as compared to 19–20 per cent of the chick.[43]

Methionine, Cystine, Homocystine and Choline. Like mammalian species, the chick has the ability to grow well if given only methionine as a source of the sulfur-containing amino acids, and, obviously, can synthesize cystine under these conditions.[37] Cystine in the diet will reduce the requisite methionine to a certain point, but not appreciably below one-half the level needed when cystine is not present.[11, 37]

The similarity in the sulfur-containing amino acid metabolism of mammalian and avian species is further carried out in the utilization of homocystine. This compound, which is a demethylated methionine, is utilized efficiently as a substitute for methionine by the chick if a sufficient level of choline is also present.[37, 56] In the absence of a methylating agent, homocystine may still serve as a substitute for cystine.[37] S-methyl cystine was found ineffective either in the methylation of homocystine or the replacement of cystine.[37]

Like the rat, the chick can utilize betaine (glycine betaine) to methylate homocystine,[9] but cannot utilize arsenocholine for this purpose.[9, 12] Arsenocholine, although non-methylating, is strongly perosis-preventing and growth-promoting,[50, 52, 54] and, like choline, may serve as a tissue structural component. When fed together with a source of methylating activity, such as betaine or methionine, arsenocholine completes the immediate functions of choline in the chick and good growth then ensues.[9]

In all probability, methionine is the specific carrier of methyl groups for the conversion of guanidoacetic acid to creatine in the chick. It has not been possible, however, to demonstrate more than a slight lowering of muscle creatine content by dietary deficiencies of methionine, or choline, or both.[13] This is a marked contrast to the withholding of arginine and glycine. These results suggest that the methylation stage of creatine formation in the chick takes a high degree of precedence over other needs for methionine, or that the methylation is a fast reaction in comparison to the prior steps in which guanidoacetic acid is formed. In support of the latter view, it may be pointed out that when guanidoacetic acid is added to a glycine- and arginine-deficient diet, creatine accumulates to a 10 or 20-fold excess over normal in the chick liver.[13, 18] The creatine is evidently formed faster than it can be carried away. This seems in harmony with the results obtained with most other species, *i.e.*, that the methylation of guanidoacetic acid takes place in the liver,[26, 27] while the formation of this compound occurs elsewhere. The pigeon appears to be exceptional in that its kidney, as well as liver tissue, is able to methylate guanidoacetic acid.[26]

Glycine. A dietary supply of glycine is necessary for optimal growth of the young chick.[8, 15, 16, 20, 47, 48] A deficiency of this simplest of amino acids in the chick results in poor growth, reduced creatine formation, a generalized weakness and muscular attenuation,[15] and poor feather formation.[47, 51] The requirement for glycine and arginine appears more acute in a more rapidly feathering breed of chickens, since feather protein contains large percentages of these amino acids.[46] The chick has a limited ability to synthesize glycine;[8, 16, 48] this is unaffected by the omission or inclusion of serine,[8] glycolic acid, betaine, β-alanine, or choline, but is apparently favored by the addition of acetates.[15]

A synthesis of glycine during embryonic development of the chick has been reported.[60] At levels higher than 2 per cent of the chick diet, glycine

appears to be harmful to growth.[20] Large doses are very toxic to hens.[61] This toxicity in chicks may be related to the nicotinic acid (niacin) content of the diet; glycine or gelatine in a niacin-deficient diet will accelerate the development of symptoms of chick pellagra,[28, 45] but the addition of sufficient niacin will enable the chick to tolerate as much as 6 per cent of glycine in the diet.[45] Niacin is evidently concerned in the metabolism of glycine, arginine and alanine in the chick.[45] It may be significant that the formation of cartilaginous tissues, which are rich in these amino acids, is deranged in the niacin-deficient chick (as manifested by perosis).

Tryptophane. The essential nature of tryptophane in the diet of the chick has been well demonstrated.[14, 38, 57] In contrast to the rat and mouse, the chick cannot utilize the unnatural form, and racemic tryptophane is, therefore, only half as active for the chick as the natural form.[38]

Tryptophane, which is most probably a precursor for niacin synthesis in mammals, is also effective in counteracting the pellagric syndrome induced by certain amino acids in niacin-deficient chick diets.[28, 28a]

Phenylalanine and Tyrosine. Chicks lose weight rapidly on diets which do not contain phenylalanine.[8, 48] Phenylalanine may meet all the requirement for these aromatic amino acids, but tyrosine has a growth-promoting effect when the phenylalanine level is not optimal.[8, 35] The relation between these amino acids in the chick is similar to that already known in other species.

Deficiency of phenylalanine is one of several causes of a peculiar structural and curable deformity of the tongue of the chick.[34]

Racemic phenylalanine is practically as active as the natural form for the chick [35] which in this respect resembles other species.

While the chick is able to synthesize vitamin C, some interrelation appears to exist between this vitamin and protein metabolism, especially of tyrosine, which may be analogous to that in other species. When tyrosine is added to a chick diet there is a reduction in the vitamin C content of the chick liver.[49]

By careful iodination, proteins such as casein may be imparted a thyroxine activity. The maximum activity is found when sufficient iodine has been combined to provide two iodine atoms per molecule of tyrosine in the protein.[62] Thyroxine has, in fact, been isolated from iodinated proteins. It has been shown that administration of iodinated protein in the diet will cause hens to maintain a higher average annual egg production, principally by counteracting the normal midsummer decline in thyroid activity.[65, 66]

Leucine. The requirement of the chick for leucine has been established.[8, 44, 48] In this case, the racemic amino acid is so effective for the chick as to suggest a high degree of utilization of the unnatural isomer.[44] In contrast, only the natural form is utilized for growth by the rat and mouse. This furnishes perhaps the only instance in which the amino acid requirements of the chick are less specific than those of other

species.[44] Leucine deficiency leads to a deformed tongue condition of the chick.[34]

Isoleucine. Isoleucine is indispensable in the diet of the chick.[8, 44, 48] Only the natural form is utilized for growth.[44] The above-mentioned deformed tongue condition is also noted with isoleucine deficiency.[34]

Valine. Valine deficiency causes a rapid decline in weight of chicks.[8, 44] Only the natural form is of value to the chick.[44]

Threonine. The status of threonine and its isomers for the chick has not been well worked out. A requirement for growth has been demonstrated and satisfied by means of racemic threonine.[8, 48]

Proline and Hydroxyproline. The deletion of these amino acids from the chick diet results in cessation of growth for a short period after which good growth was resumed.[8] Evidently, proline and hydroxyproline are dispensable in the diet of the chick.[8, 48] The time lag may have been due to a slow establishment of adequate synthesis of these amino acids. A similar time lag was noted with glycine deficiency.[8]

Serine. Chicks will grow on diets containing no serine [8, 48] without noticeable reduction of growth rate. As previously stated, serine apparently does not serve as a precursor for glycine in the chick,[8] although this function of serine has been established in the rat.

Alanine. The deletion of alanine from the chick diet caused no reduction in rate of growth.[8]

Aspartic Acid. The status of aspartic acid for the chick is similar to that of alanine.[8]

Glutamic Acid. The removal of glutamic acid from the diet of the chick resulted in a preliminary weight loss, followed by an apparently persistent period of suboptimal gain.[8] From the results reported it would appear that glutamic acid is needed to promote most rapid growth, and that glutamic acid is similar to glycine in this respect for the chick.

The Dietary Amino Acid Requirements for Normal Growth of Chicks

Qualitative Requirements. The qualitative status of the amino acids for the chick may be summarized as follows:

May be absent without detriment to growth:	Required under certain conditions:	Required for normal growth:
Alanine	*Cystine*	*Arginine*
Aspartic acid	*Tyrosine*	*Glutamic acid*
Hydroxyproline		*Glycine*
Proline		*Histidine*
Serine		*Isoleucine*
		Leucine
		Lysine
		Methionine
		Phenylalanine
		Threonine
		Tryptophane
		Valine

Quantitative Requirements. It is obvious that, when only 9 or 10 amino acids are fed, the animal must use a goodly portion of the fed amino acids in the synthesis of others for the creation of tissue proteins. Consequently, requirement data based upon such a dietary regime are very likely to be much higher than minimal requirements determined with whole proteins or a complete assortment of amino acids. The quantitative needs of the rat and chick which have been estimated on the latter basis are listed in Table 6–1.

Table 6–1. Minimum Percentage of Each Amino Acid Necessary in the Diet to Support Good Growth When All Other Amino Acids of Nutritive Importance are Provided

Amino Acid	*Rat* (%)	*Chick* (%)	*References and Remarks on Chick Requirement*
Arginine	0.2	1.2	[2,5,57]
Histidine	0.4	0.15–0.3	Between given values,[5,8,57]
Lysine	1.0	0.9	[5,17,43]
Tryptophane	0.2	0.25	[5,38]
Methionine	0.5	0.5	[5,37]
Phenylalanine	0.7	0.9	Determined with *dl*-form,[5,35]
Leucine	0.8	1.4	Either *l*- or *dl*-form,[8,44]
Isoleucine	0.5	0.6	[44]
Valine	0.7	0.8	[44]
Threonine	0.5	0.6	Between given values for half of *dl*-form,[5,8]
Glycine	0.0	1.0–1.5	[16]

The estimate of the cystine requirement (0.1 per cent) of the rat on a minimal (0.5 per cent) methionine level is probably too low. These quantities are furnished by 18 per cent casein (assuming 90 per cent protein content) and this protein source at this level is known to be favorably supplemented by added cystine for both the rat and the chick.[29, 57] From data published it would appear equally likely that the maximum cystine requirement of the rat is nearer that given for the chick. Hence, for either species, approximately 0.9 to 1.0 per cent of methionine will meet the total sulfur-containing amino acid requirement, while approximately 0.4 per cent cystine should accompany the minimum methionine requirement listed in Table 6–1. Twice the indicated amount of phenylalanine is needed by the chick if no tyrosine is present in the diet. The maximum tyrosine requirement is 0.6–0.8 per cent for the chick.[35] Except in the cases of arginine, glycine, and possibly leucine, the requirements in the diet of the young rat and the chick are very similar.

"Indispensable" Amino Acids

The rate of gain of chicks, in the suboptimal range of intake of an indispensable amino acid, becomes an almost linear function of the dietary level of that amino acid [4] and continues in this relation into the zone of negative

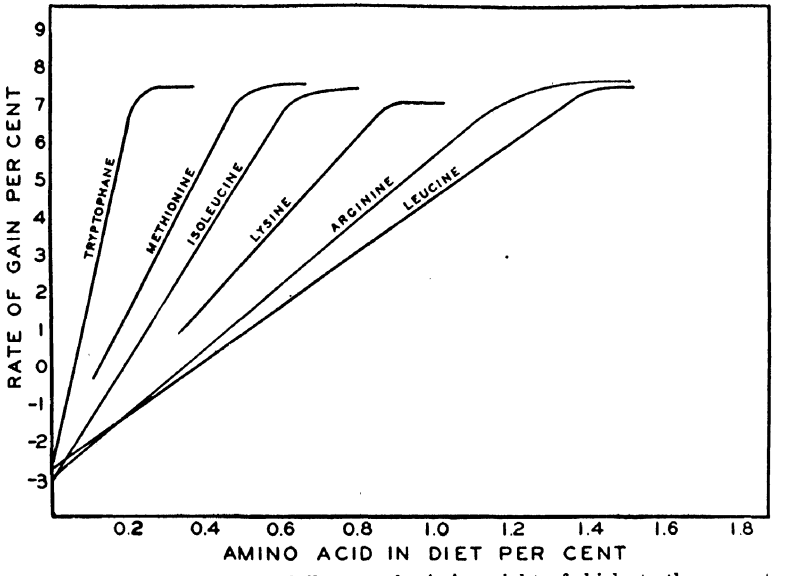

FIGURE 6–1. The relation of the daily rate of gain in weight of chicks to the percentages of certain indispensable amino acids in the diet.

gains.[5] Calculations from data available, corrected where necessary according to most recent determinations of amino acids in proteins fed, indicate the following rates of change in body weight of the chick during the complete deficiency indicated. Certain of these rates were obtained by direct experiment, while others were obtained by extrapolation of the straight lines which relate the percentage change in weight to the amino acid content in the diet in the suboptimal zones.[5] As an example, a few of these curves are presented in Figure 6–1.

Amino Acid Absent from Diet	Percentage Daily Change in Body Weight at Zero Intake of the Amino Acid
Arginine	− 3
Histidine	− 2
Isoleucine	− 2.7
Leucine	− 2.7
Lysine	− 3
Methionine	− 3
Phenylalanine	− 3.0
Threonine	− 2.5
Tryptophane	− 2.4
Valine	− 2.8

Complete cessation of protein synthesis in the chick, which is an inevitable consequence of the lack of a strictly indispensable amino acid, results in a daily weight change which approaches −3 per cent as a limit. This figure, then, is an approximate index of a state in which no new protein is being formed, while disintegration and destruction of tissues still continues. The magnitude of this figure is dependent upon the experimental conditions, which in the case of the chick experiments were substantially comparable throughout.[5] It is to be remembered, of course, that changes in body weight represent gains or losses of other components, such as water, to a greater extent than of protein. In the ultimate comparison, however, simple changes in body weight are found to be as useful as the more laborious procedures such as nitrogen balance and carcass analysis which would be necessary to put the measurements on a theoretically sounder basis.[35]

Calculations on suitable rat growth data to be found in the literature show that a similar approximate limit is approached under similar conditions for changes in body weight in the cases of extreme or complete amino acid deficiencies. In one instance, the calculation shows that an amino acid mixture supposed to contain all essential members for the rat must have inadvertently been completely lacking in one of them. That the rat and the chick will grow on only the essential amino acids for each species has been adequately proven.

In his classical review,[63] Rose has defined an indispensable amino acid as "— one which cannot be synthesized by the animal organism out of the materials ordinarily available at a speed commensurate with the demands for normal growth." Under this definition, therefore, are included all amino acids of practical importance for best growth (excluding those which exert only a sparing action). It has also been proposed to define "essential amino acids as those essential for life." [48] While this classification is probably more fundamental, it would also include those amino acids which could not be synthesized in amounts sufficient for normal needs.

The writer prefers to define an essential amino acid as one which must normally be obtained from the gastrointestinal tract in order that synthesis of body proteins may take place. From this viewpoint, the zero stage of protein synthesis is indicated by the maximum rate of loss in body weight caused by the complete lack of any one of several indispensable amino acids. Partial or complete deficiencies of other indispensable amino acids will have little bearing on this zero point, since protein synthesis may be completely stopped by a single deficiency. Any rate substantially more positive than this limiting value is evidence that some synthesis of protein and, hence, of the amino acid in question, is transpiring. Although the rate may still be negative in sign, it is then an expression of a state of affairs in which protein synthesis is not fast enough to counteract protein destruction.

In examples such as deficiencies of glycine, the prolines and glutamic

acid, where the growth rate of the chick ultimately becomes positive, it is quite clear that synthesis of these amino acids has been initiated. Under the original definition, glycine and glutamic acid are, however, "indispensable" for the chick.

The Utilization of Simple Sources of Nitrogen by the Chick

Ruminants, possessing a powerful system of microorganisms which can synthesize amino acids and proteins from simple materials, can utilize ammonia and urea as partial substitutes for protein. No appreciable ability of this nature is apparent in monogastric animals such as the rat and chick. Neither the hen [32] nor the chick [1, 23] show any retention of the nitrogen of urea. In some diets of natural feedstuffs, glycine and ammonium acetate will accelerate growth of chicks slightly. It is most probable that such acceleration is due to a correction of a glycine deficiency (see Glycine, page 223), rather than any general synthesis of amino acids.

Practical Applications

The amino acid requirements of the chick are probably known more completely than those of any other farm animal. A comparison of the chick requirements with the known supply of amino acids in feedstuff proteins shows that glycine, arginine, lysine, methionine, cystine and tryptophane need particular attention in practical rations for the chick. All the other amino acids either may be readily synthesized or are present in ample quantities so that a deficiency is quite unlikely.

The classifications given previously do not imply that the amino acids in the non-essential groups are of little or no importance in practical nutrition. Actually, all the listed amino acids are present in the tissues of the chick, and in the egg, hence all of them are physiologically essential. The chick can synthesize members of the first two groups if forced to obtain them that way, but this synthesis is conducted at the expense of the other amino acids. The dispensable amino acids are of practical importance, collectively if not individually, because they make it possible to get optimal results from smaller levels of the amino acids in the indispensable group. The latter includes several amino acids, particularly tryptophane, methionine and lysine, which are not easy to secure in more than the minimal quantities from practical feedstuffs.

Experimentally, quite a few protein concentrates have been fed as the only source of protein in the chick diet.[10, 14, 16, 17, 19, 36, 37, 40, 41] Results of these experiments have been noteworthy in showing that the chick can grow at an optimal rate regardless of source of the protein being fed, as long as that protein is readily digested and provides the fundamental amino acid requirements of the chick. This has been demonstrated with the majority of the concentrates mentioned below when fed at a level to provide 20 per cent protein in the diet, and when any characteristic amino acid

deficiencies were corrected by supplementation with pure amino acids, or by other proteins.

A problem solved in the utilization of beef blood proteins for feeding chickens may be used to illustrate the practical application of amino acid data. Blood meal is a rare example of an isoleucine deficiency, although it carries appreciable amounts of other required amino acids. The isoleucine deficiency was found to be the primary reason for the usual poor results in feeding blood meal to chicks. Another protein concentrate, corn gluten meal, carries a surplus of isoleucine but a distinct deficiency of lysine and tryptophane. When combined in a certain proportion, blood meal and corn gluten proteins compensated for the deficiencies in each other, and the mixture greatly exceeded in feeding value either concentrate when fed alone.[41]

Another such example is that of soybean and sesame meal. By analysis of sesame meal an unusually high content of methionine was found.[10] When fed as the only protein, sesame meal failed, however, to permit anything but poor chick growth. The difficulty was identified as a deficiency of lysine, only about half enough lysine being present.[10, 40] On the other hand, soybean meal has a surplus of lysine and a deficiency of methionine.[19] The combination of these two meals in a ratio of 2 soybean meal to 1 sesame meal proved to be an excellent source of protein for the chick.[10]

Corn gluten meal becomes a satisfactory source of protein when supplemented with arginine, lysine and tryptophane. Cottonseed meal was found to require an addition of methionine and lysine, while peanut meal was somewhat more deficient in methionine and less so in lysine.[36] Very few protein sources are complete for the chick at usual levels of protein in the diet. Among these may be listed high quality fish meal and sunflower seed meal,[42] as practical sources. These meals together with sesame meal are unusually rich in methionine, a feature of particular value in the supplementation of soybean meal, which has a moderate deficiency of this amino acid.

In practical diets, of course, more than one or two sources of protein are necessarily involved. The data on the individual protein sources can be used in selecting or combining feedstuff proteins so that they will not run preponderantly to one or more deficiencies. One serious amino acid deficiency is enough to cause a failure of the entire diet.

In Table 6–2 are listed the quantities in certain poultry feedstuffs of the six amino acids which require consideration in practical poultry diets. As previously stated, the remaining 14 or more amino acids are also useful in poultry feeding, but their occurrence, or the ability of the chicken to synthesize them, is such that they do not constitute a practical problem of supply, if the six listed amino acids are adequately provided by the combination of feedstuffs employed in the diet.

Table 6–2 was constructed from data provided in the comprehensive

Table 6–2. Amino Acid Composition of Feedstuffs
(Lb per 100 Lb of Feedstuff *)

	Protein	*Arginine*	*Lysine*	*Methionine*	*Cystine*	*Tryptophane*	*Glycine*
Alfalfa meal	20	1.3	1.0	0.46	0.36	0.38	?
Barley	10	0.48	0.18	0.29	0.18	0.11	?
Blood meal	82	3.5	7.2	1.0	1.5	1.1	trace
Corn	10	0.38	0.22	0.27	0.19	0.07	0.40 †
Corn gluten meal	43	1.4	0.86	1.0	0.82	0.34	1.7 †
Cottonseed meal	43	3.2	1.2	0.78	0.95	0.47	2.3
Distillers solubles, dry	40	1.1	1.4	0.64	0.40	0.20	?
Fish meal	65	3.8	3.7	1.9	0.65	0.78	4.4
Fish meal, Sardine	65	4.8	3.7	2.0	0.78	0.78	4.4
Fish solubles, Cond.	35	1.5	1.7	0.52	0.21	0.14	2.3
Linseed meal	32	2.3	0.93	0.73	0.61	0.55	?
Meat scrap	55	3.9	2.8	0.94	0.66	0.38	2.2 †
Milo	10	0.34	0.25	0.15	0.20	0.08	?
Oats	10	0.60	0.33	0.23	0.18	0.13	?
Peanut meal	44	4.4	1.3	0.57	0.70	0.40	2.5
Peas, dry	24	2.1	1.2	0.22	0.31	0.19	?
Rice, polished	8	0.58	0.26	0.27	0.11	0.10	0.82
Rye	12	0.52	0.45	0.16	0.18	0.14	?
Sesame meal	42	3.7	1.2	1.4	0.55	0.63	3.9
Skim milk, dry	35	1.4	2.6	1.0	0.42	0.46	0.2
Soybean meal	44	2.8	2.7	0.79	0.66	0.53	7.6
Sunflowerseed meal	46	3.8	2.0	1.6	0.73	0.60	1.8 †
Wheat	13	0.52	0.36	0.17	0.23	0.14	0.92
Wheat bran	16	0.96	0.53	0.19	0.27	0.24	?
Whey, dry	12	0.36	0.90	0.38	0.31	0.24	0.0
Yeast, dry	45	2.2	3.1	1.0	0.45	0.54	?
"Complete ration" (chick)	20	1.20	0.90	0.50	0.40	0.25	1.00

* Data are given to two significant figures only.
† Minimum value based on feeding tests.

compilation of Block and Bolling,[25] from many papers which have appeared subsequently, and from the work of the author.

The data represent the best or most probable values to the present date, but are subject to modification as additional information becomes available. It is unlikely that there will be any very extensive changes. The composition of a ration which is sufficiently adequate for the chick in respect to the six amino acids is also indicated at the foot of the table. This table will be most directly useful in calculating the amino acid content of mixed diets.

The amino acid requirements for egg production have not yet received much attention. The hen is able to produce at maximum capacity on a diet containing 15 to 16 per cent of good quality protein.

The amino acid composition of the proteins of the hen's egg is not noticeably altered by qualitative and quantitative changes in the protein intake of the hen, although rate of egg production may be drastically affected. The analyses which have been made in the exploration of this question have included tyrosine, tryptophane and cystine,[58] tyrosine, tryptophane, cystine, arginine, histidine and lysine,[30] arginine, cystine, histidine, lysine and glycine.[60]

A comparative study of chicken muscle and egg in respect to nine amino acids has been reported.[59] Such data afford an estimate of the *minimum* requirement of an indispensable amino acid for the production of an egg or a certain amount of muscle tissue.[59] To this must be added an unknown quota for maintenance and metabolic losses or waste.

Table 6–3, prepared on the basis of all data available, indicates the comparative amino acid composition of chicken egg and muscle protein.

Table 6–3. Amino Acid Composition of Chicken Tissues

Amino Acid	*Egg, Edible Parts* * (%)	*Chicken Muscle* * (%)
Arginine	7.0	7.0
Histidine	2.2	2.3
Lysine	6.0	8.4
Methionine	4.0	3.4
Cystine	2.4	1.2
Phenylalanine	5.8	4.6
Tyrosine	4.8	4.3
Tryptophane	1.5	1.3
Threonine	4.9	4.7

* Calculated to percentage of crude protein (16 per cent N).

There are, perhaps, three differences in these poultry products which require comment. The first is that egg protein contains distinctly more of the sulfur-containing amino acids, methionine and cystine. This would seem to indicate that for *optimal* production the hen needs a generous proportion of methionine and cystine in her protein intake. On the other hand, egg protein contains definitely less lysine than muscle protein, hence it is probable that the laying hen can make relatively better use than the meat bird of proteins, such as the cereals and many oil-cake meals, which are marginal in lysine content. These inferences, which are drawn in a circuitous manner, will require experimental testing by direct feeding.

Except in the case of lysine,[43] little is known concerning the amino acid requirements of the young turkey. Poults show no signs of a deficiency of glycine when fed a diet which will produce a distinct glycine-deficiency syndrome in chicks. In general, protein supplements of varying quality

for the chick show the same relative feeding values for poults,[7] from which it may be inferred that the amino acid requirements may be very similar in proportion but higher in quantity for the poult, because of its higher total protein requirement.

Relation of Protein Intake to Disease Resistance in Poultry

The relation of protein intake to blood protein levels, enzyme secretion, antibody formation, anemia and edema is attracting much attention on the part of investigators of mammalian nutrition and health. It is only possible here to refer the reader to a recent review dealing with protein metabolism in relation to health.[31]

Very little work of this sort has been done with fowls. One outstanding study has been published.[64] Chicks fed diets of a wide range of protein content showed a reduction in serum protein at the lower levels. After experimental injection with measured doses of avian malaria, the chicks fed the lower protein diets suffered severely from the disease, while chicks with the highest protein intake were able to overcome the parasites quickly.

It was also evident that a protein deficiency which did not lower the serum proteins noticeably still had a marked effect on the course of the disease. If the relation of disease resistance to protein intake proves to be a general phenomenon, the economical consequences in the livestock industry may be tremendous.

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Chapter 7

The Relation of Hormones to Protein Metabolism

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Introduction

The hormones are products of the endocrine or ductless glands, names applied to certain organs in the body which manufacture specific chemical substances that enter directly into the circulation and thus reach all parts of the organism. The term, *hormone*, is derived from the roots meaning, "rouse to activity," and the chemical compounds which are classed as hormones generally stimulate or alter the structure and function of one or more types of tissues. However, in certain instances hormones may exert an inhibitory influence on other organs. The name, hormone, is therefore not particularly well chosen but has come into general use.

Hormones function in the body as regulators of rates of physiological reactions, affecting processes which may proceed even without hormones, albeit at a considerably reduced rate. In the absence of one or more of the hormones, serious alterations occur in the fundamental life processes which, in certain instances, do not result in early death but may markedly change the character of the organism. Thus, the hypophysectomized or the thyroidectomized animal may live for extended periods of time, although at a considerably lower level of metabolic activity and with resulting obvious physiological modifications. On the other hand, inadequate hormone supply may cause the rate of certain fundamental cellular reactions to be reduced to a level which is incompatible with life. The severity of the physiological changes occurring following removal of the adrenal glands makes extensive postoperative prolongation of life impossible in the absence of appropriate ameliorative therapy.

It must be recognized early in the discussion that a single metabolic process, or group of processes, cannot operate in either a normal or an abnormal manner without affecting other metabolic reactions. The metabolic transformations of proteins, carbohydrates and lipids are tangential in many of their aspects, and factors influencing the direction or degree of changes involving one of these proximate principles may cause profound alterations in metabolic reactions concerned primarily with one of the other two classes of foodstuffs. Therefore, the effects of hormones on protein metabolism

may be of two broad types: (1) a primary, direct effect on a process recognized as being fundamentally one of protein metabolism; and (2) a secondary, indirect effect on some metabolic process other than one of protein metabolism, as a consequence of which protein metabolism is affected. For example, the steroid hormones of the adrenal cortex exert a direct influence on protein metabolism by increasing the rate of release of protein from tissue stores. On the other hand, the regulatory action of insulin on carbohydrate metabolism has an indirect, though profound, influence on protein metabolism.

There is a paucity of knowledge regarding the mechanism of action of hormones in metabolism. Since there are two large classes of substances in the body which influence the rates of chemical reactions, namely the hormones and the enzymes, it has been suggested [155] that some relationship of hormones to enzymes must be sought in attempts to explain the mechanism of action of the endocrine secretions. This type of relationship has recently been established [116] between insulin, the pancreatic hormone long known to be important in the regulation of carbohydrate metabolism, and purified hexokinase, the tissue enzyme catalyzing the formation of glucose-6-phosphate, a reaction involved in the peripheral utilization of glucose. Other than this single example, which is of importance in opening new fields of investigation, there is no other fundamental reaction which can be pointed to in explanation of the mode of action of a hormone. However, in certain instances experimental data exist which suggest approaches to this problem. For example, the proliferation of the mammary gland produced by direct application of estrogen [42] is a result of the catalysis of chemical events which might be capable of experimental dissection. Only when more information of this nature is available for hormones will an accurate description of their role in metabolism be possible. For the present, descriptions of the over-all processes whose rates are altered by variations in hormone concentration must suffice.

The phenomena comprising growth, either general, *i.e.*, of the entire organism, or specific, *i.e.*, hypertrophy of a particular tissue or organ, require a word of comment. It is obvious that an increase in size of a structure, either as a result of the formation of new cells or the hyperplasia of existing ones, includes the synthesis of new protein, as well as other cellular constituents. A number of hormones affect the growth of tissues, and therefore presumably the formation of protein and protein metabolism. Mammary gland proliferation, growth of Graafian follicles, hair growth, bone growth, may each be controlled in rate by specific hormones and are physiological events which include alterations in protein metabolism. It is not clear whether the endocrine products have an effect on protein anabolism through a direct action on protein formation, or whether the influence of the hormones may be exerted indirectly on some non-protein moiety and thus make available energy required for protein synthesis.

Little evidence exists at the present time for a specific effect of a hormone on a particular component of protein metabolism, *e.g.*, amino acids. Experimental results suggesting a specific influence of a hormone on an amino acid are found in the recent reports of Chaikoff and his associates [111, 112] that the pituitary thyrotrophic hormone affected the rate of synthesis of thyroxine from diiodotyrosine *in vitro*. This is an important observation, and when extended should contribute to knowledge regarding the mechanism of hormone action, since it is probably another example of the action of a hormone on an enzyme system. The reported *in vitro* inhibition by insulin of the oxidative deamination of the unnatural, or *d*-amino acids [135] is of unknown significance in protein metabolism. The discussion which follows is concerned generally with the over-all processes of protein metabolism, with measurements of tissue and urine nitrogen usually the chief criteria for assessing the extent and direction of alterations in this metabolism.

Role of the Anterior Pituitary Growth Hormone in Protein Metabolism

Of the various hormones which may exert an influence on protein metabolism, the growth hormone of the anterior pituitary has perhaps the most obvious effect. The increased rate of somatic growth, produced by injection of purified growth hormone, is a manifestation of a fairly proportional increase in all of the body tissues and structures. Not only is the rate of growth augmented, but the final body size may be greater than that normally attained. This increase in growth rate must be accompanied by an accelerated rate of protein synthesis.

The precise mechanism by which growth hormone exerts its influence on protein metabolism is still unknown. Several explanations are possible: (1) a direct peripheral effect on the amino acids available in the tissues and organs for protein synthesis; (2) a direct effect on energy-yielding systems which must satisfy the energetics of protein synthesis; and (3) an effect on one or more endocrine systems, creating a hormonal balance favoring accelerated protein synthesis. It is likely that all three of these mechanisms may operate in so profound a series of metabolic alterations which comprise growth. It is clear that normal growth requires optimal quantities of foodstuffs, vitamins and endocrine secretions. Growth may be retarded by lack of normal functioning of other endocrine glands, notably the thyroid. Evans and his associates,[31] using growth hormone-containing extracts, have demonstrated that growth of the hypophysectomized rat is more rapid in the presence of the thyroid gland than in its absence. More striking weight gains were recorded when thyroxine was administered with the pituitary extract than when the latter was given alone. These observations are illustrated in Figure 7–1.[31] The full action of growth hormone, therefore, is dependent on the normal functioning of other glands of internal secretion. Finally, it must be recognized that the pituitary growth factor is not necessary for embryonic growth.[38] Indeed, removal of all

pituitary hormones from the rat by hypophysectomy, shortly after birth, does not lead to immediate cessation of growth.

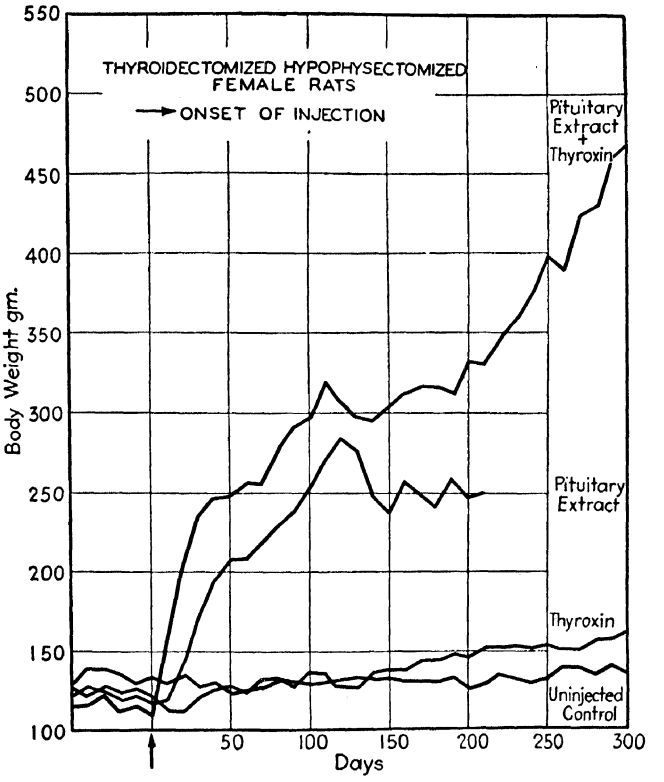

FIGURE 7–1. The role of the thyroid in augmenting the growth-promoting action of anterior pituitary extracts.

The data available concerning the metabolic effects of growth hormone have been obtained for the most part by the injection of crude anterior pituitary extracts into either normal or hypophysectomized animals. Inasmuch as a greater than normal rate of body weight gain was observed under these experimental conditions, the assumption was made that the extracts employed were rich in growth-promoting hormone. Also, analyses of tissues and body fluids for nitrogen have been used as the criteria of alterations in protein metabolism. With these limitations placed upon interpretations of the experimental data, consideration will be given to the changes in protein metabolism produced by growth hormone-containing anterior pituitary extracts, or by more highly purified preparations of the growth hormone.

An insight into the effects of growth-promoting extracts on protein metabolism has been obtained in studies of the composition of animals whose growth has been accelerated by injection of anterior pituitary ex-

tracts. Downs [24] injected mice daily for 105 days with an alkaline pituitary extract previously shown to have marked growth-promoting properties. Analysis of the animals revealed that the treated mice contained more water, protein and ash, and less fat than did the control animals. Similar studies were conducted by Wadehn [146] on mice and by Bierring and Nielsen [6] on rats. The data indicated that the injected animals contained more water and less dry matter than the controls, and that the excess weight gained by the treated animals could not be attributed to fat accumulation. A few years later, Lee and Schaffer [87] completed a somewhat more quantitative study inasmuch as adequate consideration was taken of the fact that animals treated with anterior pituitary extract generally consume more food than do untreated controls. The control and anterior pituitary extract-treated animals were pair-fed for a period of 77 days and the composition of the gain in weight determined. Some of the data of Lee and Schaffer are presented in Table 7–1. The results showed that the injection of anterior pituitary extract caused not only a greater total weight gain, but that this gain was composed largely of protein and water, to an extent of 83 per cent, while fat comprised only about 13 per cent of the total gain. In the control group, water and protein represented 58 per cent of the gain while fat formed 39 per cent.

Table 7–1. Effect of Anterior Pituitary Extract on Composition of the Gain in Weight of Normal Rats — Paired Feeding for 77 Days (Lee and Schaffer [87])

	Gain in Body Weight	*Composition of Gain in Weight*					
		Water		*Fat*		*Protein*	
	(*gm*)	(*gm*)	(%)	(*gm*)	(%)	(*gm*)	(%)
12 controls	716	324	45.0	281	39.3	96	12.6
12 treated	1217	771	63.3	162	13.3	237	19.7

It is interesting to note from the data of Lee and Schaffer that the treated animals' gain in weight was comprised to a smaller degree of fat than the controls, even though the caloric intake was the same in both groups. This may suggest that anterior pituitary extract-treated animals are using more fat to meet their energy requirements, a portion of which may be the demands for the energy of protein synthesis.

Studies of urinary nitrogen and blood nitrogenous constituents furnish additional details of the alterations produced by growth hormone. The injection of crude anterior pituitary extracts into normal or phlorhizinized dogs, or into fasted rats produced a striking reduction in the excretion of urinary nitrogen.[39, 41, 48] More recently, a similar finding has been reported in the normal female rat given relatively low doses of purified growth hormone.[101] This decrease in urinary nitrogen was a reflection of other changes

in protein metabolism. Within a few hours after hormone injection a 20 to 30 per cent decrease occurred in the non-protein nitrogen, the urea and the amino acid concentrations of the blood.[39, 48, 140] There was also a slight decrease in the urea and amino acid content of the carcass of normal rats given anterior pituitary extract, with a more striking decrease occurring in the liver.[126] In the latter organ, the urea value was reduced to less than half the initial amount, and prolonged treatment with extract maintained the amino acid and urea contents of the liver at low levels.

Although the mode of action of growth hormone on protein metabolism is, at the present time, a matter of speculation, there is some evidence that one site of action of the hormone may be the liver. Mirsky [106] has found that nitrogen retention did not occur following the injection of anterior pituitary extracts into eviscerated (liverless) animals. Frame and Russell [37] reported that the usual decrease in blood amino acids produced by anterior pituitary extract injection in rats was not evident in the eviscerated animal. Crude anterior pituitary extracts apparently exert an influence on the proteins of the liver, since Houchin [50] observed an altered protein pattern in the livers of rats given anterior pituitary extract. Injection of anterior pituitary extracts into rats or rabbits was also found [46] to cause a 50 per cent decrease in the glutathione content of the liver in 12 hours. Although many of these data are difficult to interpret at the present time, they suggest a mediating influence of the liver in the metabolic effects of the growth-promoting hormone.

In summary, it is apparent that the administration of extracts rich in growth-promoting hormone is accompanied by marked modifications in the metabolism of proteins and compounds derived from proteins in metabolism. The results obtained indicate that growth hormone promotes an increased rate of protein synthesis, or decreases the normal rate of protein catabolism. The alterations in nitrogen metabolism produced by the growth-promoting agent are so marked that Long [94] has suggested, "a fairly reasonable hypothesis can be advanced that the participation of this agent in the growth process is due to its influence on protein metabolism."

The effects of growth hormone on protein metabolism are seen not only in fed animals but also during fasting or a period of phlorhizin administration. Thus, alterations in protein metabolism can be demonstrated with growth hormone even when gain in body weight does not occur. The data further support the conclusion that the action of the growth hormone is in some manner intimately associated with protein metabolism, independent of growth *per se*. It must be emphasized, however, that most of the data which have been discussed were obtained by the use of crude extracts and may be a result, in part, of the presence of other anterior pituitary hormones. With the recent availability of a highly purified growth hormone preparation,[91] it should be possible to establish the precise effects of the growth hormone in protein metabolism.

Influence of the Adrenal Cortex in Protein Metabolism

The rate of secretion of the adrenal cortex is under the control of a trophic hormone of the anterior pituitary gland, the adrenotrophic hormone. The latter hormone, and the adrenal cortical steroids liberated as a result of its stimulatory effect on the adrenal cortex, have similar metabolic effects. Most of the data which have established a role for the adrenal cortex in protein metabolism have resulted from experiments employing potent adrenal cortical extracts or pure adrenal cortical steroids. The recent isolation of purified pituitary adrenotrophic hormone [90, 125] has made possible some studies using the pituitary principle. The latter is an effective stimulator of the adrenal cortex in normal or in hypophysectomized animals, but is, of course, devoid of this action in the absence of the adrenals. Replacement therapy in the adrenalectomized animal is obtained with adrenal cortical extracts or specific steroid hormones.

Metabolic studies of patients with Addison's disease and with adrenalectomized animals early emphasized the marked abnormalities in carbohydrate metabolism accompanying inadequate supply of adrenal cortical secretory products.[138] The most striking observation was the occurrence of hypoglycemia in the postabsorptive state or in fasting. Adrenalectomized, fasted animals also exhibited severe depletion of their liver glycogen, and, in contrast to normal fasted animals, could not replenish this glycogen by gluconeogenesis from protein. Concomitant with the failure of gluconeogenesis in the fasted, adrenalectomized animal, there was a decreased excretion of nitrogen in the urine.

Britton and his colleagues [7–11, 128] presented the first experimental evidence for the important role of the adrenal cortex in maintaining normal carbohydrate metabolism. Particularly striking was the frequency of hypoglycemia in the adrenalectomized animals. It was concluded that death from adrenal insufficiency was due to depletion of carbohydrate levels resulting from impaired carbohydrate metabolism in the tissues of the body. If animals in adrenal insufficiency were given liberal amounts of adrenal cortical extract, but no food, increases were observed in the blood glucose and in the liver and muscle glycogen.

It seemed possible that the carbohydrate which appeared in fasted adrenalectomized animals given adrenal cortical extract might arise as a consequence of an increased rate of gluconeogenesis from protein. This problem was carefully investigated by Long and his colleagues.[48, 69, 93, 94, 97] Injection of adrenal cortical extract or of certain adrenal cortical steroids, *e.g.*, corticosterone, into normal, or adrenalectomized fasted rats produced alterations in protein and carbohydrate metabolism. The most significant changes noted were: (1) a marked increase in liver glycogen content, and, to a lesser degree, a rise in blood glucose level, and (2) an increase in urine nitrogen coincident with the above absolute elevation of the carbohydrate

content of the body. The increased extent of protein catabolism was sufficient in magnitude to account for the extra carbohydrate found.[97] The conclusion was evident that under the influence of the adrenal cortical steroids, gluconeogenesis from protein was increased. It is also apparent that in the fasting animal, this can only occur as a result of an increased catabolism of protein.

The conclusion that the rate of catabolism of protein is under control of the adrenal cortex is supported by a wide variety of investigations. In 1936, Evans [25] observed that the excretion of nitrogen in phlorhizinized rats was greatly reduced following adrenalectomy. In the same year, Long and Lukens [98] made the striking observation that adrenalectomy in the depancreatized cat was followed by an amelioration of the diabetes and a decline in the high urinary nitrogen seen in the diabetic organism. The decrease in the level of urinary nitrogen following removal of the adrenals in diabetic animals has been amply confirmed by Ingle and Thorn.[62] In the adrenalectomized-depancreatized animal, administration of adrenal cortical extract caused an increase in the urinary nitrogen.

Phlorhizin diabetes has also been useful for demonstrating a role for the adrenal cortex in the regulation of the rate of protein catabolism. Administration of phlorhizin to the adrenalectomized animal produced a nitrogen excretion which was only a fraction of that observed in the non-operated, phlorhizinized control.[49, 89, 137, 147] These data are confirmatory of those of Evans,[25] mentioned above. The injection of cortical extract in phlorhizinized-adrenalectomized animals produced a rise in urinary nitrogen.[138] A similar result was obtained with those crystalline steroid hormones which are oxygenated in position 11 of the steroid nucleus.[147, 151] Those steroids which are not oxygenated in position 11, *e.g.*, 11-desoxycorticosterone and 11-desoxy-17-hydroxycorticosterone, had little or no effect upon gluconeogenesis from protein,[58] and therefore presumably on protein catabolism.

Additional evidence of a role for the adrenal cortex in protein catabolism is derived from further studies conducted in normal animals. The increased nitrogen excretion, with accelerated gluconeogenesis, produced in normal rats by injection of adrenal cortical extract [97] has been referred to above. Sprague [134] also observed an increase in the urine nitrogen of 24- or 48-hour fasted rats given potent adrenal cortical extracts. Ingle and his associates [61] have shown recently that the glycosuria produced in normal adult male rats by force-feeding a high carbohydrate diet and daily injections of 5 mg of either corticosterone or 17-hydroxy-11-dehydrocorticosterone was accompanied by an increase in urinary nitrogen. This was associated with a loss in body weight. From the evidence obtained from studies of nitrogen excretion, it is not surprising that the catabolic influence of the adrenal cortical hormones in protein metabolism may be reflected as an inhibition of growth. Ingle [54, 55] recorded the weight changes in normal rats given either desoxycorticosterone or similar quantities of 17-hydroxy-11-dehy-

drocorticosterone. The former steroid, which has no significant effect on protein catabolism, permitted young rats to make substantial weight gains, whereas the latter steroid produced weight losses and a retardation in growth of the long bones. When normal rats of 180 grams or above were treated with massive doses of 17-hydroxy-11-dehydrocorticosterone, inhibition of growth and definite loss of body weight were observed.[56] Wells and Kendall [149, 150] also studied the effects of various adrenal cortical steroids on the growth of young rats. Whereas desoxycorticosterone had no effect on body weight gain, corticosterone and 17-hydroxy-11-dehydrocorticosterone markedly inhibited the normal growth rate. The latter two steroids also retarded growth of the bone structures (tibia and femur) of the animals.

Since adrenal cortical steroids produce growth inhibition, it might be expected that stimulation of an animal's own adrenal cortex by administration of the anterior pituitary factor which normally regulates adrenal cortical secretion, the adrenotrophic hormone, should produce similar results. In 1937, Moon [109] reported that the body weight gain of young castrated rats was retarded by the injection of anterior pituitary extracts rich in the adrenotrophic factor. This stunting effect has been confirmed [27] recently with highly purified adrenotrophic hormone. The catabolic influence of the pituitary-adrenal cortical secretory mechanism on protein metabolism has been contrasted with the anabolic effects of growth hormone, using body weight gain as the criterion.[102] The growth-stimulating action of growth hormone in the hypophysectomized rat could be nullified by the simultaneous injection of purified adrenotrophic hormone. Either growth augmentation or inhibition could be readily produced, depending on the relative amounts of the two hormones (growth and adrenotrophic) which were administered. Becks, Simpson, Li and Evans [4] investigated recently the inhibitory effects of purified adrenotrophic hormone on the growth of the osseous system of young rats. The hormone produced a retardation in both chondrogenesis and osteogenesis in the region of the proximal epiphysis of the tibia, with an accompanying inhibition of body growth. These changes did not occur when adrenotrophic hormone was injected into adrenalectomized rats. Furthermore, adrenotrophic hormone was demonstrated [5] to inhibit the chondrogenic- and osteogenic-stimulating effects of growth hormone. It is apparent that the pituitary-adrenal cortical mechanism has a profound influence on catabolic processes, including those of protein metabolism.

It should be pointed out that while the catabolic actions of the adrenal cortical hormones appear to be their primary effects in the normal animal, small doses of these hormones may also have a definite growth-promoting action in the adrenalectomized animal.[14, 83] Inasmuch as desoxycorticosterone also has this effect, and since this particular steroid has no regulatory influence on protein metabolism, it seems evident that growth under

these circumstances is a result of general improvement in the condition of the operated animal.

The basis of the control which the pituitary-adrenal cortical mechanism exerts on protein metabolism has been sought by a number of investigators. The various factors concerned with gluconeogenesis have been particularly studied, since most of the evidence for a role of the adrenal cortex in protein metabolism arose from studies of carbohydrate formation from protein under a variety of conditions. The gluconeogenic reactions whose rates might be dependent on the concentration of adrenal cortical hormones are: (1) mobilization of endogenous protein; (2) deamination of amino acids; and (3) conversion of deaminized residues to glucose.

Evidence is now available which strongly suggests that at least one of the important roles of the adrenal cortex in protein metabolism is concerned with the mobilization of tissue protein. Soskin and his colleagues [133] early showed that the hypophysectomized animal could utilize exogenous protein and thus maintain his blood sugar level at near normal values. It was suggested that the influence of the anterior pituitary gland on gluconeogenesis was to facilitate the conversion or breakdown of tissue proteins to the amino acid stage. Wells and Kendall [152] observed that when adrenalectomized-phlorhizinized rats were given casein in the diet, they were able to metabolize this protein, excrete the expected amounts of glucose and nitrogen, and in every way respond as did normal phlorhizinized rats. It was concluded that the metabolism of exogenous protein is not seriously disturbed by the absence of the adrenal cortical hormones, and that it is only when the exogenous supply is lacking and endogenous protein must be mobilized that the limited capacity of the adrenalectomized animal for gluconeogenesis becomes evident. However, these authors drew the conclusion that the increased gluconeogenesis promoted by certain adrenal cortical steroids was due to their specific antagonism to insulin.

The contribution of protein by at least one type of tissue, the lymphoid structures, to protein metabolism is under control of pituitary-adrenal cortical secretion. The increased protein mobilization produced by adrenal cortical hormones, and the consequent augmented amounts of protein available for gluconeogenesis and other processes of protein metabolism, appears to be based in part on the regulatory control which the adrenal cortex exerts over the structure and function of lymphoid tissue. The existence of an inverse relationship between lymphoid tissue mass and the concentration of circulating adrenal cortical hormones has been experimentally established.[19, 28, 47, 53, 60, 63, 118, 137] Indeed, Addison,[47] in his original description of the disease which bears his name, noted the hypertrophy of lymphoid structures in his patients. The dependency of the organism on normally functioning adrenals for obtaining the protein reserves of lymphoid structures has been demonstrated recently by Dougherty and White.[21] It has long been known that partial or complete inanition produces strik-

ing involution of lymphoid tissue. Inasmuch as inanition also causes augmented pituitary-adrenal cortical secretion,[124] Dougherty and White examined the possible role of this hormonal mechanism in the lymphoid tissue involution of inanition. The results were striking. A 48-hour fast in normal mice produced approximately a 40 per cent loss of lymphoid tissue mass and of the protein content of this tissue. On the other hand, a similar period of fasting in adrenalectomized mice produced no change in lymphoid tissue mass or protein content, as compared with normal, fed controls. It is apparent that the mobilization of protein from lymphoid tissue is under the regulatory control of the adrenal cortex. In the absence of this endocrine gland, the fasted animal cannot obtain the direly needed tissue components of lymphoid structures. It may be pointed out that as the fasting period in adrenalectomized mice was extended to 96 hours, lymphoid tissue protein content did show a decrease in an occasional animal.[160] Under these circumstances, the mice were in the terminal stages of life, and it is likely that most bodily control mechanisms had broken down. From the data which have been obtained, it is evident that lymphoid tissue may constitute a portion of the reserve, or depot protein which has interested investigators for some years.[156] This protein is mobilized and catabolized under circumstances which produce augmented pituitary-adrenal cortical secretion. Long and his associates [96, 97] have shown by calculation and direct determination that the extra protein mobilized for gluconeogenesis in the fasted rat given adrenal cortical extract or hormones could not have been obtained from the liver alone and that extra hepatic tissue must have made some contribution of nitrogen. Lymphoid tissue may supply some of this protein. It is recognized that the total lymphoid mass of the organism could furnish only a small portion of the extra nitrogen eventually excreted under these circumstances. Experimental data [160] suggest that a considerable portion of this extra nitrogen may be derived from other sources in the organism, *e.g.*, carcass, and that the rate of this process is under thyroid control. However, even in the face of a continuing emphasis on protein catabolism, lymphoid tissue protein may be restored at the expense of carcass protein.[160]

Further evidence of the control which the pituitary-adrenal cortical mechanism exerts on the contributions of lymphoid tissue to protein metabolism is seen from studies of the correlation of alterations in lymphoid tissue structure with changes in the serum protein pattern. This subject has been reviewed recently by White and Dougherty.[158, 159] At a time when lymphocyte dissolution in the tissues was maximal as a consequence of augmented pituitary-adrenal cortical secretion, or because of injection of adrenal cortical steroids, there was present in the blood of rabbits elevated levels of serum beta- and gamma-globulins. These alterations were evident within a few hours after hormone injection; 24 hours later the metabolic picture had returned to normal. Lymphocytes contain as a constituent of the

cell a protein which is identical with normal serum gamma-globulin, and another protein which is probably identical with serum beta-globulin. Lymphocyte dissolution, therefore, results in an addition of these globulins to the lymph and subsequently to the systemic circulation. In the immunized animal, the lymphocytes contain antibody globulin, and the rate of release of the immune globulin is likewise controlled by pituitary-adrenal cortical secretion.[15, 159] This hormonal mechanism also appears to have other important roles in protein metabolism, since preliminary data indicate that shortly following the injection of pituitary adrenotrophic hormone in rabbits, there is a marked increase in the number of lymphocytic cells which contain increased amounts of nucleoprotein in their cytoplasm.[23] Since nucleoprotein is implicated in fundamental processes concerned with the formation of new cells, and therefore new protein, the adrenal cortex may play a hormonal role in both the mobilization of protein from reserves in the organism, and in accelerating the synthesis of new protein. The increased red cell production seen in circumstances of augmented concentrations of adrenal cortical steroids [20, 157] also suggests an effect of this hormone on new protein formation.

Adrenalectomized rats [88] and patients with Addison's disease [104] have been reported to have a low plasma albumin. These data have been obtained in circumstances of chronic adrenal insufficiency, and it is suggested [86] that they are due in part to impaired appetite. The observed increased serum globulin level after adrenalectomy [88] was not evident in later experiments.[86]

It is not unlikely that the adrenal cortex may have additional roles in protein metabolism. The deamination of amino acids appears to be retarded in the adrenalectomized animal. The rate of formation of glucose from alanine in the adrenalectomized, phlorhizinized rat has been reported [89] as less than normal. Russell and Wilhelmi,[120, 121] extending earlier work of Jimenez-Diaz [66] with kidneys of adrenalectomized rats, reported that kidney slices from adrenalectomized rats formed less than normal amounts of carbohydrate from *dl*-alanine and glutamic acid. Samuels and his colleagues [122] had earlier observed that in the absence of the adrenals, rats deposited less liver glycogen following the feeding of alanine.

In view of the evidence cited above, it is surprising that the liver tissue of adrenalectomized animals exhibited a normal rate of deamination of *dl*-alanine [26] and glutamic acid.[80] Inasmuch as the liver is one of the chief sites of deamination of amino acids, these data suggest that the decreased rate of gluconeogenesis from amino acids in adrenalectomized animals may be due to a slower conversion of the deaminized residues to glucose. This hypothesis is supported by numerous investigations [80, 120, 121, 142] which show that the normal rate of conversion of deaminized residues to glucose is decreased in the tissues of adrenalectomized animals. The liver of rats with adrenal cortical insufficiency converted fed lactic acid to liver glycogen at a decreased rate.[12] Adrenalectomized-phlorhizinized rats also had a di-

minished capacity to form glucose from lactate and pyruvate.[89] On the other hand, liver slices from rats pretreated with cortical extract were reported [80] to convert lactic and pyruvic acids to carbohydrate at a greater than normal rate.

Yet another mode of action of the adrenal cortical hormones on protein metabolism may be suggested from recent studies of the effect of these hormones on the concentration of certain tissue enzyme systems. Some of these enzymes are known to have a direct role in a particular aspect of protein metabolism, and others, while not directly concerned with protein metabolism, are important components of processes by which the cell respires and derives its energy for metabolic functions. Tipton [143] found a decrease in the cytochrome *c* oxidase concentration of the liver, heart and kidney, and in the quantity of cytochrome *c* in the kidney and liver of adrenalectomized rats. Kochakian and Vail [79] reported unexplained alterations in the concentration of the alkaline phosphatase of rat liver tissue; the enzyme content increased both following adrenalectomy and as a result of injection of adrenal cortical extract. No effect was reported on liver acid phosphatase. In the kidney, on the other hand, adrenalectomy did not influence the concentration of alkaline phosphatase but decreased acid phosphatase activity. The concentration of the latter enzyme could be restored to normal by the administration of adrenal cortical extracts. Liver arginase, an enzyme system directly concerned with certain intermediary aspects of protein metabolism, was decreased considerably in activity in adrenalectomized rats and could be increased markedly in concentration by the injection of adrenal cortical steroids in hypophysectomized or adrenalectomized rats.[36]

In summary, it seems clear that the adrenal cortex exerts a profound hormonal influence in protein metabolism. The diminished nitrogen metabolism which is evident in the organism with inadequate supply of adrenal cortical secretion is reflected secondarily in a diminished capacity to maintain carbohydrate supplies from protein by gluconeogenesis. Inasmuch as the animal without a pituitary or without adrenals is capable of metabolizing dietary protein in a normal manner, it is apparent that the aberration in protein metabolism seen in hypofunctioning of the pituitary-adrenal cortical secretory mechanism is a result of the failure to mobilize reserve or tissue protein at a normal rate. A portion of this reserve protein resides in the lymphoid structures of the organism. These structures contribute to the circulation at least one and probably two of the normal serum globulins, in addition to other components of lymphocytes. The rate of this contribution is under pituitary-adrenal cortical control, and in the absence of this hormonal mechanism the supply of protein from lymphoid tissue may be entirely unavailable, or may enter the pool of protein metabolism at a greatly diminished rate. Adrenal cortical hormones may also stimulate processes concerned with formation of new protein.

It is likely that several aspects of protein metabolism are controlled in their rates by the hormones of the adrenal cortex. The deamination of amino acids, and the conversion of the deaminized residues to glucose, appear to be influenced by the concentration of circulating adrenal cortical steroids. It remains for further investigations to establish whether this is a specific regulatory role of adrenal cortical secretion or whether the data obtained are a manifestation of a generalized tissue hypofunctioning in the absence of the adrenals. Finally, one of the factors affecting the concentration of cellular enzymes concerned with protein metabolism may be the pituitary-adrenal cortical mechanism.

Relation of the Thyroid to Protein Metabolism

The thyroid is unique among the endocrine glands in that its hormonal secretion may exert a direct metabolic influence on every cell in the body. The increased oxygen consumption, produced by either hypersecretion of the thyroid gland or injection of potent preparations of this tissue, has been accepted as evidence of an increased metabolic activity of all the cells of the organism. It is not surprising, therefore, that the thyroid gland exerts a profound influence on protein metabolism.

Inasmuch as a subnormal degree of thyroid activity, hypothyroidism, results in a marked decrease in the rates of body processes, and an above-normal degree of secretion of the thyroid, hyperthyroidism, produces augmented rates of physiological reactions, hypothyroidism and hyperthyroidism are accompanied by decreased and increased rates, respectively, of processes concerned with protein metabolism. Hypothyroid function in the early years of life causes a markedly delayed rate of growth, resulting in cretinism. Johnston and Maroney [67] reported that small amounts of thyroid produced positive nitrogen balances in growing children, suggesting an anabolic effect of the hormone. The experimental data, already referred to in the discussion of the growth hormone, together with the clinical findings, clearly establish the important role of the thyroid in somatic growth and, therefore, in processes concerned with protein synthesis. This is not unexpected in view of the deceleration of metabolic functions which occurs in hypothyroidism. Fundamental processes of the type of absorption of digestion products from the gastrointestinal tract are influenced in their rate by the thyroid gland. Russell [119] demonstrated that the decreased rate of glucose absorption in hypophysectomized rats was due to thyroid hypofunction. Treatment of the operated animals with thyroxine restored the absorption rate to normal. If the rate of supply to the organism of a large portion of its supply of energy, in the form of glucose, is under thyroid control, then energy-requiring processes of the type of protein synthesis should be influenced by thyroid function. The level of thyroid activity may affect the direction of the protein metabolism. The absence of a normal rate of protein anabolism, in hypothyroidism, may become an

abnormally high rate of protein catabolism because of either injection of excess of thyroid hormone or the presence of a hyperactive thyroid gland.

The administration of thyroid or thyroxine in a wide variety of experimental conditions has been demonstrated to produce an increased loss of nitrogen in the urine. It is clear that this nitrogen has its origin in the tissue and organ protein, and other nitrogenous constituents, which are mobilized and catabolized at an increased rate as a result of hormone administration. These effects are illustrated strikingly in a variety of diabetic circumstances, either clinical or experimental. Wilder [161] observed that in the patient, pre-existing diabetes was aggravated by the occurrence of hyperthyroidism. In these circumstances, carbohydrate formation was accelerated presumably as a consequence of the augmented mobilization of tissue protein.

A similar role for the thyroid has been demonstrated in phlorhizin diabetes.[99, 113] Fasting, thyroidectomized animals excreted much less sugar and nitrogen under the influence of phlorhizin than did phlorhizinized, unoperated controls. There was no difference between the two types of animals when they were fed protein. Thus, the deficiency evident because of the absence of the thyroid was in the mobilization and breakdown of body protein to amino acids. Investigators at the Mayo Clinic have made careful studies of the role of the thyroid in phlorhizin diabetes. Wells and Kendall [151] found that thyroidectomy diminished the amount of glucose and nitrogen excreted by normal, phlorhizinized rats and that the rate of gluconeogenesis could be restored to normal by the administration of thyroxine. Wells and Chapman [148] studied hypophysectomized-phlorhizinized rats, and observed that while adrenal replacement therapy, in the form of potent adrenal cortical steroid hormones, increased the rate of glucose and nitrogen excretion, a further augmentation in the excretion of both of these urinary constituents was obtained when the rats were also given thyrotrophic hormone. Indeed, administration of the latter together with the steroid hormones produced a degree of gluconeogenesis approaching that of normal, phlorhizinized rats. Augmented nitrogen excretion has also been reported [132] following administration of thyroxine to hypophysectomized dogs fasted for long periods of time. In these experiments the urinary nitrogen level reached that seen in fasting, normal dogs. The intravenous injection of thyroxine in dogs produced a marked increase in the concentration of amino acids in the blood plasma.[32]

These suggestions of a role for the thyroid in the mobilization of nitrogenous components of tissues are supported by Sternheimer [136] who examined the effect of a single injection of thyroxine on the carbohydrate, protein and size of rat liver. Within six hours after a single dose of thyroxine in rats, there was a loss of liver glycogen and the beginning of a rise in liver protein. These changes became more marked up to about the forty-eighth hour, and then showed a reversal in direction. By the eighty-

fourth hour, the liver glycogen reached a peak well above the original control level, while the total nitrogen of the liver, though falling, was still above the original values. The data indicated that thyroxine first causes a mobilization of protein from peripheral tissues, and also a proliferation of the liver cells. Fraenkel-Conrat, Simpson and Evans [35] found that purified thyrotrophic hormone caused an increase in the liver weight of rats.

Direct experimental evidence for a role of the thyroid in the mobilization of body nitrogen has been obtained recently by White and Dougherty.[160] Studies have been made of the content of nitrogen in the liver, lymphoid tissue, and carcass of normal mice as compared both to normal mice fasted for 48 hours, and to thyroidectomized mice fasted a similar period of time. The marked loss in carcass nitrogen seen in the normal, fasted mice did not occur in the thyroidectomized animals under the same conditions of food deprivation.

In view of the marked and well-described effects of thyroid hormone on nitrogen excretion, it is somewhat surprising that more studies have not been made of the relation of the thyroid to some of the intermediary aspects of protein metabolism. Studies have been reported of the role of the thyroid in the regulation of the pattern of serum proteins. Goldberg [45] and Levin and Leathem [88] studied the albumin to globulin ratios of the blood of rats following thyroidectomy. The salt fractionation method employed showed that the concentration of total serum globulin increased after thyroidectomy and that such globulin elevation might be prevented by thyroid replacement therapy. Hypothyroidism, produced by feeding thiourea, also led to a rise in the total globulin concentration of the plasma of rats.[85] The hyperglobulinemia of hypothyroidism was confirmed by electrophoretic examination [110] of the sera of thyroidectomized rats and rats fed thiourea. Under these conditions, a rise was reported in both the α- and γ-globulin components of the blood. The changes were not very marked, however, and the influence of possible alterations in blood volume was eliminated on the basis of a relatively few hematocrit readings. In view of the anemia of hypophysectomized rats, and the controlling influence of the thyroid on erythropoiesis,[145] hematocrit determinations as indicators of blood volume are of doubtful value under these experimental conditions.

In summary, the thyroid gland, as a consequence of its regulatory influence on the rate of metabolism of cells, exerts a marked control over the rate of protein metabolism. In the immature organism, the metabolic processes are balanced in favor of synthetic mechanisms. Hence, the acceleration of these reactions by thyroid hormone may lead to increased protein synthesis, reflected in a greater retention of fed or administered nitrogen, and in body weight gain. In the normal adult, where a dynamic balance has been established between anabolism and catabolism, thyroid hormone accelerates protein catabolism. The thyroid gland has been suggested to exert a controlling influence over the level of serum globulins and the num-

bers of circulating erythrocytes. It may be pointed out that data obtained in studies of the thyroid gland, some of which have been discussed, must be viewed with reservations, since the rate of thyroid secretion has a marked effect on the rate of release of the steroid hormones of the adrenal cortex. The role which the adrenal cortex plays is probably of prime importance among the interrelationships of the endocrine glands in protein metabolism.

Finally, the rate of hormone secretion by the thyroid gland is normally under the trophic control of the anterior pituitary thyrotrophic hormone. Although the relations of the thyroid to protein metabolism have been emphasized in the above discussion, the same physiological considerations obtain with respect to the role of the regulator of thyroid secretory rate, the anterior pituitary thyrotrophic hormone.

Influence of Insulin in Protein Metabolism

It has long been recognized that the injection of insulin decreases the urinary nitrogen excretion provided ample carbohydrate is supplied in the diet. This is true in both the normal and the diabetic organism. Further, it is well known that in the absence of an adequate supply of insulin, cessation or retardation of growth may be evident. However, the administration of large amounts of insulin may result in augmented excretion of nitrogen.

In assessing the mode of action of insulin in protein metabolism, it is difficult frequently to determine which data indicate a primary influence of the hormone, and which are the result of alterations in the endocrine balance normally obtaining among the hormones directly concerned with protein metabolism. The important role of the adrenal cortex in the direct control of protein metabolism has been discussed previously. Insulin, in common with a wide variety of other physical and chemical stimuli, produces augmented secretion of the pituitary-adrenal cortical mechanism.[124] Indeed, Latta and Henderson [84] have described alterations in lymphoid tissue histology and in the numbers of circulating lymphocytes of normal rats following a single injection of insulin which are indistinguishable from those seen following the injection of either purified adrenotrophic hormone or adrenal cortical steroids.[20, 22]

The sparing of protein which occurs in the normal organism following the administration of insulin [65, 100, 105] can be attributed to the increased utilization of carbohydrate favored by the pancreatic hormone. Under these circumstances, the proportion of the total energy requirement which must be met by protein catabolism is diminished, and there is a reduction in urinary nitrogen. On the other hand, the injection of large doses of insulin in normal animals may result in growth inhibition and weight loss, with an increased excretion of nitrogen in the urine.[59] It is not unlikely that under these circumstances the stimulatory action of insulin on the pituitary-adrenal cortical mechanism resulted in manifestations of the catabolic influence of the adrenal cortical steroid hormones.

Other views regarding the mechanism by which insulin may promote nitrogen retention have arisen as a result of studies of the effects of crude anterior pituitary extracts on protein metabolism. As has already been discussed, such extracts, which are rich in growth-promoting factor, have the capacity to reduce the excretion of nitrogen. Mirsky and his associates [105, 107, 108] suggested that an increased insulin supply might both decrease the rate of deamination of amino acids in the liver and facilitate the utilization of amino acids by the muscles for synthetic purposes. It was found that in eviscerated and nephrectomized dogs, maintained by constant injection of insulin and glucose, the blood amino acids rose more slowly, and injected glycine disappeared more rapidly, than in similar animals maintained on sugar alone. Inasmuch as the absence of the liver and kidneys precluded a loss of the amino acids by deamination, the conclusion was drawn that insulin promotes protein synthesis, from amino acids, in the muscles. With this background of evidence, Mirsky [106] interpreted the nitrogen-retaining effect of anterior pituitary extracts as being due to stimulation of the islands of Langerhans by a "pancreatrophic" hormone. Support for this hypothesis was obtained from the demonstration that whereas anterior pituitary extracts caused an increase in the rate of accumulation of non-protein nitrogen in the blood of nephrectomized-depancreatized dogs, the same extract given to nephrectomized, but otherwise normal, dogs resulted in a decreased rate of accumulation of these intermediary metabolites. Also, the injection of insulin decreased the accumulation of the blood non-protein nitrogen in normal, eviscerated, and depancreatized dogs.[105]

The data of Young [165, 166] also suggested a relation between the nitrogen-retaining action of anterior pituitary extract and insulin. When young puppies were treated with anterior pituitary extract, they responded with a marked gain in weight and increased nitrogen retention. Young pointed out that these changes were associated with an increase in the quantity of islet tissue. On the other hand, it seems clear that while insulin may play a potentiating role in the utilization of metabolic nitrogen, it is not essential for this process. Gaebler and Robinson [40] have shown that the removal of the thyroid, the adrenals and the pancreas in dogs did not prevent the nitrogen-retaining action of anterior pituitary preparations rich in growth hormone. However, the authors conceded that the presence of insulin facilitated nitrogen retention by growth-promoting extracts. Their data may again be a demonstration of the fact that while the rates of metabolic reactions may be controlled by certain hormones, these reactions do not cease entirely in the absence of the endocrine factors. It seems reasonable to conclude that an intimate connection exists between growth processes and insulin, and that other hormones may also be concerned in this relationship. If the energy for synthetic reactions is to be supplied by the concomitant metabolism of a variety of substances, then accelerated growth,

with its accompanying increased nitrogen retention, may well require additional quantities of cellular catalysts, including insulin. In this sense, insulin would promote peripheral utilization of nitrogen.

Although the suggestions of Mirsky [106] and of Young [165, 166] for a specific anterior pituitary pancreatrophic hormone have been mentioned, there is considerable doubt at the present time that such a factor need be postulated. Pancreatic islet degeneration does not follow experimental hypophysectomy. Moreover, pancreatic islet hypertrophy following injection of anterior pituitary extracts could be a secondary response caused by one of the known anterior pituitary principles. Indeed, Evans and his colleagues have reported that both purified lactogenic hormone [33] and adrenotrophic hormone [34] increased the insulin content of the pancreas of normal rats, and showed pancreatrophic activity in both normal and hypophysectomized animals.

In summary, it is not yet possible to conclude whether the marked influence of insulin in protein metabolism is a direct or an indirect one. The data which have been obtained can be interpreted in terms of either a direct action of the hormone on certain components of intermediary protein metabolism, or an indirect action on nitrogen utilization as a consequence of increased oxidation of non-nitrogenous metabolites, or both. Whatever the correct explanation, it is clear that insulin facilitates the well-known protein sparing action of carbohydrates, thus causing an increased retention of nitrogen for anabolic purposes. The augmented utilization of carbohydrate promoted by insulin would at the same time provide additional energy for protein synthesis.

Effect of the Sex Hormones on Protein Metabolism

A description of the effects of the sex hormones on protein metabolism can be obtained from the experimental evidence that the sex hormones influence somatic growth and as a consequence may favorably affect the nitrogen balance of the organism. The sex hormones to which this discussion will be limited are the male sex hormones, the androgens, and the female sex hormones, the estrogens. The two pituitary gonadotrophins, the follicle-stimulating hormone and the luteinizing or interstitial-cell stimulating principle, which exert a controlling trophic influence on androgen and estrogen secretion, have as yet not been used in extensive studies of protein metabolism. As is true with other hormones affecting metabolism, it is not yet possible to describe the exact mechanisms by which the sex hormones exert their influence in protein metabolism. The action of the sex hormones may be both a primary one directly on protein metabolism, and an indirect one mediated through some other endocrine gland or metabolic process. A particularly important factor is the influence which gonadal secretions have on the level of activity of the anterior pituitary gland. Male sex hormone, testosterone, has an inhibiting action on the

rate of anterior pituitary secretion, whereas the effect of female sex hormones, estrogens, appears to be dependent on the dose of hormone employed. Small doses of female sex hormones generally augment anterior pituitary activity, whereas large amounts of the same hormones may suppress this activity. It is not clear at the present time whether this back action of the sex hormones on the anterior pituitary relates specifically to the rate of release of gonadotrophic hormones, or whether there may be an effect on the secretion of all the anterior pituitary hormones. It is thus apparent that a wide variety of physiological results may be obtained from the administration of either male or female sex hormones, at different dose levels. In addition to these interrelationships, the male sex hormone exerts an effect on the degree of functioning of the adrenal cortex; the importance of this endocrine gland in protein metabolism has been indicated previously.

The trophic, stimulating influence of the sex hormones on the size and weight of the gonads and the accessory sex tissues [3, 129] makes it evident that these hormones are growth catalysts and therefore influence the rate of protein anabolism. The sex hormones also have a direct action on the gonads in the absence of the pituitary gland [129, 130] and their influence is therefore independent of the anterior pituitary growth hormone. Tissues other than the sex glands may also proliferate under the influence of certain of the sex hormones. Korenchevsky and his colleagues [81, 82] reported that the decreased weights of kidneys, livers and hearts of castrate male rats could be restored to normal by testosterone propionate; hypertrophy of kidney elements was also evident. These authors suggested a general anabolic property for the androgens. The effect of male sex hormone on the kidneys has been further studied by other investigators [115, 127] who observed striking renal tubular hypertrophy in mice given testosterone or testosterone propionate. The latter hormone also produced [114] hypertrophy of the temporal muscles of the castrated guinea pig.

Specific metabolic alterations as a result of administration of sex hormones were early described in experiments by Kochakian and Murlin.[74, 77, 78] Employing the castrate dog as an experimental animal, these investigators demonstrated that injection of testosterone produced a prompt and sustained reduction in urinary nitrogen excretion. Nitrogen partitions revealed that a reduced urea excretion accounted for the lowered nitrogen output, and was unaccompanied by any rise in the concentration of nitrogenous constituents of the blood. Fecal nitrogen was not affected. The maximal effect of nitrogen retention obtained (0.05 gm of nitrogen stored per kg body weight per day) could not be exceeded by increasing or prolonging the androgen dose. When hormone injection was stopped, a greater than normal excretion of nitrogen was seen in some experiments, although all the retained nitrogen was not promptly lost. Similar retention of nitrogen following injection of testosterone propionate was reported in normal dogs by Thorn and Engle.[141]

The data in animals are supported by clinical experience with testosterone. This subject has recently been reviewed extensively by Kenyon, Knowlton and Sandiford.[70] When sexually underdeveloped men and boys received intramuscular injections of testosterone propionate, notable gains in body weight occurred usually within the first few weeks of treatment. Marked retention of nitrogen was seen in the subject treated, and, as in the animal experiments, the decline in the urea fraction of the urine was responsible for the decreased nitrogen excretion. The process of weight gain and nitrogen retention was self-limiting in that despite continued treatment, a plateau was reached in the individuals studied. Simultaneous analyses of the blood revealed no alterations in plasma proteins, hemoglobin, non-protein nitrogen or urea, although other studies [72] indicated that the blood urea and non-protein nitrogen declined at the time of diminished excretion of nitrogen in the urine. The quantity of protein estimated as stored was far in excess of that conceivably used for enlarging genital accessories, indicating deposition of new protein elsewhere in the body. On cessation of hormone injections, there was no conspicuous compensatory loss of the retained nitrogen. Similar weight gains have been obtained by the oral administration of methyl testosterone.[51, 52, 68, 104] It is important to note that testosterone propionate injections also increased nitrogen retention in normal young men [13, 72] and women,[72] although the amount of nitrogen retained was less than that in the eunuchoids. These data would suggest that the normal rate of secretion of male sex hormone is not great enough to induce the maximum nitrogen retaining capacity of which the organism is capable under male hormone influence. Table 7–2 is taken from data of Kenyon and his associates [72] and illustrates the effects of male hormone on the excretion of urinary nitrogen, and other metabolic products, in a variety of subjects. The retention of other important tissue constituents during male hormone administration supports the suggestion that the nitrogen is stored as a component of new tissue mass. The marked effect of testosterone propionate on erythrocyte production [144] is again evidence of a role for the male sex hormone in the synthesis of new cells.

There has also been considerable interest in the effect of male sex hormone on one of the intermediates of protein metabolism, namely, creatine. The latter substance, which is present in the urine of both sexes until puberty and then persists only in the urine of the female, is frequently a constituent of the urine of hypogonadal individuals. Injection of testosterone propionate will reduce the level of creatine excretion of the eunuchoid either when the excretion is considerable or when it is maintained at high levels by ingestion of creatine.[70] Similar observations have been reported in the rat,[16] rabbit [164] and monkey.[64] These data also support the concept of an increase in new tissue mass resulting from hormone treatment, since creatine is an important constituent of the muscle cell. On the other hand, it has been conclusively demonstrated that while methyl testosterone may, as

Table 7–2. Comparison of the Estimated Reduction in Excretion of Several Urinary Constituents Produced by Injection of Testosterone Propionate *

Type of Subject	*Age*	*Urinary Androgens before Treatment* (*I. U. per day*) †	*Amt. of Hormone Given* (*mg per day*)	*Average Reduction in Excretion of Urinary Constituents*					
				Total Nitrogen		*Inorganic Phosphorus*	*Sodium*	*Chloride*	*Potassium*
				(*gm per day*)	(*gm/kg per day*)	(*gm per day*)	(*m. eq. per day*)	(*m. eq. per day*)	(*m. eq. per day*)
Eunuchoid I	27	13	25	5.14	0.069	—	24	19	—
Eunuchoid II	51	10	25	3.19	0.064	—	17	7	—
Eunuchoid III	27	33	25	3.27	0.053	0.170	—	—	—
Eunuchoid III	27	33	50	2.93	0.048	0.150	23	18	20
Normal man	21	83	25	1.66	0.029	0.190	21	15	9
Normal man	19	78	25	2.55	0.030	0.050	28	33	12
Normal woman	24	100	25	2.61	0.043	0.150	9	5	9
Normal woman	31	65	25	0.73	0.016	0.990	3	5	4

* Taken in part, and modified, from Table 5, reference [72].

† I.U. = International Units.

mentioned above, promote nitrogen retention in a manner similar to that of testosterone, this methylated steroid definitely augments the creatine excretion in either the underdeveloped or the normal individual. [52, 123, 139, 153, 162, 163] Apparently, methyl testosterone promotes creatine production and excretion. The divergent effects of the two male sex hormones on creatine metabolism awaits explanation.

There is little evidence at the present time of the nature of the intermediary processes of protein metabolism which are affected by male sex hormone. On the basis of data from a study of two patients with Addison's disease,[71] it seems indicated that the characteristic metabolic effect of testosterone in depressing the excretion of urinary nitrogen and other substances is not dependent on a normally functioning adrenal cortex. Kochakian and his associates, in their studies of the influence of steroid hormones on the concentration of enzyme systems, have examined [75, 76] the effects of injection of testosterone and testosterone propionate on the arginase content of liver, kidney and intestinal tissue of mice and rats. The steroid hormones produced an increase in arginase content per gram of tissue. Although this enzyme is implicated in an intermediary aspect of protein metabolism, the significance of alterations in its concentration, and of their possible control by hormones, remains for further investigations to elucidate.

Little work has been done on the direct effects of the estrogens on protein metabolism. The capacity of estrogens to cause proliferation of certain specific tissues has already been indicated. In this sense, the female sex hormones exert an anabolic function. This is also shown to a striking degree in the profound influence which estrogens exert on calcium and phosphorus retention and on new bone formation. This subject has been reviewed recently by Gardner and Pfeiffer.[43] The synthesis of bone matrix, involving new protein formation, is distinctly an important effect of estrogens on protein metabolism. It is not clear whether this is a primary influence of estrogens or a response to an altered inorganic salt metabolism. Albright and his colleagues [1, 2] have suggested that the primary action of estrogens in osteoporetic women is an anabolic one on bone matrix, in consequence of which the retention of calcium and phosphorus is possible, and secondary. This is in agreement with evidence that estrogens may cause nitrogen retention in normal dogs.[141] Also, Kenyon and his associates [73] found that estradiol benzoate, when administered in sufficient amounts to sexually under-developed men, to sexually under-developed women, and to normal women, shares several of the metabolic aspects of testosterone propionate. Retention of nitrogen, inorganic phosphorus and sodium was induced with either hormone.

It has already been pointed out that diverse effects may be produced with a particular hormone, depending on the dosage employed. This is particularly demonstrable with estrogens. Thus, the above anabolic results with

estrogens may be contrasted with the catabolic influence of large doses of female sex hormones. Large doses of estrogens have been shown to have a dwarfing effect in rats and mice.[*] Also, the diabetogenic action of estrogens in rats [57, 93] and in partially depancreatized ferrets [18] is accompanied by marked nitrogen loss. The mediation of the adrenal cortex in this effect has been suggested.[93]

In summary, it is clear that both androgens and estrogens may exert an anabolic influence on protein metabolism. This is particularly striking with the androgens which promote an increase in tissue mass of the organism. The effect of estrogens may, it appears, be anabolic or catabolic, depending on the dose of hormone employed. It is presumed that the pituitary gonadotrophins will produce effects simulating those of the products of the end organs over which they exert a trophic influence. However, this has not as yet been subjected to experimental test. Nevertheless this conclusion seems indicated from the data available from studies [70] employing preparations of chorionic gonadotrophin.

Role of Epinephrine in Protein Metabolism

Although epinephrine, the hormone of the adrenal medulla, exerts a profound influence on carbohydrate metabolism there is no evidence for a direct action of this hormone on protein metabolism. Cori and his associates,[17] in balance studies in fasted rats, found that the injection of small amounts of epinephrine (0.02 mg/100 gm body weight) produced no alteration in the excretion of nitrogen. The carbohydrate mobilized under these circumstances, therefore, was not derived from protein.

A possible indirect action of epinephrine on protein metabolism must now be recognized. This hormone, in common with a wide variety of unrelated physical and chemical stimuli, produces augmented secretion of the pituitary adrenotrophic hormone.[95, 124] As previously discussed, the latter hormone, whose physiological effects are mediated via the adrenal cortex, controls the rates of important processes in protein metabolism. It is evident that an increase in the concentration of circulating epinephrine, either as a result of adrenal medulla stimulation or injection of the hormone, may alter protein metabolism through an indirect action mediated by way of the anterior pituitary gland. In view of the rapidity with which epinephrine may induce increased secretion of pituitary adrenotrophic hormone, reinvestigation is desirable of the possible significance of the indirectly affected protein metabolism on the balance picture which has been drawn of carbohydrate metabolism.

Other Hormones Related to Protein Metabolism

Prolactin. The metabolic effects produced by prolactin indicate that this anterior pituitary hormone has a role in protein metabolism. Prolactin initiates and promotes lactation in the adequately prepared mammary

gland and causes proliferation of the pigeon's crop sac.[3] These actions must be related to aspects of protein metabolism. The exact role which prolactin may play in the synthesis of the protein constituents of milk is unknown at the present time. In addition to its role in milk secretion, prolactin has recently been shown [44, 117, 154] to be the essential anterior pituitary principle which is required, together with estrogen, to promote mammary gland duct growth and proliferation. Finally, prolactin has also been demonstrated to have a progestational action; the lactogenic hormone controls progesterone secretion from developed corpora lutea.[29, 30, 129] These functions of prolactin suggest a role for this hormone in protein metabolism.

Gastrin and Secretin. These hormones of the gastrointestinal tract probably exert an influence on protein metabolism since they control the rate of secretion of fluids concerned with protein digestion and therefore will determine the extent to which dietary proteins are broken down to nitrogenous molecules which can be absorbed from the gastrointestinal tract.

Concluding Remarks

A brief description and summary have been given of the roles for each of various hormones in protein metabolism. The integrations existing among the endocrine glands and the metabolic processes which they influence make it difficult to determine which actions of a particular hormone are primary effects and which are indirect results, mediated either through another endocrine gland or through a coupled metabolic process. The complexity of the situation is seen from a consideration of the phenomenon of growth, which in turn is a composite of many metabolic processes. Any one of the latter may be controlled by a hormone which would, therefore, influence growth, albeit indirectly. Since growth involves the formation and deposition of new protein, the hormone in question would thus exert a role in protein metabolism. Numerous illustrations have been given of an indirect action of endocrine products in protein metabolism. It seems likely that since hormones regulate the rates of metabolic reactions, and since protein metabolism is one of the fundamental categories of processes in living cells, probably every hormone in the body may exert either a direct or indirect role in protein metabolism. The more important of these roles have been outlined in the preceding pages.

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Chapter 8

Plasma Proteins and Their Relation to Nutrition

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Plasma proteins have received considerable attention during the past few years, from the point of view of both the therapeutic usefulness of some of their components and their relation to protein nutrition. Methods for the identification and quantitative determination of many of the non-protein substances found in blood have been established. Some of these methods are accurate and have proved of considerable scientific value as well as of clinical significance. A search for the presence of other unknown substances that have been suspected to be present in blood is continuously occupying the interest of investigators. A general discussion of the various constituents of blood lies beyond the scope of this chapter. In view of the various topics selected, the editor felt it incumbent to discuss certain components of plasma that are mainly concerned with protein nutrition and with resistance to infections, with particular reference to albumins and globulins. The number of components that may be identified in the plasma proteins, by either chemical, physiological or immunological means, remains far greater than the number of fractions into which it has been convenient to separate the plasma proteins.[1] Some of these fractions may be worth mentioning in order to show the magnitude of this field. Aside from albumins and globulins, those components that have been chemically characterized and some of their clinical and physiological significance realized are:

Fibrinogen. This protein has been identified and methods for its preparation have been developed. It will be briefly considered along with fibrin, thrombin and prothrombin. Thrombin has been used clinically with fibrinogen for the formation of clots in certain surgical conditions. Fibrin foams have been developed and prepared from fibrinogen and thrombin for use as hemostatics. Fibrin and fibrinogen tubes and plastics have been prepared and are used in surgery. Fibrin films have found their chief use as a covering for burns and as a dura substitute in neurosurgery.

The following components will not be discussed in detail:

Isohemagglutinins. These complicated proteins have been separated in a concentrated preparation for use in blood grouping. According to

Pillemer and his collaborators [2] they consist of a purified euglobulin fraction of plasma and represent approximately 5 per cent of its protein content. Electrophoretically, isohemagglutinins are composed mainly of γ- and β-globulins.

Complement. This fraction consists of a series of components that are of considerable significance in certain immunological phenomena. They are believed to be less stable than antibodies and constitute but a small fraction of plasma proteins.

Lipoproteins. These proteins appear to be associated with α- and β-globulins. According to Cohn and his coworkers [1] approximately two-thirds of the total plasma cholesterol seems to be associated with β-globulins.

Enzymes and Hormones. These proteins are constantly circulating in the blood stream and their presence is of considerable significance physiologically. Their concentrations may vary under certain conditions in normal individuals and more so in certain pathological disorders.

General Characterization of Plasma Proteins

An understanding of the physical and chemical properties of plasma proteins has been of considerable aid in the separation of its components. The γ-globulins occupy a unique position among the several fractions of plasma proteins since they possess the most asymmetrical charge distribution; hence they have strong interactions with other proteins and electrolytes. The albumins, on the other hand, on account of their very symmetric electrical structure, interact weakly with other proteins and electrolytes.[2]

The symmetry or asymmetry of distribution of groups capable of carrying a charge, such as the terminal carboxyl and amino groups of a protein, is of considerable importance for the behavior of a large molecule. If, for example, the groups bearing positive charges are closely spaced together on the surface of the molecule, they will give rise to an electrical field of force capable of acting on other large charged molecules and on smaller electrolytes in the system. If, however, the groups bearing positive and negative charges are evenly spaced, then the proximity of positive to negative charges will tend to cancel their electrostatic field. The distribution of these groups is expressed in terms of an overall *electric moment.*

General Methods for the Separation of the Components of Plasma Proteins

Three general methods have been devised for the separation and fractionation of plasma proteins: (1) Fractional precipitation by inorganic salts such as ammonium sulfate and sodium sulfate; (2) precipitation by organic solvents such as ethyl or methyl alcohol; and (3) electrophoretic separation by the Tiselius apparatus.

Fractionation by Ammonium Sulfate. Fractionation by ammonium sulfate has been most frequently resorted to on account of its simplicity and ease of manipulation. In order to determine the limits in ammonium sulfate

concentration for precipitation of each of the proteins, varying amounts of ammonium sulfate were dialyzed into serum through cellophane membranes. This procedure was first used by Theorell [3] and subsequently by Green, Cohn and Blanchard [4] and others.[5, 6] The principle involved in this method is the extraction of water by salt solution of predetermined concentration with subsequent removal of each fraction and reprecipitation with known amounts of ammonium sulfate and dialysis to remove the inorganic salt.[7] γ-Globulin is precipitated at 0.34 (1.39 *M*) saturated ammonium sulfate. α- and β-Globulins are soluble in 0.4 saturated ammonium sulfate (1.64 *M*) but are insoluble at 0.5 saturated ammonium sulfate (2.05 *M*). The filtrates from 2.05 *M* ammonium sulfate contain the albumins. On increasing the salt concentration to 2.57 *M*, albumins are separated in a crystalline form.[7] The order in which the proteins are precipitated by ammonium sulfate has been noted to be related to the electrophoretic mobilities of the proteins. For further details consult references.[7, 8]

Fractionation by Sodium Sulfate. Howe's clinical method [9] for the determination of albumins and globulins in plasma or in serum consisted in effecting a precipitation of globulins by the addition of a 22 per cent sodium sulfate solution. Further fractionation of globulins by this method was brought about by adding enough sodium sulfate to plasma until a concentration of 13.5 per cent was reached. The precipitate that formed was removed and termed euglobulin. This fraction is believed to be related to γ-globulins. On increasing the concentration of sodium sulfate in the filtrate to 17.4 per cent, another precipitate separated out and was called pseudoglobulin I. The third globulin fraction precipitated at 21.5 per cent concentration of sodium sulfate and was called pseudoglobulin II. These various fractions may correspond to γ-, β- and α-globulins in the order given.

Recently Majoor [10] investigated the solubility of certain of the plasma protein components in various concentrations of sodium sulfate and claimed that complete precipitation of globulin does not occur at 21.5 per cent concentration. His opinion was that a concentration of 26 per cent sodium sulfate was preferable since albumin precipitation did not take place until a concentration of 30.5 per cent was reached. He also remarked that "it is possible to separate the three serum proteins fairly completely by using concentrations of sodium sulfate equal to 190 and 260 gm per liter." Majoor's findings have not as yet been confirmed.

Fractionation by Alcohol. According to Cohn, Luetscher, Oncley, Armstrong and Davis [11] plasma proteins can be separated into four fractions by equilibration through membranes with ethanol-water mixtures of controlled ionic strength and low temperature.

Fraction I. When the concentration of alcohol reaches 15 per cent at a temperature of 0°, fibrinogen separates from the plasma proteins. It is removed by centrifugation in the cold.

Fraction II. The filtrate of fraction I is cooled to −5° and the alcohol concentration is gradually increased to 20 or 25 per cent, whereupon most of the γ-globulin is precipitated.

Fraction III. The alcohol content of the clear alcoholic filtrate of fraction II at −5° is again slowly increased until it is about 40 per cent. A precipitate forms. This largely consists of α- and β-globulins.

Fraction IV. After the removal of the precipitate formed in fraction III the clear 40 per cent alcoholic filtrate contains chiefly plasma albumin. Cohn's procedure in separating the albumin consists of removing the alcohol by evaporation, freezing the residue and drying the solids from the frozen state.

The author investigated the method of separation of albumins from globulins, using methyl alcohol as the precipitating agent. The procedure followed is essentially that of Cohn and coworkers.[11] In 1945, Pillemer and Hutchinson [121] reported essentially the same procedure for the fractionation of serum albumin and globulins at low temperature, using as reagents methanol and acetate buffer.

More recently (1947), Chow [122] investigated a novel method for the determination of human serum albumin. Chow immunized rabbits against electrophoretically pure human albumin and found that the antiserum reacted specifically with its homologous antigen and not with other plasma proteins; the amount of antibody precipitated by a given amount of albumin can be estimated either by the turbidimetric technic or by the Kjeldahl method.

Electrophoresis. Toward the end of the last century Sir William Hardy noticed that when proteins are dissolved in a suitable solvent and an external electric field of force is applied, proteins in acid solutions move in one direction and in alkaline solutions in the opposite direction. This was termed electrophoresis. Tiselius applied this principle and developed what is now known as the Tiselius electrophoretic apparatus by which, under a given electric field of force, a suitable solvent of known viscosity, and a definite pH, two or more different proteins will move at different speeds. The velocity of their motion in a unit electric field is expressed in terms of *electrophoretic mobility.*[1]

The Tiselius method of electrophoresis for fractionation of plasma protein components as described by Luetscher [12] is as follows:

"The plasma is layered in the lower half of a U-tube below buffer solution (against which the plasma has previously been dialyzed). Electrodes are placed in the buffer and direct current applied. The voltage and current are adjusted so as not to produce appreciable warming of the solution and consequent convection currents. This disturbance is minimized by running the apparatus in a bath at 1° C., a temperature just below the point of maximum density of the solutions used. Under the influence of the electric field the protein ions move toward the anode if their charge is negative and toward the cathode if their charge is positive. The speed of migration is

largely dependent on the charge of the protein ion under conditions of constant pH and salt concentration. The various fractions, combined with different amounts of base or acid at a given pH and salt concentration, migrate at different rates and gradually separate one from another, the original protein-buffer boundary breaking up into a number of boundaries representing fractions moving at various speeds.

"The optical system utilizes the fact that at each 'boundary,' where there is a change of protein concentration, there is a corresponding change of refractive index. This change produces a downward deflection of the beam of light passing through the boundary, and the deflection can be measured by the movable Schlieren diaphragm. The degree of deflection at this point is proportional to the gradient of protein concentration; in other words, to dC/dS, where C is protein concentration and S is distance along the U-tube. Now, if deflection is plotted against position along the U-tube, a peak of deflection appears at each boundary, deflection being slight between boundaries. The area under each peak,

$$\int \frac{dC}{dS}\, dS$$

is proportional to the total change of protein concentration at that boundary and hence to the concentration of the fraction which that boundary represents. Automatic cameras for recording such diagrams have been developed by Philpot,[13] Svensson,[14] and Longsworth.[15]

"It is possible to study plasma under various conditions of pH and salt concentration as well as at any dilution. In order to retain satisfactory amounts of fractions of low relative concentration in plasma and urine, it was found necessary to use only slightly diluted plasma. In these studies normal plasma was diluted with not more than 50 per cent of its volume of buffer before dialysis, and plasma with lower protein concentration was dialyzed directly against buffer without previous dilution. In order to minimize boundary disturbances at these high protein concentrations, an ionic strength of 0.20 in phosphate buffers [16, 17] was employed. The pH was maintained at 7.8. One well-known disturbance during electrophoresis is produced by concentration gradients of buffer and leads to the so-called δ-boundary.[18] This effect was minimized as a source of confusion by the use of high ionic strength. Although increasing salt concentration diminishes the mobility [19] of most proteins, it causes a relative increase in migration rate of the γ-fraction; and under the conditions used the γ-boundary is largely separated from the δ-effect. Under these conditions, electrophoresis which was essentially free from disturbance could be attained as well as a satisfactory rate of migration."

Distribution of Protein in Plasma and in Serum

Values for plasma proteins are invariably higher than those of sera by about 0.4 per cent.[20] The plasma protein content of blood of normal adults varies not only with respect to individuals but also with respect to the time

of removal of samples and the circumstances. Thus the albumin content of plasma is subject not only to such fluctuations but to the method employed for its estimation. Goettsch and Kendall [21] found that errors were caused by the adsorption of albumin by filter paper. Robinson, Price and Hogden,[22] in studying the determination of albumin and globulin fractions by the Howe procedure,[9] reported that a variable amount of albumin is adsorbed by the filter paper and that the amount adsorbed is dependent to a large extent on the type and size of filter paper used. It is therefore obvious that an error in the quantitative determination of plasma or serum albumin causes an error in the albumin-globulin ratio. Howe's method has also been modified by Kingsley.[23] Taking the necessary precautions, Gutman *et al.*[24] reported values ranging from 6.5 to 7.9 grams of protein per 100 cc of serum and the albumin content of such sera to vary between 4.7 and 5.7 grams and the globulin fractions between 1.3 and 2.5 grams per 100 cc. Luetscher [12] gave an average of 6.50 grams for total proteins, 4.06 grams for albumin and 2.44 grams for globulin per 100 cc of plasma and 63/37 albumin-globulin ratio by Tiselius' and 62/38 by Howe's method. The data presented in Table 8–1 are those reported by Cohn and his associates [25] and by Luetscher.[12] Perera and Berliner's estimates [26] are of considerable interest. They are 6.2 to 7.3 grams of protein per 100 cc of normal sera of ambulatory adults and 5.4 to 7.0 grams per 100 cc of sera for the same individuals recumbent.

Table 8–1. Distribution of Components of Human Plasma Proteins

	Amounts per 100 cc of Plasma (gm)	
	Cohn et al.[25]	*Luetscher* [12]
Proteins	6.58	6.5
Fibrinogen	0.31	0.37
Albumin	3.26	4.06
α-Globulin	1.01	0.46
β-Glubulin	1.26	0.86
γ-Glubulin	0.74	0.75
Total glubulins *	3.01	2.07
Total albumin and globulins *	6.27	6.13
A/G ratio *	52/48	66/34 †

* Fibrinogen not included.

† According to Luetscher [12] the A/G ratio is 63/37. The editor noted that in computing this ratio Luetscher included the estimate for fibrinogen in the total globulin component. In the A/G ratio of 66/34 fibrinogen was excluded.

Tiselius [27, 28] fractionated electrophoretically the components of horse serum and studied among other things albumin and α-, β- and γ-globulins. Subsequently Stenhagen [29] applied the electrophoretic technic to human

serum and noted the presence of corresponding fractions to those observed by Tiselius. Blix [30] investigated human serum globulins and reported that α- and β-globulins were above normal in individuals with pneumonia during the acute phase. MacInnes and Longsworth [31] and Longsworth, Shedlovsky and MacInnes [32] investigated plasma and urinary proteins in nephrosis and found considerable similarity in their respective components despite great variations in serum albumin and globulin; *e.g.*, α-globulin was elevated in febrile conditions while in myeloma, nephrosis, and obstructive jaundice the β-globulin was increased. According to Luetscher [12] the most striking change in the nephrotic syndrome cases is the great loss of plasma albumin. Whereas the A/G ratios as determined by Tiselius and by Howe showed close agreement in blood plasma of normal individuals, considerable variation of A/G ratios was noted in nephrotic cases. In terminal glomerulonephritis cases it was reported [12] that these patients were in a state of uremia. Plasma albumin was somewhat decreased but the β-globulin was slightly higher than was usually encountered in the normal case.

The plasma proteins of only one patient with amyloid disease was studied.[12] This patient had chronic tuberculosis of the lymphatic system preceding the development of amyloidosis. Plasma albumin was greatly reduced. Globulin in general and γ-globulin in particular were considerably increased. In cirrhosis there was a diminution of plasma albumin and α-globulin.[12]

In non-hemorrhagic degenerative Bright's disease the serum protein is markedly decreased and, according to numerous investigators,[33-38] this is almost entirely due to a deficit of serum albumin. In arteriosclerotic Bright's disease the serum proteins are generally within normal limits.[39] In hepatic disease, Butt, Snell and Keys [40] state that there is no constant relationship between the serum protein or serum colloidal osmotic pressure and the presence or absence of edema.

Fibrinogen

The transformation of a solution of the protein fibrinogen into the rigid insoluble fibrin clot is an important fundamental feature of blood plasma. This reaction is normally brought about by the action of thrombin on fibrinogen. The amount of thrombin in normal plasma is indeed insignificant; however, it is readily derived from its precursor, prothrombin, in the presence of calcium ions and of certain other complex proteins termed thromboplastins. This in brief summarizes the formation of clot.

According to Edsall and coworkers [41] the fibrinogen content of uncitrated blood plasma is approximately 0.28 per cent. These investigators have established this estimate by analyzing numerous samples of citrated human plasma by electrophoresis and by converting fibrinogen to fibrin and determining the amount of protein nitrogen in the washed fibrin clot. A correction has been made for the amount of citrate used.

The fibrinogen molecule has been reported to be larger and more elongated than any other plasma protein. It is asymmetric. Its molecular weight is about 500,000 and its molecular length is 900 Å.[41] The nature of the transformation of fibrinogen to fibrin as brought about by the action of thrombin is still obscure. Brand, Kassell and Saidel[42, 42a] have reported on the chemical composition of human fibrinogen and fibrin and bovine fibrin. Their results are found in Table 8–2.

Table 8–2. Composition of Human Fibrinogen,* Human Fibrin,† and Bovine Fibrin ‡ [42,42a]

	Human Fibrinogen (%)	*Human Fibrin* (%)	*Bovine Fibrin* (%)
Total nitrogen	16.9 *	16.9	17.0 ‡
Arginine	7.9	7.9	8.1
Histidine	2.8	2.8	2.6
Total protein sulfur	1.26	1.23	1.26
Cysteine	0.41	0.60	0.52
Half-cystine	2.32	1.81	2.02
Methionine	2.52	2.62	2.73
Tryptophane	3.29	3.22	3.37
Tyrosine	5.75	5.75	5.70
Serine	8.3	9.8	7.5
Threonine	6.6	6.5	6.8
Leucine	7.1	7.1	7.5
Glycine	5.6		5.70
Aspartic acid	13.6	13.6	13.0
Glutamic acid	14.3	14.1	14.13

* The preparation of human fibrinogen (No. 81 RI) contained about 47 per cent of protein and 53 per cent of salts; the protein was 87 per cent clottable. The values reported are calculated on the assumption that the content of total nitrogen is the same in fibrinogen as in fibrin (16.9 per cent).

† The fibrin (preparation 65A) used for analysis was obtained from a lot of fibrinogen which clotted spontaneously during purification. The clot was extracted with water until free from salts, dehydrated with alcohol, and dried at the laboratory of the Connecticut Agricultural Experiment Station. The nitrogen determination was made by Dr. Jane K. Winternitz of that laboratory.

‡ The bovine fibrin was a commercial preparation. The values given in this table are based on a total nitrogen content of 17.0.

Site of Formation of Fibrinogen. The results of numerous investigations dealing with the effect of hepatic poisons such as chloroform and phos-

phorus indicate that a decrease in blood fibrinogen accompanies liver injury.[43, 48] On the other hand, injury to other tissues not involving the liver gives rise to blood fibrinogen.[49]

In their studies of hepatectomized rabbits, Drury and McMaster [50] reported a 35 to 65 per cent decrease in blood fibrinogen, and Jones and Smith [51] a drop of 16 to 47 per cent below the control level. These losses in blood fibrinogen occurred within 13 to 20 hours after the extirpation of the liver. Inasmuch as hepatectomy is fatal to the experimental animal and may be accompanied by considerable loss of blood, a definite conclusion cannot be drawn to the effect that the liver is the only site for the fabrication of fibrinogen; however, we can safely assume that it plays an important role in the synthesis of this protein.

Prothrombin. Mellanby [52] obtained the first prothrombin preparation of high potency by precipitating it at pH 5.3 from diluted plasma. Seegers [53] employed adsorption methods and obtained a more active preparation of prothrombin. His analysis showed that it was a protein containing about 4 per cent carbohydrate. Solutions of prothrombin are unstable and lose their potency particularly at pH 4.8 and at an alkaline pH of 9 or 10.[53] Prothrombin is believed to be a euglobulin.

Thrombin. In the presence of calcium ions or with thromboplastin, prothrombin is converted into thrombin. Seegers [53–55] and Milstone [56] obtained very active preparations. Subsequently Parfentjev [57] prepared an active thrombin by fractional precipitation of rabbit's plasma with ammonium sulfate. Thrombin is considered to be a euglobulin. It is almost insoluble in water at its isoelectric point but soluble in dilute salt solutions. Whereas crude prothrombin is insoluble, thrombin is soluble at 0.45 saturated ammonium sulfate. Fairly stable dry powder preparations of thrombin have been prepared by dehydration of suitable solutions from the frozen state.

Albumin

Human plasma albumin has for the past few years been the subject of considerable interest to the layman as well as to the investigator because of (1) its usefulness in the treatment of shock, (2) its importance as a primary constituent of blood plasma, (3) its clinical importance in certain pathological diseases, and (4) its pivotal position in malnutrition and the manner in which it is affected by experimental diets. Methods [7, 8] for its isolation, purification and crystallization have been outlined earlier in this chapter. The pure albumin preparations have been subjected to rigid physical, chemical and biological tests. Attempts have also been made to gain an insight into its chemical composition. The effects of low and high nitrogen intake either as proteins or as amino-acid mixtures have been investigated in the experimental animal and in man with particular reference to plasma albumin.

Properties. Electrophoretic analyses have shown that of the six readily separable components of plasma, albumin has the most rapid electrophoretic mobility.[1] It is easily distinguished from all but the fast-moving α-globulin. It has a molecular weight of about 70,000 and on account of its very symmetric electrical structure it interacts weakly with other proteins and electrolytes.[1]

There is evidence in the literature that in horse serum there exist carbohydrate-free [5] and carbohydrate-containing fractions of albumin.[8, 58] Both of these fractions have been obtained in crystalline form. For detailed methods of fractionation and crystallization the reader is referred to the papers of McMeekin,[8] Sørensen and Haugaard,[59] Hewitt,[60] and Ferry and Oncley.[61]

According to McMeekin [8] "the carbohydrate-containing crystalline albumin is readily distinguished from the carbohydrate-free albumin even by difference in appearance. The carbohydrate-containing albumin crystals were perfectly symmetrical hexagonal disks, while the carbohydrate-free albumin crystals are rod-shaped.

"The carbohydrate-containing albumin is easily recrystallized by adding ammonium sulfate to the point of turbidity and allowing the solution to stand but the best method for recrystallization, appears to be to place the protein solution inside of a cellophane membrane and rotate it in a 2.0 *M* solution of ammonium sulfate." Solutions of the carbohydrate-containing albumin are not coagulated by heating in the presence of neutral salt or buffer at pH 4.8. However, they are precipitated by 2 per cent trichloroacetic acid.

Analytical data [8] showing the difference in nitrogen content and optical rotation are as follows:

	Albumin	
	Carbohydrate-containing component	*Carbohydrate-free component*
Nitrogen	15.1%	16.8%
Optical Rotation α_D^{20}	− 47°	− 57°

Both crystalline components were shown to be electrophoretically and ultracentrifugally homogeneous.

Chemical Composition of Albumins. Although complete data on the chemical composition and amino acid content of albumins are not as yet available, Brand, Kassell, and Saidel [42, 42a] have recently published incomplete analyses of serum albumin of man, cattle and horse. Their data are found in Table 8–3.

Table 8–3. Composition of Serum Albumin of Man, Cow and Horse [42,42a]

Amino Acids	*Albumin (%)*		
	Human *	*Bovine*	*Horse B*
Glycine	1.6	1.9	
Valine	7.7	6.5	
Leucine	11.9	13.7	10.1
Isoleucine	1.7	2.9	
Proline	5.1	5.6	
Phenylalanine	7.8	6.2	
Cysteine	0.70	1.11	1.13
Half-Cystine	5.58	5.41	5.23
Methionine	1.28	0.81	0
Tryptophane	0.19	0.58	0.30
Arginine	6.15	6.2	5.50
Histidine	3.5	3.8	4.31
Lysine	12.3	12.4	12.7
Aspartic Acid	10.4	10.6	
Glutamic Acid	17.4	16.9	
Amide NH_3	1.07	1.05	0.90
Serine	3.7	4.5	4.8
Threonine	5.0	6.5	5.97
Tyrosine	4.66	5.49	4.66

* Prepared from blood collected by the American Red Cross by the Department of Physical Chemistry, Harvard Medical School, Boston, Mass., under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

Site of Origin of Albumin. According to Madden and Whipple [49] the evidence pointing to the liver as the site of albumin and globulin formation is both clinical and experimental. Kerr, Hurwitz and Whipple [62] noted a definite lag in the regeneration of serum protein of Eck fistula dogs following acute plasma depletion. Knutti, Erickson, Madden, Rekers and Whipple [63] reported observations over a two-year period on an Eck fistula dog and showed that this dog was unable to form new plasma proteins in significant amounts on various standard diets as compared with normal dogs and at autopsy there was the usual atrophic liver.

Hepatectomized experiments have not enlightened our knowledge of the site of origin of albumin synthesis. Partial hepatectomy in rats, according to Chanutin, Hortenstine, Cole and Ludewig [64] resulted in a depression of plasma proteins within 24 hours. Warner, Brinkhous and Smith [65] reported that chloroform injury or partial hepatectomy caused a marked decrease in the plasma prothrombin.

The clinical observation made by Thompson, McQuarrie and Bell [66] on a child with edema and hypoproteinemia indicated liver atrophy with disappearance of cells in intermediate and peripheral zones in the lobule.

The other tissues were normal. In cases of cirrhosis of the liver, plasma albumin is usually decreased although total serum proteins may be within the normal range.[12] Autopsies of human cases of aplastic anemia revealed almost complete absence of all bone marrow elements,[67] yet in this disease plasma proteins were within the normal range. This is indicative that bone marrow cells are not concerned with plasma protein synthesis.

The influence of diets on hypoproteinemia and plasma protein regeneration is discussed in Chapter 9 on Protein Deficiency.

Albumin in Shock. An understanding of the pathologic physiology resulting from acute blood loss is of great significance in the management of certain clinical conditions. In the past physicians have resorted to the administration of whole blood following acute hemorrhage. In the light of our present knowledge greater emphasis has been placed on the restoration of plasma volume and total circulating plasma proteins to reestablish the hemodynamic equilibrium of the blood with respect to peripheral circulation so that the hemoglobin remaining can carry out its function of transporting oxygen to the tissues.

Numerous investigators [68–77] have shown both in the experimental animal and in man that following a single non-fatal hemorrhage there is a prompt, but gradual, inflow of fluid and protein in the circulation. The initial increase in plasma volume is apparently due to the addition of fluid relatively low in protein. This condition may exist for several hours to as much as three days. Observations on plasma proteins indicate an initial increase after hemorrhage followed by a gradual decrease of albumin which may last for several days. There seems to be considerable evidence pointing to the view that there is a continuous exchange between plasma proteins and tissue protein stores.

In experimental shock Ebert *et al.*[73] and Weston and his coworkers [78] showed the existence of a deficiency in the ability of the organism to dilute the blood and to add plasma protein to the circulation. This was substantiated by a rise of the hematocrit index. Fine, Seligman and Mark [79] and Fine and Seligman [80] attributed the progressive decline of plasma volume in hemorrhagic shock to a fall in the volume of actively circulating plasma associated with peripheral stagnation.

The intravenous administration of physiologic saline solution has been found to be ineffective in patients with surgical shock and profuse hemorrhage.[70]

Whether or not the administration of albumin in the treatment of hemorrhagic shock in man is more effective than either plasma or whole blood is a debatable question. As a war measure human plasma albumin was produced and used. Its continued application as a therapeutic agent may be considerably curtailed chiefly for economic reasons.

The use of albumin in shock was brought about through the efforts of Cohn and his associates who have not only investigated the isolation of

pure preparations of albumins but have meticulously studied the physical and chemical properties of blood proteins and enhanced our knowledge of their chemistry.

Janeway and coworkers [81] stated that properties other than the colloidal osmotic pressure of albumin had much to do with its therapeutic usefulness such as its stability and its solubility in water and in solutions of various crystalloids. Serum albumin has at least two known functions. It maintains the colloidal osmotic pressure of blood and plays a role in the nutrition of the tissues. Thus, in the treatment of shock due to considerable loss of blood, the use of such an agent is indeed valuable in the restoration of blood volume.

According to Scatchard, Batchelder and Brown [20] the corresponding osmotic pressure of a solution containing 6 grams of plasma protein is 20 mm of mercury at 25° and at pH 7.4. Therefore, if c is 6 grams of protein per cent, $1/c$ corresponds to 16.7 cc per gram. The colloidal osmotic pressure of albumin was studied by these investigators and they reported that at 25° and at pH 7.4 the volume of fluid held in the blood stream by each gram of albumin was about 18 cc but that it should vary with the protein concentration of the plasma. In their estimate each gram of albumin was equivalent to 1.2 grams of plasma proteins or 20 cc of the Red Cross citrated plasma.

Janeway and coworkers [81] investigated the administration of concentrated human albumin in shock and noted prompt hemodilution and clinical improvement. They showed that 100 cc of 25 per cent solution of albumin was equivalent to 500 cc of citrated plasma. Their experimental data indicated that concentrated albumin was not harmful in cases of shock with severe dehydration but was more effective if water and salt were also administered by any available route.

The effectiveness of concentrated albumin was to a limited extent also extended to a small group of hypoproteinemic patients. Their results [81] showed that very large amounts of albumin were needed to raise serum albumin of patients with chronic protein depletion. There were, however, two important limiting factors concerning the injection of large amounts of albumin: (1) the capacity of the circulation to adjust itself to rapid increases in plasma volume and (2) the rate at which the tissues can metabolize or store excess albumin.

The effect of parenteral injections of albumin was also studied in cases of cirrhosis of the liver.[81] There was no marked improvement noted. In nephrotic patients, the injected albumin was largely excreted in the urine.[81]

Warren and coworkers [82] injected concentrated serum albumin into six patients with circulatory failure associated with a decrease in blood volume. According to these investigators there was distinct clinical improvement in each case without any evidence of undesirable side effects. The increase in plasma volume was commensurate with the predicted osmotic effect of

the albumin. The arterial pressure of three patients was restored to approximately normal levels following the injection of adequate amounts of albumin. An increase in plasma volume and cardiac output was also noted.

Cournand and his collaborators [83] compared the administration of albumin with that of whole blood transfusion in the treatment of shock in man and reported that albumin brought about a relatively larger cardiac output. In cases of shock due to skeletal trauma or to hemorrhage, the increased cardiac output was interpreted to be a compensatory effect. Following albumin therapy these investigators noted the presence of acute anemia in many of their cases and suggested that whole blood should subsequently be administered.

Mylon, Winternitz and de Sütö-Nagy [84] investigated the use of dog plasma albumin in experimental shock and reported that intravenous infusion of dog plasma albumin of a colloid-osmotic pressure equivalent to at least that of the "total plasma" further prolongs the survival period but does not influence recovery. They stated further that the therapeutic value of plasma was not fully explained by its colloidal osmotic pressure.

Globulins

Cohn and his coworkers [25] have recently reported the results of their investigation on the distribution of the various components of human plasma proteins. Their data show an estimate of 1.01 grams for α-globulin, 1.26 grams for β-globulin and 0.74 gram for γ-globulin per 100 cc of plasma. On the basis of these figures, α-globulin represents 15.3 per cent, β-globulin, 19.2 per cent and γ-globulin, 11.2 per cent of the total plasma proteins of 6.58 grams per 100 cc.

α-Globulin. The α-globulin component is considered a pseudoglobulin.[7] Electrophoretically it has a higher mobility than either the β- or γ-globulins; hence it requires a higher concentration of either salts or alcohol for its precipitation. It has not as yet been obtained in a pure crystalline state.

β-Globulin. The β-globulin also appears to be pseudoglobulin in nature.[7] Electrophoretically it has a slower mobility than α-globulin. Owing to the closeness of its sedimentation constant to that of β-globulin it has not been possible to effect a separation of these two components by ultracentrifugal analysis.[7] For its separation from the other components of plasma protein it is first precipitated along with α-globulin at 1.7 *M* ammonium sulfate. Then by careful dialysis, adjustment of pH and fractionation by precipitation with ammonium sulfate, the β-globulin fraction is separated from the α-globulin fraction.[7]

γ-Globulin. This component of plasma protein has been given considerable attention by most investigators in this field, particularly for its intimate relationship to antibodies. It has been possible to prepare it in a high state of purity and such preparations have been shown to be electrophoretically and ultracentrifugally homogeneous.[1, 25] On the basis of its sedimentation

and diffusion constants and a partial specific volume of 0.730 its molecular weight has been estimated at 142,000.[7]

The separation of γ-globulin from the other components of plasma protein has been effected by precipitation at 0.34 saturated ammonium sulfate or by the addition of about 25 per cent ethanol to plasma at −5°.

Chemical Composition of Globulins. Calvery [85] has analyzed serum globulins and accounted for thirteen amino acids. His data are presented in Table 8–4. Brand, Kassell and Saidel [42a] have more recently reported an incomplete but interesting analysis of human γ-globulin (see Table 8–5).

Table 8–4. Estimation of Certain Amino Acids of Serum Globulin *
(According to Calvery [85])

Amino Acids	*Per Cent*
Glycine	3.5
Alanine	2.2
Leucine and Isoleucine	18.7
Histidine	0.9
Arginine	5.2
Cystine	1.0
Proline	2.8
Phenylalanine	3.8
Tyrosine	6.7
Tyrptophane	2.3
Glutamic acid	8.2
Aspartic acid	2.5
Lysine	6.2

* The nature of the serum globulin was not indicated.

Smith, Brown and Gross [86] determined the nitrogen content of pseudoglobulin of type 1 pneumococcus antibody and purified diphtheria antiserum and reported no significant difference. Hewitt [87] investigated the amide nitrogen and the cysteine, tyrosine and tryptophane content of horse serum globulin fractions and toxin-antitoxin floccules. His comparative data showed close agreement in percentages. Calvery [88] compared the amide nitrogen and arginine, histidine, cystine and lysine of normal horse serum globulin and of purified type 1 pneumococcus antibody and also was able to show close agreement in his results.

At present, information on the chemical composition of γ-globulin and antibodies is indeed meager and does not permit the formulation of any conclusion with certainty, particularly with respect to the identity of the respective molecules of globulins and of antibodies. In fact, we have no available methods capable of showing the true chemical structure of the simplest protein nor do we know the exact pattern of amino acids in the molecule as a whole.

Table 8–5. Composition and Estimation of Certain Amino Acids of Human γ-Globulin (After E. Brand)

Amino Acids	*Per Cent*	*Number of Residues (mole/mole)* *
Glycine	4.2	87
Valine	9.7	129
Leucine	9.3	111
Isoleucine	2.7	32
Proline	8.1	110
Phenylalanine	4.6	44
Cysteine	0.70	9
Half-Cystine	2.37	30
Methionine	1.06	11
Tryptophane	2.86	22
Arginine	4.80	43
Histidine	2.50	25
Lysine	8.1	86
Aspartic Acid	8.8	103
Glutamic Acid	11.8	
Amide NH_3	1.35	
Serine	11.4	169
Threonine	8.4	110
Tyrosine	6.75	58

* Corrected to the nearest integers.

Relation of Globulins to Antibodies. "One of the most astounding attributes of living things is the unerring accuracy with which specific tissue components are manufactured out of the variable mixture of amino acids circulating in the blood." [89] Thus the cell of every organ in the animal system synthesizes its own characteristic protein according to its own pattern. Given their nutrition requirements, bacteria do likewise and, as is shown in Chapter 14, certain groups produce toxins that possess remarkable specificity. When these antigens are injected into either man or animal they cause definite toxic symptoms and specific pathological changes.

There is considerable evidence in the literature to show that certain antibodies are specifically modified plasma globulins. Furthermore, the predominant trend is that γ-globulin is more intimately associated with immune bodies than any of the other globulin fractions. Enders [90] has demonstrated the presence of a large number of specific antibodies for different infectious organisms in human plasma globulin, and it is his opinion that antibody is usually but not exclusively associated with γ-globulin. Tiselius and Kabat [91] showed by electrophoretic analysis that most of the antibodies they investigated occur in the γ-globulin fraction of plasma. Tiselius [92] demonstrated by the same method that the antibody of egg

albumin serum from rabbit migrated with the γ-globulin fraction only. Immunologists welcomed this method of approach and adopted it in an effort to elucidate the mechanism of toxin-antitoxin reaction (see Chapter 14). Jameson and coworkers [93] found that γ-globulin was lacking from the serum of the new-born calf but became demonstrable in the Tiselius apparatus 18 to 24 hours after the injection of colostrum which presumably contained antibody of the colon bacillus. Tiselius and Kabat [91] showed that "rabbit and monkey antibody (pneumococcus) and rabbit anti-egg albumin antibody were contained in the γ-fraction, that removal of the antibody with antigen produced a decrease in the γ-globulin and that this decrease corresponded quantitatively to the amount of antibody removed."

In man the concentrations of total serum globulin and of the γ-fractions characterized by hypoproteinemia show an increase following infections. Cohn [94] considers the γ-globulin of human plasma as the most important fraction from the point of view of its relation to public health.

Origin of Antibodies. The site of origin of antibodies has always been of considerable interest to immunologists. Three theories have been advanced: (1) In 1939 Sabin [95] suggested that serum globulin and antibodies originate from the shedding of surface films of cytoplasmic material from monocytes or macrophages. She observed that following injection of a dye-protein into rabbits there occurred cytoplasmic changes concomitant with the appearance of antibodies. Her conclusion was that the cells of the reticulo-endothelial system produced globulin which in turn was converted into antibody globulin under the influence of an antigen. (2) Rich [96] postulated the theory that antibodies are formed not only from macrophages but also from lymphocytes. He demonstrated experimentally marked lymphoid proliferation in the spleen and regional lymph nodes associated with an increase in antibody titer following the injection of a foreign protein. Enrich and Harris [97] confirmed Rich's findings and concluded that lymphocytes were responsible for the synthesis of antibodies after microphages or macrophages had been conditioned by the antigens. (3) Dougherty, Chase and White [98] developed a different point of view regarding the site of origin of antibody globulin. These investigators are of the opinion that lymphocytes may be regarded as a storehouse of antibody, or preferably antibody carriers, and that the rate of release of antibody protein is controlled by adrenal cortical secretion.[99]

Globulin Metabolism. It has been repeatedly observed that in malnutrition, in experimental hypoproteinemia, and in diseases causing hypoproteinemia the bodily resistance to infections is considerably lowered. Plasma protein is constantly fluctuating and is said to be in dynamic equilibrium with cellular protein.[95] This concept is in accord with Schoenheimer [100] and Borsook and Dubnoff.[101] Madden and Whipple [49] state that synthesis of plasma proteins is accomplished by mechanisms of protein metabolism and must depend on the formation of complex polypeptides

from dietary amino acids. According to these authors, the synthesis of both the albumin and globulin fractions of plasma is greatly influenced by a varied assortment of dietary amino acids. In their experiments on dogs they found that the inclusion of certain proteins such as beef serum in the diet caused greater regeneration of albumin than of globulin, whereas the feeding of another protein (casein) yielded more globulin than albumin. On the other hand, Melnick, Cowgill and Burack [102] investigated serum protein, lactalbumin and casein and reported that they were approximately equal with respect to their value in meeting the requirements of nitrogen equilibrium and were of comparable effectiveness in promoting regeneration of serum protein. In the opinion of the author, plasma protein regeneration (including albumin and globulin) is dependent to a large extent on adequate intake of complete proteins containing the indispensable amino acids. In this connection Cannon [103] refers to experiments [104] which demonstrate that "the feeding of the ten 'growth' amino acids (Rose) to hypoproteinemic dogs, in adequate amounts, engenders satisfactory rates of plasma protein regeneration, including both plasma albumin and plasma globulin." It is to be noted that the emphasis is on "adequate amounts" of "growth" amino acids. There are undoubtedly chemical differences in the composition of complete proteins such as lactalbumin, casein and serum proteins. Furthermore, the exact content of indispensable amino acids of each of these proteins is not fully established, nor do we know the minimum requirement of each of these amino acids for plasma protein regeneration. Little is known of the mechanism of globulin synthesis *in vivo* or of the site of its fabrication. For example, could globulin be directly synthesized from the absorbed amino acids or must these amino acids first be converted into some cellular protein which in turn would act as a precursor to globulin? Irrespective of how globulins or antibodies are formed in the animal system, there is strong evidence that their regeneration is largely dependent on the uptake of dietary nitrogen.

Schoenheimer *et al.*[105, 106] fed isotopic amino acids to actively immune rats and rabbits. They found by this novel technic that fibrinogen, euglobulin, pseudoglobulin and albumin and antibodies participated in metabolic reactions involving the uptake of dietary nitrogen. Also from their studies with isotopic amino acids it was learned that the half life of an antibody molecule of actively immune animals was approximately two weeks. In contrast, metabolic interchanges which appear to be a part of the normal process of globulin metabolism did not occur with passively injected antibody into rabbits.[105, 106]

Protein Deficiency and Infections. There is indeed a paucity of literature on the relationship of protein deficiency to reduced resistance to bacterial infections. In 1920 Kohman [107] investigated the relationship of protein deficiency to edema and reported that pneumococcus infection was the cause of the death of her experimental animals fed a low protein-carrot

diet. Madden and Whipple [49] repeatedly observed in their hypoproteinemic dogs marked lowering of resistance to infections. Meyer [108] found that the incidence of death following the parenteral administration of diphtherial toxin was four times greater in the poorly nourished than in the well-fed rat. Watson [109] and Robertson and Doyle [110] showed that rats kept on a diet rich in dried milk or casein were considerably more resistant to typhoid and enteritis than those fed on wheat gluten or soybean flour. Sako [111] investigated the effect of multiple lethal doses of virulent pneumococci and reported that "animals which had been maintained on a very low protein intake showed a greatly decreased post-inoculation time as compared with control animals."

While the above observations are interesting, the evidence is more circumstantial than conclusive. The recent methodical research of Cannon, and Cannon *et al.*[93, 112–119] has indeed been revealing. His investigations in experimental hypoproteinemia are pertinent to the biological evaluation of proteins, the synthesis of globulin and of agglutinins, precipitins and hemolysins, the lowering of resistance to infections, and protein repletion and regeneration of globulin and antibodies.

Cannon's method for estimating the biological efficiency of proteins of different origins is discussed elsewhere in this book. However, while investigating this problem he observed that inadequate protein intake caused a marked depletion of the so-called "protein reserves" in adult rabbits and in white rats and a rapid loss of weight, whereas in young rabbits there was no loss in weight but there was marked hypoproteinemia. In such animals he studied the formation of agglutinins by the subcutaneous administration of a formaldehyde-killed suspension of typhoid bacilli and compared the agglutinin titers with those of sera of the control well-fed animals.[114]

Cannon's data, presented in Figures 8–1, 8–2, 8–3 and 8–4, are indeed significant and self-explanatory. It is thus evident that in the hypoproteinemic rabbit during the period of active growth there is markedly lowered activity of the antibody-producing mechanism. Protein deficiency in rats and the fabrication of antibodies were also investigated. Again the protein-deficient rat showed a decreased resistance to infection and a partial inhibition of antibody formation. In this connection it is noteworthy that more vigorous measures were taken to supplement the diet with the necessary vitamin intake. Cannon has shown that animals kept on protein-deficient diets supplemented with adequate mineral and vitamin intakes develop hypoproteinemia accompanied by (1) hypoalbuminemia, (2) hypoglobulinemia, and (3) a lowered resistance to infection caused by the inability of blood plasma to fabricate antibodies.

Subsequent to these investigations, Cannon fed high quality proteins to his hypoproteinemic animals and after a seven-day period administered antigens. The response was indeed startling as the output of specific antibodies paralleled that of the control animal. The increase in toxin-antitoxin

titer was concomitant with serum protein regeneration and in particular with globulin synthesis.

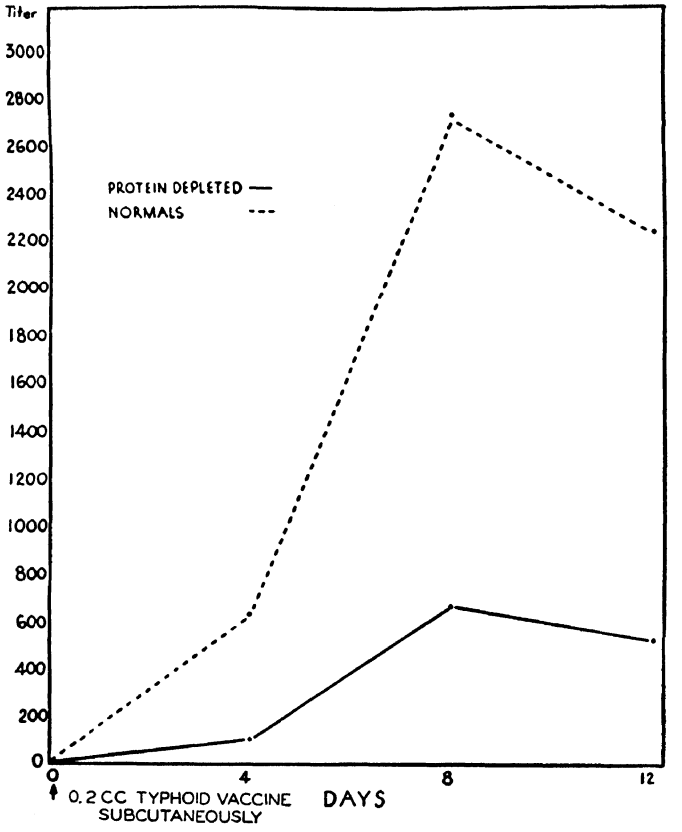

FIGURE 8–1. Average agglutinin titers of sera from young normal and protein-depleted rabbits after immunization with Eberthella typhosa. (*Courtesy of the authors* [114] *and the Journal of Immunology.*)

In conclusion, one must bear in mind that in malnutrition as well as in experimental hypoproteinemia there is lowering of hemoglobin and a decrease in both red and white cell counts. The phagocytic cells of the liver, spleen, lymph nodes, lymphoid tissues and bone marrow are also impaired. In prolonged malnutrition these cellular reserves may atrophy. According to Jackson [120] the immediate cause of death following starvation is frequently an infectious complication.

For the maintenance of our well-being and the endowment of the body with proper defense mechanisms, all the essential elements must be incorporated in our daily diet. The primary constituents are proteins, fats and carbohydrates, accessory factors, essential minerals, and water. In our discussion of high- or low-protein intake it is assumed that the remainder of the diet contains an adequate supply of all the other essential ingredients.

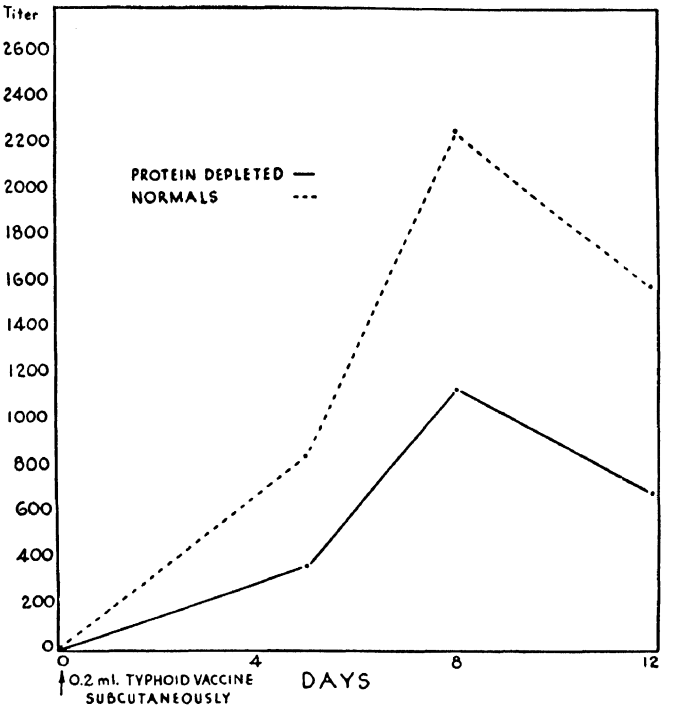

FIGURE 8–2. Average agglutinin titers of sera from young normal and protein-depleted rabbits after immunization with Eberthella typhosa. (*Courtesy of the authors* [114] *and the Journal of Immunology.*)

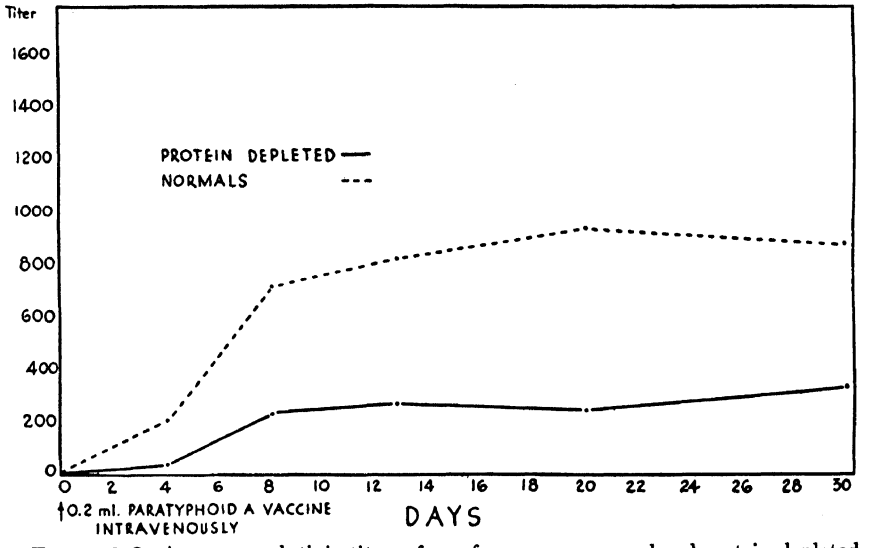

FIGURE 8–3. Average agglutinin titers of sera from young normal and protein-depleted rabbits after immunization with Paratyphoid A. (*Courtesy of the authors* [114] *and the Journal of Immunology.*)

Specific symptoms that follow vitamin or mineral deficiencies are frequently associated with a general metabolic disturbance that also affects protein metabolism. We must therefore take into consideration the state of hypoproteinemia associated with decreased resistance to infections in avitaminosis. The experimental animal deprived of vitamin A or B or C, etc., eats

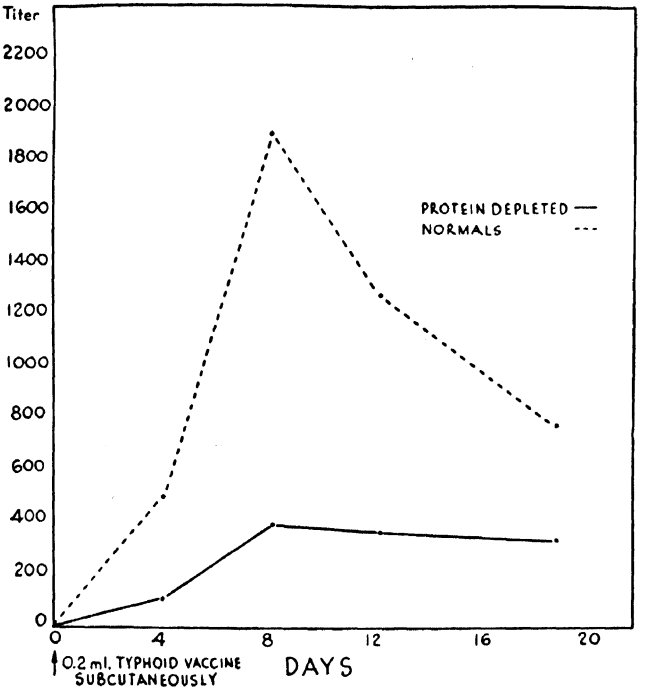

FIGURE 8–4. Average agglutinin titers of sera from adult normal and protein-depleted rabbits after immunization with Eberthella typhosa. (*Courtesy of the authors* [114] *and the Journal of Immunology.*)

his food sparingly; hence protein deficiency. A better knowledge of the pathologic changes following the intake of subminimal requirements of amino acids may lead to a clearer understanding of deficiencies and to the close metabolic relationship that exists among vitamins, minerals and proteins.

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Chapter 9

Protein Deficiency and its Relationship to Nutritional Anemia, Hypoproteinemia, Nutritional Edema, and Resistance to Infection

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Introduction

The animal body contains an infinite variety of specific proteins. The major portion of these is found in the muscular, epidermal, and connective tissues, but a sizable quantity, represented chiefly by hemoglobin and the plasma proteins, is circulated by the cardiovascular system. Quantitatively less imposing, but functionally quite as important, are the widely distributed protein enzymes and protein hormones. In common with numerous other nitrogenous, but non-protein substances, the body proteins are derived ultimately from the supply of building materials made available through the digestion and absorption of the proteins of the diet. Hence, the supply and nature of the dietary proteins require consideration.

Dietary Protein Deficiency

Recognition that native proteins vary widely in amino acid content grew with the development of new analytical technics at the turn of the century. There soon followed the observation that certain of the proteins may fail to support growth, not because they are provided in too small quantity, but because they are qualitatively inadequate in amino acid composition. No attempt will be made here to trace the earlier observations with deficient proteins and with protein hydrolysates rendered deficient by chemical means, or to outline the brilliant series of basic investigations which proved that dietary protein could be replaced with a mixture of purified amino acids and showed clearly just which of these were indispensable for growth in the laboratory rat. For an interesting historical account of these aspects of protein nutrition the reader is referred elsewhere [112] (also see Chapter 1).

Indispensable (or Essential) Amino Acids. As defined by Rose [112] an indispensable dietary component is "one which cannot be synthesized by the animal organism, out of the materials *ordinarily available*, at a speed commensurate with the demands for *normal* growth." Table 9–1 lists the common amino acids according to their conformity with this definition of

Table 9–1. Amino Acids Classified According to Their Effect Upon Growth in the Rat

Indispensable	*Dispensable*
Tryptophane	Glycine
Lysine	Alanine
Methionine	Cystine
Threonine	Serine
Phenylalanine	Tyrosine
Leucine	Norleucine
Isoleucine	Aspartic Acid
Valine	Glutamic Acid
Histidine	Hydroxyglutamic Acid
Arginine *	Proline
	Hydroxyproline

* Arginine can be synthesized by the rat, but not rapidly enough to meet the demands for maximal increase in weight.

indispensability in the rat. The non-essential character of each of the dispensable amino acids listed, except glutamic acid, proline, and hydroxyproline, has also been definitely established.[114] However, there may exist in proteins an as yet "unidentified substance which, like arginine, is not necessary for fairly rapid growth, but is required for maximum increases in weight"; unlike arginine, however, the unidentified constituent is destroyed by complete acid hydrolysis of the protein.[157] Possibly this may account for the rather common observation that rats and mice fed amino acid mixtures or completely hydrolyzed proteins seldom grow quite as rapidly as those fed native proteins. Renewed interest in this phenomenon was injected by the recent observations of Woolley [135] that hemolytic streptococci require a growth factor which is present in proteins, but destroyed upon complete acid hydrolysis; and that when a concentrate of the growth factor is prepared from tryptic digests of casein [158] and added to the diets of mice fed acid hydrolyzed casein fortified with tryptophane it also accelerates their growth. Woolley terms the substance strepogenin. It has not as yet been isolated and identified, but he thinks it may be a peptide or a peptide-like compound. In this connection it may be pertinent to recall that a somewhat similar interest in the nutrition of streptococci led Mueller some twenty years earlier to the discovery and isolation of methionine.[93] Preliminary tests on "aminoids," an enzymatic hydrolysate of casein, had produced three fractions capable of stimulating bacterial growth (and he speculates, hence possibly also important in animal metabolism). One of these, the "X" factor, like Woolley's strepogenin,[135] was precipitable with silver sulfate and barium hydroxide.[92] Unfortunately, these factors were lost when the sulfur compound was purified and Mueller was unable to return to his study of them.[93]

Species Differences in Requirements for Growth and Nitrogen Equilibrium. Growth tests in the young rat have shown that the removal from the diet of any one of the tabulated indispensable amino acids, except arginine, causes a prompt and profound loss of weight, in some instances ultimately death; and conversely, that correction of the deficiency invariably induces an abrupt and dramatic growth response. Dietary arginine is not required for maintenance in the adult,[155] or even for moderate growth in the immature [112] rat. Its need becomes apparent only under conditions which favor maximal growth. One may speculate that differences in rates of weight loss upon removal of the various other essential amino acids from the diet and differences in incidence of death may possibly be attributable in part to variations in rates of synthesis much too slow to provide for maintenance.

The young mouse is apparently able to manufacture arginine with considerable facility, but whether it can do so rapidly enough to support maximal growth must await further tests in which maximal growth is attainable when dietary arginine is supplied. The amino acid requirements of the growing mouse are otherwise qualitatively the same as those of the growing rat.[12] A dietary source of arginine is not required for maintenance in the adult dog [117] or the adult human subject.[66, 115] With this exception, the amino acid needs seem to be qualitatively the same for maintenance in the adult rat and the adult dog as for growth in the young rat.

Curiously, the adult human subject needs no dietary source of histidine, either for the maintenance of nitrogen equilibrium or for its restoration after a short period of marked negative nitrogen balance induced by the simultaneous removal of isoleucine and phenylalanine.[116] Whether the rapidly growing infant or child must have a dietary supply of histidine is not yet known. Except for arginine and histidine, the amino acids essential for growth in the rat are also indispensable for maintenance in the human adult.[4, 5, 33, 67, 111, 115, 116] That the amino acids tabulated as dispensable in the rat are also dispensable in the human adult is indicated by the fact that maintenance occurs on diets lacking them.[115] The dispensability of histidine [7] and cystine [5] in the human adult has been confirmed.

The amino acid requirements of less closely related species may differ even more widely. This is clear from observations that the chick's need of arginine is critical and that, in addition to the ten amino acids essential for maximal growth in the rat, the chick must also have dietary supplies of glycine and glutamic acid.[10, 55] (See Chapter 6.)

Dispensable Amino Acids. It is a mistake to ignore the dispensable amino acids on the ground that they are unimportant dietary components. Like the indispensable amino acids, they are involved normally in the fabrication of tissue proteins. They differ from them in that, when they are not supplied ready-made, the lack can usually be overcome by synthesis. Obviously such manufacture requires raw materials. This being true, a critical shortage of their precursors could block their synthesis and seriously

retard the processes of protein construction and tissue fabrication. Such interruption has been clearly demonstrated in at least one instance. When the dispensable amino acid, cystine, is not supplied in the diet, a portion of the indispensable dietary methionine is apparently commandeered to provide the sulfur needed to manufacture cystine.[118] If the amount of methionine allotted the young rat is inadequate to meet both this need of sulfur and the need of methionine, as such, growth is curtailed. It can again be accelerated by fortifying the diet further, either with cystine or with still more methionine.[156] Tyrosine probably has its origin biosynthetically in phenylalanine.[12, 90] Histidine is converted to glutamic acid by the enzyme, histidase; [42] it therefore seems possible, but is by no means certain, that such transformation may occur preferentially if glutamic acid is not provided in the diet. Further experimental work will obviously be required to clarify some suggested interrelationships and to indicate what others may exist.

Deficiency Syndrome

In the Animal. Growth in the young animal and maintenance of nitrogen equilibrium in the adult (or the lack of them) are only gross indications of nutritional adequacy (or deficiency). In a few instances, disturbances relatively specific in character have been attributed to dietary deficiencies in one or another of the indispensable amino acids. Thus, prolonged maintenance of rats on tryptophane-deficient diets has been known for some time to cause the development of cataracts.[34] Corneal vascularization, less marked, but detectable with the biomicroscope, has more recently been observed to occur in the rat after 9 to 20 days on diets deficient in lysine, methionine, or protein.[140] Failure of female rats to cast litters when transferred to a tryptophane-deficient diet immediately after their insemination has led to the suggestion that poor reproduction on low protein diets may be due specifically to an inadequate intake of tryptophane; [9] it seems fair, however, to question whether the same result might not also reasonably be expected to obtain if the diet were markedly deficient in any other of the several amino acids critically needed for growth. Recently the claim has also been made that a dietary deficiency of tryptophane of only 3 to 18 days' duration in rats 28 to 48 days old at the outset is responsible for a subsequent permanent sterility in both sexes; [71] tests in our laboratory have failed to confirm this observation.[14] Valine deprivation renders the rat extremely sensitive to touch and induces a severe lack of coordination in movement; the anomaly can apparently be readily remedied by valine restoration.[113] Lack of threonine in the diet of the mouse induces marked edema and ascites which are not observed in mice fed diets deficient in other essential amino acids for even longer periods of time.[12]

In Man. Deficiency of lysine in the diet has been reported to produce nausea, dizziness, a hypersensitivity to metallic sounds, and a sharp rise in

excretion of non-keto acids in the human subject.[6] Unfortunately the chief source of nitrogen in the lysine-deficient diets used in these studies was a hydrolysate prepared from casein which had been deaminized to destroy the terminal amino groups of lysine and thus render it nutritionally ineffective. Since the organic acid residues produced in the deamination were not removed from the diet, it is quite likely that their presence and not the lysine deficiency *per se* was responsible for the non-keto acid excretion. If this be true, the diminution in the output of non-keto acids, when lysine was provided, may simply reflect a greater capacity of the properly nourished organism to oxidize these abnormal deaminized residues. Although arginine deprivation does not prevent the maintenance of nitrogen equilibrium in adult human males, spermatogenesis is said to be markedly impaired after nine days on an arginine-deficient diet. The spermatogenic tissue is assumed to undergo atrophy to provide the arginine needed for nitrogen equilibrium.[66] The experimental details of this study are not available, and the observations have not as yet been confirmed by other workers.

Protein Synthesis in the Body

The processes involved in the fabrication of proteins *in vivo* from the amino acids available in the diet are not well understood, but it seems advisable to sketch the essential features of what is known to provide a background against which to view protein deficiency in the body.

Amino Acids in the Blood. The non-protein amino nitrogen content of the blood of the normal human subject is never very high. In the post-absorptive state it ranges between 5 and 8 mg per 100 cc and during the active digestion of protein food the increment is small (around 2 mg).[47] In other mammalian species the post-absorptive range is about the same as in the human being, sometimes a little higher, but the ingestion of meat, as in the dog,[145] or of a large dose of an amino acid, as in the rat,[78] may cause the concentration to double. Incidentally, the blood of the bird contains considerably higher concentrations of amino nitrogen [20] than that of mammals.

Classical experiments on the dog suggest that amino acids are rapidly withdrawn from the systemic blood into the tissues without undergoing immediate change. Thus, in 5 minutes after the intravenous injection of 12 gm of alanine (which required 5 minutes) Van Slyke and Meyer [146] found that only 1.5 gm remained in circulation; in 35 minutes, less than 0.5 gm could be found and only 1.5 gm could be recovered from the urine. Numerous studies of changes in the concentrations of amino acid and urea nitrogen of the blood have been conducted, particularly after the administration of single amino acids. No advantage would be gained by reviewing these here, but it may be well to emphasize what should be obvious yet is apparently often overlooked. When only one amino acid is provided, anabolic phases of metabolism, such as protein synthesis and repair which require the presence

simultaneously of most of the amino acids, certainly must be greatly inhibited. Under such circumstances the course of metabolism is limited largely to catabolism and is bound to differ widely from the course it would follow if the amino acid were a component of a well-balanced mixture. Our own studies of tryptophane metabolism convince us that it is not always possible to predict from results obtained in one situation what may characterize the other.[13] It is commonly assumed that under normal circumstances each tissue has the capacity to withdraw the amino acids it needs to fashion or repair its own proteins. Some tissues are, of course, also able to synthesize and elaborate proteins or non-protein nitrogenous substances, or both, for extraneous use.

The non-protein amino nitrogen content of the blood is not reduced significantly by fasting, even after several weeks; on the contrary, it may rise.[147] Presumably autolysis of expendable proteins sets in to provide energy or to insure conservation or replacement of the vitally needed proteins (or vitally needed non-protein nitrogeneous constituents). The protein lost from the body of the albino rat during a 7-day fast is largely that of the muscle, skin, and skeleton. The other organs furnish various, but smaller, amounts. In proportion to original content, however, the liver loses the most protein (40 per cent) of any tissue.[2] In only 2 days of fasting the loss from the liver is less, but its share in the total loss is even more striking.[3]

Protein Storage. The ingestion of high-protein diets has long been known to induce enlargement of the liver and to increase its content of protein. Appropriate histological examination of sections of the livers of rabbits and salamanders fed protein or protein hydrolysates reveals pyraninophilic granules in the cytoplasm.[15, 120] The fact that this granular material increases or decreases with intake of protein has led to the assumption that it constitutes a "reserve" or a "cell inclusion" protein.[15] In other critical histochemical studies the granular structures have been characterized as lipoprotein-ribonucleic acid complexes.[75] Physicochemical fractionation has failed to show the presence of a reserve protein which differs from the proteins which are always found, presumably because they are basic structural components.[79] Present evidence may therefore be said to indicate that the quantity of protein deposited in the liver depends in part upon the protein intake; but also to suggest that if indeed a distinctive reserve protein is formed, the quantity of such produced must be relatively unimportant. Quantitative variation with diet has also been observed in the protein content of other organs and tissues. In rats fed diets containing 16 per cent of protein, all of the tissues and organs examined contained more protein than did the corresponding tissues and organs in rats fed 11 or 6 per cent of dietary protein. However, maximal protein content was attained in some organs when the diet contained 16 per cent of protein, in others when it contained 27 per cent, and in still others when it contained 43 per cent.[1]

Degradation in the Liver. The strategic position of the liver astride the path which the amino acids take from the gastrointestinal tract to the peripheral tissues allows this organ very early contact with them. According to Van Slyke,[144] the greatly increased concentration of urea observed in the blood of the hepatic vein indicates that a large part of the amino acids absorbed at the height of digestion appears to be captured and destroyed by the liver and never to have a chance to reach and nourish the tissues. Evidence of urea production cannot alone lend this inference incontrovertible support. The process of deamination which yields the nitrogen for the synthesis of the urea also produces carbon residues, probably chiefly ketonic acids, and several such have been shown to serve effectively in the growing rat as substitutes for the corresponding essential amino acids.[112] To preclude possible nourishment of the tissues, it must therefore also be shown that the deaminized residues are altered sufficiently to render their use for resynthesis of the original amino acids no longer possible.

However extensively the nutritional utility of the amino acids absorbed from the alimentary tract may be affected by changes which occur in their passage through the liver, it is obvious that the complexion of the metabolic mixture which does result will depend largely upon the amino acid composition and the quantity of the protein ingested in the diet, and on the degree to which the amino acid units in the protein are rendered available by digestion. Some proteins, like the keratins, resist enzymolysis in their native state. Others may have their digestibility influenced favorably or unfavorably enough by processing to enhance or lower their biological values.[89] Rate of liberation of the individual amino acids during digestion, some promptly, others only after long delay, may be an additional factor of some consequence.[85]

Dynamic Interrelationships of the Body Proteins

Experimental evidence that the organs and tissues of the rat lose protein in varying degrees during starvation has already been mentioned. Though given no food, the dog which is deprived of a goodly share of its hemoglobin is nevertheless able to synthesize new hemoglobin; when iron and sugar are provided, synthesis of as much as 150 gm may occur in 2 weeks.[36] The fasted dog robbed of plasma proteins by plasmapheresis (removal of blood with return of the red cells) is able to manufacture new plasma protein to the extent of half the original total.[72] Analogous observations have been made in the rat.[35] Obviously the new blood proteins produced under these circumstances must have been manufactured from materials supplied by the body tissues, presumably in response to the urgency of maintaining efficient commerce over the vascular and interstitial traffic lanes. Conversely, laked red blood cells (of dogs) given intraperitoneally as virtually the sole source of nitrogen can be used to maintain approximate nitrogen balance, at least for periods as long as 20 days in normal dogs receiving

essentially protein-free diets by mouth;[87] when similarly administered, plasma is even more effective than this.[37, 87]

Experiments of the type cited in the previous paragraph show quite clearly that there is an interdependence among the various proteins of the body, one upon the others. They lend strong support to the view of Whipple that: "A part of the body protein forms a *reserve against adversity* in the sense that it can be circumspectly depleted without apparent injury to the body. The supplies of amino acids coming from outside the body and the demand for protein materials within the body are in a constant state of balance with these protein stores, a *dynamic equilibrium*." [80] What seems to be incontrovertible proof of the fluid or "dynamic state" of the body constituents has come from the work of Schoenheimer and his associates, which has been summarized in a series of well-written lectures available in book form.[127] In the case of proteins, the experimental work has involved tracing the fate of amino acids and metabolic products whose molecules have been marked by the incorporation in them of deuterium or heavy nitrogen or both. When such substances are fed to an experimental animal the isotopes serve to identify tissue components or metabolites produced from them. The concept of the "dynamic state" of body constituents, as it pertains to protein, has been summarized succinctly by Schoenheimer, Ratner, and Rittenberg [128] as follows: ". . . nitrogenous groupings of tissue proteins are constantly involved in chemical reactions; peptide linkages open, the amino acids liberated mix with others of the same species of whatever source, diet, or tissue. The mixture of amino acid molecules, while in the free state, takes part in a variety of chemical reactions: some reenter directly into vacant positions left open by the rupture of peptide linkages; others transfer their nitrogen to deaminated molecules to form new amino acids. These in turn continuously enter the same chemical cycles which render the source of the nitrogen indistinguishable. Some body constituents like glutamic and aspartic acids and some proteins like those of liver, serum, and other organs are more actively involved than others in this general metabolic mixing process. The excreted nitrogen may be considered as part of the metabolic pool originating from interaction of dietary nitrogen with the relatively large quantities of reactive tissue nitrogen." With the view of these authors that "it is scarcely possible to reconcile" findings of the type described with a theory which draws distinctions between exogenous and endogenous metabolism not everyone would agree. Proof of interchange between tissue nitrogen and dietary nitrogen certainly makes revision of the original concept of endogenous and exogenous metabolism essential, but it does not seem to necessitate complete abandonment, any more than the evidence that body proteins are being subjected to continuous modification requires abandonment of the concept that marked specificity is nevertheless maintained in the tissue proteins. The change needed is rather one of viewpoint. What was once considered static is now proved

only to have appeared so. The state which actually obtains is one of equilibrium, involving the summation of a complex array of interdependent equilibria between opposing dynamic forces.

Protein Deficiency and Nutritional Anemia

The blood, lymph and interstitial fluid are the media through which the tissues of the body establish contact with each other and with the external environment of the organism. Chiefly to the blood falls the responsibility of facilitating the exchange of useful and waste products and of maintaining the fluid balance. Its capacity to discharge these and its other physiological functions depends largely upon the electrolytes and the proteins it contains.

Almost half (42 to 45 per cent) of the volume of the blood of the normal human adult is composed of erythrocytes. These contain approximately 40 per cent of solid matter, three-fourths of which is usually protein. Of this electrophoretic analysis indicates that 95 per cent is the conjugate, hemoglobin,[138] upon which the tissues depend for their supply of oxygen and, directly or indirectly, for the disposal of carbon dioxide.

Anemia is a term which may be applied to any condition in which the red cell count or the amount of hemoglobin in the blood is subnormal. The erythrocytes may vary in number, in size, and in concentration of hemoglobin. The number of these cells in the circulating blood depends upon the balance which is maintained between their formation in the bone marrow and their destruction in the liver, spleen and lymph nodes. If, as seems probable, the healthy human subject normally destroys and replaces about 1 per cent of his erythrocytes each day, the average life of the red cell must be around 100 days.[50] Failure to fabricate erythrocytes or synthesize hemoglobin at the usual rate, loss of blood by hemorrhage, and abnormal red cell destruction are all factors which may singly or collectively upset the balance which prevails normally and thus ultimately produce anemia. The most common nutritional cause of anemia is the lack of enough iron to build hemoglobin. Among other nutritional factors, deficiency of protein is particularly pertinent to the present discussion.

As has already been intimated, there is considerable evidence that hemoglobin occupies a favored position among the body proteins. Dogs maintained on diets low in protein, high in iron, and adequate in all other factors, but bled to deplete the hemoglobin and the plasma proteins, have been observed to produce 1.5 to 4 times as much hemoglobin as plasma protein; this was true even when digests of dog plasma or serum were given intravenously.[87, 110] In contrast with proteins of the plasma and of most of the tissues of the body, the proteins of the erythrocytes take up much less isotopic nitrogen, thus supporting the assumption that the circulating hemoglobin is involved in a comparatively slow cycle of synthesis and destruction.[129] Hence protein malnutrition alone should not be expected to produce anemia very promptly.

Lysine Deficiency. Some years ago (in 1934) Hogan and Ritchie [64] observed that albino rats which survived the rapid losses of weight which occurred when they were placed on deaminized casein diets became severely anemic in 6 or 8 weeks. The animals assumed a waxy white appearance and their red cell counts dropped to 2,000,000 or less per cubic millimeter before their death. The anemia appeared to have been due, at least in part, to the deficiency of lysine. Resumption of growth and recovery from the anemia occurred when adequate amounts of lysine were added to the diet, but the amount of lysine required was almost twice that needed to promote comparable growth in the non-anemic rat.[65] This observation is consistent with the fact that the lysine content of vertebrate hemoglobin is high. Block records the content as 8 per cent,[18] but this value is based on 16 gm of hemoglobin nitrogen. Ready synthesis of a protein containing approximately this much of any indispensable amino acid could obviously not occur unless the dietary supply of the amino acid were liberal.

Pigeons injected with lysine or with leucine [94] showed a marked increase in reticulocytes (immature red blood cells). Examination of the bone marrow indicated that stimulation and proliferation of red blood cells and an extension of the blood forming tissue had occurred.

Trytophane Deficiency. Several reports have indicated that anemia is produced in rats fed a tryptophane-deficient diet, among them a paper by Albanese *et al.*[48] which reports that 50 days or more are required for the anemia to develop in the young animal, and that a still longer time is needed in the adult. The anemia was hypochromic in type, *i.e.*, the red cell count was approximately normal, but the hemoglobin level was reduced. Similar diets supplemented with tryptophane prevented the anemia even when the amount of diet fed was barely adequate for maintenance. The changes in hemoglobin were paralleled by changes in plasma proteins. Block records the tryptophane content of hemoglobin as 1 to 2 per cent.[18]

Methionine Deficiency. After 100 days or more, rats on a methionine- and cystine-deficient diet showed marked drops in hemoglobin values, but not in red blood cell counts. A casein hydrolysate, fortified with tryptophane, but containing only 0.05 ± 0.02 per cent of cystine failed to induce a similar response. Since hemoglobin contains very little cystine, the hypochromic anemia which developed on the doubly deficient diet was assumed to have been due to deficiency in methionine.[8]

Isoleucine Deficiency. Quite likely a diet low enough in any of the essential amino acids found in the hemoglobin characteristic of the species tested would ultimately produce anemia. Brand and Grantham have recently noted[21] that the hemoglobins of man, the horse, and the cow contain no isoleucine, whereas fetal bovine hemoglobin contains 0.63 per cent and dog hemoglobin 1.36 per cent. On the other hand, dog hemoglobin contains only 0.42 per cent of methionine, fetal bovine, human, and adult bovine hemoglobins 0.97, 1.32, and 1.76 per cent, respectively. These data correlate

well with the several observations that rats fail to grow when fed unsupplemented human or beef globin as the dietary protein, but respond when isoleucine is added; [40] they also explain why supplementation with this amino acid does not improve the utilization of dog hemoglobin in the dog, but supplementation with methionine does.[86]

Hemoglobin Regeneration. Experimental evidence from several laboratories has indicated that hemoglobin regeneration in rats and dogs rendered anemic by bleeding is more rapid when the protein intake is adequate than when it is low.[69] Orten and Orten [100] observed that normal young rats fed for 76 days on diets which contained only 3.5 per cent of lactalbumin, but ample calories, minerals, and vitamins, showed a mild chronic anemia characterized by a low hemoglobin value, a normal number of erythrocytes, but an increased reticulocyte count. Increasing either the caloric or the iron allotment produced no beneficial effect, but the anemia could be prevented or cured by increasing the lactalbumin intake without altering the allotment of the other dietary constituents.

Some time ago, Fontes and Thivolle [49] made the claim that an extra supply of histidine produced excessive formation of hemoglobin and erythrocytes in the blood of the dog, even though the dog lost weight. In the dog with hemorrhagic anemia, the individual amino acids seem to differ appreciably in their capacity to stimulate hemoglobin synthesis.[109] On the other hand, no consistent, sustained increase was noted in the hemoglobin values of rats rendered anemic on 3.5 per cent lactalbumin diets when these diets were supplemented with a single one of the ten essential, or with any one of the several non-essential amino acids, in amount equal to the extra supply of the amino acid which the diet would have provided had its lactalbumin content been raised to 18 per cent. There was no basis in the latter tests for assuming that any single amino acid functioned as a "key" acid in hemoglobin synthesis.[98] At present one can only speculate as to why the results of tests with individual amino acids have differed so widely among the various laboratories. Difference in body stores of labile protein in the test animals, differences in animal species, and differences in experimental technics or in other factors which may affect hematopoiesis are all possibilities. Level of protein intake is known to influence riboflavin storage [125] and nicotinic acid synthesis in the rat.[119] Deficiencies in either of these factors may produce anemia.

Clinical Incidence. According to Youmans,[160] mild anemias are commonly observed clinically, but rarely can they be traced to uncomplicated protein deficiency. Usually they can be ascribed to other deficiencies, and are often associated with a deficiency of iron. In only a few cases does the anemia seem to be caused directly by a low protein intake. Both microcytic and macrocytic (small and large cell) anemias have been noted. Occasionally hemoconcentration masks the true condition and produces red cell counts and hemoglobin values which lie within the normal range; but these drop when the anhydremia is overcome.

Protein Deficiency and Hypoproteinemia

The plasma of the blood contains approximately 8 or 9 per cent of solids, of which about four-fifths are proteins. The fractionation of these and the properties of the various individual proteins have been discussed in a previous chapter (Chapter 8). Several of the physiological functions which they serve collectively and individually will be considered in the discussions which follow.

Hypoproteinemia, a lower than normal content of protein in the circulating plasma, may be caused by a number of factors. Hemorrhages, urinary excretion, and the formation of exudates and transudates promote losses which, if persistent, may overwhelm the capacity of replacement. Febrile and other states in which metabolism is greatly accelerated induce excessive tissue breakdown. Impaired capacity to synthesize plasma protein may be involved or adequate synthesis may fail to occur because the building materials provided in the diet are inadequate in quality or quantity, or are rendered so by abnormal digestion and absorption.

Rate of Depletion and Repletion. Interdependence among the various tissues of the body tends to prevent marked degradation from occurring in one without involving the others. Weech, Goettsch, and Reeves [151] estimated that the plasma protein lost in the dog during chronic depletion represented only 4 per cent of the total loss of tissue nitrogen. Sachar, Horvitz, and Elman [123] suggest that the total loss or gain of protein in the human adult is about 30 times the loss or gain of albumin in the plasma. What this implies can perhaps be made clearer by example. The plasma volume of a 70-kg man approximates 3.3 liters. Assuming that the volume does not change, a loss of 1 gm of albumin per 100 cc of plasma would represent not only a loss of 33 gm of plasma albumin but a total loss from the body of 990 gm of protein. It is evident that depletion or repletion involving this much protein is unlikely to occur rapidly. Peters and Van Slyke have observed [103] that patients with Bright's disease of the degenerative type may excrete albumin at the rate of 25 gm per day for months, without appreciable alteration of the plasma albumin content. It is well to recall that in contrast with chronic depletion, an acute reduction in plasma proteins, as by plasmapheresis, can be met fairly completely and promptly, even in the fasting animal.[35, 72]

Whipple and Madden [154] believe that the circulating plasma protein serves as a medium of exchange and that the passage of large protein molecules across cell borders, such as those of an active liver cell, must certainly occur to and fro, from cell to blood and reverse. Co Tui, Barcham, and Shafiroff postulate that the lymphatic system and the thoracic duct constitute an important pathway for the return of protein from the capillary filtrate to the blood and for the mobilization of protein from the protein depots of the body.[32] This assumption is based on evidence that after a hemorrhage, an increase in protein concentration occurs in thoracic duct

lymph. Furthermore, in dogs with ligated thoracic ducts, a period of 8 days is required to show the same regeneration of plasma proteins after a hemorrhage as that which occurs in a day or two in animals with unligated ducts.

Hypoalbuminemia. There seems to be ample evidence that, in chronic protein depletion, the albumin fraction of the plasma is particularly susceptible to reduction. Weech [150] observed that the fall in total protein in the blood serum of dogs subjected to virtual protein starvation was due entirely to diminution in albumin. In 3 weeks about 0.9 gm of albumin was lost per 100 cc, and in 11 weeks, twice that much. Hypoalbuminemia has become rather generally accepted clinically as indicative of protein deficiency associated with chronic malnutrition.[102, 149] By the use of electrophoretic analysis, Zeldis *et al.*[162] have shown that the decrease in concentration of plasma albumin in the dog fed a low-protein diet for several weeks was accompanied by an increase in the α-globulin concentration, but by no significant change in the concentrations of the β- or γ-globulins or the fibrinogen. Plasma volume was not estimated and absolute changes were therefore not established. A significant decrease in volume would presumably have masked moderate decreases in absolute values and have made them appear as normal, or even as elevated concentrations. When the plasma proteins are depleted by repeated plasmapheresis, or their depletion by low-protein feeding is thus hastened, the plasma albumin is again the more markedly reduced.[80, 149] Electrophoretic analysis in the latter type of depletion discloses a sharp drop in total circulating albumin, slight drops in the β- and γ-globulins, but no change or only a slight increase in total circulating α-globulin.[30]

A fasting dog which is given whole dog plasma by vein to supply its protein needs shows no change in its albumin to globulin ratio.[105] Hence it seems unlikely that a preferential utilization of albumin causes its more rapid depletion. From studies with isotopic nitrogen in the rabbit and the rat,[130] the dog,[46] and the human being,[107] the half-life of the average serum protein molecule has been estimated to be about two weeks. Hence the processes of degradation and synthesis must be relatively active ones. Quite likely, differential increases or decreases in the plasma proteins are the result primarily of their relative ease of synthesis.

Site as a Probable Factor n Rate of Synthesis of Plasma Protein. It is fairly generally accepted that fibrinogen,[81] prothrombin,[134] and albumin [80] are probably synthesized in the liver. Whether the liver is even the chief site of globulin synthesis [80] is uncertain. The cells of the reticulo-endothelial system, including the Kupffer cells in the liver, apparently produce at least part of the serum globulin and when under the influence of an antigen, also antibody globulin.[122] Recently the lymphocytes have been observed to contain antibodies,[41, 43] either because they produce them or because they take them up from the lymph. If appreciable synthesis of globulins can occur extrahepatically one might speculate that this may possibly favor

their more rapid synthesis than albumin under conditions known to affect the liver.

In animals subjected to protein depletion the liver not only loses protein,[2, 44] but decreases in size,[29, 95] and becomes fatty [106] and soft.[44] Complete restoration of the liver protein apparently does not occur until the total body protein is also replenished.[54] In liver disorders [106] or liver injury [80] the manufacture of albumin and prothrombin is impaired and albumin to globulin ratios are usually low.

Miller and Whipple [88] observed that the sulfur content of the liver decreased more rapidly during protein depletion than did the nitrogen content. They found that ingestion of methionine or of cystine and choline protected the organ against chloroform poisoning. Hock and Fink [63] noted a high percentage of deaths in rats fed yeast as the nitrogen source. Autopsy revealed evidence of extensive hemorrhage and necrosis in the liver; in chronic cases there was evidence of cirrhosis. These changes, which were assumed to have been due to a lack of adequate cystine in the yeast, were corrected by incorporation of cystine in the diet. Himsworth and Glynn [60] have produced massive acute necrosis of the liver in 40 to 60 days in 90- to 120-gm rats receiving an intake of 500 mg each of protein per day. The proteins employed were casein and yeast, both poor in cystine and containing insufficient methionine at the level fed to overcome the cystine deficiency. Supplements of 20 mg of methionine per day prevented development of the lesions, but daily supplements of only 2.2 mg of cystine did not. Diffuse hepatic fibrosis (cirrhosis) was not observed until after 150 days. Even then it did not develop in the rats receiving protein as yeast, although such a diet favored the appearance of necrosis. The fibrosis could be prevented or delayed by providing choline or additional casein. Chemically, the necrotic livers showed increased contents of water and protein, an absence of glycogen and no significant change in fat content.[61] Some of the water and protein were from the blood plasma. The changes in composition appeared suddenly at the onset of cellular damage. In some respects these findings resembled those made earlier by Weichselbaum in rats fed cystine-deficient diets; the symptoms could be prevented by either methionine or cystine, but once established could be relieved only by cystine.[102] Using diets low in casein György and Goldblatt [53] noted that choline reduced the incidence of cirrhosis, but did not always prevent the development of necrosis. Either choline plus cystine, or methionine alone, was effective in preventing the liver injury. Blumberg and McCollum reported [19] that rats which were fed diets containing 20 per cent of protein showed normal livers when the protein was fed as casein to provide a relatively high intake of methionine but a relatively low intake of cystine. Fatty livers, but no cirrhosis, occurred when the protein was fed as arachin to provide a diet low in methionine but intermediate in cystine content. Moderately fatty livers, with cirrhosis, were observed in all survivors when the protein was fed as glycinin to afford

an intermediate amount of both cystine and methionine. When the dietary protein of the latter animals was changed to casein, the cirrhotic process was arrested.

In adult rats subjected to protein depletion by maintenance on a diet in which the chief source of protein was carrots, Cannon *et al.*[28] found no evidence of injury to the serum-producing mechanism in periods of 60 to 94 days. Madden and Whipple have concluded [80] that in dogs "prolonged hypoproteinemia *per se* causes no damage to the protein-forming mechanism." On the other hand, Weech has suggested that the slower overall rate of regeneration of albumin in the dog after prolonged depletion probably is the result of injury to the synthetic mechanism, presumably in the liver.[149]

Amino Acid Needs for Synthesis of Plasma Proteins. It is of interest to note that in three different laboratories beef serum protein has been found the most effective of all of the food proteins for the formation of plasma protein in the depleted dog.[80, 84, 149] Its biologic value for dogs exceeds slightly that of casein, but is inferior to that of lactalbumin.[83] In the rat, 18 per cent of protein fed as beef blood solids promoted very poor growth.[99] Growth in the rat fed dried human plasma was also meager when compared with that in the rat fed casein,[57] but the addition of 0.5 per cent of isoleucine to the diet which contained the human plasma proteins rendered it as effective as the casein diet. In adult protein-depleted rats [28] comparisons of bovine albumin and γ-globulin and of several purified fractions of human plasma as sources of dietary protein showed that better weight recoveries and more effective regeneration of serum protein occurred in the animals fed fibrinogen and globulin than in those fed purified albumin or fractions high in albumin. Several fractions of human plasma have been assayed for amino acid content [22] and the analyses of human, bovine and horse (B) albumin have been compared. All three albumins were very low in tryptophane (0.19, 0.58, and 0.30 per cent respectively); that of the horse was entirely lacking in methionine; and the human albumin contained considerably less isoleucine (1.7 per cent) than the bovine (2.9 per cent). Human γ-globulin contained more tryptophane (2.86 per cent) and isoleucine (2.7) but less methionine (1.06 *vs* 1.28) than human serum albumin. Hence, it seems quite probable that differences in the composition of the plasma proteins may account for differences in rate of regeneration or rate of depletion in different species on the same type of diet. Hegsted, Hay and Stare [56] found that when plasma proteins were fed to young rats at 20 per cent levels, fibrin promoted as good growth as did a skim-milk control diet, albumin failed to support growth, and globulin was intermediate. When the albumin diet was supplemented with *l*-tryptophane and *dl*-isoleucine, growth occurred. Calculations indicated that increasing the *l*-tryptophane content to 0.42 per cent and the *l*-isoleucine content to 3.9 per cent of the protein afforded optimal growth. Interestingly enough, in the adult dog maintained

for ten days on a "nitrogen-free" ration, 0.14 gm of unsupplemented albumin nitrogen per kilogram was adequate for positive nitrogen balance. Comparisons of these data on the adult dog with those previously cited on the adult rat [28] again strongly suggest that marked species differences may exist in the relative amounts of the various essential amino acids needed, even for similar purposes.

Whipple [80] has long regarded cystine as a "key" amino acid in plasma protein synthesis. In the rat fed ample methionine, cystine can apparently be synthesized rapidly enough to meet the need, but when the diet is deficient both in methionine and cystine, hypoproteinemia is produced.[8]

Nutritional Edema

Half a century ago Starling [137] suggested that normally much of the responsibility for the maintenance of the fluid balance between the blood and the tissues resides in the protein components of the body fluids. In comparison with the blood plasma, the protein concentration of the lymph and the interstitial fluid is relatively small. On the arterial side of the capillary bed, extra hydrostatic pressure is produced by the work of the heart. This operates to overcome the osmotic differential resulting from the higher concentration of proteins in the plasma than in the interstitial fluid and thereby induces fluid to move from the blood into the interstitial spaces. On the venous side of the capillary bed the osmotic differential is virtually unopposed and hence the direction of the fluid transfer is reversed. Conditions which lower considerably the concentration of plasma proteins narrow the osmotic differential between the blood plasma and the interstitial fluid and thus may upset the balance enough to permit fluid to leak into the interstitial spaces. Other factors, such as increased capillary blood pressure, increased capillary permeability, and decreased lymphatic drainage or lymphatic obstruction may produce the same effect. An exaggerated extravascular accumulation of fluid is known as edema. When the spaces of the soft subcutaneous tissues are involved and the condition is sufficiently marked, digital pressure will leave pits in the skin. In some instances the accumulated fluid may be tapped. A similar accumulation of fluid in the abdominal cavity is known as ascites, and in the pleural cavity as hydrothorax.

The term "nutritional edema" is applied when the condition is caused by hypoproteinemia induced by a diet deficient in protein, and probably invariably inadequate also in calories. According to Youmans,[160] the first clinical sign of protein deficiency is a transitory or constant edema of the dependent extremities. Such a condition has long been associated with famines and wars, during which its incidence has reached epidemic proportions. In World War I it commanded considerable attention. The historical aspects of "war edema," "prison dropsy," or "hunger swelling" have been summarized by Maver.[82] Although resolution of edema had been known for some time to occur with improvement of the diet, the probable

association of protein impoverishment and protein replenishment with the incidence and subsidence of the condition was not clearly recognized until it had been demonstrated by animal experimentation. Denton and Kohman [39] were apparently the first to show that edema could be produced in animals (rats) by restricting their protein intake severely for a relatively long period of time. They noted that maintenance of weight was barely possible on a diet consisting exclusively of carrots. Addition of fat or starch to the carrot diet effectively lowered its already low nitrogen content and induced dropsy in some, but not all, of the rats. Maver [82] reports having confirmed this observation in rats and guinea pigs, and Kohman [74] subsequently published the results of more extensive studies which verified her earlier assumption that protein deficiency was responsible and showed that the edema could be alleviated only by increasing the protein content of the diet. Her conclusions were confirmed by Frisch, Mendel, and Peters,[51] who noted further that serum proteins were reduced in the animals which failed to develop edema, as well as in the animals which became edematous on the protein-poor diet of carrots, starch, lard, and salt mixture.

Edema Production by Plasmapheresis. Leiter [77] produced edema in dogs by removing 400 to 500 cc of blood from them twice daily, suspending the erythrocytes in a corresponding volume of modified Locke's solution, and reinjecting them intravenously. He also gave 1500 cc of 0.85 per cent sodium chloride solution daily, by stomach tube. Edema usually appeared in the soft tissues on the fifth day, becoming detectable when the plasma protein had dropped to about 3 per cent; ascites, hydrothorax, and pulmonary edema developed subsequently. Following a similar technic, Barker and Kirk [11] confirmed Leiter's observations. In both studies diuresis and a tendency of the edema to clear were noted when the plasmapheresis was omitted for a day or two.

Protein Content of Edema Fluid. Shelburne and Egloff [132] were able to produce edema in dogs fed diets low in protein, but adequate in carbohydrate and fat. They noted that administration of sodium chloride and sodium bicarbonate tended to increase the edema, but that administration of potassium chloride did not. In his extensive studies on hypoproteinemia in the dog, Weech reports that edema was rarely observed [151] until the albumin of the blood had dropped below 2 per cent. When the albumin was between 1 and 2 per cent edema was more often found than not, and below 1 per cent it was always present. Association of the edema with hypoglobulinemia was not apparent. Its correlation with low concentrations of total proteins was attributable to the changes in the albumin fraction. The protein content of the subcutaneous edema fluid ranged from 0.02 to 0.72 gm per 100 cc, with an average of 0.23 and a median of 0.17. Shelburne and Egloff [132] record 0.13 to 0.22 gm per 100 cc. Evidence obtained by the use of the precipitin reaction with specific rabbit antisera [52] suggest that the albumin and the globulin of lymph and edema fluid originate from the

plasma by filtration. Since the capillary is more readily permeable to the smaller albumin molecule, these transudates contain a higher proportion of albumin to globulin than do the sera from which they are derived. When the albumin to globulin ratio of the transudate is divided by that of the corresponding serum, the resulting factor, called the permeability index by Weech, is therefore usually above 1. In an extensive series of tests the average index was found to be 1.35. Three indices on ascitic fluid showed still higher values (1.82, 1.98, and 2.18), probably because it was necessary for the proteins to traverse the peritoneum as well as the capillary endothelium.[149]

Complicating Factors. Bloomfield [17] has called attention to the fact that in most of the studies in which nutritional hypoproteinemia and nutritional edema have been produced experimentally, carrots have been used as the source of the protein. He observed that rats placed on a diet which furnished 2.6 per cent of protein as yeast and 0.3 per cent as alfalfa lost over a fourth of their initial weight in 21 weeks, but showed only a small initial drop in total serum protein from 6.25 to 5.14 per cent. When he repeated the study with desiccated carrots, he obtained no edema or ascites in the 21-week period, as had Frisch, Mendel and Peters.[51] He suggests that the animals of the latter may have been made more susceptible to edema by frequent sampling of the blood for analysis.[17] On the other hand, many of the rats which Bloomfield fed exclusively on undesiccated carrots did show edema, some even ascites and hydrothorax, but the response varied greatly. Bloomfield stresses the probability that no single factor can alone produce the condition with any consistency. Individual responses vary greatly and inadequate caloric intake, ill-balanced diet, ingestion of electrolytes, and a large fluid intake all may be involved.

Nutritional surveys conducted in this country among the economically handicapped, of which one directed by Youmans [161] may be taken as an example, usually show only a small incidence of edema. In this survey, conducted in middle Tennessee, little relationship was observed between the edema and the serum protein concentrations. Of 37 subjects with edema when examined, only 5 had abnormally low serum albumin or abnormally low total protein, and in all but 6 of the 32 with normal serum proteins there were other possible causes of the edema. Of the 95 persons with hypoalbuminemia only 11 had edema or a significant history of it; in 85 of the 95 cases, the hypoalbuminemia was slight and was accompanied by a globulinemia sufficiently high to produce an oncotic pressure adequate to prevent the appearance of edema. A number of secondary factors may also operate to determine the level of hypoproteinemia at which edema will appear or disappear, among them the salt and water intake; posture, which induces variations in hydrostatic, hence in capillary, pressure and in the appearance and location of the edema; and environmental temperature, through modification of arteriolar and capillary dilatation. By increasing

the caloric requirements, hard physical work and cold and exposure may divert the already inadequate dietary protein to use for fuel instead of tissue conservation.

When reports of surveys made in the "occupied" countries and prison camps immediately following the allied invasion become available, they may throw more light on the problem of nutritional edema and its correction in man. In general, the relationship of the edema to hypoproteinemia has not been clear-cut. Davidson, Wilcke and Reiner [38] have reported observations on 171 young men in a group of Germans who had been imprisoned in upper Austria for 2 to 3 months. Their daily food intake was deficient in calories, which ranged between 650 and 850, and in protein. In 41 of the men (24 per cent) varying degrees of dependent "hunger edema" were noted. Total serum protein determinations were made on 18 of these men and on 33 without edema. The values averaged 5.7 gm per 100 cc, but 36 per cent were 5.5 gm or below. Of the men without edema 29 per cent showed values in this range; of the men with edema, 47 per cent. In attempting to account for the lack of correlation between edema and level of serum protein, increases in the globulin fraction were not excluded. Had such increases occurred they might have masked decreases in albumin in the instances in which the total blood protein seemed too high to account for the edema.

A carefully conducted series of observations on 34 men, volunteers from civilian public service, has been published in preliminary form by Keys, Taylor, Mickelsen and Henschel.[73] After a control period of 3 months these men were fed a European type of famine diet for 6 months. Whole cereals, potatoes, turnips, etc., provided an average of 49 gm of protein daily. During this subsistence period the men lost a fourth of their original body weights and the ratio of extracellular water to cellular tissue weight, as determined by the thiocyanate method, approximately doubled. The plasma protein concentration fell an average of only 0.73 gm per 100 cc and assay by the Tiselius electrophoretic procedure showed only a slight decrease in the albumin to globulin ratio. There were no signs of thiamine deficiency or renal or cardiac failure. Direct measurement showed a drop of 50 per cent in the venous pressure. There was moderate arterial hypotension and tissue pressure did not seem abnormal on digital examination. The authors conclude that the blood plasma is not in simple equilibrium with the interstitial fluid as is usually postulated, hence that famine edema is not a result simply of hypoproteinemia. Evidence from famine areas seems to support them in this. The authors suggest "that there is a dynamic nonequilibrium state of the capillary wall."

Protein Depletion and Resistance to Infections

The idea that the well-nourished individual is better equipped to resist infection sounds so plausible that it is commonly accepted with little reservation. Inquiry reveals that clinical evidence bearing on the subject,

though generally favorable, is largely circumstantial and that experimental evidence is rather meager. At the present state of development of the complex field of immunology this is not surprising.

Numerous incidental observations have indicated the possibility that proteins play an important role in immunity. Kohman observed that the rats restricted to her carrot-cornstarch-lard-salt diet for 8 to 12 weeks frequently developed pneumonic lungs.[74] Whipple and his associates have repeatedly called attention to the greater susceptibility of plasma-depleted dogs to infections, intoxication, and arsenicals, and to the removal of this susceptibility by protein feeding.[153] Mice showed a greater susceptibility to natural contact infection on a diet from which dried skim milk was removed.[148]

An interesting comparison of the physique and the health of two African tribes of the same original stock brings out the fact that members of the Masai tribe, whose diets contained considerable meat and milk, were more robust than the Kikuyus, whose diets consisted chiefly of cereals, with some legumes and tubers.[97] In the latter tribe, parasitic infestation and chronic respiratory disease were much more prevalent, general health was lower, and mortality rate was higher. Members of the Kikuyu tribe who were provided a more satisfactory diet showed prompt improvement.

Phagocytosis. According to one school of thought the first line of defense against an invading microorganism is the phagocyte. By virtue of their transport in the intravascular system and their capacity to penetrate and crawl through the capillary wall to reach the infected area, the polymorphonuclear leucocytes (neutrophilic) and the monocytes, which together usually constitute over half of the white cells of the blood, bear the brunt of such responsibility. If the phagocytic cells are able to ingest and destroy the microorganisms rapidly enough to prevent them from entrenching themselves in the tissues of the host, the infection may be speedily arrested. If the infective agent reaches the blood stream, the fixed phagocytic cells of the reticuloendothelial system may also participate. Most of the phagocytic cells originate, or undergo maturation, in the bone-marrow, spleen, liver, lymph-nodes and lymphoid tissues. Since these tissues are known to undergo marked atrophy during a period of prolonged protein deficiency, it would seem reasonable to assume that the stage might eventually be reached where the usually abundant reserves of phagocytic cells can no longer be sustained quantitatively or qualitatively.

Evidence of Cottingham and Mills [31] suggests that such may be the case. Young white male mice were fed various diets, among them diets varying from 6 per cent of protein to 36 per cent. Samples of blood withdrawn by heart puncture were mixed with a standard bacterial suspension of *Micrococcus candidus* and films were prepared and stained to permit counting the number of ingested bacteria in 40 or 50 unruptured and unclumped neutrophils. In the mice housed at 68° F the best growth and the best phagocy-

tosis were obtained when the diet contained 18 per cent of casein. At 90° F and 60 to 70 per cent relative humidity, growth and phagocytic activity increased from the 6 to the 36 per cent casein level. The data are difficult to evaluate accurately, partly because no information is available on food intake and partly because the number of cells counted was lower than is usually considered desirable, but they do show a trend encouraging enough to warrant careful further study of the possible correlation of phagocytosis with protein intake.

Berry, Davis and Spies [16] conducted a somewhat similar study in which rats were fed a basal diet containing unenriched white flour, corn meal, pork fat, and cane sugar, unsupplemented; supplemented with vitamin-free casein; with a mixture of the B vitamins; with minerals; or with all three. The best two-month growth occurred on the most completely supplemented diet. The leucocyte counts were only a third as great on the basal as on the complete diet and the granular leucocytes engulfed a third less of the test organism. The average agglutination titer for a mixed typhoid-paratyphoid vaccine was less than a fourth as large for males and less than half as large for females on the basal as for those on the complete diet. The differences were statistically significant. On the other supplemented diets the differences from those on the basal diet were less striking.

Production of Antibodies. The most extensive studies bearing upon the relationship between protein depletion and resistance to infection have come from Cannon's laboratory. Foreign proteins, whether introduced into the body as free protein or as components or products of invading microorganisms, act as antigens. They stimulate the production of antibodies capable of clumping (agglutinins) or lysing (cytolysins) the antigenic cell; precipitating the antigenic protein (precipitins); or counteracting the toxicity of products elaborated by the organism (antitoxins). Some authorities also list as antibodies the opsonins, factors which appear to render the invading organism susceptable to engulfment by the phagocyte. Depending on a variety of circumstances, antibodies may or may not remain in large amounts after the invasion has been resisted, but the capacity to form them tends to persist and to respond in accelerated degree to reinfection. The intriguing feature about using antibody production as an index of resistance to infection is that a number of specific antibodies can be measured quantitatively with reasonable accuracy (see Chapter 14).

There is good evidence that purified antibodies have the properties of serum globulins.[58] Concentration of the γ-globulin fraction of the blood plasma has yielded antibodies 15 to 30 times as concentrated as in the original pooled plasma, and in some cases the resulting concentration has exceeded that of corresponding convalescent sera. Incidentally, it should not be assumed that antibodies are found exclusively in the γ-globulin fraction [45] or that all of the γ-globulin is antibody.[70] It is of interest, however,

to note that the γ-globulin fraction of pooled plasma has been used successfully for the prevention or attenuation of measles.[96, 139]

The macrophages [122] and the lymphocytes [41, 43] were mentioned earlier as possible sites of antibody formation. The most common suggestion as to mode of synthesis is that the antigen, acting at the site of globulin fabrication, leaves its structural imprint on the globulins produced to make them react readily with antigen of the same type.[23, 91, 101] Other suggestions have been that the antigen modifies the enzyme responsible for the globulin synthesis [24] and that antigen ingestion by the macrophages alters the character of the globulins produced and shed as surface films.[122]

If the antibodies are merely modified globulins, there is every reason to assume that their synthesis should be governed by factors essentially similar to those that influence the production of the other plasma proteins. Of interest in this connection is the fact that antibodies produced in the actively immunized rabbit or rat take up heavy nitrogen in the same way as the globulins, show a similar distribution of N^{15} among their constituent amino acids and have approximately the same half life.[130] Incidentally, passively injected antibody does not show a similar incorporation of dietary nitrogen.[59, 98, 129]

Young animals are known to be less resistant to infection than adults, to have an inferior capacity to produce antibodies, and to have a low globulin concentration in their sera.[142, 143] The new-born calf is highly susceptible to colon bacillus infection.[68] Its blood is deficient in γ-globulin, but within 24 hours after the ingestion of colostrum appreciable γ-globulin appears.[133] Coincidentally the calf becomes less susceptible to infection.[68] Cannon, Chase and Wissler [27] observed that young rabbits fed a low protein diet developed considerably less capacity to form agglutinins when injected with a formaldehyde killed suspension of typhoid bacilli than did well-fed rabbits of the same age. Similar results were obtained in comparisons made between adult rabbits rendered hypoproteinemic by protein deficiency and plasmapheresis and adult rabbits that were well-fed.[27] These findings have been substantiated by tests on rats in which the control animals were fed adequate diets, but the experimental animals were rendered markedly hypoproteinemic by maintenance on diets low in protein. All rats were then injected with a suspension of washed sheep's erythrocytes. Titrations of their sera for hemolysins six to eight days later indicated that the well-fed animals had fabricated about ten times as much antibody as the protein-depleted animals. Moreover, the depleted animals showed a much greater tendency to develop spontaneous infections. A subsequent study from the same laboratory [159] has corroborated these observations and has indicated further that repletion by feeding a high quality protein for as short a period as two days before antigenic stimulation leads to a detectable increase in antibody production; in only seven days the capacity approximates that of

the control animals. In severely depleted rats the authors noted atrophy and simplification of the bone marrow and atrophy of the lymphatic elements, particularly the spleen. Prompt restoration of normal capacity to produce antibodies even after 191 days on low-protein intake suggested that the antibody-producing mechanism could not have suffered serious permanent damage in that period.

Of relative interest is the recent case history [76] of a 15-year old girl suffering from hypoproteinemia produced by malnutrition. The subject had a total plasma protein level of 3.5 per cent and was edematous. Particularly striking was the extreme depression of the γ-globulin content to 0.16 per cent and its elevation after two months on a high protein-high calorie diet to 0.68 per cent.

The work of Cannon has been criticized on the grounds that in his experiments resistance to bacterial infection or response to virulent pathogenic organisms was not tested, but that antibody production, as he uses the term, refers primarily to the response obtained in the application of a technical procedure. In a field as complex and varied as this one, however, such a beginning seems at least to provide a foothold. In a recent review, Cannon indicates that one of his associates (Wissler) has obtained unpublished data which show that hypoproteinemic rabbits and rats have an impaired capacity to acquire resistance to infection with virulent pneumococci [25] (see Chapter 8). Sako has reported that young albino mice maintained on high protein diets for six weeks before being injected with a standard multiple lethal dose of pneumococci showed a much longer survival time than rats fed low protein diets.[124]

On the basis of evidence that mice fed commercial mouse feeds were much more susceptible to fatal infection with type I pneumococcus than were mice fed a standard purified basal diet, Hitchings and Falco [62] have suggested that there may be dietary factors which favor infection by stimulating the propagation of the infective agent.

The relation of protein deficiency to avian malaria has been studied in chicks fed diets varying in protein content from 1 to 32 per cent, beginning 24 hours after hatching.[131] On the eleventh day half of each group was infected with Plasmodium lophurae. In the infected chicks fed the high protein diet the course of the infection was mild; in the protein-deficient chicks it was severe. Diets low in protein, but not sufficiently so to decrease the blood protein level, nevertheless markedly influenced the course of the disease.

Resistance to Virus Infections. Since infective agents differ widely in nature and mode of action, and specific agents may show mutations in virulence, the mechanism of resistance to infection may also be expected to vary widely. Thus, resistance to virus infections, according to the limited evidence at present available, seems often to be enhanced, rather than impaired, by nutritional deficiency. This was early shown to be true of sus-

ceptibility to the sarcoma virus in chickens [121] and more recently to spontaneous or induced tumors in mice.[141] Unhealthy or malnourished rabbits [108] or rabbits on a starvation diet show fewer and smaller skin lesions after infection with vaccinia virus than do well-fed animals.[136] Most of the rather sparse evidence available is concerned with the effect of vitamin deficiencies rather than with a deficiency in protein.

The virus requires the presence of living tissue for its propagation and there is evidence that it attaches itself to the structural elements of the infected cell.[104] In a discussion of the associations between a virus and its host, Pirie [104] observes that interest in the properties of viruses has directed attention away from the problem of their state and method of multiplication in the host and from the problem of the mechanism by which they induce their characteristic symptoms. Viruses which have been modified or partially inactivated by detergents, exposure to ultraviolet light, treatment with formaldehyde or some other suitable procedure are used to produce immunity to virus diseases. Killed viruses are ineffective. Since the virus is apparently exclusively parasitic, one may speculate that anchorage within a host cell frees it from danger of attack by the phagocytes, but renders it entirely dependent for its growth upon the nutriment that its host cell is able to provide.[26]

Variations in Resistance of the Host to Infection. Some of the experimental studies on resistance to infection have been criticized because the animals employed were not carefully selected. Schneider and Webster [126] have called attention to the importance of the genetic constitution of the host in a series of tests conducted to determine the effect of infection with *Salmonella enteritidis* in mice of various pure inbred strains entirely free of the infection. The resistance to infection on a "natural" diet consisting mainly of cereal grain and dried milk was compared with resistance to infection on a synthetic diet. The survival on the "natural" diet was significantly greater, but the influence of the genetic constitution of the mice upon their survival outweighed the influence of the diet. This is but another of the troublesome variations which emphasize the complexity of the field. It has, of course, long been known that natural resistance to a number of infective agents varies greatly in intensity and pattern with species or race (or strain), and that differences in capacities may be modified by such factors as age, chilling, overheating, and fatigue, all of which add to the problem of determining definitely the extent to which the supply of body protein may be involved in resistance to infection.

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Chapter 10

Protein and Amino Acid Nutrition in Pediatrics and in Pregnancy

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Introduction

Proteins and their component amino acids are of basic importance to animals and plants because they are the main structural units of protoplasm and they subserve many physiologic functions. Their fabrication by the animal organism requires a continuing supply of incoming essential amino acids. With adequate intake, qualitative and quantitative, the healthy animal synthesizes species-specific and tissue-distinctive body proteins estimated to number 1600 in man [62] and to comprise roughly 16 per cent of the body weight. The existence in the body of labile protein reserves is still controversial; its dimensions, speculative.

The structural specificity of cellular and plasma proteins is reflected in a diversity of functions: serum albumin in osmotic relations; fibrinogen and prothrombin in blood clotting; γ-globulin in antibody production; hemoglobulin in oxygen transport; enzymes in digestion; hormones in metabolic processes; creatine (a derivative of arginine, glycine and methionine) in muscular contraction; melanin (a derivative of tyrosine) in pigment formation; conjugated proteins in detoxicating mechanisms. Besides these and other specific functions doubtless related to distinctive amino acid configurations, the protein molecule as a whole serves the more general function of providing energy. By means of isotopically marked nutrients, Schoenheimer [79] has shown that the products of exogenous (dietary) and endogenous (body) protein metabolism are in dynamic equilibrium and mutually replaceable. From the general protein pool, hemoglobin, plasma proteins and tissue proteins are anabolized or proteins are catabolized for energy as the needs arise.

Maintenance requirements for healthy adults, commonly estimated not to exceed one gram of good quality protein per kilogram of body weight, are greatly increased in pregnancy, growth, lactation and in disease. In the physiologic states, extra protein above replacement quotas is needed to build new tissues for fetal and post-natal growth and to manufacture milk proteins. In illness and convalescence, the extra protein serves to meet a heightened catabolism and abnormal losses and to restore body depletion.

This chapter reviews the role of proteins and amino acids: (1) in children during normal growth and in childhood disease, and (2) in women during pregnancy, normal and complicated.

Nutrition in Pediatrics

Growth Quota in Health

Protein needs for human growth are both qualitative and quantitative. Evidence based on animal studies and human observations embracing dietary surveys, nitrogen balance, creatinine excretion and growth curves indicates a wide flexibility of the growth quota and its modifiability by a number of conditioning factors. They include such dietary variables as the biologic value of protein, coefficient of digestibility, preparatory heating, caloric, vitamin and mineral intake, non-protein roughage and sulfonamide medication; such physiologic factors as age, sex, nutritional status, puberty and the menarche; and abnormal states characterized by an altered protein metabolism. Besides these known variables, other more equivocal issues impair the reliability of accepted protein standards for human growth: (1) the questionable validity of transposing data derived from young animals and human adults to infants and children; (2) lack of clinical criteria for defining optimal as against average or standard growth; (3) paucity of information of the quantitative requirements of each amino acid, singly and in combination, and of the relative potency of natural and unnatural isomers, in terms of human growth-promoting effects; (4) the existence of unidentified growth factors in different proteins, separate and distinct from the known amino acids; (5) irregularities in bacterial synthesis of amino acids in the child's intestine; (6) possible dissimilarities in action of hormones as protein-anabolic and growth-promoting agents in individual children.

Bearing these reservations in mind, an adequate protein intake for the infant and child may be defined as one which contains all the known essential (and some or perhaps all the nonessential) amino acids in palatable and digestible form and in sufficient amounts to meet maintenance needs and to furnish the surplus amino acids for deposition in the body in quantities and proportions compatible with a normal rate and composition of growth.

Qualitative Needs — Amino Acids. Chemically, proteins are large organic aggregates of linked amino acids. They are broken down in the normal processes of digestion into their constituent amino acids. The absorbed amino acids are in part reconverted to structural proteins and in part deaminated to provide energy with the excretion in the urine of non-protein nitrogen. The ratio of anabolized to catabolized amino acid fractions depends on the quality and quantity of ingested proteins and the total caloric intake, and on the energy exchange and nutritional state of the subject. An essential amino acid is one that cannot be synthesized by the animal in

sufficient amounts to meet the requirements for maintenance, growth and physiologic function. The biologic value of a protein in turn largely depends on its amino acid composition and is graded by the ratio of body weight gain to protein consumed or more reliably by the ratio of body protein gain (nitrogen balance) to protein absorbed.

The reader is referred to Chapter 9 for Rose's classification [73, 75] of dispensable and indispensable amino acids with reference to their growth-promoting effects in the young white rat. Rose's findings have since been confirmed by numerous investigators in rats [41, 97] and in mice.[4]

The applicability of Rose's findings to the child must await direct human observations since the requirements for amino acids and other growth factors in man may differ both qualitatively and quantitatively from those of the mouse and rat. Pending long term studies of growth curves and physical fitness of infants and children receiving mixtures of pure amino acids, fortified protein hydrolysates and natural protein foods, final proof is lacking of the minimal quantitative requirements of essential amino acids conducive to optimal human growth and of the nutritive value of the rest of the protein molecule with its twelve or more constituent amino acids and other postulated but as yet unidentified growth factors. Reliance for growth in health remains with the natural protein foodstuffs; use of substitutes is for the present reserved for therapy.

Fortunately, from the practical standpoint of feeding infants and children, the protein foodstuffs of animal origin in common usage (milk and milk products, egg, meat, fowl and fish) contain all the essential amino acids in high concentrations. Although the vegetable proteins (whole grain cereals, bread, potato, nuts and legumes) are generally of lower biological value, often being low in lysine, and have lower coefficients of digestibility, they are valuable adjuvants in dietary supplementation. The content of essential amino acids in some of the common foods used by infants and children is listed in Table 10–1.

Quantitative Needs — Proteins. The quantity as well as the quality of dietary proteins must be adequate to cover (1) maintenance needs — the wear and tear quota and fecal loss * and (2) the growth quota. The wear and tear quota is a measure of catabolized protein and is estimated by analysis of urinary non-protein nitrogen ($\times$ 6.25) under stable conditions. Fecal loss indirectly measures the apparent digestibility of dietary proteins and is determined by nitrogen analysis of the stools ($\times$ 6.25). In health and with customary diets, fecal loss of protein averages 10 per cent of the intake at all ages during the growth period. (For further details on fecal nitrogen loss, see Chapter 2.) The difference between intake and combined

* The specific dynamic action of protein is a by-product and not an intrinsic need. On the customary diets of infants and children, this effect raises the basal metabolic rate by 5 or 10 per cent. Similarly, exercise does not increase the protein requirements, provided the total caloric intake is adequate.

Table 10–1. Essential Amino Acids in Proteins of Some Foods Used by Infants and Children (in Per Cent) (Calculated to 16.0 per cent Nitrogen) [8]

	Milk *		*Egg*	*Meat*	*Fish*	*Wheat*		*Rice*
	Lac-talbumin	*Casein*				*Flour*	*Bread*	*Polished*
Arginine	3.5	4.1	7.0	7.2	5.6	3.9	3.5	7.2
Histidine	2.0	2.5	2.4	2.1	1.9	2.2	2.3	1.5
Isoleucine	4.5	6.5	5.3	3.4		3.7	2.8	5.1
Leucine	12.2	12.1	19.0	12.1		12.0	11.2	7.7
Lysine	8.0	6.9	6.0	7.6	6.8	1.9	2.8	3.2
Methionine	2.8	3.5	3.5	3.2	3.4	3.0	2.3	3.4
Phenylalanine	5.6	5.2	5.2	4.5	4.5	5.5	5.1	6.3
Threonine	5.3	3.9	4.9	5.3	4.4	2.7	2.8	3.9
Tryptophane	2.3	1.8	1.6	1.2	1.3	0.8	1.3	1.3
Valine	4.0	7.0	4.4	3.4		3.4	3.1	6.4

* The protein content of human milk averages 1.25 per cent (0.2 per cent nitrogen), the casein fraction comprising 0.5 per cent and the whey proteins (chiefly lactalbumin) 0.75 per cent. Corresponding values for cow's milk are 3.5 per cent protein (0.56 per cent nitrogen), 2.8 per cent casein and 0.7 per cent whey proteins.

outgo in urine and feces (nitrogen balance × 6.25) provides an estimate of the protein deposited in the body for replacement, for physical growth and presumably for other anabolic functions. Positive nitrogen balances are a requisite for growth.

Besides the magnitude of nitrogen balances, adequacy of protein intake for growth is also judged by the rate and composition of gain in body weight. The latter may be estimated from the ratio of retained nitrogen to retained sulfur (14.2:1), phosphorus (3.6:1) and potassium (3.0:1) or more directly from the ratio of deposited protein to total weight accretion. Normal growth curves conforming to standard tables with concurrent deposition of protein per unit of weight gain in amounts approximating the nitrogen content of the body by chemical analysis (2.0 per cent in the newborn to 2.6 per cent in the adult) [92] presumably characterizes an optimal state of qualitative and quantitative growth and nutrition.

Based on available evidence, the Food and Nutrition Board of the National Research Council [66] has proposed daily protein allowances for infants and children which have been amplified to include premature infants and broken down into shorter age intervals for the infantile period.[51] These allowances, as modified, appear in Table 10–2.

The high protein intake advocated for small premature infants is explained by their rapid growth impulse in the face of frequent difficulty in digesting and absorbing fat.[29] This defect results in the preferential use of dietary carbohydrate and protein for energy. The amounts of human milk and of cow's milk mixtures needed to cover their energy requirements for rapid growth, 120 calories per kg (55 per pound), are shown in Table 10–3. As noted in the table, the latter may be prepared in isocaloric amounts to provide less fluid and fat, higher protein (and calcium and phosphorus) and equivalent or higher carbohydrate. For these and other reasons,[6, 17] heated partially skimmed cow's milk mixtures are preferred in the routine institutional feeding of small premature infants.

Carefully controlled clinical observations [30] and laboratory studies [31] of such mixtures and of human milk favored the former feedings. Average daily weight gains were higher, nitrogen balances were greater and coefficients of digestibility were not lowered by the more liberal protein intakes. Moreover, at equivalent levels of dietary protein, heated human and cow's milk yielded absolute and percentile retentions of nitrogen of similar magnitude. These results are in accord with expectation in view of the similar amino acid composition of human and diluted cow's milk, reproduced in Table 10–4. For older and heavier premature infants and for full term newborn infants able to nurse and to tolerate higher fat and fluid intakes, breast milk remains the food of choice for psychologic, economic, bacteriologic and other reasons.

As growth decelerates in later infancy, the protein intake may be progressively reduced to 3.5 gm per kg per day. As might be expected, the

Table 10–2. Recommended Daily Allowances for Protein [66] Expanded for the Growing Period [51]

Subject	*Age*	*Protein in Grams* *			*% of Dietary Calories Average*
		Total (1)	*per kg* (2)	*per lb* (3)	
Premature †	1 week to 1 month		6.0–4.4	2.7–2.0	17
Premature ‡	1 week to 1 month	3.5	5.0–4.4	2.3–2.0	15
Premature	1 to 3 months	per	4.4–3.3	2.0–1.5	13
Full term	2 days to 3 months	kg.	4.4–3.3	2.0–1.5	13
All infants	4 months to 1 year		4.0–3.0	1.8–1.4	13
Toddlers	1 through 3 years	40	(4.2–2.9)	(1.9–1.3)	(13)
Preschool	4 through 6 years	50	(3.3–2.5)	(1.5–1.1)	(13)
School	7 through 9 years	60	(2.6–2.1)	(1.2–1.0)	(12)
School	10 through 12 years	70	(2.2–1.8)	(1.0–0.8)	(11)
Youths, female	13 through 15 years	80	(1.8–1.5)	(0.8–0.7)	(11)
Youths, male	13 through 15 years	85	(2.0–1.7)	(0.9–0.8)	(11)
Youths, female	16 through 20 years	75	(1.6–1.4)	(0.7–0.6)	(13)
Youths, male	16 through 20 years	100	(2.1–1.7)	(1.0–0.8)	(11)

* Column (1) gives the allowances recommended by the Food and Nutrition Board, columns (2) and (3) the suggested modifications for infants. The figures in parentheses in these columns, beyond 1 year, represent the total allowances in the original recommendations [column (1)] per unit of body weight on the basis of average weights for age groups derived from the tables of Baldwin and Wood.

† Premature infants weighing less than 2,000 gm (4 pounds 6 ounces).

‡ Premature infants weighing 2,000 gm and over.

Table 10–3. Formulas for Feeding Premature Infants per Kilogram of Body Weight and in Percentages of Dietary Calories [51]

Milk				*Protein*		*Fat*		*Carbohydrate*		*Calories*
	Amount (*cc*)	*Sugar* (*gm*)	*Water* (*cc*)	(*gm*)	(%)	(*gm*)	(%)	(*gm*)	(%)	
Human	180	—	0	2.2	7	6.7	50	12.9	43	120
Cow's										
Whole	100	13	50	3.5	13	3.5	27	17.8	60	120
Lactic acid	140	6	—	4.8	16	5.5	41	12.9	43	120
Evaporated	70	6	80	4.8	16	5.5	41	12.9	43	120
Powdered half skimmed	18	11	150	6.0	20	2.2	16	19.4	64	120

Table 10–4. Amino Acid Content (Mg/100 cc) of Cow's Milk and Human Milk from Their Protein Analysis [99]

	Milk		
	Cow's	*Diluted Cow's (1 : 1)*	*Human*
Casein %	2.8	1.4	0.5
Lactalbumin %	0.5	0.25	1.0
Essential			
Arginine	127	64	67
Histidine	63	32	25
Isoleucine	167	83	75
Leucine	490	245	228
Lysine	200	100	94
Methionine	99	50	29
Phenylalanine	177	88	77
Threonine	151	76	63
Tryptophane	47	24	31
Valine	171	86	66
Nonessential			
Alanine	75	37	35
Aspartic Acid	166	83	116
Cystine	27	14	41
Glutamic Acid	680	340	230
Glycine	11	6	0
Proline	250	125	80
Serine	160	80	69
Tyrosine	172	86	73

recommended levels of dietary protein yield declining nitrogen balances with advancing age and decreased growth impulse. Nitrogen retentions averaged 0.3 gm per kg and 50 per cent of the intake in premature infants; [31, 61a] 0.2 gm per kg and less than 40 per cent of the intake in full term infants under 3 months; [61b] and 0.15 gm per kg and 15 per cent of the intake in infants of 5 months and older.[52] These figures in infants contrast with reported nitrogen retention in children from 3 to 4½ years of age [54] and from 4 to 12 years [58] on protein intakes from 2.0 to 3.5 gm per kg. The retentions in the younger group averaged 0.033 gm per kg and 7 per cent of the intake; in the older group, 0.027 gm per kg and 6 per cent of the intake. Despite the declining nitrogen balances with age, the ratio of retained protein to total increment of weight gain approximates the rising nitrogen content of the body [64, 85] in the growing period. Protein intakes under 2.2 gm per kg (1 ounce per pound) in infants may lead to negative nitrogen balances. Intakes above the recommended levels are wasteful: body depots for reserve protein are scanty, excess amino acids entering interchangeably

with fat and carbohydrate into the energy exchange; extra dietary protein may tax the liver and kidneys by increased demands for deamination and excretion of nitrogenous end products; some incoming amino acids (phenylalanine and tyrosine) may be incompletely metabolized by the young infant in the absence of vitamin C supplements.[53]

With further deceleration in the growth curve with advancing age, lower levels of dietary protein per unit of body weight are required for nitrogen retention and body deposition. The daily allowances recommended by the National Research Council are generally acceptable. Based on average weights for each age group, these allowances progressively fall from levels of 3 to 4 gm per kg at 1 year of age to 1.4 gm per kg for girls of 20 years. The percentage of dietary calories derived from protein at these levels remains constant between 11 and 13 per cent at all ages. The bulk of available evidence indicates that these allowances are compatible with normal qualitative and quantitative nutrition as judged by dietary surveys, nitrogen balance studies, urinary excretion of creatinine, growth curves and clinical appraisement.[20, 49, 60, 75, 93] Some observers report improved measurements in children of pre-school and school age on higher protein intakes of 4 gm versus 3 gm per kg.[37] Whether such acceleration is desirable in health awaits further study but it seems valid to state that in under-nutrition from any cause, the higher allowances are preferable.[94]

Dietary Prescription. Human or cow's milk ordinarily comprises the sole protein-containing food in early infancy. Since the protein content of cow's milk (3.5 per cent) exceeds that of human milk (1.5 per cent) and since casein and the whey proteins contain abundant and comparable amounts (per gram of protein) of all the amino acids essential for growth in young animals and presumably in infants (Tables 10–1 and 10–4), the protein needs for growth are automatically met by providing sufficient milk in either form. The earlier concept of a higher biologic value of human milk, erroneously postulated on the basis of a deficiency of cystine in casein, has been discarded with the demonstration that methionine and not cystine is the sulfur-containing amino acid required for growth [76, 102] and that cow's milk contains this amino acid in higher concentrations than human milk (Table 10–4). From the standpoint of protein alone, it seems fair to conclude that in equivalent amounts above maintenance levels, the two milks are equally nutritious and yield comparable gains in weight per gram of nitrogen intake.

A daily intake (from the first few days after birth) of 175 to 200 cc of human milk per kg (2½ to 3 ounces per pound) or of 100 to 130 cc of heated cow's milk (1½ to 2 ounces per pound) covers the protein needs of young full term infants. The daily allowance of cow's milk for young premature infants, as previously indicated, is better set at somewhat higher levels (130 to 140 cc per kg). When the infant is old enough to take additional foods, usually around 4 months of age, other proteins of animal origin (eggs,

meat, fowl, cheese, fish) and some vegetable proteins (cereals, bread, potato, legumes) progressively replace milk as the sole source of protein. In children beyond infancy, it has been recommended that from two-thirds to three-fourths of the protein intake come from animal sources; approximately 50 per cent as milk (750 cc or 1½ pints), 25 per cent as meat and eggs, 15 per cent as bread, cereals and potatoes and the remaining 10 per cent as fruit, vegetables and other foods.[10, 13] With a wide variety in the choice of foods which is good nutritional practice, larger proportions of well chosen vegetable proteins will satisfactorily meet the protein needs for growth. Recent work has shown that supplementation of vitamin-and mineral-enriched wheat, corn or rice proteins with wheat germ, corn germ, soy bean flour, dried cultured or brewer's yeast notably increases the rate of growth of rats.[87, 89] Dietary surveys reveal that the amount and distribution of protein in the daily diets of growing children are below the recommended allowances in many parts of the country.[67]

Table 10–5 lists the protein content of foods commonly used in the feeding of infants and children; and Table 10–6, the daily amounts of each required to furnish the 100 gm recommended by the National Research Council for adolescent boys, 16 to 20 years of age. The recommended allowances for younger children may readily be provided by the same foods in smaller portions. It is of interest, as shown at the bottom of Table 10–6, that the content of each essential amino acid in this mixture of protein foods approximates the optimal [8] and greatly exceeds the minimal [84] calculated daily requirements of the human adult. As previously stated, conclusions with respect to the growing child must be held in abeyance.

Proteins and Amino Acids in Disease

Many childhood (and adult) illnesses, medical and surgical, are accompanied by an altered protein metabolism and nutrition. The common disturbance is body protein depletion and hypoproteinemia (hypoalbuminemia); a less frequent accompaniment is hyperproteinemia (hyperglobulinemia). In a few abnormal states, the pathogenic agent itself is protein in nature.

Body Protein Deficit and Hypoproteinemia (Hypoalbuminemia).

Methods of Assay. Direct measurement in man of body protein content is not practical. Since plasma proteins represent a circulating reservoir in dynamic equilibrium with the more fixed tissue proteins, determinations of their concentrations afford an indirect but usually reliable index of body protein nutrition. Valid assessment, however, requires consideration of the following factors: distortions in total blood volume; infection (hyperglobulinemia); abnormal permeability of capillaries to protein; sequential irregularities, especially in acute deficits, of loss and repair of plasma versus body proteins (see Chapters 2, 8, and 9).

Table 10–5. Protein Content of Common Foods (Edible Portion) *[91]

Of Animal Origin	*Per Cent*
Milk	
whole	3.5
evaporated	7.0
dried	25.8
Cheese	
cream	7.1
cottage	19.2
American (cheddar)	23.9
Egg	12.8
Meat	
bacon	9.1
pork chops	11.9
veal chops	19.2
steak	18.8
ham (fresh)	15.2
liver (beef)	19.8
lamb chops	18.0
Chicken	20.2
Fish	
haddock	17.2
halibut	18.6
mackerel	18.7
salmon (canned)	20.6
tuna (canned)	23.9

Of Vegetable Origin	*Per Cent*
Bread	
rolls	8.2
white	8.5
whole wheat	9.5
Crackers	
graham	8.0
saltines	9.2
soda	9.6
zweibach	10.9
Cereals	
rice (uncooked)	7.6
hominy	8.5
farina	11.5
oatmeal (dry)	14.2
wheat germ	25.2
soybean flour	42.5
spaghetti	13.0
Legumes	
green peas	6.7
lima beans (green)	7.5
lima beans (dried)	20.7
Vegetables (fresh)	
carrots	1.2
potatoes (white)	2.0
spinach	2.3
beans (green, string, wax)	2.4
celery	1.3
lettuce	1.2
tomatoes	1.0
Fruits — all common ones (fresh)	1.0 or less
Nuts — pecans, brazil, cashew, pea	9 to 27

* Tables of Food Composition, U. S. Department of Agriculture, Miscellaneous Publication, No. 572, 1945.

Table 10–6. Amounts Supplying 100 Grams of Protein and Essential Amino Acid Content, Compared with Daily Needs Calculated for Man (Adult)

Milk	1 quart (1 liter)	35
Eggs	2	12
Meat (cooked)	4 oz. (120 gm)	24
Bread	6 slices (200 gm)	18
Potatoes	3 oz. (90 gm)	2
Peas or Beans	2 oz. (60 gm)	4
Vegetables	3 servings (180 gm)	3
Fruit	2 servings (200 gm)	2
	Total	100

	Amino Acids in †					*Average Daily Needs for Man Calculated*	
	Milk 1 liter	*Meat 120 gm*	*Bread 220 gm*	*Eggs 2*	*Total*	* *Minimal*	† *Optimal*
Protein (gm)	35	24	20	12	91		
Arginine	1.5	1.8	0.8	0.8	4.9	1.8	3.5
Histidine	0.9	0.5	0.4	0.2	2.0	0.5	2.0
Isoleucine	1.5	0.8	0.7	0.6	3.6	1.3	3.3
Leucine	5.7	3.0	2.4	1.6	12.7	3.6	9.1
Lysine	2.6	2.0	0.4	0.6	5.6	1.4	5.2
Methionine	1.4	1.0	0.8	0.8	4.0	1.9	3.8
Phenylalanine	2.0	1.1	1.1	0.6	4.8	1.4	4.4
Threonine	1.6	1.1	0.5	0.5	3.7	1.2	3.5
Tryptophane	0.6	0.3	0.2	0.2	1.3	0.4	1.1
Valine	1.9	0.8	0.6	0.6	3.9	1.3	3.8

* Calculated [84] on basis of minimal requirements for maintenance in adults of 0.4 gm of egg protein per kg of weight.

† Calculated and averaged (page 309, ref. [8]) from data of Rose, W. C., Macy, I. C., and Block, R. J.

It has been estimated [77] that in chronic deficiency states, the ratio of fall in total circulating plasma albumin to loss of body proteins is 30:1. If this relation holds for the child, a gradual fall in plasma albumin concentration from 4.5 to 3.5 gm per 100 cc in a youngster weighing 20 kg with a plasma volume of 1000 cc represents a loss of body albumin totaling 300 gm. Reductions of circulating albumin of this order and of (calculated) body loss are not uncommon in chronic childhood illness. These figures gain importance in the face of evidence that the capacity of the human body to regenerate albumin may not greatly exceed a daily rate of 25 gm.[70]

Pathogenesis. The causes and mechanisms contributing to depletion of body and plasma proteins are the same in childhood as in adult illness. They are listed in Table 10–7. Since identical mechanisms operate to produce general malnutrition and since most foods rich in protein are also rich in other nutrients (minerals and the vitamin B complex), pure protein deficiency states are rarely encountered in pediatric (and medical) practice. Coexisting nutritional deficiencies almost always require general dietetic management in addition to specific correction of the protein deficit.

Table 10–7. Pathogenesis of Protein Deficit in Disease

Causes	*Mechanisms*
(1) Inadequate Intake — Qualitative or quantitative	deprivation from poverty or ignorance, intentional restriction, difficult swallowing, anorexia
(2) Impaired Digestion or Absorption Increased fecal loss	deficient digestive enzymes, mechanical interference with absorption due to edema, inflammation or tumors of mucosa, operative resections and short circuiting, hyperperistalsis with diarrhea, excess putrefaction, fistulas, melena, pus in stools
(3) Excessive Loss in Urine Proteinuria (albuminuria)	increased glomerular permeability, decreased tubular reabsorption
(4) Losses through Abnormal Channels	vomitus, exudates, abscesses, transudates, fistulas, ascites, hemorrhage, plasmapheresis, increased capillary permeability
(5) Increased Catabolism Excess non-protein nitrogen in urine.	elevated basal metabolism, fever, poisons, trauma, "toxic destruction"
(6) Impaired Fabrication of Plasma Plasma proteins — hypoproteinemia	tissue damage (liver, reticuloendothelial system)

Although the major cause of body protein depletion in all illness is absolute or relative protein starvation, its magnitude is dependent on the combined action of a number of other mechanisms: faulty digestion and absorption with increased fecal loss; excessive loss in urine (proteinuria); protein loss through abnormal channels; increased protein catabolism; and impaired synthesis of tissue and plasma proteins (Table 10–7).

Consequences. Table 10–8 records the major effects of body and plasma protein depletion in animals and man. In all states of protein deficit, irrespective of the cause, the albumin fraction falls first and to a greater extent than globulin. The sick child is especially susceptible to severe deficits because the heightened protein needs of the illness itself are superimposed on his already high requirements of growth. The latter must continue to be met in illness despite: (1) a normally lower content of body and plasma proteins; (2) a higher content of body water; (3) a relatively higher metabolism; (4) a frequently coexisting hypochlorhydria which may render dietary protein less assimilable.

Table 10–8. Major Consequences of Tissue and Plasma Protein Deficit

(1) Disturbances of Water Balance
  (a) Reduced blood volume — shock
  (b) Increased interstitial fluid volume — edema
(2) Retardation of Wound Healing
  (a) Delayed cellular proliferation — wounds, ulcers (trauma, surgery)
  (b) Delayed epidermization — burns
  (c) Delayed callus formation — fractures
(3) Lowered Resistance to Infection
  (a) Impaired cellular defenses — phagocytosis
  (b) Impaired humoral immunity — antibody formation
(4) Lowered Resistance to Intoxications (Poisons, Anesthetics)
  (a) Impaired deamination of amino acids
  (b) Retarded synthesis of conjugated and plasma proteins
(5) Anemia
(6) Disturbances Based on Specific Amino Acid Deficiencies
(7) Muscle Atrophy and Fatiguability
(8) Retardation of Growth

These predisposing factors undoubtedly contribute to the high incidence of hypoproteinemia, anemia, nutritional edema, muscular weakness and stunting of growth observed in sick children and in those receiving faulty diets.[23] This predisposition and the lowered resistance of premature infants to infection and intoxication may also be related to their physiologic hypoproteinemia. Reported concentrations of plasma proteins in these subjects [22] range below the low levels of full term infants at birth: total proteins, 5.5 gm per cent; albumin, 3.8 gm per cent; globulin, 1.7 gm per cent. Adult levels of 7.0 gm, 5.0 gm and 2.0 gm, respectively, are reached at around two years of age. Chronic protein deficiency of mild degree is doubtless more common in infants and children throughout the growing period than is generally recognized.

The newer knowledge of the important role of protein nutrition in trauma (Chapter 13), surgical conditions (Chapter 11), infections (Chapter 9),

intoxications (Chapter 14) and anemia (Chapter 9) is amply presented elsewhere and its pediatric implications are obvious. Although direct observations of the practical value of liberal protein intakes on the course and outcome of these conditions in children are sparse, the accumulated evidence derived from studies in animals and human adults [68] points to the importance of restoring body protein deficits and maintaining good protein nutrition in all protracted and debilitating diseases of infancy and childhood.

The nutritional anemias of infancy are usually a sequel of iron deficiency but the contributory role of protein deficit cannot be neglected since: (1) the protein, globin, comprises 95 per cent of the hemoglobin molecule; (2) proteins and lipids constitute the stromal network of red blood cells; (3) the extrinsic factor in erythrocyte maturation is presumably protein in nature.[7] It is of pediatric interest that the proteins and amino acids best suited for hemoglobin formation, erythrocyte production, granulocyte stimulation and plasma protein regeneration were also found to be best suited for somatic growth in the young rat.[50, 69, 72]

Only a few amino acid deficiency syndromes, based on lack of a specific amino acid, are known. Available evidence points to the importance of valine lack in the production of marked muscular weakness; of arginine lack in azoospermia; [40] of methionine lack in corneal opacity and vascularization; [88] of cystine lack in hair growth; of lysine lack in feather pigmentation [26] and of tryptophane lack in cataract formation and in the production of both male and female sterility.[45] The major roles of histidine and tyrosine in antigenic specificity, of tyrosine (diiodotyrosine) in thyroxin, adrenalin and melanin formation, and of methionine and cystine in the fabrication of the growth and adrenocorticotropic hormones of the anterior pituitary [56] have also been demonstrated. As the precise role of each amino acid, based on specific physiologic function, becomes known, its contribution to the bodily enzyme and hormone systems concerned with biocatalytic reactions and detoxicating mechanisms will be recognized and a larger number of specific amino acid deficiency syndromes identified in man (see Chapter 7).

A serious consequence of tissue protein depletion is muscle wasting. Since such losses antedate and usually exceed the fall in plasma proteins, weakness, fatigability, lassitude, bradycardia, lowered basal metabolism and mental depression may occur in the absence of hypoproteinemic edema. Conversely, the latter when present may mask a coexisting emaciation.

It is not surprising that inadequate provision of proteins of high quality results in marked stunting of linear growth. Both fetal growth *in utero* [12] and postnatal growth are notably impaired [42] by maternal and childhood low protein diets. Childhood dwarfism, as seen in the clinic, is usually not the direct result of protein inanition but irrespective of other types of therapy (hormonal), abundant dietary protein of high quality is basic in treatment. Utilization of ingested protein for body deposition and growth

has been shown to be enhanced in these conditions by the administration of the growth hormone of the anterior pituitary and especially by the androgenic steroids, methyl testosterone, testosterone propionate and free testosterone. They are potent protein-anabolic and growth-stimulating agents in man.[46, 98]

Illnesses. The conditions of pediatric interest most frequently complicated by protein deficits are listed in Table 10–9.

Table 10–9. Common Illnesses with Protein Deficit

(1) Of Dietary Origin
- (a) "Famine" or nutritional edema
- (b) Anorexia nervosa
- (c) Prescribed diets
  - obesity
  - allergy
  - kidney disease
  - vegetarian regime

(2) Alimentary Disease
- (a) Esophageal anomalies
- (b) Pylorospasm and stenosis
- (c) Peptic ulcer
- (d) Enteritis and colitis
- (e) Infantile diarrhea
  - fermentative
  - putrefactive
- (f) Celiac disease
- (g) Pancreatic fibrosis

(3) Infections
- (a) Acute
- (b) Chronic
- (c) Convalescence

(4) Metabolic Disease
- (a) Thyrotoxicosis
- (b) Diabetes mellitus

(5) Kidney Disease
- (a) Benign albuminuria
- (b) Nephritis
- (c) Nephrosis

(6) Liver Disease
- (a) Hepatitis
- (b) Cirrhosis

(7) Trauma
- (a) Wounds
- (b) Fractures
- (c) Burns
- (d) Hemorrhage
- (e) Shock

(8) Other Surgical Conditions
- (a) Preoperative
- (b) Anesthesia
- (c) Operative procedures esp. of G. I. tract
- (d) Postoperative

In the healthy infant and child, the coefficient of digestibility of protein is high, only about 10 per cent of the intake being lost in the feces. This high tolerance for protein is least impaired of all the organic foodstuffs in systemic and most digestive disorders. This fact, doubtless ascribable to the high levels even in early infancy of gastric rennin and pepsin, pancreatic trypsin and chymotrypsin and intestinal peptidases, explains the early use of feedings high in protein and low in fat and carbohydrate, in the recovery period of the listed alimentary diseases of infants, except for putrefactive diarrhea. This infantile disturbance, due to abnormal break-

down of amino acids by intestinal bacteria with the production of toxic amines (tyramine, histamine, indole, skatole) or of fatty acids (formic, acetic, propionic and other volatile acids), inducing intestinal hypermotility, is rarely productive of severe body protein depletion.

The minimal amounts of unaltered protein normally absorbed into the blood stream have been shown to be increased in digestive disturbances of early life.[2] For this reason, predigested protein in the form of protein hydrolysates or amino acid mixtures is in theory the food of choice in gastrointestinal disorders of infancy and in practice it is gradually replacing native proteins in treatment during the acute stage of many of these conditions.[18, 35, 81, 82] This form of therapy has the advantage of minimizing the hazard of subsequent hypersensitiveness.

A multiplicity of factors operates to deplete body and plasma proteins in kidney disease. In acute nephritis, anorexia, vomiting, abnormal capillary permeability, hematuria and heightened protein catabolism are the initiating factors. On these are superimposed albuminuria, altered qualitative [28] and impaired quantitative regeneration of plasma albumin and often ill conceived therapeutic restriction of protein intake in the chronic and nephrotic stages of disease.

Although impaired synthesis of plasma proteins is seen primarily in liver disease, congenital or acquired, it has also been implicated in greater or lesser degree in the hypoproteinemias encountered in the nephrotic syndrome,[28] hyperthyroidism, infections, surgical anesthesia, intoxications [32, 48] and even as a late effect in simple nutritional edema arising from protein starvation.[95]

Dietotherapy. The dietary regimen should contain abundant amounts of all the known nutrients and sufficient protein to maintain positive nitrogen balances in all chronic and in most acute diseases. In contrast to adults, children usually require from 2.5 to 5.0 gm or more of dietary protein or its equivalent per kg of body weight per day, depending on age and the extent of body protein deficit. Only in acute hemorrhagic nephritis with marked hypertension and azotemia are lower levels of intake temporarily indicated.

Oral feeding with natural protein foods is the most satisfying and effective means of supplying amino acids in illness as in health. Even high intake of protein foods of good quality or protein concentrates may fail to meet the increased needs of illness, or poor appetite and disturbed digestive function may interfere with sufficient ingestion and absorption of native proteins. In these instances, supplements by mouth, gastric gavage or jejunal tube, of fortified protein hydrolysates are required. Rectal alimentation is of little value in children because of variabilities in absorption.

When enteral feeding is impossible, undesirable or inadequate and in acute states of plasma protein deficits,[24] resort is made to parenteral administration. The protein products adaptable for this purpose include whole

blood, "modified globin," human plasma or concentrated serum albumin solutions (salt-free or salt-poor), protein hydrolysates and pure amino acid mixtures.

Whole blood transfusions are least suited for correction of protein deficits uncomplicated by anemia since hemoglobin is not strictly a nutritional protein and it does not contribute freely to the protein pool.[96] "Modified globin" has been used with more promising results but verification of its value awaits further observations.[86]

The special role in therapy of human blood plasma and albumin solutions [43] is the correction of acute intravascular protein deficits occurring in shock due to hemorrhage, burns, intestinal obstruction, idiopathic or secondary impairment of plasma protein fabrication. These solutions directly replace plasma proteins without preliminary amino acid synthesis by the liver. Concentrated salt-free or salt-poor plasma albumin solutions are especially indicated in severe liver and kidney disease with ascites and edema because of their diuretic action as well as their replacement value. Their use in children over protracted periods as the sole source of protein requires fortification with isoleucine in the case of human plasma and with isoleucine and tryptophane in the case of plasma albumin since, unfortified, they do not support growth in young animals.[38]

Protein hydrolysates in a 5 per cent solution combined with dextrose by intravenous or if necessary by subcutaneous route are valuable for restoring tissue and plasma protein deficits of long standing. In adequate amounts, orally and parenterally, they have been shown to yield positive nitrogen balances not only in animal and human adults but in sick infants and children in a variety of conditions: nutritional edema, allergic states, malnutrition, liver disease, colitis, nephritis and nephrosis, hyperthyroidism, burns, peptic ulcers and in other medical and surgical conditions requiring replenishment of body and plasma proteins.[5, 14, 25, 35, 36, 47, 82, 83] By-effects including flushing, warmth, abdominal pain, nausea, vomiting and venous thrombosis, as well as excess loss of amino acids in urine, may be largely prevented in children by regulation of the rate and intervals of injection to not more than 10 or at most 20 gm of amino acids per hour. Purified amino acid mixtures by mouth or in a 5 per cent solution by parenteral route are well tolerated by animals and man. With the exceptions of aspartic and glutamic acids they may be given more rapidly by intravenous injection and they cause less clinical disturbance than hydrolyzed proteins.[19, 59] They are, however, not yet freely available and the therapy is too costly for extensive use.

The reader is referred to the appropriate chapters for details of dosage, routes and technics of administration in the dietetic management of individual diseases.

Hyperproteinemia (Hyperglobulinemia). Hyperproteinemia or plasma protein concentrations above 8.0 gm per 100 cc in the absence of hemocon-

centration is a rare accompaniment of illness and is invariably due to an elevation of the globulin fraction. Its incidence in adults is less than 1 per cent and it is even lower in children.[15] Hyperglobulinemia of mild degree is common in systemic infections as part of the body defense mechanisms (antibody formation) and is less frequent in diseases of the liver and of the recticuloendothelial system elsewhere in the body. A concomitant hypoalbuminemia of nutritional origin often masks the rise and results in seemingly normal concentrations of total plasma proteins. The infections most consistently accompanied by elevations in plasma globulin are all rare in children: Boeck's sarcoid, leprosy, kalazar, schistosomiasis, chronic suppuration, malaria, tuberculosis, syphilis, trypanosomiasis, rheumatoid arthritis and particularly lymphogranuloma venereum and subacute bacterial endocarditis. The non-infectious illnesses frequently exhibiting hyperglobulinemia such as multiple myeloma and cirrhosis of the liver are also rare in children. The slightly elevated globulin levels sometimes seen in lymphoid and myeloid leukemia are of no diagnostic value.

Diseases of Protein Origin. In a few abnormal states, the inciting factor itself is of protein origin: protein hypersensitiveness, congenital defect in plasma protein synthesis, inborn errors of amino acid intermediary metabolism.

Allergy. Food allergies are more common in infants and young children than in later life. This predilection is probably explained by the greater permeability of the intestinal tract of these subjects to unaltered protein.[57, 71, 100] The most commonly implicated protein foods are milk, egg, wheat, meat and fish. In order of increasing age, clinical hypersensitivity is manifested by infantile eczema, angioneurotic edema, mucous colitis and bronchial asthma. Detection of the responsible protein or proteins is made preferably by therapeutic test (ingestion) or by skin tests, the latter yielding reliable results in roughly one half of the cases. If possible, avoidance of the specific foodstuff with the substitution of other protein foods is the therapeutic method of choice. If sensitivity to multiple proteins is present, elimination diets may be so low in this foodstuff as to lead to body protein depletion. This eventuality may be obviated by attempts at immunization (desensitization) which are often unsatisfactory or preferably by resort to protein hydrolysates which have lost their biologic specificity. When amino acid mixtures become more freely available, they will undoubtedly serve an increasingly important therapeutic role in food allergies.[39]

Congenital Hypoproteinemia. Occasional reports have appeared in which the existence of a persistent hypoproteinemia and edema is not explained by "lack or loss" of protein. These patients apparently suffer from a congenital defect in plasma protein synthesis. Only 2 of the 13 reported patients with this syndrome have been described in children.[78, 90] Impaired liver function was detected during life or liver damage found at necropsy in the majority of the reported cases.

Inborn Errors of Metabolism. A limited number of congenital aberrations of the intermediary metabolism of proteins or more accurately of specific amino acids are known. Normal metabolic processes are either not carried out or are incomplete. The disorder is generally recognized by the persistent excretion of abnormal urinary constituents. They are usually unassociated with constitutional symptoms in early life (except for phenylpyruvic oligophrenia) and they occur more commonly in males.[27] No specific therapy is at hand.

Cystinuria is a rare anomaly, characterized by the constant excretion of abnormally large amounts of cystine in the urine, appearing as clear, colorless, hexagonal crystals. The relative insolubility of cystine at the pH of normal urine (acid) predisposes to the formation of calculi. Avoidance of urinary infection, rather than restriction of protein intake or alkalinization of the urine, is the prophylactic method of choice for combating the tendency to calculi formation. Since the administration of methionine and cysteine leads to an augmented excretion of cystine in a cystinuric patient, whereas cystine itself is completely oxidized, the fundamental defect may be in cysteine catabolism.[11, 55]

Alkaptonuria is an inborn error of phenylalanine and tyrosine metabolism transmitted by a recessive Mendelian character, in which the intermediary metabolite, hemogentisic acid, is constantly excreted in the urine, causing it to darken on standing (alkalinization or oxidation). Reduction with Fehling's solution and an ammoniacal solution of silver nitrate differentiates it from melanuria (also a dark urine); and the color of the urine, from glycosuria. Constitutional symptoms are usually absent in early life but with increasing age there is a tendency to abnormal discoloration of the ears, sclerae, cartilage, fibrous tissue and tendons (ochronosis) and osteoarthritis. The absence of high levels of aminoaciduria in conjunction with large outputs of total phenol and organic acids (not homogentisic acid) suggests to recent observers that other unidentified phenolic (but not keto) organic acids are present in the urine.[33]

Tyrosinosis has been reported in only one adult patient.[63] The urinary excretion of tyrosine and its keto acid, *p*-hydroxyphenylpyruvic acid, characteristic of this anomaly, is augmented by the ingestion of proteins or of the aromatic amino acids, phenylalanine and tyrosine. The metabolic difficulty appears to be an inability to convert the keto-acid to its next degradation product, 2,5-dihydroxyphenylpyruvic acid, and the absence of homogentisic acid (2,5-hydroxyphenylacetic acid).

Phenylpyruvic oligophrenia is manifested by the constant excretion of phenylpyruvic acid in the urine. This anomaly of phenylalanine metabolism differs from the other inborn metabolic errors in that it is always associated clinically with marked mental retardation, and often athetosis. Blond hair and complexion, physical attractiveness and susceptibility to eczema are frequent accompaniments. The incidence is somewhat less than

one per cent of all feebleminded institutionalized patients. It is transmitted by a single autosomal recessive gene. The defect is limited to the metabolism of phenylalanine and its derivatives, phenylpyruvic and phenylacetic acid; tyrosine and its derivatives having no effect on urinary output. Vitamin C (ascorbic acid) is ineffective in reducing the output of aromatic metabolites.[21] It is generally believed that the basic defect is an inability to dispose of phenylalanine at a normal rate rather than a failure to catabolize phenylpyruvic acid.[44]

In addition to these inborn metabolic errors, it has recently been discovered that the immature infant (and the scorbutic guinea pig)[80] is unable to catabolize completely the aromatic amino acids, phenylalanine and tyrosine, in the absence of supplemental vitamin C. Tyrosine and its derivatives (*p*-hydroxyphenyllactic and *p*-hydroxyphenylpyruvic acids) appear in the urine when the protein intake of 4 or 5 gm per kg is exceeded. They disappear promptly when ascorbic acid is added to the diet. This aberration can also be brought out in full term infants by exhibition of the pure amino acids.[53] Recent studies indicate that the point of action of the vitamin in scorbutic guinea pigs precedes the breakdown to the tyrosine keto acid since, of a substantial number of derivatives and intermediates tested, phenylalanine, phenylpyruvic acid and *l*-tyrosine were the only compounds found to be dependent for their metabolism on sufficient ascorbic acid.[3]

Nutrition in Pregnancy

The importance of maternal nutrition, preconceptual and prenatal, has gained expanding recognition. It is now generally accepted by obstetricians and pediatrists that the mother's diet is a major factor affecting not only her own health in pregnancy, labor, delivery, the puerperium and lactation but also the growth and well being of her developing fetus. If the increased nutritional requirements of pregnancy are not met by an augmented intake, the mother, her infant or both may suffer serious consequences, manifested in the mother by: (1) impaired maternal health; (2) prenatal deficiency disease; (3) complications of pregnancy; (4) difficult labor and delivery; and in the infant by: (1) increased morbidity and mortality; and perhaps (2) fetal malformations.

The precise role of proteins and their component amino acids in promoting good nutrition in human pregnancy is difficult to assay since pure protein deficiency states rarely occur in man, deficiencies of other important nutrients frequently coexisting. With this reservation in mind, this section reviews the available evidence on the subject.

Normal Requirements

Extra dietary protein above maintenance requirements is needed in pregnancy: (1) to meet an increased maternal metabolism; (2) to cover systemic growth of maternal tissues; (3) to promote growth of breasts and prepare

for lactation; (4) to build tissues of the fetus *in utero* and of other products of conception.[46]

The heightened energy exchange can be satisfied by adequate dietary calories as carbohydrates and fat without added protein, *per se*. In preparation for later tissue growth and hormonal development, body deposition of nitrogen, however, begins as early as the tenth week of gestation [49] in amounts apparently exceeding immediate maternal and fetal needs. The net gain of body nitrogen throughout pregnancy has been calculated to range around 250 grams with extreme values of perhaps 200 to 400 grams.[21] Accepting these calculations, a large balance is made available to the maternal organism for direct utilization since the nitrogen content of the infant's body at birth is less than 100 grams.[42] Despite these high nitrogen balances, the extra maternal demands for protein produce a physiologic lowering of plasma proteins from preconceptual average levels of 7.0 gm per 100 cc to 6.2 gm by the sixth month of pregnancy.[34] The subsequent rise in the last trimester of pregnancy is in turn followed by a fall after delivery and a tendency to negative nitrogen balances in the puerperal period, presumably arising from post-partum bleeding, placental extrusion and involutional changes of the uterus and other pelvic organs. Return to normal levels usually takes place around the seventh post-partum day.[35] The formation of milk proteins for lactation during this period emphasizes the need for abundant dietary protein of high biologic value.

Based on available evidence, the Food and Nutrition Board of the National Research Council [30] has recommended that the mother's daily diet contain 85 grams (1.5 gm per kg) of good quality proteins during the latter half of pregnancy and that this intake be further raised to 100 grams (2.0 gm per kg) during lactation. From one half to three fourths of the dietary protein should be of animal origin. Presupposing a preexisting state of good protein nutrition, these allowances appear to be wholly acceptable. Unfortunately, a number of dietary surveys have revealed the high incidence of protein intakes below the recommended levels in all parts of the country.[3, 7, 14, 17] In roughly one half of the reported cases, the daily intake was less than 60 grams of protein (1.0 gm per kg). These inadequate diets are due to poor economic status, ignorance, food habits or idiosyncrasies, ill-conceived therapeutic restriction, aversion to food and anorexia, nausea and vomiting, acting singly or in combination. Superimposed on inadequate intake and heightened requirements a coexisting physiologic hypo- or achlorhydria,[23, 37] most marked in the early months of pregnancy, may further impair effective digestion of already poor diets. Under the stress of pregnancy, borderline states of nutrition may be precipitated into overt clinical deficiencies.[31] The data collectively demonstrate that satisfactory prenatal diets, including abundant amounts of high quality proteins, reduce the complications of pregnancy and labor and promote the health and growth of the infant at birth and in the neonatal period.

Undernutrition

When the protein intake does not satisfy the dual demands of mother and fetus, both organisms suffer. The old concept of a prior claim by the fetus on available nutrients has been largely invalidated by recent work.[43]

Effects on Mother. Satisfactory evidence is at hand to implicate poor protein nutrition in such complications of pregnancy as hypoproteinemia and edema, macrocytic anemia, preeclampsia and eclampsia and perhaps pernicious vomiting. Moreover, less specific syndromes are doubtless related to the same cause.

Hypoproteinemia and Edema. Nutritional edema with frequent involvement of the vulva, and attributable to hypoproteinemia, is more common in women during pregnancy than in the general population.[4, 9, 45] Methods of assay, pathogenesis, consequences and dietotherapy do not differ in pregnancy from hydrostatic edematous states in other conditions (see preceding section on Nutrition in Pediatrics, and Chapter 9).

Anemias of Pregnancy. The available evidence indicates that hypochromic anemias are common and that they result from the combined effects of lowered intake, increased needs and impaired digestion and utilization of iron chiefly, and to a lesser degree of protein.

Macrocytic anemias, resembling or in severe forms simulating pernicious anemia, are ascribed by different authors to defective fabrication of extrinsic factor due chiefly to deficiency of vitamin B complex [15] or to intrinsic factors or both. Irrespective of the basic mechanisms, insufficient protein is generally conceded to play a major contributory role experimentally in animals (rats and monkeys) [22, 48] and clinically in humans.[3, 5, 22] Bethell and his coworkers found a consistent relation in their patients between the intake of animal proteins, especially when the daily content was under 50 gm, and the incidence and severity of macrocytic anemia. Improvement always followed an augmented intake of proteins of animal origin. These results are confirmed in a recent hematologic study [24] of three groups of women receiving, respectively, daily supplements of meat (5 oz, 83 gm total protein), vitamin B complex (58 gm protein), and self-chosen diets without supplements (58 gm protein) from 4 months before to 3 months after delivery. The first group had significantly higher hemoglobin and red cell values and less edema throughout the periods of observation than the last two groups. They also nursed their infants with more success.

Toxemias of Pregnancy — Preeclampsia and Eclampsia. Little support remains for the old belief that high protein intakes predispose to toxemias of pregnancy, unrelated to preexisting hypertension and cardio-renal disease. Recent evidence, except for one author,[11] points in the opposite direction. Hypoproteinemia (hypoalbuminemia) is a frequent accompaniment of the edema initiated by increased venous pressure in preeclampsia and eclampsia.[6, 12, 13, 37, 38] Whether plasma protein deficit is a primary or

secondary factor in initiating toxemic states, the evidence is suggestive that low protein intakes predispose to and abundant protein intakes correct an already existing hypoproteinemia and edema.[10, 12, 18, 38] Clinical studies show that toxemia, mild hypertension, edema and preeclampsia occur in rising order many times more in pregnant women receiving low protein diets (50 to 70 gm daily) than in those on high protein diets (110 to 120 gm daily).[7, 20, 41] The fallacy of dietary restriction of protein as a prophylactic or therapeutic regimen in the toxemias of pregnancy except in the presence of impaired renal function with nitrogen retention is established. The use of high protein diets as an important accessory measure in treatment does not minimize the established value of salt restriction and other measures.

Reproduction. Only data derived from animal studies are available. Rats fed diets containing 5 per cent or less of protein were found to be incapable of ovulation and reproduction.[17, 32] Not only quantitatively low protein maternal diets but those of poor quality adversely affect fertility in these animals.[26, 33] Supplementation with milk (proteins and minerals) results in the rearing of higher proportions of young per litter than in control animals.[36] The effect of poor maternal protein nutrition on reproductive capacity is apparently less marked in other animal species, such as the rabbit,[16] pig,[8] sheep [19] and horse.[2]

Lactation. Insurance of an adequate supply of breast milk begins in pregnancy. Sufficient dietary protein (100 gm or more daily) of high quality is required: (1) to provide for growth of mammary tissues, approximated at 17 grams of nitrogen or over 100 grams of protein;[27] (2) to supply amino acids for fabrication of the lactogenic hormone of the anterior pituitary, prolactin, required for continued milk secretion;[25] and (3) to furnish the amino acid precursors of the milk proteins, casein, lactalbumin and lactglobulin.[40] According to Macy *et al.*,[27] maintenance of nitrogen equilibrium in the lactating mother requires a daily intake of 2 grams of nitrogen for every gram of nitrogen in the milk. Both in animals [29] and humans,[1, 7, 14, 24] successful milk secretion varies directly with levels and quality of protein intake in pregnancy as well as during lactation. Even calcium utilization by the mother for subsequent lactation has been shown to be affected adversely by inadequate protein intake, calcium absorption at constant levels of intake varying directly with the protein content of the diet.[28] A daily diet containing 1 quart of milk, 2 eggs, ¼ pound of meat or fish will provide 75 gm of animal protein; the remaining 25 gm may readily be supplied from vegetable sources (fruits, vegetables, bread, cereals, legumes).

Other Effects. Besides the relation of maternal protein nutrition to the above specific conditions, the established importance of this foodstuff in regulation of fluid balance, in resistance to infections, wound healing, detoxication of poisons, maintenance of muscle tore and other functions applies with equal force to the period of pregnancy (see section on Nutri-

Table 10–10. Relationship of Birth Weight * and Birth Length to Total Protein in Mother's Diet During Pregnancy (Fourth Through Ninth Month) [7]

	Average Total Protein (gm)					
	Under 45	45 to 54	55 to 64	65 to 74	75 to 84	85 or more
	Birth Weight in Pounds and Ounces					
Boys	6, 8	7, 0	7, 7	8, 0	8, 5	9, 2
Girls	5, 14	6, 14	7, 8	7, 12	8, 1	8, 8
	Birth Length in Centimeters					
Boys	47.6	49.3	50.2	51.4	52.0	53.3
Girls	46.8	48.7	49.9	50.3	51.4	52.4

*No infants under 5 pounds in weight were included in this distribution.

Table 10–11. Relation of Birth Lengths and Birth Weights to Pediatric Ratings Assigned to Infants at Birth and within the First Two Weeks of Life [7]

	Norm *	*Pediatric Ratings* †			
		"Superior"	"Good"	"Fair"	"Poorest"
	Mean Weight in Pounds and Ounces				
Boys	7, 10	8, 6	8, 0	7, 4	6, 11
Girls	7, 8	7, 15	7, 12	7, 1	6, 15
	Mean Length in Centimeters				
Boys	50.6	51.5	51.1	49.9	49.1
Girls	50.1	50.8	50.2	49.2	49.4

* Vickers, V. S., and Stuart, H. C., *J. Pediat.* **22,** 155 (1943).

† The pediatric rating "superior" refers to all of the infants in the group of 216 against whom there was no physical count of any kind at birth, or within the first two weeks of life. "Good" includes all infants in the group who were considered in good condition, except for one or two minor physical counts. "Poorest" includes all infants who were stillborn, died within a few hours or days of life, had a marked congenital defect, were premature, or functionally immature, except that in this table we have excluded those who were premature (weight under 5 pounds). All infants not in one of these three classifications have been termed "fair" (within this group are some infants who were in the "fair to good" range and others in the "fair to poor" range in physical condition).

tion in Pediatrics). The entire course from conception through the puerperium is facilitated by a good dietary regimen containing liberal amounts of high quality proteins. Such intakes are especially indicated in the puerperal period, complicated by post-partum hemorrhage, subinvolution of pelvic organs or albuminuria.

Effects on Fetus and Infant. As previously noted, damage to the fetus and infant may be a sequel of poor maternal nutrition. If extreme, dietary deficiencies may result in resorption, abortion, stillborn infants, premature delivery, full term infants below par at birth and in the neonatal period, and congenital malformations.

Congenital Malformations. In the animal field, striking evidence exists of fetal damage and malformations resulting from inadequacies in the maternal diet.[44] No such evidence is available in man, either with respect to general dietary inadequacy or deficiencies of specific nutrients. Even in animals, data are lacking of the specific role of pure protein deficiencies on the production of congenital anomalies. It may be stated with some assurance, however, that if congenital anomalies in the human are ever of nutritional origin, the dietary deficiency must be exhibited in early pregnancy at the time of most rapid embryologic development and differentiation.

Health and Growth of Infants. The nature of the human problem precludes precise assessment of the role of pure protein deficiency states in the mother on the health and well being of her offspring. Natural protein foods contain a number of essential nutrients (minerals, vitamin B complex) other than protein. Until human studies employing purified protein or amino acid mixtures are made in pregnancy, the results can only be suggestive. The careful studies of Burke, Stuart and their associates indicate a significant relation between the protein content of the mother's diet during pregnancy and the length, weight and general physical condition of her infant at birth. Diets containing less than 75 grams of protein daily and presumably poor in other nutrients, in the latter half of pregnancy resulted in short, thin infants with poor pediatric ratings.[7] Table 10–10 and 10–11, reproduced from their study, summarize their results. Subsequent analysis of the data by Stuart [39] revealed that a closer relationship existed between the protein than calcium content of the diet of the mother, and osseous development and state of calcification of unerupted teeth in the infant. These findings confirm the earlier work of Ebbs.[14]

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Chapter 11

Protein Nutrition in Surgical Patients *

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Introduction

Individuals who are afflicted with serious diseases are almost invariably in a poor state of nutrition, involving protein deficiency. This condition is a consequence of metabolic disorders arising from the disease itself and is aggravated by surgical operative procedures when they have to be performed. It has been recognized that the incidence of mortality is high in malnourished individuals but only recently has there been a clear understanding of some of the basic responsible factors.

Protein deficiency in man and in the experimental animal results in decreased resistance to shock and infection, delayed wound healing, impaired gastrointestinal and liver function, progressive loss of weight and, not infrequently, edema. When such conditions prevail in the surgical patient convalescence is prolonged, complications are frequent and the mortality rate is high. It is therefore imperative that every possible means be utilized to provide the seriously ill patient with good nutrition to correct this state of deficiency, particularly in those patients who must undergo surgery. Otherwise even the best surgical technic cannot accomplish its purpose nor shorten the patient's period of convalescence and hasten his resumption of full activity. These facts are not as yet fully appreciated. Duncan [1] has stated, "I know of no therapeutically effective measure which is more often disregarded than that of maintaining a positive nutritional balance during the course of chronic and acute illness."

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Causes of Protein Deficiency in Surgical Patients

Protein deficiency may result from inadequate intake of food, impairment of digestion or absorption, excessive loss by extra intestinal routes, an abnormally high demand, or because of some failure of utilization. Inadequate intake, either quantitative or qualitative, is the most important and frequent factor leading to nutritional deficiency. The biological utilization of proteins and the protein requirements of healthy individuals and of animals have been described in previous chapters. In surgical patients (in addition to a possible reduced intake for economic or faddist reasons or special dietary restriction), the food intake may be considerably decreased because of apprehension, pain, oversedation, and poor appetite. When gastrointestinal disturbances are present, most often secondary to disorders of the gastrointestinal tract, including diseases of the liver, pancreas, and biliary tract, the net food intake is further reduced due to anorexia, vomiting, improper digestion and absorption, distention and diarrhea. If liver function is abnormal, as is often the case in patients with primary hepatic, pancreatic or biliary tract diseases, or malignancy of the gastrointestinal tract, the synthesis of plasma proteins (particularly albumin and fibrinogen) may be impaired. In some cases, even when a normal supply of protein is ingested and absorbed, there may be a relative inadequacy when the demand is abnormally high, utilization faulty, or losses excessive. These latter considerations have received considerable study in the past few years.

An increased urinary excretion of nitrogenous products following injury, particularly fractures and major burns,[2–9] major operations [10, 11] and serious acute illnesses,[12, 13] has been demonstrated by many investigators. Increased nitrogen excretion, which accompanies the catabolic phase, begins very shortly after the onset of the illness. It reaches its peak during the first week, persisting for two to six weeks, and is then followed by an anabolic period. By and large, the intensity and duration of the catabolic response parallels the severity of the injury. Excretions of nitrogen as high as 45 grams a day have been reported. The urinary nitrogen in most instances is chiefly in the form of urea, but in certain cases a large proportion of "residual" nitrogen has been reported.[7] This so-called catabolic reaction is exhibited markedly by previously well nourished adult males and to a lesser extent by females and children. It is not seen in previously malnourished individuals. The mechanism of the extensive nitrogen excretion is not fully understood. It is probable that there is more than one precipitating factor. In individuals with considerable tissue destruction, such as patients with extensive deep burns, absorption of the autolyzed tissue protein may contribute to the nitrogen products excreted. Atrophy of disuse will account for only a small part of the nitrogen loss.[3] Fever alone cannot account for the increased catabolism.[3, 8, 12] However, the "basal" meta-

bolic rate, which is increased following injury [14] over and above the rise associated with fever, possibly is due to the absorption of specific substances from the injured area [15] which may in part lead to increased protein catabolism. It has also been suggested [16, 17] that augmented gluconeogenesis, brought about perhaps by increased production of the so-called "S" hormone by the adrenal cortex, with a concomitant decrease in the "N" hormone, may account for increased protein catabolism. In any event, whether or not this theory is valid, it has been shown that abnormalities of carbohydrate metabolism, as exemplified by hyperglycemia and hyperlactacidemia, occur in acutely ill animals [18] and in man.[14, 19, 20] A deficiency in one or more essential amino acids is known to evoke negative nitrogen balance. Recently Croft and Peters [21] have shown in the burned rat that methionine will lessen the catabolic reaction. This has not yet been confirmed in man. Attempts to reduce the catabolic response by the administration of testosterone have not been conclusively successful.[9] Similarly, there is no universal agreement that the administration of very high caloric, high protein intake during this period will reestablish positive nitrogen balance in the patient. However, since the length of the catabolic response is variable, it is advisable to institute early intake of diets rich in proteins and calories to take advantage of a possible early transition to the anabolic phase.

In addition to the loss of nitrogenous products in the urine there may be a larger protein loss from the injured surfaces in patients with infected wounds, such as osteomyelitis, pleuropulmonary infection, and burns (see Chapter 13).[22–25] This nitrogen deficit continues until healing is complete. Co Tui [26] has measured losses in wound exudates and from tube drainage of the upper gastrointestinal tract. Losses from surfaces and wounds have not been considered, in the past, in calculating conventional nitrogen balances. It must therefore be realized that errors of great magnitude may be introduced into such studies if these factors are not taken into consideration.

Casten [27] has found hypoproteinemia to be associated with anesthesia. His studies have not been carried far enough to determine whether protein lost from the blood stream in anesthesia is excreted or merely shifted temporarily into the tissues. He also reported a steady reduction in the protein level in the plasma concomitant with any anesthetic agent. If the liver function was impaired preoperatively as demonstrated by a hippuric acid test, reduction of plasma proteins followed even after short operations. Further studies of the effect of anesthesia in protein metabolism are indicated.

Results of Protein Deficiency in Surgical Patients

The negative nitrogen balance occurring in the surgical patient under the conditions described may have in a few days serious effects in the

patients with already depleted body protein and in a few weeks in those with previously excellent protein nutrition. Unless the increased nutritional requirements are met, complications ensue, convalescence is prolonged and a fatal outcome may result.

Body Weight, Hypoproteinemia and Edema. The first objective manifestation of protein deficiency is loss of body weight which chiefly represents body fat. Although this loss is not significant, before the stores of body fat are completely depleted, tissue protein begins to decrease. This is most evident externally by progressive muscular atrophy which is concomitant with a general decrease of protein in all the tissues. This depletion leads to the development of pathological (functional and anatomical) changes in various body organs.

Keys [28] has reported a study on a group of adult males, previously healthy, who were maintained on a low caloric (1760), low protein (49 grams) diet for six months. The mean weight loss at the end of this period was 37 pounds, or 24 per cent of the original body weight. The actual loss of body tissue was greater than accounted for because of the presence of 12 to 16 pounds of excess water in the form of hydremia and edema. Edema occurred relatively late and frequently it was not closely related to the hypoproteinemia.

Furthermore, one encounters hypotension, tachycardia, lowered basal metabolic rate, polyuria, lack of appetite, weakness and mental changes, including confusion, lethargy and depression, in protein-deficient individuals. Similar findings have been reported by Stare [29] and Davidson [30] in studies of malnourished individuals in Europe shortly after the close of the war. A full discussion of hypoproteinemia and edema following protein depletion is presented in Chapter 9. The rate of development and severity of the changes following protein depletion are accentuated in patients with injury or disease. In a survey of routine postoperative cases treated by the usual dietary regime at the Barnes Hospital in St. Louis, Elman [10] observed that loss of weight was universal and often surprisingly great. One patient, for example, suffered a decline of 25 pounds in weight after a relatively uncomplicated cholecystectomy. Riegel [11] reported similar findings in a group of surgical patients treated at the Pennsylvania Hospital. There have also been numerous reports [6, 7, 9] of extensive weight losses in patients with serious burns. Lyons [31] and Sprinz,[32] and many others, observed a weight loss of one-third to one-half of the original weight in American battle casualties with infected wounds or paraplegia. Hypoproteinemia has been reported to occur much earlier in these "surgical" patients than in the uncomplicated protein deficient individuals studied by Keys.

Wound Healing. In 1919, Clark [33] reported an increased "lag" period in the healing of experimental wounds in dogs on a low protein diet, as compared to dogs on a high protein diet. In 1930, Harvey and Howes [34]

showed that wounds in rats on a low protein diet healed significantly slower than in rats on a high protein intake. Shortly thereafter Howes and McKeown[35] found a delay in the healing time and strength of fractures in rats kept on a low protein intake. This was confirmed in dogs by Rhoads and Kasinkas [36] who noted that as long as 76 days after the division of a bone in hypoproteinemic animals there was little evidence of callus formation, whereas animals with normal serum proteins showed good callus formation at the end of 39 days. Hypoproteinemia is known to interfere with the fibroblastic repair that normally precedes the deposition of osteoid repair. Thompson, Ravdin and Frank [37] showed that in 70 per cent of their hypoproteinemic dogs there was marked delay in fibroblastic proliferation and subsequent delay in wound healing. Similar observations have been made in man.[38, 39, 40] It has also been observed that failure of skin grafts is associated with depleted protein stores.[41] It is obvious, therefore, that for wound healing adequate protein intake must be provided. It is noteworthy that the patient who is deficient in protein is usually deficient in other nutrients such as vitamin A, ascorbic acid and riboflavin, which are also concerned with tissue formation.

Gastrointestinal Function. Impaired gastrointestinal function leads to protein deficiency. Protein deficiency from any cause may result in marked disturbances in gastrointestinal function.

Reporting on a series of autopsies on patients with severe malnutrition, Sprinz [32] stated that there was one common finding — an edema of the wall of the stomach, and of the small intestine, particularly the latter. It is well known that an edematous gastrointestinal tract does not function normally.

Prolongation of the emptying time of the stomach [42, 43] and abnormal movement and absorption of food in the small bowel [44, 45] are found in patients with hypoproteinemia. Postoperative intestinal obstruction due to edema of the stoma at the site of an intestinal or of a gastrointestinal anastomosis has been reported in the presence of hypoproteinemia. Reduction of the sodium intake and the intravenous infusion of concentrated human albumin should be useful in such an emergency.

Peptic Ulcers. Several investigators have observed ulceration in the forestomach of rats [46, 47] and in the stomach of dogs [48] in protein-deficient animals. Although peptic ulcers were not observed in significantly increased numbers in malnourished individuals,[29, 30] patients with peptic ulcers frequently have protein deficiencies often to the point of hypoproteinemia. Co Tui [49] investigated the use of protein hydrolysate in a series of 93 patients with intractable peptic ulcers and reported excellent results and healing of ulcers as evidenced by x-ray examination in 47 cases. He suggests that neutralization of the acidity of the gastric juice by the strong buffer action of the protein hydrolysates, relief of digestive effort on the part of the gastrointestinal tract, and the provision of essential protein

constituents for tissue repair are the factors responsible for the prompt response to this hyperalimentation regime. The usual initial Sippy diet furnishes only about one-sixth the amount of protein and one-half the calories that are furnished in Co Tui's regime. Lewis [50] has also reported marked alleviation of symptoms and roentgenologic improvement in patients with peptic ulcers treated in a manner similar to Co Tui's.

Chronic Ulcers of the Skin. Altshuler *et al.*[51] found that hypoproteinemia was present in the majority of patients with chronic ulceration of the skin associated with phlebitis or varicose veins. The administration of an adequate diet supplemented by additional protein hydrolysate resulted in a return of the plasma protein concentration to normal and a concomitant healing of the ulcers. Similar findings in decubitus ulcers have been reported by Mulholland,[52] Riegel [11] and Sprinz.[32] Mulholland [52] found hypoproteinemia in 35 random cases of bed sores, and in 8 of these selected for study he observed gain of weight, rise in plasma protein concentration, and healing of the ulcers when the nitrogen balance was reversed from negative to positive by amino acid therapy. Sprinz [32] states, "even the largest bed sores we have seen among our patients with spinal cord injuries healed rapidly. Without any specific chemotherapy the granulation tissue changes in character from sloughing to healing and epithelialization begins."

Liver. It has long been recognized that diet has a marked effect in exaggerating or mitigating damage to the liver resulting from exposure to definite hepatic poisons. Ravdin [53] demonstrated that the protein component of the diet was of great importance in this respect. In 1935 Weichselbaum [54] showed that liver injury can be initiated by dietary factors alone. Since then several investigators have produced liver damage by dietetic means and it has been suggested that several other factors of dietary origin are also involved. Elman and Heifetz [55] demonstrated impairment of liver function (excretion of iso-iodeikon) as early as the sixteenth day in dogs on low protein diets, and morphological changes in three weeks. Kosterlitz [56] has shown that the losses in liver protein, phospholipids and nucleic acids during protein deficiency are due to loss of liver cytoplasm. Goettsch *et al.*[57] demonstrated impairment of deaminization by the liver of dogs on low protein diets. This occurred after only one week on the low protein diet and increased progressively during the course of protein depletion. Himsworth and Glynn [58–60] have studied the problem of liver injury due to dietary deficiency very carefully and have concluded that there are two distinct types of liver damage following deficient diets. "One is a massive acute necrosis which either kills or leads to post-necrotic scarring and nodular hyperplasia; the other is a diffuse hepatic fibrosis of insidious development which closely resembles portal cirrhosis. The common feature of diets which cause this latter lesion is that they all produce and maintain heavy, fatty infiltration of the liver. Such a deficient diet may be either rich in fat or low in lipotrophic factors. On the other hand,

the massive hepatic necrosis is dependent on the amount of dietary protein consumed and neither the vitamin, choline, fat or carbohydrate content of the diet influences the result. The sole factor is the amount of protein consumed. Different proteins vary in their ability to prevent the lesion, and it has been demonstrated that cystine is essential. Methionine deficiency produces anorexia, arrest of growth, wasting and hypoproteinemia and anemia, but not massive hepatic necrosis of the liver." Himsworth and Glynn feel that the hypothesis that massive hepatic necrosis in man may result from an inadequate supply of protein, whether this inadequacy is brought about directly or indirectly, must be considered seriously.

Shock. Protein-deficient animals [61] and human beings [62] have long been recognized as being particularly susceptible to shock following trauma or operation. It is believed that the reduction in blood volume which may accompany hypoproteinemia is one of the most important factors in the pathogenesis of traumatic shock. Elman and Davey [63] have recently shown that dogs on a high horse meat diet may be more resistant to hemorrhagic shock than dogs on a low or "normal" meat intake. No definite conclusions as to a particular effective ingredient of the meat could be made, but it was suggested that the difference in protein intake was the responsible factor.

Anemia. The interrelationship of hemoglobin and other body proteins has been clearly demonstrated by the work of Whipple *et al.*[64] Hence it is not surprising that individuals with marked protein deficiency exhibit marked anemia and as the patient's nutritional status is improved the anemia disappears. Himsworth and Glynn [59] have shown that rats on a diet deficient in methionine develop hypoproteinemia and anemia. The latter syndrome is characterized by anisocytosis, polychromasia, and poikilocytosis and has the tendency to be hyperchromic and macrocytic. It is accompanied by a conspicuous reticulocytosis with proliferation of type A normoblastic cells in the bone marrow. Hypoproteinemia and anemia have also been observed in animals kept on diets deficient in lysine [65] and phenylalanine.[66]

Infection. The importance of good nutrition in resistance to infectious diseases has long been recognized, but it is only recently that consideration has been given to the functions of proteins in imparting resistance to surgical infections. For further details see Chapters 8 and 9.

The occurrence in hypoproteinemic individuals of postoperative progressive synergistic bacterial gangrene, as described by Meleney,[67] and the increase in local exudation noted by Levenson *et al.*[41] in patients with extensive deep burn, fit in with Cannon's view.[68] Cannon points out that both natural and acquired resistance is affected by the state of protein nutrition. "It is to be expected that profound undernutrition and its concomitant depletion of the protein reserves should influence adversely the mechanism of natural resistance because a protracted period of protein deficiency leads

eventually to marked atrophy of the liver, spleen, bone marrow and lymphoid tissue, and from these tissues most of the phagocytic cells originate. Moreover, inasmuch as protein is the basic material from which all tissues ultimately are constructed, the absence of protein stores necessary for proper construction of leucocytic reserves might also reduce the continued production of phagocytes."

The Evaluation of Protein Deficiency in Surgical Patients

One can readily recognize the existence of protein depletion in an individual at a time when there is marked weakness, weight loss, hypoproteinemia and edema, but to wait for this stage to develop before a diagnosis of deficiency is made is analogous to waiting for an absent pulse and blood pressure before making the diagnosis of shock. Nutritional disturbances may never develop to the stage where gross anatomic changes occur, and yet it is certain that physiological functions are impaired early in the course of depletion. In the past clinicians have often set their sights on anatomical or pathological changes as indicative of deficiencies. This is entirely too late. The diagnosis of deficiency or impending deficiency must be made early if subsequent ill effects are to be avoided.

There are many factors which must be considered in evaluating the state of protein nutrition of an individual. Among them are (1) dietary history; (2) the optimum, previous, and observed weights of the patient; (3) the plasma and total blood volume; (4) the concentration of the total and various plasma protein fractions; (5) the hemoglobin concentration; (6) the nitrogen intake and nitrogen output; (7) estimation of the patient's "strength," *e.g.*, by ergograph tests, etc. It is not often possible to obtain all this data, and in most clinical work judgment must be based chiefly on a consideration of the nutritional history, an approximation of the patient's intake and probable nitrogen loss, serial determinations of the patient's weight and plasma protein concentration. By and large, a persistent and progressive loss in body weight is a fairly reliable guide as to the development of protein deficiency. Abrupt and transient changes in body weight are apt to be due to acute changes in water balance and not necessarily to significant disturbances in protein nutrition (see Chapter 12). Serial weights can be readily obtained in ambulatory patients. In patients confined to bed, weights may be obtained by putting the patient on a weighed stretcher, each end of which is placed on a scale. Slight but persistent changes in weight are often the first indication of changes in the patient's nutritional state. But a gain in weight does not necessarily mean improvement. On the contrary, the patient may be actually deteriorating and the gain in weight may indicate the development of edema. On the other hand, a loss in weight may mean an improvement in the patient's nutritional status, as it may represent the loss of edema fluid coincident with an increase in the tissue and plasma proteins. Large losses in weight may occur

rapidly in seriously ill surgical patients. Daily losses of one to one and one-half pounds and overall losses totaling fifty pounds or more have been reported following major injuries, burns and severe infections.

The significance of plasma protein concentrations in evaluating the state of protein nutrition of the burned patient has been discussed fully in Chapter 13. It should be stressed that the interpretation of the plasma protein concentration data may often be difficult. The state of hydration, the plasma volume, acute plasma and blood losses, and the relative values of the various protein fractions must all be borne in mind. Finally, it must be observed that, except in acute loss, the plasma proteins fall only after long continued depletion, and, conversely, in recovery from protein deficiency the plasma protein concentration may return to normal long before the tissue deficit is made up.

Treatment of Protein Deficiency in Surgical Cases

It is clear from the foregoing discussion of the role of protein nutrition in surgical cases that, in addition to correcting the underlying disorder of the patient, it is imperative to anticipate the nutritional requirements and to meet them before severe malnutrition occurs. The principle of supplying adequate food to patients is simple and easy, but the actual carrying out of this principle is often difficult. An "all out" effort must be made to maintain adequate nutrition. At the start, it must be emphasized that prophylaxis is better than treatment and that the food provided must be a composite mixture providing, in addition to adequate amounts of good protein, sufficient fats, carbohydrates, vitamins, essential minerals and water (see Chapter 3).

Calories. DuBois [69] has pointed out that an adequate caloric intake is just as important now as it ever was, despite the fact that emphasis in recent years has been placed on the protein and vitamin contents of diets. Calories are required to maintain warmth and to furnish enough energy for bodily activity. The caloric requirements of the individual vary according to the balance between total heat production and heat loss. An increase in metabolic rate is characteristic of many diseases. Prominent among these is thyrotoxicosis. However, depending on the nature and severity of injury, an increase in metabolic rate may be as high as plus 50 per cent in addition to the elevation of metabolic rates resulting from any fever which may be present (there is an elevation of about 7 per cent in general metabolism for each 1° in body temperature). It is also noteworthy that the metabolic rate of patients receiving large quantities of protein may be significantly increased as a consequence of the specific dynamic action of the food.

With these factors in mind, it may be appreciated that the caloric requirements of the acutely ill patient are considerably higher than those of the healthy individual. Thus, while the healthy individual at rest may require about 2000 calories daily, the bed-ridden individual may need 3000

to 5000 calories daily. Calories are provided by carbohydrate, protein and fat. It is disadvantageous to attempt to supply a diet composed chiefly of protein since a considerable portion of the protein will be metabolized mainly for its caloric value and very little of it will be utilized for replacement and tissue repair. As a rule, most of the calories should be supplied in the form of carbohydrates since fat is not well tolerated by the gastrointestinal tract of the sick individual. If large amounts of soluble carbohydrates are administered orally, lactose is advocated in preference to glucose since it does not have as great a tendency to form gas in the gastrointestinal tract and cause distention of the bowels.

The manner in which the carbohydrate is given is worthy of consideration. Cuthbertson and Munro [70] fed human adults the same diets and the same number of calories in two regimes. In one, fat, carbohydrate and protein were given at each meal and in the other, the protein and carbohydrate were given at alternate meals. In the individuals kept on the latter regime, there was negative nitrogen balance irrespective of the frequency of meals administered. It is therefore necessary to give the carbohydrate and protein together to achieve the protein-sparing action of the former.

DuBois [69] has also emphasized that it is not sufficient only to prescribe adequate caloric intake to the patient but that the physician is responsible for the actual consumption of the prescribed diet. Heat loss is an important factor. Thus, the temperature of the room and movement of air should be adjusted so that there is no excessive heat waste.

When the patient has recovered from his illness so that he is no longer suffering from any condition besides simple starvation, the experience of Murray [71] may be of value. He found from observations of individuals liberated from German prison camps that they would tolerate enormous diets after their diarrhea had ceased. He allowed the quantity of food to be guided by the appetite and gave rich diets of between 7500 and 9000 calories per day. He feels that an optimum diet of this kind should contain 275 grams of protein, 225 grams of fat, and 1000 grams of carbohydrate. On this regime more than 95 per cent of the patients gained weight quickly and no untoward results developed.

Protein. It has been shown that there is a definite reason for the great increase in the protein intake of the surgical patient. Unless this requirement is met there is progressive depletion of body protein. The treatment of body depletion, with or without hypoproteinemia, depends on increasing the protein intake until the patient is in positive nitrogen balance. This must continue until the protein deficit is fully met. Under optimal conditions it may require a long period of time. Keys [28] found that months elapsed before his experimentally "starved," but otherwise healthy, subjects regained most of their weight and strength.

Just as caution must be exercised in the interpretation of plasma protein concentration data during the development of protein deficiency, so must

we be conservative in the interpretation of this data during recovery. The return of plasma albumin to normal indicates that the treatment is effective but not necessarily complete nor that body proteins are reestablished. The high protein food intake should be maintained until normal weight and strength are regained.

The biologic value of protein foodstuffs is dependent not only on the presence of all indispensable amino acids among their constituents but on the relative amounts of amino acids that are absorbed from the gastrointestinal tract. Generally, proteins of animal origin, such as those in meat, milk, eggs, fowl and fish, are of higher nutritional value than the commonly used vegetable proteins since they are more "complete" from the point of view of their essential amino acid content. However, vegetable proteins supply many of the essential amino acids and in properly selected diets will supplement each other and result in the ingestion of a complete protein food. Several studies [72] in man have shown that nitrogen equilibrium and apparent good health are obtained when only about one-tenth of the total protein in the diet is of animal origin. For further details see Chapter 2 and 3.

Method of Supplying Foods. The most satisfactory method for the maintenance of good nutrition consists of supplying food by mouth. The diet prescribed by the physician must be checked to conform to the prescription and every effort must be made to induce the patient to eat it.[73]

The amount of food ingested by a sick patient is often limited by loss of appetite. However, a surprisingly large amount of food may be eaten even in the absence of appetite, provided attention is paid to the special likes and dislikes of the patient and an effort is made to serve well prepared, appetizing meals when the patient is "hungry" rather than at stated intervals. In most instances diets in which the greater amount of protein and calories are in the form of liquid "drinks" are better tolerated than "solid" food.

One of the simplest and most useful diets is made by suspending 100 grams of skim milk powder in one quart of milk with suitable flavoring. This roughly doubles the protein content of the milk, yielding a preparation containing about 65 grams of protein and 980 calories per quart. Most patients can take one to two quarts of this preparation readily. If more concentrated "drinks" are necessary, many of the oral protein "hydrolysate" preparations can be used. These have been described elsewhere.[50,74–76] Many patients can tolerate larger quantities of these preparations than of "whole protein." Some of these preparations contain fairly large amounts of vitamins, either added or derived from the original source. Protein hydrolysates are not very palatable but can be taken by some patients. They are best given by gavage. Recently, a partially hydrolyzed preparation of lactalbumin of a high biologic value and a very low sodium content [77] has been introduced. It has little tendency to cause gastrointestinal difficulties. Having no particular taste of its own, it can be readily incorporated in drinks flavored to the patient's taste. However,

this product tends to sediment and must be shaken immediately before it is administered to the patient.

In the event that food cannot be given by mouth, either because of lack of appetite, pain or other similar factors, gavage feedings through an inlying gastric tube should be instituted. Once a gastric tube is in place, there is temptation to "pour" down at one time large quantities of food. If this is done, marked gastrointestinal upsets will ensue. It is therefore imperative to attain the desired number, concentration and total quantity of gavage feedings by a gradual increase, extending over several days, in the amount of food administered at one time. A suitable mixture for beginning gavage feedings consists of 200 cc of an equal mixture of skim milk and water every two hours. We have found it most convenient to leave the gastric tube in place continuously, removing it only for cleaning every third or fourth day. Mild sedation of the first day will enable many patients to tolerate tube feeding. Instead of supplying the mixture in intermittent doses, a drip apparatus may be used which, after a short period of training, can be regulated by the patient himself. Completely hydrolyzed protein preparations are very useful in gavage mixtures because of their high solubility and tolerance. They are particularly useful in patients with decreased gastric secretions and those with extensive pancreatic disease. These mixtures may also be used by feeding through inlying double lumen tubes after upper gastrointestinal tract operations and through tubes inserted into the stomach or jejunum.

Forcing the diet orally is at times not beneficial to sick patients. Nausea, vomiting, distention and diarrhea are limiting factors. Under these conditions supplementation by the intravenous route is indicated. Intravenous alimentation is also resorted to where the function of the gastrointestinal tract is impaired (as in intestinal obstruction or edema of the bowel wall), or when it is desired to put the bowel at relative rest, as in ulcerative colitis and peritonitis. At the present time there is no suitable preparation of fat or fatty acids available for intravenous use. Water, salt, vitamins, carbohydrate, protein hydrolysates, and amino acids can be given parenterally. The water and salt requirements have been discussed in Chapter 12. It is preferable to give the carbohydrates as 15 or 20 per cent rather than as 5 or 10 per cent glucose solutions. The purpose is to increase the caloric intake without increasing the amount of fluid. To avoid excess glycosuria, glucose should not be infused at a rate greater than 0.8 gram per kilogram of body weight per hour. It is usually preferable to give the glucose along with or just before or after the administration of protein or amino acids.

Certain precautions must be observed in using protein hydrolysates or amino acid mixtures. First, extreme care must be exercised not to contaminate the solutions before use. Once a bottle is opened, it should be used immediately. If there is any cloudiness in the liquid, the bottle should be discarded. As a rule, two or three hours are required for the infusion of

about 50 grams of a protein hydrolysate. If these solutions are given at a faster rate, nausea and vomiting result. Recently these symptoms have been attributed to the presence of glutamic acid in these hydrolysates.[78] A glutamic acid free amino acid mixture has been investigated by Silber, Seeler and Howe.[79] It has also been reported that small doses of barbiturate [80] increase the tolerance of dogs to infused glutamic acid.

Amino acid preparations containing the ten essential amino acids [81] are under investigation at present but are not yet available for general clinical use. These can maintain nitrogen balance and may be infused more rapidly than the less completely hydrolyzed preparations.

A discussion of the use of whole blood, blood plasma and plasma albumin preparations is given elsewhere in this volume.

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Chapter 12

The Relation of Fluid and Mineral Balance to Protein Metabolism

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A discussion of the intimate relationship that exists between the intake and output of mineral salts and fluid is of considerable importance to our understanding of protein and amino acids in nutrition.

Intake and Output of Fluid and Mineral Salts

The intake and output of fluid and mineral salts in the normal human adult is summarized in Figure 12–1.[1–8] However, in abnormal and even in normal states there are marked quantitative variations. The magnitude of some of the changes which occur under certain circumstances are presented in parentheses in this figure.

Figure 12–1 also shows that somewhere around 8200 cc of fluid is excreted into the gastrointestinal tract daily.[1] This amount varies between 5000 and 10,000 cc [9] and it has been generally accepted that it is derived from the extracellular body fluid and is normally almost entirely reabsorbed. It is evident therefore that when any of this fluid is lost due to a diseased or abnormal state (vomiting, excessive perspiration, diarrhea, drainage from a fistula or intestinal tube) the body may be rapidly depleted of large amounts of water and salt.

Functional Divisions of the Body Fluids

Table 12–1 demonstrates the approximate amount and distribution of the body fluids in an adult but again it should be remembered that fairly large changes may occur under abnormal conditions. Some studies indicate that in many diseased states the amount of gastrointestinal fluid formed may be increased (during vomiting and diarrhea).[10, 11] On the other hand it has been demonstrated that if the gastrointestinal tract is put to rest (by withholding everything by mouth) there is an appreciable fall in the volume of fluid excreted.[12–15] These facts are important in diseased states since too often the loss of fluid and salt is increased when specific measures to reduce the quantity of fluid excreted by the gastrointestinal tract are not taken. If it is necessary to give fluid while a patient is on high suction, care should be taken that it is made isotonic with salt. Crider and

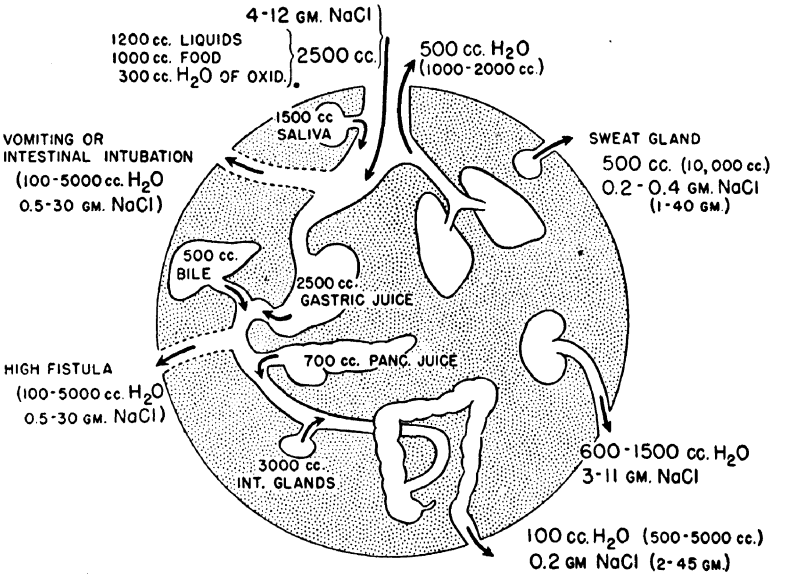

FIGURE 12–1. The normal and abnormal intake and output of fluid and salt. (The possible abnormal loss of fluid and salt is shown in parentheses.)

Thomas [16] have demonstrated that isotonic solutions of glucose or saline when placed in the gastrointestinal tract elicit no secretory response from the intestinal glands or pancreas.

Table 12–1. The Approximate Amount and Division of the Body Fluid

Normal Body Fluid Compartments	*% of Body Weight*	*cc in 154-lb or 70-kg Man*
Extracellular		
Plasma	4–5	3,000
Interstitial	12–18	11,000
Intracellular	40–50	35,000
Total Body Water	Approx. 70%	49,000 cc

General Considerations of the Amount, Movement and Distribution of Body Fluids. The movement of fluid, protein and salts within the body occurs quite rapidly [17] and yet except for small fluctuations the amount and distribution of the body fluid remain relatively constant in the normal individual.

The control of the amount and distribution of these constituents is dependent on many factors, which will be subsequently discussed. However, it should be kept in mind that the function of the kidneys is not limited to

the removal of certain nitrogenous waste products but is largely responsible for the ultimate adjustment or maintenance of a proper fluid and acid base balance.[2] When one considers that 100–200 liters of water and relatively large quantities of inorganic solutes are excreted by the glomeruli daily and that much of these materials is reabsorbed, the importance of the kidneys can be appreciated.

The Solutes of the Body Fluid

The solutes of the body fluids can be divided into three main categories:[1, 2, 4, 8, 18] (1) the organic or colloidal electrolytes; (2) the inorganic electrolytes; and (3) the organic solutes of small molecular weight.

Since these three groups of solutes remain fairly constant in the normal individual and since the osmotic pressure in almost all body media is the same and is dependent on the solute concentration, it can be seen that the amount, distribution and transfer of fluid is dependent to a great extent on these substances. Because there is free passage of water between the various body fluid compartments it naturally follows that the transfer of water and the amount present is largely determined by the hydrostatic and osmotic forces within each compartment.

Organic or Colloidal Electrolytes. For the past ten years much emphasis has been placed on the importance of the plasma protein fractions and the role they play in the determination of the size of the plasma volume. While the amount and concentration of plasma albumin and the other proteins are important in the exchange of fluid to and from the circulatory system their effect on the maintenance of total body water is almost negligible.[19, 20] More recent studies[7, 8, 21] indicate that in diseased or abnormal states, the role of the plasma proteins in maintaining the plasma volume and in governing the exchange of fluid is not as great as it is in the normal individual.

Thus, while the blood volume can usually be temporarily increased by raising the colloid osmotic pressure, the correction of plasma protein deficiencies is frequently difficult in abnormal states because the colloids are being lost from the vascular system at abnormal rates.[22, 23] The amount of fluid in the extracellular and intracellular compartments is important and the factors governing the maintenance of these compartments will be discussed along with those influencing the size of the plasma volume.

Since the blood lipoids come under the general heading of organic electrolytes, their effect on the osmotic pressure should be mentioned. Man and Peters[24] concluded that cholesterol, fatty acids and lipoid phosphorus are restrained by the capillary wall to the same extent as are the proteins. Keys and Butt[25] confirmed their findings and demonstrated that plasma lipoids play but a negligible role in the mechanism of the colloidal osmotic pressure system.

Davis,[26, 27] in discussing the significance of the binding of electrolytes by

plasma proteins, emphasizes the importance of this factor in the transport of molecules from one part of the body to another. This hypothesis was first proposed by Bennhold [28] who believed that the binding property permitted the transportation and distribution of molecules just as hemoglobin carries oxygen. Davis [27] elaborates on this function and points out that the plasma proteins aid in the conservation and excretion of bound molecules by the kidney and that they also protect the body from toxic substances which if not bound would exert a deleterious effect.

Inorganic Electrolytes. It has been established that the inorganic electrolytes exert by far the greatest total osmotic pressure [1–8, 18–20] and, therefore, largely govern the quantity of body water and also to a certain extent the exchange of fluid. Since sodium is the most important extracellular ion and potassium the most important intracellular ion, these two cations have a marked influence on the state and distribution of body water. It is noteworthy that in most instances these two cations remain in their respective compartments.

The fact that sodium rarely enters the cell has led to the generally accepted theory that this cation largely controls the quantity of extracellular fluid.[1, 2, 5, 8, 18] The other extracellular fluid cations (Ca, Mg, etc.) are present in such small amounts as compared to sodium that their osmotic effect is slight. It has also been pointed out that calcium, magnesium and some of the other solutes present in small amounts are partially bound to albumin, and thus the bound solutes are osmotically inactive. On the other hand, changes in the ion concentration of these constituents may affect the permeability of membranes and influence cardiac function. It seems worth reiterating that while several of the anions (Cl, HCO_3) are present in fairly large quantities they do not exert much effect on the amount or distribution of fluid since there is a reciprocal rise or fall of either anion when the other decreases or increases. While the anions do not influence the total quantity of body water they are important in the acid-base balance and this latter factor does affect the distribution of fluid. Van Slyke [29] demonstrated that an intracellular shift of fluid occurred in acidosis and a transfer of fluid from the cell to the extracellular phase occurred during alkalosis. This transfer is due to the greater concentration of proteins in the cell, since the acidification of a protein sol increases the osmotic pressure of the system.[29, 30]

Potassium is located in large amounts in the intracellular compartment and, therefore, largely governs the quantity of fluid present in the cell. Animal experiments support these views and demonstrate that osmotic equilibrium in the extra- and intracellular fluid compartments is generally obtained by the transfer of water rather than solutes.[31–33] Thus, if all cell membranes permit the free exchange of water, but not of sodium and potassium, changes in the concentration of either of these two solutes frequently causes a transfer of water to or from the cell. The aforementioned

experimental studies [31–33] confirm the fact that in many instances when the concentration of sodium decreases, the cells swell due to an intracellular transfer of water, thus decreasing the intracellular potassium concentration and restoring equilibrium. If large amounts of sodium are given which are not excreted or if the extracellular sodium concentration increases due to the loss of fluid, the cell shrinks due to the loss of water.

Recently Darrow [5, 34] and others [35–37] have shown that these accepted theories are not always true. On occasions sodium does enter the cell when potassium has been withdrawn. Darrow [34] also points out that normal or high serum or plasma potassium levels may occur when a deficiency exists and that the intravenous administration of a solution containing this ion may at times be of great benefit if the cells are depleted of potassium.

Organic Solutes of Small Molecular Size. These solutes (glucose, urea, creatine, etc.) are normally of little importance to fluid exchange as they diffuse freely through all cell membranes and thus like water are permitted unobstructed passage into any of the body fluid compartments. Since they may be increased in amount in certain abnormal states the general level of the osmotic pressure of the body as a whole might thus be raised with a resulting accumulation of water. Such an increase would not affect the distribution of fluid between compartments, but would lead to a generalized increase in it.

Factors Affecting the Transfer of Fluid Across Capillary Membranes

The factors influencing the size of the plasma volume in the normal person were first clearly defined in 1895, by Starling.[19] He pointed out that the filtration pressure in the arterial end of the capillaries was greater than the colloid osmotic pressure and thus of sufficient magnitude to force fluid out of the vascular system. Since the plasma proteins are almost entirely retained, the concentration of these solutes is increased as the fluid decreases. Thus, with the increasing osmotic pressure and falling infiltration pressure, fluid is gradually attracted back into the vascular system. This occurs in the venous end of the capillary because the osmotic pressure has exceeded the filtration pressure. Starling [19] also proposed two additional factors: tissue tension and the colloid osmotic pressure of the interstitial fluid. The former factor tends to keep fluid in the vascular system and the latter attracts it away. This hypothesis has been confirmed by other workers [38–40] and emphasis has been placed on the importance of all of the items and their interrelationship with one another.

In the normal individual who assumes the upright position a temporary readjustment occurs. The filtration pressure below the level of the heart is increased due to a slight increase in the force of the arterial stream and a decrease in venous return. Following this the colloid osmotic pressure and tissue tension increase. Thus, very rapidly a readjustment is made. The report of Landis and Gibbon [41] substantiates this view and demon-

strates that a decrease in the plasma volume, an increase in the plasma protein concentration and a slight rise in tissue tension result.

Factors Influencing the Transfer of Fluid in Abnormal States

Although the same factors that influence fluid transfer in normal states are present in abnormal states, nevertheless their importance is often superseded by the following conditions:

(1) Changes in the normal hemodynamics due to alterations in cardiac function, viscosity of the blood and in the peripheral capillary resistance (size, number, and permeability of vessels).

(2) Abnormal states of hydration caused by large or small losses or excessive or reduced intakes of fluid, protein or salt.

(3) Endocrine or kidney dysfunction causing an excessive loss or retention of fluid, salt or protein.

Recently so much emphasis has been placed on the effect of the plasma proteins that attempts to restore a normal circulating blood volume have on occasions been limited to the administration of plasma or albumin solutions. Under certain circumstances this mode of therapy may be satisfactory, but frequently it in itself is inadequate. For example, in patients who have been burned, the capillary permeability in the injured area is greatly increased and plasma proteins have been shown to leak out of the vascular system rapidly.[22, 23] Therefore, while the administration of plasma might produce a temporary increase in the blood volume, the continued administration of fluids or the restoration of the capillary permeability to normal would be preferable. In such states the increased capillary permeability does not return to normal for at least several days.[42] The administration of an electrolyte solution resembling interstitial fluid has been advocated [21, 43] since it passes into the traumatized area and rapidly increases tissue tension. Thus under such circumstances it is logical to believe that by increasing the tissue tension sufficiently a further diminution in the blood volume will be prevented. Some workers [44, 45] have demonstrated that the application of external pressure accomplishes this, especially where injuries of the extremities have occurred. In burned patients, Berman, Pierce, and Best [46] have advocated injecting a salt solution into the injured area, thus increasing the tension and preventing the further loss of fluid from the circulatory system.

In other instances, the abnormal loss of fluid may occur due to a strangulated abdominal organ or to increased venous pressure (cardiac compression, cirrhosis, varicosities, compression or occlusion of veins due to tumor or inflammation). Many such diseased states show reflex changes in arterial flow (leading to anoxia) and also may exhibit a lowering of the plasma protein concentration due to the resulting increased capillary permeability. The ultimate success in the management of such patients is dependent on

the removal of the various causative agents and a restoration of both the arterial and venous circulations to normal.

Butt, Snell and Keys [47] have investigated the accumulation of ascitic fluid in cases of cirrhosis and conclude that it can probably be explained "by the increased venous pressure in the portal system in addition to the reduction in the colloid osmotic pressure and the possible injury of the capillary membrane." In their study the colloid osmotic pressure was determined and it was found that there was no constant relation between the level of serum protein and the colloid osmotic pressure although, as would be expected, a better correlation was found between the osmotic pressure and the serum albumin concentration.

This study again demonstrates the interrelationship of the various factors and it also makes one wonder if the loss of fluid from the vascular system which occurs in certain instances is due to a low protein or albumin level or to an alteration of capillary permeability produced by stasis and anoxia. Undoubtedly the importance of each would vary, depending on the diseased state, but generally in most abnormal conditions, reduced arterial flow, venous stasis and an increased capillary permeability would be the most important factors. In malnutrition edema, the plasma protein concentrations are of far greater concern than in most other abnormal states.[48–51] In cardiac failure and compression, shock and infection, the presence of an impaired venous return, anoxia or of substances increasing capillary permeability which in turn lead to the loss of protein and fluid, are more significant than the finding of a low protein concentration.

The Effects of Changes in Temperature and Humidity. Burch [52] has recently presented interesting studies on the effect of temperature and humidity on congestive heart failure, and other workers [53–56] have discussed the importance of the physiological changes resulting in normal individuals.

Burch [52] pointed out that the rate of water loss from the skin was markedly impaired in patients with severe congestive heart failure in a hot and humid environment. He also demonstrated that on occasions the symptoms of heart failure were precipitated or increased by such an environment and that the rate of sweating returned to normal when failure was mild or absent.

Talbott and his associates [53] stated that there were three clinical entities associated with exposure to high temperatures, (1) heat cramps, (2) heat prostration, and (3) heat pyrexia. The patients with heat cramps showed a depletion of body water and sodium chloride and an increased concentration of hemoglobin and protein. The other disorders were not associated with demonstrable disturbances of electrolyte equilibrium.

Pitts and his colleagues [55] have demonstrated that when young men on a daily diet were fully acclimatized they worked most efficiently when the water loss by sweating was replaced hour by hour. The addition of salt or glucose was of little if any advantage. Taylor, Henschel, Mickelsen and

Keys [56] point out the desirability of giving an adequate daily salt intake (15 to 30 gm) to men working in hot climates and state that if they are maintained on a "low" (6 gm) intake, deficits of sodium chloride occur and the incidence of complications is greater than in those on the higher salt intake.

Conley and Nickerson [57] showed that there was a pronounced and prolonged increase in the plasma volume when a normal individual was exposed to heat, and a reduction in the plasma volume with a tendency to return toward the normal on exposure to cold. These subjects were on an adequate diet and exercise was limited. Thus while these findings are of interest it appears that the fluid volume alterations under such conditions are largely dependent on the amount of salt and water consumed and the extent of the fluid and salt lost.

The maintenance of an adequate caloric, salt and water intake in a hot environment or during fever are important and yet are often not fully understood or carefully considered in combating abnormal or diseased states.

Factors Influencing the Volume of Urine

The influence of the protein and salt intake on the water consumed and upon the urinary volume is of interest. Gamble, Putman and McKhann [58] fed animals certain inorganic electrolytes and urea and recorded the effect on the fluid consumed. They showed that with each added increment of urea or salt the fluid intake rose but when urea was given with one of the salts, the water intake was not equal to the combined sum of both but was greater than either one independently. A later study by Gamble, McKhann, Butler and Tuthill [59] demonstrated that the volume of urine excreted was also dependent to a large extent on the protein and inorganic electrolyte intake.

Effect of Diet on the Urine Volume and Body Water. From the aforementioned studies [58, 59] it seems evident that in dehydration the urine volume is largely governed by the amount of urea and other nitrogenous metabolites to be excreted. Thus if the intake of water is low the body at first gives up its water for the excretion of waste products. Under such circumstances intra- and extracellular salts are eliminated in the urine so as to maintain normal solute concentrations. Eventually the body can spare no more fluid so the blood concentrations of the nitrogenous waste products increase [60] (especially urea nitrogen since it usually constitutes 70 to 90 per cent of the total blood non-protein and urinary nitrogen). For the same reason a more rapid dehydration and a decrease in the survival time occur when a moderate to large dry protein intake is given than when no food or water is consumed.[61] Ordinarily these findings are of little importance, but are of significance in attempting to select the best and most compact dry ration for stranded soldiers. A dry protein regime increased the metabolism

and thus more nitrogenous end-products were formed which ultimately has to be excreted in the urine. This in turn necessitated the withdrawal of body fluid for a larger urine volume even though dehydration was already present.[61]

The administration of small amounts of protein does not influence the rate of dehydration appreciably while the consumption of sugar decreases it and hence prolongs the survival time. Gamble and Butler,[62] and Winkler, *et al.*[60] demonstrated the beneficial effects of glucose even when given in small amounts (100 gm daily) and emphasized its ability to protect the extracellular fluid volume during periods of dehydration.

Thus it is evident that in dehydrated states the extent of dehydration is dependent not only on a reduced intake of water and salt, or an abnormal loss of these constituents, but also on the amount of protein metabolized. Since shock and dehydration accelerate the rate of protein catabolism [63–68] it naturally follows that additional body water and salts might be lost because the body attempts to rid itself of the nitrogenous waste products. If the condition is marked, however, the urine volume decreases due to a reduction in the rate of renal blood flow and under such circumstances there is a rise in the blood non-protein nitrogen concentration.

Campbell, Berry and Iob [69] investigated the effect of intravenously administered glucose in apparently normal hydrated postoperative individuals and found that when large amounts of salt and water were given, the addition of glucose did not influence the rate of sodium excretion. They concluded that this was contrary to the hypothesis of Gamble and Butler.[62] However, their experiments were of a different nature, and while interesting, do not disprove the aforementioned work. The point of practical importance is that in states where the body water is being depleted or where adequate water and salt are not available, the loss can be minimized by carbohydrates (and to some extent fats) because of its protein sparing effect and to a lesser extent from the water formed during oxidation. It should be remembered however that dextrose, or dextrose and water, will not entirely prevent dehydration nor will they alone correct such a state once it exists since the body is dependent on inorganic electrolytes to maintain a proper water balance in the various compartments.

Allen and Cope [70] studied the effect of anesthesia and a high salt, water or protein intake on the kidneys. They pointed out that in hypertensive animals the kidney decreased in size during etherization. This, they attributed to a reduced renal blood flow. Other studies [71–74] indicate that shock and dehydration also lead to a decrease in renal blood flow and a diminished urinary output because of reduction in the amount of fluid available (pre-renal azotemia).

Allen and Cope [70] further demonstrated that an increase in the kidney size occurs from a continued water and salt overload primarily due to hyperemia. The kidney is also enlarged following a prolonged high protein

intake and it is their opinion that this is fairly permanent (actual renal hypertrophy).

The relationship of these studies to clinical problems is not fully understood but it does seem applicable to the management of the burned patient. Following a moderate to severe thermal injury, animals and patients may show an inability to excrete salt and in some cases water in a normal fashion.[21, 74, 75] Similar changes have also been noted in other abnormal conditions.[76–78] Since a negative nitrogen balance also accompanies a burn, and other injuries, it has been felt that the use of extremely high protein intakes might aid in eliminating or minimizing the nitrogen deficit. However, more recent studies [68, 79, 80] show that when such a regimen is started during the first few days after injury, it so influences the quantity of fluid consumed that the amount of water retained by the body might be deleterious. In some abnormal states it therefore seems wise not to increase the work of the kidneys too rapidly but rather to give them time to get readjusted before additional or excessive loads are added.

Value of Blood and Plasma Solute Concentrations as a Guide to State of Hydration or Nutrition. In the past ten years many workers [81–85] have advocated certain blood determinations as clinical guides to the state of hydration or nutrition, or as an index of the type of therapy needed in shock and dehydrated states. In 1923 Marriott [86] pointed out that this was not feasible and Gamble and McIver,[87] Peters [88] and others [7, 8, 89, 90] have emphasized these facts.

Figure 12–2 demonstrates how misleading the hematocrit and plasma protein concentration may be. This figure shows that deficient or normal red cell volumes may be encountered when low, normal or high hematocrits are present. The plasma protein concentration may vary markedly or be within the normal range when the total circulating proteins are increased or decreased. Since the red cell and protein concentrations are largely responsible for the specific gravity of whole blood and the protein concentration accounts for the specific gravity of plasma, it is evident that while such tests indicate the state of the particular sample taken, they would not accurately reflect the state of hydration or nutrition of the body as a whole or the need for specific therapy.

The hematocrit or hemoglobin concentration has been advocated not only as a means of determining the state of hydration but has been considered by some to be a useful method of estimating the amount of plasma needed to combat shock.[85, 92, 93] Experimental [94] and clinical studies [21] have demonstrated how misleading this might be (due to a pre-existing anemia, the destruction or loss of red cells, etc.). In a study [95] of 63 apparently normal males the hematocrit varied between 42 and 53 with a mean of 48.2. While these values were slightly higher than those obtained by Gibson *et al.*[96] the extent of the normal range was about the same. Thus it seems clear that with such a large deviation in the normal individual, and

since anemia or a loss of red cells may occur, these determinations could not be employed as a means of accurately estimating deficits.

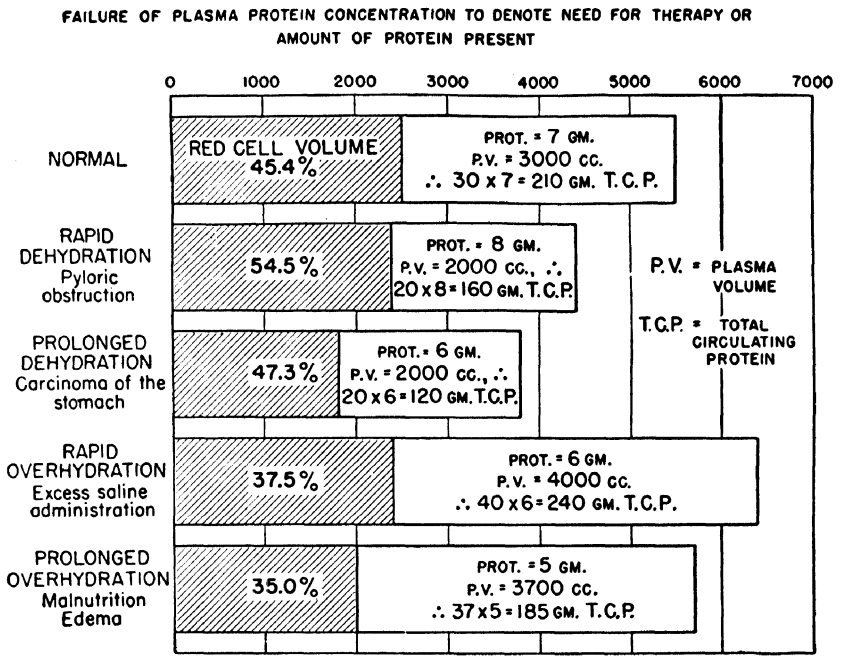

FIGURE 12-2. The failure of the plasma protein concentration to denote the need for therapy, or the amount of total circulating plasma protein present. (The cross-hatched area equals the red cell mass, and the open area of the bar, the extent of the plasma volume.)

In burned patients [21] and also in experimental animals [97] with intestinal obstruction, hematocrit and plasma protein concentrations (or specific gravity determinations) gave little indication of the exact type and amount of therapy needed. Similarly in patients [90] or animals [98] in a more chronic state of dehydration the fall in the total mass of red blood cells or protein often parallels the decrease in the plasma volume so that blood concentrations are of little value and indeed might be quite confusing if the above facts are not kept in mind.

Phillips, Yeomans, Dole, Farr and Van Slyke [99] have recently stated that the blood volume can be estimated within plus or minus 13 per cent of normal by determining the hemoglobin concentration or the specific gravity of whole blood before and after the administration of a known amount of plasma. While such methods may occasionally be of value there is reason to believe that the importance of determining the blood volume in abnormal states has been overemphasized. This is true because there is a fairly large error in the estimated normal plasma or blood volumes

when they are calculated from height, weight or surface area.[95, 96] Also since the accuracy of such determinations is based on the fact that the circulating red cell mass remains relatively constant (although following burns and various other forms of trauma such is rarely the case), and since the test gives no knowledge of the rapidity of loss of plasma and blood cells the blood volume determination would probably be of little clinical value, where control values were not available. Experience has also shown that in shock and hemorrhage the amounts of blood or plasma needed to treat effectively an injured or diseased patient is frequently greater than the quantity lost (due to continued loss, stasis and pooling of blood and changes in the capacity of the vascular system).

The serum or plasma concentrations of the various inorganic solutes (Na, K, Cl, etc.) are likewise frequently not indicative of the state of hydration nor the need for therapy. Table 12–2 demonstrates some theoretical examples which show that high, normal or low plasma chloride levels may accompany deficiencies or increases in the total body chloride. Similar changes in sodium and potassium may occur. Because blood levels frequently do not indicate the specific need for therapy, they should be considered only in relation to the general state of nutrition and hydration and volume and specific gravity of the urine. It is therefore advisable that therapy be guided by a careful evaluation of each individual patient (duration, type, extent and severity of illness, the findings of the physical examination with special emphasis being placed on eyeball tension, appearance of the tongue and turgor of the skin and the blood pressure and renal function). A knowledge of the physiologic and chemical changes that occur are thus important so that the laboratory findings may be interpreted in the light of the aforementioned data.

It has been pointed out that under certain circumstances high plasma potassium levels are encountered in patients with intestinal obstruction [100] or ileus,[101] intestinal fistula,[102] burns,[103] and occasionally in diarrhea.[104] Govan and Darrow [104] stated that remarkable clinical improvement occurred in infants with diarrhea following the administration of potassium if proper precautions were taken. It seems evident, therefore, when high potassium levels are encountered, that this cation is probably leaving the cells in abnormally large amounts and because of dehydration or shock (with a reduced renal blood flow) it is not being excreted rapidly enough. According to Tabor and Rosenthal [105] the administration of potassium shortly after an experimental thermal injury is accompanied by toxic changes and a high mortality rate. This would be expected because, following a burn, potassium enters the extracellular fluid in relatively large amounts and kidney function is frequently reduced. Since in some instances potassium deficits exist for several days after a burn, it might be of benefit to give potassium after kidney function was restored, especially in those cases where the intake of food by mouth was low. Darrow [5, 34] has stressed

Table 12-2. The Failure of the Plasma, Chloride Level to Denote the Total Amount of Extracellular Chloride *

Type of Case	*Plasma Chloride Conc. m. eq. (per Liter)*	*Extracellular Fluid* (cc)	*Total m. eq. of Chloride in the Extracellular Fluid*
Normal	105	14,000	1470
Pancreatic, biliary or high intestinal fistula	118	10,000	1180
Pyloric obstruction	88	10,000	880
Malnutrition, burn and cardiac edema	88	20,000	1760
Obstructions of the jejunum, ileum or colon	105	10,000	1050
		or	
	90	10,000	900

* It should be pointed out that the findings shown in this table are not uniformly encountered. There are variations in normal as well as in abnormal states. The point to be stressed is that regardless of the concentration of the particular inorganic solute in question there is a decrease in the presence of dehydration and an increase in overhydration. The magnitude of this change is dependent on the two factors: volume and concentration.

the importance of establishing an adequate renal function before the intravenous administration of potassium and it is probable that his work will lead the way to a further understanding of the potassium needs in other diseased states. In acute or chronic dehydrated states increased or normal concentrations of hemoglobin, red blood cells, protein, chloride, potassium, etc., are often encountered. These are most frequently found when a reduction in the total amount has occurred.

Effect of Diminished Electrolytes on Nitrogen Balance. Deficiencies of potassium and sodium not only lead to dehydration, but it has also been shown that an inadequate intake of these constituents is accompanied by a negative nitrogen balance and, if prolonged, a reduction in the rate of growth.[86, 106–109] This fact has generally received little consideration. It appears that, while changes in the electrolyte and water content of the body may lead to a negative nitrogen balance, the latter alteration is often of secondary importance.

Studies done on patients convalescing from fractures and burns show that a fairly marked nitrogen deficit may persist for 25 to 35 days and yet the individual will survive.[66–68] On the other hand, if persistent increases or decreases in the body water or salt occur, death often results after several days.

Relationship of Hormones to Fluid, Mineral, and Protein Metabolism

Selye [63, 64] and Albright [65] believe that the changes encountered following many abnormal states are primarily a result of alterations in the hormonal output which result from injury or disease. While further study of the problem is indicated, it seems evident that a characteristic metabolic and hormone response occurs in such states as they describe. The changes occurring in carbohydrate and protein metabolism following the so-called "alarm reaction" have been discussed elsewhere;[68] however the simultaneous effect on water, protein, and salt metabolisn should not be overlooked. As aforementioned, the body can tolerate fairly prolonged periods of a negative nitrogen balance but it does not respond well to acute alterations in dehydration or in the acid-base balance. For example, patients with burns, intestinal obstruction, diarrhea and other diseases leading to dehydration exhibit a negative nitrogen balance and, while this fact needs consideration in the treatment of such cases, a restoration of normal blood pressure, kidney function, and correction of the state of hydration and acid-base balance are of far greater immediate importance.

In chronically ill patients the administration of large caloric and protein intakes are not only beneficial but usually well tolerated.[110–112] In contrast, in the acutely ill (previously healthy) patient, the giving of extremely large food intakes might be detrimental and would also be of questionable value.[68] Under the latter circumstances the very high caloric diets should

probably be temporarily delayed, for several days and preferably until the catabolic phase of the alarm reaction is subsiding (6 to 12 days).

Overhydration. In patients who are overhydrated due to a deficient protein intake, salt and water play an important part in the formation of edema as Jones, Eaton and White [51] and others [2, 88, 113, 114] have shown. The correction of such a state can be accomplished by proper parenteral therapy but regardless of what methods are employed the restriction of the salt intake is important. Studies by Jones and Eaton,[115] and Barden, Ravdin and Frazier [116] have demonstrated that the gastrointestinal tract in malnourished individuals does not function normally. The ingestion of an adequate caloric and protein diet by mouth is not only difficult in such cases but may result in vomiting and diarrhea if the disorder is fairly far advanced. Thus several days of salt restriction and the promoting of a diuresis should suffice to correct the edema. Subsequently the oral intake of a high protein, low salt diet will then be well tolerated and the negative nitrogen balance and hypoproteinemia corrected.[113]

Too often in caring for debilitated patients, attempts are made to correct or prevent low plasma protein levels by plasma transfusions while at the same time other factors are neglected. Such therapy may be of aid, but as a rule it is inadequate. If more attention is placed on the dietary intake of the patient, the presence of anemia or of infection, the administration of adequate but not excessive inorganic electrolytes, etc., the occurrence of such cases would be infrequent and the response to treatment would be enhanced. The administration of plasma alone provides a deficient caloric and protein intake to such patients in whom anemia often exists. The giving of whole blood is preferable under these circumstances.

The increased tendency for edema to occur in the presence of anemia has been demonstrated.[117, 118] The giving of whole blood to malnourished patients is in harmony with Peters and Eisenman's [117] findings, namely: "only a slight reduction of the protein osmotic pressure is required to induce edema in patients with anemia."

Earlier studies [3, 47–51] emphasized the fact that many factors lead to malnutrition edema, and more recent work [8, 113] has clarified the problem and treatment and reemphasized an overall consideration of the problem. It seems evident that the plasma protein concentration *per se* is frequently of secondary consideration [113, 114, 119] since the edema is often eliminated prior to any significant change in the plasma protein concentration. The presence of infection not only causes some reduction in the total plasma protein concentration and hemoglobin but often leads to a greater decrease in the albumin concentration than in the globulin level. Cannon [120] emphasizes the importance of γ-globulin in resisting infection and points out that the antibody or γ-globulin fraction is often reduced when the total globulin concentration is normal. Under such abnormal circumstances when total circulating proteins are determined they may be low, normal or

even elevated. Lyons [121] has pointed out that the extracellular fluid volume increases in infection. It seems evident, therefore, that when infection is present more consideration should be given to the alterations in capillary permeability, abnormal protein losses (from the accumulation of pus, etc.), the retention of salt and water and the general state of protein metabolism as a whole than to the low plasma protein concentration. Certainly, the patient would benefit if the physician sees that an adequate caloric and protein intake is not only ordered but administered to the patient (orally, or if not feasible, parenterally) and that proper measures are instituted to combat infection. The effect of an adequate protein intake in malnutrition edema is probably greater from the natural diuretic effect of protein and the restoration of normal capillary and cell function than it is from the plasma protein concentration. As was previously pointed out, the edema in such cases may disappear long before the protein concentration is restored to normal. In discussing the results of treatment in one of their patients, Spence, Evans and Forbes [114] state: "Probably the greatest factor in the loss of edema was the low sodium chloride regime rather than the actual gain of plasma protein which was in reality quite small."

The importance of the use of high protein, high caloric diets has been recently stressed by Varco [110] and others.[111, 112] In most instances the malnutrition existing in patients with debilitating diseases can be largely corrected preoperatively by a proper dietary regime. If this is done, extensive surgery can be carried out if necessary and the incidence of postoperative complications greatly reduced.

In considering overhydrated states, it should be reemphasized that the elimination of water and salt by the kidneys is an important factor. A decrease in the ability of the kidneys to excrete water and salt has been observed in cardiac failure,[76, 77] endocrine disorders,[65, 122] burns,[21, 87] following trauma [74] and following the administration of desoxycorticosterone acetate.[123] Increases above normal in the plasma and extracellular fluid volumes occur from such a retention of salt and water.[124, 125] It has also been pointed out that, postoperatively, when moderately large amounts of salt solutions are given a retention of sodium chloride and some water occurs which might be detrimental.[7, 8, 90, 126, 127] The retention of fluid and salts by the extravascular system may be due to an increased capillary permeability with a resulting loss of fluid and salts before the kidney can excrete it or because of an endocrine or kidney dysfunction. Such possibilities should be constantly kept in mind and treatment modified accordingly, if lower mortality and morbidity rates are to be had.

Evidence has accumulated indicating that some of the toxemic states observed may be in part due to abnormal hydration.[8, 21, 128] While further proof is needed it should be kept in mind that electrolytes and water changes may occur within the cell while relatively normal blood or plasma concentrations exist. The occurrence of epileptiform seizures, insomnia, and per-

sonality changes have on occasions been thought to be due to deviations from the normal fluid and salt concentrations and while these observations are inconclusive such possibilities should not be abandoned without further investigation.

Dieckmann [129] lists the possible causes of edema in pre-eclampsia and eclampsia (decreased colloid osmotic pressure, increased capillary permeability and increased capillary pressure) and also the contributing factors (decreased tissue tension, increased colloid osmotic pressure of the interstitial fluid, warm environment, impaired excretion of water and sodium chloride and abnormal hormone metabolism). He stresses the fact that it is difficult to determine one causative factor. Such is usually the case, for the edema observed in normal and in abnormal pregnancy as well as in other diseased states usually results from a multiplicity of these factors rather than from any one of them alone.

In normal states [124] and in apparently well nourished cardiac patients,[125] it has been demonstrated that when sodium is given in large amounts, or retained even when relatively normal amounts are consumed, the total circulating plasma proteins show a marked increase. In such instances one should bear in mind that the rise in the plasma volume does not follow the increase in the plasma proteins but is due to the administration of sodium salts. In abnormal or hypoproteinemic states the mobilization of protein is not as marked, hence following the administration of saline solutions there is a decrease of plasma protein concentration due to dilution. This may in part account for the precipitous fall in the plasma protein concentration reported following some major surgical operations.[130–132] It does demonstrate the presence of a plasma protein reserve, as Whipple [133] advocated or at least points out that proteins can be mobilized in an attempt to maintain a normal concentration. The administration of plasma to normally hydrated and nourished animals [134] or individuals [135] is followed by the rapid removal of most of the injected protein. Since the administration of a concentrated albumin solution alone does not restore the plasma volume to normal levels, in the presence of dehydration, the administration of an electrolyte solution is essential under such circumstances.

Dehydration. The experimental studies of Hartwell and Hoguet [136] first emphasized the importance of the administration of saline in high intestinal obstruction. Other studies [97, 137] have demonstrated that fairly marked decreases occur in the plasma and extracellular fluid volumes in animals with low intestinal obstruction as well. Coller and Maddock [138] showed that dehydration might be severe if water was withheld, and Nadal *et al.*[139] showed severe dehydration to occur if salts were removed by high intestinal intubation. These studies [97, 137–139] and other experimental work [33, 140, 141] demonstrated that, even though the plasma concentration may increase during periods of dehydration, a restoration of the plasma volume does not occur unless sufficient extracellular fluid is present.

Peters [13, 14] and Foster [15] have emphasized that when gastric or high intestinal suction is employed the loss of fluid and salts will be almost negligible if nothing is given by mouth. It has also been demonstrated [14, 16] that the intake of limited amounts of isotonic salt solution not only satisfies the patient's thirst and prevents abnormalities in hydration and acid-base balance but also leads to the excretion of smaller amounts of gastrointestinal fluid than would normally be formed. Apparently the reason that problems are not encountered more frequently in patients on high suction is that the usual apparatus does not work efficiently at all times or that the treatment is discontinued before complications occur. When large amounts of gastric or intestinal drainage are obtained, dehydration may be prevented if parenteral salt solutions are given in proper amounts. However, since it is difficult to estimate the patient's exact needs and because the giving of salt solutions would either increase the amount or tonicity of the fluid intake and thus reduce the quantity of carbohydrate and amino acids that could be given parenterally, it seems inadvisable to give food or water when high intestinal or gastric suction is being employed.

When dehydration is present, the injection of sterile solutions of amino acids or of carbohydrates does not correct the deficit of extracellular fluid that exists; however, it is important nutritionally and it helps to decrease the rate of further salt and water loss.

The depletion of sodium and potassium may be marked in diabetic acidosis and in obstructions of the gastrointestinal tract, or in patients with external or internal (gastrocolic) fistulas as well as in diarrhea. The main objectives of treatment with parenteral solutions should be to:

(1) Combat a state of hypotension or peripheral vascular collapse.
(2) Correct a state of acidosis or alkalosis.
(3) Return the state of hydration to normal.
    (*a*) Provide the necessary fluid and salts for dehydrated individuals, (*b*) remove excesses of fluid or to restore the intra- and extracellular compartments to normal, and (*c*) provide water for the removal of waste products by the kidneys.
(4) Correct the state of nutrition by:
    (*a*) Supplying adequate protein, carbohydrate, fats, minerals, vitamins, and water, and (*b*) administering whole blood when indicated or when anemias exist.

Darrow [5] has recently discussed the role of sodium and potassium in alkalosis and he and others have previously emphasized the fact that, while the total amount of body water may be normal, intra- and extracellular derangements might exist which ultimately produce serious complications.

Holler [142] demonstrated the need of potassium in a case of diabetic acidosis and undoubtedly a more complete understanding of these needs will aid in the treatment of other dehydrated subjects.

Since sodium-containing solutions have on occasions been shown to play

an important role in preventing peripheral vascular collapse, or combating it while it is still in the reversible stage, it is evident that the maintenance of a normal circulating blood volume is dependent on many factors (amount of plasma protein, rate of loss, tissue tension, etc.), which should be considered in outlining the treatment.

Emphasis has been made of the importance of determining and correcting the plasma protein fractions in shock, due to hemorrhage, burns, tissue trauma and intestinal obstruction, and again while this is important it is only one of several factors to be considered collectively. It should be kept in mind that hemoconcentration is often accompanied by a diminution in the red cell mass and therefore the administration of blood is frequently of greater benefit than plasma. The importance of combating anoxia in states of peripheral vascular collapse have been stressed, and while the administration of plasma may restore the blood pressure to normal, whole blood is usually preferable as it provides a greater oxygen-carrying power. The amount of protein present in the total circulating hemoglobin is twice as great as in the total circulating plasma protein. It is therefore important to give whole blood not only to reduce the incidence of anemia but also in the hope of sparing amino acids, which could then be used for the formation of plasma and tissue proteins instead of hemoglobin.

An attempt has been made to emphasize some of the important facts involving protein metabolism and certain problems which are intimately connected with it. This presentation was not prepared with the idea of minimizing the importance of protein nutrition but for the elucidation of some misconceptions which might occur.

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Chapter 13

Proteins as Related to Burns

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Introduction

The main object of this chapter is to point out the importance of protein nutrition in thermal burns. Burns are a severe form of trauma. They are accompanied by numerous metabolic changes, particularly those involving loss of nitrogen which immediately follow the injury and loss of protein from the large open surfaces of the burned areas. Studies of such protein losses have been made in several series of burn cases and not infrequently it has been noted that the presence of toxemia causes considerable difficulties in alimentation and proper absorption of nutrients. Thus the degree of protein deficit in patients who have suffered severe burns is often the determining factor of life or death.

The severely burned patient is not only seriously injured but is suffering from excruciating pain that requires immediate medical care. The shock that ensues shortly after the injury may last from 48 to 72 hours. During this phase there is considerable loss of blood and tissue fluids concomitant with a fall in blood pressure. If during this period whole blood or plasma is parenterally administered to replace the blood constituents lost into the tissues or if the patient is able to resist or correct this commonly occurring disorder, the patient appears to be in a fairly normal physiological state, except for the burned area. While the extent of the thermal injury is usually a good measure of the physiologic and hematologic changes that are bound to occur in the patient, this is not always directly proportional. The severity of the symptoms is, however, so nearly related to the amount of surface area involved that it is customary to describe a burn not only in terms of depth but also in terms of the burned surface area. Many formulas have been devised for rapid estimation of the surface area. The simplest one is that of Berkow [5] which gives the following percentages for various segments of the body: trunk 38 per cent, lower extremities 38 per cent, upper extremities 18 per cent, and the head and neck 6 per cent. Recently, Lund, Levenson and Taylor [21] have considered that for their careful studies

of burns a more detailed estimation of the surface area was required to permit measurement of the areas of the smaller segments unaccounted for by the Berkow formula. According to Harkins [5a] "the importance of the proportionate surface area burned is that it may serve as a rough guide to treatment, especially as to the plasma needs during the first 24 hours." This investigator [15] has attempted to relate the degree of hemoconcentration to the amount of fluid needed to correct the loss from the blood stream. Such a relationship is not considered accurate enough to be of much value and it is necessary to use other criteria for determining the amount and kind of fluid to be replaced.

The burned patient who survives the initial phase of the injury has in store other serious complications. Infection is one of the common hazards in burn mortality which resists all attempts at adequate protection and treatment. As a rule, edema accompanies all second and third degree burns. It is usually one of the most outstanding results of such trauma. According to Drinker,[10a] "Abnormal leakages from the capillaries is the dominant continuing characteristic of the burn lesion." The functioning of such important organs as the lungs, the kidneys and the liver is impaired. The vascular system and gastrointestinal tract are often affected as a result of absorption of toxins produced either from autolysis of dead tissues or from faulty treatment. Thus under such circumstances the maintenance of adequate nutrition in the burned patient is rendered difficult not only because of increased catabolism but because of inadequate alimentation and impaired absorption.

These introductory remarks serve to indicate the complexity of the burn problem and the many factors which are involved. A more detailed discussion of some of these factors, with particular emphasis on nutrition, is worthy of consideration.

Pathology

The pathology of burns is of basic importance. Cells may be injured or killed. Injured cells are always to be found in the superficial skin layers of mild burns and in the deeper tissues of severe burns. The injured cell membranes undergo changes in permeability. Adjacent capillaries, arterioles, and venules are dilated and blood flow soon increases. With more severe injury, capillary walls are damaged, and plasma leaks into the tissues. The tissue spaces become distended with fluid, which may appear either on the surface as blisters or gradually be absorbed into the lymphatics. Both lymph and blister fluid are similar in composition to plasma.[3, 25] The amount of fluid lost in this manner may be large. If the burn is a deep one, red blood cells in involved capillaries may be damaged or destroyed early and hemoglobinemia and hemoglobinuria may occur.

The process of repair begins immediately after the burn has occurred. If the damage is slight, fluid is absorbed, blood vessels assume their normal

caliber, and dead cells are removed by autolysis and phagocytosis. In deeper burns, this process occurs between living and dead cells, the latter gradually separating off as a slough. At the same time, vascular fibroplasia occurs beneath the separating layer, while epithelial cells begin to grow in from the borders of the wound. This process continues until healthy granulations replace the dead sloughing cells and scar epithelium covers the surface. However, epithelialization is slow and incomplete, and the surface layer thus formed is thin and delicate. Therefore, for satisfactory end results, corrective procedures including skin grafting are required whenever the burn is very extensive.

Besides the local changes just described, distant organs are affected.[2, 6, 15, 21, 29, 33] The early outpouring of fluid decreases the circulating blood volume. This, together with absorption of toxins from the burned area, results in a general constriction of the small arterioles throughout the body; and in the more severe cases, surgical shock supervenes, with fall in blood pressure and attendant phenomena. There is also impairment of renal function, with oliguria and azotemia. Failure to eliminate adequately nitrogenous wastes at this period is probably due to a diminished effective blood flow through the kidney.[19] Elimination of the products of hemolysis and of red blood cells from the burned area, by the kidney, tends to increase renal damage. The most consistent anatomic changes seen in kidneys at post mortem consist of necrosis of the tubules, particularly the ascending and descending portions of Henle's loops, and the occurrence of pigmented casts within these tubules.

Liver necrosis also follows severe burns. There may be a diffuse injury to all the liver cells, or the lesion may be localized to the central zone of each lobule.[4, 6] However, there is good evidence that, in certain cases, the latter may be more directly attributable to the use of tannic acid in treatment than to the burn itself.[2, 24, 32] Lesions of the gastrointestinal tract (Curling's ulcer [16]), and changes in the adrenals, spleen, lymph nodes, heart and central nervous system may be found [21, 29f] and, although of importance, do not directly concern us in this discussion.

Clinical Course

Clinically, burns are classified according to depth as well as to the extent of the injured surface area. Thus, first degree burns involve only the epidermis, and grossly present the erythematous appearance of a sunburn; second degree burns destroy part of the skin and are accompanied by blister formation; while those of third degree involve loss of the full thickness of the skin with or without death of underlying tissues. The course of the burned patient in general follows a fairly consistent pattern. After the initial injury there appears the stage of burn shock. This is chiefly due to local loss of fluid and is aggravated by poorly understood toxic phenomena. As a rule, burns which are properly treated and do not involve a loss of

the full thickness of the skin usually heal in about two weeks. Deeper burns, in separating dead from living tissues, always show some evidence of infection, and the course of healing is likely to be prolonged. This is painful and distressing to both patient and physician before the final reconstructive stage of repair has been drawn to a successful conclusion.

Metabolic changes, particularly those involving protein metabolism, which occur in each phase of progress of the burned patient, are of such importance that they may make the difference between life and death. Therefore this subject requires special consideration.

The Initial Injury. When various thicknesses of skin with or without underlying tissues are destroyed, destruction of protein stores occurs throughout the body. As early as 1930, Cuthbertson [10] was able to show experimentally that in simple bone and soft tissue damage, extensive metabolic changes occurred, with negative nitrogen balance persisting for many days after the original thermal injury. More recently, Howard [18] conducted carefully controlled studies of nitrogen and potassium balance on several patients who had had simple or compound fractures. His conclusions are particularly valid because preoperative and postoperative studies of patients who had operative section of the femur (femoral osteotomy) were used as controls. He found that, in a group of six fracture cases, the average loss of nitrogen was greater than 220 grams (1400 gm protein) or, in terms of muscle tissue, 15 pounds. The excess catabolism reached its maximum in six days with equilibrium after 36 days, but only a slow replenishment of depleted body nitrogen occurred after that time. The slight changes in metabolism that he noted in operative control cases served to eliminate such factors as bed rest, cast immobilization, delayed absorption or increased demand for calories. Fever was not found to be an important feature of these cases. The failure of correlation between potassium and nitrogen balance further indicated that starvation was a relatively insignificant factor. With the elimination of these various possibilities, it was concluded that increased catabolism of protein followed this type of injury, and it was felt that the usual sparing effect of dietary caloric and protein increases was small or absent. Although the burn presents a rather different type of tissue destruction, there is no question that the actual number of cells destroyed is far greater than following a fracture. It therefore seems reasonable to assume that the direct tissue damage resulting from a burn may initiate a process of increased body protein breakdown, which persists for several weeks, and that this portion of the total nitrogen deficit will probably not be corrected by the oral administration of high caloric, high protein foods.

In addition to the immediate death of skin and subcutaneous tissue resulting from a burn, one must consider the associated destruction of red blood cells and other components of the blood in the burned area, which often results in anemia. Although this syndrome is only indirectly asso-

ciated with protein metabolism, it requires early recognition and correction for the adequate care of the patient.

Thus the initial injury produces deleterious effects which may not become evident to the clinician until several days have passed, and which then may be masked by the more extensive metabolic changes due to fluid exudation, toxic absorption, and infection.

The Stage of Shock. Almost immediately following the burn there commences the outpouring of a plasma-like fluid from the burned surface, as already mentioned.[3] At this stage, most clinicians prefer to consider the burn as a large open wound and treat it accordingly. The seepage of this protein-rich fluid from the surface or into the dressings may assume large proportions within a few hours. For example, Hirshfeld and coworkers [17] developed a method for the quantitative collection and determination of the nitrogen lost in the exudate from a burned surface. They performed complete nitrogen studies on six patients with burns and found that the exudate contained 3 to 25 per cent of the total nitrogen output.

The tannic acid method of producing an eschar over the burned area (formerly widely used) served to eliminate most of the surface loss of fluid. However, it is to be remembered that this exudation occurs both from the surface and directly into the underlying damaged tissues, and that the latter exudate may constitute just as important a loss of protein-rich fluid from the body as a whole as does the former. Indeed, in deep burns no surface loss whatever may occur until after the slough has separated. Thus, the loss into the tissue is the only important local one early in the course of these cases. Obviously, the surface eschar formed by tanning has no effect on the loss of fluid within the tissues. This is one of the reasons why tanning more recently has been abandoned in favor of the application of pressure dressings [1] which tend to eliminate or minimize fluid loss in both directions.

Local loss of fluid is of great importance, and is accepted by most workers as an exceedingly important factor in the production of shock which is the early cause of death in burns. Thus, the adequate replacement of this lost plasma is frequently a life-saving measure. In our own experience it has sometimes been found necessary to give as much as 6 to 8 liters of plasma intravenously within the first 24-hour period to compensate for the fluid lost from the blood stream.

The patient's condition at this point is complicated further by the absorption of toxins from the burned area.[16, 26] The toxin problem in burns is a very controversial one, yet there is little doubt that burn toxemia is a real factor and that toxins absorbed from the burned area do have a considerable deleterious effect on the patient. For example, (*a*) Christophe,[8] in cross-circulation experiments in dogs, was able to show that toxins liberated in the burned animals resulted in death of the uninjured ones, and (*b*) Prinzmetal and coworkers,[30] working with rats, showed that severe

burns of the limbs were not accompanied by visible edema, and did not show enough local fluid loss to produce shock, yet the animals were found to die of shock, presumably toxic in origin. Whatever the toxins may be, their absorption makes a seriously ill patient worse, so that there tends to be produced disorientation and other clinical manifestations associated with toxemia. Of particular importance are gastrointestinal disturbances with vomiting. The negative nitrogen balance present at this time is therefore aggravated by the inability of certain patients to assimilate food taken by mouth and by impaired absorption of nutrients, as well as by the inability of the damaged liver to perform adequately its important role in protein metabolism.

Moreover, still other factors are concerned in the general disturbance occurring shortly after a severe burn. There is a disturbance in electrolyte balance. Fox,[12] using radioactive sodium, pointed out that sodium loss from and into the burned surface is important, and stressed the necessity for its adequate replacement. Such a view is amplified further by Moyer *et al.*[27] who obtained best results in the treatment of early burns by administration of whole blood intravenously plus salt solutions by mouth. Mineral balance studies are undergoing continued investigation and will doubtless assume their proper place in therapy with the passage of time. It is safe, however, to state categorically, that the loss of protein-rich fluid from the blood stream is the most important single factor in the production of shock following a burn.

The Late Deep Burn — Infection. Should the patient with a severe burn survive the initial injury with its resulting shock and toxemia, his general condition is likely to be poor in spite of heroic supportive measures, and his ability to withstand the final important and exhausting complication, namely infection, is sure to be grossly jeopardized. Infection is a constant accompaniment of deep burns. It usually makes its appearance within a week to ten days after the injury and its hazard persists until healing is complete. Even with the aid of the newer chemotherapeutic agents, penicillin, and other antibiotics, its successful management requires a maximum of patience and painstaking care. In this connection it may be pointed out that loss of fluid and concomitant infection in the burned area is so serious that debridement and early skin grafting is advocated whenever possible.[8a, 8b]

Local measures include the continued treatment of the burn as an open wound, in the manner already described, care being taken to observe the principles of surgical cleanliness in the changing of the pressure dressings in order to minimize infection. It has been shown by numerous workers that exudation of fluid from the granulating infected surfaces of late deep burns is considerable. These exudates, like those occurring shortly after the burn, contain large amounts of nitrogen, so that the factor of fluid and nitrogen loss from the wound constantly must be kept in mind. Since

infection persists for a long time (months often elapse before complete healing occurs), large amounts of nitrogen may be lost from the granulating surfaces, and it is important that adequate protein replacement be supplied over this prolonged period.

The interference of chronic infection with protein metabolism further complicates the picture. Nutritional problems in patients with long standing infections have been appreciated for years. This was stressed by Graham in consideration of the treatment of empyema thoracis during World War I. In certain cases with streptococcal empyema, Bell [13] performed metabolic studies, and found that unless special attention was paid to their diets, the patients were likely to develop a negative nitrogen balance amounting to a deficit of as much as 21 grams a day. It was further shown that low caloric diets were a constant accompaniment of negative nitrogen balance in these cases, and that a positive balance could be assured by the oral intake of a mixed diet containing 3300 to 3500 calories per day, with weight gain rather than weight loss. The additional finding of only about 2 grams of nitrogen loss in pleural exudates per day, compared to 20 or 30 grams in the urine, indicated that the catabolic process was probably of a general rather than a local nature. Although these workers assumed that the negative nitrogen balance was due to increased breakdown rather than to delayed synthesis of body protein, Madden and Clay [23] have obtained experimental evidence which tends to prove that the excess urinary nitrogen associated with inflammatory processes actually is due to increased catabolism of body protein rather than to inhibition of anabolism. Their experiments also show that some protection at least could be afforded by an adequate oral intake of protein. The presence of fever, with its increased metabolic rate, appears to account for only a small part of the increased rate of nitrogen loss, most of which, therefore, must be related directly to the effects of the infective process.

More recent evidence of the importance of protein metabolism in chronic infections of many types is abundant.[7, 22, 28] Mulholland and coworkers [28] studied 35 cases with bed sores, and found protein deficiency with negative nitrogen balance to be present in all cases. Once they were able to restore a positive balance, healing of the ulcers and general improvement in the patient's condition became manifest. Their conclusion that impaired vitality from protein deficiency played a major role in these cases appears justifiable. Other experimental evidence, accumulated by Cannon *et al.*[7] brings out more clearly the relationship between protein metabolism and resistance to infection. It was demonstrated by these workers that the frequency and severity of postoperative infection depended largely upon the capacity of the individual to mobilize protective forces of natural and acquired resistance, and that the latter were related directly to ingested or reserve protein. They produced evidence to show that, through the γ-glob-

ulin fraction of serum protein, protein deficiency may result in the poor production of antibodies.

From the foregoing remarks, it is easily understood that unless feeding is forced to the patient with a late burn, a type of nutritional vicious cycle may become established — debility, loss of appetite, further nitrogen loss, more weight loss, greater debility, and so on. In this way weight loss, a constant feature of the late burn, tends to become extreme, and, once present, is most difficult to correct. Levenson and coworkers [20] performed careful metabolic studies on some 30 patients burned in the Boston Coconut Grove disaster. Their case reports are complete and illustrate well the clinical problems that occur in these patients, particularly the duration of illness and the associated debility with weight loss. The measures that they used successfully to combat these complications are described in detail.

Chronic protein deficiency with infection leads to the continuance of the secondary anemia usually present in these patients, and to other important changes in the circulating blood. The most significant of these is hypoproteinemia, and particularly hypoalbuminemia. Elman and coworkers [31] performed a series of controlled nutritional experiments on dogs, and from their results calculated that a deficiency of one gram of plasma protein is equivalent to a loss of approximately 30 grams of body protein. Thus, by computing the total plasma protein deficit and multiplying this figure by 30, a rough measure of the total protein deficit may be reached; and although large repeated infusions of plasma or amino acid preparations may temporarily raise the plasma protein level to nearly normal, it is only after the total tissue protein loss has been replaced that a permanent elevation of plasma proteins can be expected. In this connection, it has been clearly demonstrated both in the experimental animal and in man that positive nitrogen balance can be maintained after depletion of body proteins, by the intravenous administration of amino acid preparations.[11] This is important in the management of the chronically ill patient, who may be unable to take adequate nourishment by mouth, even with the aid of an indwelling tube to the stomach.

The general disturbances of protein metabolism in the patient suffering from a chronic infection are accompanied by deleterious changes in the electrolyte and protein exchange between blood, tissue fluids, and cells themselves. Nutritional edema is an example of such disturbances. Although frank edema is seldom noted in young, otherwise healthy patients, subclinical edema is doubtless of frequent occurrence. Lyons [22] in a study of the effects of penicillin on chronic infections, particularly of bone, also undertook estimations of the approximate size of blood, plasma and extracellular volumes. It was found that at times the amount of extracellular fluid was greatly increased, the degree of increase being roughly proportional to the general debility of the patient. However, as the infection was brought

under control and nitrogen stores replenished, excess extracellular fluid diminished along with improvement in the condition of the patient. There was no evidence of specificity of infection resulting from the changes occurring in nitrogen metabolism, but such changes might be anticipated in any long standing infection, and do occur in the severely burned patient. It is thus apparent that attention to protein metabolism is of extreme importance, both to promote recovery of the individual and to prepare for the extensive reconstructive procedures frequently required.

The Stage of Reconstruction. Reconstruction surgery forms the final page in the chapter on burn management. The individual who has weathered successfully the storm of a severe deep burn finds himself afflicted with many raw granulating surfaces which must be covered. Such procedures, of course, are postponed until infection is at a minimum and the patient's general condition is as good as can be obtained. Many days or weeks may elapse before such a situation is realized, and depletion all too often has left its mark on the burned individual. Surgeons handling reconstructive problems in burn cases are familiar with these points and are always cautious to proceed. It has been shown by Co Tui [9] and others that the patient whose protein stores are diminished tolerates operative procedures poorly. Skin grafts may fail to take, blood loss tends to be excessive, and operative shock is seen on occasion during or after relatively insignificant procedures. The maintenance of adequate nutrition during the earlier stages of burn care is therefore doubly important in the preparation of the individual for the necessary final surgical procedures. The staging of such plastic procedures into multiple, relatively minor operations offers further protection to the patient.

Summary

The severe burn presents a complex problem in its effects on protein metabolism. The course of the burned patient has been divided conveniently into several consecutive stages, each of which exerts its own particular effect, although the clinical picture is a summation of effects. These stages comprise the initial injury, and the stages of shock with toxemia, infection, and finally repair and reconstruction.

The initial injury results in marked catabolism of nitrogen, the mechanism of which is not clear, and the prevention of which is impossible. The stage of shock is due chiefly to the exudation and seepage of large amounts of fluid rich in protein from and into the burned surface, and is aggravated by the absorption of toxins with resulting liver damage and gastrointestinal disturbances. Lastly, chronic infection is accompanied constantly by depletion of protein reserves, thus not only endangering the life of the patient, but also decreasing the ultimate chance of successful surgical repair and reconstruction of the burned areas.

The severe burn is a continuous problem from inception until final healing. Present trends in treatment are the result of prolonged research and scientific cooperation between the biochemist, physiologist, and clinician. Careful attention to nutritional problems, and especially to protein metabolism, is most important; advances in this field constitute one of the reasons for the gradual lowering of mortality from burns, which has been observed in recent years.

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Chapter 14

The Protein Nature of Toxins, Antitoxins and Related Substances *

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Toxins

Highly poisonous, non-dialyzable, antigenic substances occur widely distributed in nature and are termed *toxins*.[1, 2] Such substances, which in minute amounts show lethal properties for man and animals or which may cause destruction of cells, are present in filtrates from cultures of a number of bacteria, including certain staphylococci, streptococci, the clostridia, and the diphtheria bacillus,[1–4] in extracts of the seeds of certain plants, such as *Ricinus communis*, *Abrus precatorius*, *Croton tiglium*,[2] and in the salivary secretions (venom) of a number of snakes.[2, 5] In a number of bacterial infections, such as diphtheria, tetanus, gas gangrene, these toxins may be largely responsible for the characteristic symptoms and pathology of the disease. The presence of toxins in certain foods may result in botulism or in other forms of food poisoning.

Each of these substances gives rise in animals to its own typical symptom complex and pathological lesions. Thus, tetanus toxin affects the motor nerve cells; diphtheria toxin causes acute fatty degeneration of cardiac muscle, hemorrhages in the adrenal gland, and paralysis. The toxins of the staphylococcus and certain toxins of *Cl. welchii* are lethal and also cause hemolysis and necrosis of skin. Ricin, the toxin obtained from the castor bean, produces hemorrhagic lesions, congestion and edema throughout the body especially in the omentum, intestine, and mesentery, with degenerative changes and focal necrosis in the liver, spleen and lymph nodes.[6] The venom of the rattlesnake causes hemolysis and affects the nerve cells.[5] In addition, bacteria synthesize a variety of substances which specifically affect certain cells. For example, hemolytic streptococci produce a soluble hemolysin which causes solution of red blood cells [7] and soluble substances capable of causing hemagglutination are also found in certain beans and, in some instances, are associated with toxins.[2]

Many species of bacteria, chiefly the Gram negative organisms, show intrinsic toxicity and, frequently, old culture filtrates may be toxic due to

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their content of bacterial debris or products of autolysis. These substances, *endotoxins*, are the somatic antigens of the organism. They are generally distinguished from the true bacterial toxins, *exotoxins*, which appear to be metabolic products of bacteria rather than portions of the bacterial cell.[1, 2] The endotoxins are much less toxic than the exotoxins. They are complex antigens containing lipid, carbohydrate, and protein and will not be considered further (*cf.*[2, 8, 8a]).

The toxins, hemolysins, hemagglutinins, etc., were first recognized by their unique biological activity. When several types of biological activity were present in a single solution, it was frequently not known whether one substance was responsible for all of the biological activities. The problem of purification and characterization of the toxins, although facilitated by the possibility of assaying for biological activity at each step in the procedure, was complicated by the presence of numerous other substances of complex chemical composition occurring in crude toxin-containing materials. With bacterial toxins, difficulties in purification were, until recently, multiplied because of the necessity for including in the culture media, tissue extracts, proteoses, peptones, and other poorly-defined chemical entities. Despite these difficulties earlier studies served to make it clear that the biological activity of toxins, venoms, etc. was closely associated with protein, and partially purified toxic concentrates were obtained by precipitation with acid or with ammonium sulfate, alcohol and acetone, by dialysis or ultrafiltration, etc., and by adsorption on magnesium and aluminium hydroxides and subsequent elution.[3]

Purification of the toxins was materially facilitated by the development of media of well-defined chemical composition for the cultivation of toxin-producing bacteria with the resultant elimination of protein and protein-like substances derived from the culture media.[3, 9] During the same period the introduction and widespread adoption of physicochemical methods for characterizing proteins, such as electrophoretic [10–12] and ultracentrifugal analyses,[13] made it possible to determine by these technics whether or not purified toxin preparations contained more than one protein component and to establish with which component the toxicity was associated. Also, the development of quantitative immunochemical methods for the estimation of antigens and antibodies (*cf.*[8, 14, 15]) permitted the characterization of toxins by measurement of the capacity of various preparations to flocculate with their homologous antitoxins.[16, 17] During the past few years, a considerable body of data on several toxins has been accumulated.

Assay Methods. An essential prerequisite to any attempt to purify a toxin or other biologically active material is a precise method for assay of the activity. A reliable assay method makes it possible to estimate the yield at each step in the purification procedure and to detect any deleterious effects of the chemical treatment on the activity. When activity assays are correlated with nitrogen analyses or determinations of dry weight, it also

becomes possible to express the degree of purification at each step as a ratio of activity per unit of nitrogen or per unit of weight. If crude products exhibit several biological activities, it is frequently of added value to carry out parallel assays for each of the activities. For example, in the purification of ricin from the castor bean, it has been found that toxicity and hemagglutinating potency are contained in the same fractions.[18, 19] With the venom of the rattlesnake, however, the toxic, hemolytic and lecithinase activity can be separated from the blood clotting activity.[20] Bernheimer [21] has presented evidence indicating that the lethal and hemolytic properties of culture filtrates of *Cl. perfringens* are due to a single substance.

In general, assay methods measure the minimum amount of the substance or the highest dilution of the solution which will produce a given biological effect. For example, the highest dilution of a culture filtrate which is uniformly lethal to a given experimental animal — the minimum lethal dose, or MLD — may be measured. Similarly, the minimum amount of substance or solution giving a definite skin reaction, or causing detectable hemagglutination or hemolysis of a given number of erythrocytes may be determined. Many workers prefer an endpoint at which only 50 per cent of the inoculated animals die (LD_{50}) or at which 50 per cent hemolysis occurs, since such endpoints are more precisely located (*cf.*[22]). In all assays it is usually desirable to include a toxin of known potency to check on the reproducibility of the determinations. (For details and principles of various methods consult reference.[23]) Assay methods based on the reaction with antitoxins are given under antitoxins.

Purification of Toxins. During the past decade a considerable number of toxins have been purified and several have been obtained in crystalline form. In all instances, purification has been achieved by the use of standard procedures such as fractional precipitation with ammonium or sodium sulfate, precipitation with acid, or adsorption and elution methods.[2, 3, 4, 7, 17–20, 24–34] A representative procedure for the preparation of a bacterial toxin in crystalline form is given.

Botulinus toxin has been purified and crystallized by two groups of workers.[28, 29] The procedure of Abrams, Kegeles and Hottle [28] employs an initial precipitation of the toxin grown on a "pepticase" cornsteep liquor medium by acidification to pH 3.5 as described by Sommer.[27] The washed precipitate is dissolved in water at pH 6.8, and precipitated at 0.4 saturation with sodium sulfate. The precipitate is taken up in water at pH 5, reprecipitated at pH 3.5, centrifuged, and the toxin dissolved in phosphate buffer at pH 6.8, and reprecipitated at 0.3 saturation with sodium sulfate. The precipitate is again taken up in phosphate buffer at pH 6.8, and the acidity adjusted to pH 4.7. The precipitate which forms is discarded, and the toxin in the supernatant is precipitated by 0.3 saturation with sodium sulfate. The precipitate is dissolved in phosphate buffer at pH 6.8 and reprecipitated by 0.5 saturation with ammonium sulfate.

It is then crystallized as follows: The precipitate is dissolved in phosphate buffer at pH 6.8, the solution is clarified by centrifugation and dialyzed at 4° at pH 6.8 against 0.1 saturated ammonium sulfate. The salt concentration outside the membrane is slowly increased until opalescence and crystallization of the toxin occurs.

High yields of tetanus toxin suitable for production of toxoid for human immunization have been obtained by Taylor [32] and by Mueller and Miller [33] with a medium consisting of an autolysate of hog stomach and a veal infusion. Toxins with five to ten times the potency of those grown on a peptone-free medium [34] (*cf.*[35]) were obtainable and a strain of *Cl. tetani* was used which did not require accurate adjustment of the iron concentration. Pickett *et al.*[30] were able to effect considerable purification of tetanus toxin by precipitation with cadmium chloride (*cf.*[24]) and ammonium sulfate. Pillemer *et al.*[36] have recently obtained tetanus toxin in crystalline form by fractionation with methyl alcohol and crystallization at −8° from 25 per cent methanol at pH 5.1 and ionic strength of 0.02.

Crotoxin, the active neurotoxic and hemolytic principle in rattlesnake venom, has been purified [20, 37] by procedures involving removal of inert protein by heating to 70° at pH 4.1, fractional precipitation between 0.45 and 0.62 saturation with ammonium sulfate and crystallization by dissolving in dilute acetic acid and adjusting the acidity to pH 4.4 with pyridine.

The neurotoxin of the Shiga bacillus has been partially purified by precipitation with 2 per cent trichloroacetic acid, or with cadmium chloride [38, 39] or by digestion with trypsin.[39a] Impurities could also be removed by precipitation with protamine sulfate.[39, 39a] Products with 10.6 per cent nitrogen had a potency of 550 L_f units per mg of nitrogen; 25 μg was about the minimum lethal dose for a mouse. For other studies on the Shiga neurotoxin the reader is referred to Weil's paper.[40]

Ricin has been purified from castor bean extracts by precipitation at about 0.36 saturation with sodium sulfate.[19] On repeated precipitation, preparations (B1) which are electrophoretically and ultracentrifugally homogeneous have been obtained. Ricin has also been obtained in crystalline form by Kunitz (*cf.*[19]) and by Cannan (*cf.*[19]). The B1 fractions consistently showed only about two-thirds the toxicity of crystalline ricin.[19]

Properties of Purified Toxins. Some of the physical, chemical, biological, and immunological properties of several purified toxins are summarized in Table 14–1. Of these, diphtheria toxin has been most extensively studied.[16, 24, 25] Although it has not been crystallized, there is much evidence to indicate that a high degree of purity has been attained. It is ultracentrifugally homogeneous [41, 42] and on electrophoresis 95 to 97 per cent of the material is associated with the toxin.[25] Immunochemical evidence of the high purity is provided by the close agreement between the nitrogen per flocculating unit for the purified toxin and that calculated from the quantitative study of the flocculation reaction between diphtheria

Table 14–1. Chemical, Physical and Biological Properties of Purified Toxins

Toxin	*Diphtheria* [16, 25]	*Botulinus* [9, 28, 29]	*Tetanus* [36]	*Scarlatinal Erythrogenic* [17]		*Streptolysin O* [7]	*Ricin* [18, 19]	*Crotoxin* [20, 37]	*Shiga neurotoxin* [39]
Source	Culture filtrates	Culture filtrates	Culture filtrates	Strain 594 Culture filtrates	Strain NY5 Culture filtrates	Culture filtrates	Castor beans	Rattlesnake venom	Autolysates or culture filtrates.
Method of Purification	Adsorption on alumina; $(NH_4)_2SO_4$ precipitation	Acid precipitation $(NH_4)_2SO_4$ fractionation and crystallization	Methanol fractionation and crystallization	$(NH_4)_2SO_4$ fractionation		$(NH_4)_2SO_4$ precipitation; adsorption on $Ca_3(PO_4)_2$	Precipitation with Na_2SO_4 or $(NH_4)_2SO_4$ and crystallization	Precipitation of inert protein at 70° C., $(NH_4)_2SO_4$ fractionation and crystallization	Trichloroacetic acid and calcium chloride precipitation
Nitrogen %	16.1	14.1		14.0	12.5	16.8			10.6
Sulfur %	0.7	0.1				2.3		4.0	
$[\alpha]_D$ degrees	− 36 to − 40						− 26		− 36
Sedimentation constant Svedbergs	4.6 *						4.8 (5.3 ††)	3.13	
Diffusion constant $D \times 10^{-7}$ cm²/sec	6.0	2.1					6.0 ††	8.59	
Partial specific volume	0.736	0.760						0.704	
Molecular weight	74,000	1,130,000 **					77,000(85,000††)	30,000	
Frictional ratio f/fo	1.2	1.45					1.2	1.2	
Ratio of major to minor axes	4.7	8.3					4–5	4.7	
Electrophoretic homogeneity	95–97% †	100%							
Isoelectric point pH	4.1	5.6					5.4 −5.5(5.2 ††)		
MLD (per animal) μg	0.1 (guinea pig)	0.00003 (mouse)	0.0001–0.0002 (mouse)			44 (mouse)	0.25 ‡‡ (mouse)	7 (mouse)	25 (mouse) ¶¶
N per activity unit of preparation μg	0.46(Lf)	0.48(Lf)	0.25–0.29 (Lf)	0.54(Lf)	0.35(Lf)	0.54 (hemolytic)			2(Lf)
MLD per unit	30(Lf)	110,000(Lf)	18,000–19,000 (Lf)						
Skin test dose per mg N				5×10^7 ‖	25×10^7 ‖				
pH stability range	5.6–10.1 ¶	1.0–6.0 §						5.0–10.0	
N per Lf unit from flocculation reaction	0.46		0.30	0.23	0.23				
Amt. precipitable by antitoxin %	100 ±		100 ±	43	66				
Other biological properties						hemolytic	hemagglutination	hemolytic; lecithinase	

* Single homogeneous component
† 2–5 per cent of a faster component, probably bacterial protein present.
¶ From sedimentation and activity studies.
§ At room temperature; by activity measurements.
‖ Pure scarlet fever toxin was calculated to contain 13×10^9 skin test doses per mg N and 0.23 μg N per L_f unit.[17]

** From diffusion and viscosity measurements.[28] A value of 900,000 was obtained from sedimentation and diffusion measurements.
†† On electrophoretically and ultracentrifugally homogeneous fraction B1.[19] Contains a mixture of about 65% toxic and 35% non-toxic ricin.
‡‡ Lethal for 20 gm. mouse in 24 hours.
¶¶ Proportions showing only 1 to 3 μg per mouse [28a] LD_{50} were recently reported.[39a]

toxin and antitoxin. This latter procedure is independent of the purity of the toxin used [16, 25] (see below). Direct immunological tests for other proteins of the diphtheria bacillus with specific antisera show that 98 to 99 per cent of the bacterial protein are removed during purification.

Botulinus toxin has not as yet been extensively studied. However, the toxin has been crystallized and crystalline preparations have been found to be electrophoretically homogeneous over a wide range of pH. Variations in the toxicity of different crystalline preparations have been encountered and attributed to the partial inactivation of toxin.

The erythrogenic toxin of the hemolytic streptococcus has not been obtained in pure form. At present the best preparations consist of about one-half to two-thirds toxin [17] as determined by comparison of the nitrogen content per flocculating (L_f) unit of the preparations and the calculated value from the flocculation reaction (Table 14–1) between scarlatinal toxin and antitoxin.[43]

The physical and chemical properties of crotoxin have been studied by Gralen and Svedberg.[44] Their data are given in Table 14–1. The sulfur content of crotoxin is about 4 per cent and about 88 per cent of the sulfur is present as cystine and approximately 7 per cent as methionine.[37] Treatment of crotoxin with cystine splits the disulfide linkage and results in a marked loss of toxicity. Crystalline crotoxin shows the neurotoxic and hemolytic properties and the lecithinase activity but does not have any of the blood coagulating properties of the whole venom.

Ricin has a molecular weight of about 77,000 to 85,000. No significant differences in electrophoretic and ultracentrifugal properties of crystalline samples and those obtained by sodium sulfate precipitation (B1) have been found. The two products are also immunochemically identical since equal amounts yield the same quantities of specific precipitate with rabbit antiricin sera.[19] Since crystalline ricin is more toxic than B1, Kabat, Heidelberger and Bezer [19] concluded that B1 was composed of one-third nontoxic and two-thirds toxic ricin.[19] This non-toxic ricin was shown by parallel assays for immunological potency and for toxicity to be present in crude aqueous extracts of castor beans in about the same proportions as in fraction B1.[19]

Mode of Action of Toxins. The hope of many workers that preparation of toxins in pure form and their chemical characterization would throw some light on the chemical basis for their toxicity has not been realized. No evidence for the existence of any prosthetic group has been found and thus far toxicity appears to be a function of the intact protein molecule.

Two current concepts of the action of toxins which serve as working hypotheses have been clearly expressed by MacFarlane and Knight.[45] One of these assumes that the toxins may "block a metabolic reaction in the host by competing with the normal substrate for the enzyme catalyzing the reaction" in a manner similar to that proposed by Fildes [46] to explain the action

of chemotherapeutic agents. The second hypothesis postulates that the toxin may itself be an enzyme "exerting its toxic function by attacking, according to its degree of enzyme specificity, one or more substances which are normal constituents of a cell. It consequently distorts the metabolism of the cell in one or other particular direction, either primarily by destruction of an essential structure or the inhibition of a metabolic mechanism or secondarily by the production from a normal cell constituent of a toxic substance with such powers."

Both of these approaches have yielded valuable information. Wooldridge and Higginbottom [47] found that the α-toxin of *Cl. welchii* could inhibit the aerobic oxidation of succinate by minced guinea pig tissues and that this inhibition could be reversed to a considerable extent by addition of antitoxin. No indication of any effect of diphtheria toxin on tissue respiration or on dehydrogenase or phosphorylase systems was observed.[48]

The second hypothesis had previously been shown to be applicable, at least in part, to the snake venoms. Thus, the hemolytic action of the venom may be attributed to its lecithinase activity. Studies by von Dungern and Coca [49] and others (*cf.* Sevag [50]) established that a strongly hemolytic substance, desoleolecithin or lysolecithin, was formed by the action of the lecithinase of the venom on lecithin with the splitting off of oleic acid. More recent studies by Feldberg and Kellaway [51] and by Feldberg, Holden and Kellaway [52] with cobra venom indicate that lysolecithin is in part responsible for the toxic action of the venom in that much of the pharmacologic effects of cobra venom can be produced by purified lysolecithin. Both venom and lysolecithin were found to liberate histamine (*cf.* Essex [53]).

Snake venoms have also been found to hydrolyze nucleic acid [54] but this is not definitely known to be a property of the toxic protein; however, the nuclease activity is inhibited by antivenom. Roy [55] has recently suggested that some venoms may be intrinsically hemolytic and thus toxicity may not be due to the formation of lysolecithin.

The α-toxin of *Cl. welchii* has also been shown to possess lecithinase activity. This discovery resulted from an observation by Nagler [56] and by Seiffert [57] that when human serum was incubated with *Cl. welchii* under anaerobic conditions, the medium became opalescent "with the uppermost layer more opaque than the rest of the medium." On centrifugation, three zones were noted: The bacteria at the bottom, an opalescent middle layer of serum, and a top layer of fat-like material. Nagler demonstrated that this effect on human serum was caused by the lethal α-toxin, and was specifically inhibited by antitoxin. Both toxin and antitoxin could be titrated by this reaction.[56] The reaction could also be used to measure the combining capacity of type A toxoids of *Cl. welchii*.[58]

The chemical nature of the reaction described by Nagler and Seiffert was further clarified by the demonstration that the α-toxin could produce opalescence of egg lecithovitellin even in the cold and that extraction of

the lecithovitellin with ether reduced the degree of opalescence.[59] No effect on lecithovitellin could be obtained from the β-, δ-, ϵ- and θ-toxins. The reaction was also shown to require calcium ions and to be inhibited by antiserum.

MacFarlane and Knight [45] subsequently established that the α-toxin reacted with an aqueous emulsion of lecithin to form phosphorylcholine and a diglyceride and attributed the action of the toxin in producing opalescence of egg yolk to this lecithinase action. The lecithinase was found to have no action on diphenyl, monophenyl or sodium β-glycerol phosphates or on nucleic acid. It was relatively heat stable, showing only 55 per cent loss of activity after 10 minutes at 100° in borate buffer at pH 7.6, but was more rapidly inactivated at a more acid pH. It was activated by calcium ions. The lecithinase activity could be inhibited by antitoxin, and the potency of antitoxic sera in inhibiting the enzymatic reaction paralleled their capacity to protect against the lethal effects of the α-toxin. MacFarlane and Knight [45] suggested that the lecithinase activity could account for the toxicity since one mouse lethal dose could hydrolyze the entire blood lecithin of a mouse in two to three hours. Recently, Zamecnik and Lipmann [60] introduced a simple manometric method for the measurement of the lecithinase activity of the toxin. The reaction was carried out in a bicarbonate buffered medium. The phosphorylcholine liberated displaced CO_2 from the medium which was measured in a Warburg manometer. This procedure makes it possible to study the kinetics of the reaction. It has also been used for measurements on the combination of toxin with antitoxin. These workers found that cephalin, phosphatidylserine, sphingomyelin, and glycerophosphorylcholine were not hydrolyzed by the enzyme. The lecithinase activity of the toxin was enhanced by calcium, cobalt, zinc, manganese, and magnesium and was inhibited by barium, iron, aluminium, cadmium, strontium, and copper.

Apart from our present knowledge that crotoxin and the α-toxin of *Cl. welchii* possess lecithinase activity and the evidence that the toxicity of these two toxins may be attributed, at least in part, to their enzymatic nature, little else is known of the underlying basis for the unique biological properties of the other toxins. Observations by Kellaway and Trethewie [61,62] that snake venoms and several of the toxins of *Cl. welchii* liberate histamine and adenyl compounds may prove of importance in further elucidation of the action of toxins.

Effects of Chemical and Physical Agents on Toxins. Toxins, in general, show the same type of behavior on treatment with various chemical and physical agents as do other proteins (*cf.*[63]) and these changes will not be considered in detail. However, their toxicity or other biological activity may frequently be destroyed under relatively mild conditions without producing apparent changes in some of their other properties. Procedures of this type have proven of value in the preparation of detoxified products which still

retain their antigenicity (toxoids). Similar types of studies have been carried out on viruses (*cf.*[64]).

The most widely used method for the production of toxoids from toxins consists in treatment with dilute aqueous formaldehyde.[64] The reaction is dependent upon pH and with diphtheria toxin only one one-hundredth as much formaldehyde is required for detoxication at pH 8.6 as at pH 6.[24] Toxoid formation by formaldehyde is different from the reaction involved in the usual formol titration in that it is much slower and is irreversible.[64] Much less formaldehyde is required for toxoid formation when purified toxins are used as compared with that for detoxifying crude toxins. Formaldehyde has been used successfully to effect almost complete detoxification of diphtheria,[3, 24] tetanus [33, 34] and botulinus toxins,[28, 29] the Shiganeurotoxin [39] and of crotoxin and other snake venoms,[53] without marked impairment of antigenicity. Ricin, however, has proven relatively resistant to the action of formaldehyde [19, 65] and concentrations as high as 10 per cent of formalin * over long periods only reduced but did not abolish toxicity. More drastic treatment resulted in impaired antigenicity.[65]

MacFadyen [66] has developed a method suitable for the estimation of free and combined formaldehyde in vaccines, toxoids and other biological products.

Eaton has shown that unlike the toxin, diphtheria toxoid is more stable to heat, and does not remain insoluble after precipitation at pH 5 or after treatment with acetone.[24] The capacity of toxoid to flocculate with antitoxin as well as the amount of nitrogen per flocculating unit is unchanged. It has also been found to resist the action of copper and neutralized ascorbic acid.[67] Madsen, Jensen and Ipsen,[68] however, reported that, *in vivo*, diphtheria toxin shows a higher binding capacity than does toxoid.

Staphylococcal toxin has been found to show a 50 per cent reduction in combining capacity for antitoxin after treatment with formalin.[3]

Diphtheria toxin has been acetylated with ketene in an attempt to produce a satisfactory toxoid.[69] After 30 to 50 per cent of the amino groups were acetylated some reduction in toxicity occurred without loss of flocculating power, but with more prolonged treatment both properties were destroyed.

Other methods including heat, partial oxidation with permanganate, and ultraviolet irradiation have been employed by Carmichael [70] in efforts to detoxify ricin. Several of these produced somewhat more rapid detoxication than destruction of immunizing potency. By photooxidation in the presence of methylene blue, staphylococcal toxin [71] was completely, and diphtheria toxin [72] partially, detoxified. The products obtained were equal in immunizing potency to formolized toxoid. Carbon disulfide has been found to detoxify tetanus and botulinus toxins. These toxoids retained at least some of their antigenic properties.[73]

* Formalin refers to a 40 per cent aqueous solution of formaldehyde.

Soaps, lipids and sterols also (*cf.*[3]) neutralize bacterial toxins. The reaction is largely reversible and appears to be due to fixation of the toxin at the site of injection. Frazer and Walsh [74] and more recently Halbert, Smolens, and Mudd [75] have obtained similar results by administration of toxins as an emulsion with mineral oil.

Digestion with trypsin destroys the toxicity of both diphtheria [76] and tetanus toxins [77]; no studies on the antigenicity of diphtheria toxin after digestion were reported. Tryptic digests of tetanus toxin showed great impairment of antigenic power.

The application of quantitative methods to the study of the toxin-antitoxin reaction could be used to determine more precisely whether any alterations in combining capacity for antitoxin occur as a result of toxoid formation or enzymatic digestion. For example, recent studies on ricin have shown that the formation of dialyzable nitrogen or of nitrogen not precipitable by trichloroacetic acid or half-saturation with sodium sulfate on digestion with pepsin or trypsin corresponded closely to the decrease in material which reacted with antiricin sera [19] and also with loss of toxicity.[18, 19]

Antitoxins

Repeated injection of sublethal doses of various toxins into susceptible animals results in the development of a tolerance or resistance so that after a series of injections, the animals can generally withstand many times the dose lethal for an untreated animal. The development of this resistance or immunity has been shown to be due to the formation of specific antibodies to the toxin. These antibodies or antitoxins are usually present in the serum of immune animals and, when added to the corresponding toxin in suitable proportions, are capable of neutralizing the toxicity. In many instances they also specifically precipitate or flocculate when mixed with toxin. If given to a susceptible animal in sufficient amounts, they confer protection against otherwise lethal quantities of toxin.

The production of antitoxin in the experimental animal does not differ fundamentally from the production of antibodies to any antigen, except that if native toxin is used its injurious effects place an upper limit on the amounts which can be given in the initial injections. After some degree of tolerance has been developed, however, immunization with toxins may be continued with amounts of material comparable to those used with non-toxic proteins. To avoid the use of minute initial doses of native toxin in immunization, it is customary to immunize with partially or completely detoxified toxins (toxoids), prepared by chemical treatment as outlined above. For purposes of immunization, administration of the toxoid as an alum precipitate has been found to give better antibody response.[78, 79] With other protein antigens, precipitation with alum has also been found to give enhanced antibody production.[80, 81] This is generally attributed to the

superiority of particulate antigens over antigens in solution in stimulating antibody formation.

Assay of Antitoxic Potency. (*Cf.*[1, 22, 81]) The potency of an antitoxic serum may be measured in several ways. The most significant of these is the estimation of its ability to neutralize or protect against toxin. Theoretically, the most direct measure of antitoxin strength would be to determine the number of MLD of toxin neutralized by 1 ml of antitoxin. In practice, however, this presents many difficulties, since culture filtrates may contain both toxin and toxoid.[82] On standing, many crude toxins may lose some of their toxicity and still retain their capacity to combine with antitoxin, so that the use of toxicity as a primary standard is frequently not possible.[22, 81] To eliminate this difficulty a given antitoxin is arbitrarily set up as a standard and assigned a definite potency (*i.e.* 100 units) and the potency of other antitoxins is measured in terms of the standard. As generally performed, neutralization tests involve the preparation of a series of mixtures consisting of increasing amounts of toxin and a constant amount of antitoxin. After an arbitrary interval each of the mixtures is injected into a number of suitable susceptible animals and death or survival over a given time interval is noted. The amount of toxin which, when added to one unit of antitoxin and injected, will just fail to give any observable reaction under the standard conditions of the test is called the Lo dose; the amount of toxin which, when added to one unit of antitoxin, will just suffice to cause death under the conditions specified is called the L+ dose; for diphtheria antitoxin assays, 250-gram guinea pigs are used and observed for 4 days after subcutaneous injection of the mixtures. The L+ dose offers a more reliable end point since it can be more precisely established than the end point of complete neutralization. In assaying the potency of an unknown antitoxin, its potency is compared with the standard by assaying both with the same toxin. If only one-half as much toxin is required to reach the L+ dose of the unknown, then it contains only one-half the number of antitoxic units.

Protection tests usually involve an initial injection of toxin into a series of animals followed after an arbitrary interval by the antitoxin.

In addition to these *in vivo* methods of measuring antitoxic potency, *in vitro* methods have come into widespread use.[1, 8, 22] The best known of these is the Ramon flocculation test [83] in which decreasing amounts of antitoxin are added to a constant quantity of toxin and the proportions for which flocculation is most rapid are determined. Results are expressed in L_f units. The L_f unit denotes the amount of toxin giving most rapid flocculation with one standard antitoxic unit. Tests based upon rate of flocculation in mixtures of antigen and antibody are also employed in studies on antigens other than toxins and their antibodies.[84] Crude toxins, which contain other antigens derived from the bacteria, may give rise to anomalous zones of flocculation; [85] removal from the antitoxic serum of

antibodies to these substances has been used to eliminate these difficulties.[85] The time required for flocculation at 42° is termed K_f. It is frequently determined as a measure of damage to toxins during purification. K_f is usually reported with a subscript denoting the number of L_f units per ml used in the test.

The quantitative absolute methods for the estimation of antibodies, so widely used in studies of other antigen-antibody systems,[8, 14, 15] have also been applied to toxin-antitoxin systems.[16, 17] These methods permit the standardization of antitoxins on the basis of their content of antitoxin nitrogen. This method is considered in detail below under toxin-antitoxin reactions.

When toxins exhibit biological properties, such as hemagglutinating, hemolytic, lecithinase activity, etc., or with other non-toxic proteins showing such behavior, it is also possible to standardize antisera by measuring their capacity to inhibit these effects. For instance, antitoxins to *Cl. welchii* could be standardized by measuring their capacity either to neutralize toxicity or to inhibit lecithinase activity.[45, 56, 59] Antisera to ricin may be standardized by their capacity to inhibit hemagglutination or to neutralize the toxicity of ricin.[19] Indeed the finding that, with a number of antitoxic sera of varying potency, there is a correlation between the capacity of antitoxins to neutralize toxic effects and to inhibit some other biological activity, provides strong evidence for the association of both biological properties with the same molecule.[19, 21, 45, 59] It is indeed unlikely that several animals would all produce the same relative amounts of antibody to each of two distinct antigens.

Purification and Properties of Antitoxins. Antitoxins, like other antibodies, are definitely established as modified serum globulins (*cf.*[2, 8, 14, 15, 86]). They may be concentrated by precipitating the globulin fraction with such reagents as sodium or ammonium sulfate or alcohol. They may, however, be separated specifically from the other serum proteins by the addition of homologous toxin. The preparation of washed toxin-antitoxin floccules offers one of the best methods for the separation of antitoxins. At present, specific methods for recovery of native antitoxin from such precipitates are not widely employed. Ramon [87] and Locke and Main [88] were able to recover antitoxin from diphtheria toxin-antitoxin floccules by heating them in dilute acetic acid. Solutions were obtained in which 0.012 mg protein or less contained one antitoxin unit. From quantitative studies on the toxin-antitoxin reaction,[16] one unit of diphtheria horse antitoxin was found to contain 0.0016 mg N or 0.010 mg protein, indicating that the purified antitoxins obtained by Ramon [87] and Locke and Main [88] were about 80 per cent pure. These procedures have not been used in recent years, and very little is known about the physical and chemical properties of such antitoxins.

Antitoxins from horse sera are found in the pseudoglobulin fraction.

Table 14–2. Physico-Chemical Properties of Purified Native and Digested Diphtheria Antitoxins

	Antitoxic Pseudoglobulin			*Crystallized after Dissociation from Floccules by Tryptic Digestion*
	Untreated	*Pepsin-digested*	*Dissociated from Floccules by Peptic Digestion*	
Reference	[41,93]	[93]	[93]	[95,96]
Specifically precipitable nitrogen %	33	33	77	92
Partial specific volume	(0.745)	(0.745)	(0.745)	(0.749)
Sedimentation constant Svedbergs	7.2	5.7*	5.7	5.5
Diffusion constant $\times 10^7$ cm²/sec	3.9	5.8	5.0	5.8
Molecular weight	184,000	98,000	113,000	90,500
f/fo	1.38	1.14	1.26	1.23
Ratio of major to minor axes	7.0	3.3	5.0 †	4.7

( ) assumed.

* Tiselius and Dahl obtained similar values for the sedimentation constant of pepsin-digested antitoxin.[97]

† Reported as 5.3 in [93]; value of 1.26 for f/fo corresponds to 5.0 for oblong ellipsoid in [98].

Pappenheimer, Lundgren and Williams [41] have prepared a concentrated pseudoglobulin solution by fractionation with ammonium sulfate from which 33 to 35 per cent of the total nitrogen could be specifically precipitated by toxin. This pseudoglobulin showed two components in the Tiselius electrophoresis apparatus. One component contained no antitoxin and corresponded in mobility to the α-globulin fraction of serum. The other had a mobility intermediate between that of the β- and γ-globulins.[41] Separation of the α-globulin by electrophoresis yielded an electrophoretically and ultracentrifugally homogeneous product from which 43.5 per cent of the nitrogen could be specifically precipitated by toxin. Some of the physical and chemical properties of antitoxic pseudoglobulin are shown in Table 14–2 and indicate that the antitoxin has about the same molecular weight as does normal γ-globulin.

Horse antitoxins have been reported to exhibit peculiar behavior when treated with certain proteolytic enzymes. After purification the enzyme-treated antitoxin seems to have a higher neutralizing potency on the basis of its nitrogen content than does the untreated antitoxin. Thus digestion of horse plasma or of pseudoglobulin concentrates containing diphtheria antitoxin or of specific precipitates of toxin and antitoxin with pepsin at pH 3 to 4 has been investigated.[89–94] By digestion of floccules with pepsin [94] or with trypsin at pH 3.5 [95] and subsequent fractional precipitation with ammonium sulfate, Pope and Healey and Northrop were able to obtain antitoxins which were 90–100 per cent precipitable by toxin. Northrop succeeded in crystallizing his preparation, which was obtained by fractional precipitation between 0.33 and 0.50 saturated ammonium sulfate. The crystalline antitoxin was electrophoretically and ultracentrifugally homogeneous [96] but did not show a constant solubility.[95] The products obtained by Pope and Healey and by Northrop were about equal in potency, containing 850 and 700 to 900 flocculating units per milligram N respectively.

Comparison of the physical and chemical properties of native antitoxic pseudoglobulin, of pepsin digested pseudoglobulin, of antitoxin from toxin-antitoxin floccules and of crystalline antitoxin (Table 14–2) shows that a portion of the native antitoxin molecule has been split off as a consequence of the enzymatic action, since the digested products have a molecular weight of approximately 100,000 as compared with 180,000 for the native antitoxin. Both pepsin and trypsin gave products of essentially identical properties.[93, 95, 96] Petermann and Pappenheimer [93] have calculated that digestion with pepsin yields a product possessing the same number of flocculating units per mole of antitoxin as originally found in the native antitoxin. This indicates that an inert portion amounting to 36 per cent of the weight of the native antitoxin is split off on digestion. Hence it would appear that the groups on the antitoxin molecule responsible for its activity are asymmetrically distributed.[41] This was supported by the finding that the digested antitoxin showed an increase in carbohydrate

content corresponding to that which would have been expected if the inert portion removed by digestion had contained no carbohydrate. The change in the axial ratios of native and digested antitoxins indicates that the inert portion was split from the native antitoxin in a plane normal to the major axis.[93]

Bourdillon [99] has reported that antitoxic pseudoglobulin solutions exhibit spontaneous splitting of inert protein on storage for a number of years in the absence of added enzyme. These solutions are similar to the digested antitoxins in that they flocculate more rapidly with toxin and "their lower molecular weight is reflected in the smaller amount of antitoxin nitrogen that combines with toxin in optimal ratio." [99] Digested or spontaneously split antitoxic pseudoglobulin has been found to form a compound with pepsin which is insoluble in salt-free solutions at moderately acid pH. It contains two to three molecules of pepsin per one molecule of antitoxin.[99]

Papain has also been found to split antitoxins into two components, one of which flocculates with toxin. Further digestion with papain yields quarter molecules none of which flocculates with toxin.[100] Prolonged digestion of normal human γ-globulin with papain or bromelin has been found to split the γ-globulin into quarter molecules. Kalmanson and Bronfenbrenner [100a] have been able to reactivate botulinus toxin from neutral mixtures with antitoxin by digestion with papain. Wright [101] has shown that diphtheria antitoxic pseudoglobulin is inactivated by urea. In 7.5 *M* urea at pH 7.82 half of the neutralizing capacity for toxin is destroyed in 24 hours. This partial inactivation is increased either at more acid or alkaline pH and is independent of the concentration of protein. The urea-treated antitoxin flocculates with toxin and the amount of precipitate formed varies with the protein concentration at which the urea treatment is carried out and is much greater than that obtained from an equal amount of untreated antitoxin.[101] This appears to be attributable to the formation of complexes of the antitoxin with non-antibody protein under the influence of urea. Similar effects have been obtained with other antibodies after exposure to heat, acid, ultraviolet light, etc. (for references *cf.*[8]).

In horse serum, the antitoxins have been found to be associated with two electrophoretic components. In diphtheria and tetanus antitoxic horse sera, a new electrophoretic peak (*T*) has been found migrating between the β- and γ-globulins.[41, 102, 103] A similar component is also present in antisera to botulinus, scarlatinal, staphylococcal toxins and in sera containing antitoxin to a variety of strains of clostridia.[104] Some investigators have reported this component as an increase in the β-globulin fraction.[105, 106] In addition, antitoxin is frequently associated with the γ-globulin component in horse serum. Kekwick and Record [106] found both types of diphtheria antitoxin in the same serum. During the course of immunization, the proportion of the two components has been found to vary. The γ type of

antitoxin is produced first but on prolonged immunization the T or β type appears and rises until it constitutes the bulk of the antitoxin in the serum. Similar conclusions may be drawn from the electrophoretic patterns reported for tetanal antitoxic sera.[103, 104] The two types of antitoxin were separated electrophoretically. They showed differences (1) in flocculation time, (2) in their combining proportions with toxin, and (3) in their ratios of protective power to flocculating activity.[107] The relation of these two types of antitoxin to the purified products obtained after digestion has not as yet been established. Digestion of antitoxic sera with pepsin has been shown to cause the disappearance of the albumin and antitoxin peaks with the formation of a large amount of substance migrating with the γ-globulin component.[108] Other inhomogeneously migrating materials are formed but decrease on prolonged digestion.

Digestion with pepsin has been found to result in a pronounced decrease in the antigenicity of diphtheria antitoxin.[109] The low residual antigenicity has been attributed to a small amount of undigested antitoxin. Takadiastase, a crude enzyme preparation containing a mixture of proteolytic and carbohydrate splitting enzymes, has also been used for the preparation of antitoxins of lowered antigenicity.[110] The action of takadiastase is believed to be primarily on the carbohydrate in the antitoxin. The takadiastase-treated products have been stated to contain less carbohydrate but to have the same sedimentation constant and electrophoretic mobility as the untreated antitoxin.[110]

Physical and chemical methods have also been employed in efforts to destroy or reduce the antigenicity of antitoxins and other horse antisera with a view to minimizing the danger from serum sickness and other allergic manifestations which may accompany serotherapy. Thus heat, acid, alkali, acetylation, treatment with diazotized aromatic amines, and ultraviolet irradiation have all been employed with varying degrees of success. Recent studies indicate that under suitable conditions ultraviolet irradiation offers considerable promise [111] (*cf.*[8]).

There is a paucity of literature concerning the physicochemical properties of antitoxins formed in animal species other than the horse. (For data on the properties of other types of antibodies consult references.[2, 8, 15, 22])

Toxin-Antitoxin Reaction. The interaction between toxin and antitoxin has frequently been thought of as a distinct type of immune reaction characteristic of toxic proteins. In the light of our present knowledge about the kinds of antibodies formed in various animal species, it is apparent that the toxin-antitoxin reaction is a special case of the precipitin reaction in that flocculation occurs only over a relatively narrow range, and in that the specific precipitate is soluble in an excess of either antibody or antigen (*cf.*[8, 14]). This type of precipitin reaction is exhibited by certain antibodies formed in the horse. It is not required that the antigen be a toxin since egg albumin,[112] rabbit serum albumin [113] and hemocyanin [114] give rise in

the horse to antibodies which react with their homologous antigens in the same manner as do the antitoxins. The horse antibodies which show the antitoxin type of reactivity are antigenically distinct from those horse antibodies, such as the antipneumococcal antibodies, which show the precipitin type of behavior although they do cross-react to some extent.[115, 116]

In the toxin-antitoxin reaction, as in the precipitin reaction, antigen and antibody can combine in multiple proportions. However, the toxin-antitoxin reaction shows a much narrower range of combining proportions (*cf.*[8, 14]). For example, Healey and Pinfield [117] found that one L_f unit of toxin could combine with one or two units of antitoxin. The floccules of toxin and antitoxin were also shown not to carry down non-specific protein.[118]

Pappenheimer and Robinson [16] applied the quantitative absolute methods for the estimation of precipitins (*cf.*[8, 14, 15]) to the study of the course of the toxin-antitoxin reaction and to the quantitative assay of antitoxic sera for their antitoxin nitrogen content.

In the study of the toxin-antitoxin reaction [16] increasing amounts of toxin are added to a constant volume of antitoxin and the total volume is kept constant. After three hours' incubation at 42°, the tubes are placed in a refrigerator over night. They are then centrifuged in the cold and the precipitates are washed three times with chilled saline to remove excess serum protein and analyzed for nitrogen by the micro-Kjeldahl method. The supernatants are tested for excess toxin or antitoxin by the Ramon flocculation reaction or by intracutaneous tests in rabbits.

Table 14–3 and Figure 14–1 (curve A) show the type of data and curves obtained in the reaction between diphtheria toxin and homologous horse antitoxin.[16] Curve B of Figure 14–1 shows a similar curve for the course of the reaction between scarlatinal toxin and antitoxin.[17] Crotoxin has also been found by Bier [118a] to give the same type of curves in reacting with its homologous horse antivenom. Flocculation begins with 150 units of toxin and the amount of precipitate increases to a maximum at 400 units of toxin. Larger amounts of toxin result in less precipitation and at 600 units or more complete inhibition occurs (Table 14–3). Tests on supernatants show that throughout the entire range in which flocculation is complete, all the toxin and antitoxin is contained in the precipitate since neither can be detected by intracutaneous tests in rabbits.

The amount of toxin nitrogen corresponding to the number of units added can be estimated from the difference in total nitrogen precipitated by addition of 200 and 400 units of toxin (Table 14–3). Thus, this difference 0.097 mg N corresponds to 200 L_f units of toxin and 1 L_f unit of toxin equals 0.00048 mg N; the average of six determinations is 0.46 μg N per L_f unit.[16] The amount of antitoxin N in the precipitate is obtained by subtracting the toxin N corresponding to the number of L_f units added from the total N precipitated. The data in Table 14–3, column 5, indicate that

Table 14–3. Addition of Increasing Amounts of Diphtheric Toxin to 300 Units of Antitoxin [16]

(A — antitoxin 621 — 300 units and 8.11 mg nitrogen per ml. T — toxin GH3–4 — 0.00055 mg nitrogen per L_f unit.)

I	*II*	*III*		*IV*	*V*	*VI* **
L_f *Units Toxin*	*Toxin* * *Nitrogen*	*Supernates* ¶		*Nitrogen* ‖ *in Precipitate*	*Antitoxin-Nitrogen in Precipitate (IV–II)*	*Ratio A–Nitrogen / T–Nitrogen (V: II)*
		Toxin, Total L_f	*Antitoxin Total Units*			
	(*mg*)			(*mg*)		
50 †	0.023		190 §	0	0	
100 †	0.046		95 §	0	0	
150 ‡	0.069			0.386	(0.474)	(6.9)
175	0.081		Trace ¶	0.554	0.473	5.8
200	0.092			0.564	0.472	5.1
225	0.103			0.579	0.476	4.6
300	0.138			0.612	0.474	3.4
400	0.184	Trace ¶		0.661	0.477	2.6
425						(2.4)
450	0.207	Trace ¶		0.652		
500 ‡	0.230	100 §		0.359		
600 †	0.276	240 §		0	0	

* The nitrogen (column IV) precipitated by 200 L_f of toxin subtracted from that precipitated by 400 L_f and divided by 200 gives 0.00048 mg nitrogen per L_f of toxin. The figure used in column II, however, is 0.00046 mg nitrogen per L_f, the average obtained from six titrations including the above.

† No flocculation.

‡ Incomplete flocculation.

§ Determined by flocculation.

¶ By intracutaneous rabbit-test.

‖ Average of duplicates.

** Figures in parentheses were calculated for the ends of the neutral zone assuming 150 L_f and 425 L_f and complete flocculation.

the same amount of antitoxin is precipitated throughout the region in which flocculation is complete and up to the point at which toxin first appears in the supernatant.

As indicated in the section on purification of toxins, the ratio of the amount of N per L_f unit found for a purified toxin preparation to that calculated from quantitative studies on the flocculation reaction affords a method for estimating the degree of purity of the preparation (Table 14–1).

The antitoxin content of a serum can be estimated by determining the nitrogen content of the washed precipitates formed at two points in the flocculation zone and demonstrating the absence of toxin and antitoxin in the supernatants. From the difference in total N precipitated between the

two points, the toxin N content of each of the specific precipitates may be calculated and that of the antitoxin in the precipitates obtained by difference.[16] The amount of antitoxin N per antitoxin unit is also readily obtained and is indicative of the degree of purity of the preparation of antitoxin (see above). Photoelectric measurements of the flocculation reaction have given curves similar to those obtained by nitrogen analysis of specific precipitates.[94]

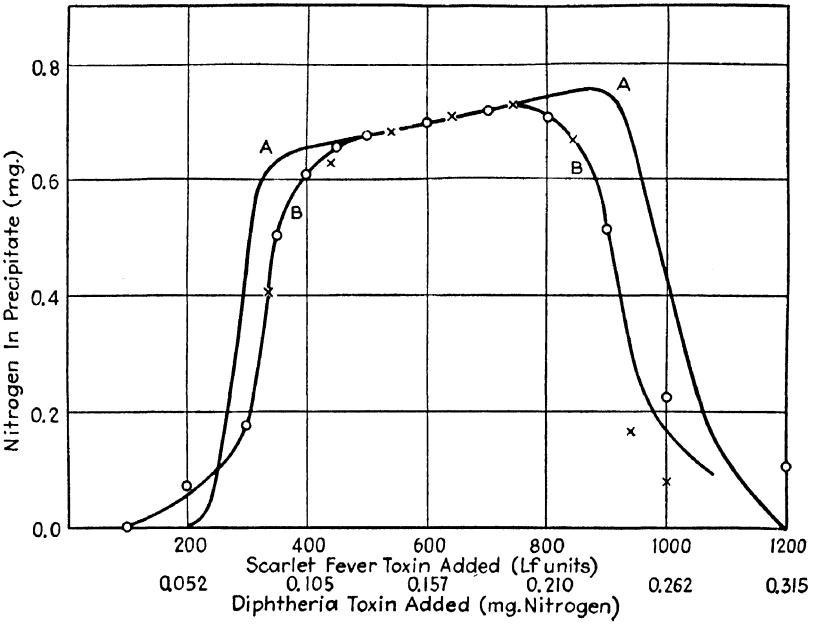

FIGURE 14–1. Quantitative toxin-antitoxin flocculation reaction. *Curve* A, diphtheria system.[16] *Curve* B, scarlet fever system. o = 594 toxin. x = NY5 toxin.[17]

Theoretical considerations of the mechanisms of the precipitin and toxin-antitoxin reactions also lend support to the inclusion of the toxin-antitoxin reaction as a special case of the precipitin reaction. Kendall [119] formulated a comprehensive theory which is in good agreement with the experimental data for both types of systems. The only assumption required by the theory to explain the differences between the precipitin and toxin-antitoxin reaction is that the precipitin type of antibody has two similar kinds and the antitoxin two different reactive groups per molecule. By considering toxin to have a valence of 4 and the dissociation constants of the compounds formed by the two types of antitoxin to be 10^{-4} and 10^{-8} and plotting the results, a curve was obtained [119] which satisfied the data of Pappenheimer and Robinson [16] up to the point where toxin appeared in excess in the supernatant.

The reaction of ricin with homologous rabbit antisera gives the usual

precipitin type of curve.[19] Ricin, however, precipitates non-specifically with normal serum proteins and specific precipitates of ricin and anti-ricin contain coprecipitated normal serum protein. For this reason antisera to ricin cannot be standardized on the basis of their content of specifically precipitable nitrogen.[19]

It is also of interest to consider the neutralization of toxins by antitoxins in relation to the reaction of other substances with biological activity, such as enzymes and viruses, with their corresponding antibodies. Little has as yet been offered to explain the observations that mixtures of toxin and antitoxin, or of virus and antivirus, in suitable proportions, fail to exhibit the characteristic toxicity of the antigen. It has frequently been assumed that the antibody exerts its neutralizing effects by combination with certain reactive groups on the antigen molecule which are essential to toxicity. A detailed discussion may be found in Sevag.[50] This concept tacitly involves the assumption that antitoxic specificity is directed toward a different portion of the antigen molecule than is the specificity of antibodies to other antigens showing biological activity such as enzymes, which are not neutralized by combination with antibody, or that the groups involved in toxicity are the same as those responsible for antigenicity. This latter view is difficult to sustain since toxins are readily detoxified without affecting their antigenicity. The former view seemed reasonable, at least for toxins, until studies of Pappenheimer [112] and of Hooker and Boyd [114] showed that the immunochemical characteristics of toxin-antitoxin reactions were not uniquely limited to toxic proteins but could be obtained with anti-egg albumin and anti-hemocyanin formed in the horse.

In contrast to the complete neutralization of toxins and viruses by their homologous antibodies over a wide range of combining proportions, it has been found that with a considerable number of enzymes, including urease,[120] ribonuclease,[121] tyrosinase,[122] emulsin (*cf.*[50]), and invertase (*cf.*[50]), only relatively slight inhibition of enzymatic activity occurred when the enzyme was allowed to react with antibody. Complete inhibition by antienzyme was not obtained. Washed specific precipitates of enzyme and antienzyme possessed as much as 75 per cent of the activity as that of the enzyme originally added.[120] This reduction in activity could be attributable to the reduced state of dispersion of the enzyme. It is noteworthy that the above mentioned enzymes act upon substrates which are of relatively low particle size and which might readily pass through antigen-antibody aggregates and combine with their enzymes. Indeed, even protein molecules appear to pass into specific precipitates, since the uptake of complement by such specific precipitates is almost as great as when they are formed in the presence of complement.[123]

The only well-documented instances in which it has been shown that antibody to an enzyme completely inhibits its activity appear to be that for malt amylase (*cf.*[50]) and for the lecithinase activity of the snake venoms

and of the α-toxin of *Cl. welchii*.[45, 59, 60] In each instance, the substrates, starch and lecithin are relatively huge particles and inhibition may be attributable in part to the formation of a three phase system in which the rate of reaction between two large aggregates (substrate and antigen-antibody) would be negligible and in part to the steric effects of the antibody in preventing the active groups of the enzyme from coming into sufficient proximity to the substrate to exert its effect. This hypothesis could also serve to explain satisfactorily the neutralization of toxin by antitoxin or virus by antivirus as observed in the usual *in vivo* assays if it be assumed that the primary step required for a toxin or a virus to exert its effect as an enzyme [45, 46] is to combine with substrates of the size of lecithin or starch particles or with living cells. Similarly, if the toxin or virus is to function as an enzyme inhibitor its combination with enzymes in or on cells is to be anticipated. It is generally conceded that the combination of viruses with cells is essential for infection and for propagation. Indeed, a number of toxins, such as ricin, abrin, etc., as well as several viruses are known to combine with erythrocytes to cause hemagglutination. This reaction may be readily demonstrated *in vitro*. The specific antibody might then exert its effect sterically merely by preventing the combination of toxin or virus with substrate or with the cell. Thus, the neutralizing effect of antibody would be largely the mechanical one of preventing the antigen from reaching its site of action.

This hypothesis offers the advantage of providing a unified concept in that it attributes to all antibodies the same type of reactivity, explaining differences between the observed effects of specific antibodies on various enzymes, and on viruses and toxins on the basis of the size of their substrates and their sites of action. It is also consistent with the recent findings of Zamecnik and Lipmann [60] that addition of antitoxin to the α-toxin of *Cl. welchii* before addition of the lecithin substrate completely inhibits the enzymatic action of the toxin. On the other hand, the addition of antitoxin to a mixture of substrate and toxin in which hydrolysis is taking place only produces a gradual slowing down of the reaction rate. In this latter instance, the antitoxin might be considered to be sterically hindered from combination with toxin while the latter was combined with the substrate. It also provides a reasonable explanation for the observation that neutralization of toxicity or viral infectivity by antibody parallels inhibition of hemagglutination or enzymatic activity.

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Chapter 15

Protein Nature of Filtrable Viruses

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Discovery of Viruses. Even though some virus diseases have been known since antiquity, the existence of viruses as entities distinct from other etiologic agents has been recognized only during the past half century.*, [89, 91] In 1892, Iwanowski found that the infectious principle in the juice of plants diseased with tobacco mosaic was able to pass through a filter which would retain bacteria. However, he failed to interpret this result to mean that the causative agent of tobacco mosaic differed from ordinary bacteria. When Beijerinck repeated this observation in 1898, he realized that the infectious entity of tobacco mosaic differed from ordinary bacteria and described it as being a contagious living fluid. In the same year, Loeffler and Frosch announced that the virus of the foot and mouth disease of cattle was caused by a filtrable agent. Four years later, Reed and associates identified yellow fever as a virus disease of man. Since that time, many diseases of plants, animals and man have been found to be caused by viruses.

During the four decades following the first recognition of viruses, very little real progress was made in the determination of the nature of these agents. However, in 1935, Stanley [87] focused attention upon the virus problem when he announced the purification and crystallization of tobacco mosaic virus and showed that it was a high molecular weight protein. The methods used to obtain purified tobacco mosaic virus have since been modified so as to be useful in obtaining numerous other viruses in purified or crystalline form. Thus, during the past decade considerable progress has been realized in the elucidation of the nature of viruses as entities, principally through studies on such purified preparations.

Knowledge concerning the nature of viruses has developed to the extent that a reasonably satisfactory definition of the term, virus, can be formulated. Viruses are disease-producing agents which resemble pathogenic bacteria in many respects. They are multiplied in the tissues of an infected host, and they lose their viability under conditions comparable to those

* Several completely documented review articles on viruses, published in widely circulated journals, [39, 46, 83, 89, 91] are cited when possible in place of original references. In such a case, original citation can be obtained from the review quoted.

under which bacteria are killed. However, they differ from most bacteria in two important respects; no virus has ever been grown in the absence of living tissue of the host, and viruses are almost always smaller than bacteria, small enough to pass through bacterial filters and too small to be seen with the optical microscope. Some viruses are of a sufficient degree of simplicity to warrant the assumption that they are protein molecules; others are almost as complex as some of the simple bacteria.

Estimation of Virus Infectivity. The epoch-making discovery that tobacco mosaic virus can be crystallized as a nucleoprotein was possible only because a reasonably reliable method had been developed for quantitative estimation of virus concentration. This provided a means for determining with precision the effects of various procedures upon the concentration of virus activity. It might be a valid generalization to state that progress in the elucidation of the properties of any virus depends upon the availability of a reasonably accurate method for measuring that virus.

Quantitative estimation of virus infectivity depends upon the nature of the relationship between the percentage response of inoculated organisms and the magnitude of the dose of virus applied. In general, one finds that, with very small doses, virtually no response is obtained and, with very large doses, virtually complete response is obtained. Usually, some intermediate dose can be found which results in partial response. As a rule, when one plots percentage response against logarithm of dose, a sigmoidal graph results.[44] If one plots logarithm of percentage response against logarithm of dose, one usually obtains a graph which is linear for small and intermediate doses and curvilinear for higher doses.

The case of tobacco mosaic virus affords an interesting illustration of the methods useful for the determination of virus activity.[89, 91] In 1929, Holmes observed that local necrotic lesions were produced on leaves of several species of Nicotiana when they were rubbed with tobacco mosaic virus. Price showed in the next year that similar lesions were developed on certain varieties of the garden bean. In both cases, the number of lesions is largely dependent upon the concentration of the inoculating dose. However, other factors such as variability among plants also influence the response. Such factors can be overcome to a considerable extent by the employment of proper experimental arrangements. Samuel and Bald introduced the procedure of comparing virus preparations on opposite halves of the same leaves. The next step was the application of statistical procedures to determine the accuracy with which differences in virus concentration could be detected. Beale demonstrated that the half leaf method could be relied upon to distinguish between virus preparations which differed by 50 per cent in virus concentration, and Loring noted that, under more favorable conditions, differences as little as 10 per cent were detectable.

Price and Spencer [70, 84] developed a method which made it possible, first,

to evaluate quantitatively the relative activity of two virus samples and, second, to provide an approximation of the probable error of that estimate. Their method is based upon the assumption that the logarithm of the number of lesions obtained on bean or other test plants is an essentially linear function of the logarithm of the virus concentration applied. The method consists of applying each preparation of virus at two different concentrations to the leaves of the test plant. The ratio, r, of the infectivity of the two virus solutions is then calculated by using the following simplified formula:

$$\log r = \frac{dI}{B}$$

The symbol, d, is equal to $(S_1 + S_2) - (U_1 + U_2)$, where S_1 and S_2 are the logarithms of the numbers of lesions obtained with the standard virus prep-

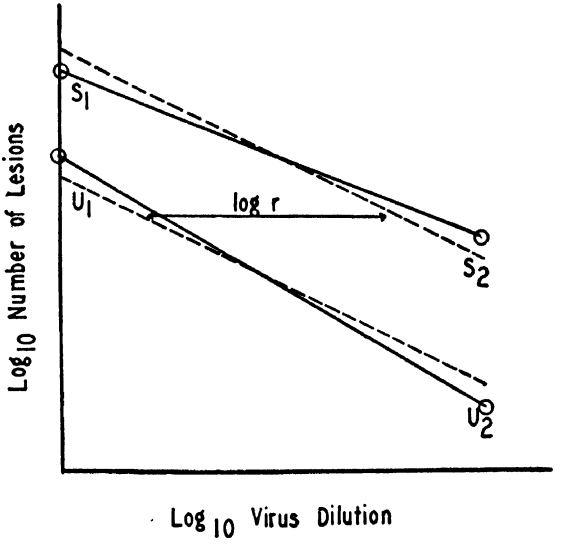

FIGURE 15–1. Graphical interpretation of the method of Price and Spencer for determining relative virus activities. U_1 and U_2 are the logarithms of the numbers of lesions obtained with the unknown virus solution at the higher and the lower concentrations, respectively. S_1 and S_2 are the logarithms of the numbers of lesions obtained with the standard virus solution at higher and lower concentrations respectively, and r is the ratio of the infectivities of the two preparations.

aration at the higher and the lower concentrations, respectively, and U_1 and U_2 are the logarithms of the numbers of lesions obtained with the unknown virus. The symbol, B, is equal to $(S_1 + U_1) - (S_2 + U_2)$, and the symbol, I, represents the interval on a logarithmic scale between the high and the low dilutions of each virus sample. The sort of data obtained can be represented graphically as the two solid lines in Figure 15–1. The algebraic process described above is the equivalent of finding the lateral separation between those two parallel lines which represent the best possible

fit of the data, the broken graphs in the figure. This lateral separation obviously represents the logarithm of the relative concentrations of the two solutions. Price and Spencer have shown that this method can be used to determine the relative activities of virus solutions, that it can be applied to many viruses, and that a statistical procedure can be utilized to provide a reliable estimate of the error.

A somewhat different approach has been followed in the development of methods for measuring the activity of animal viruses. The underlying philosophy can be illustrated by a consideration of the way in which mouse infectivity titers of influenza virus are determined. As was stated previously, when percentage response is plotted against logarithm of dose, a sigmoidal relationship is obtained. In studies on elementary bodies of vaccinia, Parker pointed out that the concentration of virus which produces a 50 per cent response in the host could be used as a satisfactory measure of virus infectivity.[83] In utilizing this method in the case of influenza virus, a series of virus suspensions is prepared at concentrations differing by tenfold factors, and each dilution is intranasally inoculated into 5 to 10 mice. One then finds the concentration which causes the death of approximately 50 per cent of the inoculated mice within some reasonable period of time. Reed and Muench pointed out that one can achieve greater accuracy in the estimation of the 50 per cent end point if all of the data obtained in an inoculation series are employed instead of merely those at the concentration giving most nearly 50 per cent response.[83] In essence, their method consists of carrying out the experiment just as described before and then in accumulating the fraction of positive responses from the more dilute to the more concentrated inoculum and also the fraction of negative responses from the more concentrated to the more dilute inoculum. The concentration at which the accumulated positive and accumulated negative responses are equal is then found by either algebraic or graphical means. This concentration is regarded as the 50 per cent end point. The method depends upon the implicit assumptions that the graphical relationship between percentage response and logarithm of dose is a symmetrical sigmoidal curve and that the interval between dose concentrations is constant. Under such circumstances, accumulating the positive responses on the low concentration side of the dilution series is the equivalent of measuring the area between the dose response curve and the base line from zero concentration to the highest concentration involved in the accumulation. Similarly, accumulating the negative response from the highest concentration toward the lowest is the equivalent of determining the corresponding area between the dose response curve and the asymptote corresponding to 100 per cent response. It can be shown readily that at the 50 per cent end point the indicated area below the curve is equal to the corresponding area above the curve. The method of Reed and Muench simply involves the determination of the point at which these two areas are equal. It represents the best estimate of the 50 per cent

end point which can be obtained from the available data. A statistical study of the accuracy of the 50 per cent mortality end point for the titration of influenza virus was made by the author and Miller.[42] They determined the precision which could be expected when five mice were inoculated with each of six virus solutions differing in concentration by tenfold factors. From this they determined that a difference of five- to sixfold must be obtained between the end points of two different virus samples in order to insure a probability of 0.95 that the two samples actually differ in virus activity.

Another type of measurement of virus infectivity can be illustrated by the studies of Bryan and Beard on rabbit papilloma virus.[12] These workers showed that there is a high correlation between the concentration of virus inoculum and the length of time required for the development of observable symptoms. This relationship was used in subsequent studies in order to estimate virus concentrations.

Virus activity can be of two sorts, the ability to produce infection, and secondary manifestations such as immunochemical reactions and the ability to agglutinate red blood cells. These secondary activities can sometimes be utilized as bases for precise measurements, as illustrated by the red blood cell agglutination of influenza virus. It was observed independently by Hirst and by McClellan and Hare that influenza virus is capable of agglutinating or precipitating red blood cells of chickens and other animals.[39] Hirst and Pickels showed that this property can be used for determining the amount of influenza virus in a suspension. They defined the unit of red cell agglutinating activity as that amount of virus which will precipitate half of the red cells from a 0.75 per cent suspension in 75 minutes. They observed that this 50 per cent point could be found by using a photoelectric colorimeter. Miller and Stanley [60] determined by statistical procedures that a difference of about 8 per cent between the averages of two sets of duplicate determinations has a probability of 0.95 of being significant. The sole limitation to an indirect method such as this is the fact that it is necessary to assume a correlation between the biological activity and infectivity. In the case of influenza virus, it has been shown by sedimentation and electrophoresis experiments that the red blood cell agglutinating ability is associated with the same particles as the infectivity.[39]

Purification and Crystallization of Plant Viruses. The availability of viruses in pure or highly purified form has been responsible for much of the progress of the past decade in the elucidation of their nature. The efforts to obtain purified viruses received their greatest impetus by the crystallization, in 1935, of tobacco mosaic virus. Thus far, five viruses have been crystallized and many more have been obtained in purified and concentrated forms.

The crystallization of tobacco mosaic virus was of such great significance that the methods used in the original investigation are discussed in a little

detail. Methods for the partial purification of this virus had been worked out by Vinson and Petre.[89] However, it was obtained in the pure form for the first time by Stanley from starting material consisting of the juice extracted, after freezing, grinding and thawing, from Turkish tobacco plants diseased with tobacco mosaic.[87] This juice was adjusted to a pH between 6 and 7, and the virus was precipitated by adding about 20 per cent ammonium sulfate. It was collected by filtration and was redissolved and reprecipitated several times. Finally, a brown solution remained. The pigment was removed by treatment with lead subacetate. The reagent was added at pH 8.8, the solution was filtered, and the filtrate was acidified to pH 4.5.

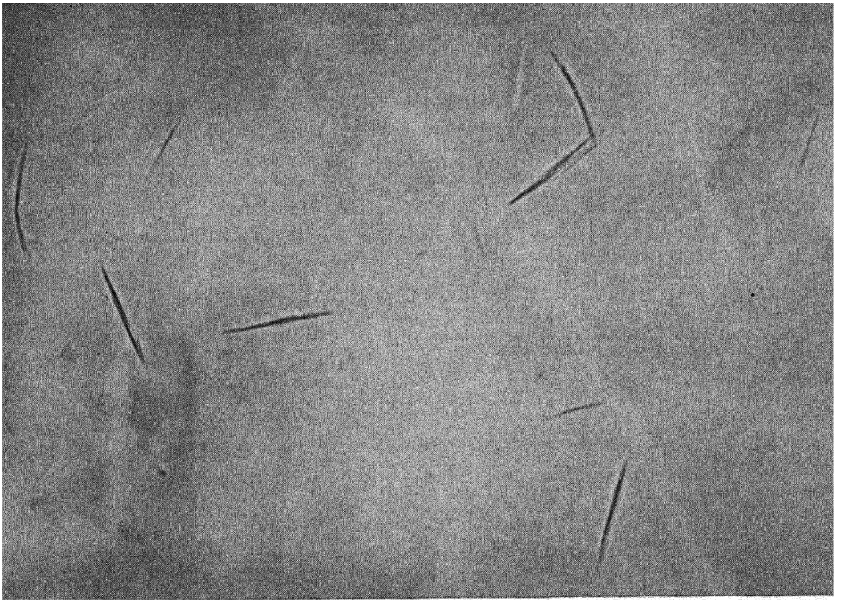

FIGURE 15–2. Crystals of tobacco mosaic virus. *From Stanley.*[88]

Under these circumstances, the virus precipitated, and much of the remaining pigment stayed in solution. The virus was filtered off and then redissolved. Ammonium sulfate was added until crystallization began, and then 5 per cent glacial acetic acid in ammonium sulfate was added to complete the crystallization process. Needle-like crystals, such as those shown in Figure 15–2, were obtained. In subsequent studies, it was shown that a calcium oxide suspension could be used for the purpose of getting rid of pigment, and under favorable conditions, this decolorizing step could be omitted entirely. Numerous strains of tobacco mosaic virus have since been obtained in similar crystalline forms.

Soon after tobacco mosaic virus was crystallized, it was learned that the virus is a protein of very high molecular weight. This discovery led

Wyckoff, Biscoe and Stanley [89, 91] to attempt the purification of tobacco mosaic virus by means of high speed centrifugation. This procedure was highly successful and paved the way for the subsequent purification of many viruses.

It is now known that dried tobacco mosaic virus particles are rod-shaped bodies with a diameter of 15.2 mμ and a length of about 280 mμ.[38] X-ray studies on tobacco mosaic virus crystals carried out by Bernal and Fankuchen[9]

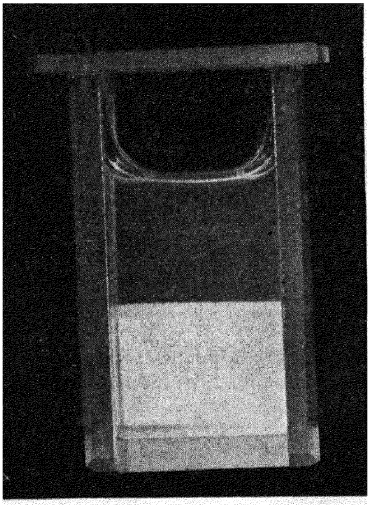

FIGURE 15–3. The layering phenomenon shown by tobacco mosaic virus. Cell photographed between crossed Polaroid plates. *From Lauffer.*[38]

have shown that in the crystals the rod-like virus particles are lined up parallel to each other with perfect hexagonal regularity with respect to cross section but without regularity in the direction of the long axis. Crystals with such two-dimensional regularity are often referred to as paracrystals. Because the virus is composed of long rod-shaped particles, it is possible for its solution to form liquid crystals. When a one or two per cent solution of tobacco mosaic virus is allowed to stand, it may separate into two phases, one of which is isotropic and the other of which is birefringent or liquid crystalline, as illustrated in Figure 15–3. Concentrated gels of the virus material are almost invariably liquid crystalline. The x-ray studies of Bernal and Fankuchen have shown that, in the liquid crystalline state, the virus particles are lined up parallel with the same degree of regularity as in the solid state. In many cases, the parallel virus rods in the liquid crystals are separated by several hundred millimicrons. The intervening space is, of course, occupied by solvent. This fact has given rise to the conclusion that long range forces are exerted by tobacco mosaic virus particles.

Other viruses have since been obtained in crystalline form. Bawden and Pirie [4] purified the virus causing the bushy stunt disease of tomatoes and showed that it could be obtained in the form of dodecahedric crystals. Stanley [92] later purified this same virus by centrifugal means and obtained

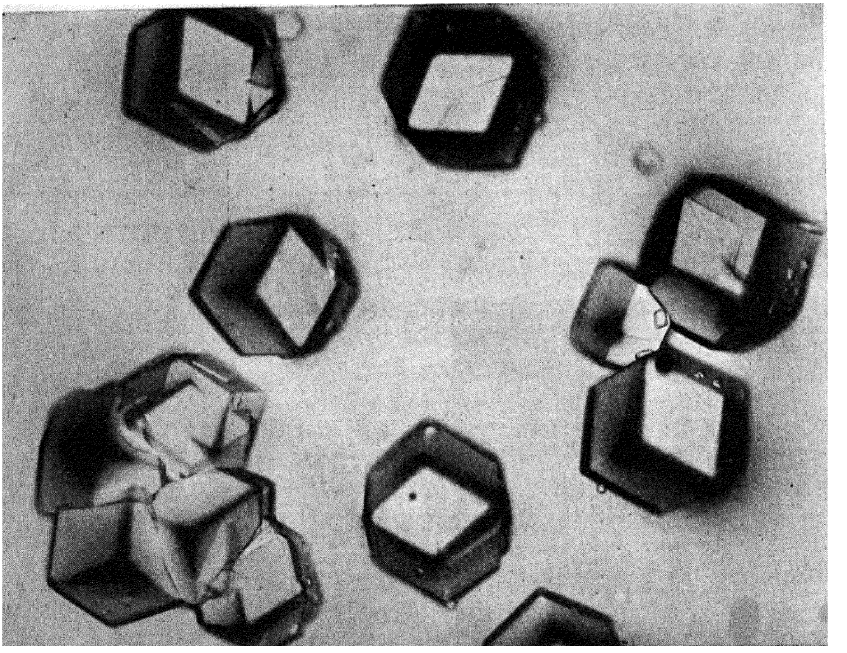

FIGURE 15–4. Crystals of tomato bushy stunt virus. *From Stanley.*[12]

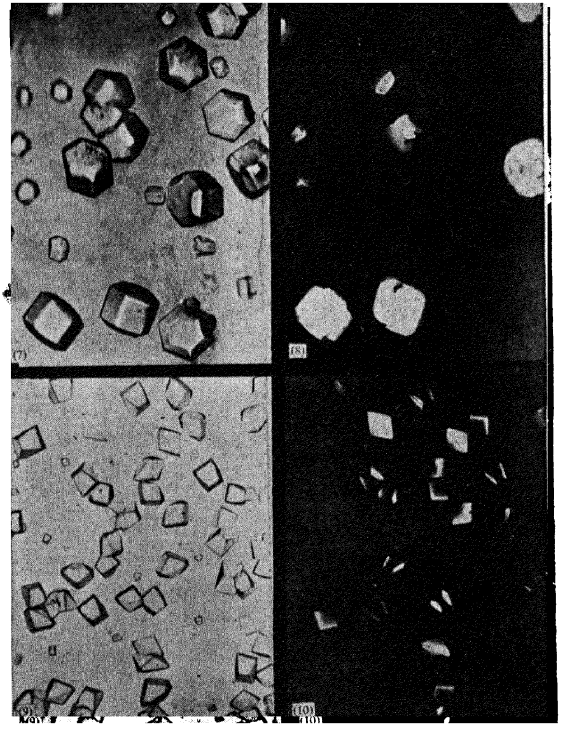

FIGURE 15–5. Crystals of tobacco necrosis virus. *From Bawden and Pirie.*[6]

the same type of crystals. Cohen [15] showed that a different crystalline form could be obtained when hydrophyllic colloids were used to induce crystallization. Representative crystals of the virus are shown in Figure 15–4. Tobacco necrosis virus was next subjected to crystallization by Pirie, Smith, Spooner and McClement [67] and later by Bawden and Pirie.[6] Two types of crystals are shown in Figure 15–5. The virus causing southern bean mosaic was purified by chemical and centrifugal means by Price.[69] It was found

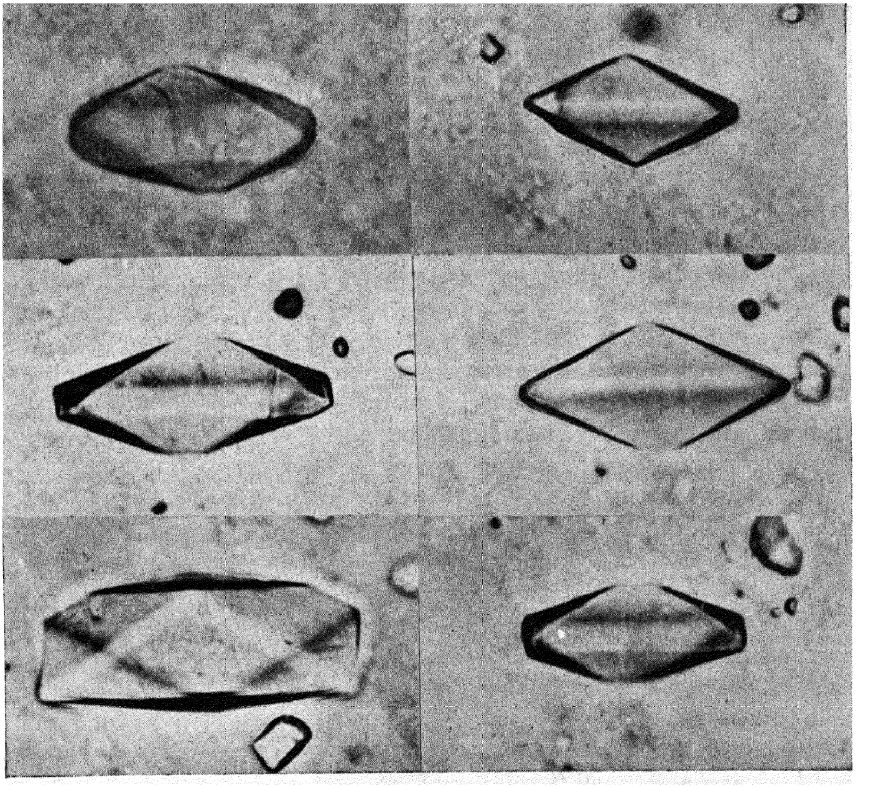

FIGURE 15–6. Crystals of southern bean **mosaic virus.** *From Price.*[69]

possible to crystallize it in different forms by different technics. Pictures of crystals of this virus are shown in Figure 15–6. Recently Markham and Smith [56] have announced the crystallization of a virus causing turnip yellow mosaic. Several plant viruses have been obtained in purified form even though it was not found possible to crystallize them. Stanley and Wyckoff have described preparations of ring-spot virus and of severe etch virus,[89] and Loring and Wyckoff [89, 91] have purified latent mosaic virus.

Purification of Animal Viruses. Work on the purification and concentration of animal viruses in reality predates the crystallization of tobacco mosaic virus.[83] In 1923, MacCallum and Oppenheimer showed that the

elementary bodies of vaccinia could be concentrated by means of centrifugation. In 1932, Craige described a detailed method for the purification of elementary bodies of vaccinia through the use of differential centrifugation. This method was modified somewhat by Parker and Rivers. The virus is obtained from skin lesions of inoculated rabbits. After the rabbit is sacrificed, the skin is cleaned, covered with a buffer solution and then scraped. The virus is liberated in this process and may then be purified by alternate centrifugation for five minutes at 3,000 rpm and one hour at 4,500 rpm. After several cycles, a reasonably purified virus preparation is obtained. Rabbit papilloma virus was concentrated and purified by Beard and associates [8] from warts occurring on cotton-tail rabbits. Wyckoff [104] and Beard and associates [98] reported the concentration of the virus causing equine encephalomyelitis. In recent years, influenza virus has been obtained in highly purified form in several laboratories.[20, 49, 82, 99, 100] A number of reports are available on the concentration and purification of poliomyelitis virus. A representative one is that of Loring and associates [54] in which the concentration of the Lansing strain from brains and spinal cords of diseased cotton rats is described. Gard [21] reported experiments on the purification and concentration of Theiler's mouse encephalitis virus. Claude [14] described the concentration of mouse sarcoma virus, and Janssen [26] described the purification of foot and mouth disease virus. Several of the bacterial viruses have been obtained in the purified form. A representative study is that by Taylor and other associates of Beard.[25, 80, 97]

Identification of the Virus. In order to be justified in considering a preparation obtained by crystallization or other procedure as actually being the virus, it is necessary to bring many types of evidence to bear upon that supposed identity. Of the many viruses which have been isolated thus far, three have been subjected to really critical tests for the identity of the virus and the preparation. The most exhaustively studied is tobacco mosaic virus. The early work of Stanley [89, 91] and of other investigators has shown that tobacco mosaic virus protein is always found associated with the infectious agent, for it has been isolated from many different batches of diseased tobacco plants and from other plant species infected with the mosaic disease. Similar but characteristic proteins have been obtained from plants infected with related diseases. Entirely different proteins have been isolated from tobacco and other plants infected with unrelated diseases. The ultraviolet absorption spectrum of tobacco mosaic virus protein coincides with the range for the destruction of infectivity. The pH stability range of the protein coincides with the stability range of virus infectivity. Immunochemical studies have failed to demonstrate the presence of appreciable amounts of impurity in highly purified preparations. Attempts to separate virus activity from virus protein by chemical fractionation have invariably failed. Attempts to fractionate by high speed centrifugation have likewise failed. Virus preparations were found to be essentially homogeneous with

respect to sedimentation rate and electrophoretic mobility. Finally, the sedimentation rate of the infectious principle of tobacco mosaic virus was determined through studies carried out with the separation cell in the ultracentrifuge. It was found to be the same as that of the virus protein within a probable error of 6 per cent.[36] This constitutes a highly sensitive test for the identity of the virus particle with the virus protein molecule. All of these studies leave little doubt that tobacco mosaic virus protein is the virus itself.

Among the viruses causing diseases of man and animals for which extensive evidence of the identity of the preparation with the virulent agent is available, influenza virus and vaccinia virus are conspicuous. Influenza virus preparations have been shown by means of electron microscopy and ultracentrifugation to consist largely of spherical particles with a diameter of around 115 mμ.[39] Highly purified preparations homogeneous with respect to sedimentation rate and electrophoretic mobility have been obtained. Such materials have been isolated from the allantoic fluid of infected chicken embryos and from the lungs of diseased mice. Invariably, 115-mμ particles have been found, even though in all cases other types of particles have also been present. Elford, Andrewes and Tang [19] inferred from filtration studies that the infectious particles were between 80 and 120 mμ in diameter. Similar results were obtained by Friedewald and Pickels.[20] Thus, the size of the infectious entity and of the 115-mμ spheres in preparations agree. Elford and Andrewes carried out sedimentation studies on the infectious principle and derived data from which it can be computed that the sedimentation constant is between 840 and 1090 Svedberg units.[18] Friedewald and Pickels [20] found from sedimentation experiments in sucrose gradients in an angle centrifuge that the sedimentation rates of the infectious principles of several strains of influenza virus fall somewhere between 600 and 1000 Svedberg units. Sedimentation studies carried out by Beard and associates led to essentially the same conclusions.[82, 99, 100] The sedimentation constants of the principal components of purified preparations of various strains of influenza virus are in the range from 700 to 800 Svedbergs. Stanley succeeded in obtaining fractions of influenza virus preparations consisting largely of small particles and other fractions consisting largely of the 115-mμ particles.[39] He was able to show that the fraction composed of the large particles had a much higher specific virus infectivity than the fraction consisting largely of the smaller particles. Results of relatively high precision were obtained by Lauffer and Miller through the use of the separation cell in the ultracentrifuge. These results show that the sedimentation rates of the infectious principle and the red blood cell agglutinating ability agree with the sedimentation rate of the 115-mμ particles within the probable error of the experiment. Similarly, the electrophoretic mobility of the chicken red blood cell agglutinating activity was found to coincide with that of the 115-mμ particle. When all of the evidence is assem-

bled, it seems highly probable that the infectious principle and the hemagglutinin of influenza virus are associated with the 115-mμ spherical particles which predominate in all preparations of the virus. However, the question as to whether or not any influenza virus preparation has ever been obtained chemically pure is still open. It is known that several types of particles characteristic of normal chicken embryos are present in the preparations obtained by routine methods. These can be demonstrated by means of the ultracentrifuge and the electron microscope and even by viscometry. It is possible to fractionate influenza virus preparations with the ultracentrifuge in such a way as to get rid of most of the contaminating material. However, Knight's [31] immunochemical studies have indicated that influenza virus preparations contain perhaps 30 to 50 per cent of antigens characteristic of the host from which the virus was obtained. He has interpreted this result to mean that the 115-mμ spheres are composed of essentially equal portions of constituents derived from the host and of constituents characteristic of the virus.

Much evidence has also been brought to bear upon the question of the identity of the elementary body of vaccinia and the virus causing vaccinia.[83] Highly purified preparations of elementary bodies have been shown to be of uniform chemical composition. They are essentially homogeneous with respect to sedimentation rate and electrophoretic mobility. Here again, filtration studies indicate that the virus is associated with particles of essentially the size which ultraviolet microscopy and electron microscopy indicate for the elementary body. Sedimentation studies also indicate that the sedimentation rate of the infectious principle is of the same order of magnitude as that of the elementary bodies. Highly significant evidence was obtained by Parker and by Smadel, Rivers and Pickels when they showed that a single infectious unit of vaccinia virus of a highly virulent strain is capable of inducing an infection in a rabbit, and that the number of infectious units in a given preparation, as determined by infectivity titrations, is of the same order of magnitude as the number of elementary bodies, as determined by direct analysis of the suspension.

Many of the other virus preparations which have been obtained are presumed by investigators in the field to represent reasonably pure virus suspensions. There is no good reason to regard these beliefs as being entirely unwarranted, but it must be remembered that very few viruses have been identified with the virus preparations with anything approximating the degree of assurance obtained in the case of tobacco mosaic, influenza, and vaccinia viruses.

Chemical Nature of Viruses. Even though many viruses have been subjected to chemical analyses, the most extensive results obtained thus far have been on tobacco mosaic virus.[89, 91] Elementary analyses have shown that tobacco mosaic virus protein contains about 16 per cent nitrogen, 48 per cent carbon, 7.3 per cent hydrogen, 0.56 per cent phosphorus and 0.2

per cent sulfur. Positive color tests are given with Millon, Sakaguchi, biuret, xanthoproteic, glyoxylic acid, and Folin's tyrosine reagents. The Fehling test is negative and the Molisch test is faint and delayed. Tobacco mosaic virus is precipitated by trichloroacetic acid, phosphotungstic acid, tannic acid, lead acetate, lead subacetate, alcohol, acetone and common salts. The virus can be precipitated at its isoelectric point, and also by pyridine,[5] by heparin and by other hydrophyllic colloids.[16] It can form crystalline complexes with oppositely charged proteins, [28a, 38a] and it can also be crystallized by proteins like serum albumin when the charge is of the same sign as that of the virus.[38a]

Tobacco mosaic virus protein is a nucleoprotein. The isolation of nucleic acid was reported by Bawden and associates [7] and by Stanley.[90] Subsequently, the nucleic acid was isolated in quantity and analyzed by Loring.[53] He demonstrated that guanine, adenine, pyrimidine and a crystalline brucine salt with the characteristic solubility of the brucine salt of uridylic acid could be recovered after hydrolysis. Bawden and Pirie [3] did not obtain a positive test for desoxypentose with Schiff's reagent. They found that the phosphorus was liberated as phosphate on acid hydrolysis in the same manner as in the case of yeast nucleic acid. On the basis of these results, it can be concluded that the nucleic acid of tobacco mosaic virus is comparable in many ways to yeast nucleic acid. Loring, however, observed a somewhat higher specific rotation. One can calculate from the phosphorus analyses that the virus contains about 5 per cent nucleic acid. This result has been confirmed by Schramm and Dannenberg,[75] who studied the ultraviolet absorption spectrum of tobacco mosaic virus and of the nucleic acid after liberation by denaturation. On the assumption that tobacco mosaic virus nucleic acid has the same absorption as yeast nucleic acid, they calculated that the virus contains 4.9 per cent of the acid. Nucleic acid can be liberated from tobacco mosaic virus protein by denaturation by strong acids, strong bases, urea, or at high temperatures or high pressures. However, simple treatment with neutral salts does not result in a separation. Cohen and Stanley [17] found that a nucleophosphatase obtained from intestinal mucosa would hydrolyze virus nucleic acid. However, they found that this enzyme had no effect whatever on the intact virus. The latter was in contradiction with an earlier report of Schramm.[73] Cohen and Stanley isolated preparations of nucleic acid from heat denatured virus. Through physical studies with the ultracentrifuge, the electron microscope, and other tools, they found that freshly isolated nucleic acid consists of fibers about 70 mμ long with an average molecular weight of about 300,000. This material decomposes spontaneously into asymmetric particles with a molecular weight of about 61,000. By the action of cold alkali, the material is converted further into a substance with a molecular weight of about 15,000. The dimensions for these smallest particles were estimated to be about 15.0 $\times$ 1.5 millimicrons.

Several experiments designed to detect the presence of enzyme or partial enzyme systems in tobacco mosaic virus have been reported. Sprince and Schoenbach found a mere trace of biotin and no evidence of riboflavin or pantothenic acid.[85] Williams, Schlenk and Eppright [103] found traces of thiamine, niacin, pantothenic acid, pyridoxin and biotin and virtually no inositol and folic acid. The amounts were comparable to those in egg albumin and hemoglobin preparations. They are much smaller than they should be if tobacco mosaic virus were to possess its own functioning enzyme systems.

Amino Acid Content of Tobacco Mosaic Virus. Considerable work has been done on the amino acid composition of tobacco mosaic virus by Ross,[72] by Knight and Stanley [29, 32] and by other investigators.[11, 23, 24, 52, 62, 95, 101] The various values reported from the several laboratories are listed in Table 15–1.

Some of the studies were carried out by various chemical assay methods,[11, 24, 29, 32, 62, 72, 101] others by microbiological methods.[23, 52, 95]

Amino acid analyses afford a chemical basis for the estimation of the molecular weight of the virus or of some repeating unit within the virus, provided that the repeat units are all identical and invariable structures. The minimum molecular weight of the repeat unit must be high enough to contain one residue of the least concentrated amino acid. Because of the fact that the number of free amino groups in tobacco mosaic virus seems to be just about the same as the number of lysine residues,[59] one can conclude that there are not many free ends of peptide chains. Thus, the residue weight may be approximated by subtracting the molecular weight of water from that of the amino acid in question. Along with the analyses presented in Table 15–1 are listed the molecular weights of the units which would contain at least one residue. A value of 18,000 can be seen to be approximately a simple multiple of each value listed. Thus, one can conclude that a repeat unit with a molecular weight of about 18,000 is indicated by the amino acid analyses at present available. It is not unlikely that this value will have to be changed when more precise data become available, for the sum of all of the residue percentages and the nucleic acid percentage at present accounts for only about 80 per cent of the virus particle.

Chemical Differences between Strains of Tobacco Mosaic Virus. It is obvious that the biological differences between two strains of tobacco mosaic virus must be reflected in some differences in structure of the virus protein. Results obtained by Pfankuch, Kausche and Stubbe [66] seemed to indicate that irradiation-induced mutations may result in slight changes in the nucleic acid content. Extensive studies were carried out by Knight and Stanley,[29, 32] on eight different naturally occurring strains of tobacco mosaic virus. Numerous analyses were made on each strain. Their data are shown in Table 15–2. It can be observed that no appreciable differences were found in phosphorus content. Therefore, presumably there are no differences in nucleic acid content in the various strains, in contrast with

Table 15–1. Amino Acid Composition of Tobacco Mosaic Virus

Amino Acid	*Per Cent*	*Residue * Per Cent*	*Residue Weight*	*Minimum Mol. Wt.*	*Approx. No. per 18,000*
Arginine	9.0,[a] 8.9 [c]	8.07	156.2	1,935	9
Lysine	1.36 [c]	1.19	128.2	10,800	2
Histidine	0.02 [c]	—	137.2	—	—
Phenylalanine	6.0,[a] 6.0,[b] 6.8 [c]	6.06	147.2	2,430	7
Tyrosine	3.9,[a] 3.8,[b] 3.4,[c] 3.6,[f] 3.6[h]	3.51	163.2	4,650	4
Tryptophane	4.5,[a] 4.5,[b] 2.3,[c] 3.7 [f]	2.10	186.2	8,860	2
Proline	4.6 [a]	3.88	97.1	2,500	7
Cysteine	0.7,[a] 0.68,[e] 0.73 [f]	0.60	103.1	17,200	1
Methionine	0.06 [c]	—	131.2	—	—
Aspartic acid	2.6 [a]	2.25	115.1	5,115	4
Glutamic acid	5.3,[a] 12.5,[d] 17.0 [g]	14.91	129.1	865	2
Alanine	2.4 [a]	1.915	71.1	3,722	5
Serine	6.4 [a]	6.01	87.1	1,645	11
Valine	3.9,[a] 7.0 [c]	5.92	99.1	1,673	11
Leucine	6.1,[a] 7.5 [c]	6.46	113.2	1,750	10
Isoleucine	5.7 [c]	4.91	113.2	2,307	8
Threonine	5.3,[a] 8.7 [c]	7.38	85.1	1,154	16

[a] Ross [72]
[b] Knight and Stanley [32]
[c] Stokes *et al.* [23, 95]
[d] Lewis and Olcott [52]
[e] Hess *et al.* [24]
[f] Best and Lugg [11]
[g] Olcott [62]
[h] Thomas [101]

* Computed from underscored amino acid analyses in second column.

Table 15–2. Aromatic and Basic Amino Acids and Phosphorus in Strains of Tobacco Mosaic Virus [29, 32]

Virus	*Tyrosine* (%)	*Tryptophane* (%)	*Phenylalanine* (%)	*Arginine* (%)	*Histidine* (%)	*P* (%)
Tobacco mosaic	3.8	4.5	6.0	9.2	0.02	0.56
Yellow aucuba	3.9	4.2	6.3	10.0	—	0.52
Green aucuba	3.9	4.2	6.1	10.0	—	0.54
Holmes' ribgrass	6.4	3.5	4.3	9.1	0.55	0.53
Holmes' masked	3.9	4.3	6.1	9.1	—	0.54
J14D1	3.8	4.4	6.1	9.2	—	0.55
Cucumber virus 4	3.8	1.4	10.2	8.7	—	0.54
Cucumber virus 3	4.0	1.5	10.0	8.7	—	0.56

the report mentioned above. However, other chemical differences were observed in several instances. For example, the Holmes ribgrass strain of tobacco mosaic virus was found to have very much more tyrosine and histidine and significantly lower amounts of both tryptophane and phenylalanine than the normal strain. Also, it was found that cucumber mosaic viruses 3 and 4 have much less tryptophane and much more phenylalanine than the other strains. The two aucuba strains seem to have more arginine than the others. Subsequent studies by Knight [30] have shown that the ribgrass strain contains about 0.62 per cent sulfur as contrasted with 0.2 per cent in the normal strain. However, the cysteine content of the ribgrass virus was found to be about 0.68 per cent, virtually the same as in the ordinary strain. The additional sulfur of the ribgrass strain seems to be associated with methionine, which is either absent or present in only very low concentration in the normal strain. These results indicate that the minor differences in biological properties, shown by the related strains of a virus, are reflected in definite differences in the structures of the virus proteins.

Knight [31a] recently reported chemical and microbiological analyses for 19 amino acids on purified preparations of 8 different strains of tobacco mosaic virus. In general, those strains which appeared to be most distantly related showed the greatest differences in amino acid composition. Gaw and Stanley [21a] analyzed purified preparations of the ordinary and the ribgrass strains of tobacco mosaic virus obtained from both Turkish tobacco and phlox plants. Values for tyrosine, tryptophane and phenylalanine indistinguishable from those shown in Table 15–2 were obtained for the two strains, regardless of the source. Thus, the amino acid composition was shown to be characteristic of the virus strain and not influenced by the nature of the host.

Relation of Infectivity to Alteration of Chemical Structure of Tobacco Mosaic Virus. Tobacco mosaic virus can be modified chemically to a certain extent without alteration of its infectiousness. However, extensive chemical changes are paralleled by inactivation and other changes in the virus particle. Ross and Stanley studied the reaction of tobacco mosaic virus with formaldehyde.[91] They found that the virus was inactivated and that the extent of inactivation was correlated with the extent of decrease of amino groups as indicated by the Van Slyke method and the ninhydrin method. Inactivation was also accompanied by a decrease in the number of groups which react with Folin's reagent. Ross and Stanley found that an apparent reactivation, as indicated by an increase in the number of lesions produced on *Nicotiana glutinosa* plants, could be achieved by dialyzing inactivated virus at pH 3. Kassanis and Kleczkowski [27] failed to confirm either the parallelism between activity and amino groups or the apparent reactivation. However, results recently obtained by a student in the author's laboratory indicate that the apparent reactivation can be confirmed.

Anson and Stanley [1] found that the SH groups in tobacco mosaic virus protein can be oxidized by treatment with iodine dissolved in hydrogen iodide. This oxidation can be detected by the failure of the virus protein to give a positive nitroprusside test in guanidine and hydrochloric acid. Their experiments showed that when only the SH groups have been oxidized, the virus still retains its normal biological activity as shown by the number of lesions produced on *Nicotiana glutinosa*. When Turkish tobacco plants were inoculated with virus containing oxidized SH groups, the virus eventually isolated from these diseased plants contained normal SH groups. When the reaction with iodine was carried out under more strenuous conditions, the tyrosine groups in the virus protein molecule were converted into diiodotyrosine. This was indicated by the failure of such viruses to give a test with Millon reagent. Such virus was not infectious.

Schramm and Müller [76] reported that the amino groups of tobacco mosaic virus could be completely covered by treatment with ketene or phenyl isocyanate without a decrease in virus activity. When this problem was reinvestigated by Miller and Stanley,[59] it was found that when as many as 70 per cent of the amino groups were covered, virus activity remained essentially normal. When more than 70 per cent coverage was achieved, virus activity decreased. Electrophoretic analyses carried out on virus which had 70 per cent of its amino groups covered by acetylation or by treatment with phenylisocyanate showed single boundaries indicating that 70 per cent of the groups in each molecule were covered rather than that 70 per cent of the molecules had reacted. Miller and Stanley also tested for decrease in tyrosine and tryptophane groups with Folin's phenol reagent by Herriott's method. They found that from 20 to 40 per cent of these groups could be covered without decrease in infectivity. When Turkish tobacco plants were infected with treated virus and then allowed to propagate, normal tobacco mosaic virus was isolated from the plants. Essentially similar results were obtained when tobacco mosaic virus was allowed to react with carbobenzoxy chloride, *p*-chlorobenzoyl chloride, and benzene sulfonyl chloride. One alteration in biological activity was observed, however. A number of the derivatives exhibited a significantly lower specific activity when tested on leaves of the common bean, *Phaseolus vulgaris*, than when tested on leaves of *Nicotiana glutinosa*.

Very few plant virus proteins have been subjected to the extensive chemical studies just considered for tobacco mosaic virus. However, elementary analyses on some of the other virus proteins are available. Representative figures for the amounts of carbon, hydrogen, nitrogen, sulfur, phosphorus, ash and carbohydrate have been assembled from the literature in Table 15–3. It can be observed that the analyses for the various strains of tobacco mosaic virus are virtually identical, with the exception that the ribgrass strain has much more sulfur than the other strains. As judged by phosphorus and carbohydrate analyses, the tobacco necrosis, bushy stunt

and southern bean mosaic viruses contain considerably more nucleic acid than tobacco mosaic virus.

Table 15–3. Elementary Analyses of Several Selected Plant Viruses

Virus	*C*	*H*	*N*	*S*	*P*	*Ash*	*Carbohydrate*
Tobacco mosaic	48	7.3	16.0	0.20	0.56	2.0	2.5
Aucuba mosaic	51	7.1	16.7	0.24	0.52	1.5	2.5
Enstion mosaic	51	7.1	16.7	0.3	0.5	1.0	2.5
Cucumber mosaic	50	7.4	15.6	1.3	0.55	1.5	2.4
Ribgrass mosaic	50	7.0	15.7	0.64	0.53	2.3	2.4
Bushy stunt virus	49	7.7	16.1	0.6	1.4	3.0	5.5
Tobacco necrosis	45	6.5	16.3	1.6	1.6	7.0	6.5
Southern bean mosaic	46	6.5	17.0	1.3	1.9	5.7	—
Latent mosaic	49	7.4	16.0	1.1	0.56	—	3.6

Biochemical Studies on Vaccinia Virus. Fairly extensive biochemical studies on elementary bodies of vaccinia have been reported. The most recent data were obtained by Hoagland and associates.[83] The elementary composition of vaccinia virus is about the same as that for tobacco mosaic virus, with the exception that elementary bodies regularly contain between 5 and 6 per cent lipid. The cholesterol fraction of the lipid can be removed readily by cold ether extraction. Since this can be accomplished without loss of activity, it is apparent that cholesterol is not an integral part of the virus. The remaining lipid, however, cannot be freed without destruction of the virus, even by incubation with pancreatic lipase. Since this procedure removes adsorbed lipid readily, it is assumed that the lipid other than cholesterol is an integral part of the elementary body. The nucleic acid of the elementary body differs from that of tobacco mosaic virus. Smadel, Dubos and Lavin showed that an extract of elementary bodies gave a positive Feulgen reaction, indicating the presence of thymus nucleic acid. Hoagland and associates isolated 15 mg of nucleic acid and showed that this material also gave a strongly positive Feulgen reaction. Adenine and guanine were recovered and a qualitative test for thymine with *ortho*-aminobenzaldehyde was positive. The nucleic acid was not depolymerized by crystalline ribonuclease. Yeast nucleic acid, on the other hand, underwent rapid depolymerization. These data leave relatively little doubt that the nucleic acid of the elementary body is thymus nucleic acid and not yeast nucleic acid. A colorimetric test involving the reaction of diphenylamine with thymus nucleic acid was applied directly to vaccinia virus and showed an average of 5.6 per cent nucleic acid.

Relatively little work has been done on the amino acid composition of elementary bodies. Some studies have been carried out on the digestibility of this virus by proteolytic enzymes. Enzymes of animal origin failed to

digest the elementary bodies. However, papain attacked the virus vigorously, resulting in structural disintegration, destruction of infectivity, loss of antigenicity and release of free amino groups. The enzyme, ficin, was without effect.

In contrast with the results obtained with tobacco mosaic virus, vaccinia virus was found to contain several constituents known to play a role in enzyme systems. The virus apparently does not contain cytochrome-c. However, a material was identified which has the absorption spectrum of riboflavin. Microbiological tests indicated that the amount of this material ranged between 1.1 and 1.5 milligrams per hundred grams of virus. All attempts to remove the flavin by methods which do not inactivate the virus failed. Thus, there is some reason to presume that the flavin may be an essential constituent. Copper was found to be present in the virus at a concentration of 0.05 per cent. Attempts to remove copper by electrodialysis, ultrafiltration, and repeated washings invariably failed. On the other hand, other metals present in crude virus preparations could always be eliminated readily by purification technics. It is believed, therefore, that the copper in the elementary body is an essential constituent. Microbiological tests indicate that the elementary bodies contain a considerable amount of biotin. A fivefold increase can be obtained upon partial hydrolysis. The possibility exists that these materials may indicate the presence of enzyme systems within the elementary body and may indicate that this virus is not wholly dependent upon the host for some phases of its metabolism.

Hydration of Viruses. The amount of water associated with a virus particle is a factor in the evaluation of physical data in terms of the size and shape of the particle. By using the x-ray technic, Bernal, Fankuchen and Riley [10] showed that crystals of tomato bushy stunt virus contained water which could be removed by drying. From the shrinkage of the unit cell when this water was removed, they were able to determine that the hydrated bushy stunt virus crystals contain about 40 per cent water.

Studies on viruses have pointed the way to the successful measurement of the hydration of particles in the dissolved state. The anhydrous molecular weight, M, of a protein or virus can be determined by using the equation, $M = RTs/D\,(1 - V\rho)$, in which R is the gas constant, T, the absolute temperature, s, the sedimentation constant, D, the diffusion constant, V, the partial specific volume, and ρ, the density of the medium. From the anhydrous molecular weight, it is a simple problem to calculate the diffusion constant, D_0, that the particle would have if it were an unhydrated sphere. The ratio of this hypothetical diffusion constant to the actual diffusion constant, symbolized by D_0/D, is known as the diffusion ratio. When the diffusion ratio for a particle is unity, one can conclude that the particle may indeed be an unhydrated sphere. However, when the diffusion ratio is greater than one, as is usually the case, it is possible to conclude

that the particle is hydrated, that it deviates from the spherical shape, or both. Bushy stunt virus particles have been shown to be spherical by the electron micrographs of Price, Williams and Wyckoff.[71] From diffusion studies carried out by Neurath and Cooper [61] and sedimentation studies by the author and Stanley,[47] the diffusion ratio was evaluated to be 1.27. Since the virus is known to be essentially spherical, this diffusion ratio can be interpreted to indicate that hydrated bushy stunt virus particles contain about 43 per cent by weight of water. This value for the hydration of the particle in suspension agrees remarkably well with the value of the hydration in the crystal as obtained by x-ray analysis.

Similar data are available for southern bean mosaic virus. The electron micrographs of Price, Williams and Wyckoff [71] indicate clearly that the southern bean mosaic virus particle is practically spherical. Yet, from the diffusion and sedimentation data obtained by Miller and Price,[58] one can calculate that the diffusion ratio of this virus is 1.25. This can be interpreted to mean that the hydrated southern bean mosaic virus particle contains about 40 per cent by weight of water.

A method of somewhat more general applicability has been developed. The sedimentation equation presented above can be rewritten in the following form: $s = K(\rho - \rho_0)$. This equation shows that the rate of sedimentation is directly proportional to the difference between the density of the suspended particles, ρ, and that of the suspension medium ρ_0. If a particle is suspended in a medium which has the same density as the particle itself, the rate of sedimentation will be zero. Thus the density of a particle in suspension can be determined by finding a medium of such density that the sedimentation rate is zero. By comparing this value with the dry density, which can be obtained by familiar methods, the degree of hydration of the suspended particles can be evaluated.[39] This method of approach was used by MacCallum and Oppenheimer to estimate the density of vaccinia virus. It was used later by Elford and Andrewes on both vaccinia and influenza viruses. More extended studies were carried out by Smadel, Pickels and Shedlovsky on the sedimentation of vaccinia virus in media containing various amounts of sucrose and of glycerol.[83] Similar sedimentation studies were carried out by the author and Stanley on influenza virus in sucrose solutions.[49] When the rate of sedimentation of influenza virus was plotted against the density of the medium, the results illustrated in Figure 15–7 were obtained. It can be observed that the sedimentation rate was zero in a sugar solution with a density of 1.18. However, the relationship between the sedimentation rate and the solvent density was not linear; thus the results were not in accord with the simplified equation quoted above. This is probably due, at least in part, to the withdrawal of water from the virus by osmosis. Sharp and other associates of Beard [81] studied the sedimentation of influenza virus in serum albumin solutions of various densities. The advantage of the serum albumin is that it has a low osmotic

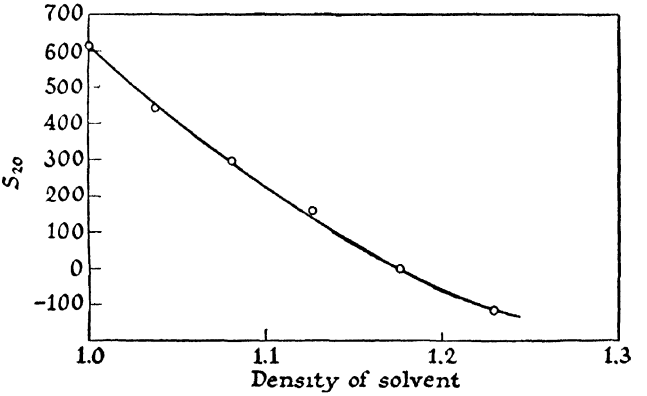

FIGURE 15–7. Graph showing the sedimentation rate of PR8 influenza A virus as a function of the density of sucrose solutions used as solvents. *From Lauffer and Stanley.*[49]

activity. Their results indicated that a linear relationship can be obtained between sedimentation rate and density of suspension medium, as illustrated in Figure 15–8. From data of this sort, it is possible to show that influenza virus particles in solution would just fail to sediment in a solvent with a density of 1.10. Thus the density of hydrated influenza virus is 1.10.

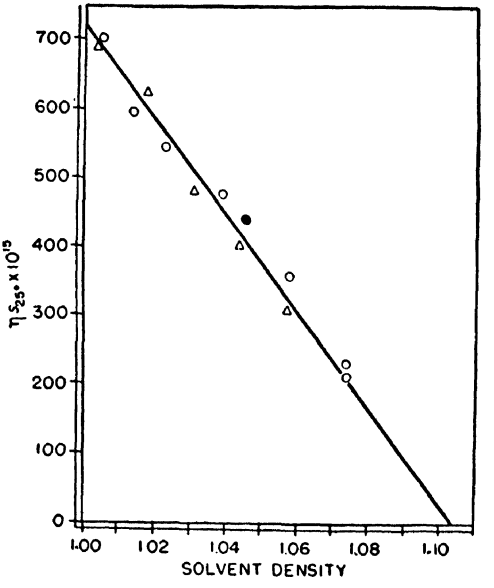

FIGURE 15–8. Graph showing the sedimentation rate of PR8 influenza A virus as a function of the density of serum albumin solutions used as solvents. *From Sharp et al.*[81] (*Science.*)

The dry density of influenza virus was shown to be 1.26.[49] From these results, it can be calculated that hydrated influenza virus contains about 57 per cent water. Similar data have been obtained by Sharp and collaborators on rabbit papilloma virus.[78] Preliminary results obtained by Schachman and the author on tobacco mosaic virus sedimenting in serum

albumin solutions indicate that it too has a hydrated density of about 1.1. Since the dry density of tobacco mosaic virus is 1.37, one can conclude that this virus may be hydrated to the extent of about 67 per cent on a wet basis.

The finding that tobacco mosaic virus particles may be hydrated in solution might force a reinterpretation of the x-ray data of Bernal and Fankuchen.[9] These workers observed two types of x-ray pattern for oriented tobacco mosaic virus particles, a low angle scattering and a high angle scattering. The spacings in low angle patterns were found to increase as the water content of oriented gels was increased. This was interpreted as being characteristic of the distance between oriented rods lying parallel. However, spacings of the high angle scattering were found to be constant, regardless of the amount of water in the gel. This was originally interpreted to mean that these patterns are characteristic of the interior of the virus particles and that no internal swelling due to hydration is possible. If this view is correct, the hydration must be external. The low angle scattering shows that the lateral distance between oriented virus rods in gels is exactly proportional to the reciprocal of the square root of concentration. This must mean that any swelling which takes place within a virus rod as well as that which takes place between rods must be in the directions perpendicular to the long axis of the rods. Thus, there will still be constant spacings in the direction of the long axis. Internal arrangements can be postulated which would also allow certain lateral spacings to remain constant, even though internal hydration took place. It would seem desirable to subject the interpretation of the x-ray data to critical review to determine whether internal hydration is possible.

Still another method is available for the determination of hydration of particles known to be of spherical shape. According to the Einstein equation, the specific viscosity,

$$\left(\frac{\eta}{\eta_0} - 1\right)$$

of a dilute suspension of spheres should be directly proportional to the volume concentration, G, of those spheres;

$$\frac{\eta}{\eta_0} - 1 = 2.5\,G$$

The Einstein equation has been verified experimentally. Thus, if one has a pure suspension of particles known to be spherical, one can calculate their volume concentration from viscosity measurements. From the volume concentration so calculated and the weight concentration of the suspension, it is possible to evaluate the hydration. Viscosity studies carried out by the author and Stanley [49] on influenza virus would indicate a degree of hydration in reasonable agreement with that found from sedimentation studies in serum albumin.

Size and Shape of Virus Particles. Numerous direct and indirect methods have been developed for the determination of the size and shape of virus

particles. The estimation of the size and shape of tobacco mosaic virus is of unusual difficulty and of unusual interest, because the particles deviate greatly from the spherical shape. The rod-like shape of tobacco mosaic virus was first indicated by double refraction of flow studies carried out by Takahashi and Rawlins. When the double refraction of flow of tobacco mosaic virus was reinvestigated by the author and Stanley, all possible

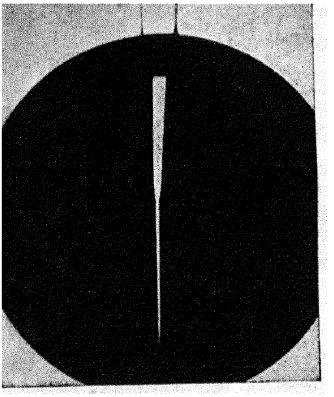

FIGURE 15–9. Stream of tobacco mosaic virus solution flowing from the end of a pipette photographed between crossed Polaroid plates. *From Lauffer and Stanley.*[46]

interpretations of the phenomenon, excepting that the virus particles are rod shaped, were eliminated.[46] Figure 15–9 illustrates the double refraction of flow of tobacco mosaic virus by showing a stream of the virus flowing from the end of a pipette, photographed between crossed Polaroid plates. Subsequent studies by the author showed that double refraction of flow disappears when the virus rods are suspended in mixtures of glycerin, aniline and water with refractive indices approaching that of the dry virus.[33] This can be interpreted to mean that the virus rods possess very little if any intrinsic double refraction, and that the double refraction of solutions of oriented particles is due largely to the shape of the particles. The author was also able to show that the virus particles can be induced to exhibit double refraction by orientation in an electric field.[34]

Filtration studies carried out on tobacco mosaic virus by Thornbery indicated that the virus would just fail to pass through filters with a pore size of 25 millimicrons.[46] Since it can be assumed that some virus particles would pass through a filter lengthwise, the filtration end point would tend to be a measure of the diameter of the virus rod. A value of about 15 millimicrons for the diameter is indicated by these data. The rod-like shape of tobacco mosaic virus was confirmed when pictures were obtained by means of the electron microscope. Figure 15–10 is an electron micrograph of tobacco mosaic virus obtained by the shadowing technic by Oster and Stanley.[64] Figure 15–11 is a histogram showing the distribution of lengths of the rods in a tobacco mosaic virus preparation studied by the author.[33] It can be seen that the virus particles were not all of exactly the same length in

the author's preparation. However, the degree of uniformity obtained by Oster and Stanley was much better.

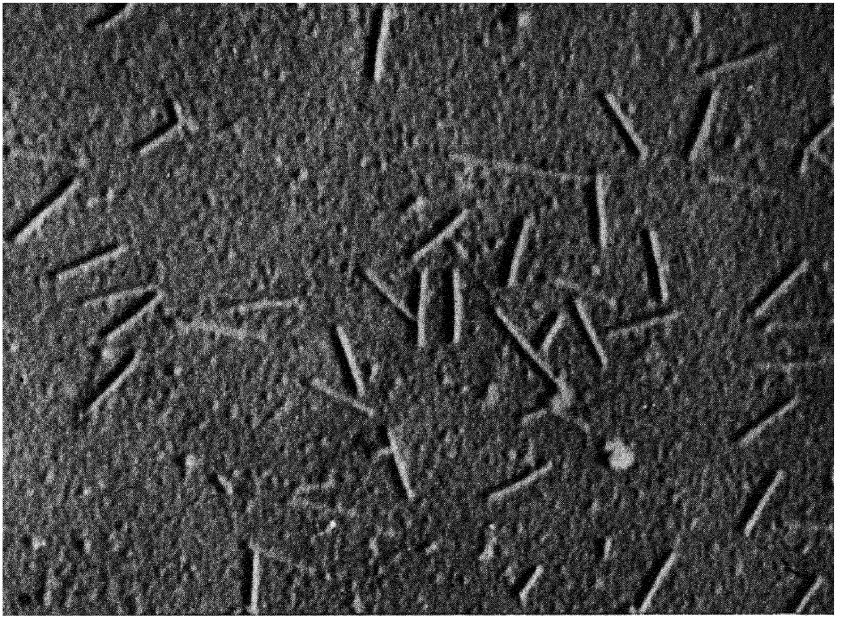

FIGURE 15–10. Electron micrograph of tobacco mosaic virus particles made by the shadow casting technic. *From Oster and Stanley.*[64]

The dimensions of the tobacco mosaic virus particles can be calculated from data obtained by various indirect physical methods. Viscosity data obtained by the author [38] were interpreted in terms of the ratio of the length to the thickness of the rod-like particles according to an equation

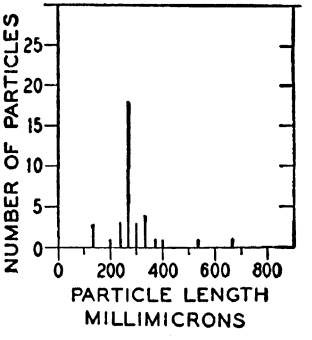

FIGURE 15–11. Histogram showing the distribution of particle lengths for a preparation of tobacco mosaic virus. *From Lauffer.*[38]

derived by Simha and also by Onsager. When it was assumed that the virus particles are unhydrated rods, the viscosity data indicated a ratio of 20.3. However, if it is assumed that the virus particles are hydrated to the extent of 67 per cent on a wet weight basis, then the viscosity data indicate

an axial ratio of the hydrated virus rod of 8.2. Actually both of these values are in agreement with the findings of the electron microscope. Studies with the electron microscope indicate that the virus particles are about 270 or 280 millimicrons long.[38, 64] X-ray data obtained by Bernal and Fankuchen [9] show that the anhydrous virus particle has a diameter of 15.2 millimicrons. Thus, if the tobacco mosaic virus particle is anhydrous, the axial ratio from electron microscope and x-ray data is 17.8. When the fact that any swelling which takes place within the virus particle must be only in the directions perpendicular to the long axis is taken into account, one can calculate from the electron microscope and x-ray data that a tobacco mosaic virus particle hydrated to the extent of 67 per cent on a wet weight basis should have a length of 270 millimicrons and a wet diameter of 29.4 millimicrons. This would correspond to an axial ratio of 9.2, a value still in reasonable agreement with that obtained from viscosity data.

When the shape of the virus particle and its degree of hydration are known, it is possible to interpret sedimentation and viscosity data in terms of the molecular weight and the actual dimension of the particle. If it is assumed that the virus in solution is unhydrated, one can calculate from sedimentation and viscosity data that the virus has a molecular weight of 33.2 million and that it is a particle 13.6 millimicrons in diameter and 276 millimicrons in length. If it is assumed that the virus particle is hydrated to the extent of 67 per cent on a wet weight basis, it can be calculated that the hydrated virus particle is a body 248 millimicrons in length and about 30.2 millimicrons in diameter. The values from viscosity and sedimentation agree with those from electron microscopy and x-ray diffraction about equally well for assumptions of high hydration and of no hydration.

On theoretical grounds, it is possible to determine the size and shape from diffusion and sedimentation data and also from diffusion and viscosity data. If it can be assumed that the tobacco mosaic virus particle is anhydrous, the results obtained by these two methods agree with those obtained from viscosity and sedimentation and from electron microscopy and x-ray analyses. The data of Table 15–4 illustrate this fact.[38] However, when it is assumed that the tobacco mosaic virus is highly hydrated, the estimation of the size and shape derived from diffusion data do not agree with those

Table 15–4. Dimensions of Tobacco Mosaic Virus Particles Calculated on the Assumption of No Hydration [38]

Methods	*Diameter,* (μ)	*Length,* (μ)	*Mol Wt* ($\times 10^{-7}$)
Sedimentation and viscosity	13.6	276	3.32
Sedimentation and diffusion	13.8	256	3.16
Viscosity and diffusion	14.0	283	3.60
Electron microscope and x-ray	15.2	270	4.0

obtained by the other means. This result might lead one to question somewhat the accuracy of even the best diffusion constant thus far obtained for tobacco mosaic virus.

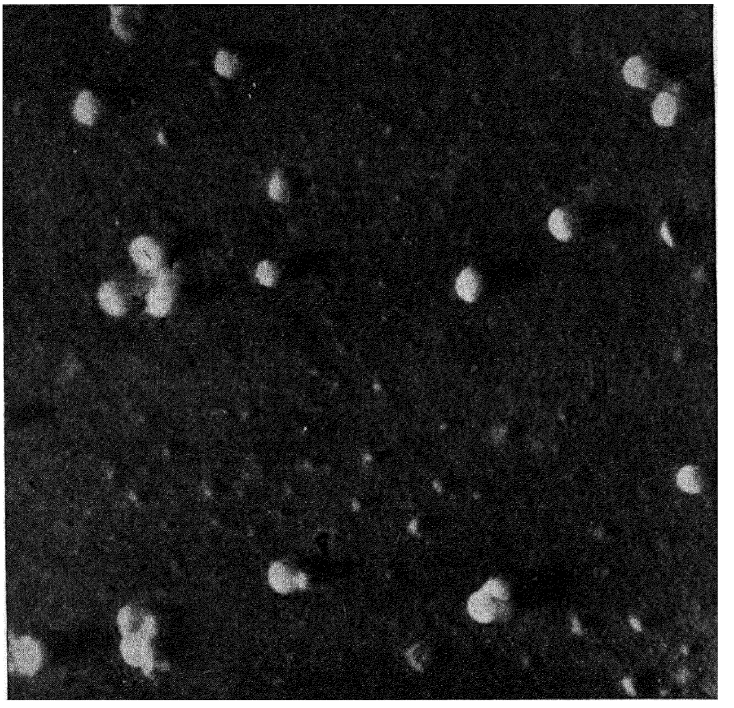

FIGURE 15–12. Electron micrograph of PR8 influenza A virus made by the shadow casting technic. *From Williams and Wyckoff.*[102]

The size and shape of influenza virus has been determined by sedimentation and electron microscope studies. Electron micrographs obtained by numerous investigators have shown a predominance of particles with a size of about 115 millimicrons.[39] Figure 15–12 is a micrograph obtained by the shadowing method.[102] Sedimentation studies on various strains of influenza virus were carried out. The author and Stanley [49] found that the PR8 strain of influenza A virus had a sedimentation constant of 740 Svedberg units. Slightly different values were obtained by Stanley and the author [94] and by Beard and associates [82, 99, 100] on other strains. Since it is known that the influenza virus particle is spherical and that it has a wet density of 1.10, it is possible to determine from the sedimentation constant that the diameter of the hydrated PR8 influenza virus particle is 114 millimicrons. This value is in agreement with that obtained by electron microscopy. Oster determined the size of influenza virus by a light scattering method.[63] A value of 97 mμ was found for the diameter.

The anhydrous molecular weight of bushy stunt virus has been estimated from sedimentation [47] and diffusion [61] experiments to be 10.6 million and from x-ray diffraction experiments [10] to be 12.8 million. The value obtained by Oster from light scattering experiments was 9 million.[63] These values

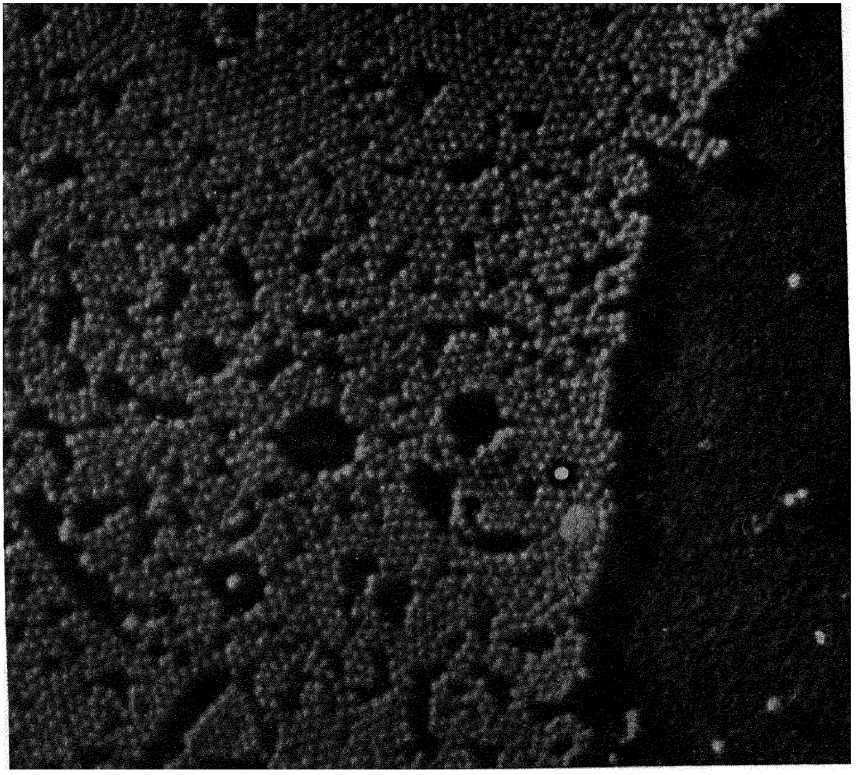

FIGURE 15–13. Electron micrograph of tomato bushy stunt virus made by the shadow casting technic. *From Price, Williams and Wyckoff.*[71]

correspond to anhydrous spheres with diameters of 29.2, 31.0 and 27.6 mμ, respectively. Measurements from the electron micrographs of Price, Williams and Wyckoff [71] indicate values between 25 and 27 millimicrons. A detailed analysis of the boundary obtained in an ultracentrifugation experiment on bushy stunt virus showed that the particles of this virus are either absolutely uniform in size or so nearly uniform that the standard deviation of the distribution of diameters is less than 1 per cent of the mean diameter.[35] An electron micrograph of bushy stunt virus particles is shown in Figure 15–13. Diffusion and sedimentation data [58] and electron micrographs [71] are also available for southern bean mosaic virus. The anhydrous molecular weight was calculated from the diffusion and sedimentation data to be 6.6 million. This would correspond to a dry sphere with a diameter of

24.4 mμ. The diameter indicated by the electron micrographs is 25 mμ. An electron micrograph of southern bean mosaic virus is shown in Figure 15–14.

FIGURE 15–14. Electron micrograph of southern bean mosaic virus made by the shadow casting technic. *From Price, Williams and Wyckoff* [71]

Several other viruses have been studied with the electron microscope. Among them are vaccinia, bacteriophage, and rabbit papilloma. The approximate sizes of these viruses are: 300, 100 and 50 millimicrons, respec-

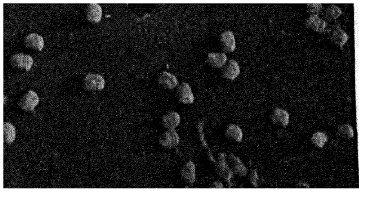

FIGURE 15–15. Electron micrograph of elementary bodies of vaccinia made by the shadow casting technic. *From Sharp et al.*[79]

tively. Electron micrographs are reproduced in Figures 15–15, 15–16 and 15–17.[79]

Inactivation of Viruses. Attempts have been made to determine the size of virus units by studying the rate of inactivation with known intensities

of radiation. It is believed that viruses are inactivated by radiation because of the production of ion clusters due to collision of photons with atoms. The number of ion clusters produced by a dose of radiation can be determined independently. If the volume of a virus particle is known, it is

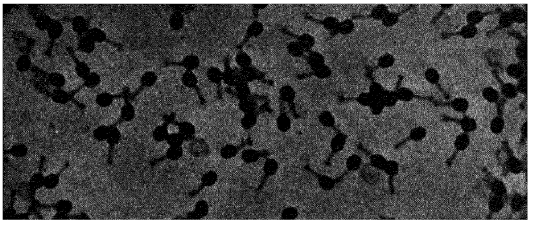

FIGURE 15-16. Electron micrograph of the T_2 bacteriophage of E. coli. *From Sharp et al.*[79]

possible to calculate, from a simple application of the theory of probability, the chance that one or more ion clusters will be induced within that small volume during the subjection of the material to a known amount of radiation. It turns out that the inactivation due to ion production should be an exponential function of the dose of radiation. Conversely, if the percentage inactivation following radiation by a given dose is measured, it should be possible to calculate the radiation sensitive volume of the virus. If only

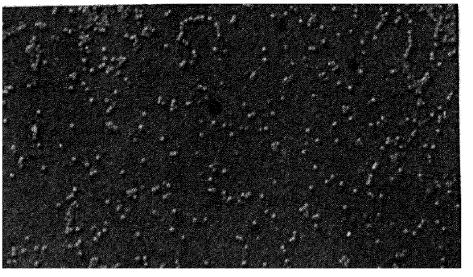

FIGURE 15-17. Electron micrograph of rabbit papilloma virus made by the shadow casting technic. *From Sharp et al.*[79]

certain portions of the virus particle are sensitive to radiation, then this method should determine the sensitive volume rather than the total volume. It is clear that the sensitive volume can be less than, but never greater than, the total volume. In studies of this sort on bacteriophage, Luria and Exner [55] were able to show that the sensitive volume was of the same order of magnitude as the volume determined by physical means. This shows conclusively that the bacteriophage does not exist in the form of aggregates of smaller dissociable active units. Studies of this sort have also been carried out on tobacco mosaic virus by Lea and Smith [51] and by other investigators.[22, 57] In this case, the sensitive volume turns out to be a small fraction of the total volume.

Virologists have long regarded the thermal death point as a characteristic

by which to identify viruses. Today, most workers realize that the thermal death point is not an absolute constant. The inactivation of viruses is now recognized as one aspect of the denaturation of proteins. Such reactions almost invariably have high temperature coefficients. The thermal death point is merely that temperature at which the inactivation proceeds rapidly enough to destroy most of the virus activity within the time of the experiment. Extensive studies on the kinetics of the inactivation of tobacco mosaic virus and other plant viruses were carried out by Price.[68] The author and Price [43] found that the thermal denaturation of tobacco mosaic virus protein follows the course of a first order process. It was shown that the reaction velocity varies with the temperature according to the Arrhenius equation. The energy of activation was shown to be 153,000 calories per mole. The author and Dow [41] showed that the denaturation of tobacco mosaic virus protein at a pressure of 7500 kilograms per square centimeter proceeds at 30° also according to the law of a first order process. The specific reaction rate is about 10^{12} times the value at atmospheric pressure obtained by extrapolating the data of Lauffer and Price to a temperature of 30°. From thermodynamics, one can derive the following equation:

$$\frac{d \log k}{dP} = \frac{\Delta \overline{V}}{RT}$$

P is the pressure, k is the specific reaction velocity, and $\Delta \overline{V}$ is the change in partial molar volume. From the data obtained at high pressure and at atmospheric pressure, one can calculate that the change in molar volume, when tobacco mosaic virus protein passes from the normal state into the activated complex, is a decrease of 97,000 cubic centimeters per mole. This represents a shrinkage of about ¼ of 1 per cent. The denaturation of tobacco mosaic virus in strong solutions of urea was found by the author and Stanley [37, 48, 93] to be a reaction of the first order. This reaction was observed to proceed with a minimum rate at room temperature and more rapidly at higher temperatures and also at lower temperatures. This behavior is illustrated in Figure 15–18. Bawden and Pirie [5] confirmed this unusual temperature effect for tobacco mosaic virus and showed that several other viruses exhibit the same behavior. The variation with temperature can be interpreted to mean that tobacco mosaic virus reacts reversibly with urea to form virus urea complexes of at least two sorts. These complexes then denature by parallel reactions. The effect of temperature is twofold; first, to dissociate the virus urea complex, and, second, to speed up the denaturation of the complex. If it is postulated that, for at least one complex, the effect of temperature on the rate of denaturation is greater than the effect upon the dissociation and that, in the case of at least one other parallel reaction, the reverse is true, one can rationalize the unusual effect of temperature on the rate of virus denaturation.

The kinetics of the loss of red blood cell agglutinating activity and of

infectivity by influenza virus has also been studied by the author and associates.[40, 45, 50, 77] It was found that the loss of agglutinating activity seems to proceed as though the reaction were a process of the three-halves order. At any rate, the law of a three-halves order process could be used to evaluate a rate constant characteristic of the process. This rate constant

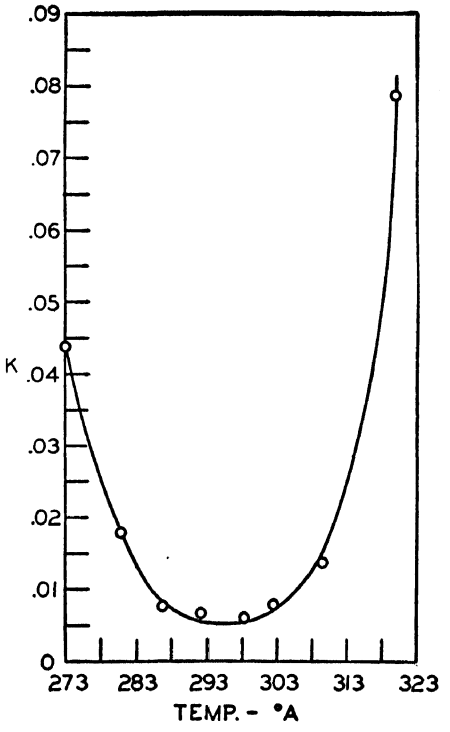

FIGURE 15–18. Graph showing the variation with temperature of the rate of denaturation of tobacco mosaic virus in urea. *From Lauffer.*[37]

varied with the initial concentration of virus. This fact and the unusual apparent order of the reaction can be accounted for on the assumption that influenza virus is inhomogeneous with respect to the rate at which hemagglutinin is destroyed, that is, that the hemagglutinin of some particles is destroyed more readily than that of others. The approximate energy of activation for the destruction of hemagglutinin was found to be 110,000 calories per mole, and the reaction was found to have a minimum velocity at pH 8.4. Urea changes both the energy of activation and the entropy of activation for the denaturation process. In contrast with these results, the destruction of influenza virus infectivity seems to proceed as a first order process. The data obtained in this case indicate clearly that the destruction of infectivity proceeds at a given temperature very much more rapidly than the destruction of hemagglutinin. The energy of activation for the destruction of infectivity was found to be 54,000 calories per mole.

Kinetic studies such as these may eventually shed some light upon the

nature of the bonds which hold virus particles together. The results with influenza virus suggest that a killed vaccine could be prepared by heating the virus at a temperature at which the inactivation proceeds rapidly but at which the other biological attributes of the virus are still stable. Hints concerning conditions most favorable for the storage of vaccines might also be derived from these data.

Are Viruses Protein Molecules? The most challenging concept to arise from chemical and physical studies on viruses is that viruses are protein molecules. It is possible to provide strong defense for the assumption that tomato bushy stunt virus and certain other plant viruses may be molecules. Much of the evidence obtained in the study of tobacco mosaic virus leads to the same conclusion, but the apparent inhomogeneity with respect to particle length has forced the adoption of a cautious attitude. Nevertheless, recent studies carried out by refined technics have provided evidence of far less inhomogeneity than obtained in former studies.[64] It is entirely possible that tobacco mosaic virus preparations will be obtained in the future which will fulfill even the most rigorous criteria of homogeneity and which therefore will present no obstacle to the assumption of molecular nature. Some other viruses, however, are almost certainly not nucleoprotein molecules. The evidence reviewed in this chapter relative to elementary bodies of vaccinia demonstrates clearly that these bodies resemble small living organisms more than protein molecules. When it is remembered that viruses are differentiated from other disease producing agents solely on the bases of size and inability to carry on wholly independent metabolism, it is not surprising that viruses may differ from one another in even fundamental respects.

Conclusion

In view of the theory that some viruses may be molecules, the ultimate goal of research on the chemistry and physics of viruses is the determination of the structure of a virus molecule. It would be entirely premature to attempt to assign details of structure on the basis of present evidence, even in the case of tobacco mosaic virus. However, sufficient is known to permit the formulation of a few tentative ideas.

The most obvious attribute of the structure of a virus is the overall size and shape. Tobacco mosaic virus particles have been shown to be rod shaped objects about 270 or 280 mμ long and 15 mμ in diameter. The particles probably exist in solution in a hydrated state, and this hydration probably causes swelling only in directions perpendicular to the length.

Amino acid analyses lead one to anticipate a protein repeat unit with a molecular weight of the order of magnitude of 18,000. Because of the incomplete status of the amino acid analyses, this figure must be accepted with caution. Nevertheless, degradation studies tend to confirm a unit of this size. When tobacco mosaic virus is disintegrated by the action of heat,

high pressure, urea, acids, bases and ultrasonic vibration,[28, 86, 96] fragments of various sizes are formed. The smallest ever found had a sedimentation constant compatible with a molecular weight of about 18,000.[48] It is interesting to observe that, while the virus particles themselves are soluble in neutral aqueous solvents, the fragments are insoluble. This probably means that those portions of fragments adjacent to other fragments in the intact virus particle have a higher percentage of nonpolar residues than the portions exposed to the surface of the virus particle. The fragments are also devoid of nucleic acid. This leads one to suspect that in the virus structure the nucleic acid is not a portion of the protein repeat unit.

Independent evidence seems to substantiate this conclusion. The author and Dow [41] obtained inactive anisometric nucleoprotein fragments with a sedimentation constant of about 35×10^{-13} by subjecting the virus to high pressures. By the action of alkali and of pyridine, Pfankuch and Pieckenbrock [65] isolated nucleic acid free fragments with about the same sedimentation rate. In his early studies, Stanley obtained preparations of tobacco mosaic virus which showed the same general solubility and physical behavior as subsequent preparations, but which were devoid of phosphorus, and, therefore, presumably of nucleic acid.[87] Schramm [74] has reported obtaining inactive tobacco mosaic virus protein preparations which are free of nucleic acid but which nevertheless possess about the same physical characteristics as the normal virus. These were produced by the aggregation of smaller units obtained by denaturing the virus at pH 9.6. These smaller nucleic acid free fragments were found to have a sedimentation constant of 8.7×10^{-13} and a diffusion constant of 2.2×10^{-7}. These values correspond to a particle with a slightly greater friction ratio than the intact virus and about 1/100 the molecular weight. Schramm also isolated similar particles which still possessed their nucleic acid. Since no smaller nucleoprotein fragment has been reported, it seems reasonable to assume that one unit of nucleic acid is associated with one such unit of protein. The results of Cohen and Stanley [17] showed that the nucleic acid split off from tobacco mosaic virus particles by heating could be broken up into fragments with dimensions of about $15 \times 1.5 \, m\mu$, each of which could account for about one per cent of the total nucleic acid in the virus particle. Thus, it is the right amount to be associated with one of the small rods isolated by Schramm.

The only way the nucleic acid could fit into the small rod shaped nucleoprotein particle would be for it to lie parallel along the long axis of the rod. A similar conclusion can be derived from light absorption experiments carried out by Butenandt *et al.*[13] The absorption spectrum of oriented tobacco mosaic virus rods in polarized light of wavelengths between 2300 and 3000 Å was found to be practically identical with the spectrum of a mixture of 5 per cent yeast nucleic acid and 4.5 per cent tryptophane, when

the electric vector of the polarized light was in the plane perpendicular to the long axis of the virus particles. A totally different spectrum was obtained when the electric vector vibrated in the plane including the long axis. Previous studies on aromatic ring systems had shown that these compounds absorb light when the electric vector vibrates in the plane of the ring. Thus, Butenandt *et al.* concluded that the purine and pyrimidine rings of the nucleic acid, as well as the indole ring of tryptophane, are all oriented perpendicular to the long axis of the virus. Astbury and Bell [2] had previously deduced from x-ray diffraction studies that, in thymus nucleic acid, the nucleotides were tightly packed flat plates lying perpendicular to the long axis of the nucleic acid thread. If ribose nucleic acid is of similar structure, the results of Butenandt *et al.* could therefore be interpreted to mean that the threads of nucleic acid lie parallel to the long axis of the virus, a conclusion in agreement with the deduction from the results of Cohen and Stanley.

All of the pertinent data can be rationalized by assuming tobacco mosaic virus is composed of about a hundred sub units with dimensions in the anhydrous state of about 70×3 mμ. The virus rod could be made up by the end to end aggregation of four bundles, each composed of about 25 of the small rods oriented parallel. Each fragment seems to be associated with a small thread of nucleic acid lying parallel to the long axis of the protein fragment. The protein fragments may be composed of about 20 ultimate units of molecular weight of 18,000. These are probably fairly symmetrical in shape, about 3 mμ in each dimension. However, the solubility behavior suggests that the polar groups are concentrated in those surfaces which are not involved in the aggregation into the rods.

Whether or not subsequent studies will bear out the tentative conclusions outlined here remains to be seen. It must be pointed out that these conclusions are not consistent with the interpretation of Bernal and Fankuchen [9] of the high angle x-ray diffraction pattern of tobacco mosaic virus. It is important to observe, however, that a beginning has been made in the elucidation of the structure of the tobacco mosaic virus particle, an entity which can be regarded with some justification as providing a bridge between the simplest forms of living matter and the molecules familiar to the chemist.

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APPENDIX

Table 1

Proximate Composition of American Food Materials

Table 1

Table 1* gives average values on the consumption of an extensive list of natural and processed foods of animal and plant origin. Information regarding the variability of various foods is not included but in many instances maximum and minimum values representing the range in composition are stated. Therefore the table will serve as a satisfactory working basis for dietary estimates.

In general, the figures are based on direct analyses of the food in question. Of the values not based on direct analyses, some are calculations from recipes or commercial formulas, while others are essentially arbitrary.

Explanation of Table and Meaning of Terms

Food

Meats and Poultry. The edible portion in the fresh and cured meats is the lean meat and visible or separable fat, with the exceptions noted as "lean meat only." The refuse is bone or in a few cases, especially pork, it is bone and skin. The data on meat refer to wholesale cuts.

Lean meats from animals other than those included in the table can be estimated from the general figures for game animals.

In rabbits the allowance to be made for refuse depends on the basis of purchase, whether purchased with or without the edible giblets and whether purchased on the live-, drawn-, or dressed-weight basis. On the live-weight basis refuse is relatively high since it includes entrails, pelt, head, and feet in addition to the bones. Drawn weight, in the case of rabbits, refers to the weight after removal of the entrails, and on this basis refuse consists of pelt, head, feet, and bones. Dressed weight refers to the weight of the drawn, skinned carcass with head and feet removed, and refuse in this case is essentially bone.

Because of the difference in fatness and proportion of refuse and edible fat of various classes of chickens no single set of figures for all chickens is offered in the table. If approximate values must be used in diet estimates for want of information on the class or on the weight or age of the chicken, then the values for roasters are probably the best choice. The basis of purchase determines the allowances that should be made for refuse or waste. Live weight is self-explanatory. Dressed weight is the weight after removal of blood and feathers, either before or after chilling. Drawn weight refers to the weight of dressed and drawn fowl, that is after the blood, feathers,

* From Chatfield, C., and Adams, G., "Proximate Composition of American Food Materials," *U. S. Dept. Agr. Circ. No. 549*, 1940.

head, feet, and inedible viscera have been removed. "Total edible" refers to the total of flesh or muscle, the skin with any external fat, the edible viscera or giblets, and the internal fat.

The composition of cooked meats depends not so much on the kind of meat or cut as on the fatness and the degree of doneness. The values for cooked meats and poultry are presented, therefore, without reference to cut and without designation of kind or species.

Fish and Shellfish. Data are given on separate species of fish, or on combinations of certain related species. In addition, general figures are given for two classes of fresh fish. Fish of class 1 are fairly low in fat and have about 19 per cent of protein. Examples: Atlantic black sea bass, striped bass, carp, muskellunge, white perch, etc. Fish of class 2 are very low in fat and somewhat lower in protein, as a whole, than those in class 1. Examples: Cod, haddock, etc. Although the generalized figures for classes 1 and 2 are fairly satisfactory as estimates on the edible portion, they are less so as estimates on the as-purchased basis. Refuse figures for the particular kind are in general to be preferred. The generalized figures will necessarily be used, however, in diet studies when the record does not indicate the kind of fish eaten.

Cereals and Cereal Products. Cereals and their products vary so widely in their composition that for the purpose of this table only crude approximations for the main types of the several kinds of products can be offered.

Fruits and Vegetables. The figures for dried fruits are applicable to products prepared by any of the conventional methods of drying, that is, to sun-dried, dehydrated, or evaporated fruits. The analyses of canned fruits and vegetables refer in every case to the total contents of the can.

Miscellaneous Products — Soups. The figures for soups apply either to canned soups or to those prepared in the home.

The term "fresh" is used to designate foods in an essentially fresh state; it is not meant to exclude foods that have been subjected to storage or freezing, if these conditions have not grossly altered the proximate composition.

The chemical terms used in the headings relate, in general, to the constituents as they are usually determined by the prevailing methods for food analyses.

Basis

Edible Portion (E.P.). This term is usually self-explanatory, meaning the part most commonly eaten.

As Purchased (A.P.). "As purchased" is usually defined unless it is obvious. In some cases where it is not defined and not obvious, it can be inferred from information on the edible portion and refuse. The chemical data that are given on the as-purchased basis relate only to the edible portion.

Refuse

The term "refuse" relates to the portion that is commonly discarded in preparation, that is, the portion of the purchased material not usually eaten.

Water

Water, as reported, indicates the amount of free moisture in the food, and represents the substance that would be lost in drying the material under specified conditions of analysis.

Protein

Protein, in nearly all cases, is total nitrogen times some factor that is considered appropriate to use for the particular food. In a great many cases this factor is 6.25. There are, however, numerous exceptions, as for example patent wheat flour which is conventionally calculated as N × 5.7. Other foods in which factors other than 6.25 have been used include cereals and cereal products, certain nuts and oilseeds, gelatin, milk, and milk products. In foods that are mixtures of several materials it is customary to use the factor 6.25, and such a practice was followed in the majority of cases. In a few foods where much of the nitrogen is nonprotein in character it would overestimate the protein to calculate it as total nitrogen times some factor. In these cases, designated by parentheses, a more reasonable protein value has been estimated either somewhat arbitrarily or on the basis of biological experiments.

Fat

Fat, determined as ether extract, includes not only true fats but various other ether-soluble substances, such as fatty acids, lecithin, and plant pigments.

Ash

Ash is the residue from burning the dry substance until it is free from carbon.

Carbohydrates

"Total" Carbohydrate. In the majority of cases the total carbohydrate is reckoned as carbohydrate by difference; that is, as the difference between 100 per cent and the sum of the percentages of water, protein, fat, and ash. This measure includes starch, dextrin, and sugars, and is to this extent an approximate measure of the total carbohydrate that can be utilized by the body. However, it tends to overestimate the available carbohydrate since it also includes crude fiber and organic acids, when present, and any undetermined solids. Certain values in this column are not calculated by difference; these particular figures are enclosed in parentheses. They represent essentially the quantity of carbohydrates and organic acids that are available to the body. Total carbohydrate is reported as zero for fresh

muscular meats and fish. Although it is frequently present in these tissues, it is generally less than 0.5 per cent as indicated by direct determination in numerous specimens. "Nitrogen-free extract," that is, total carbohydrate excluding fiber, is not given in these tables.

Fiber. The fiber, determined chemically as crude fiber, gives an approximate measure of the fibrous portion of plant foods. It is usually counted in the total carbohydrate and, therefore, as contributing to the total calories, although it is admitted that the fibrous portion is not readily available to the body. Fiber is not present in any of the animal foods.

Sugars. Sugars are not reported on a strictly uniform basis, partly because the data in the original reports did not permit this, and partly because foods of different classes differ in the predominating sugar. For most fresh and dried fruits and vegetables and for many other foods the values for sugar represent total sugar as invert or as dextrose; for many foods they represent the direct sum of sucrose and reducing sugars. In milk and milk products the predominating sugar is lactose. For these the values reported represent lactose by difference and include, therefore, not only lactose but lactic acid and any undetermined solids.

Starch. The starch values represent results from determinations by conventional analytical methods. Dextrins are often included in the portion reported as starch.

Acid

Acid is the total free acid, calculated in most foods as malic (m) or citric (c) acid, depending on which was considered to predominate. In the few foods where lactic (L) acid predominates, total acid is calculated to that basis, and in vinegar it is counted as acetic (a) acid.

Fuel Value

Fuel value is expressed in calories and is calculated on the basis of the conventional physiological values; that is, 4 calories per gram of protein and of carbohydrate, and 9 per gram of fat. The values are given to the nearest *calorie per 100 grams.* The *calories per pound* are given to the nearest 5 for all foods except meats, poultry, game, meat organs, and meat products which are reported to the nearest 10 calories per pound.

Other Symbols

Parenthetical Values. In the protein and carbohydrate columns of the table, values in parentheses are substitutions in place of more direct analytical values and relate in general to the quantity of the constituent estimated to be utilized by the body.

Blanks. Where no figure is given it sometimes signifies that no satisfactory value was available for the constituent in question, although there is reason to suppose it to be present. In other cases the constituent is assumed to be absent.

Zero. A zero indicates that a particular constituent has been reported as absent or is low enough, usually under 1 per cent, to justify neglecting it in diet calculations. In a few instances, zero appears in the tables as a parenthetical value to indicate that the constituent, though present in appreciable quantities, should not be reported.

Key to Abbreviations

a = *acetic*
A.P. = *as purchased*
c = *citric*
Do. = *ditto*
E.P. = *edible portion*
Excl. = *excluding*
Incl. = *including*
L = *lactic*
m = *malic*
Ref. = *refuse*
Tot. = *total by difference, including fiber*
Wt. = *weight*
( ) = *see text for discussion of parenthetical values, p. 464*

Table 1. Proximate Composition of American Food Materials

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Abalone:														
Fresh or canned solids	E. P., meat	E. P.		74.0	21.7	0.5	1.4	2.4					101	460
Agar-agar (see Algae).														
Albacore:														
Raw	E. P., flesh	E. P.		66.2	25.3	7.6	1.3	0.					170	770
Alewife:														
Raw	do	E. P.		74.4	19.4	4.9	1.5	0.					122	550
	A. P., whole	A. P.	51	36.5	9.5	2.4	.7	0.					60	270
Algae:														
Agar-agar		E. P.		17.8	(0.)	.2	3.3	(0.)						
Irish moss		E. P.		19.8	(0.)	.9	14.9	(0.)						
Kelp		E. P.		23.6	(0.)	1.1	20.6	(0.)						
Laver or sloke		E. P.		17.8	(0.)	.8	12.2	(0.)						
Almonds:														
Dried, unblanched	E. P., kernels	E. P.		4.7	18.6	54.1	3.0	19.6	2.7	4.4			640	2,900
	Ref., shells	A. P.	49	2.4	9.5	27.6	1.5	10.0	1.4				326	1,480
Almond meal, partially defatted.		E. P.		7.2	39.5	18.3	6.1	28.9	2.3		0.0		438	1,990
Amaranth:														
Fresh	E. P., leaves and stems	E. P.		88.6	3.0	.6	2.23	5.6	1.0		5		40	180
	Ref., tough stems	A. P.	29	62.9	2.1	.4	1.6	4.0	.7				28	125
Anchovies:														
Pickled, not heavily salted	Prepared with or without added oil	E. P.		58.6	19.2	10.3	11.6	.3					171	775
Anchovy paste		E. P.		56.5	20.2	11.6	7.4	4.3			2.5		202	920
Anserine		E. P.		84.2	3.8	.7	3.0	8.3	2.6	.3	1.4		55	250
Apples:														
Fresh:														
All	E. P., flesh or flesh and skin	E. P.		84.1	.3	.4	.29	14.9	1.0	11.1		0.47m	64	290
	Ref., skins and core	A. P.	12	74.0	.3	.4	.3	13.0	.9				57	260
Early (summer)		E. P.		86.5	.3	.4	.30	12.5		9.4		.70m	55	250

Medium (fall)		E. P.		86.4	.3	.3	.25	13.8	1.1	10.4		0.45m	59	270
Late (winter)		E. P.		83.6	.3	.3	.28	15.5	.9	11.2		.46m	66	300
Canned (see Applesauce).														
Dried		E. P.		23.	1.4	1.0	1.4	73.2	4.6	54.0		2.3m	307	1,395
Apple juice:														
Fresh		E. P.		87.1	.1	.0	.25	12.5		10.5		.52m	50	230
Applesauce:														
Canned:														
Unsweetened		E. P.		88.4	.2	.2	.3	10.9	.6	7.9		.3m	46	210
Juice pack		E. P.		87.3	.2	.2	.3	12.0	.6				51	230
Sweetened		E. P.		79.8	.2	.1	.2	19.7	.6	17.6		.4m	80	365
Apricots:														
Fresh	E. P., flesh or flesh and skin	E. P.		85.4	1.0	.1	.59	12.9	.6	10.4		1.19m	56	255
	Ref., pits	A. P.	6	80.3	.9	.1	.6	12.1	.6				53	240
Candied		E. P.		12.	.6	.2	.7	86.5	.6				350	1,590
Canned:														
Water pack	E. P., contents of can	E. P.		90.9	.5	.1	.4	8.1	.3	6.4			35	160
Juice pack	do	E. P.		86.8	.5	.2	.7	11.8	.4	9.4			51	230
In syrup	E. P., contents of can (except pits)	E. P.		77.3	.6	.1	.6	21.4	.4				89	405
	Ref. (if canned whole), pits	A. P.	4	74.2	.6	.1	.6	20.5	.4				85	385
Sieved, unsweetened		E. P.		85.8	.9	.2	.7	12.4	.7	8.7		.6m	55	250
Sieved, sweetened		E. P.		76.6	1.0	.1	.8	21.5	.6	15.8		1.2m	91	410
Dried		E. P.		24.	5.2	.4	3.5	66.9	3.2	46.0		5.0m	292	1,325
Artichokes, globe or French:														
Fresh	E. P., base and soft part of leaves	E. P.		83.7	2.9	.4	1.1	11.9	3.2				63	285
	A. P., entire bud	A. P.	52	40.2	1.4	.2	.5	5.7	1.5				30	135
Artichokes, Jerusalem (see Jerusalem-artichokes).														
Asparagus:														
Fresh	E. P., tender shoots	E. P.		93.0	2.2	.2	.67	3.9	.7	1.3	.4		26	120
	A. P., butt ends	A. P.	25	69.8	1.6	.2	.5	2.9	.5				20	90
Canned	E. P., contents of can	E. P.		93.9	1.7	.1	1.3	3.0	.5	1.6	1.0		20	90
Canned, sieved		E. P.		92.	2.	.1	1.4	4.5	.5	1.5			27	120
Asparagus-beans:														
Fresh:														
Young green pods		E. P.		84.5	3.4	.3	1.3	10.5	2.0	5.1	2.7		58	265
Sprouted seeds		E. P.		92.8	2.4	.4	.4	4.0	.7				29	130
Dry seeds (see Cowpeas).														

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
Avocados:			*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Fresh:														
Fuerte	E. P., flesh	E. P.		65.4	1.7	26.4	1.42	5.1	1.8	0.6			265	1,200
	Ref., seeds and skins	A. P.	25	49.0	1.3	19.8	1.1	3.8	1.4				199	905
Guatemalan (type)	E. P., flesh	E. P.		74.1	2.0	17.2	1.28	5.4	1.4	.7			184	835
	Ref., seeds and skins	A. P.	30	51.9	1.4	12.0	.9	3.8	1.0				129	585
Mexican (type)	E. P., flesh	E. P.		66.7	2.0	23.2	1.38	6.7					244	1,105
	Ref., seeds and skins	A. P.	31	46.0	1.4	16.0	1.0	4.6					168	760
West Indian (type)	E. P., flesh	E. P.		82.2	1.3	7.7	.98	7.8	1.2				106	480
	Ref., seeds and skins	A. P.	24	62.5	1.0	5.9	.7	5.9	.9				81	365
Bacon:														
Raw:														
Lean		E. P.		29.	12.2	53.	4.7	(1.4)		1.4			531	2,410
	Ref., rind	A. P.	8	26.	11.2	49.	4.3	(1.3)					489	2,220
Medium		E. P.		20.	9.1	65.	4.3	(1.1)		1.1			626	2,840
	Ref., rind	A. P.	6	19.	8.6	61.	4.0	(1.0)					588	2,670
Fat		E. P.		13.	6.2	76.	3.8	(.7)		.7			712	3,230
	Ref., rind	A. P.	4	13.	6.0	73.	3.6	(.7)					683	3,100
Broiled	Medium fat, crisp, drained	E. P.		13.	25.	55.	6.	1.		1.			599	2,720
Bacon, Canadian: Raw (see Pork, cured, raw).														
Bamboo shoots:														
Fresh	E. P., tender inside part	E. P.		91.3	2.5	.3	.79	5.1	.8				33	150
	Ref., sheath	A. P.	71	26.5	.7	.1	.2	1.5	.2				10	45
Bananas:														
Fresh	E. P., flesh	E. P.		74.8	1.2	.2	.84	23.0	.6	19.2		0.39m	99	445
	Ref., skins	A. P.	33	50.1	.8	.1	.6	15.4	.4				66	300
Dried		E. P.		23.	3.6	.3	2.5	70.6	1.7			1.4m	300	1,360

Banana flour		E. P.		10.	3.9	.7	2.6	82.8	1.4				353	1,600
Barley:														
Flour		E. P.		10.0	10.2	1.7	1.2	76.9	.7				364	1,650
Pearled:														
Pot or Scotch		E. P.		10.8	8.7	1.0	1.2	78.3	.8				357	1,620
Light		E. P.		11.1	8.2	1.0	.9	78.8	.5				357	1,620
Whole	Hull-less type	E. P.		10.2	12.8	2.1	2.1	72.8	2.7				361	1,640
Barracuda, California:														
Raw	E. P., flesh	E. P.		75.0	21.2	3.1	1.3	0.					113	510
Basella:														
Fresh	E. P., leaves and stems	E. P.		93.2	2.0	.3	1.5	3.0	.6				23	105
Bass, Atlantic black sea:														
Raw	E. P., flesh	E. P.		79.3	19.2	1.2	1.2	0.					98	395
	A. P., whole	A. P.	61	30.9	7.5	.5	.5	0.					34	155
Bass, black, large- and small-mouthed:														
Raw	E. P., flesh	E. P.		76.7	20.6	1.8	1.2	0.					99	445
	A. P., whole	A. P.	56	33.7	9.1	.8	.5	0.					43	195
Bass, California white sea:														
Raw	E. P., flesh	E. P.		76.3	21.4	.5	1.4	0.					90	410
Bass, striped:														
Raw	do	E. P.		77.7	18.9	2.7	1.2	0.					100	455
	A. P., whole	A. P.	57	33.4	8.1	1.2	.5	0.					43	195
	A. P., entrails removed	A. P.	51	35.1	9.3	1.3	.6	0.					49	220
Beans:														
Fresh:														
Asparagus-beans (see Asparagus-beans).														
Broadbeans:														
Shelled	E. P., immature seeds	E. P.		74.1	8.1	.6	1.4	15.8	2.0				101	460
	Ref., shells	A. P.	66	25.2	2.8	.2	.5	5.3	.7				34	155
Green pods	E. P., trimmed pods	E. P.		84.0	3.0	.3	.8	11.9					62	285
Hyacinth-beans:														
Green pods	do	E. P.		89.9	2.8	.2	.8	6.3	1.7				38	175
Lima:														
Shelled	E. P., immature seeds	E. P.		66.5	7.5	.8	1.71	23.5	1.5				131	595
	Ref., shells	A. P.	60	26.6	3.0	.3	.7	9.4	.6				52	235
Mung bean sprouts	E. P., fresh sprouted seeds	E. P.		92.4	2.9	.3	.44	4.0	.7				30	135
Scarlet runner:														
Green pods	E. P., trimmed pods	E. P.		92.3	1.4	.1	.7	5.5	.8		0.1		28	130
	Ref., ends and strings	A. P.	8	84.9	1.3	.1	.6	5.1	.7				26	120
Snap beans, green or wax (common or kidney)	E. P., trimmed pods	E. P.		88.9	2.4	.2	.77	7.7	1.4	.4	2.2		42	190
	Ref., ends and strings	A. P.	10	80.0	2.2	.2	.7	6.9	1.3				38	170
Soybeans (see Soybeans).														
String (see Beans, snap).														

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
Beans—Continued.			*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Canned:														
Baked, with pork	E. P., contents of can	E. P.		71.0	5.7	2.0	2.3	19.0	1.0				117	530
Baked, without pork	do	E. P.		72.5	6.0	.4	2.3	18.8	1.0				103	465
Lima	do	E. P.		80.	5.	.5	1.5	13.	1.				76	345
Red kidney	do	E. P.		76.0	5.7	.4	1.5	16.4	.9				92	415
Snap, green or wax	do	E. P.		91.3	1.0	.1	1.3	3.3	.6	1.2	0.7		18	80
Snap, green or wax, sieved (see Snap).	do	E. P.		93.3	1.3	.1	.6	4.7	.7	1.7	.9		25	115
Dry seeds:														
Broadbeans	E. P., whole mature seeds	E. P.		12.0	25.1	1.8	3.4	57.7	6.6				347	1,575
Common or kidney (includes navy, pea beans, pinto, red, others).	do	E. P.		10.5	22.0	1.5	3.9	62.1	3.9	3.6	35.8		350	1,585
Lima, green	do	E. P.		12.6	20.7	1.3	3.8	61.6	4.3				341	1,545
Mung	do	E. P.		11.0	24.4	1.4	3.5	59.7	4.5	3.0	46.8		349	1,585
Soybeans (see Soybeans).														
Bean flour, lima		E. P.		10.5	21.5	1.4	3.6	63.0	2.				351	1,590
Bean sprouts (see Beans, fresh, mung, and Soybeans).														
Beechnuts	E. P., kernels	E. P.		4.0	20.0	57.4	3.6	15.0					657	2,980
	Ref., shells	A. P.	39	2.4	12.2	35.0	2.2	9.2					401	1,815
Beef:														
Fresh:														
Carcass or sides including kidney fat:														
Thin (common grade)	E. P., 86 percent lean	E. P.		66.	18.8	14.	.97	0.					201	910
	A. P., 70 percent lean	A. P.	19	54.	15.2	11.	.8	0.					163	740
Medium (medium grade)	E. P., 79 percent lean	E. P.		60.	17.5	22.	.87	0.					268	1,220
	A. P., 66 percent lean	A. P.	16	50.	14.7	18.	.7	0.					225	1,020

Fat (good grade)	E. P., 73 percent lean	E. P.		55.	16.3	28.	.79	0.					317	1,440
	A. P., 62 percent lean	A. P.	15	47.	13.9	24.	.7	0.					270	1,220
Very fat (choice and prime grades)	E. P., 62 percent lean	E. P.		47.	13.7	39.	.65	0.					406	1,840
	A. P., 55 percent lean	A. P.	12	41.	12.1	34.	.6	0.					357	1,620
Wholesale cuts:														
Chuck:														
Thin	E. P., 92 percent lean	E. P.		71.	19.2	9.	.94	0.					158	720
	A. P., 75 percent lean	A. P.	19	57.	15.6	7.	.8	0.					128	580
Medium	E. P., 87 percent lean	E. P.		65.	18.6	16.	.88	0.					218	990
	A. P., 72 percent lean	A. P.	17	54.	15.4	13.	.7	0.					181	820
Fat	E. P., 83 percent lean	E. P.		60.	17.6	22.	.82	0.					268	1,220
	A. P., 71 percent lean	A. P.	15	51.	15.0	19.	.7	0.					228	1,030
Very fat	E. P., 76 percent lean	E. P.		52.	15.0	32.	.74	0.					348	1,580
	A. P., 66 percent lean	A. P.	13	45.	13.0	28.	.6	0.					303	1,370
Flank:														
Thin	E. P., 60 percent lean	E. P.		52.	17.0	30.	.77	0.					338	1,530
	A. P., 59 percent lean	A. P.	1	52.	16.8	30.	.76	0.					335	1,520
Medium	E. P., 51 percent lean	E. P.		45.	14.6	40.	.61	0.					418	1,900
	A. P., 50 percent lean	A. P.	1	44.	14.5	40.	.63	0.					414	1,880
Fat	E. P., 44 percent lean	E. P.		39.	12.7	48.	.54	0.					483	2,190
	A. P., 44 percent lean	A. P.	1	39.	12.6	48.	.53	0.					478	2,170
Very fat	E. P., 32 percent lean	E. P.		28.	9.3	62.	.36	0.					595	2,700
	A. P., 32 percent lean	A. P.	1	28.	9.2	61.	.36	0.					589	2,670
Kidney fat (suet):														
Thin		E. P.		9.	3.0	88.	.16	0.					804	3,650
Medium		E. P.		5.	1.7	93.	.12	0.					844	3,830
Fat		E. P.		4.	1.5	94.	.11	0.					852	3,860
Very fat		E. P.		4.	1.5	94.	.11	0.					852	3,860
Loin, excluding kidney knob:														
Thin	E. P., 85 percent lean	E. P.		64.	18.6	16.	.95	0.					218	990
	A. P., 71 percent lean	A. P.	16	54.	15.6	13.	.8	0.					183	830
Medium	E. P., 76 percent lean	E. P.		57.	16.9	25.	.84	0.					293	1,330
	A. P., 65 percent lean	A. P.	14	49.	14.5	22.	.7	0.					252	1,140
Fat	E. P., 70 percent lean	E. P.		53.	15.6	31.	.77	0.					341	1,550
	A. P., 62 percent lean	A. P.	12	46.	13.7	27.	.7	0.					300	1,360
Very fat	E. P., 59 percent lean	E. P.		44.	12.8	43.	.62	0.					438	1,990
	A. P., 53 percent lean	A. P.	10	39.	11.5	39.	.6	0.					394	1,790

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
Beef—Continued.														
Fresh—Continued.														
Wholesale cuts—Continued.														
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Neck:														
Thin	E. P., 88 percent lean	E. P.		69.	19.1	11.	0.92	0.					175	800
	A. P., 64 percent lean	A. P.	27	50.	13.9	8.	.7	0.					128	580
Medium	E. P., 83 percent lean	E. P.		62.	18.2	19.	.85	0.					244	1,110
	A. P., 61 percent lean	A. P.	26	46.	13.5	14.	.6	0.					180	820
Fat	E. P., 78 percent lean	E. P.		57.	17.0	25.	.80	0.					293	1,330
	A. P., 58 percent lean	A. P.	25	43.	12.8	19.	.6	0.					220	1,000
Very fat	E. P., 71 percent lean	E. P.		50.	14.0	35.	.71	0.					371	1,680
	A. P., 54 percent lean	A. P.	24	38.	10.6	27.	.5	0.					282	1,280
Plate and brisket:														
Thin	E. P., 83 percent lean	E. P.		60.	17.9	21.	.87	0.					261	1,180
	A. P., 65 percent lean	A. P.	22	47.	14.0	16.	.7	0.					203	920
Medium	E. P., 73 percent lean	E. P.		53.	15.8	30.	.75	0.					333	1,510
	A. P., 60 percent lean	A. P.	18	44.	13.0	25.	.6	0.					273	1,240
Fat	E. P., 66 percent lean	E. P.		47.	14.0	38.	.65	0.					398	1,810
	A. P., 56 percent lean	A. P.	15	40.	11.9	32.	.6	0.					338	1,530
Very fat	E. P., 53 percent lean	E. P.		38.	11.0	51.	.48	0.					503	2,280
	A. P., 47 percent lean	A. P.	11	33.	9.8	45.	.4	0.					448	2,030
Rib:														
Thin	E. P., 92 percent lean	E. P.		66.	10.0	14.	.94	0.					202	920
	A. P., 69 percent lean	A. P.	25	50.	14.2	10.	.7	0.					152	690
Medium	E. P., 82 percent lean	E. P.		59.	17.4	23.	.83	0.					277	1,250
	A. P., 65 percent lean	A. P.	21	46.	13.7	18.	.7	0.					219	990
Fat	E. P., 76 percent lean	E. P.		52.	15.8	31.	.74	0.					342	1,550
	A. P., 62 percent lean	A. P.	18	43.	13.0	25.	.6	0.					281	1,270
Very fat	E. P., 62 percent lean	E. P.		43.	12.7	44.	.59	0.					447	2,030
	A. P., 53 percent lean	A. P.	14	37.	10.9	38.	.5	0.					384	1,740

Round:															
Thin	E. P., 92 percent lean	E. P.		71.	19.7	8.	1.00	0.					151	680
	A. P., 81 percent lean	A. P.	12	63.	17.3	7.	.9	0.					133	600
Medium	E. P., 87 percent lean	E. P.		67.	19.3	13.	.95	0.					194	880
	A. P., 77 percent lean	A. P.	11	59.	17.2	12.	.8	0.					173	780
Fat	E. P., 84 percent lean	E. P.		63.	18.7	17.	.90	0.					228	1,030
	A. P., 76 percent lean	A. P.	10	57.	16.8	15.	.8	0.					205	930
Very fat	E. P., 78 percent lean	E. P.		58.	17.4	24.	.82	0.					286	1,300
	A. P., 71 percent lean	A. P.	9	53.	15.8	22.	.7	0.					260	1,180
Rump:															
Thin	E. P., 75 percent lean	E. P.		60.	17.4	22.	.88	0.					268	1,210
	A. P., 55 percent lean	A. P.	27	44.	12.7	16.	.6	0.					195	890
Medium	E. P., 67 percent lean	E. P.		53.	15.5	31.	.77	0.					341	1,550
	A. P., 51 percent lean	A. P.	24	40.	11.8	24.	.6	0.					259	1,180
Fat	E. P., 61 percent lean	E. P.		48.	14.2	37.	.69	0.					390	1,770
	A. P., 48 percent lean	A. P.	22	38.	11.1	29.	.5	0.					304	1,380
Very fat	E. P., 50 percent lean	E. P.		40.	11.4	48.	.56	0.					478	2,170
	A. P., 40 percent lean	A. P.	19	32.	9.2	39.	.5	0.					387	1,750
Shank, fore:															
Thin	E. P., 93 percent lean	E. P.		72.	21.0	6.	.98	0.					138	630
	A. P., 55 percent lean	A. P.	41	42.	12.4	4.	.6	0.					81	370
Medium	E. P., 90 percent lean	E. P.		70.	20.4	9.	.94	0.					163	740
	A. P., 53 percent lean	A. P.	41	41.	12.0	5.	.6	0.					96	440
Fat	E. P., 88 percent lean	E. P.		67.	19.7	12.	.90	0.					187	850
	A. P., 53 percent lean	A. P.	40	40.	11.8	7.	.5	0.					112	510
Very fat	E. P., 82 percent lean	E. P.		63.	18.2	18.	.83	0.					235	1,070
	A. P., 51 percent lean	A. P.	38	39.	11.3	11.	.5	0.					146	660
Shank, hind:															
Thin	E. P., 92 percent lean	E. P.		71.	20.8	7.	.96	0.					146	660
	A. P., 38 percent lean	A. P.	59	29.	8.5	3.	.4	0.					60	270
Medium	E. P., 88 percent lean	E. P.		69.	20.1	10.	.93	0.					170	770
	A. P., 36 percent lean	A. P.	59	28.	8.2	4.	.4	0.					70	320
Fat	E. P., 83 percent lean	E. P.		66.	19.2	14.	.88	0.					203	920
	A. P., 36 percent lean	A. P.	57	28.	8.3	6.	.4	0.					87	400
Very fat	E. P., 74 percent lean	E. P.		59.	17.1	23.	.76	0.					275	1,250
	A. P., 33 percent lean	A. P.	55	27.	7.7	10.	.3	0.					124	560

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
Beef—Continued.			*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Canned:														
Corned beef hash		E. P.		70.7	12.8	5.5	2.3	8.7	0.2				136	610
Roast beef		E. P.		60.	25.	13.	2.	0.					217	980
Cooked (see Meat and poultry, cooked).														
Corned:														
Very lean		E. P.		66.4	19.4	8.	6.2	0.					150	680
Lean		E. P.		62.8	18.4	13.	5.8	0.					191	860
Medium		E. P.		54.2	15.8	25.	5.0	0.					288	1,310
Fat		E. P.		46.2	13.5	36.	4.3	0.					378	1,710
Corned, canned:														
Lean		E. P.		62.0	26.4	8.	3.6	0.					178	810
Medium		E. P.		59.3	25.3	12.	3.4	0.					209	950
Fat		E. P.		55.3	23.5	18.	3.2	0.					256	1,160
Dried (salted and smoked)		E. P.		47.7	34.3	6.3	11.6	0.					194	880
Beef organs (see Liver, etc.)														
Beer	Average alcohol content, 4 percent	E. P.		90.2	.6	.0	.2	(4.4)		4.0		0.2L	48	220
Beets, common red:														
Fresh	E. P., peeled root	E. P.		87.6	1.6	.1	1.11	9.6	.9				46	205
	A. P., without tops	A. P.	25	65.7	1.2	.1	.8	7.2	.7				34	155
	A. P., with tops	A. P.	47	46.4	.8	.1	.6	5.1	.5				24	110
Canned	E. P., contents of can	E. P.		85.5	1.5	.1	1.4	11.5	.9				53	240
Canned, sieved		E. P.		89.3	1.3	.1	.8	8.5	.7	5.7	0.4		40	180

Beet greens:														
Fresh:														
Common		E. P.		90.4	2.0	.3	1.7	5.6	1.4	.5			33	150
Sugar beet		E. P.		87.5	2.2	.1	1.3	8.9	1.3				45	205
Beverages, carbonated:														
Ginger ale	About 11 calories per fluid ounce	E. P.		91.				9.					36	165
Others, including kola type	About 14 calories per fluid ounce	E. P.		88.				12.					48	220
Biscuits, baking powder or soda		E. P.		31.	7.3	13.	2.5	46.2	.2				331	1,500
Blackberries:														
Fresh		E. P.		85.3	1.2	1.1	.47	11.9	4.1	6.1		.91c	62	285
Canned:														
Water pack	E. P., contents of can	E. P.		88.7	.9	.7	.3	9.4	2.0	5.2			48	215
Juice pack	do	E. P.		87.2	.8	.3	.4	11.3	2.8				51	230
In sirup	do	E. P.		79.2	.7	.7	.3	19.1	2.5	15.6		.6c	86	390
Blackberry juice:														
Fresh		E. P.		91.4	.3	.0	.39	7.9		6.0		1.13c	33	150
Black-eyed peas (see Cowpeas).														
Black-salsify:														
Fresh	E. P., scraped root	E. P.		77.2	3.1	.3	.81	18.6	2.3				90	405
	Ref., scrapings	A. P.	20	61.8	2.5	.2	.6	14.9	1.8				71	320
Blueberries:														
Fresh		E. P.		83.4	.6	.6	.28	15.1	1.2	9.7		.67c	68	310
Canned:														
Water pack	E. P., contents of can	E. P.		90.	.4	.4	.2	9.	1.				41	185
Juice pack	do	E. P.		88.	.4	.4	.2	11.	1.				49	225
In sirup	do	E. P.		73.	.4	.4	.2	26.	1.				109	495
Blueberry juice:														
Fresh		E. P.		85.9	.1	.0	.19	13.8		12.4		.19c	56	250
Bluefish or tailor														
Raw	E. P., flesh	E. P.		74.6	20.5	4.0	1.2	0.					118	535
	A. P., whole or entrails removed	A. P.	49	38.0	10.5	2.0	.6	0.					60	275
Bonito, including California, Atlantic, and striped														
Raw	E. P., flesh	E. P.		67.6	24.0	7.3	1.4	0.					162	735
	A. P., whole	A. P.	42	39.2	13.9	4.2	.8	0.					94	425
Borage:														
Fresh	E. P., leaves and stems	E. P.		89.4	2.8	.4	1.5	5.9		.1	.2		38	175
Bouillon		E. P.		95.	(1.)	0.	1.5	(0.)	.0				4	20
Bouillon cubes		E. P.		5.	(5.)	2.5	68.	(0.)	0.				46	210

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Brains:														
Fresh:														
Beef		E. P.		77.9	10.5	8.8	1.4	1.4					127	580
Calf		E. P.		80.6	10.0	8.3	1.3	0.					115	520
Hog		E. P.		78.2	10.6	9.0	1.5	.7					126	570
Sheep		E. P.		78.8	10.5	8.3	1.4	1.0					121	550
Brazil nuts	E. P., kernels	E. P.		5.3	14.4	65.9	3.4	11.0	2.1	1.5	2.2		695	3,150
	Ref., shells	A. P.	50	2.6	7.2	33.0	1.7	5.5	1.0				347	1,575
Breads:														
Biscuits		E. P.		31.	7.3	13.	2.5	46.2	.2				331	1,500
Boston brown bread		E. P.		43.4	4.9	2.5	3.0	41.2	.5				207	940
Buns, cinnamon		E. P.		29.6	7.8	5.4	1.2	56.0	.3				304	1,380
French bread	No milk or shortening	E. P.		34.1	8.4	1.0	1.3	55.2	.3				263	1,195
Gluten bread		E. P.		39.1	25.1	3.8	1.8	30.2	.3	1.8	24.9		255	1,160
Graham breads	Made with milk	E. P.		37.	10.	4.	2.	47.	1.				264	1,200
	Made with some milk	E. P.		37.	9.5	3.5	2.	48.	1.				262	1,185
	Made with water	E. P.		37.	9.	3.	2.	49.	1.				259	1,175
Partly graham bread	Containing some milk	E. P.		37.	9.	3.	1.6	49.4	.7				261	1,180
Raisin bread		E. P.		33.	9.	3.	2.	53.	.8				275	1,245
Rolls		E. P.		29.4	8.2	6.1	2.2	54.1	.2				304	1,380
Rusks (toasted)		E. P.		6.8	13.1	7.4	1.5	71.2	1.0				404	1,830

Rye bread, American	Half rye, half patent wheat flour	E. P.		37.6	8.9	2.0	1.8	49.7	.5				252	1,145
Rye bread, black or pumpernickel		E. P.		40.5	6.7	1.2	1.9	49.7	1.3				236	1,070
Salt-rising bread		E. P.		38.	7.1	3.3	1.2	50.4	.3				260	1,180
Toast, Melba		E. P.		5.	12.6	3.0	1.9	77.5	.4				387	1,755
Toast, plain		E. P.		21.0	10.1	2.4	1.5	62.0	.4				310	1,405
Vienna bread	No milk or shortening	E. P.		34.1	8.4	1.0	1.3	55.2	.3				263	1,195
White, milk	"All milk"	E. P.		36.0	9.	3.6	1.6	49.8	.3				268	1,215
White, commercial	Containing some milk solids	E. P.		35.9	8.5	2.0	1.3	52.3	.3				261	1,185
Zwieback		E. P.		4.9	10.9	8.6	1.3	74.3	.3				418	1,895
Breakfast foods (see Corn, Oatmeal, etc.).														
Broccoli:														
Fresh	E. P., flower stalks	E.P.		89.9	3.3	.2	1.1	5.5	1.3	1.9			37	170
	Ref., leaves and tough stalks	A. P.	53	42.3	1.6	.1	.5	2.5	.6				17	75
Brussels sprouts:														
Fresh		E. P.		84.9	4.4	.5	1.28	8.9	1.3				58	260
	Ref., outer leaves	A. P.	23	65.4	3.4	.4	1.0	6.8	1.0				44	200
Buckwheat flour:														
Dark and very dark		E. P.		12.	12.4	2.4	1.6	71.6	1.0				358	1,620
Light and very light		E. P.		12.	6.3	1.1	.9	79.7	.4				354	1,605
Pancake	Prepared, self-rising	E. P.		10.9	11.3	2.2	5.6	70.0	1.3				345	1,565
Burdock:														
Fresh	E. P., roots	E. P.		72.4	3.0	.1	1.14	23.4	2.3	7.3	.3		106	485
	Ref., scrapings and trimmings	A. P.	34	47.8	2.0	.1	.8	15.3	1.5				70	320
Butter		E. P.		15.5	.6	81.0	2.5	.4					733	3,325
Butterfish or dollarfish:														
Raw	E. P., flesh	E. P.		71.4	18.1	10.2	1.4	0.					164	745
	A. P., whole	A. P.	49	36.4	9.2	5.2	.7	0.					84	380
Buttermilk:														
Genuine		E. P.		90.7	3.5	.5	.7	4.6					37	165
Artificially cultured	Made from skim milk	E. P.		90.5	3.5	.2	.8	5.0					36	160
Butternuts	E. P., kernels	E. P.		3.8	23.7	61.2	2.9	8.4					679	3,080
	Ref., shells	A. P.	86	.5	3.3	8.6	.4	1.2					95	430
Cabbage:														
Fresh	Including green, white, red, and savoy	E. P.		92.4	1.4	.2	.75	5.3	1.0	3.5			29	130
	Ref., outer leaves and core	A. P.	27	67.5	1.0	.1	.5	3.9	.7				20	90

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
			Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Calories*	*Calories*
Cabbage, Chinese:														
Fresh		E. P.		95.2	1.4	0.1	0.89	2.4	0.6	0.9	0.2		16	75
	Ref., outer leaves	A. P.	13	82.8	1.2	.1	.8	2.1	.5				14	65
Cakes:														
Angel		E. P.		31.6	8.4	.3	1.0	58.7	.0				271	1,230
Foundation		E. P.		25.1	5.9	11.7	1.4	55.9	.1				352	1,600
Foundation, frosted		E. P.		24.1	5.0	9.3	1.2	60.4	.1				345	1,565
Fruit, dark		E. P.		22.9	5.2	13.8	2.2	55.9	1.2				369	1,670
Plain		E. P.		26.8	6.4	8.2	1.6	57.0	.1				327	1,485
Plain, frosted		E. P.		25.2	5.2	6.2	1.3	62.1	.1				325	1,475
Pound		E. P.		19.3	7.1	23.5	.8	49.3	.1				437	1,985
Rich		E. P.		21.6	5.0	17.7	1.5	54.2	.1				396	1,795
Rich, frosted		E. P.		21.4	4.4	14.7	1.3	58.2	.0				383	1,735
Sponge		E. P.		31.8	7.9	5.0	.9	54.4	.2				294	1,335
Candy:														
Candied or glace peel, fruits, etc.:														
Apricots		E. P.		12.	.6	.2	.7	86.5	.6				350	1,590
Cherries		E. P.		12.	.5	.2	.6	86.7	.5				351	1,590
Citron		E. P.		18.0	.2	.3	1.3	80.2	1.4				324	1,470
Figs		E. P.		21.	3.5	.2	1.6	73.7					311	1,410
Gingerroot	Crystallized	E. P.		12.	.3	.2	.4	87.1	.7				351	1,595
Lemon, orange, or grapefruit peel		E. P.		17.4	.4	.3	1.3	80.6	2.3			0.2c	327	1,480

Pears		E. P.		21.0	1.3	.6	1.2	75.9					314	1,425
Pineapple		E. P.		18.	.8	.4	.8	80.	.8	76.5			327	1,480
Butterscotch		E. P.		5.	0.	12.	1.	82.					436	1,980
Caramels		E. P.		7.	2.	12.	1.	78.					428	1,940
Chocolate, bitter, sweet, milk, with nuts (see Chocolate).														
Chocolate creams		E. P.		9.	4.	14.	1.	72.					430	1,950
Fondant		E. P.		8.	0.	0.	1.	91.					364	1,650
Fudge, plain		E. P.		5.	2.	4.	1.	88.					396	1,795
Hard		E. P.		1.	0.	0.	0.	99.					396	1,795
Marshmallows		E. P.		15.	3.	0.	1.	81.					336	1,525
Peanut brittle		E. P.		2.	12.	18.	1.	67.					478	2,170
Cane (see Sirups, and Sugars).														
Cantaloup (see Muskmelons).														
Cape-gooseberry (see Ground-cherry).														
Carissa or Natal plum:														
Fresh	E. P., pulp	E. P.		79.9	.5	1.3	.4	17.9	.9	9.0		1.7c	85	385
	Ref., seeds and skin	A. P.	18	65.5	.4	1.1	.3	14.7	.7				70	315
Carp or German carp:														
Raw	E. P., flesh	E. P.		77.9	18.2	2.2	1.2	0.					93	420
Carp sucker:														
Raw	do	E. P.		76.2	19.2	3.2	1.2	0.					106	480
	A. P., whole	A. P.	61	29.7	7.5	1.2	.5	0.					41	185
Carrots:														
Fresh	E. P., roots	E. P.		88.2	1.2	.3	1.02	9.3	1.1	7.5			45	205
	Ref., tops and scrapings	A. P.	37	55.6	.8	.2	.6	5.8	.7				28	125
	Ref., tops only	A. P.	27	64.4	.9	.2	.7	6.8	.8				33	150
	Ref., scrapings only (if purchased without tops).	A. P.	12	77.6	1.1	.3	.9	8.2	1.0				39	180
Canned	E. P., contents of can	E. P.		89.6	1.0	.3	1.5	7.6	1.0				37	170
Canned, sieved		E. P.		91.9	.7	.1	.6	6.7	.6	3.9	.3		30	140
Cashew nuts	E. P., roasted or cooked seed	E. P.		4.1	19.6	47.2	2.7	26.4	1.0	6.8	10.7		609	2,760
Catchup, tomato	Salt, 2.5 percent	E. P.		69.5	2.0	.4	3.6	24.5	.4			1.5c	110	495
Catfish:														
Raw (see Fish, class 1).														

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Cauliflower:														
Fresh	A. P., with leafstalks:													
	E. P., bud	E. P.		91.7	2.4	0.2	0.85	4.9	0.9	2.6			31	140
	Ref., main stalk	A. P.	43	52.3	1.4	.1	.5	2.7	.5				18	80
	Ref., main stalk and leafstalks	A. P.	55	41.3	1.1	.1	.4	2.1	.4				14	65
Canned	E. P., contents of can	E. P.		94.5	1.	.2	1.3	3.0	.6				18	80
Caviar, sturgeon:														
Granular		E. P.		46.0	26.9	15.0	8.3	(0.)					243	1,100
Pressed		E. P.		36.0	34.4	16.7	8.0	(0.)					288	1,305
Celeriac:														
Fresh	E. P., roots	E. P.		88.3	1.7	.3	.9	8.8	1.4	.8	0.1		45	205
	Ref., parings	A. P.	14	75.9	1.5	.3	.7	7.6	1.2				39	175
Celery:														
Fresh	E. P., stalks	E. P.		93.7	1.3	.2	1.08	3.7	.7	1.2			22	100
	Ref., leaves and trimmings	A. P.	37	59 0	.8	.1	.7	2.4	.4				14	65
Canned, sieved		E. P.		93.8	.9	.2	1.0	4.1	.8				22	100
Chard:														
Fresh:														
Leaves only		E. P.		91.0	2.6	.4	1.20	4.8	.8	.8	.1		33	150
Stalks only		E. P.		95.2	1.0	.1	.8	2.9	.4	1.1	.7		16	75
Leaves and stalks		E. P.		91.8	1.4	.2	2.2	4.4	.9				25	115
Chayote:														
Fresh	E. P., fruit flesh	E. P.		91.6	1.0	.1	.48	6.8	.8	3.1	1.6		32	145
	Ref., skin of fruit	A. P.	15	77.9	.8	.1	.4	5.8	.7				27	120
Chayote roots		E. P.		77.4	1.8	.1	1.1	19.6	.8	.5	20.0		86	390
Chayote leaves		E. P.		91.0	3.2	.7	1.2	3.9	1.4	1.1			35	155
Cheese:														
Camembert		E. P.		51.0	19.7	25.2	4.1	.0					306	1,385
Cheddar, American		E. P.		39.	23.9	32.3	3.1	1.7					393	1,785

Cheddar, processed		E. P.		40.	22.3	30.2	5.8	1.7					368	1,670
Cottage (from skim milk)		E. P.		74.0	19.2	.8	1.7	4.3					101	460
Cream (Neufchâtel type)		E. P.		53.3	7.1	36.9	1.0	1.7		1.2		0.75L	367	1,665
Edam		E. P.		43.3	27.0	20.1	5.6	4.0					305	1,385
Emmenthaler	Similar to Swiss													
Gruyère	do													
Liederkranz		E. P.		53.3	15.8	25.6	4.2	1.1					298	1,350
Limburger		E. P.		38.3	23.5	32.4	5.1	.7					388	1,760
Limburger, processed		E. P.		48.	21.2	26.4	3.7	.7					325	1,475
Mysost		E. P.		28.3	9.3	1.4	6.6	54.4					267	1,215
Parmesan		E. P.		28.9	36.3	27.4	5.1	2.3					401	1,820
Roquefort		E. P.		37.4	21.7	33.2	6.3	1.4					391	1,775
Swiss		E.P.		34.0	28.6	31.3	4.2	1.9					404	1,830
Swiss, processed		E. P.		42.	23.8	26.1	6.5	1.6					336	1,525
Cherimoya:														
Fresh	E. P., pulp	E. P.		68.6	1.4	.4	.9	28.7	4.3	14.0		.2c	124	560
	Ref., seeds and skin	A. P.	29	48.7	1.0	.3	.6	20.4	3.1				88	400
Cherries:														
Fresh:														
Sour, sweet, and hybrid	E. P., flesh	E. P.		83.0	1.1	.5	.55	14.8	.3				68	310
	Ref., pits	A. P.	6	78.0	1.0	.5	.5	14.0	.3				64	290
Sour	E. P., flesh	E. P.		84.4	1.3	.5	.51	13.3		9.5		1.38m	63	285
	Ref., pits	A. P.	5	80.2	1.2	.5	.5	12.6					60	270
Sweet	E. P. flesh	E. P.		80.0	1.1	.5	.6	17.8	.4	11.6		.68m	80	365
	Ref., pits	A. P.	6	75.2	1.0	.5	.6	16.7	.4				75	340
Candied		E. P.		12.	.5	.2	.6	86.7	.5				351	1,590
Canned:														
Water pack:														
Red and white	E. P., contents of can (except pits)	E. P.		89.0	.6	.2	.4	9.8	.2	6.7			43	195
	Ref., pits	A. P.	4	85.4	.6	.2	.4	9.4	.2				42	190
Black	E. P., contents of can (except pits)	E. P.		82.5	.7	.3	.5	16.	.2				70	315
	Ref., pits	A. P.	4	79.2	.7	.3	.5	15.	.2				67	305

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
Cherries—Continued.			*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Calo-ries*	*Calo-ries*
Canned—Continued.														
Juice pack:														
Red and white	E. P., contents of can (except pits)	E. P.	.....	85.7	0.8	0.4	0.5	12.6	0.2	11.3	.....	.....	57	260
	Ref., pits	A. P.	4	82.3	.8	.4	.5	12.1	.2	.....	.....	.....	55	250
Black	E. P., contents of can (except pits)	E. P.	.....	80.5	.7	.3	.5	18.	.2	.....	.....	.....	78	350
	Ref., pits	A. P.	4	77.3	.7	.3	.5	17.	.2	.....	.....	.....	74	335
In sirup:														
Red, pitted	E. P., contents of can	E. P.	.....	70.2	.6	.1	.6	28.5	.2	.....	.....	.....	117	530
White	E. P., contents of can (except pits)	E. P.	.....	78.1	.6	.1	.4	20.8	.2	.....	.....	.....	86	390
	Ref., pits	A. P.	4	75.0	.6	.1	.4	20.0	.2	.....	.....	.....	83	375
Maraschino	E. P., drained solids	E. P.	.....	47.4	.4	.1	.2	51.9	.1	49.7	.....	.....	210	955
Chervil:														
Fresh	E. P., leaves	E. P.	.....	80.7	3.4	.9	3.5	11.5	.....	.....	.....	.....	68	305
Chestnuts:														
Fresh	E. P., kernels (with brown skins)	E. P.	.....	53.2	2.8	1.5	1.0	41.5	1.1	6.4	14.8	.....	191	865
	Ref., shells	A. P.	19	43.1	2.3	1.2	.8	33.6	.9	.....	.....	.....	154	700
Dried	E. P., kernels (with brown skins)	E. P.	.....	8.4	6.7	4.1	2.2	78.6	2.5	14.4	41.1	.....	378	1,715
	Ref., shells	A. P.	18	6.9	5.5	3.4	1.8	64.4	2.0	.....	.....	.....	310	1,405
Chestnut flour		E. P.	.....	11.7	6.1	3.7	2.6	75.9	2.0	.....	.....	.....	361	1,640
Chicken:														
Fresh:														
Squab broilers (¾-1¼ pounds live weight).	Dressed weight is 88 percent of live weight; drawn weight 63 percent.													
Total edible	E. P., flesh, fat, skin, and giblets	E. P.	.....	74.9	21.6	2.7	1.4	0.	.....	.....	.....	.....	111	500
	A. P., live	A. P.	58	31.5	9.1	1.1	.6	0.	.....	.....	.....	.....	46	210
	A. P., dressed	A. P.	52	36.0	10.4	1.3	.7	0.	.....	.....	.....	.....	53	240
	A. P., drawn	A. P.	32	50.9	14.7	1.8	1.0	0.	.....	.....	.....	.....	75	340
Broilers (1½-2½ pounds live weight; about 8-12 weeks old).	Dressed weight is 88 percent of live weight; drawn weight 65 percent.													

Total edible	E. P., flesh, fat, skin, and giblets	E. P.		71.2	20.2	7.2	1.1	0.					146	660
	A. P., live	A. P.	51	34.9	9.9	3.5	.5	0.					71	320
	A. P., dressed	A. P.	45	39.2	11.1	4.0	.6	0.					80	360
	A. P., drawn	A. P.	25	53.4	16.2	5.4	.8	0.					109	500
Flesh only		E. P.		74.0	20.6	4.4	1.1	0.					122	550
Giblets		E. P.		73.7	19.2	4.5	1.3	0.					117	530
Fryers (2½–3¼ pounds live weight; about 14–20 weeks old).	Dressed weight is 88 percent of live weight; drawn weight 68 percent.													
Total edible	E. P., flesh, fat, skin, and giblets	E. P.		67.6	20.0	11.0	1.0	0.					179	810
	A. P., live	A. P.	47	35.8	10.6	5.8	.5	0.					95	430
	A. P., dressed	A. P.	40	40.6	12.0	6.6	.6	0.					107	490
	A. P., drawn	A. P.	22	52.7	15.6	8.6	.8	0.					140	630
Flesh, skin, and giblets		E. P.		67.9	19.1	11.7	1.1	0.					182	820
Flesh only		E. P.		73.4	20.6	4.8	1.1	0.					126	570
Giblets		E. P.		74.0	19.7	3.5	1.3	0.					110	500
Roasters (over 3½ pounds live weight; about 5–9 months old).	Dressed weight is 89 percent of live weight; drawn weight 71 percent.													
Total edible	E. P., flesh, fat, skin, and giblets	E. P.		66.0	20.2	12.6	1.0	0.					194	880
	A. P., live	A. P.	46	35.6	10.9	6.8	.5	0.					105	480
	A. P., dressed	A. P.	39	40.3	12.3	7.7	.6	0.					118	540
	A. P., drawn	A. P.	23	50.8	15.6	9.7	.8	0.					150	680
Flesh, skin, and giblets		E. P.		67.2	19.6	11.7	1.0	0.					184	830
Flesh only		E. P.		72.8	21.1	4.5	1.1	0.					125	570
Giblets		E. P.		72.4	19.8	4.8	1.3	0.					122	560
Hens and cocks (mature birds).	Dressed weight is 90 percent of live weight; drawn weight 72 percent.													
Total edible	E. P., flesh, fat, skin, and giblets	E. P.		55.9	13.0	25.0	1.1	0.					297	1,350
	A. P., live	A. P.	42	32.4	10.4	14.5	.6	0.					172	780
	A. P., dressed	A. P.	36	35.8	11.5	16.0	.7	0.					190	860
	A. P., drawn	A. P.	20	44.7	14.4	20.0	.9	0.					238	1,080
Flesh, skin, and giblets		E. P.		59.7	18.1	21.1	1.0	0.					262	1,190
	A. P., live	A. P.	49	30.4	9.2	10.8	.5	0.					134	610
	A. P., dressed	A. P.	43	34.0	10.3	12.0	.6	0.					150	680
	A. P., drawn	A. P.	29	42.4	12.9	15.0	.7	0.					186	840
Flesh only		E. P.		70.3	21.3	7.1	1.1	0.					149	680
	A. P., live	A. P.	63	26.0	7.9	2.6	.4	0.					55	250
	A. P., dressed	A. P.	59	28.8	8.7	2.9	.5	0.					61	280
	A. P., drawn	A. P.	49	35.9	10.9	3.6	.6	0.					76	340
Giblets		E. P.		66.8	18.6	11.6	1.2	0.					179	810

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
Chicken—Continued.														
Fresh—Continued.														
Capons (over 4 pounds live weight; usually 7–10 months old).	Dressed weight is 90 percent of live weight; drawn weight 73 percent.		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Calories*	*Calories*
Total edible	E. P., flesh, fat, skin, and giblets	E. P.	.....	56.6	21.4	21.2	1.2	0.	.....	.....	.....	.....	276	1,250
	A. P., live	A. P.	40	34.0	12.8	12.7	.7	0.	.....	.....	.....	.....	166	750
	A. P., dressed	A. P.	34	37.4	14.1	14.0	.8	0.	.....	.....	.....	.....	182	830
	A. P., drawn	A. P.	17	47.0	17.8	17.6	1.0	0.	.....	.....	.....	.....	229	1,040
Flesh, fat, and skin		E. P.	.....	55.8	21.6	22.0	1.2	0.	.....	.....	.....	.....	284	1,290
	A. P., live	A. P.	51	27.3	10.6	10.8	.6	0.	.....	.....	.....	.....	139	630
	A. P., dressed	A. P.	46	30.1	11.7	11.9	.6	0.	.....	.....	.....	.....	154	700
	A. P., drawn	A. P.	33	37.4	14.5	14.7	.8	0.	.....	.....	.....	.....	191	860
All classes:														
Light meat only		E. P.	.....	72.5	23.3	3.2	1.2	0.	.....	.....	.....	.....	122	550
Dark meat only		E. P.	.....	73.0	21.0	4.7	1.1	0.	.....	.....	.....	.....	126	570
Canned:														
Meat only		E. P.	.....	61.9	29.8	8.0	2.4	0.	.....	.....	.....	.....	191	870
Meat and broth		E. P.	.....	70.2	23.2	3.4	1.6	0.	.....	.....	.....	.....	123	560
Cooked (see Meat and poultry, cooked).														
Potted		E. P.	.....	58.2	18.8	18.8	2.6	0.	.....	.....	.....	.....	244	1,110
Chickpeas:														
Dry	Whole seeds	E. P.	.....	10.6	20.8	4.7	3.0	60.9	5.3	.....	.....	.....	369	1,675
Chicory or "French endive":														
Fresh	E. P., leaves	E. P.	.....	94.2	1.6	.3	1.0	2.9	.8	0.2	.....	.....	21	95
	Ref., outer leaves	A. P.	11	83.8	1.4	.3	.9	2.6	.7	.....	.....	.....	19	85
Chili sauce		E. P.	.....	68.7	2.8	.4	4.4	23.7	.7	22.	.....	1.7a	110	495
Chives:														
Fresh	E. P., bulbs and tops	E. P.	.....	86.0	3.8	.6	1.8	7.8	2.0	.....	.....	.....	52	235
Chocolate:														
Bitter or unsweetened		E. P.	.....	2.3	(5.5)	52.9	3.2	(18)	2.6	.....	.....	.....	570	2,585

Sweetened:															
Plain		E. P.		1.4	(2.)	29.8	1.4	(60.)	1.4	52.			516	2,340
Milk		E. P.		1.1	(6.)	33.5	1.7	(54.)	.5	49.			542	2,455
Milk, with almonds		E. P.		.6	(8.)	33.6	1.8	(51.)	.6	44.			583	2,645
Cider, sweet		E. P.		87.1	.1	.0	.25	12.5		10.5		.52m	50	230
Cisco (see Herring, lake).														
Citron:														
Candied		E. P.		18.0	.2	.3	1.3	80.2	1.4				324	1,470
Clams:														
Fresh:														
Long:														
Meat only		E. P.		80.6	13.6	1.7	2.0	2.1					78	355
	Ref., shells and liquor	A. P.	65	28.2	4.8	.6	.7	.7					27	125
Meat and liquor (59 percent solids; 41 percent liquor).		E. P.		85.8	8.6	1.0	2.6	2.0					51	235
	Ref., shells	A. P.	42	49.8	5.0	.6	1.5	1.2					30	135
Round:														
Meat only		E. P.		79.8	11.1	.9	2.3	5.9					76	345
	Ref., shells and liquor	A. P.	83	13.6	1.9	.2	.4	.9					13	60
Meat and liquor (52 percent solids; 48 percent liquor).		E. P.		86.2	6.5	.4	2.7	4.2					46	210
	Ref., shells	A. P.	68	27.6	2.1	.1	.9	1.3					15	65
Long and round:														
Meat only		E. P.		80.3	12.8	1.4	2.1	3.4					77	350
Meat and liquor (58 percent solids; 42 percent liquor).		E. P.		85.9	8.1	.9	2.6	2.5					50	230
Canned:														
Long and round:														
Meat only (discarding liquor).		E. P.		76.8	15.8	2.5	2.8	2.1					94	425
		A. P.	59	31.5	6.5	1.0	1.1	.9					39	175
Liquor		E. P.		93.7	2.2	.1	1.9	2.1					18	80
Meat and liquor		E. P.		86.7	7.9	1.	2.3	2.1					49	220
Clam bouillon, juice, or nectar:														
Canned		E. P.		94.8	1.4	.0	2.3	1.5					12	55
Cocoa:														
Plain:														
All	Dry powder	E. P.		4.3	(9.)	13.8	5.2	(31.)	4.8				329	1,495
Breakfast	....do	E. P.		3.9	(8.)	23.8	5.0	(29.)	4.6				362	1,645
Soluble	....do	E. P.		5.5	(7.5)	22.8	7.4	(29.)	4.5				351	1,595

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
Coconut:			*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Fresh														
Meat and milk	A. P., nut with shell and milk:	E. P.		60.2	2.5	25.0	0.9	11.4	2.3	5.0			281	1,275
	Ref., shell only	A. P.	26	44.5	1.9	18.5	.7	8.4	1.7				208	940
Meat (with brown skin)		E. P.		46.9	3.4	34.7	1.0	14.0	3.2	5.1			382	1,730
	Ref., shell and milk	A. P.	47	24.9	1.8	18.4	.5	7.4	1.7				202	920
Milk only		E. P.		93.6	.3	.4	.7	5.0		4.8			25	110
Prepared, sweetened:														
Moist, shredded		E. P.		17.3	3.7	28.6	.8	49.6	4.2	32.			471	2,135
Dried, shredded		E. P.		3.3	3.6	39.1	.8	53.2	4.1	36.			579	2,625
Coconut bars or cookies		E. P.		4.0	6.2	16.7	1.5	71.6					462	2,095
Cod:														
Raw	E. P., flesh	E. P.		82.6	16.5	.4	1.2	0.					70	315
	A. P., whole	A. P.	52	39.6	7.9	.2	.6	0.					33	150
	A. P., dressed	A. P.	31	57.0	11.4	.3	.8	0.					48	220
	A. P., steaks	A. P.	9	75.2	15.0	.4	1.1	0.					63	285
Canned (see Cod, raw, E. P.).														
Dried		E. P.		12.3	81.8	2.8	7.0	0.					352	1,600
Salted		E. P.		52.4	29.0	.7	19.7	0.					122	555
Cod, black (see Sablefish)														
Cod roe:														
Fresh		E. P.		70.6	24.3	1.8	2.0	(0.)					113	515
Collards:														
Fresh	E. P., leaves	E. P.		86.6	3.9	.6	1.70	7.2	1.2	1.2	0.2		50	225
	Ref., tough stalks and some leaves	A. P.	55	39.0	1.8	.3	.8	3.1	.5				22	100
Consomme		E. P.		95.	(1.)	0.	1.5	(0.)	.0				4	20
Cookies:														
Crisp, thin, rich	Any flavor, including chocolate	E. P.		3.	7.8	18.0	1.4	69.8	.2				472	2,145
Soft, thick	do	E. P.		7.8	6.8	10.5	1.9	73.0	.2				414	1,875

Sandwich-type, commercial	do	E. P.		2.3	5.0	19.6	.9	72.2	.3				485	2,200
Iced thinly	do	E. P.		4.2	5.7	24.9	1.8	63.4	.3				500	2,270
Frosted thickly	do	E. P.		10.	4.	10.	1.	75.	.3				406	1,840
Coconut bars		E. P.		4.0	6.2	16.7	1.5	71.6					462	2,095
Fig bars		E. P.		13.8	4.2	4.8	1.4	75.8	1.7				363	1,645
Gingersnaps		E. P.		5.5	6.4	8.9	2.5	76.7	.4				412	1,870
Macaroons		E. P.		10.5	6.3	16.9	.9	65.4	1.1				439	1,990
Molasses cookies		E. P.		5.5	6.4	8.9	2.5	76.7	.4				412	1,870
Peanut cookies		E. P.		2.6	14.0	27.5	2.4	53.5	.8				518	2,345
Shortbread		E. P.		4.2	5.8	23.0	1.4	65.6	.1				493	2,235
Vanilla wafers		E. P.		5.6	6.1	14.9	1.0	72.4	.3				448	2,035
Corn:														
Field corn:														
Dry	Whole grain, white and yellow	E. P.		11.	10.0	4.3	1.3	73.4	2.1				372	1,690
Popcorn:														
Popped		E. P.		4.0	11.4	5.2	1.6	77.8	1.7				404	1,830
Unpopped		E. P.		9.8	11.9	4.7	1.5	72.1	2.1				378	1,715
Sweet corn:														
Fresh:														
All	E. P., kernels	E. P.		73.9	3.7	1.2	.66	20.5	.8	4.3	14.6		108	490
	A. P., with husks	A. P.	62	28.1	1.4	.5	.3	7.7	.3				41	185
	A. P., without husks	A. P.	43	42.1	2.1	.7	.4	11.7	.5				61	280
Young		E. P.		80.3	2.9	.8	.56	15.4	.6	5.2	8.7		80	365
	A. P., with husks	A. P.	70	24.1	.9	.2	.2	4.6	.2				24	110
Medium		E. P.		72.4	3.7	1.1	.8	22.0	.9	4.0	16.1		113	510
	A. P., with husks	A. P.	53	30.4	1.6	.5	.3	9.2	.4				48	220
Old		E. P.		65.7	4.5	1.8	1.	27.	.8	3.2	21.6		142	645
	A. P., with husks	A. P.	52	31.5	2.2	.9	.5	13.	.4				68	310
Canned	E. P., contents of can	E. P.		76.0	2.5	.9	1.0	19.6	.4				96	440
Dried		E. P.		9.6	12.7	7.3	2.0	68.4	2.0				390	1,770
Corn flakes		E. P.		9.3	7.9	.7	1.8	80.3	.5				359	1,630
Corn flour		E. P.		12.1	7.9	2.2	.8	77.0	.7				359	1,630

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Corn germ, commercially milled	Containing some bran and flour	E. P.		9.1	14.5	20.8	5.7	49.9	5.1				445	2,020
Corn, hominy (see Hominy).														
Corn meal:														
Whole ground:														
White or yellow		E. P.		12.	9.1	3.7	1.3	73.9	2.0				365	1,655
Bolted, degerminated:														
White		E. P.		12.	7.5	1.1	.6	78.8	.8				355	1,610
Yellow		E. P.		12.	8.3	1.2	.5	78.0	.7				356	1,615
Corn oil (see Oils, salad).														
Corn sirup (see Sirups).														
Corn sugar (see Sugars).														
Cornsalad:														
Fresh	E. P., leaves and stems	E. P.		92.8	2.0	.4	1.2	3.6	.8				26	120
	Ref., roots	A. P.	4	89.1	1.9	.4	1.2	3.4	.8				25	115
Cottonseed flour		E. P.		6.8	42.3	10.8	5.8	34.3	4.1		2.2		404	1,830
Cowpeas:														
Fresh:														
Shelled	Immature seeds	E. P.		65.9	9.4	.6	1.4	22.7					134	605
Young pods, as green vegetable (see Asparagus-beans).														
Dry	Whole mature seeds	F. P.		10.6	22.9	1.4	3.5	61.6	4.2	5.9	23.8		351	1,590
Crab apples:														
Fresh		E. P.		81.1	.4	.3	.42	17.8	.6	12.6		0.93m	76	340
Crab apple juice:														
Fresh		E. P.		85.0				(12.0)		11.2		.77m		
Crabs, Atlantic and Pacific, hard-shell:														
Fresh or cooked	E. P., meat	E. P.		80.0	16.1	1.6	1.7	.6					81	370
	Ref., shells	A. P.	52	38.4	7.7	.8	.8	.3					39	175
Canned		E. P.		77.2	16.9	2.9	1.7	1.3					99	450

Crackers:														
Butter		E. P.		4.9	9.8	12.4	2.1	70.8	.3				434	1,970
Cheese		E. P.		5.9	15.5	16.4	3.8	58.4	.1				443	2,010
Fat-rich		E. P.		3.1	8.7	17.9	1.7	68.6	.4				470	2,135
Graham		E. P.		5.5	8.0	10.0	2.2	74.3	.8				419	1,900
Oatmeal		E. P.		6.5	8.7	11.5	1.8	71.5	.3				424	1,925
Oyster (see Crackers, soda, plain).														
Saltines		E. P.		4.6	9.2	11.8	3.3	71.1	.4				427	1,940
Soda, plain		E. P.		5.7	9.6	9.6	2.4	72.7	.2				416	1,885
Sweet, tea biscuit		E. P.		4.6	7.7	11.0	1.2	75.5	.3				432	1,960
Water crackers, unshortened		E. P.		6.4	10.7	.3	.5	82.1	.4				374	1,695
Water crackers, shortened		E. P.		7.0	10.1	4.8	1.3	76.8	.5				391	1,775
Whole-wheat wafers		E. P.		7.3	9.4	7.4	2.2	73.7	.8				399	1,810
Cracker meal (see Crackers, soda, plain).														
Cranberries:														
Fresh		E. P.		87.4	.4	.7	.20	11.3	1.4	4.2		2.36c	53	240
Cranberry sauce:														
Canned	Sweetened	E. P.		48.1	.1	.3	.1	51.4	.4	43.		.9c	209	945
Crayfish, freshwater:														
Raw	E. P., flesh	E. P.		81.2	16.0	.5	1.3	1.0					72	330
	A. P., whole	A. P.	88	9.7	1.9	.1	.2	.1					9	40
Cream:														
Light, table, or coffee		E. P.		72.5	2.9	20.	.6	4.0					208	940
Heavy or whipping		E. P.		59.	2.3	35.	.5	3.2					337	1,530
Cress, garden:														
Fresh	E. P., leaves and stems	E. P.		87.2	4.2	1.4	1.9	5.3	1.2				51	230
	Ref., stalks and outer leaves	A. P.	37	54.9	2.6	.9	1.2	3.4	.8				32	145
Cress, water:														
Fresh	E. P., leaves and stems	E. P.		93.6	1.7	.3	1.09	3.3	.5				23	105
Croaker:														
Raw	E. P., flesh	E. P.		77.4	17.8	2.2	1.3	0.					91	415
	A. P., whole	A. P.	66	26.3	6.1	.7	.4	0.					31	140
Croaker, yellow-fin:														
Raw	E. P., flesh	E. P.		79.0	19.2	.8	1.2	0.					84	380
Cucumber:														
Fresh		E. P.		96.1	.7	.1	.44	2.7	.5	2.6			14	65
	Ref., parings	A. P.	30	67.3	.5	.1	.3	1.8	.4				10	45
Cultus, Pacific:														
Raw	E. P., flesh	E. P.		79.7	17.9	.8	1.2	0.					79	355
	A. P., whole	A. P.	50	39.8	9.0	.4	.6	0.					39	180

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
								Carbohydrates						
			Refuse	Water	Protein	Fat	Ash	Total	Fiber	Sugars	Starch	Acid	Per 100 grams	Per pound
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Currants, red, white, and black:														
Fresh		E. P.		84.7	1.6	0.4	0.61	12.7	3.2	5.7		2.30c	61	275
Currant juice:														
Fresh:														
Red		E. P.		89.1	.3	.0	.54	10.1		6.2		2.00c	42	190
Black		E. P.			.5		.68	(13.8)		10.9		2.87c		
"Currants":														
Dried (see Raisins).														
Cusk, Atlantic:														
Raw	E. P., flesh	E. P.		82.0	17.0	.2	.9	0.					70	315
	A. P., entrails removed	A. P.	42	47.6	9.9	.1	.5	0.					40	185
Dandelion greens:														
Fresh		E. P.		85.8	2.7	.7	2.0	8.8	1.8	.7	0.2		52	235
Dasheens:														
Fresh	E. P., corms and tubers	E. P.		66.6	2.9	.2	1.42	28.9	.7	1.7	21.8		129	585
	Ref., skins	A. P.	16	55.9	2.4	.2	1.2	24.3	.6				109	495
Dasheen leaves and stems:														
Fresh		E. P.		87.8	2.7	.7	1.6	7.2	1.4		.4		46	210
Dates:														
"Fresh" and dried	E. P., flesh and skin	E. P.		20.	2.2	.6	1.8	75.4	2.4	61.2			316	1,430
	Ref., pits	A. P.	13	17.	1.9	.5	1.6	65.6	2.1				275	1,245
Deviled ham:														
Canned		E. P.		31.	19.	43.	7.	0.					463	2,100
Dock or sorrel:														
Fresh	E. P., leaves and stems	E. P.		93.3	2.1	.3	.95	3.4	.8	.0	.1		25	110
	Ref., stalks	A. P.	30	65.3	1.5	.2	.7	2.3	.6				17	75
Dollarfish (see Butterfish).														
Doughnuts		E. P.		18.7	6.6	21.0	1.0	52.7	.2				426	1,935
Drum, red:														
Raw	E. P., flesh	E. P.		80.2	18.0	.4	1.3	0.					76	345
	A. P., whole	A. P.	59	32.9	7.4	.2	.5	0.					31	140

Duck, domesticated:														
Fresh:														
Total edible	E. P., flesh, skin, giblets and most of fat	E. P.		54.3	16.0	28.6	1.0	0.					321	1,460
	A. P., dressed	A. P.	36	34.8	10.2	18.3	.6	0.					206	930
	A. P., drawn	A. P.	16	45.6	13.4	24.0	.8	0.					270	1,220
Flesh only		E. P.		68.8	21.4	8.2	1.2	0.					159	720
Cooked (see Meat and poultry, cooked).														
Duck, wild:														
Fresh:														
Total edible	E. P., flesh, skin, and giblets	E. P.		61.1	21.1	15.8	1.1	0.					227	1,030
	A. P., dressed	A. P.	42	35.4	12.2	9.2	.6	0.					131	600
Flesh only		E. P.		70.8	21.3	5.2	1.3	0.					132	600
Cooked (see Meat and poultry, cooked).														
Eels, American:														
Raw		E. P.		71.6	18.6	9.1	1.0	0.					156	710
	A. P., head, skin, and entrails removed	A. P.	24	54.4	14.1	6.9	.8	0.					119	540
Eels:														
Smoked		E. P.		50.2	18.6	27.8	2.4	0.					325	1,470
Eggplant:														
Fresh		E. P.		92.7	1.1	.2	.54	5.5	.9				28	130
	Ref., calyx and parings	A. P.	13	80.6	1.0	.2	.5	4.7	.8				25	115
	Ref., calyx only	A. P.	4	89.0	1.1	.2	.52	5.3	.9				27	125
Eggs:														
Fresh, stored, or frozen:														
Hen:														
Total edible	E. P., white and yolk	E. P.		74.0	12.8	11.5	1.0	.7		.3			158	715
	Ref., shell	A. P.	11	65.9	11.4	10.2	.9	.6					140	635
White only		E. P.		87.8	10.8	.0	.6	.8		.4			46	210
Yolk only		E. P.		49.4	16.3	31.9	1.7	.7		.2			355	1,610
Duck:														
Total edible	E. P., white and yolk	E. P.		70.8	13.1	14.3	1.0	.8		.3			184	835
	Ref., shell	A. P.	11	63.0	11.7	12.7	.9	.7					164	745
Goose:														
Total edible	E. P., white and yolk	E. P.		70.4	13.9	13.3	1.1	1.3		.4			180	820
	Ref., shell	A. P.	13	61.2	12.1	11.6	1.0	1.1					157	710
Turkey:														
Total edible	E. P., white and yolk	E. P.		72.6	13.1	11.8	.8	1.7		.4			165	750
	Ref., shell	A. P.	12	63.9	11.5	10.4	.7	1.5					146	660
Boiled (see Eggs, fresh, stored, or frozen).														
Endive:														
Fresh	E. P., leaves	E. P.		93.3	1.6	.2	.89	4.0	.8				24	110
	A. P., stalks and outer leaves	A. P.	48	48.5	.8	.1	.5	2.1	.4				12	55
Endive, French," or chicory:														
Fresh	E. P., leaves	E. P.		94.2	1.6	.3	1.0	2.9	.8	.2			21	95
	Ref., outer leaves	A. P.	11	83.8	1.4	.3	.9	2.6	.7				19	85

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Fuel value	
								Total	Fiber	Sugars	Starch		Per 100 grams	Per pound
Escarole (see Endive).			*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Calo-ries*	*Calo-ries*
Farina		E. P.		11.	11.5	1.0	0.4	76.1	0.3				359	1,630
Fats, cooking	Vegetable or animal	E. P.				100.							900	4,080
Feijoa:														
Fresh		E. P.		84.5	.9	.2	.5	13.9	3.4	6.6		0.3c	61	275
	Ref., skin	A. P.	13	73.5	.8	.2	.4	12.1	3.0				53	240
Fennel:														
Fresh	E. P., stems	E. P.		92.8	1.9	.2	1.5	3.6	.8				24	110
	A. P., leaves	A. P.	7	86.3	1.8	.2	1.4	3.3	.7				22	100
Figs:														
Fresh		E. P.		78.0	1.4	.4	.64	19.6	1.7	16.2		.17c	88	395
Dried		E. P.		24.	4.0	1.2	2.4	68.4	5.8	55.0		.6m	300	1,365
Candied		E. P.		21.	3.5	.2	1.6	73.7					311	1,410
Canned:														
Water pack	E. P., contents of can	E. P.		85.5	.5	.1	.4	13.5	.6				57	260
In sirup	do	E. P.		68.5	.8	.3	.4	30.	.9	28.		.1c	126	570
Fig bars		E. P.		13.8	4.2	4.8	1.4	75.8	1.7				363	1,645
Filberts (see Hazelnuts).														
Finnan haddie or haddock:														
Smoked		E. P.		72.6	23.2	.4	3.1	0.					96	435
Fish:														
Raw:														
Class 1, medium composition (see also kind as Alewife, Bass, etc.).	E. P., flesh	E. P.		77.2	19.0	2.5	1.3	0.					98	445
	A. P., whole	A. P.	55	34.7	8.6	1.1	.6	0.					44	200
	A. P., drawn	A. P.	52	37.1	9.1	1.2	.6	0.					47	215
	A. P., dressed	A. P.	33	51.7	12.7	1.7	.9	0.					66	300
	A. P., steaks or sections	A. P.	16	64.8	16.0	2.1	1.1	0.					83	375

Class 2, low fat, low protein (see also kind as Cod, Haddock, etc.):	E. P., flesh	E. P.		81.8	16.4	.5	1.3	0.					70	320
	A. P., whole	A. P.	55	36.8	7.4	.2	.6	0.					32	145
	A. P., drawn	A. P.	52	39.3	7.9	.2	.6	0.					34	150
	A. P., dressed	A. P.	33	54.8	11.0	.3	.9	0.					47	215
	A. P., steaks or sections	A. P.	16	68.7	13.8	.4	1.1	0.					59	265
Class 3, higher protein and fat (see individual kind, as Salmon, Mackerel, Tuna).														
Cooked:														
Boiled or steamed:														
Lean or medium-fat fish		E. P.		75.	21.	2.	1.5	0.					102	465
Fatter fish only		E. P.		65.	21.5	11.	2.0	0.					185	840
Baked or broiled		E. P.		60.	24.	12.5	2.5	0.					208	945
Fried in meal, flour, batter, etc.:														
Moderate fat absorption	Most fried fish	E. P.		60.	19.5	11.5	2.5	6.5					207	940
High fat absorption	Small fish, as smelts and sprats	E. P.		37.	24.5	30.	3.5	5.0					388	1,760
Flounders, southern:														
Raw	E. P., flesh	E. P.		77.6	21.3	.2	1.3	0.					87	395
	A. P., whole	A. P.	46	41.9	11.5	.1	.7	0.					47	215
Flounders, summer and winter:														
Raw	E. P., flesh	E. P.		82.7	14.9	.5	1.3	0.					64	290
	A. P., whole	A. P.	61	32.3	5.8	.2	.5	0.					25	115
	A. P., entrails removed	A. P.	59	33.9	6.1	.2	.5	0.					26	120
Flour (see Wheat, Rye, etc.)														
Flour, pancake, prepared, self-rising.	From wheat and mixed cereals	E. P.		11.2	10.3	1.5	4.7	72.3	.6				344	1,560
Frog's legs:														
Fresh		E. P.		81.9	16.4	.3	1.1	0.					68	310
	Ref., bones	A. P.	35	53.2	10.7	.2	.7	0.					44	200
Frozen custard		E. P.		63.	4.	9.	1.	18.					169	765
Game animals:														
Fresh	Lean meat	E. P.		73.	20.	6.	1.	0.					134	610
Garbanzos or chickpeas:														
Dry	Whole seeds	E. P.		10.6	20.8	4.7	3.0	60.9	5.3				369	1,675
Garlic:														
Fresh	E. P., bulbs	E. P.		74.2	4.4	.2	1.18	20.0	1.0				99	450
	Ref., skins	A. P.	8	68.3	4.0	.2	1.1	18.4	.9				91	415
Gelatin:														
Plain, dry		E. P.		13.0	85.6	.1	1.3	0.					343	1,555
Dessert powders	Sweetened, flavored	E. P.		1.6	9.4	.0	.3	88.7		87.			392	1,780
Ginger:														
Fresh	E. P., roots	E. P.		84.6	1.8	1.5	1.3	10.8	1.0				64	290
Candied		E. P.		12.	.3	.2	.4	87.1	.7				351	1,595

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Ginger ale	About 11 calories per fluid ounce	E. P.		91.				9.					36	165
Gingerbread		E. P.		30.4	4.2	11.9	2.1	51.4	0.1				330	1,495
Gingersnaps		E. P.		5.5	6.4	8.9	2.5	76.7	.4				412	1,870
Gizzard:														
Fresh:														
Chicken		E. P.		71.1	23.1	3.8	1.4	.6					129	590
Duck		E. P.		73.3	21.3	3.7	1.1	.6					121	550
Goose		E. P.		73.0	21.4	5.3	1.0	0.					133	600
Turkey		E. P.		66.6	20.5	10.6	1.0	1.3					183	830
Gluten flour		E. P.		8.5	41.4	1.9	1.0	47.2	.4	4.4	36.8		372	1,685
Goose, domesticated:														
Fresh:														
Total edible	E. P., flesh, skin, and giblets	E. P.		51.1	16.4	31.5	.9	0.					349	1,580
	A. P., dressed	A. P.	41	30.1	9.7	18.6	.5	0.					206	930
	A. P., drawn	A. P.	10	46.0	14.8	28.4	.8	0.					314	1,430
Flesh and skin		E. P.		49.7	15.9	33.6	.9	0.					366	1,660
Flesh only		E. P.		68.3	22.3	7.1	1.1	0.					153	690
Gooseberries:														
Fresh	Ripe and underripe	E. P.		88.3	.8	.4	.39	10.1	2.5	4.2		2.32c	47	215
Canned:														
Water pack	E. P., contents of can	E. P.		93.	.5	.2	.3	6.	1.5				28	125
In sirup	do	E. P.		80.5	.5	.2	.3	18.5	1.5				78	355
Granadilla, purple, or passion fruit:														
Fresh	E. P., juice	E. P.		80.6	1.2	.0	.5	17.7	0.	11.5		2.2c	76	345
	Ref., rind and seeds	A. P.	71	23.4	.3	.0	.1	5.2	0.				22	100

Grapefruit:														
Fresh:														
All		E. P.		88.8	.5	.2	.42	10.1	.3	6.5			44	200
	Ref., rinds and seeds	A. P.	34	58.6	.3	.1	.3	6.7	.2				29	130
California-grown		E. P.		87.7	.5	.2	.4	11.2	.3	6.6		2.23c	49	220
	Ref., rinds and seeds	A. P.	34	57.9	.3	.1	.3	7.4	.2				32	145
Florida-grown		E. P.		90.1	.5	.2	.54	8.7		6.5		1.16c	39	175
	Ref., rinds and seeds	A. P.	31	62.2	.3	.1	.4	6.0					26	120
Canned:														
Water pack	E. P., contents of can	E. P.		91.	.5	.2	.3	8.	.2				36	160
Juice pack	do	E. P.		90.	.5	.2	.3	9.	.2				40	180
In sirup	do	E. P.		85.5	.5	.2	.3	13.5	.2				58	260
Grapefruit juice:														
Fresh:														
Florida-grown fruit		E. P.		90.1			.4	(8.1)		6.7		1.42c		
California-grown fruit		E. P.		89.3	.4	.1	.4	9.8		7.0		1.77c	42	190
Arizona-grown fruit		E. P.		89.9				(8.3)		6.7		1.61c		
Canned:														
Unsweetened		E. P.		88.	.4	.1	.4	11.1		8.5		1.6c	47	215
Sweetened		E. P.		83.	.4	.1	.4	16.1		14.		1.6c	67	305
Grapefruit peel:														
Candied		E. P.		17.4	.4	.3	1.3	80.6	2.3			.2c	327	1,480
Grapes:														
Fresh:														
American type (slip skin) as Concord, Delaware, Niagara, and Scuppernong.	E. P., pulp or pulp and skin	E. P.		81.9	1.4	1.4	.45	14.9	.5	11.5		1.21m	78	355
	Ref., seeds only	A. P.	6	77.0	1.3	1.3	.4	14.0	.5				73	330
	Ref. skins and seeds (No data on stems).	A. P.	22	63.9	1.1	1.1	.4	11.5	.4				60	270
European type (adherent skin) as Malaga, Muscat, Sultanina (Thompson Seedless), and Flame Tokay.	E. P., pulp and skin	E. P.		81.6	.8	.4	.46	16.7	.5	14.9		.47m	74	335
	Ref., seeds and stems	A. P.	3	79.2	.8	.4	.4	16.2	.5				72	325
Canned (European type):														
Water pack	E. P., contents of can	E. P.		85.5	.6	.6	.3	13.					60	270
Grape juice:														
Fresh:														
American type:														
All		E. P.		80.7	.4	.0	.39	18.5		16.8		.80m	76	345
Catawba		E. P.		79.1	.4	.0	.30	20.2		17.9		.89m	82	375
Concord		E. P.		82.1	.3	.0	.33	17.3		15.7		.63m	70	320

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
Grape juice—Continued.														
Fresh—Continued.														
American type—Continued.														
Delaware		E. P.		77.3	0.3	0.0	0.32	22.1		19.9		0.65m	90	405
Muscadine		E. P.		86.7	.1	.0	2	13.0		12.5		.69m	52	240
European type:														
All		E. P.		77.1	.4			(20.4)		19.8		.61m		
Table and juice grapes		E. P.		78.3	.4			(18.9)		18.3		.63m		
Raisin grapes		E. P.		73.6	.5			(25.2)		24.6		.55m		
Bottled commercial, any type		E. P.		81.	.4	.0	4	18.2		16.8		.8m	74	335
Grayfish (a shark):														
Raw	E. P., flesh	E. P.		72.3	17.6	9.0	.0	0.					151	685
Greenland halibut (see Turbot).														
Groundcherry (including poha and Cape-gooseberry):														
Fresh	E. P., fruit pulp	E. P.		82.9	2.1	8	.9	13.3	3.4	8.2		1.4c	69	310
	Ref., husks and stems	A. P.	7	77.1	2.0	.7	.8	12.4	3.2				64	290
Grouper, spotted hind:														
Raw	E. P., flesh	E. P.		77.5	19.1	1.2	1.3	0.					87	395
	A. P., whole	A. P.	55	34.9	8.6	.5	.6	0.					39	180
Guavas:														
Fresh:														
Common	E. P., pulp including seeds or pulp only	E. P.		80.6	1.0	.6	.70	17.1	5.5	6.1		.62c	78	355
	Ref., skins only	A. P.	13	70.1	.9	.5	.6	14.9	4.8				68	305
	Ref., skins and seeds	A. P.	18	66.1	.8	.5	.6	14.0	4.5				64	290
Strawberry	E. P., pulp including seeds	E. P.		79.3	1.2	.6	.73	18.2	6.5	6.7		1.1c	83	375
	Ref., skins	A. P.	14	68.2	1.0	.5	.6	15.7	5.6				71	320
Guinea hen:														
Fresh:														
Total edible	E. P., flesh, skin, and giblets	E. P.		69.0	23.1	6.4	1.2	0.					150	680
	A. P., live	A. P.	50	34.5	11.6	3.2	.6	0.					75	340
	A. P., drawn	A. P.	16	58.0	19.4	5.4	1.0	0.					126	570

Haddock:															
Raw	E. P., flesh	E. P.		81.7	17.2	.3	1.2	0.					72	325
	A. P., whole or entrails removed	A. P.	52	39.2	8.3	.1	.6	0.					34	155
Smoked		E. P.		72.6	23.2	.4	3.1	0.					96	435
Hakes (including Boston hake, squirrel hake, and silver hake or whiting):															
Raw	E. P., flesh	E. P.		81.6	16.3	1.1	1.1	0.					75	340
	A. P., whole	A. P.	58	34.3	6.8	.5	.5	0.					32	145
	A. P., entrails removed	A. P.	52	39.2	7.8	.5	.5	0.					36	165
Halibut:															
Raw	E. P., flesh	E. P.		75.4	18.6	5.2	1.0	0.					121	550
	A. P., steak or section	A. P.	19	61.1	15.1	4.2	.8	0.					98	445
Smoked		E. P.		49.4	20.8	15.0	15.0	0.					218	990
Halibut, California:															
Raw	E. P., flesh	E. P.		77.8	19.8	1.4	1.3	0.					92	415
Ham (see Pork, cured and Pork, fresh).															
Haw, scarlet:															
Fresh	E. P., flesh and skin	E. P.		75.8	2.0	.6	.8	20.8	2.1				97	440
	Ref., core	A. P.	20	60.6	1.6	.5	.6	16.7	1.7				78	355
Hazelnuts (including filberts)	E. P., kernel	E. P.		6.0	12.7	60.9	2.7	17.7	3.4	3.2	1.6		670	3,040
	Ref., shell	A. P.	53	2.8	6.0	28.6	1.3	8.3	1.6				315	1,430
Heart:															
Fresh:															
Beef, lean		E. P.		77.6	16.9	3.7	1.1	.7					104	470
Beef, lean with visible fat		E. P.		63.0	15.4	20.7	.8	.1					248	1,130
Chicken		E. P.		69.6	20.5	7.0	1.3	1.6					151	690
Pork		E. P.		76.8	16.9	4.8	1.1	.4					112	510
Sheep		E. P.		71.6	16.8	9.6	1.0	1.0					158	710
Turkey		E. P.		69.8	16.2	12.7	1.1	.2					180	820
Veal		E. P.		75.6	15.4	7.1	1.1	.8					129	580
Herring, Atlantic:															
Raw	E. P., flesh	E. P.		73.0	19.0	6.7	1.6	0.					136	620
	A. P., whole	A. P.	49	37.2	9.7	3.4	.8	0.					70	315
Herring, lake, or cisco:															
Raw	E. P., flesh	E. P.		74.0	18.5	6.8	1.1	0.					135	615
	A. P., whole	A. P.	44	41.4	10.4	3.8	.6	0.					76	345
Herring, Pacific:															
Raw	E. P., flesh	E. P.		79.6	16.6	2.6	1.3	0.					90	405

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									
								Carbohydrates					Fuel value
			Refuse	Water	Protein	Fat	Ash	Total	Fiber	Sugars	Starch	Acid	Per 100 grams	Per pound
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Herring:														
Canned:														
Plain		E. P.		63.1	20.7	12.4	3.9	0.					194	880
In tomato sauce		E. P.		66.7	15.8	10.5	3.3	3.7					172	780
Pickled, Bismarck type		E. P.		59.4	20.4	15.1	4.0	0.					218	985
Salted, or brined		E. P.		58.1	19.6	11.3	12.0	0.					180	815
Smoked:														
Bloaters		E. P.		64.0	19.6	12.4	3.2	0.					190	860
Hard		E. P.		34.6	36.9	15.8	13.2	0.					290	1,315
Kippered		E. P.		61.0	22.2	12.9	4.0	0.					205	930
Herring roe:														
Fresh		E. P.		69.2	26.3	4.2	1.4	(0.)					143	650
Hickory nuts	E. P., kernels	E. P.		3.5	13.9	67.4	2.0	13.2	2.2				715	3,245
	Ref., shells	A. P.	65	1.2	4.9	23.6	.7	4.6	.8				250	1,135
Hominy:														
Dry		E. P.		11.4	8.5	.8	.4	78.9	.4				357	1,620
Cooked or canned		E. P.		82.6	1.8	.2	.5	14.9	.1				69	310
Honey	Strained or extracted	E. P.		20.	.3	0.	.2	79.5		76.			319	1,450
Horse mackerel, Pacific:														
Raw	E. P., flesh	E. P.		71.4	21.6	5.6	1.2	0.					137	620
Horseradish:														
Fresh		E. P.		73.4	3.2	.2	1.8	21.4	2.4				100	455
	Ref., parings	A. P.	27	53.6	2.3	.1	1.3	15.7	1.8				73	330
Prepared		E. P.		85.	1.4	.1	1.5	12.	1.0				54	245

Huckleberries (see Blueberries).														
Ice cream and frozen desserts:														
Ice cream:														
Plain		E. P.		62.	3.9	13.	.8	20.3					214	970
Rich		E. P.		54.8	3.5	23.	.7	18.0					293	1,330
Ices	Fruit juice, sugar, and water	E. P.		72.5	.1	.1	.3	27.					109	495
Frozen custard		E. P.		68.	4.	9.	1.	18.					169	765
Sherbet		E. P.		69.4	2.	3.	.6	25.					135	610
Iceland moss		E. P.		13.7	(0.)	2.4	1.5	(0.)						
Irish moss (see Algae).														
Jams and preserves:														
Commercial		E. P.		28.	.5	.3	.4	70.8	.6				288	1,305
Home-cooked		E. P.		40.	4	.3	.4	58.9	1.2				240	1,090
Jellies		E. P.		34.5	.2	.0	3	65.	.0	63.		0.7c	261	1,185
Jerusalem-artichokes:														
Fresh		E. P.		79.5	2.2	.1	1.17	17.0	.8				78	350
	Ref., parings	A. P.	31	54.9	1.5	.1	.8	11.7	.6				54	245
Jew's mallow:														
Fresh	E. P., leaves and stems	E. P.		85.0	5.1	.5	2.	7.4					54	245
Jujube:														
Fresh		E. P.		63.9	1.2	.3	.9	33.7	1.5	25.6		.4c	142	645
	Ref., seeds	A. P.	7	59.4	1.1	.3	.8	31.3	1.4				132	600
Dried		E. P.		16.8	3.9	.9	2.1	76.3	3.1	60.0		1.2c	329	1,490
	Ref., seeds	A. P.	12	14.8	3.4	.8	1.8	67.1	2.7				289	1,315
Junket powder:														
Chocolate flavor		E. P.		1.	3.	5.	2.	89.					413	1,875
Other than chocolate		E. P.		.3	.1	.1	.8	98.7		97.7			396	1,795
Kale:														
Fresh	E. P., leaves	E. P.		86.6	3.9	.6	1.70	7.2	1.2	1.2	0.2		50	225
	Ref., stalks, outer leaves, in some cases midribs.	A. P.	36	55.4	2.5	.4	1.09	4.6	.8				32	145
Kelp (see Algae).														
Kidneys:														
Fresh:														
Beef		E. P.		74.9	15.0	8.1	1.08	.9					136	620
Pork		E. P.		77.1	16.3	4.6	1.2	.8					110	500
Sheep		E. P.		77.8	16.6	3.3	1.3	1.0					100	450
Veal		E. P.		76.4	16.8	5.2	1.4	.2					115	520
Kingfish, Pacific:														
Raw	E. P., flesh	E. P.		79.7	18.0	.8	1.3	0.					79	360

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
			Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Calories*	*Calories*
King whiting:														
Raw	E. P., flesh	E. P.		77.3	18.3	3.0	1.3	0.					100	455
	A. P., whole	A. P.	56	34.0	8.1	1.3	.6	0.					44	200
Kohlrabi:														
Fresh	E. P., stem (bulb-like)	E. P.		90.1	2.1	.1	1.05	6.7	1.1	2.2			36	165
	Ref., tops and parings	A. P.	46	48.7	1.1	.1	.6	3.5	.6				19	85
Kumquats:														
Fresh	E. P., pulp and skin	E. P.		81.3	.9	.1	.6	17.1	3.7	14.2		1.0c	73	330
	Ref., seeds	A. P.	7	75.6	.8	.1	.6	15.9	3.4				68	310
Lake trout:														
Raw	E. P., flesh	E. P.		70.8	17.8	10.3	1.2	0.					164	745
	A. P., whole	A. P.	57	30.4	7.7	4.4	.5	0.					70	320
	A. P., entrails removed	A. P.	36	45.3	11.4	6.6	.8	0.					105	475
Lamb:														
Fresh:														
Carcass or side:														
Thin	E. P., 84 percent lean	E. P.		66.3	17.1	14.8	.9	0.					202	910
	A. P., 58 percent lean	A. P.	31	45.7	11.8	10.2	.6	0.					139	630
Intermediate	E. P., 72 percent lean	E. P.		55.8	15.7	27.7	.8	0.					312	1,420
	A. P., 56 percent lean	A. P.	22	43.5	12.2	21.6	.6	0.					243	1,100
Fat	E. P., 59 percent lean	E. P.		46.2	13.0	39.8	.7	0.					410	1,860
	A. P., 48 percent lean	A. P.	19	37.4	10.5	32.2	.6	0.					332	1,510
Wholesale cuts:														
Breast:														
Thin	E. P., 80 percent lean	E. P.												
	A. P., 49 percent lean	A. P.	39											
Intermediate	E. P., 64 percent lean	E. P.												
	A. P., 46 percent lean	A. P.	28											
Fat	E. P., 53 percent lean	E. P.												
	A. P., 39 percent lean	A. P.	26											

Leg, trimmed:															
Thin	E. P., 90 percent lean	E. P.		71.0	18.4	9.1	1.0	0.					156	710
	A. P., 70 percent lean	A. P.	23	54.7	14.2	7.0	.8	0.					120	540
Intermediate	E. P., 83 percent lean	E. P.		63.7	18.0	17.5	.9	0.					230	1,040
	A. P., 69 percent lean	A. P.	17	52.9	14.9	14.5	.7	0.					190	860
Fat	E. P., 78 percent lean	E. P.		59.8	16.7	22.4	.8	0.					268	1,220
	A. P., 65 percent lean	A. P.	16	50.2	14.0	18.8	.7	0.					225	1,020
Loin:															
Thin	E. P., 85 percent lean														
	A. P., 65 percent lean	A. P.	23												
Intermediate	E. P., 72 percent lean														
	A. P., 61 percent lean	A. P.	15												
Fat	E. P., 56 percent lean														
	A. P., 50 percent lean	A. P.	12												
Neck:															
Thin	E. P., 91 percent lean														
	A. P., 54 percent lean	A. P.	41												
Intermediate	E. P., 78 percent lean														
	A. P., 53 percent lean	A. P.	32												
Fat	E. P., 60 percent lean														
	A. P., 43 percent lean	A. P.	28												
Rib cut (9 ribs):															
Thin	E. P., 85 percent lean	E. P.		65.3	17.7	15.6	.9	0.					211	960
	A. P., 55 percent lean	A. P.	35	42.4	11.5	10.1	.6	0.					137	620
Intermediate	E. P., 68 percent lean	E. P.		51.9	14.9	32.4	.8	0.					351	1,590
	A. P., 52 percent lean	A. P.	24	39.4	11.3	24.6	.6	0.					267	1,210
Fat	E. P., 51 percent lean	E. P.		38.7	11.2	49.2	.6	0.					488	2,210
	A. P., 41 percent lean	A. P.	18	31.7	9.2	40.3	.5	0.					400	1,810
Shoulder (3 ribs):															
Thin	E. P., 84 percent lean	E. P.		67.2	16.7	14.7	.9	0.					199	900
	A. P., 62 percent lean	A. P.	27	49.1	12.2	10.7	.7	0.					145	660
Intermediate	E. P., 78 percent lean	E. P.		58.3	15.6	25.3	.8	0.					290	1,320
	A. P., 62 percent lean	A. P.	20	46.6	12.5	20.2	.6	0.					232	1,050
Fat	E. P., 67 percent lean	E. P.		51.3	13.6	34.3	.7	0.					363	1,650
	A. P., 55 percent lean	A. P.	18	42.1	11.2	28.1	.6	0.					298	1,350
Cooked (see Meat and poultry, cooked).															
Lamb organs (see Liver, etc.).															
Lambsquarters:															
Fresh	E. P., leaves and stems	E. P.		84.2	3.8	.7	3.0	8.3	2.6	.3	1.4		55	250
Lambsquarters, Algerian:															
Fresh	E. P., leafy shoots	E. P.		76.7	7.6	.9	4.3	10.5					80	365

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Lard		E. P.				100.							900	4,080
Laver (see Algae).														
Leeks:														
Fresh	E. P., bulbs and some leaf	E. P.		83.2	2.5	.4	1.03	7.9	1.3	2.6	1.4		45	205
	Ref., tops and rootlets	A. P.	48	45.9	1.3	.2	.5	4.1	.7				23	105
Lemons:														
Fresh		E. P.		89.3	.9	.6	.54	8.7	.9	2.2		5.07c	44	200
	Ref., rind and seeds	A. P.	38	55.4	.6	.4	.3	5.3	.6				27	120
Lemon juice:														
Fresh		E. P.		89.4			.33	(8.3)		2.3		5.96c		
Canned		E. P.		91.	.4	.3	.3	8.		2.		5.0	36	165
Lemon peel:														
Candied		E. P.		17.4	.4	.3	1.3	80.6	2.3			.2c	327	1,480
Lentils:														
Dry:														
Whole	Entire seed	E. P.		11.2	24.7	1.0	3.2	59.9	3.3				347	1,575
Split	Without seed coat	E. P.		12.2	24.0	1.2	2.2	60.4	1.7				348	1,580
Lettuce:														
Fresh	E. P., inner leaves	E. P.		94.8	1.2	.2	.91	2.9	.6	1.6			18	85
	Ref., stalks and outer leaves	A. P.	31	65.4	.8	.1	.6	2.1	.4				12	55
Lichens (see Iceland moss).														
Lima bean flour		E. P.		10.5	21.5	1.4	3.6	63.0	2.				351	1,590
Limes:														
Fresh		E. P.		86.0	.8	.1	.8	12.3		.5		5.9c	53	240
	Ref., rind and seeds	A. P.	24	65.4	.6	.1	.6	9.3					40	180
Lime juice:														
Fresh		E. P.		91.0	.4	.0	.3	8.3		1.4		6.9c	35	160
Limes, sweet:														
Fresh		E. P.		89.6	.8	.1	.6	8.9	.3	6.0		.16c	40	180
	Ref., rind and seeds	A. P.	23	69.0	.6	.1	.5	6.8	.2				30	135
Litchi fruits:														
Dried	E. P., fruit flesh	E. P.		24.	3.6	.5	1.9	70.0	3.2	65.8			299	1,355
	Ref., thin shell and seed	A. P.	54	11.	1.7	.2	.9	32.2	1.5				137	625

Liver:														
Fresh:														
Beef		E. P.		69.7	19.7	3.2	1.4	6.0					132	600
Calf		E. P.		70.8	19.0	4.9	1.3	4.0					136	620
Chicken		E. P.		69.6	22.1	4.0	1.7	2.6					135	610
Goose		E. P.		66.9	16.5	10.0	1.2	5.4					178	810
Hog		E. P.		72.3	19.7	4.8	1.5	1.7					129	580
Sheep or lamb		E. P.		70.8	21.0	3.9	1.4	2.9					131	590
Turkey		E. P.		70.9	22.0	4.8	1.6	.7					134	610
Liver paste or paté de foie gras		E. P.		37.0	11.4	43.8	3.0	4.8					459	2,080
Lobster:														
Fresh or boiled	E. P., flesh	E. P.		79.2	16.2	1.9	2.2	.5					84	380
	A. P., whole	A. P.	64	28.5	5.8	.7	.8	.2					30	135
Canned		E. P.		77.2	18.4	1.3	2.7	.4					87	395
Lobster or shrimp paste		E. P.		61.3	20.8	9.4	7.0	1.5					174	790
Logan blackberries or loganberries:														
Fresh		E. P.		82.9	1.0	.6	.52	15.0	1.4	6.0		2.18c	69	315
Canned:														
Water pack	E. P., contents of can	E. P.		87.3	1.0	.6	.4	10.7	1.9	4.6			52	235
Juice pack	do	E. P.		86.	1.	.1	.4	12.5	2.				55	250
Logan blackberry juice:														
Fresh or canned		E. P.		88.9	.6	.0	.40	10.1		6.5		1.89c	43	195
Loquats:														
Fresh	E. P., flesh	E. P.		86.5	.4	.2	.5	12.4	.5	8.5		1.4c	53	240
	Ref., skin and seeds	A. P.	38	53.6	.2	.1	.3	7.8	.3				33	150
Lungs:														
Fresh:														
Beef		E. P.		78.8	17.6	2.3	1.0	0.					92	420
Calf		E. P.		77.4	16.8	3.8	1.2	0.					105	470
Sheep		E. P.		76.7	19.3	2.3	1.2	0.					100	450
Macadamia nuts	E. P., kernels	E. P.		3.1	8.7	71.4	1.7	15.1	2.5	2.7			738	3,345
	Ref., shells	A. P.	69	1.0	2.7	22.1	.5	4.7	.8				229	1,035
Macaroni:														
Dry, uncooked		E. P.		11.	13.	1.4	.7	73.9	.4				360	1,635
Cooked, plain		E. P.		75.	3.7	.4	1.5	19.4	.1				96	435
Macaroons		E. P.		10.5	6.3	16.9	.9	65.4	1.1				439	1,990

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Mackerel, common Atlantic:														
Raw	E. P., flesh	E. P.	.....	68.1	18.7	12.	1.2	0.	.....	.....	.....	.....	183	830
	A. P., whole	A. P.	46	36.8	10.1	6.	.6	0.	.....	.....	.....	.....	99	450
	A. P., entrails removed	A. P.	43	38.8	10.7	7.	.7	0.	.....	.....	.....	.....	104	475
Mackerel, Pacific coast:														
Raw	E. P., flesh	E. P.	.....	69.4	22.2	7.6	1.4	0.	.....	.....	.....	.....	157	715
Mackerel, Spanish (see Spanish mackerel).														
Mackerel:														
Canned	E. P., contents of can	E. P.	.....	66.6	22.6	7.9	2.9	0	.....	.....	.....	.....	162	735
Salted	.....	E. P.	.....	43.0	18.5	25.1	13.0	0.	.....	.....	.....	.....	300	1,360
Smoked	.....	E. P.	.....	59.4	23.8	13.0	2.	0.	.....	.....	.....	.....	212	965
Malt breakfast food	Miscellaneous cereals	E. P.	.....	5.	12.3	1.4	2.1	79.2	1.5	.....	.....	.....	379	1,715
Mamey or mammee apple:														
Fresh	E. P., flesh	E. P.	.....	86.5	.5	.8	.3	11.9	2.6	7.8	.....	0.5c	57	260
	Ref., seeds and skin	A. P.	38	53.6	.3	.5	.2	7.4	1.6	.....	.....	.....	35	160
Mangos:														
Fresh	.....	E. P.	.....	81.4	.7	.2	.48	17.2	1.0	13.7	.....	.50c	73	335
	Ref., seeds and skin	A. P.	34	53.7	.5	.1	.3	11.4	.7	.....	.....	.....	48	220
Manioca starch or tapioca	.....	E. P.	.....	12.6	.6	.2	.2	86.4	.1	.....	85.4	.....	350	1,585
Maple (see Sirups and Sugars).														
Margarine	.....	E. P.	.....	15.5	.6	81.	2.5	.4	.....	.....	.....	.....	733	3,325
Marmalade (see Jams and preserves).														
Marmalade plum (see Sapote).														
Mayonnaise	.....	E. P.	.....	16.	1.5	78	1.5	3.0	.....	.....	.....	.5a	720	3,265
Meat and poultry:														
Cooked:														
Lean:														
Dry, or "overdone"	.....	E. P.	.....	59.	34.	6	1.4	0.	.....	.....	.....	.....	190	860
Medium-done	.....	E. P.	.....	63.	30.	6.	1.2	0.	.....	.....	.....	.....	174	790

Rare		E. P.		66.	27.	6.	1.1	0.					162	730
Medium fat:														
Dry, or "overdone"		E. P.		51.	39.	18.	1.2	0.					282	1,280
Medium-done		E. P.		54.	27.	18.	1.1	0.					270	1,220
Rare		E. P.		58.	23.	18.	.9	0.					254	1,150
Fat:														
Medium-done		E. P.		47.	22.	30.	.9	0.					358	1,620
Very fat:														
Medium-done		E. P.		37.	17.	45.	.7	0.					473	1,150
Milk:														
Cow:														
Fresh:														
Whole		E. P.		87.0	3.5	3.9	.7	4.9					69	310
Skim		E. P.		90.5	3.5	.2	.8	5.0					36	160
Canned:														
Evaporated (unsweetened)		E. P.		73.7	7.0	7.9	1.5	9.9					139	630
Condensed (sweetened)	Added sugar about 42 percent	E. P.		27.0	8.1	8.4	1.7	54.8					327	1,485
Dry:														
Skim		E. P.		3.5	35.6	1.0	7.9	52.0					359	1,630
Whole		E. P.		3.5	25.8	26.7	6.0	38.0					496	2,250
Malted, plain	Dry powder	E. P.		2.6	14.6	8.5	3.6	70.7	.3				418	1,895
Goat:														
Fresh		E. P.		87.0	3.3	4.2	.7	4.8					70	320
Human:														
Fresh		E. P.		87.5	1.4	3.7	.2	7.2					68	305
Reindeer:														
Fresh		E. P.		63.7	10.3	19.7	1.5	4.8					238	1,080
Sheep:														
Fresh		E. P.		82.6	5.5	6.5	.9	4.5					98	445
Molasses, cane:														
Light		E. P.		24.			3.0	(65.)		65.			260	1,180
Medium		E. P.		24.			4.5	(60.)		60.			240	1,090
Dark		E. P.		24.			5.0	(55.)		55.			220	1,000
Molasses cookies		E. P.		5.5	6.4	8.9	2.5	76.7	.4				412	1,870
Mulberries, black, white, and red:														
Fresh		E. P.		82.8	1.2	.6	.84	14.6	2.0	9.4		.95m	69	310
Mullet, common:														
Raw	E. P., flesh (with and without roe)	E. P.		75.1	19.3	4.4	1.2	0.					117	530
	A. P., whole	A. P.	47	39.8	10.2	2.3	.6	0.					62	280
Mung beans (see Beans).														

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
Mushrooms:			*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Fresh:														
All		E. P.		91.1	(0.)	0.3	1.14	(0.)	0.9					
		A. P.	9											
Truffles		E. P.		72.5	(0.)	.6	1.7	(0.)						
Dried		E. P.		12.	(0.)	3.0	11.3	(0.)						
Canned		E. P.		93.0	(0.)	.2	1.0	(0.)						
Muskellunge:														
Raw	E. P., flesh	E. P.		76.3	20.2	2.5	1.6	0.					103	470
	A. P., whole	A. P.	51	37.4	9.9	1.2	.8	0.					51	230
Muskmelons:														
Fresh:														
All	E. P., flesh	E. P.		92.7	.6	.2	.6	5.9	.5	5.4			28	125
Honeydew, casaba, Spanish		E. P.		90.6	.6	.2	.6	8.0	.5	7.0			36	165
	Ref., rind and cavity contents	A. P.	37	57.1	.4	.1	.4	5.0	.3				23	105
Others including cantaloup		E. P.		94.0	.6	.2	.6	4.6	.6	4.2			23	105
	Ref., rind and cavity contents	A. P.	53	44.2	.3	.1	.3	2.1	.3				11	50
Muskmelon juice:														
Fresh	Honeydew and California cantaloup	E. P.		87.2				(9.3)		9.1		0.18c		
Mussels:														
Fresh (Atlantic and Pacific):														
Solids only		E. P.		77.2	14.4	2.3	1.6	4.5					96	435
	Ref., shells, "beards," and liquor	A. P.	71	22.4	4.2	.7	.5	1.2					28	125
Solids and liquor (57 percent solids; 43 percent liquor)		E. P.		83.8	9.6	1.4	2.1	3.1					63	290
	Ref., shells and "beards"	A. P.	49	42.7	4.9	.7	1.1	1.6					32	145
Canned (Pacific):														
Drained solids		E. P.		74.6	18.2	3.3	2.4	1.5					108	490
Mustard greens:														
Fresh		E. P.		92.2	2.3	.3	1.21	4.0	.8	.4			28	125
	Ref., stalks and lower leaves	A. P.	27	67.3	1.7	.2	.9	2.9	.6				20	90

Mutton:														
Wholesale cuts (see Lamb).														
Cooked (see Meat and poultry, cooked).														
Natal plum (see Carissa).														
Nectarines:														
Fresh	E. P., flesh	E. P.		82.9	.5	.1	.5	16.0	.4	11.8		1.15m	67	305
	Ref., pits only	A. P.	8	76.3	.5	.1	.5	14.7	.4				62	280
	Ref., pits and skins	A. P.	13	72.1	.4	.1	.4	14.0	.3				58	265
Nettle:														
Fresh	E. P., leafy shoots	E. P.		82.4	5.5	.7	2.3	9.1	2.0				65	295
Noodles:														
Dry	Containing egg	E. P.		9.1	14.3	5.0	1.0	70.6	.3				385	1,745
Noodles, plain (see Macaroni).														
Oatmeal or rolled oats:														
Dry, uncooked		E. P.		8.3	14.2	7.4	1.9	68.2	1.2				396	1,795
Cooked		E. P.		84.8	2.3	1.2	.7	11.0	.2				64	290
Oca:														
Fresh	E. P., tubers	E. P.		80.9	1.4	.2	1.0	16.5		3.5	11.2	.26c	73	335
Oils, salad		E. P.				100.							900	4,080
Okra:														
Fresh		E. P.		89.8	1.8	.2	.84	7.4	1.0				39	175
	Ref., stem ends	A. P.	12	79.0	1.6	.2	.7	6.5	.9				34	155
Oleomargarine		E. P.		15.5	.6	81.	2.5	.4					733	3,325
Olives:														
Green, pickled	E. P., flesh	E. P.		75.2	1.5	13.5	5.8	4.0	1.2				144	650
	Ref., pits	A. P.	21	59.4	1.2	10.7	4.6	3.2	.9				113	515
Ripe, pickled	E. P., flesh	E. P.		73.4	1.6	19.0	3.0	3.0	1.9				189	860
	Ref., pits	A. P.	16	61.7	1.3	16.0	2.5	2.5	1.6				159	720
Ripe, pickled, Greek process	E. P., flesh	E. P.		43.4	2.1	32.3	7.3	14.9	3.8				359	1,625
	Ref., pits	A. P.	27	31.7	1.5	23.6	5.3	10.9	2.8				262	1,190
Onions:														
Fresh:														
All		E. P.		87.5	1.4	.2	.58	10.3	.8	6.7	.5		49	220
	Ref. (mature kinds), skins and rootlets	A. P.	6	82.2	1.3	.2	.5	9.8	.8				46	210
Top onions		E. P.		81.5	2.1	.2	.7	15.5	.7				72	325
Young, green	E. P., tender part	E. P.		87.6	1.0	.2	.6	10.6	1.8	3.7	.5		48	220
	Ref., tops, skins, and roots	A. P.	59	35.9	.4	.1	.3	4.3	.7				20	90
Onions, Welsh:														
Fresh	E. P., tender part	E. P.		89.2	2.2	.6	.8	7.2	.9				43	195
	Ref., tops and roots	A. P.	34	58.9	1.5	.4	.6	4.6	.6				28	125
Opossum	E. P., total edible	E. P.		46.	13.	40.	.7	0.					412	1,870
	A. P., live	A. P.	50	23.	6.5	20.	.4	0.					206	930

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion										
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Fuel value	
								Total	Fiber	Sugars	Starch		Per 100 grams	Per pound
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Orach, garden:														
Fresh	E. P., leaves and stems	E. P.	.....	88.0	4.5	0.4	2.4	4.7	1.0	0.1	0.2	.....	40	185
Orach, Peruvian, or quinoa:														
Fresh	E. P., leafy shoots	E. P.	.....	92.7	2.4	.2	1.	3.7	.....	.....	.....	.....	26	120
Oranges:														
Fresh		E. P.	.....	87.2	.9	.2	.47	11.2	.6	8.8	.....	0.68c	50	230
	Ref., rind and seeds	A. P.	28	62.8	.6	.1	.3	8.2	.4	.....	.....	.....	36	165
Canned	E. P., contents of can	E. P.	.....	83.	.8	.2	.5	15.5	.5	.....	.....	.....	67	305
Orange juice:														
Fresh:														
All	.....	E. P.	.....	.....	.....	.....	.....	(10.1)	.....	9.0	.....	1.16c	.....	.....
California-grown fruit	.....	E. P.	.....	85.7	.6	.0	.58	13.1	.....	9.1	.....	1.23c	55	250
Florida-grown fruit	.....	E. P.	.....	.....	.....	.....	.....	(9.4)	.....	8.4	.....	.99c	.....	.....
Canned	.....	E. P.	.....	86.	.6	.1	.4	12.9	.....	9.	.....	1.c	55	250
Orange, mandarin type (loose-skinned oranges, including King and Satsuma, and tangerines):														
Fresh		E. P.	.....	87.3	.8	.3	.66	10.9	1.0	8.7	.....	.93c	50	225
	Ref., rind and seeds	A. P.	29	62.0	.6	.2	.5	7.7	.7	.....	.....	.....	35	160
Orange, mandarin type, juice:														
Fresh	.....	E. P.	.....	89.2	.9	.3	.4	9.2	.....	7.8	.....	.83c	43	195
Oranges, Seville or sour:														
Fresh		E. P.	.....	87.0	.8	.2	.6	11.4	.....	6.0	.....	2.61c	51	230
	Ref., rind and seeds	A. P.	41	51.3	.5	.1	.4	6.7	.....	.....	.....	.....	30	135
Orange peel:														
Candied	.....	E. P.	.....	17.4	.4	.3	1.3	80.6	2.3	.....	.....	.2c	327	1,480
Oysters:														
Fresh:														
Solids only	.....	E. P.	.....	80.3	9.8	2.0	2.0	5.9	.....	.....	.....	.....	81	365
	Ref., shells and liquor	A. P.	90	8.0	1.0	.2	.2	.6	.....	.....	.....	.....	8	35

Solids and liquor (55 percent solids; 45 percent liquor)		E. P.		87.1	6.0	1.2	2.0	3.7					50	225
	Ref., shells	A. P.	82	15.7	1.1	.2	.4	.6					9	40
Canned.														
Drained solids		E. P.		80.3	9.8	2.0	2.0	5.9					81	365
Solids and liquid	E. P., contents of can	E. P.		87.1	6.0	1.2	2.0	3.7					50	225
Palmetto or Palmetto cabbage:														
Fresh	E. P., buds	E. P.		87.2	3.3	.6	1.7	7.2	.9	1.0	1.0		47	215
Pancake flour (see Flour, pancake; also Buckwheat flour).														
Pancreas:														
Fresh:														
Beef:														
Thin		E. P.		67.	14.9	17.	1.3	0.					213	960
Medium		E. P.		60.	13.5	25.	1.2	0.					279	1,270
Fat		E. P.		57.	12.8	29.	1.1	0.					312	1,420
Very fat		E. P.		53.	11.8	34.	1.1	0.					353	1,600
Calf		E. P.		69.7	19.2	8.8	1.4	0.					156	710
Hog ("Hog sweetbread")		E. P.		60.0	14.5	23.8	1.1	0.					272	1,230
Papaws, native:														
Fresh		E. P.		76.6	5.2	.9	.5	16.8					96	435
	Ref., rind and seeds	A. P.	25	57.4	3.9	.7	.4	12.6					72	325
Papayas:														
Fresh		E. P.		88.7	.6	.1	.62	10.0	.9	9.0		.14c	43	195
	Ref., rind and seeds	A. P.	32	60.3	.4	.1	.4	6.8	.6				30	135
Parsley:														
Fresh:														
Common	E. P., leaves	E. P.		83.9	3.7	1.0	2.4	9.0	1.8				60	270
Hamburg	E. P., roots	E. P.		87.6	2.1	.2	.9	9.2	1.1	2.8			47	215
Parsnips:														
Fresh	do	E. P.		78.6	1.5	.5	1.15	18.2	2.2	9.5	2.4		83	380
	Ref., scrapings	A. P.	22	61.3	1.2	.4	.9	14.2	1.7				65	295
Passion fruit (see Granadilla).														
Paté de foie gras or liver paste		E. P.		37.0	11.4	43.8	3.0	4.8					459	2,080
Peaches:														
Fresh:														
All		E. P.		86.9	.5	.1	.47	12.0	.6	8.8		.64m	51	230
	Ref., pits and skins	A. P.	12	76.5	.4	.1	.4	10.6	.5				45	205
	Ref., pits only	A. P.	8	79.9	.5	.1	.4	11.1	.6				47	210
Georgia grown		E. P.		85.8				(10.0)		9.4		.65m		
North Carolina grown		E. P.		86.4				(9.8)		9.2		.62m		

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
Peaches—Continued.			*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Fresh—Continued.														
Maryland grown		E. P.		87.1				(9.2)		8.6		0.62m		
New Jersey grown		E. P.		88.8				(8.2)		7.6		.58m		
Canned:														
Water pack	E. P., contents of can	E. P.		92.3	0.5	0.1	0.3	6.8	0.3	4.6		.2m	30	135
Juice pack	do	E. P.		89.6	.4	.2	.4	9.4	.2	7.3			41	185
In sirup	do	E. P.		80.9	.4	.1	.4	18.2	.4				75	340
Sieved, unsweetened		E. P.		87.	.5	.1	.4	12.	.5				51	230
Dried		E. P.		24.	3.0	.6	3.0	69.4	3.5	51.0		3.0m	295	1,340
Peach juice:														
Fresh		E. P.		86.5	.2	.0	.5	12.8		11.8		.56m	52	235
Peanuts:														
Raw:														
Spanish type	E. P., kernels with skins	E. P.		5.1	27.6	48.5	2.3	16.5	2.5	3.5	1.9		613	2,780
	Ref., shells	A. P.	25	3.8	20.7	36.4	1.7	12.4	1.9				460	2,085
Virginia type	E. P., kernels with skins	E. P.		4.0	26.2	42.8	2.7	24.3	2.6				587	2,665
	Ref., shells	A. P.	29	2.8	18.6	30.4	1.9	17.3	1.8				417	1,890
Roasted in shell:														
Virginia type	E. P., kernels without skins	E. P.		2.6	26.9	44.2	2.7	23.6	2.4				600	2,720
	Ref., shells and red skins	A. P.	28	1.9	19.4	31.8	1.9	17.0	1.7				432	1,960
Peanut butter		E. P.		1.7	26.1	47.8	3.4	21.0	2.0		4.7		619	2,805
Peanut cookies		E. P.		2.6	14.0	27.5	2.4	53.5	.8				518	2,345
Peanut flour		E. P.		2.6	51.2	5.0	4.7	36.5	4.4				396	1,795
Pears:														
Fresh:														
All		E. P.		82.7	.7	.4	.39	15.8	1.4	8.9		.29c	70	315
	Ref., skins and cores	A. P.	17	68.6	.6	.3	.3	13.2	1.2				58	265

Bartlett		E. P.		83.5	.4	.4	.3	15.4		8.3		.32c	67	305
Beurre Bosc		E. P.		81.0				(10.3)		10.1		.22c		
Candied		E. P.		21.0	1.3	.6	1.2	75.9					314	1,425
Canned:														
Water pack	E. P., contents of can	E. P.		91.2	.3	.1	.2	8.2	.7	4.1			35	160
Juice pack	do	E. P.		87.3	.2	.1	.3	12.1	.6	8.0			50	225
In sirup	do	E. P.		81.1	.2	.1	.2	18.4	.8				75	340
Dried		E. P.		24.	2.3	.4	1.7	71.6	6.1	36.0		1.5m	299	1,355
Peas:														
Fresh:														
Shelled:														
All	E. P., immature seeds	E. P.		74.3	6.7	.4	.92	17.7	2.2	3.2	8.2		101	460
	Ref., shells	A. P.	55	33.4	3.0	.2	.4	8.0	1.0				46	210
Young		E. P.		81.4	5.4	.3	.77	12.1	1.8	3.3	3.9		73	330
Medium		E. P.		75.8	6.5	.4	.93	16.4	2.2	3.8	5.2		95	430
	Ref., shells	A. P.	50	37.9	3.2	.2	.5	8.2	1.1				47	215
Old		E. P.		65.0	8.2	.4	1.05	25.4	2.5	2.3	12.3		138	625
Edible podded	E. P., entire green pods	E. P.		83.9	3.5	.3	1.0	11.3	1.4				62	280
Canned	E. P., contents of can	E. P.		85.4	3.3	.2	1.0	10.1	1.3	4.5			55	250
Canned, sieved		E. P.		85.7	4.0	.4	.6	9.3	.7	3.2	3.9		57	260
Dry:														
Whole	Mature seeds, entire	E. P.		11.6	23.8	1.4	3.0	60.2	5.4		45.1		349	1,580
Split	Mature seeds without seed coat	E. P.		10.0	24.5	1.0	2.8	61.7	1.2				354	1,605
Peas, black-eyed (see Cowpeas).														
Pecans	E. P., kernels	E. P.		3.0	9.4	73.0	1.6	13.0	2.2	3.9	.0		747	3,385
	Ref., shells	A. P.	48	1.6	4.9	38.0	.8	6.8	1.1				388	1,760
Peppers or redpeppers, sweet and pungent varieties:														
Fresh:														
All, immature and ripe	E. P., empty pods	E. P.		91.5	1.4	.4	.53	6.2	1.6	2.1	4.2		34	155
	Ref., stem ends, seeds, and cores	A. P.	18	75.0	1.1	.3	.4	5.2	1.3				28	125
Green, or immature	E. P., empty pods	E. P.		92.4	1.2	.2	.5	5.7	1.4	1.7	4.2		29	135
	Ref., stem ends, seeds, and cores	A. P.	16	77.6	1.0	.2	.4	4.8	1.2				25	115
Red, or ripe	E. P., empty pods	E. P.		89.2	1.3	.7	.7	8.1	1.6	3.0			44	200
	Ref., stem ends, seeds, and cores	A. P.	20	71.4	1.0	.6	.5	6.5	1.3				35	160

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Perch, white:														
Raw	E. P., flesh	E. P.		75.7	19.3	4.0	1.2	0.					113	515
	A. P., whole	A. P.	64	27.3	6.9	1.4	.4	0.					41	185
Perch, yellow:														
Raw	E. P., flesh	E. P.		79.8	18.7	.8	1.2	0.					82	370
	A. P., whole	A. P.	63	29.3	6.9	.3	.4	0.					30	135
	A. P., dressed	A. P.	39	48.4	11.4	.5	.7	0.					50	225
Persimmons:														
Fresh:														
Japanese, or kaki	E. P., pulp only	E. P.		78.2	.8	.4	.6	20.0	1.9	15.9		0.12m	87	395
	A. P., "seedless" kind	A. P.	3	75.9	.8	.4	.6	19.4	1.8				84	380
	A. P., kinds with seeds	A. P.	24	59.4	.6	.3	.5	15.2	1.4				66	300
Native	E. P., pulp	E. P.		64.4	.8	.4	.9	33.5	1.5	18.9		.19m	141	640
	Ref., seeds	A. P.	16	54.1	.7	.3	.8	28.1	1.3				118	535
Pheasant:														
Fresh:														
Total edible	E. P., flesh, skin, and giblets	E. P.		69.2	24.3	5.2	1.2	0.					144	650
	A. P., dressed	A. P.	34	45.7	16.0	3.4	.8	0.					95	430
	A. P., drawn	A. P.	13	60.2	21.1	4.5	1.0	0.					125	570
Pickerel, common eastern:														
Raw	E. P., flesh	E. P.		79.7	18.7	.5	1.2	0.					79	360
	A. P., whole	A. P.	49	40.6	9.5	.3	.6	0.					40	185
Pickles:														
Cucumber:														
Sweet		E. P.		77.1	.4	.1	1.7	20.7					85	385
Sour and dill	Dill pickles contain 0.25 percent lactic acid.	E. P.		95.2	.5	.2	2.2	1.9	.4				11	50
Mixed:														
Sweet		E. P.		72.	1.	.2	1.8	25.					106	480
Sour		E. P.		93.8	1.1	.4	.7	4.0					24	110
Pig's feet:														
Pickled	E. P., skin, muscle, tendon, and fat	E. P.		66.9	16.7	14.8	1.7	(0.)					200	910
	Ref., bone and gristle	A. P.	44	37.5	9.4	8.3	1.0	(0.)					112	510

Pigweeds (see Amaranth, Lambsquarters, and Quinoa).														
Pike, common:														
Raw	E. P., flesh	E. P.		79.8	18.7	.6	1.0	0.					80	365
	A. P., entrails removed	A. P.	44	44.7	10.5	.3	.6	0.					45	205
Pike, sauger:														
Raw	E. P., flesh	E. P.		80.8	17.9	.8	1.1	0.					79	355
	A. P., whole	A. P.	65	28.3	6.3	.3	.4	0.					28	125
Pike, wall-eyed:														
Raw	E. P., flesh	E. P.		79.7	18.6	.5	1.4	0.					79	360
	A. P., whole	A. P.	59	32.7	7.6	.2	.6	0.					32	145
Pimientos:														
Canned	E. P., contents of can	E. P.		91.8	1.0	.5	.5	6.2	.6	4.2	0.3		33	180
Pineapple:														
Fresh	E. P., flesh	E. P.		85.3	.4	.2	.42	13.7	.4	11.9		.72c	58	265
	Ref., crown, core, and parings	A. P.	47	45.2	.2	.1	.22	7.3	.2				31	140
Candied		E. P.		18.	.8	.4	.8	80.	.8	76.5			327	1,480
Canned:														
Water pack	E. P., contents of can	E. P.		86.4	.3	.1	.3	12.9	.3	10.1		.6c	54	245
Juice pack	do	E. P.		84.6	.4	.1	.4	14.5	.4	12.5		.9c	60	275
In sirup, sliced or crushed	do	E. P.		78.0	.4	.1	.4	21.1	.3	18.6		.8c	87	395
Pineapple juice:														
Fresh		E. P.		86.2				(12.8)		11.8		1.02c		
Canned		E. P.		86.2	.3	.1	.4	13.0		12.		1.c	54	245
Pine nuts:														
Pignolias	E. P., kernels	E. P.		4.9	31.2	48.4	4.3	11.2	1.0	4.3	.0		605	2,745
Piñon	do	E. P.		3.1	12.5	60.6	2.8	21.0					679	3,080
	Ref., shells	A. P.	42	1.8	7.2	35.1	1.6	12.2					394	1,790
Pistachio nut	E. P., kernels	E. P.		5.6	19.6	53.2	3.0	18.6	2.2	6.1	.0		632	2,865
Pitanga (see Surinam-cherry).														
Plantains or baking bananas:														
Fresh	E. P. flesh	E. P.		64.7	1.3	.4	.8	32.8	.4	25.3		.55m	140	635
	Ref., skins	A. P.	23	49.8	1.0	.3	.6	25.3	.3				108	490
Plums:														
Fresh (excluding prunes):														
All		E. P.		85.7	.7	.2	.51	12.9	.5	8.3		1.60m	56	255
	Ref., pits only	A. P.	5	81.4	.7	.2	.5	12.3	.5				53	240
	Ref., pits and skins	A. P.	15	72.8	.6	.2	.4	11.0	.4				48	220
European type, excluding damsons.		E. P.		86.1	.7	.1	.47	12.6		8.5		1.46m	54	245
	Ref., pits only	A. P.	5	81.8	.7	.1	.4	12.0					51	235
	Ref., pits and skins	A. P.	15	73.2	.6	.1	.4	10.7					46	210
Damsons		E. P.		78.8			.67	(13.5)	.4	8.7		2.02m		

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion										
								Carbohydrates					Fuel value	
			Refuse	Water	Protein	Fat	Ash	Total	Fiber	Sugars	Starch	Acid	Per 100 grams	Per pound
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Plums—Continued.														
Fresh (excluding prunes)—Con.														
Japanese type		E. P.		83.9	0.7	0.1	0.4	14.9		8.0		1.69m	63	285
Native American hybrids:														
Compass		E. P.		87.6	.5	.3	.42	11.2	0.4				50	225
Canned:														
Water pack:														
Other than prunes	E. P., contents of can except pits	E. P.		92.0	.4	.1	.3	7.2	.3	4.5		.9m	31	140
	Ref., pits	A. P.	3	89.2	.4	.1	.3	7.0	.3				30	140
Prunes	E. P., contents of can except pits	E. P.		88.9	.4	.1	.3	10.3	.2	6.4			44	200
	Ref., pits	A. P.	4	85.3	.4	.1	.3	9.9	.2				42	190
Juice pack:														
Prunes	E. P., contents of can except pits	E. P.		80.	.4	.1	.5	19.	.2				78	355
	Ref., pits	A. P.	4	77.	.4	.1	.5	18.	.2				75	340
In sirup:														
Plums including prunes	E. P., contents of can except pits	E. P.		78.6	.4	.1	.5	20.4	.3				84	380
	Ref., pits	A. P.	4	75.5	.4	.1	.5	19.6	.3				81	365
Sieved prunes		E. P.		69.7	1.1	.3	.8	28.1	.7	19.1		.6m	120	540
Poha (see Groundcherry).														
Pokeberry or poke:														
Fresh	E. P., shoots	E. P.		91.6	2.6	.4	1.7	3.7			0.2		29	130
Pollock:														
Raw	E. P., flesh	E. P.		76.0	21.6	.8	1.5	0.					94	425
	A. P., dressed	A. P.	30	53.2	15.1	.6	1.0	0.					66	295
Pomegranate:														
Fresh	E. P., pulp only	E. P.		81.0	.6	.2	.5	17.7	.3	13.3		1.05c	75	340
	Ref., skin and seeds	A. P.	44	45.4	.3	.1	.3	9.9	.2				42	190
	E. P., pulp and seeds	E. P.		75.8	1.5	1.2	.6	20.9	3.6	11.9		1.11c	100	455
	Ref., skin	A. P.	36	48.5	1.0	.8	.4	13.3	2.3				64	290
Pompano, common:														
Raw	E. P., flesh	E. P.		70.9	18.8	9.5	1.1	0.					161	730
	A. P., whole	A. P.	44	39.7	10.5	5.3	.6	0.					90	410
Popcorn (see Corn, popcorn).														

Porgy, Atlantic, or jolthead:															
Raw	E. P., flesh	E. P.		76.2	21.4	.9	1.5	0.					94	425
	A. P., whole	A. P.	50	38.1	10.7	.4	.8	0.					47	210
Pork, fresh:															
Raw:															
Carcass or side:															
Packers':															
Thin	E. P., 65 percent lean	E. P.		50.	14.1	35.	.8	0.					371	1,680
	A. P., 53 percent lean	A. P.	18	41.	11.6	29.	.6	0.					305	1,380
Medium	E. P., 55 percent lean	E. P.		42.	11.9	45.	.6	0.					453	2,050
	A. P., 48 percent lean	A. P.	12	37.	10.5	40.	.6	0.					398	1,810
Fat	E. P., 45 percent lean	E. P.		35.	9.8	55.	.5	0.					534	2,420
	A. P., 41 percent lean	A. P.	10	31.	8.8	50.	.5	0.					481	2,180
Shippers':															
Thin	E. P., 65 percent lean	E. P.		50.	14.1	35.	.8	0.					371	1,680
	A. P., 49 percent lean	A. P.	24	38.	10.7	27.	.6	0.					282	1,280
Medium	E. P., 55 percent lean	E. P.		42.	11.9	45.	.6	0.					453	2,050
	A. P., 45 percent lean	A. P.	19	34.	9.6	36.	.5	0.					367	1,660
Fat	E. P., 45 percent lean	E. P.		35.	9.8	55.	.5	0.					534	2,420
	A. P., 39 percent lean	A. P.	14	30.	8.4	47.	.4	0.					459	2,080
Wholesale cuts:															
Belly:															
Thin		E. P.		43.	11.4	45.	.6	0.					451	2,040
		A. P.	9	39.	10.4	41.	.5	0.					410	1,860
Medium		E. P.		34.	9.1	56.	.5	0.					540	2,450
		A. P.	7	32.	8.5	52.	.4	0.					503	2,280
Fat		E. P.		25.	6.6	68.	.4	0.					638	2,900
		A. P.	6	24.	6.2	64.	.3	0.					600	2,720
Boston:															
Thin		E. P.		63.	17.6	18.	1.0	0.					232	1,050
		A. P.	8	58.	16.2	17.	.9	0.					214	970
Medium		E. P.		60.	16.6	23.	.9	0.					273	1,240
		A. P.	5	57.	15.8	22.	.8	0.					260	1,180
Fat		E. P.		55.	15.3	29.	.8	0.					322	1,460
		A. P.	4	53.	14.7	28.	.8	0.					309	1,400
Clear plate:															
Thin		A. P.	12												
Medium		E. P.		16.	3.9	80.	.2	0.					736	3,340
		A. P.	9	14.	3.5	73.	.2	0.					669	3,040
Fat		A. P.	8												

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Acid	Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates						Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch				
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Pork, fresh—Continued.															
Raw—Continued.															
Wholesale cuts—Continued.															
Feet:															
Medium		E. P.		57.	20.2	22.	0.8	0.						279	1,260
		A. P.	65	20.	7.1	8.	.3	0						98	440
Ham:															
Thin		E. P.		60.	17.2	22.	.9	0.						267	1,210
		A. P.	18	49.	14.1	18.	.7	0.						219	990
Medium		E. P.		53.	15.2	31.	.8	0						340	1,540
		A. P.	14	46.	13.1	27.	.7	0						292	1,330
Fat		E. P.		46.	13.2	40.	.7	0.						413	1,870
		A. P.	11	41.	11.7	36.	.6	0.						367	1,670
Head, including jowl:															
Thin		E. P.		47.	13.2	39.	.7	0.						404	1,830
		A. P.	53	22.	6.2	18.	.3	0.						190	860
Medium		E. P.		37.	10.4	52.	6	0.						510	2,310
		A. P.	43	21.	5.9	30.	.3	0.						290	1,320
Fat		E. P.		28.	7.8	64.	.4	0.						607	2,750
		A. P.	36	18.	5.0	41.	.3	0.						389	1,760
Jowl:															
Thin		E. P.		39.	11.1	49.	.6	0.						485	2,200
		A. P.	10	35.	10.0	44.	.5	0						437	1,980
Medium		E. P.		32.	8.9	59.	.5	0.						567	2,570
		A. P.	8	29.	8.2	54.	.4	0.						521	2,360
Fat		E. P.		24.	6.7	69.	.4	0.						648	2,940
		A. P.	7	22.	6.2	64.	.3	0.						602	2,730
Leaf fat:															
Thin		E. P.		6.	2.3	92.	.1	0.						837	3,800
Medium		E. P.		5.	2.0	93.	.1	0						845	3,830

Loin:															
Thin		E. P.		63.	17.9	18.	1.0	0.					234	1,060
		A. P.	24	48.	13.6	14.	.7	0.					178	810
Medium		E. P.		58.	16.4	25.	.9	0.					291	1,320
		A. P.	19	47.	13.3	20.	.7	0.					235	1,070
Fat		E. P.		52.	14.8	32.	.8	0.					347	1,570
		A. P.	16	44.	12.4	27.	.7	0.					292	1,320
Neck bones:															
Thin		E. P.		60.	16.7	22.	.9	0.					265	1,200
		A. P.	71	18.	4.8	6.	.3	0.					77	350
Medium		E. P.		57.	15.8	26.	.9	0.					297	1,350
		A. P.	63	21.	5.8	10.	.3	0.					110	500
Fat		E. P.		54.	15.0	30.	.8	0.					330	1,500
		A. P.	55	24.	6.8	14.	.4	0.					148	670
Picnic:															
Thin		E. P.		59.	16.7	23.	.9	0.					274	1,240
		A. P.	21	47.	13.2	18.	.7	0.					216	980
Medium		E. P.		52.	14.8	32.	.8	0.					347	1,570
		A. P.	18	43.	12.1	26.	.6	0.					285	1,290
Fat		E. P.		46.	13.0	40.	.7	0.					412	1,870
		A. P.	16	39.	10.9	34.	.6	0.					346	1,570
Shoulder, New York style:															
Thin		E. P.		55.	15.3	29.	.8	0.					322	1,460
		A. P.	16	46.	12.9	24.	.7	0.					271	1,230
Medium		E. P.		49.	13.5	37.	.7	0.					387	1,760
		A. P.	12	43.	11.9	33.	.6	0.					341	1,540
Fat		E. P.		42.	11.6	46.	.6	0.					460	2,090
		A. P.	10	38.	10.4	41.	.6	0.					414	1,880
Shoulder butt:															
Thin		E. P.		52.	14.4	33.	.8	0.					355	1,610
		A. P.	10	47.	13.0	30.	.7	0.					319	1,450
Medium		E. P.		45.	12.5	42.	.7	0.					428	1,940
		A. P.	7	42.	11.6	39.	.6	0.					398	1,810
Fat		E. P.		37.	10.3	52.	.6	0.					509	2,310
		A. P.	5	35.	9.8	49.	.5	0.					484	2,190
Spareribs:															
Thin		E. P.		57.	15.8	26.	.9	0.					297	1,350
		A. P.	48	30.	8.2	14.	.5	0.					155	700
Medium		E. P.		53.	14.6	32.	.8	0.					346	1,570
		A. P.	40	32.	8.8	19.	.5	0.					208	940
Fat		E. P.		47.	13.1	39.	.7	0.					403	1,830
		A. P.	34	31.	8.6	26.	.5	0.					266	1,210

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
Pork, fresh—Continued.														
Raw—Continued.														
Wholesale cuts—Continued.														
Tail:			*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Calo-ries*	*Calo-ries*
Medium		E. P.		26.	7.7	66.	0.4	0.					625	2,830
		A. P.	17	21.	6.4	55.	.3	0.					519	2,350
Tenderloin, muscle:														
Thin		E. P.		74.	20.3	5.	1.1	0.					126	570
Medium		E. P.		72.	19.9	7.	1.1	0.					143	650
Fat		E. P.		70.	19.3	10.	1.1	0.					167	760
Cooked (see Meat and poultry, cooked).														
Pork, cured:														
Raw:														
Ham, smoked:														
Very lean (also Canadian bacon).		E. P.		56.	22.1	15.	6.2	(.3)		0.3			225	1,020
Lean		E. P.		49.	19.5	25.	5.8	(.3)		.3			304	1,380
		A. P.	14	42.	16.8	22.	5.0	(.3)					262	1,190
Medium		E. P.		42.	16.9	35.	5.4	(.3)		.3			384	1,740
		A. P.	13	37.	14.7	30.	4.7	(.3)					334	1,510
Fat		E. P.		36.	14.6	44.	5.1	(.3)		.3			456	2,070
		A. P.	11	32.	13.0	39.	4.5	(.3)					405	1,840
Shoulder, smoked:														
Lean		E. P.		42.	16.9	35.	5.4	(.3)		.3			384	1,740
		A. P.	14	36.	14.5	30.	4.6	(.3)					330	1,500
Medium		E. P.		36.	14.6	44.	5.1	(.3)		.3			456	2,070
		A. P.	12	32.	12.8	39.	4.5	(.3)					401	1,820
Fat		E. P.		30.	12.2	53.	4.7	(.3)		.3			527	2,390
		A. P.	10	27	11.0	48.	4.2	(.3)					474	2,150

Salt pork:														
Medium		E. P.		14.	6.2	76.	3.8	0.					709	3,220
		A. P.	7	13.	5.8	71.	3.5	0.					659	2,990
Fat, with little or no lean		E. P.		8.	3.9	85.	3.5	0.					781	3,540
		A. P.	4	7.	3.7	82.	3.4	0.					749	3,400
Cooked (see Meat and poultry, cooked).														
Deviled ham:														
Canned		E. P.		31.	19.	43.	7.	0.					463	2,100
Pork organs (see Liver, etc.).														
Potatoes:														
Fresh	E. P., tubers, whole or peeled	E. P.		77.8	2.0	.1	.99	19.1	0.4	.9	14.7		85	385
	Ref., parings	A. P.	16	65.4	1.7	.1	.8	16.0	.3				72	325
Potato chips		E. P.		3.1	6.7	37.1	4.0	49.1			37.1		557	2,525
Potato flour		E. P.		7.	8.5	.5	4.0	80.0	1.7				358	1,625
Preserves (see Jams and preserves).														
Pretzels		E. P.		8.0	8.8	3.2	5.5	74.5	.3				362	1,640
Prickly pear:														
Fresh	E. P., pulp	E. P.		88.6	.5	.1	.42	10.4		8.8		0.25m	44	200
	Ref., seeds and rind	A. P.	56	39.0	.2	.0	.18	4.6					20	90
Prunes:														
Fresh	E. P., flesh and skin	E. P.		76.5	.9	.2	.6	21.8	.5	13.3		.98m	93	420
	Ref., pits only	A. P.	6	71.9	.8	.2	.6	20.5	.5				87	395
Canned (see Plums, canned).														
Dried	E. P., flesh and skin	E. P.		24.	2.3	.6	2.1	71.0	1.6	41.5		1.7m	299	1,355
	Ref., pits (medium sized prunes)	A. P.	15	20.	2.0	.5	1.8	60.4	1.4				254	1,150
	Ref., pits (large prunes)	A. P.	12	21.	2.0	.5	1.8	62.5	1.4				263	1,190
	Ref., pits (small prunes)	A. P.	18	20.	1.9	.5	1.7	58.2	1.3				245	1,110
Prune juice:														
Canned		E. P.		80.	.4	0.	.3	19.3		13.		.2c	79	355
Pumpernickel		E. P.		40.5	6.7	1.2	1.9	49.7	1.3				236	1,070
Pumpkin:														
Fresh:														
Mature	E. P., flesh only	E. P.		90.5	1.2	.2	.82	7.3	1.3	2.5	2.6		36	160
	Ref., rind and contents of cavity	A. P.	31	62.4	.8	.1	6	5.1	.9				24	110
Immature (see Squash, fresh, summer).														
Canned		E. P.		90.2	1.0	.3	.6	7.9	1.2	3.9	.3		38	175
Purslane:														
Fresh	E. P., leaves and stems	E. P.		93.2	1.6	.4	1.48	3.3	.8				23	105
Quail:														
Fresh:														
Total edible	E. P., flesh, skin, and giblets	E. P.		65.9	25.0	6.8	1.6	0.					161	730
	A. P., live	A. P.	33	44.2	16.8	4.6	1.1						108	490
Quinces:														
Fresh	E. P., flesh	E. P.		85.3	.3	.1	.38	13.9	1.8	6.3		.87m	58	260
Quince juice:														
Fresh		E. P.			.3		.36	(10.3)		9.1		1.2m		

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Quinoa:														
Fresh	E. P., leafy shoots	E. P.		92.7	2.4	0.2	1.	3.7					26	120
Rabbit, domesticated:														
Fresh	Drawn weight (including giblets) is 73 percent of live weight; dressed weight (including giblets) 56 percent; giblets, 5 percent; head and skin, 17 percent.													
Total edible (rabbit purchased with giblets).	E. P., flesh, fat, and giblets	E. P.		67.9	20.8	10.2	1.1	0.					175	795
	A. P., live	A. P.	54	31.2	9.6	4.7	.5	0.					80	365
	A. P., drawn	A. P.	37	42.8	13.1	6.4	.7	0.					110	500
	A. P., dressed (i. e., drawn, skinned, head and feet off).	A. P.	18	55.7	17.1	8.4	.9	0.					144	650
Flesh only (rabbit purchased without giblets).		E. P.		67.9	20.8	10.2	1.1	0.					175	795
	A. P., drawn	A. P.	40	40.7	12.5	6.1	.7	0.					105	475
	A. P., dressed (i. e., drawn, skinned, head and feet off).	A. P.	20	54.3	16.6	8.2	.9	0.					140	635
Cooked (see Meat and poultry, cooked).														
Rabbit, wild:														
Fresh	Drawn weight (including giblets) is 73 percent of live weight; dressed weight (including giblets), 56 percent; giblets, 5 percent; head and skin, 17 percent.													
Total edible (rabbit purchased with giblets)	E. P., flesh, fat, and giblets	E. P.		73.	21.	5.	1.	0.					129	585
	A. P., live	A. P.	54	34.	10.	2.	.5	0.					59	270
	A. P., drawn	A. P.	37	46.	13.	3.	.6	0.					81	370
	A. P., dressed (i. e., drawn, skinned, head and feet off).	A. P.	18	60.	17.	4.	.8	0.					106	480
Flesh only (rabbit purchased without giblets)		E. P.		73.	21.	5.	1.	0.					129	585
	A. P., drawn	A. P.	40	44.	13.	3.	.6	0.					77	350
	A. P., dressed (i. e. drawn, skinned, head and feet off).	A. P.	20	58.	17.	4.	.8	0.					103	470
Cooked (see Meat and poultry, cooked).														

Radishes:														
Fresh		E. P.		93.6	1.2	.1	.95	4.2	.7	3.4			22	100
	Ref., tops and rootlets	A. P.	51	45.9	.6	.0	.5	2.0	.3				11	50
Raisins	Seeded and seedless, including dried "currants"	E. P.		24.	2.3	.5	2.0	71.2		63.0		1.8m	298	1,355
Raspberries:														
Fresh:														
Black		E. P.		80.7	1.5	1.6	.65	15.6	3.5	7.9		1.16c	83	375
Red		E. P.		83.4	1.1	.6	.47	14.4	2.8	7.2		1.34c	67	305
Canned:														
Red or black:														
Water pack	E. P., contents of can	E. P.		87.7	.9	.9	.8	10.0	2.5	4.8			52	235
Juice pack	....do	E. P.		85.2	.8	.6	.4	13.0	2.4	7.3			61	275
In sirup	....do	E. P.		70.6	.6	.5	.5	27.8	1.9				118	535
Raspberry juice:														
Fresh:														
Black		E. P.		88.4	.2	.0	.7	10.7		7.6		1.04c	44	200
Red		E. P.		90.8	.4	.0	.46	8.3		7.3		1.40c	35	160
Ray or skate:														
Raw		E. P.		77.8	21.5	.7	1.2	0.					92	420
Red grouper:														
Raw	E. P., flesh	E. P.		79.6	19.4	.4	1.2	0.					81	370
	A. P., whole	A. P.	58	33.4	8.1	.2	.5	0.					34	155
	A. P., entrails removed	A. P.	56	35.0	8.5	.2	.5	0.					36	160
Red snapper:														
Raw	E. P., flesh	E. P.		78.4	19.8	.9	1.3	0.					87	395
	A. P., whole or dressed	A. P.	48	40.8	10.3	.5	.7	0.					45	205
Reindeer:														
Fresh:														
Lean meat only		E. P.		73.	20.	6.	1.	0.					134	610
Side		E. P.		63.3	20.5	14.4	1.0	0.					212	960
		A. P.	20	50.6	16.4	11.5	.8	0.					169	770
Forequarter		E. P.		67.4	21.8	9.4	1.1	0.					172	780
		A. P.	26	49.9	16.1	7.0	.8	0.					127	580
Hindquarter		E. P.		59.6	19.4	19.2	.9	0.					250	1,140
		A. P.	14	51.3	16.7	16.5	.8	0.					215	980
Rhubarb:														
Fresh	E. P., stems only	E. P.		94.9	.5	.1	.72	3.8	.7	.4			18	80
	Ref., leaves	A. P.	32	64.5	.3	.1	.5	2.6	.5				12	55
Canned:														
Water pack	E. P., contents of can	E. P.		95.6	.4	.4	.6	3.0	.8		1.9		17	80

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Rice:														
Brown:														
Uncooked		E. P.		12.0	7.5	1.7	1.1	77.7	0.6				356	1,615
White:														
Uncooked		E. P.		12.3	7.6	.3	.4	79.4	.2				351	1,590
Boiled		E. P.		74.4	2.2	.1	.1	23.2	.1				102	465
Rice, puffed		E. P.		9.3	6.7	.3	.4	83.3	.3				363	1,645
Rice flakes		E. P.		8.3	7.7	.5	1.5	82.0	.7				363	1,650
Rice flour		E. P.		12.1	7.4	.5	.5	79.5	.4				352	1,595
Rice polishings		E. P.		9.3	11.6	10.1	5.0	64.0	2.2				393	1,785
Rice bran		E. P.		10.7	12.5	14.4	9.9	52.5	10.7				390	1,765
Rice, wild (see Wild rice).														
Rock cod, Pacific:														
Raw	E. P., flesh	E. P.		79.7	18.3	1.0	1.2	0.					82	375
Roe, fish:														
Fresh:														
Cod		E. P.		70.6	24.3	1.8	2.0	(0.)					113	515
Other than cod, including carp, shad, herring, salmon.		E. P.		66.8	26.2	3.1	1.6	(0.)					133	600
Rose apple:														
Fresh	E. P., pulp	E. P.		86.4	.5	.3	.3	12.5	.8	10.6		0.4c	55	250
	Ref., seeds and skin	A. P.	33	57.9	.3	.2	.2	8.4	.5				37	165
Rusks (toasted)		E. P.		6.8	13.1	7.4	1.5	71.2	1.0				404	1,830
Rutabagas:														
Fresh	E. P., roots	E. P.		89.1	1.1	.1	.83	8.9	1.3	6.7			41	185
	Ref., parings	A. P.	15	75.7	.9	.1	.7	7.6	1.1				35	160
Rutabaga tops (see Turnip tops).														
Rye bread (see Breads).														

Rye flour:														
Dark		E. P.		11.	14.9	2.1	2.0	70.0	2.1				358	1,625
Medium		E. P.		11.	11.0	1.2	1.0	75.8	1.5				358	1,625
Light		E. P.		11.	8.9	.9	.7	78.5	1.1				358	1,625
Rye meal or whole grain		E. P.		10.	11.2	1.7	1.9	75.2	2.0				361	1,635
Rye wafers or "Swedish health bread."		E. P.		6.8	12.9	1.6	3.3	75.4	1.8				368	1,665
Sablefish or black cod:														
Raw	E. P., flesh	E. P.		75.8	15.0	7.5	1.3	0.					128	580
Sago meal		E. P.		14.6	.9	.2	.4	83.9	.2				341	1,545
Salad dressings:														
Mayonnaise		E. P.		16.	1.5	78.	1.5	3.0				.5a	720	3,265
Mineral oil (mayonnaise type)	Containing 75 percent mineral oil	E. P.		16.	1.5	(2.7)	1.5	(3.0)					42	190
Other than mayonnaise:														
Commercial:														
Plain		E. P.		44.7	1.1	36.8	3.46	13.9					391	1,775
French and Thousand Island.		E. P.		38.3	.8	39.0	4.6	17.3	.4				423	1,920
Home-cooked	Boiled	E. P.		68.	4.5	10.	2.5	15.				.5a	168	760
Salad-rocket:														
Fresh	E. P., leaves and stems	E. P.		92.2	1.1	.2	.9	5.6	.5		0.3		29	130
	Ref., tough stems	A. P.	8	84.8	1.0	.2	.8	5.2	.5				27	120
Salmon, Atlantic:														
Raw	E. P., flesh	E. P.		63.6	22.5	13.4	1.4	0.					211	955
	A. P., whole	A. P.	35	41.3	14.6	8.7	.9	0.					137	620
	A. P., entrails removed	A. P.	24	48.3	17.1	10.2	1.1	0.					160	725
Salmon, Pacific:														
Raw:														
Chinook or king	A. P., anterior section:													
	E. P., flesh	E. P.		63.4	17.4	16.5	1.0	0.					218	990
	Ref., bones and skin	A. P.	11	56.4	15.5	14.7	.9	0.					194	880
Canned:														
All kinds		E. P.		67.4	20.6	9.6	2.4	0.					169	765
	Ref., bones	A. P.	2	66.1	20.2	9.4	2.4	0.					165	750
Chinook or king		E. P.		64.7	19.7	13.2	2.4	0.					198	895
Chum		E. P.		70.8	21.5	5.2	2.6	0.					133	600
	Ref., bones	A. P.	2	69.4	21.1	5.1	2.5	0.					130	590
Coho or silver		E. P.		67.6	21.1	8.4	1.7	0.					160	725
	Ref. bones	A. P.	2	66.2	20.7	8.2	1.7	0.					157	710
Pink or humpback		E. P.		70.0	20.5	6.2	2.6	0.					138	625
	Ref., bones	A. P.	2	68.6	20.1	6.1	2.5	0.					135	610

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
Salmon, Pacific—Continued.			*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Canned—Continued.														
Sockeye or red		E. P.		67.2	20.2	9.6	3.0	0.					167	760
"Steelhead salmon" (see Steelhead trout).														
Smoked		E. P.		58.9	21.6	9.3	9.4	0.					170	770
Salsify (see Vegetable-oyster).														
Sand dab, California:														
Raw	E. P., flesh	E. P.		82.1	16.8	.2	.9	0.					69	315
Sapodilla or Sapota:														
Fresh	E. P., pulp	E. P.		76.7	.5	1.0	.5	21.3	3.5	12.2		0.2c	96	435
	Ref., skin and seeds	A. P.	20	61.4	.4	.8	.4	17.0	2.8				77	350
Sapote or marmalade plum:														
Fresh	E. P., pulp	E. P.		66.0	1.4	.7	1.1	30.8	1.9	19.8		.2c	135	615
	Ref., skin and seed	A. P.	24	50.2	1.1	.5	.8	23.4	1.4				103	465
Sardines, California:														
Raw	E. P., flesh	E. P.		70.8	19.3	8.6		0.					155	700
Sardines:														
Canned:														
In oil	E. P., drained solids	E. P.		57.4	25.7	11.0	4.7	1.2					207	935
	Ref., oil	A. P.	18	47.1	21.1	9.0	3.9	1.0					169	770
	E. P., contents of can	E. P.		49.7	20.5	25.4	3.9	.5					313	1,420
In mustard or souse sauce	do	E. P.		62.5	20.0	11.8	3.5	2.2					195	885
In tomato sauce	do	E. P.		65.3	20.7	8.7	3.9	1.4					167	755
Sauerkraut:														
Bulk		E. P.		91.2	1.3	.2	2.4	4.9	1.4	.3		1.6L	27	120
Canned	E P., contents of can	E P.		93.2	1.1	.2	2.1	3.4	.7	.3		1.3L	20	90
Sausage:														
Beef and pork	Link	E. P.		44.8	11.3	41.2	2.5	0.					416	1,890
Blood sausage and blood pudding.		E. P.		47.1	14.8	34.6	2.3	0.					371	1,680
Bockwurst		E. P.		63.5	11.7	21.8	2.4	0.					243	1,100

Bologna:														
All meat		E. P.		64.0	14.4	17.8	3.0	0.					218	990
With added cereal		E. P.		62.4	14.8	15.9	3.3	3.6			1.8		217	980
Braunschweiger		E. P.		56.2	15.4	23.8	2.7	0.					276	1,250
Capacola or Capicola		E. P.		26.2	20.2	45.8	7.9	0.					493	2,240
		A. P.	9	23.8	18.4	41.7	7.2	0.					449	2,030
Cervelat:														
Dried		E. P.		34.6	23.3	35.0	6.7	0.					408	1,850
		A. P.	3	33.6	22.6	34.0	6.5	0.					396	1,800
Semidried (see Sausage, summer sausage, semidried).														
Country-style		E. P.		51.7	16.2	27.4	3.9	0.					311	1,410
Frankfurt:														
All meat		E. P.		61.1	14.1	20.8	2.8	0.					244	1,100
With added cereal		E. P.		64.3	15.2	14.1	3.1	3.3			1.2		201	910
Headcheese		E. P.		62.0	15.0	20.3	2.3	0.					243	1,100
Liver sausage and liver pudding.		E. P.		59.0	16.7	20.6	2.2	1.5					258	1,170
Luncheon roll		E. P.		56.4	15.9	23.8	3.4	0.					278	1,260
Mortadella (see Sausage, summer sausage, semidried).														
Polish-style sausage		E. P.		56.0	16.4	23.1	3.6	0.					274	1,240
Pork sausage, pure	Link or bulk	E. P.		41.9	10.8	44.8	2.1	0.					446	2,020
Salami		E. P.		31.1	23.9	36.8	7.0	0.					427	1,940
		A. P.	8	28.6	22.0	33.9	6.4	0.					393	1,780
Souse		E. P.		72.9	13.2	12.3	1.9	0.					164	740
Summer sausage:														
Dried		E. P.		29.7	24.5	37.3	7.1	0.					434	1,970
		A. P.	7	27.6	22.8	34.7	6.6	0.					403	1,830
Semidried		E. P.		51.6	18.9	23.8	4.7	0.					290	1,310
All types		E. P.		33.6	23.5	34.9	6.8	0.					408	1,850
		A. P.	7	31.2	21.9	32.5	6.3	0.					380	1,720
Vienna (see Sausage, Frankfurt).														
Wienerwurst (see Sausage, Frankfurt).														
Scallops:														
Fresh	E. P., edible muscle	E. P.		80.3	14.8	.1	1.4	3.4					74	335
Scup or porgy:														
Raw	E. P., flesh	E. P.		75.4	18.6	4.5	1.4	0.					115	520
	A. P., whole	A. P.	62	28.7	7.1	1.7	.5	0.					44	200

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Seakale:														
Fresh	E. P., shoots	E. P.	-------	93.4	1.5	0.2	0.6	4.3	0.8	-------	-------	-------	25	115
	Ref., root and waste leaves	A. P.	23	71.9	1.2	.2	.5	3.2	.6	-------	-------	-------	19	85
Seaweed (see Algae).														
Sesame seed:														
Whole seed	Thin-shelled type	E. P.	-------	5.8	19.3	51.1	5.7	18.1	3.2	-------	-------	-------	610	2,765
Decorticated	Thick- and thin-shelled types	E. P.	-------	5.6	17.9	53.2	6.2	17.1	2.7	-------	-------	-------	619	2,805
Shad or American shad:														
Raw	E. P., flesh	E. P.	-------	70.2	18.7	9.8	1.4	0.	-------	-------	-------	-------	163	740
	A. P., whole	A. P.	52	33.7	9.0	4.7	.7	0.	-------	-------	-------	-------	78	355
Shad roe:														
Fresh		E. P.	-------	71.2	20.9	3.8	1.5	(0.)	-------	-------	-------	-------	118	535
Shallot:														
Fresh	E. P., bulbs	E. P.	-------	80.9	1.2	.2	.4	17.3	-------	-------	-------	-------	76	345
Sheepshead, Atlantic:														
Raw	E. P., flesh	E. P.	-------	75.9	20.6	2.8	1.3	0.	-------	-------	-------	-------	108	490
	A. P., whole	A. P.	64	27.3	7.4	1.0	.5	0.	-------	-------	-------	-------	39	175
	A. P., entrails removed	A. P.	58	31.9	8.7	1.2	.5	0.	-------	-------	-------	-------	45	205
Sherbet		E. P.	-------	69.4	2.	3.	.6	25.	-------	-------	-------	-------	135	610
Shortbread		E. P.	-------	4.2	5.8	23.0	1.4	65.6	.1	-------	-------	-------	493	2,235
Shrimp:														
Canned	Dry pack or drained solids of wet pack	E. P.	-------	78.3	17.8	.8	2.3	.8	-------	-------	-------	-------	82	370
Cooked (see Canned).														
Shrimp or lobster paste		E. P.	-------	61.3	20.8	9.4	7.0	1.5	-------	-------	-------	-------	174	790
Sirups:														
Cane	Concentrated cane juice	E. P.	-------	27.	-------	-------	1.5	(67.)	-------	67.	-------	-------	268	1,215
Corn:														
Table-mixtures	Light and dark mixtures, chiefly corn sirup	E. P.	-------	25.	-------	-------	.6	(74.)	-------	-------	-------	-------	296	1,345
Commerical	For manufacturing purposes	E. P.	-------	19.1	-------	-------	.3	80.6	-------	-------	-------	-------	322	1,460
Maple		E. P.	-------	34.	-------	-------	.7	(64.)	-------	64.	-------	-------	256	1,160
Sorghum		E. P.	-------	23.	-------	-------	2.5	(67.)	-------	67.	-------	-------	263	1,215

Skate or ray:															
Raw		E. P.		77.8	21.5	.7	1.2	0.					92	420
Sloke (see Algae).															
Smelt, Atlantic:															
Raw	E. P., flesh	E. P.		79.2	17.6	1.8	1.7	0.					87	395
	A. P., whole	A. P.	45	43.6	9.7	1.0	.9	0.					48	215
Smelt, jack:															
Raw	E. P., flesh	E. P.		76.7	20.6	1.4	1.2	0.					95	430
Smelt, Pacific bay:															
Raw	do	E. P.		77.4	19.8	.7	1.2	0.					86	390
Sole, California:															
Raw	do	E. P.		80.4	17.4	.7	1.7	0.					76	345
Sorghum sirup		E. P.		23.			2.5	(67.)		67.			268	1,215
Sorrel (see Dock).															
Soups (ready to serve):															
Bean		E. P.		84.1	4.3	2.7	1.4	7.5	.4				72	325
Bouillon, broth, and consommé		E. P.		95.	(1.)	0.	1.5	(0.)	.0				4	20
Clam chowder		E. P.		87.9	2.1	1.9	1.6	6.5	.1				52	235
Creamed, vegetable		E. P.		85.8	2.3	3.8	1.6	6.5	.1				69	315
Noodle, rice, or barley		E. P.		93.0	1.7	1.2	1.3	2.8	.0				29	130
Vegetable, with beef or chicken stock		E. P.		91.1	2.1	1.0	1.2	4.6	.1				36	160
Vegetable, strained	Includes some cereal	E. P.		88.4	2.0	.1	.9	8.6	.5				43	195
Soybeans:															
Fresh:															
Shelled	E. P., immature seeds	E. P.		67.	12.5	6.5	1.5	(6.)	1.5	3.0	1.5		132	600
	Ref., shells	A. P.	47	36.	6.6	3.4	.8	(3.)	.8				70	320
Dry:															
Seeds of edible varieties	E. P., whole, mature seeds	E. P.		7.5	34.9	18.1	4.7	(12.)	5.0	8.4	2.1		350	1,590
Soybean sprouts	E. P., fresh sprouted seeds	E. P.		82.3	8.5	1.8	1.07	6.3	.9				75	340
Soybean curd or cheese		E. P.		84.1	7.5	4.2	.8	3.4	.1				81	370
Soybean flour:															
High fat content		E. P.		6.9	37.3	20.2	4.7	(12.)	2.5	9.3			379	1,720
From press cake		E. P.		7.5	43.4	7.8	5.8	(12.)	2.6	11.4			292	1,325
Soybean meal:															
Ground whole beans		E. P.		8.9	35.7	18.0	4.6	(12.)	5.4	8.9			353	1,600
From press cake		E. P.		6.2	41.8	5.7	5.8	(15.)	5.3	11.0	.5		278	1,265
Soybean milk		E. P.		92.6	3.4	1.5	.5	2.0	.0	.7			35	160
Soy sauce	Domestic or oriental	E. P.		65.	(2.)	1.	20.	(9.)		8.		1. L	53	240

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Spaghetti (see Macaroni).														
Spanish mackerel:														
Raw	E. P., flesh	E. P.		66.1	19.8	13.3	1.3	0.					199	900
	A. P., whole	A. P.	39	40.3	12.1	8.1	.8	0.					121	550
Spinach:														
Fresh	E. P., leaves	E. P.		92.7	2.3	.3	1.53	3.2	0.6	0.3			25	110
	Ref., main stalk and outer leaves	A. P.	18	76.0	1.9	.2	1.3	2.6	.5				20	90
Canned	E. P., contents of can	E. P.		91.8	2.3	.5	1.9	3.5	.7	.8	1.7		28	125
Canned, sieved		E. P.		93.7	2.0	.3	1.4	2.6	.6	.5	.4		21	95
Spinach, New Zealand:														
Fresh	E. P., leaves and stems	E. P.		91.4	2.2	.2	2.11	4.1	.8	.6	.3		27	120
Spleen:														
Fresh:														
Beef and veal		E. P.		76.9	18.1	3.0	1.4	0.					99	450
Hog		E. P.		77.4	17.1	3.8	1.4	0.					103	470
Sheep		E. P.		74.4	18.8	3.9	1.6	0.					110	500
Squab (pigeons):														
Fresh:														
Total edible	E. P., flesh, skin, and giblets	E. P.		58.0	18.6	22.1	1.5	0.					273	1,240
	A. P., dressed	A. P.	40	34.8	11.2	13.3	.9	0.					164	740
Flesh	E. P., breast muscle without skin	E. P.		74.0	20.4	4.2	1.2	0.					119	540
Squash:														
Fresh:														
Cushaw (including Canada crookneck)	E. P., flesh	E. P.		90.4	1.2	.3	.76	7.3	1.2	4.5	.6		37	165
	Ref., rind and contents of cavity	A. P.	21	71.4	.9	.2	.6	5.9	.9				29	130
Summer	E. P., tender part	E. P.		95.0	.6	.1	.44	3.9	.5	1.0	.2		19	85
	Ref., stem end	A. P.	3	92.2	.6	.1	.4	3.7	.5				18	80
	Ref., stem end, skin, and seed part	A. P.	35	61.8	.4	.1	.3	2.5	.3				12	55
Winter	E. P., flesh only	E. P.		88.6	1.5	.3	.83	8.8	1.4	3.9	1.0		44	200
	Ref., rind and contents of cavity	A. P.	25	65.6	1.1	.2	.6	6.5	1.0				32	145
Canned		E. P.		90.2	1.0	.3	.6	7.9	1.2	3.9	.3		38	175

Squeateague, gray, *or* weakfish:														
Raw	E. P., flesh	E. P.		79.4	17.8	1.7	1.2	0.					86	390
	A. P., whole	A. P.	52	38.1	8.5	.8	.6	0.					42	190
Starch (including corn, arrowroot, etc.), pure		E. P.		12.	.5	.2	.3	87.	.1				352	1,595
Steelhead trout:														
Canned		E. P.		63.2	20.6	13.4	2.4	0.					203	920
	Ref., bones	A. P.	2	61.9	20.2	13.1	2.4	0.					199	900
Stomach:														
Beef (see Tripe, beef).														
Hog:														
Cooked		E. P.		74.0	16.5	9.0	.6	0.					147	670
Strawberries:														
Fresh		E. P.		90.0	.8	.6	.50	8.1	1.2	5.3		1.09c	41	185
	Ref., stems and caps	A. P.	4	86.4	.8	.6	.5	7.7	1.2				39	175
Canned:														
Water pack	E. P., contents of can	E. P.		92.8	.6	.4	.4	5.8	.9	3.6			29	130
Juice pack	do	E. P.		90.	.8	.8	.4	8.	.8	7.			42	190
In sirup	do	E. P.		70.8	.5	.2	.5	28.0	.7				116	525
Strawberry juice:														
Fresh		E. P.		94.2	.2	.0	.45	5.1		3.63		1.01c	21	95
Sturgeon:														
Raw	A. P., anterior section:													
	E. P., flesh	E. P.		78.7	18.1	1.9	1.4	0.					90	405
	Ref., bones and skin	A. P.	15	66.9	15.4	1.6	1.2	0.					76	345
Smoked		E. P.		63.7	31.2	1.8	1.9	0.					141	640
Sucker, white-nosed:														
Raw	E. P., flesh	E. P.		78.6	18.0	2.3	1.2	0.					93	420
	A. P., entrails removed	A. P.	54	36.2	8.3	1.1	.6	0.					43	195
Suet (see Beef, fresh, wholesale cuts, kidney fat).														
Sugar-apple or sweetsop:														
Fresh	E. P., pulp	E. P.		73.5	1.8	.5	.9	23.3	1.6	16.4		.4c	105	475
	Ref., skin and seeds	A. P.	59	30.1	.7	.2	.4	9.6	.7				43	195
Sugars:														
Granulated	Cane or beet	E. P.		.5				99.5		99.5			398	1,805
Powdered		E. P.		.5				99.5		99.5			398	1,805
Brown	Light or dark	E. P.		3.			1.2	(95.5)		95.5			382	1,735
Corn sugar, unrefined		E. P.		7.5			.3	(90.)		90.			360	1,635
Dextrose (including refined corn sugar):														
Anhydrous		E. P.		.5				99.5		99.5			398	1,805
Crystallized		E. P.		10.				90.		90.			360	1,635
Maple		E. P.		7.5			.9	(90.)		90.			360	1,635

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Surinam-cherry or pitanga:														
Fresh		E. P.		85.8	0.8	0.4	0.5	12.5	0.6	7.0		2.0c	57	260
	Ref., stem, blossom end, and seeds	A. P.	19	69.5	.6	.3	.4	10.1	.5				46	210
"Swedish health bread" or rye wafers		E. P.		6.8	12.9	1.6	3.3	75.4	1.8				368	1,665
Sweetbreads:														
Fresh:														
Beef:														
Thin	From common grade beef	E. P.		65.	14.4	19.	1.48	0.					229	1,040
Medium	From medium-grade beef	E. P.		54.	11.8	33.	1.11	0.					344	1,560
Fat	From good-grade beef	E. P.		48.	10.3	41.	.94	0.					410	1,860
Very fat	From choice- and prime-grade beef	E. P.		40.	8.5	51.	.75	0.					493	2,240
Calf		E. P.		75.4	19.6	3.1	1.9	0.					106	480
Lamb		E. P.		79.5	14.1	3.8	1.27	0.					91	410
Sweetpotatoes:														
Fresh	E. P., root	E. P.		68.5	1.8	.7	1.07	27.9	1.0	5.4	20.2		125	565
	Ref., parings	A. P.	14	58.9	1.5	.6	.9	24.1	.9				103	490
Canned		E. P.		66.7	1.5	.2	.9	30.7	.6				131	590
Sweetpotato tops, common and oriental types:														
Fresh		E. P.		89.6	2.3	.3	1.55	6.3	1.2				37	170
	Ref., waste stems and leaves	A. P.	24	68.1	1.7	.2	1.2	4.8	.9				23	125
Sweetsop (see Sugar-apple).														
Swordfish:														
Raw	E. P., flesh	E. P.		74.9	18.8	4.4	1.5	0.					115	520
Tailor (see Bluefish).														
Tangerines (see Oranges, mandarin type).														
Tapioca:														
Dry		E. P.		12.6	.6	.2	.2	86.4	.1		85.4		350	1,585

Taros:														
Fresh:														
Dasheens	E. P., peeled corms and tubers	E. P.		66.6	2.9	.2	1.42	28.9	.7	1.7	21.8		129	585
	Ref., skins	A. P.	16	55.9	2.4	.2	1.2	24.3	.6				109	495
Others	E. P., peeled corms and tubers	E. P.		75.1	2.0	.2	1.17	21.5	.8	1.4	18.2		96	435
	Ref., skins	A. P.	18	61.6	1.6	.2	1.0	17.6	.7				79	360
Taro leaves and stems (including dasheens)		E. P.		87.8	2.7	.7	1.6	7.2	1.4		.4		46	210
Taro shoots (excluding dasheens)		E. P.		95.4	.9	.1	.8	2.8	.6				16	70
Tautog:														
Raw	E. P., flesh	E. P.		79.3	18.6	1.1	1.1	0.					84	380
	A. P., whole	A. P.	63	29.3	6.9	.4	.4	0.					31	140
	A. P., entrails removed	A. P.	57	34.1	8.0	.5	.5	0.					36	165
Terrapin:														
Fresh	E. P., muscle	E. P.		74.5	21.2	3.5	1.0	(0.)					116	530
		A. P.	79	15.6	4.5	.7	.2	(0.)					24	110
Tilefish:														
Raw	E. P., flesh	E. P.		80.3	17.5	.5	1.4	0.					74	340
	A. P., whole	A. P.	49	41.0	8.9	.3	.7	0.					38	170
Tomatoes:														
Fresh:														
Red		E. P.		94.1	1.0	.3	.57	4.0	.6	3.4		.51c	23	105
	Ref., skin or stem end	A. P.	2	92.2	1.0	.3	.6	3.9	.6				22	100
Green or unripe		E. P.		94.7	1.2	.2	.6	3.3	.4	1.2	.4		20	90
Canned	E. P., contents of can	E. P.		94.2	1.0	.2	.7	3.9	.4			.4c	21	95
Tomato juice:														
Fresh		E. P.		94.	1.0	.2	.5	4.3	.2				23	105
Canned	Salt, 0.5 percent	E. P.		93.5	1.0	.2	1.0	4.3	.2	3.4		.4c	23	105
Tomato catchup	Salt, 2.5 percent	E. P.		69.5	2.0	.4	3.6	24.5	.4			1.5c	110	495
Tomato paste:														
Canned		E. P.		71.7	4.7	1.4	3.34	18.7	.9	15.8		2.4c	106	480
Tomato puree:														
Canned		E. P.		89.2	1.8	.5	1.26	7.2	.4	6.0		.91c	40	185
Tomcod, Atlantic:														
Raw	E. P., flesh	E. P.		81.5	17.2	.4	1.0	0.					72	330
	A. P., whole	A. P.	61	31.8	6.7	.2	.4	0.					28	130
Tongue:														
Fresh:														
Beef:														
Lean		E. P.		70.	17.4	11.	.92	.4					170	770
Medium		E. P.		68.	16.4	15.	.86	.4					202	920
Fat		E. P.		65.	15.7	18.	.82	.4					226	1,030
Very fat		E. P.		62.	14.4	23.	.74	.4					266	1,210

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
Tongue—Continued.			*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Fresh—Continued.														
Calf		E. P.		74.3	18.5	5.3	1.0	0.9					125	570
Lamb		E. P.		69.5	13.9	15.3	.80	.5					195	890
Pork		E. P.		66.1	16.8	15.6	1.02	.5					210	950
Sheep		E. P.		61.0	13.7	21.8	1.1	2.4					261	1,180
Canned or cured:														
Whole, canned or pickled	Beef, lamb, pork, etc	E. P.		56.6	19.3	20.3	3.5	.3					261	1,180
Potted or deviled	do	E. P.		52.8	18.6	23.0	4.9	.7					284	1,290
Tripe:														
Beef:														
Commercial		E. P.		79.1	19.1	2.0	.4	0.					94	430
Pickled		E. P.		86.5	11.8	1.3	.3	0.					59	270
Hog (see Stomach, hog).														
Trout, eastern brook:														
Raw	E. P., flesh	E. P.		77.7	19.2	2.1	1.2	0.					96	435
	A. P., whole	A. P.	51	38.1	9.4	1.0	.6	0.					47	215
Truffles (see Mushrooms).														
Tuna, blue-fin:														
Raw	E. P., flesh	E. P.		69.1	24.8	5.2	1.4	0.					146	660
Tuna, yellow-fin:														
Raw	do	E. P.		71.5	24.7	3.0	1.4	0.					126	570
Tuna:														
Canned	Canned with or without added oil	E. P.		63.1	24.2	10.8	2.0	0.					194	880
Turbot or Greenland halibut:														
Raw	E. P., flesh	E. P.		71.4	14.8	14.4	1.3	0.					189	855
	A. P., whole	A. P.	48	37.1	7.7	7.5	.7	0.					98	445
Turkey:														
Fresh:														
Medium-fat birds:														
Total edible	E. P., flesh, skin, giblets, and fat	E. P.		58.3	20.1	20.2	1.0	0.					262	1,190
	A. P., live	A. P.	39	35.6	12.3	12.3	.6	0.					160	730
	A. P., dressed	A. P.	33	39.1	13.5	13.5	.7	0.					176	800
	A. P., drawn	A. P.	19	47.2	16.3	16.4	.8	0.					212	960

Flesh and skin		E. P.		63.0	22.8	13.0	1.1	0.					208	940
Flesh only		E. P.		68.6	24.0	6.7	1.1	0.					156	710
Light meat only		E. P.		69.2	24.5	4.6	1.2	0.					139	630
Dark meat only		E. P.		68.0	23.2	9.4	1.1	0.					177	800
Fat birds:														
Total edible	E. P., flesh, skin, giblets, and fat	E. P.		50.7	18.4	29.3	.9	0.					337	1,530
Thin, young birds:														
Total edible	do	E. P.		69.9	20.6	7.8	1.1	0.					153	690
Cooked (see Meat and poultry, cooked).														
Turnips:														
Fresh	E. P., roots	E. P.		90.9	1.1	.2	.73	7.1	1.1	4.6			35	155
	Ref., parings	A. P.	13	79.1	1.0	.2	.6	6.1	1.0				30	135
	Ref., tops and parings	A. P.	34	60.0	.7	.1	.5	4.7	.7				23	105
Turnip tops (also rutabaga tops):														
Fresh		E. P.		89.5	2.9	.4	1.76	5.4	1.2				37	165
	Ref., discarded leaves	A. P.	16	75.2	2.4	.3	1.5	4.6	1.0				31	140
Turtle, green:														
Fresh	E. P., muscle	E. P.		79.8	19.8	.5	1.2	(0.)					84	380
		A. P.	76	19.2	4.8	.1	.3	(0.)					20	90
Turtle meat (muscle):														
Canned	E. P., contents of can	E. P.		75.0	23.4	.7	.9	(0.)					100	455
Udo:														
Fresh	E. P., shoots	E. P.		95.0	1.0	.2	.64	3.2	.8	1.1			19	85
Veal:														
Fresh:														
Carcass or sides excluding kidney and kidney fat:														
Thin	E. P., 88 percent lean	E. P.		71.	19.7	8.	1.0	0.					151	680
	A. P., 68 percent lean	A. P.	23	55.	15.2	6.	.8	0.					116	530
Medium	E. P., 84 percent lean	E. P.		68.	19.1	12.	1.0	0.					184	840
	A. P., 66 percent lean	A. P.	21	54.	15.1	9.	.8	0.					146	660
Fat	E. P., 79 percent lean	E. P.		65.	18.5	16.	.9	0.					218	990
	A. P., 64 percent lean	A. P.	19	52.	15.0	13.	.7	0.					177	800
Carcass or sides, including kidney and kidney fat:														
Thin	E. P., 86 percent lean	E. P.		70.	19.4	10.	1.0	0.					168	760
	A. P., 67 percent lean	A. P.	22	54.	15.1	8.	.8	0.					131	590
Medium	E. P., 81 percent lean	E. P.		66.	18.8	14.	1.0	0.					201	910
	A. P., 64 percent lean	A. P.	21	52.	14.9	11.	.8	0.					159	720
Fat	E. P., 76 percent lean	E. P.		62.	18.0	19.	.9	0.					243	1,100
	A. P., 62 percent lean	A. P.	19	50.	14.6	15.	.7	0.					197	890

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion										
								Carbohydrates					Fuel value	
			Refuse	Water	Protein	Fat	Ash	Total	Fiber	Sugars	Starch	Acid	Per 100 grams	Per pound
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Veal—Continued.														
Fresh—Continued.														
Wholesale cuts:														
Chuck, including neck:														
Thin	E. P., 90 percent lean	E. P.		73.	19.9	6.	1.1	0.					134	610
	A. P., 70 percent lean	A. P.	22	57.	15.5	5.	.9	0.					104	470
Medium	E. P., 86 percent lean	E. P.		70.	19.4	10.	1.0	0.					168	760
	A. P., 69 percent lean	A. P.	20	56.	15.5	8.	.8	0.					134	610
Fat	E. P., 83 percent lean	E. P.		67.	19.0	13.	1.0	0.					193	880
	A. P., 68 percent lean	A. P.	18	55.	15.6	11.	.8	0.					158	720
Flank:														
Thin	E. P., 73 percent lean	E. P.		63.	18.1	18.	.9	0.					234	1,060
	A. P., 72 percent lean	A. P.	1	62.	17.9	18.	.9	0.					232	1,050
Medium	E. P., 61 percent lean	E. P.		56.	16.5	27.	.8	0.					309	1,400
	A. P., 60 percent lean	A. P.	1	55.	16.3	27.	.8	0.					306	1,390
Fat	E. P., 49 percent lean	E. P.		49.	14.5	36.	.7	0.					382	1,730
	A. P., 49 percent lean	A. P.	1	48.	14.4	36.	.7	0.					378	1,720
Loin, excluding kidney and kidney fat:														
Thin	E. P., 89 percent lean	E. P.		71.	19.7	8.	1.0	0.					151	680
	A. P., 72 percent lean	A. P.	19	58.	16.0	6.	.8	0.					122	550
Medium	E. P., 85 percent lean	E. P.		69.	19.2	11.	1.0	0.					176	800
	A. P., 71 percent lean	A. P.	17	57.	15.9	9.	.8	0.					146	660
Fat	E. P., 80 percent lean	E. P.		65.	18.6	15.	1.0	0.					209	950
	A. P., 67 percent lean	A. P.	16	55.	15.6	13.	.8	0.					176	800
Plate:														
Thin	E. P., 82 percent lean	E. P.		68.	19.1	12.	1.0	0.					184	840
	A. P., 63 percent lean	A. P.	23	52.	14.7	9.	.8	0.					142	640
Medium	E. P., 74 percent lean	E. P.		64.	18.3	17.	.9	0.					226	1,030
	A. P., 58 percent lean	A. P.	21	50.	14.5	13.	.7	0.					179	810

Fat	E. P., 66 percent lean	E. P.		59.	17.3	23.	.9	0.					276	1,280
	A. P., 53 percent lean	A. P.	19	48.	14.0	19.	.7	0.					224	1,010
Rib:														
Thin	E. P., 87 percent lean	E. P.		70.	19.5	9.	1.0	0.					159	720
	A. P., 65 percent lean	A. P.	25	53.	14.6	7.	.8	0.					119	540
Medium	E. P., 82 percent lean	E. P.		66.	18.8	14.	1.0	0.					201	910
	A. P., 63 percent lean	A. P.	23	51.	14.5	11.	.8	0.					155	700
Fat	E. P., 76 percent lean	E. P.		62.	18.0	19.	.9	0.					243	1,100
	A. P., 59 percent lean	A. P.	22	48.	14.0	15.	.7	0.					190	860
Round, with rump:														
Thin	E. P., 91 percent lean	E. P.		73.	19.9	6.	1.1	0.					134	610
	A. P., 68 percent lean	A. P.	25	55.	14.9	4.	.8	0.					100	450
Medium	E. P., 87 percent lean	E. P.		70.	19.5	9.	1.0	0.					159	720
	A. P., 67 percent lean	A. P.	23	54.	15.0	7.	.8	0.					122	560
Fat	E. P., 84 percent lean	E. P.		68.	19.1	12.	1.0	0.					184	840
	A. P., 66 percent lean	A. P.	22	53.	14.9	9.	.8	0.					144	650
Shank, fore:														
Thin	E. P., 91 percent lean	E. P.		74.	20.1	5.	1.1	0.					125	570
	A. P., 46 percent lean	A. P.	49	38.	10.3	3.	.6	0.					64	290
Medium	E. P., 87 percent lean	E. P.		71.	19.7	8.	1.0	0.					151	680
	A. P., 45 percent lean	A. P.	48	37.	10.2	4.	.5	0.					78	360
Fat	E. P., 84 percent lean	E. P.		70.	19.4	10.	1.0	0.					168	760
	A. P., 45 percent lean	A. P.	47	37.	10.3	5.	.5	0.					89	400
Quarter, fore:														
Thin	E. P., 88 percent lean	E. P.		71.	19.7	8.	1.0	0.					151	680
	A. P., 66 percent lean	A. P.	25	53.	14.8	6.	.8	0.					113	510
Medium	E. P., 84 percent lean	E. P.		68.	19.1	12.	1.0	0.					184	840
	A. P., 65 percent lean	A. P.	23	52.	14.7	9.	.8	0.					142	640
Fat	E. P., 79 percent lean	E. P.		65.	18.5	16.	.9	0.					218	990
	A. P., 62 percent lean	A. P.	21	51.	14.6	13.	.7	0.					172	780
Quarter, hind, excluding kidney and kidney fat:														
Thin	E. P., 88 percent lean	E. P.		71.	19.7	8.	1.0	0.					151	680
	A. P., 70 percent lean	A. P.	21	56.	15.6	6.	.8	0.					119	540
Medium	E. P., 84 percent lean	E. P.		68.	19.1	12.	1.0	0.					184	840
	A. P., 68 percent lean	A. P.	19	55.	15.5	10.	.8	0.					149	680
Fat	E. P., 79 percent lean	E. P.		65.	18.5	16.	.9	0.					218	990
	A. P., 66 percent lean	A. P.	17	54.	16.4	13.	.7	0.					181	820

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
			Refuse	Water	Protein	Fat	Ash	Carbohydrates				Acid	Per 100 grams	Per pound
								Total	Fiber	Sugars	Starch			
Veal—Continued.														
Fresh—Continued.														
Wholesale cuts—Continued.														
Quarter, hind, including kidney and kidney fat:			*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Thin	E. P., 84 percent lean	E. P.		68.	19.1	12.	1.0	0.					184	840
	A. P., 68 percent lean	A. P.	19	55.	15.5	10.	.8	0.					149	680
Medium	E. P., 79 percent lean	E. P.		65.	18.5	16.	.9	0.					218	990
	A. P., 65 percent lean	A. P.	18	53.	15.2	13.	.7	0.					179	810
Fat	E. P., 73 percent lean	E. P.		61.	17.6	21.	.9	0.					259	1,180
	A. P., 61 percent lean	A. P.	16	51.	14.8	18.	.8	0.					218	990
Cooked (see Meat and poultry, cooked).														
Vegetable marrow (see Squash, fresh, summer).														
Vegetable-oyster or salsify:														
Fresh	E. P., roots	E. P.		79.1	3.5	1.0	.88	15.5	1.8				85	385
	Ref., parings	A. P.	24	60.1	2.7	.8	.7	11.7	1.4				65	295
Venison:														
Raw	E. P., lean meat	E. P.		73.	20.	6.	1.	0.					134	610
Vermicelli (see Macaroni).														
Vinegar		E. P.			.0		3	(5.0)		0.4		4.6a		
Vinespinach (see Basella).														
Walnuts:														
Black	E. P., kernels	E. P.		2.7	18.3	58.2	2.1	18.7	1.9				672	3,045
	Ref., shells	A. P.	78	.6	4.0	12.8	.5	4.1	.4				148	670
Persian or English	E. P., kernels	E. P.		3.3	15.0	64.4	1.7	15.6	2.1				702	3,185
	Ref., shells	A. P.	55	1.5	6.8	29.0	.8	6.9	.9				316	1,435
Water cress:														
Fresh	E. P., leaves and stems	E. P.		93.6	1.7	.3	1.09	3.3	.5				23	105
Watermelons:														
Fresh	E. P., flesh	E. P.		92.1	.5	.2	.27	6.9	.6	6.0		.03m	31	140
	Ref., rinds and seeds	A. P.	54	42.4	.2	.1	.1	3.2	.3				14	65

Waternut (a tuber):														
Fresh	E. P., tubers	E. P.		77.1	1.5	.1	1.10	20.2	.8	8.8	7.7		88	405
	Ref., skins	A. P.	22	60.1	1.2	.1	.9	15.7	.6				68	310
Wheat (see Wheat flours, graham).														
Wheat flours:														
Graham:														
All types		E. P.		11.	13.	2.	1.6	72.4	1.8				360	1,630
Hard red wheat		E. P.		11.	13.9	2.1	1.6	71.4	2.3				360	1,635
Soft red wheat		E. P.		11.	11.4	2.0	1.7	73.9	2.3				359	1,630
White wheat		E. P.		11.	10.7	2.0	1.7	74.6	1.9				359	1,630
Straight:														
All types		E. P.		12.	11.2	1.1	.5	75.2	.4				356	1,615
Hard red		E. P.		12.	12.0	1.2	.5	74.3	.4				356	1,615
Soft red		E. P.		12.	10.8	1.0	.5	75.7	.4				355	1,610
White		E. P.		12.	9.3	1.0	.5	77.2	.4				355	1,610
Patent:														
All-purpose		E. P.		12.	10.8	.9	.4	75.9	.3				355	1,610
Bread		E. P.		12.	11.8	1.1	.5	74.6	.3				356	1,615
Cake or pastry		E. P.		12.	8.3	.8	.4	78.5	.2				354	1,610
Self-rising (salt and leavening added):														
All		E. P.		12.	10.2	.9	4.0	72.9	.4				340	1,545
Patent, soft		E. P.		12.	7.9	.8	3.9	75.4	.2				340	1,545
Straight		E. P.		12.	10.2	.9	4.0	72.9	.4				340	1,545
Clear		E. P.		12.	10.9	1.4	4.1	71.6	.4				343	1,555
Prepared, ready to mix	Shortening added	E. P.		9.5	8.3	11.9	4.0	66.3	.3				406	1,840
Pancake (see Flour, pancake).														
Wheat breakfast foods:														
Bran, flakes	Bran with other parts of grain	E. P.		6.2	13.0	1.9	4.0	74.9	3.1				369	1,670
Farina		E. P.		11.	11.5	1.0	.4	76.1	.3				359	1,630
Puffed wheat		E. P.		7.8	13.4	1.7	1.5	75.6	1.8				371	1,685
Shredded wheat		E. P.		7.7	10.4	1.4	1.8	78.7	2.1				369	1,675
Wheat flakes		E. P.		4.7	10.4	1.3	3.7	79.9	1.6				373	1,690
Wheat meals	Ground, cut, or cracked grain	E. P.		8.7	11.7	2.0	1.8	75.8	1.8				368	1,670

Table 1. Proximate Composition of American Food Materials — (*Continued*)

Food	Nature of sample and refuse	Basis	As purchased	Constituents of the edible portion									Fuel value	
								Carbohydrates						
			Refuse	Water	Protein	Fat	Ash	Total	Fiber	Sugars	Starch	Acid	Per 100 grams	Per pound
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*	*Calories*
Wheat bran:														
Crude		E. P.		10.1	16.6	3.7	6.1	63.5	10.3	7.2	9.0		354	1,605
Packaged	Almost wholly bran	E. P.		7.4	15.9	4.2	6.3	66.2	8.4	5.2	17.4		366	1,660
Washed		E. P.		6.6	16.0	5.2	4.9	67.3	17.1	1.0	3.7		380	1,725
Wheat germ, commercially milled.	Containing some bran and flour	E. P.		11.0	25.2	10.0	4.3	49.5	2.5				389	1,765
Whey		E. P.		93.0	1.0	.3	.6	5.1					27	125
Whitefish, Great Lakes:														
Raw	E. P., flesh	E. P.		69.8	22.9	6.5	1.6	0.					150	690
	A. P., whole	A. P.	54	32.1	10.5	3.0	.7	0.					69	315
Whiting (see Hakes).														
Wild rice:														
Parched or sun-dried	E. P., hulled grain	E. P.		8.6	13.8	.8	1.3	75.5	1.4		62.4		364	1,655
Witloof (see Chicory).														
Yams, winged:														
Fresh	E. P., tubers	E. P.		72.6	2.1	.2	.98	24.1	.8	1.0	17.7		107	485
Yeast:														
Compressed		E. P.		70.9	13.3	.4	2.4	13.0	.3				109	495
Dried (brewer's and baker's)		E. P.		7.0	46.1	1.6	7.9	37.4	.8				348	1,580
Yellowtail:														
Raw	E. P., flesh	E. P.		72.7	21.0	5.4	1.3	0.					133	600
Zwieback		E. P.		4.9	10.9	8.6	1.3	74.3	.3				418	1,895

APPENDIX

Table 2

Nutritive Value of 100 Grams of Selected Foods, Edible Portion

Table 2

Table 2* supplements Table 1 by furnishing values for three minerals (calcium, phosphorus and iron) and the better known vitamins (vitamin A, thiamine, riboflavin, niacin and ascorbic acid). The data in this table are given on a 100-gram basis.

Explanation of Table and Meaning of Terms

For the sake of uniformity the mineral values and the values for all the vitamins except vitamin A are expressed in terms of *milligrams*.

The word "*trace*" is used to represent small values that would have rounded to zero.

Parentheses are used to denote values imputed usually from some other form of the same food or from similar foods. Parentheses also indicate values of nutrients covered by specifications for enrichment, such as vitamin A in margarine.

Dashes have been used in the few cases where no reliable data were available but where there was reason to suppose a measurable amount of a nutrient to be present.

Army ration components are identified by an *asterisk*.

* From "Tables of Food Composition in Terms of Eleven Nutrients," prepared by the Bureau of Human Nutrition and Home Economics, U. S. Department of Agriculture, in cooperation with the National Research Council, *U. S. Dept. Agr. Misc. Pub. No. 572*, 1945.

Table 2. Nutritive Value of 100 Grams of Selected Foods, Edible Portion

Food item	Water	Food energy	Protein	Fat	Carbohydrate	Calcium	Phosphorus	Iron	Vitamin A value	Thiamine	Riboflavin	Niacin	Ascorbic acid
MILK, CREAM, ICE CREAM, CHEESE													
Milk:	*Percent*	*Calories*	*Grams*	*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*	*International Units*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
1. Buttermilk, cultured	90.5	35	3.5	0.1	5.1	(118)	(93)	(0.07)	(Trace)	(0.04)	(0.18)	(0.1)	(1)
2. Chocolate flavored [1]	83.0	75	3.2	2.2	10.6	109	91	.07	90	.03	.16	.1	0
3. Condensed, sweetened	27.0	327	8.1	8.4	54.8	273	228	(.20)	(430)	(.05)	(.39)	(.2)	(1)
4. Dry skim	3.5	359	35.6	1.0	52.0	1,300	1,030	.58	(40)	.35	1.96	1.1	7
5. Dry whole	3.5	496	25.8	26.7	38.0	949	728	.58	1,400	.30	1.46	.7	6
6. Evaporated, unsweetened	73.7	139	7.0	7.9	9.9	243	195	.17	400	.05	.36	.2	1
7. Fresh skim	90.5	35	3.5	.1	5.1	(118)	(93)	(.07)	(Trace)	.04	(.18)	(.1)	(1)
8. Fresh whole	87.0	69	3.5	3.9	4.9	118	93	.07	(160)	.04	.17	.1	1
Cream; ice cream:													
9. Cream (20 percent), sweet or sour	72.5	208	2.9	20.0	4.0	(97)	(77)	(.06)	(830)	(.03)	(.14)	(.1)	(1)
10. Ice cream; plain [1]	62.0	210	4.0	12.3	20.8	132	104	.10	540	.04	.19	.1	Trace
Cheese:													
11. Cheddar type	39	393	23.9	32.3	1.7	873	610	(.57)	1,740	.04	.50	(.2)	(0)
12. Cottage	74.0	101	19.2	.8	4.3	82	263	(.46)	(30)	.02	.29	(.1)	(0)
13. Cream	53.3	367	7.1	36.9	1.7	(298)	(208)	(.17)	2,210	(.01)	.14	.1	(0)
14. *Processed, canned [2]	37.5	382	21.9	31.8	2.0	716	831	.76	1,260	.03	.43	.1	(0)
15. All other	(39)	393	(23.9)	(32.3)	(1.7)	(873)	(610)	(.57)	2,050	.04	.52	.2	(0)
FATS, OILS													
16. *Army spread, canned [3]	27.8	562	5.2	56.7	7.7	244	241	.5	2,820	.03	.19	.1	0
17. *Bacon, canned	12.6	704	7.9	74	1.6	14	38	.9	(0)	.26	.10	1.5	0
18. Bacon, medium fat	20	626	9.1	65	(1.1)	13	108	.8	(0)	(.42)	(.10)	(2.1)	0
19. Butter	15.5	733	.6	81	.4	16	16	.2	[4] 3,300	Trace	.01	.1	0
20. French dressing	38.3	423	.8	39	17.3	(5)	(5)	.1	0	0	0	0	0
21. Lard, other shortening	0	900	0	100	0	0	0	0	0	(0)	(0)	(0)	0
22. Margarine with vitamin A added	15.5	733	.6	81	.4	(2)	(15)	(.2)	[5] (1,980)	(0)	(0)	(0)	0

Note: Asterisk indicates Army ration component; parentheses, imputed value.

[1] Calculated from ingredients.
[2] Cheddar type.
[3] Not less than 56 percent butter fat on dry solids basis, cheese curd, skim milk powder.
[4] Year-round average.
[5] Plain margarine is considered to have no vitamin A value.

Table 2. Nutritive Value of 100 Grams of Selected Foods, Edible Portion — (*Continued*)

	Food item	Water	Food energy	Protein	Fat	Carbohydrate	Calcium	Phosphorus	Iron	Vitamin A value	Thiamine	Riboflavin	Niacin	Ascorbic acid
	FATS, OILS—Continued	*Percent*	*Calories*	*Grams*	*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*	*International Units*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
23.	Mayonnaise	16	720	1.5	78	3.0	(19)	(60)	(1.0)	(210)	(0.04)	(0.04)	(0)	(0)
24.	Salad dressing	44.7	391	1.1	36.8	13.9	(9)	(30)	(.4)	(140)	(.02)	(.03)	(0)	(0)
25.	Salad or cooking oil	0	900	0	100	0	0	0	0	0	0	0	0	0
26.	Salt pork, fat	8	781	3.9	85	0	2	42	.6	(0)	(.18)	(.04)	(.9)	0
	EGGS													
27.	Egg yolk, fresh	49.4	355	16.3	31.9	.7	147	586	7.2	3,210	.32	.52	----------	0
28.	*Eggs, whole, dried	2	593	(48.2)	(43.3)	(2.6)	187	800	8.7	4,460	.35	1.23	.2	0
29.	Eggs, whole, fresh	74.0	158	12.8	11.5	.7	54	210	2.7	1,140	.12	.34	.1	0
	MEAT, POULTRY, FISH													
	Beef:													
	Thin—Utility, Grade C:													
30.	Carcass; side, including kidney fat	66	201	18.8	14	0	11	203	2.8	(0)	.12	.15	5.1	0
	Medium—Commercial, Grade B:													
31.	Carcass; side, including kidney fat	60	268	17.5	22	0	10	189	2.6	(0)	.11	.14	4.7	0
32.	Carcass trimmed to retail basis	63	235	18.2	18	0	11	196	2.7	(0)	.11	.14	4.9	0
33.	*Chopped meat [6]	54	325	16.1	29	0	9	174	2.4	(0)	.10	.13	4.4	0
34.	*Roasting meat [6]	67	193	18.9	13	0	11	204	2.8	(0)	.12	.15	5.1	0
35.	*Stewing meat [6]	63	235	18.2	18	0	11	196	2.7	(0)	.11	.14	4.9	0
	Fat—Good, Grade A:													
36.	Carcass; side, including kidney fat	55	317	16.3	28	0	10	176	2.4	(0)	.10	.13	4.4	0
	Very fat—Choice, Prime, Grade AA:													
37.	Carcass; side, including kidney fat	47	406	13.7	39	0	8	148	2.1	(0)	.08	.11	3.7	0
	Retail items: [7]													
38.	Chuck roast (wholesale chuck)	65	218	18.6	16	0	11	200	2.8	(0)	.12	.15	5.0	0
39.	*Corned beef, canned	57.3	232	24.4	15	0	29	113	4.0	(0)	.02	.19	2.7	0
40.	Corned beef, medium	54.2	288	15.8	25	0	9	170	2.4	(0)	.05	.10	1.7	0
41.	Dried or chipped	47.7	194	34.3	6.3	0	20	370	5.1	(0)	.11	.22	3.7	0

42.	Hamburger	55	316	16	28	0	9	172	2.4	(0)	.10	.13	4.3	0
43.	Loin steaks (wholesale loin)	57	293	16.9	25	0	10	182	2.5	(0)	.10	.13	4.6	0
44.	Rib roast or steak (wholesale rib)	59	277	17.4	23	0	10	188	2.6	(0)	.11	.14	4.7	0
45.	*Roast, canned	60.0	217	25	13	0	9	164	2.2	(0)	.02	.24	4.5	0
46.	Round steak (wholesale round)	67	194	19.3	13	0	11	208	2.9	(0)	.12	.15	5.2	0
47.	Rump roast (wholesale rump)	53	341	15.5	31	0	9	167	2.3	(0)	.10	.12	4.2	0
48.	Soup meat (wholesale shanks)	70	162	20.3	9	0	12	219	3.0	(0)	.13	.16	5.5	0
49.	Stew meat (73 percent lean)	53	333	15.8	30	0	9	170	2.4	(0)	.10	.12	4.3	0
	Lamb:													
	Carcass; side:													
50.	Thin	66.3	202	17.1	14.8	0	10	184	2.6	(0)	.20	.25	5.6	0
51.	Intermediate	55.8	312	15.7	27.7	0	9	169	2.4	(0)	.18	.23	5.2	0
52.	Fat	46.2	410	13.0	39.8	0	8	140	2.0	(0)	.15	.19	4.3	0
	Retail items, [7] intermediate grade:													
53.	Leg roast (wholesale leg)	63.7	230	18.0	17.5	0	10	194	2.7	(0)	.21	.26	5.9	0
54.	Shoulder roast (wholesale 3-rib shoulder)	58.3	290	15.6	25.3	0	9	168	2.3	(0)	.18	.23	5.2	0
55.	Sirloin chop (wholesale leg)	63.7	230	18.0	17.5	0	10	194	2.7	(0)	.21	.26	5.9	0
	Pork:													
	Packers' carcass; side:													
56.	Thin	50	371	14.1	35	0	8	152	2.1	(0)	.89	.18	3.8	0
57.	Medium	42	453	11.9	45	0	7	128	1.8	(0)	.75	.15	3.2	0
58.	Fat	35	534	9.8	55	0	6	106	1.5	(0)	.62	.12	2.6	0
59.	Miscellaneous lean cuts [8]	52	352	14.5	32.7	0	8	156	2.2	(0)	.92	.18	3.9	0
	Retail items: [7]													
	Bacon. See Fats, Oils.													
60.	Boston butt	60	273	16.6	23	0	10	179	2.5	(0)	1.05	.21	4.5	0
61.	Ham, fresh	53	340	15.2	31	0	9	164	2.3	(0)	.96	.19	4.1	0
62.	Ham, smoked	42	384	16.9	35	(.3)	10	182	2.5	(0)	.78	.19	3.8	0
63.	Loin	58	291	16.4	25	0	10	177	2.5	(0)	1.04	.20	4.4	0
64.	Picnic	52	347	14.8	32	0	9	160	2.2	(0)	.94	.18	4.0	0
65.	Pork links; sausage	41.9	446	10.8	44.8	0	6	116	1.6	(0)	.22	.15	2.3	0
	Salt pork. See Fats, Oils.													
66.	Spareribs	53	346	14.6	32	0	8	157	2.2	(0)	.92	.18	3.9	0

NOTE: Asterisk indicates Army ration component; parentheses, imputed value.

[6] Average values for composition of all cuts in a boned and trimmed carcass of commercial grade generally used for (a) chopped meat, (b) roasting and broiling, (c) stewing and boiling.

[7] Values for fresh items are from the medium fat wholesale cuts considered to be nearest approximations for corresponding retail items.

[8] Lean cuts from medium fat carcass weighted according to civilian supply, 1944. Excludes bacon, lard, salt side, fat back.

Table 2. Nutritive Value of 100 Grams of Selected Foods, Edible Portion — (*Continued*)

	Food item	Water	Food energy	Protein	Fat	Carbohydrate	Calcium	Phosphorus	Iron	Vitamin A value	Thiamine	Riboflavin	Niacin	Ascorbic acid
	MEAT, POULTRY, FISH—Continued													
	Veal:													
	Carcass; side, excluding kidney fat:	*Percent*	*Calories*	*Grams*	*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*	*International Units*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
67.	Thin	71	151	19.7	8	0	11	212	3.0	(0)	0.18	0.28	6.5	0
68.	Medium	68	184	19.1	12	0	11	206	2.9	(0)	.17	.27	6.3	0
69.	Fat	65	218	18.5	16	0	11	199	2.8	(0)	.17	.26	6.1	0
	Retail items, [7] medium fat:													
70.	Chops (wholesale loin)	69	176	19.2	11	0	11	207	2.9	(0)	.18	.27	6.3	0
71.	Cutlet (wholesale round)	70	159	19.5	9	0	11	210	2.9	(0)	.18	.28	6.4	0
72.	Leg roast or steak (wholesale leg)	(68)	186	(19.1)	(12.2)	0	11	206	2.9	(0)	.17	.27	6.3	0
73.	Stew meat (74 percent lean)	64	226	18.3	17	0	11	197	2.7	(0)	.17	.26	6.0	0
	Variety meats; meat mixtures:													
74.	*Beef and gravy, canned [9]	65.3	188	19.4	11.7	1.3	19	122	2.7	(30)	.09	.19	2.7	0
75.	Bologna	62.4	217	14.8	15.9	3.6	9	160	2.2	(0)	.31	.30	3.0	0
76.	*Chile con carne, without beans, canned [10]	66.3	198	10.2	14.6	6.4	21	152	.7	160	.01	.10	2.1	0
77.	Frankfurters	64.3	201	15.2	14.1	3.3	9	164	2.3	(0)	.19	.23	2.4	0
78.	*Ham and eggs, canned [11]	63.9	227	14.4	18.3	1.2	43	166	2.2	500	.16	.24	1.7	0
79.	*Hash, corned beef, canned [12]	69.4	143	15.1	6.1	7.0	26	(90)	1.3	(0)	.02	.13	2.4	0
80.	*Hash, meat and vegetable, canned [13]	73.3	122	10.0	5.0	9.3	14	(66)	1.2	(0)	.04	.11	2.5	6
81.	Heart, fresh	75.4	126	(16.5)	(6.3)	(.7)	10	236	6.2	(0)	.54	.90	6.8	14
82.	Liver, fresh	70.9	131	(19.8)	(4.2)	(3.6)	8	373	12.1	19,200	.27	2.80	16.1	31
83.	Liver sausage	59.0	258	16.7	20.6	1.5	9	238	5.4	(5,750)	.17	1.12	4.6	(0)
84.	*Luncheon meat, canned [14]	56.3	270	15.2	22.5	1.7	21	170	1.4	(0)	.29	.21	2.7	0
85.	*Pork and gravy, canned [15]	64.9	206	15.4	15.2	1.9	16	162	1.6	(0)	.19	.24	2.7	0
86.	*Pork sausage, bulk, canned	57.0	280	16.0	24.0	0	17	131	2.2	(0)	.19	.21	2.8	0
87.	*Spaghetti with meat, canned [16]	71.0	142	9.8	3.9	10.2	38	97	1.8	480	.02	.12	2.2	----------
88.	*Stew, meat and vegetable, canned [17]	72.9	127	11.6	5.5	7.8	36	(136)	1.4	1,780	.04	.12	2.4	4
89.	Tongue, fresh, medium fat	68	202	16.4	15	.4	30	119	6.9	(0)	.22	.27	5.0	0
90.	*Vienna sausage, canned	64.1	210	16.0	16.2	0	19	(164)	.6	(0)	.07	.14	3.1	0

Poultry:													
91. Chicken, boned, canned	67.1	175	21.8	9.8	0	32	(218)	(1.9)	Trace	.01	.15	3.7	2
92. Chicken, roasters [18]	66.0	194	20.2	12.6	0	16	218	1.9	Trace	.11	.18	8.6	----------
93. Turkey, medium fat [18]	58.3	262	20.1	20.2	0	23	320	3.8	Trace	.12	.19	7.9	----------
Fish and shellfish:													
94. Cod	82.6	70	16.5	.4	0	18	189	.9	----------	.04	.05	2.3	2
95. Fish, miscellaneous, medium fat	77.2	98	19.0	2.5	0	21	218	1.0	----------	.07	.07	4.2	(2)
96. Oysters, solids and liquor	87.1	50	6.0	1.2	3.7	68	172	7.1	----------	.18	.23	1.2	----------
97. Salmon, canned	67.4	169	20.6	9.6	0	67	286	1.3	[19] 80	.03	.18	6.5	0
98. Sardines, canned in oil, drained solids	57.4	207	25.7	11.0	1.2	35	365	1.8	290	.06	.12	5.2	0
99. Sardines, canned in oil, total contents of can	47.1	331	21.1	27	1.0	29	299	1.5	710	.05	.10	4.3	0
100. Shrimp, canned	78.3	82	17.8	.8	.8	(75)	(210)	(2.0)	60	.01	.03	1.9	0
101. Tuna fish, canned, drained solids	57.7	217	27.7	11.8	0	34	290	1.7	70	.04	.13	10.6	0
102. Tuna fish, canned, total contents of can	51.4	294	23.9	22.1	0	30	252	1.5	130	.04	.11	9.2	0
DRY BEANS AND PEAS, NUTS													
Dry beans and peas:													
103. *Bean soup, navy, dehydrated [20]	7.2	332	17.6	1.2	62.7	(148)	(463)	(10.3)	(3)	.46	.22	2.4	1
104. Beans, canned, baked	71.0	117	5.7	2.0	19.0	(49)	(154)	(3.4)	[21] 70	.05	.05	.8	[21] 4
105. Beans, common or kidney, dry seed	10.5	350	22.0	1.5	62.1	148	463	10.3	0	.60	.24	2.1	2
106. Beans, lima, dry seed	12.6	341	20.7	1.3	61.6	68	381	7.5	0	.60	.24	2.1	2
107. Chickpeas	10.6	369	20.8	4.7	60.9	92	375	7.1	Trace	.35	.15	1.4	(2)
108. Cowpeas	10.6	351	22.9	1.4	61.6	80	450	7.8	0	.83	.23	2.2	2
109. *Pea soup, dehydrated [22]	7.2	336	20.4	1.2	60.8	(73)	(397)	(6.0)	220	.62	.21	3.1	2
110. Peas, split	10.0	354	24.5	1.0	61.7	73	397	6.0	370	.87	.29	3.0	2
111. Soybeans, whole, mature	7.5	351	34.9	18.1	[23](12.0)	227	586	8.0	110	1.14	.31	2.1	Trace
Soy flour; flakes; grits:													
112. Low fat	11	246	44.7	1.1	[23](14.2)	265	623	13.0	70	1.10	.35	2.9	(0)
113. Medium fat	9	283	42.5	6.5	[23](13.6)	244	610	13.0	110	.82	.34	2.6	(0)
114. Full fat	9	375	35.9	20.6	[23](11.4)	195	553	12.1	140	.77	.28	2.2	(0)

NOTE: Asterisk indicates Army ration component; parentheses, imputed value.

7 Values for fresh items are from the medium fat wholesale cuts considered to be nearest approximations for corresponding retail items.

9 90 percent beef, 10 percent tomato gravy.

10 Not less than 60 percent meat, not more than 8 percent cereals, seasonings.

11 50 percent ham, 50 percent whole eggs.

12 72 percent beef, 28 percent potatoes.

13 50 percent meat, 48 percent potatoes, 2 percent onions.

14 Pork.

15 90 percent pork, 10 percent gravy.

16 50 percent meat, 10 percent dry spaghetti, 30 percent tomato puree, 5 percent cheese, 5 percent onions.

17 50 percent meat, 15 percent potatoes, 15 percent carrots, 8 percent dry beans, 12 percent tomato puree.

18 Vitamin values based on muscle meat only.

19 Based on pink salmon. Canned red salmon may have a value several times higher.

20 Navy bean meal, farinaceous flour up to 15 percent.

21 Contributed by tomatoes.

22 Pea meal, farinaceous flour up to 15 percent.

23 "Available" carbohydrate.

Table 2. Nutritive Value of 100 Grams of Selected Foods, Edible Portion — (*Continued*)

Food item	Water	Food energy	Protein	Fat	Carbohydrate	Calcium	Phosphorus	Iron	Vitamin A value	Thiamine	Riboflavin	Niacin	Ascorbic acid
DRY BEANS AND PEAS, NUTS—Continued													
Nuts:	*Percent*	*Calories*	*Grams*	*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*	*International Units*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
115. Almonds	4.7	640	18.6	54.1	19.6	254	475	4.4	0	0.25	0.67	4.6	Trace
116. Peanut butter	1.7	619	26.1	47.8	21.0	74	393	1.9	0	.20	.16	16.2	(0)
117. Peanuts, roasted	2.6	600	26.9	44.2	23.6	74	393	1.9	0	[24] .30	.16	16.2	(0)
118. Pecans	3.0	747	9.4	73.0	13.0	74	324	2.4	50	.72	.11	.9	2
119. Walnuts, English	3.3	702	15.0	64.4	15.6	83	380	2.1	30	.48	.13	1.2	3
VEGETABLES													
Fresh:													
120. Asparagus	93.0	26	2.2	.2	3.9	21	62	.9	1,000	.16	.17	1.2	33
121. Beans, lima, green	66.5	131	7.5	.8	23.5	63	158	2.3	280	.25	.14	.9	32
122. Beans, snap	88.9	42	2.4	.2	7.7	65	44	1.1	630	.08	.10	.6	19
123. Beet greens	90.4	33	2.0	.3	5.6	[25]	45	3.2	6,700	.05	.17	.3	34
124. Beets	87.6	46	1.6	.1	9.6	27	43	1.0	20	.03	.05	.4	10
125. Broccoli	89.9	37	3.3	.2	5.5	130	76	1.3	3,500	.09	.21	.9	118
126. Brussels sprouts	84.9	58	4.4	.5	8.9	34	78	1.3	400	.11	(.06)	(.3)	94
127. Cabbage	92.4	29	1.4	.2	5.3	46	31	.5	80	.07	.06	.3	52
128. Carrots	88.2	45	1.2	.3	9.3	39	37	.8	12,000	.07	.06	.5	6
129. Cauliflower	91.7	31	2.4	.2	4.9	22	72	1.1	90	.10	.11	.6	69
130. Celery	93.7	22	1.3	.2	3.7	50	40	.5	0	.03	.04	.3	7
131. Chard	91.8	25	1.4	.2	4.4	[26]	36	4.0	2,800	.06	.13	.2	38
132. Collards	86.6	50	3.9	.6	7.2	249	58	1.6	6,870	.22	(.20)	(.8)	100
133. Corn, sweet, white or yellow	73.9	108	3.7	1.2	20.5	9	120	.5	[27] 390	.15	.14	1.4	12
134. Cucumbers	96.1	14	.7	.1	2.7	10	21	.3	[28] 0	.04	.09	.2	8
135. Dandelion greens	85.8	52	2.7	.7	8.8	187	70	3.1	13,650	.19	.14	(.8)	36
136. Eggplant	92.7	28	1.1	.2	5.5	15	37	.4	30	.07	.06	.8	5
137. Kale	86.6	50	3.9	.6	7.2	225	62	2.2	7,540	.12	.35	(.8)	115
138. Lettuce, headed	94.8	18	1.2	.2	3.9	22	25	.5	540	.06	.07	.2	8

139.	Lettuce, all other	94.8	18	1.2	.2	2.9	62	20	1.1	1,620	.06	.07	.2	18
140.	Mustard greens	92.2	28	2.3	.3	4.0	220	38	2.9	6,460	.09	.20	.8	102
141.	Okra	89.8	39	1.8	.2	7.4	82	62	.7	740	.12	.10	.7	30
142.	Onions, mature	87.5	49	1.4	.2	10.3	32	44	.5	50	.03	.02	.1	[29] 9
143.	Parsnips	78.6	83	1.5	.5	18.2	57	80	.7	0	.11	.09	.2	18
144.	Peas, green	74.3	101	6.7	.4	17.7	22	122	1.9	680	.36	.18	2.1	26
145.	Peppers, green	92.4	29	1.2	.2	5.7	11	25	.4	630	.07	.04	.4	120
146.	Potatoes	77.8	85	2.0	.1	19.1	11	56	.7	20	.11	.04	1.2	17
147.	Pumpkin	90.5	36	1.2	.2	7.3	21	44	.8	(3,400)	(.05)	(.08)	(.6)	8
148.	Radishes	93.6	22	1.2	.1	4.2	37	31	1.0	30	.04	.04	.1	24
49.	Rutabagas	89.1	41	1.1	.1	8.9	55	41	.4	330	.06	.06	.5	36
50.	Spinach	92.7	25	2.3	.3	3.2	[30]	55	3.0	9,420	.12	.24	.7	59
151.	Squash, summer	95.0	19	.6	.1	3.9	15	15	.4	260	.04	.05	1.1	17
152.	Squash, winter	88.6	44	1.5	.3	8.8	19	28	.6	4,950	.05	.08	.6	8
153.	Sweetpotatoes	68.5	125	1.8	.7	27.9	30	49	.7	[31] 7,700	.10	.06	.7	22
154.	Tomatoes	94.1	23	1.0	.3	4.0	11	27	.6	1,100	.06	.04	.6	23
155.	Turnip greens	89.5	37	2.9	.4	5.4	259	50	2.4	9,540	.10	.56	.8	136
156.	Turnips	90.9	35	1.1	.2	7.1	40	34	.5	Trace	.06	.06	.5	28
	Canned:													
157.	Asparagus	93.6	21	1.6	.3	3.0	20	34	1.0	[32] 600	.06	.09	.8	15
158.	Beans, lima	80.9	72	3.8	.3	13.5	27	73	1.7	130	.03	.05	.5	8
159.	Beans, snap	94.0	19	1.0	0	3.8	27	19	1.4	410	.03	.05	.3	4
160.	Beets	89.4	39	1.0	0	8.7	15	29	.6	20	.01	.03	.1	5
161.	Carrots	92.2	30	.5	.4	6.1	22	24	.6	12,000	.03	.02	.3	2
162.	Corn, white or yellow	80.5	77	2.0	.5	16.1	4	51	.5	[27] 200	.02	.05	.8	5
163.	Peas, green	82.3	69	3.4	.4	12.9	25	67	1.8	540	.11	.06	.9	8
164.	Pumpkin	90.2	38	1.0	.3	7.9	(20)	(36)	(.7)	3,400	.02	.06	.5	(0)
165.	Sauerkraut	93.2	20	1.1	.2	3.4	(46)	(31)	(.5)	Trace	.03	.20	.2	[33] 18
166.	Spinach	92.3	25	2.3	.4	3.0	[34]	33	1.6	6,790	.02	.08	.3	14

NOTE: Asterisk indicates Army ration component; parentheses, imputed value.

[24] Based on peanuts without skins; when skins are included the thiamine value is higher.
[25] 118 mg.; may not be available because of presence of oxalic acid.
[26] 105 mg.; may not be available because of presence of oxalic acid.
[27] Based on yellow corn; white corn contains only a trace.
[28] Based on pared cucumber; unpared contains about 260 I. U. vitamin A per 100 gm.
[29] Green bunching onions contain about 23 mg. ascorbic acid per 100 gm.
[30] 81 mg.; may not be available because of presence of oxalic acid.
[31] If pale varieties only were used, value would be very much lower.
[32] Based on green products; bleached products contain only a trace.
[33] Drained solids only.
[34] 90 mg.; may not be available because of presence of oxalic acid.

Table 2. Nutritive Value of 100 Grams of Selected Foods, Edible Portion — (*Continued*)

Food item	Water	Food energy	Protein	Fat	Carbohydrate	Calcium	Phosphorus	Iron	Vitamin A value	Thiamine	Riboflavin	Niacin	Ascorbic acid
VEGETABLES—Continued													
Canned—Continued:	*Percent*	*Calories*	*Grams*	*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*	*International Units*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
167. Tomato catsup	69.5	110	2.0	0.4	24.5	12	18	0.8	(1,880)	0.09	0.07	2.2	11
168. Tomato juice	93.5	23	1.0	.2	4.3	(7)	(15)	(.4)	1,050	.05	.03	.7	16
169. Tomato puree	89.2	40	1.8	.5	7.2	(11)	(37)	(1.1)	1,880	.09	(.07)	1.8	28
170. Tomatoes	94.2	21	1.0	.2	3.9	(11)	(27)	(.6)	1,050	.05	.03	.7	16
Dehydrated: [35]													
171. *Cabbage, unsulfited [36]	8.8	346	13.7	1.8	68.8	374	274	4.7	520	.41	.37	2.4	189
172. *Carrots	5.6	361	4.0	1.4	83.1	(242)	(102)	(5.9)	117,000	.29	.28	3.2	11
173. *Onions	9.9	350	10.1	1.0	75.2	158	256	3.1	20	.23	.15	1.1	37
174. *Potatoes	7.2	363	7.1	.7	82.0	25	103	3.7	(0)	.25	.10	4.8	26
175. *Sweetpotatoes	5.3	373	5.1	.9	86.1	(76)	(75)	(2.3)	21,900	.18	.14	1.9	34
FRUIT													
Fresh:													
176. Apples	84.1	64	.3	.4	14.9	6	10	.3	90	.04	.02	.2	5
177. Apricots	85.4	56	1.0	.1	12.9	16	23	.5	2,790	.03	.04	.7	4
178. Avocados	65.4	265	1.7	26.4	5.1	10	38	.8	290	.12	.15	1.1	16
179. Bananas	74.8	99	1.2	.2	23	8	28	.6	430	.09	.06	.6	10
Berries:													
180. Blueberries	83.4	68	.6	.6	15.1	16	13	.8	280	(.03)	(.07)	(.3)	16
181. Strawberries	90.0	41	.8	.6	8.1	28	27	.8	60	.03	.07	.3	60
182. Other berries	84.4	65	1.2	.8	13.2	36	34	.9	320	.03	(.07)	(.3)	23
183. Cantaloups	94.0	23	.6	.2	4.6	17	16	.4	[37] 3,420	.06	.04	.8	33
184. Grapefruit	88.8	44	.5	.2	10.1	17	18	.3	Trace	.04	.02	.2	40
185. Grapes	81.6	74	.8	.4	16.7	17	21	.6	80	.05	.03	.4	4
186. Lemons	89.3	44	.9	.6	8.7	(14)	(10)	(.1)	0	.04	Trace	.1	45
187. Limes	86.0	53	.8	.1	12.3	(14)	(10)	(.1)	0	(.04)	(Trace)	(.1)	27
188. Oranges	87.2	50	.9	.2	11.2	33	23	.4	(190)	.08	.03	.2	49
189. Peaches	86.9	51	.5	.1	12.0	8	22	.6	880	.02	.05	.9	8

190.	Pears	82.7	70	.7	.4	15.8	13	16	.3	20	.02	.04	.1	4
191.	Pineapples	85.3	58	.4	.2	13.7	16	11	.3	130	.08	(.02)	(.2)	24
192.	Plums	85.7	56	.7	.2	12.9	17	20	.5	350	15	(.03)	.6	5
193.	Rhubarb	94.9	18	.5	.1	3.8	[38]	25	.5	30	.01	----------	.1	9
194.	Tangerines; other mandarin type oranges	87.3	50	.8	.3	10.9	(33)	(23)	(.4)	(420)	.07	(.03)	(.2)	31
195.	Watermelons	92.1	31	.5	.2	6.9	7	12	.2	590	.05	.05	.2	6
	Canned:													
196.	Apples; applesauce	79.8	80	.2	.1	19.7	(4)	(6)	(.2)	(60)	.01	.01	Trace	1
197.	Apricots	77.3	89	.6	.1	21.4	(10)	(15)	(.3)	1,350	.02	.02	.3	4
198.	Cherries	78.1	86	.6	.1	20.8	(11)	(14)	(.3)	(430)	.03	.02	.2	3
199.	Cranberry sauce	48.1	209	.1	.3	51.4	(8)	(7)	(.3)	(30)	----------	(.04)	----------	2
200.	Fruit cocktail	(80.6)	78	(.4)	(.2)	(18.6)	(9)	(12)	(.4)	160	.01	.01	.4	2
201.	Grapefruit juice	89.4	41	.5	.2	9.4	8	12	.4	Trace	.03	.02	.2	35
202.	Grapefruit segments	79.8	81	.6	.2	19.1	13	14	.3	Trace	.03	.02	.2	30
203.	Orange juice	86	55	.6	.1	12.9	(33)	(23)	(.4)	(100)	.07	.02	.2	42
204.	Peaches	80.9	75	.4	.1	18.2	(5)	(14)	(.4)	450	.01	.02	.7	4
205.	Pears	81.1	75	.2	.1	18.4	(8)	(10)	(.2)	Trace	.01	.02	.1	2
206.	Pineapple juice	86.2	54	.3	.1	13.0	15	8	.5	80	.05	.02	.2	9
207.	Pineapples	78.0	87	.4	.1	21.1	29	7	.6	80	.07	.02	.2	9
208.	Plums; Italian prunes	78.6	84	.4	.1	20.4	8	12	1.1	(230)	.03	.03	.4	1
	Dried:													
209.	*Apple nuggets	1.6	390	1.4	1.0	93.9	24	42	4.1	(0)	.05	.08	.5	11
210.	Apricots [39]	24	292	5.2	.4	66.9	86	119	4.9	7,430	.01	.16	3.3	12
211.	*Cranberries	4.9	409	2.9	6.6	84.4	82	22	3.4	660	.19	.18	.9	33
212.	Peaches [39]	24	295	3.0	.6	69.4	44	126	6.9	3,250	.01	.20	5.4	19
213.	Prunes [40]	24	299	2.3	.6	71.0	54	85	3.9	1,890	.10	.16	1.7	3
214.	Raisins [40]	24	298	2.3	.5	71.2	78	129	3.3	50	.15	.08	.5	Trace
	Synthetic fruit powders, canned:													
215.	*Grape juice [41]	.2	[42] 250	.1	.5	3.1	132	65	.1	(0)	0	(0)	(0)	600
216.	*Lemon juice [43]	1.7	[42] 336	.4	.3	60.8	60	33	1.5	(0)	(0)	(0)	(0)	876
217.	*Orange juice [44]	1.9	[42] 341	1.1	.2	65.1	180	101	2.2	(0)	(0)	(0)	(0)	927

NOTE: Asterisk indicates Army ration component; parentheses, imputed value.

[35] Freshly dehydrated products; some loss of vitamins is to be expected during storage.
[36] If sulfited, the thiamine value would be much lower, and the ascorbic acid value would be about double.
[37] Based on deeply colored varieties.
[38] 51 mg.; may not be available because of presence of oxalic acid.
[39] Sulfured.
[40] Unsulfured.
[41] Citric acid, dextrose, coloring, flavoring, ascorbic acid.
[42] Caloric value of organic acids included.
[43] Powdered lemon juice and corn sirup, dextrose, citric acid, oil of lemon, ascorbic acid.
[44] Powdered orange juice, lemon juice, and corn sirup, dextrose, citric acid, oil of orange, ascorbic acid.

Table 2. Nutritive Value of 100 Grams of Selected Foods, Edible Portion — (*Continued*)

	Food item	Water	Food energy	Protein	Fat	Carbohydrate	Calcium	Phosphorus	Iron	Vitamin A value	Thiamine	Riboflavin	Niacin	Ascorbic acid
	GRAIN PRODUCTS													
	Flour, meal:													
	Corn meal:	*Percent*	*Calories*	*Grams*	*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*	*International Units*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
218.	White, degerminated	12	355	7.5	1.1	78.8	10	140	1.0	(0)	0.16	0.09	0.9	0
219.	White, whole-grain	12	365	9.1	3.7	73.9	18	248	2.7	(0)	.41	.12	1.7	0
220.	Yellow, degerminated	12	356	8.3	1.2	78.0	10	140	1.0	300	.15	.06	.9	0
221.	Yellow, whole-grain	12	365	9.1	3.7	73.9	18	276	2.7	510	.45	.17	2.1	0
222.	Cornstarch	12	352	.5	.2	87.0	Trace	Trace	Trace	(0)	(0)	(0)	(0)	0
	Flour:													
223.	Buckwheat, light	12	354	6.3	1.1	79.7	11	88	1.0	(0)	[45].31	[45].08	[45]2.1	0
224.	Rye, light	11	358	8.9	.9	78.5	18	278	1.3	(0)	.15	.07	.9	0
225.	Rye, whole-grain	10	361	11.2	1.7	75.2	61	369	4.8	(0)	.47	.21	1.7	0
	Soy. See Dry Beans and Peas.													
226.	Wheat, patent	12	355	10.8	.9	75.9	19	93	.7	(0)	.07	.03	.8	0
227.	Wheat, patent, enriched	12	355	10.8	.9	75.9	19	93	(2.9)	(0)	(.44)	(.26)	(3.5)	0
228.	Wheat, self-rising	12	340	10.2	.9	72.9	220	330	.6	(0)	.02	.02	.7	0
229.	Wheat, self-rising, enriched	12	340	10.2	.9	72.9	220	330	(2.9)	(0)	(.44)	(.26)	(3.5)	0
230.	Whole wheat	11	360	13.0	2.0	72.4	38	385	3.8	(0)	.56	.12	5.6	0
	Baked goods:													
	Bread:													
231.	Rye, light	37.6	263	(6.4)	(3.4)	(51.7)	(22)	(96)	(.8)	(0)	.16	(.04)	(1.1)	0
232.	White, enriched	35.9	261	8.5	2.0	52.3	(56)	(100)	(1.8)	(0)	(.24)	(.15)	(2.2)	0
233.	Whole wheat	37	262	9.5	3.5	48.0	(60)	370	2.6	(0)	.28	.15	3.5	0
234.	Cake, light batter type	26.8	327	6.4	8.2	57.0	62	(126)	2.0	----------	.03	.10	.7	0
235.	Cookies, assorted, plain	4.8	438	6.0	12.7	75.0	(22)	(65)	(.6)	(0)	(.04)	(.04)	(.5)	0
236.	Cracker meal; crackers, assorted	4.5	422	9.5	10.3	72.7	22	102	1.5	(0)	(.07)	(0)	(.6)	0
237.	Crackers, graham	5.5	419	8.0	10.0	74.3	20	203	1.9	(0)	.36	.12	1.5	0
238.	Fig bars	13.8	363	4.2	4.8	75.8	(69)	(69)	(1.3)	(0)	(.02)	(.06)	(.9)	0
239.	Pie, apple	----------	266	(2.9)	(9.6)	(42.0)	(11)	(22)	1.9	(0)	(.05)	(.04)	.4	(0)
240.	Pie, cream	----------	223	(2.8)	(9.8)	(31.0)	20	(38)	.5	(0)	.03	.08	.2	(0)
241.	Rolls, plain, enriched	29.4	304	8.2	6.1	54.1	(56)	(100)	(1.8)	(0)	(.24)	(.15)	(2.2)	0
242.	Rolls, sweet, unenriched	29.6	304	7.8	5.4	56.0	(56)	(100)	.5	(0)	.08	.13	.8	0

	Breakfast cereals:													
243.	Corn flakes	9.3	359	7.9	.7	80.3	(10)	56	(1.0)	(0)	(.16)	.08	1.6	0
	Corn flakes, restored.													
244.	Oatmeal	8.3	896	14.2	7.4	68.2	54	365	5.2	(0)	.55	.14	1.1	0
245.	Rice flakes; puffed rice	8.8	363	7.2	.4	82.6	(9)	(92)	.9	(0)	(.05)	(.03)	(1.4)	0
	Rice flakes; puffed rice, restored.													
	Wheat cereals:													
246.	Farina	11	359	11.5	1.0	76.1	21	125	.8	(0)	.06	.06	1.0	0
247.	Farina, enriched	11	359	11.5	1.0	76.1	21	125	(1.3)	(0)	(.37)	(.26)	(1.3)	0
248.	Flakes; puffed wheat	6.2	372	11.9	1.5	77.7	33	353	3.7	(0)	.15	.12	4.2	0
	Flakes; puffed wheat, restored.													
249.	Shredded wheat	7.7	369	10.4	1.4	78.7	(38)	(385)	(3.8)	(0)	.20	.14	4.2	0
250.	Whole-grain, uncooked	8.7	368	11.7	2.0	75.8	38	385	3.8	(0)	.45	.13	4.6	0
	Other cereals:													
251.	Barley, pearled, light	11.1	357	8.2	1.0	78.8	16	189	(2.0)	(0)	.12	.08	3.1	0
252.	Hominy	11.4	357	8.5	.8	78.9	11	70	1.0	(0)	.15	.05	(.9)	0
253.	Macaroni; spaghetti	11	360	13	1.4	73.9	22	144	1.2	(0)	.13	.08	2.1	0
254.	Noodles	9.1	385	14.3	5.0	70.6	24	156	1.9	(200)	(.13)	(.12)	(2.1)	0
	Rice:													
255.	Brown	12.0	356	7.5	1.7	77.7	39	303	5.5	(0)	.29	.05	4.6	0
256.	Converted	(12.3)	351	(7.6)	(.3)	(79.4)	(9)	(92)	(.7)	(0)	.23	.04	3.8	0
257.	White	12.3	351	7.6	.3	79.4	9	92	.7	(0)	.05	.03	1.4	0
258.	Tapioca	12.6	350	6	.2	86.4	12	12	(1.0)	(0)	0	(0)	(0)	0
	SUGARS, SWEETS													
259.	Honey	20	319	.3	0	79.5	5	16	.9	(0)	Trace	.04	.2	4
260.	Jams; marmalades	28	288	.5	.3	70.8	12	12	(.3)	10	.02	.02	.2	6
261.	Jellies	34.5	261	.2	0	65.0	(12)	(12)	(.3)	(10)	(.02)	(.02)	(.2)	4
262.	Molasses, cane	24	240	(0)	(0)	(60)	273	51	6.7	(0)	.08	.16	2.8	(0)
263.	Sirup, table blends	25	296	(0)	(0)	(74)	46	16	4.1	0	0	.01	.1	(0)
264.	Sugar, brown	3	382	(0)	(0)	(95.5)	[46] 76	[46] 37	2.6	(0)	(0)	(0)	(0)	(0)
265.	Sugar, granulated or powdered	.5	398	(0)	(0)	99.5	(0)	(0)	.1	(0)	(0)	(0)	(0)	0

NOTE: Asterisk indicates Army ration component; parentheses, imputed value.

[45] Whole-grain buckwheat flour has approximately 0.61 mg. thiamine; 0.16 mg. riboflavin; and 4.2 mg. niacin per 100 gm.

[46] Based on dark brown sugar; lower values for light brown sugar.

Table 2. Nutritive Value of 100 Grams of Selected Foods, Edible Portion — (*Continued*)

Food item	Water	Food energy	Protein	Fat	Carbohydrate	Calcium	Phosphorus	Iron	Vitamin A value	Thiamine	Riboflavin	Niacin	Ascorbic acid
MISCELLANEOUS	*Percent*	*Calories*	*Grams*	*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*	*International Units*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
266. *Bouillon cubes	(3)	259	17.7	0	47.0	40	510	9.2	(0)	0.03	0.83	[47].6	(0)
267. Chocolate, unsweetened	2.3	570	(5.5)	52.9	(18)	[48]	343	2.5	(0)	Trace	.24	1.1	(0)
268. Cocoa	4.3	329	(9.0)	18.8	(31.0)	[49]	709	2.7	(0)	Trace	(.39)	(2.3)	(0)
269. Coconut, dry, shredded	3.3	579	3.6	39.1	53.2	43	191	3.6	0	Trace	Trace	Trace	(0)
270. Gelatin dessert powder	1.6	392	9.4	0	88.7	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
271. Olives, green	75.2	144	1.5	13.5	4.0	101	15	2.0	420	Trace			
272. Pickles, cucumber	95.2	11	.5	.2	1.9	24	22	.9	190	.01	.02	Trace	7
273. Wheat germ	11.0	389	25.2	10.0	49.5	84	1,096	8.1	(0)	2.05	.80	4.6	(0)
274. Yeast, compressed, baker's	70.9	109	13.3	.4	13.0	25	605	4.9	(0)	.45	2.07	28.2	(0)
275. Yeast, dried, brewer's	7.0	348	46.1	1.6	37.4	106	1,893	18.2	(0)	9.69	5.45	36.2	(0)

NOTE: Asterisk indicates Army ration component; parentheses, imputed value.

[47] Based on vegetable extract type; meat extract type may have up to 27.0 mg. of niacin per 100 gm.

[48] 95 mg.; may not be available because of presence of oxalic acid.

[49] 160 mg.; may not be available because of presence of oxalic acid.

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