Lee Russell McDowell

Vitamins in Animal and Human Nutrition

SECOND EDITION

Iowa State University Press / Ames

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This book is dedicated, with appreciation, to my parents; my wife, Lorraine; my daughters, Suzannah, Joanna, and Teresa and their husbands and children; and to Tony J. Cunha (deceased), the former head of the Department of Animal Science at the University of Florida, for his practical knowledge of the livestock industry and for his support and encouragement to write books.

CONTENTS

PREFACE

Vitamins in Animal and Human Nutrition contains 19 chapters of concise, up-to-date information on vitamin nutrition for both animals and humans. The first chapter deals with the definition of vitamins, general considerations, and the fascinating history of these nutrients. Chapters 2 through 16 discuss the 15 established vitamins in relation to history; chemical structure, properties, and antagonists; analytical procedures; metabolism; functions; requirements; sources; deficiency; supplementation; and toxicity. Chapter 17 deals with other vitamin-like substances, and Chapter 18 reviews the importance of essential fatty acids. The final chapter discusses vitamin supplementation considerations.

An earlier edition of this book with a somewhat similar title was published by Academic Press in 1989. The present book has been completely and vigorously revised with one additional chapter. In the last 10 years, a great deal of new information has been generated in the field of vitamins; this is reflected by the fact that more than half of all the references have been published since the first edition. It is hoped that this book will be of worldwide use and will continue, as the first edition, to be used as a textbook and as an authoritative reference book for use by research and extension specialists, feed manufacturers, teachers, students, and others. An attempt has been made to provide a balance between animal nutrition and clinical human nutrition. Likewise, a comparison between the balance of chemical, metabolic, and functional aspects of vitamins and their practical and applied considerations has been made.

A unique feature is the description of the practical implications of vitamin deficiencies and excesses and the conditions that might occur with various animal species and humans. A large number of photographs illustrate vitamin deficiencies in farm livestock, laboratory animals, and humans. Unlike other textbooks, this one places strong emphasis on vitamin supplementation in each chapter and devotes the last chapter to this subject.

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VITAMINS IN ANIMAL AND HUMAN NUTRITION

chapter one

INTRODUCTION AND HISTORICAL CONSIDERATIONS

DEFINITION OF VITAMINS

Vitamins are defined as a group of complex organic compounds present in minute amounts in natural foodstuffs that are essential to normal metabolism and lack of which in the diet causes deficiency diseases. Vitamins consist of a mixed group of chemical compounds and are not related to each other as are proteins, carbohydrates, and fats. Their classification together depends not on chemical characteristics but on function. Vitamins are differentiated from the trace elements, also present in the diet in small quantities, by their organic nature.

Vitamins are required in trace amounts (micrograms to milligrams per day) in the diet for health, growth, and reproduction. Omission of a single vitamin from the diet of a species that requires it will produce deficiency signs and symptoms. Many of the vitamins function as coenzymes (metabolic catalysts); others have no such role, but perform certain essential functions.

Some vitamins deviate from the preceding definition in that they do not always need to be constituents of food. Certain substances that are considered to be vitamins are synthesized by intestinal tract bacteria in quantities that are often adequate for body needs. However, a clear distinction is made between vitamins and substances that are synthesized in tissues of the body. Ascorbic acid, for example, can be synthesized by most species of animals, except when they are young or under stress conditions. Likewise, in most species, niacin can be synthesized from the amino acid tryptophan and vitamin D from action of ultraviolet light on precursor compounds in the skin. Thus, under certain conditions and for specific species, vitamin C, niacin, and vitamin D would not always fit the classic definition of a vitamin.

CLASSIFICATION OF VITAMINS

Classically, vitamins have been divided into two groups based on their solubilities in fat solvents or in water. Thus, fat-soluble vitamins include A, D, E, and K, while vitamins of the B-complex and C are classified water soluble. Fat-soluble vitamins are found in foodstuffs in association with lipids. The fat-soluble vitamins are absorbed along with dietary fats, apparently by mechanisms similar to those involved in fat absorption. Conditions favorable to fat absorption, such as adequate bile flow and good micelle formation, also favor absorption of the fat-soluble vitamins (Scott et al., 1982). Water-soluble vitamins are not associated with fats, and alterations in fat absorption do not affect their absorption. Three of the four fat-soluble vitamins (vitamins A, D, and E) are well stored in appreciable amounts in the animal body. Except for vitamin B_{12}, water-soluble vitamins are not well stored, and excesses are rapidly excreted. A continual dietary supply of the water-soluble vitamins and vitamin K is needed to avoid deficiencies. Fat-soluble vitamins are excreted primarily in the feces via the bile, whereas water-soluble vitamins are excreted mainly in the urine. Water-soluble vitamins are relatively nontoxic, but excesses of fat-soluble vitamins A and D can cause serious problems. Fat-soluble vitamins consist only of carbon, hydrogen, and oxygen, whereas some of the water-soluble vitamins also contain nitrogen, sulfur, or cobalt.

Table 1.1 lists 14 vitamins classified as either fat or water soluble. The number of compounds justifiably classified as vitamins is controversial. The term vitamin has been applied to many substances that do not meet the definition or criteria for vitamin status. Of the 14 vitamins listed, choline is only tentatively classified as one of the B-complex vitamins. Unlike other B vitamins, choline can be synthesized in the body, is required in larger amounts, and apparently functions as a structural constituent rather than as a coenzyme. *Myo*-inositol and carnitine are not listed in Table 1.1 even though they could fit the vitamin category but apparently for only several species. Chapters 2 through 15 in this book concern the 14 vitamins listed in Table 1.1; Chapter 16 is about carnitine; Chapter 17 concerns vitamin-like substances; and Chapter 18 considers essential fatty acids. The essential fatty acids are not vitamins, but a deficiency disease does result that is similar to vitamin deficiency. The final chapter deals with vitamin supplementation considerations.

■ **Table 1.1 Fat- and Water-Soluble Vitamins with Synonym Names**

Vitamin	Synonym
Fat soluble	
Vitamin A_1	Retinol, retinal, retinoic acid
Vitamin A_2	Dehydroretinol
Vitamin D_2	Ergocalciferol
Vitamin D_3	Cholecalciferol
Vitamin E	Tocopherol, tocotrienols
Vitamin K_1	Phylloquinone
Vitamin K_2	Menaquinone
Vitamin K_3	Menadione[a]
Water soluble	
Thiamin	Vitamin B_1
Riboflavin	Vitamin B_2
Niacin	Vitamin pp, vitamin B_3
Vitamin B_6	Pyridoxol, pyridoxal, pyridoxamine
Pantothenic acid	Vitamin B_5
Biotin	Vitamin H
Folacin	Folic acid, folate, vitamin M, vitamin B_c
Vitamin B_{12}	Cobalamin
Choline	Gossypine
Vitamin C	Ascorbic acid

[a]The synthetic form is water soluble.

VITAMIN NOMENCLATURE

When the vitamins were originally discovered, they were isolated from certain foods. During these early years, the chemical composition of the essential factors was unknown; therefore, these factors were assigned letters of the alphabet. The system of alphabetizing became complicated when it was discovered that activity attributed to a single vitamin was instead the result of several of the essential factors. In this way, the designation of groups of vitamins appeared (e.g., the vitamin "B" group). Additional chemical studies showed that variations in chemical structure occurred within compounds having the same vitamin activity but in different species. To overcome this, a system of suffixes was adopted (e.g., vitamin D_2 and D_3). The original letter system of designation thus became excessively complicated.

With the determination of the chemical structure of the individual vitamins, letter designations were sometimes replaced with chemical-structure names (e.g., thiamin, riboflavin, and niacin). Vitamins have also been identified by describing a function or its source. The term vitamin H (biotin) was used because the factor protected the *haut*, the

German word for skin. Likewise, vitamin K was derived from the Danish word *koagulation* (coagulation). The vitamin pantothenic acid refers to its source, as it is derived from the Greek word *pantos*, meaning "found everywhere."

The Committee on Nomenclature of the American Institute of Nutrition (CNAIN, 1981) has provided definite rules for the nomenclature of the vitamins. This nomenclature is used in this book. The official and major synonym names of vitamins are given in Table 1.1 and in the respective vitamin chapters.

VITAMIN REQUIREMENTS

Vitamin requirements for animals and humans are listed in the Appendix tables at the end of this book and in the appropriate chapter. While metabolic needs are similar, dietary needs for vitamins differ widely among species. Some vitamins are metabolic essentials, but not dietary essentials, for certain species, because they can be synthesized readily from other food or metabolic constituents.

Poultry, swine, and other monogastric animals are dependent on their diet for vitamins to a much greater degree than are ruminants. Tradition has it that ruminants in which the rumen is fully functioning cannot suffer from a deficiency of B vitamins. It is generally assumed that ruminants can always satisfy their needs from the B vitamins naturally present in their feed, plus that synthesized by symbiotic microorganisms. However, under specific conditions relating to stress and high productivity, ruminants have more recently been shown to have requirements, particularly for the B vitamins thiamin (see Chapter 6) and niacin (see Chapter 8). Likewise, vitamin B_{12} cannot be synthesized in the rumen if the essential building block cobalt is lacking in the diet.

The rumen does not become functional with respect to vitamin synthesis for some time after birth. For the first few days of life, the young ruminant resembles a nonruminant in requiring dietary sources of the B vitamins. Beginning as early as 8 days, and certainly by 2 months of age, ruminal flora have developed to the point of contributing significant amounts of the B vitamins (Smith, 1970). Production of these vitamins at the proximal end of the gastrointestinal tract is indeed fortunate for they become available to the host as they pass down the tract through areas of efficient digestion and absorption.

In monogastric animals, including humans, intestinal synthesis of many B vitamins is considerable (Mickelsen, 1956) though not as extensive or as efficiently utilized as in ruminants. Low efficiency of utiliza-

tion is probably related to several factors. Intestinal synthesis in nonruminants occurs in the lower intestinal tract, an area of poor absorption. The horse, with a large production of B vitamins in the large intestine, is apparently able to meet most of its requirements for these vitamins in spite of the poor absorption from this area. Intestinally produced vitamins are more available to those animals (rabbit, rat, and others) that habitually practice coprophagy and thus recycle products of the lower gut. This behavior yields significant amounts of B vitamins to the host animal.

VITAMIN OCCURRENCE

Vitamins originate primarily in plant tissues and are present in animal tissue only as a consequence of consumption of plants, or because the animal harbors microorganisms that synthesize them. Vitamin B_{12} is unique in that it occurs in plant tissues as a result of microbial synthesis. Two of the four fat-soluble vitamins, A and D, differ from the water-soluble B vitamins in that they occur in plant tissue in the form of a provitamin (a precursor of the vitamin), which can be converted into a vitamin in the animal body. No provitamins are known for any water-soluble vitamin. However, the amino acid tryptophan can be converted to niacin for most species. In addition, fat- and water-soluble vitamins differ in that water-soluble B vitamins are universally distributed in all living tissues, whereas fat-soluble vitamins are completely absent from some tissues.

HISTORY OF THE VITAMINS

The history of the discovery of the vitamins is an inspirational and exciting reflection of the ingenuity, dedication, and self-sacrifice of many individuals. Excellent reviews of vitamin history with appropriate references include Funk (1922), McCollum (1957), Wagner and Folkers (1962), Maynard et al. (1979), Scott et al. (1982), Widdowson (1986), and Loosli (1991). Important books that describe the historical discovery of three specific vitamin deficiency diseases include the following: Eijkman, 1890–1896; Funk, 1911; Williams, 1961 (beriberi); Hess, 1920; Carpenter, 1986 (scurvy); Harris, 1919; and Carpenter, 1981 (pellagra). A brief sketch of important events emphasizing early history of vitamins is outlined in Table 1.2.

The existence of nutritive factors, such as vitamins, was not recognized until about the start of the twentieth century. The word "vitamin"

■ **Table 1.2 Brief History of Vitamins (Ancient History–1951)**

2697 B.C.	Beriberi was recognized in China and is probably the earliest documented deficiency disorder.
1500 B.C.	Scurvy, night blindness, and xerophthalmia were described in ancient Egypt. Liver consumption was found to be curative for night blindness and xerophthalmia.
130–200 A.D	Soranus Ephesius provided classical descriptions of rickets.
1492-1600	World exploration threatened by scurvy: Magellan lost four-fifths of his crew. Vasco da Gama lost 100 of his 160 men.
1747	Lind performed controlled shipboard experiments on the preventive effect of oranges and lemons on scurvy. He also developed a method of preserving citrus juice by evaporation and conserving in acid form.
1768–1771	Captain Cook demonstrated that prolonged sea voyages were possible without ravages of scurvy.
1816	Magendie described xerophthalmia in dogs fed carbohydrate and olive oil.
1824	Combe described a fatal anemia (pernicious anemia) and suggested that it could be related to a disorder of the digestive tract.
1849	Choline was isolated by Streker from the bile of pigs.
1881	Lunin reported that animals did not survive on diets composed solely of purified fat, protein, carbohydrate, salts, and water.
1880s	Japanese physician Takaki prevented beriberi in the Japanese Navy by substituting other foods for polished rice.
1897	Eijkman showed that beriberi (thiamin deficiency) from polished rice consumption could be cured by adding rice polishings back into the diet.
1901	Grijins concluded that beriberi was caused by a vitally important food constituent.
1906	Hopkins suggested that substances in natural foods, termed "accessory food factors," were indispensable and did not fall into the categories of carbohydrate, fat, protein, or mineral.
1907	Holst and Frolich produced experimental scurvy in guinea pigs by feeding a deficient diet, with pathological changes resembling those in humans.
1909	Hopkins reported a rat growth factor in some fats.
1910	Vedder was convinced that beriberi was caused by a nutritional deficiency and saved many lives in the Philippines by feeding rice polishings.
1911	The term "vitamine" was first used by the Polish biochemist Funk to describe an accessory food factor.
1913	McCollum and Davis discovered fat-soluble A in butter that was associated with growth.
1919	Steenbock reported that the yellow color (carotene) of vegetables was vitamin A.
1919	Mellanby produced rickets in dogs, which responded to a fat-soluble vitamin in cod liver oil.
1920	Goldberger reported that pellagra was not caused by bacterial infection, but rather was an ill-balanced diet high in corn.
1922	McCollum established vitamin D as independent of vitamin A by preventing rickets after destroying vitamin A activity when bubbling oxygen through cod liver oil.
1923	Evans and Bishop discovered vitamin E. The deficiency caused female rats to abort, while male rats became sterile.
1926	Jansen and Donath isolated thiamin in crystalline form from rice bran.
1926	Minot and Murphy showed that large amounts of raw liver given by mouth daily would alleviate pernicious anemia.

■ **Table 1.2** *continued*

1926	Steenbock showed that irradiation of foods as well as animals produced vitamin D.
1926	Goldberger and Lillie described a rat syndrome, later shown to be riboflavin deficiency.
1928	Bechtel and coworkers established that rumen bacteria of cattle synthesized B vitamins.
1928	Szent-Györgyi isolated hexuronic acid (ascorbic acid, vitamin C) from orange juice, cabbage juice, and cattle adrenal glands.
1929	Moore proved that the animal body converts carotene to vitamin A.
1929	Norris and coworkers reported a curled-toe paralysis (riboflavin deficiency) in chicks.
1929	Castle showed that pernicious anemia resulted from the interaction of a dietary (extrinsic) factor and an intrinsic factor produced by the stomach.
1930	Norris and Ringrose described a pellagra-like dermatitis in the chick, later established as a pantothenic acid deficiency.
1931	Pappenheimer and Goettsch showed that vitamin E is required for prevention of encephalomalacia of chicks and nutritional muscular dystrophy in rabbits and guinea pigs.
1931	Willis demonstrated that a factor from yeast was active in treating a tropical macrocytic anemia seen in women of India.
1932	Choline was discovered to be the active component of pure lecithin previously shown to prevent fatty livers in rats.
1933	Williams and associates fractionated a growth factor from yeast and named it pantothenic acid.
1934	György named a factor that would cure dermatitis in young rats, vitamin B_6.
1934	Dam and Schönheyder described a nutritional disease of chickens characterized by bleeding, thus a new fat-soluble vitamin was discovered (vitamin K).
1935	Wald demonstrated the relation of vitamin A to night blindness and vision.
1935	Kuhn in Germany and Karrer in Switzerland synthesized riboflavin.
1935	Warburg and coworkers first demonstrated a biochemical function for nicotinic acid when they isolated it from an enzyme (NADP).
1935–1937	Cobalt, the central ion in vitamin B_{12}, was shown to be a dietary essential for cattle and sheep by Underwood and coworkers in Australia and in Florida by Becker and associates.
1936	Biotin was the name given to a substance isolated from egg yolk by Kogl and Tonnis that was necessary for the growth of yeast.
1936	Williams and colleagues determined the structure of thiamin and synthesized the vitamin.
1937	Elvehjem and associates found that nicotinic acid cured black tongue in dogs. It was quickly shown to be effective for pellagra in humans.
1939	Vitamin K was isolated by Dam and Karrer of Europe and a few months later in the U.S. from three different laboratories.
1940	Harris and associates completed the first synthesis of biotin.
1942	Baxter and Robeson crystallized vitamin A.
1943–1946	Chemists from the Lederle group crystallized and later synthesized folacin.
1948	Rickes and coworkers in the U.S. and Smith in England isolated vitamin B_{12}.
1951	Smith and coworkers showed that cobalt deficiency in sheep could be prevented by vitamin B_{12} injection.

had not been coined yet. However, what were to be later known as vitamin-deficiency diseases, such as scurvy, beriberi, night blindness, xerophthalmia, and pellagra, had plagued the world at least since the existence of written records. Records of medical science from antiquity attesting to human association of certain foods with either the cause or prevention of disease and infirmity are considered the nebulous beginnings of the concept of essential nutrients (Wagner and Folkers, 1962). Even so, at the beginning of the twentieth century, the value of food in human nutrition was expressed solely in terms of its ability to provide energy and basic building units necessary for life.

In the late 1800s and early 1900s, some scientists believed that life could be supported with chemically defined diets. In 1860, Louis Pasteur reported that yeast could grow on a medium of sugar, ammonium salts, and ash of yeast. Justus von Liebig observed that certain yeasts were unable to grow at all under these conditions, while others grew only very slowly. The ensuing arguments between Liebig and Pasteur did not solve the question. Pasteur's (1822–1895) research showing that bacteria caused disease led scientists trained in medicine to be reluctant to believe the "vitamin theory" that certain diseases resulted from a shortage of specific nutrients in foods (Loosli, 1991). Guggenheim (1995) suggested that the immensely successful germ theory of disease, with the related toxin theory and success of using antisepsis and vaccination, occupied the thoughts of scientists at that time with the idea that only a positive agent could cause a disease.

The first phase leading to the "vitamin hypothesis" began with gradual recognition that the cause of diseases such as night blindness, scurvy, beriberi, and rickets could be related to diet. Although the true cause, nutritional deficiency, was not suspected, these results marked the first uncertainty in the germ and infection theories of origin for these diseases. Finally, in the early 1900s, many scientists in the field of nutrition almost simultaneously began to realize that a diet could not be adequately defined in terms of carbohydrate, fat, protein, and salts. At that time, it became evident that other organic compounds had to be present in the diet if health was to be maintained.

Beriberi was probably the earliest documented deficiency disorder, being recognized in China as early as 2600 B.C. Scurvy, night blindness, and xerophthalmia were described in the ancient Egyptian literature around 1500 B.C. Substances rich in vitamin A as remedies for night blindness were used very early by the Chinese, and livers were recommended as curative agents for night blindness and xerophthalmia by

Hippocrates around 400 B.C. In 1536, Canadian Indians cured Jacques Cartier's men of scurvy with a broth of evergreen needles. In 1747, James Lind, a British naval surgeon, showed that the juice of citrus fruits was a cure for scurvy, but its routine use was not started in the British Navy until 1795. Cod liver oil was used as a specific treatment for rickets long before anything was known about the cause of this disease, and was fed to farm animals as early as 1824. In the 1880s, the Japanese physician Takaki recognized the cause of beriberi in the Japanese Navy as stemming from an unbalanced white rice diet, and virtually eliminated this condition by increasing the consumption of vegetables, fish, and meat and by substituting barley for rice.

The period before the close of the nineteenth century was characterized by the discovery of diseases of nutritional origin in animals, which opened the way for controlled experimental studies of nutritional causes and cures for diseases that were common to both humans and the lower animals. The rat undoubtedly contributed most to the discovery of vitamins from 1900 through the 1920s, although chickens, pigeons, guinea pigs, mice, and dogs also played their part (Widdowson, 1986). In 1890, Christiaan Eijkman, a Dutch physician working in a military hospital in Java, found that chickens fed almost exclusively on polished rice developed polyneuritic signs bearing a marked resemblance to those of beriberi in humans. A new head cook at the hospital discontinued the supply of "military" rice (polished), and thereafter the birds were fed on whole-grain "civilian" rice, with the result being that they recovered. He also noted that beriberi in prisoners eating polished rice tended to disappear when a less highly milled product was fed. Many great advances in science have started from such chance observations pursued by men and women of inspiration.

Beginning in the middle 1850s, German scientists were recognized as leaders of nutrition. In the late 1800s, Professor C. von Bunge (Dorpat, Estonia, Germany, and then at Basel) had graduate students experimenting with purified diets for small animals (Wolf and Carpenter, 1997). In 1881, N. Lunin, a Russian student studying in von Bunge's laboratory, observed that mice died (16–36 days) when fed a diet composed solely of purified fat, protein, carbohydrate, salts, and water. Lunin proposed that natural foods such as milk contain small quantities of as yet "unknown substances essential to life." Other researchers from von Bunge's school and under his influence had essentially the same results as Lunin; these researchers included C.A. Socin (1891), W.S. Hall (1896), W. Falta (1906), and C.T. Noeggerath (1906).

Von Bunge explained away results of these experiments as he was inclined to disbelieve the existence of unknown nutritional factors. Von Bunge believed that iron and phosphorus must be present in preformed organic combinations, which was his explanation for the deaths of laboratory animals consuming purified diets (Wolf and Carpenter, 1997).

In 1906, Frederick Hopkins in England suggested that unknown nutrients were essential for animal life and used the term "accessory growth factors." Hopkins was responsible for opening up a new field of discovery that largely depended on the use of the rat. When Hopkins later discovered that he was not the first to suggest that unknown nutrients were essential, or to conduct animal experiments, he was anxious to share his Nobel prize with Eijkman in 1929.

In 1911, Casimir Funk proposed the "vitamine theory." He had reviewed the literature and made the important conclusion that beriberi could be prevented or cured by a protective factor present in natural food, which he had isolated from rice by-products (Funk, 1911). Funk named the distinct factor that prevented beriberi a "vitamine." This word was derived from "vital amine." Later, when it became evident that not all "vitamines" contained nitrogen (amine), the term became "vitamin." Funk had not believed that all "vitamines" were amines; rather, the name was chosen as a catchword to create interest in the new emerging field of nutrition.

After reviewing the literature between 1873 and 1906, in which small animals had been fed restricted diets of isolated proteins, fats, and carbohydrates, E.V. McCollum of the United States noted that the animals rapidly failed in health and concluded that the most important problem in nutrition was to discover what was lacking in such diets. By 1915, McCollum and M. Davis of Wisconsin discovered that the rat required at least two essential growth factors: a "fat-soluble A" factor and a "water-soluble B" factor. In addition to being required as factors for normal growth, the "fat-soluble A" factor was found to cure xeropththalmia, and the "water-soluble B" factor cured beriberi. At the same time as their work in Wisconsin, T.B. Osborne and L.B. Mendel of Connecticut also established the importance of what was later named vitamin A.

With the pioneer work of Eijkman, Hopkins, Funk, McCollum, and others, scientists began to seriously consider the new class of essential nutrients. The brilliant research of scientists in the first half of the twentieth century led to the isolation of more than a dozen vitamins as pure chemical substances. The golden age of vitamin research was mainly in

the 1930s and 1940s. For vitamin discovery, the general procedure employed was first to study the effects of a deficient diet on a laboratory animal and then to find a food that would prevent the deficiency. Using a variety of chemical manipulations, the particular nutrient involved was gradually concentrated from the food, and its potency was tested at each stage of concentration on further groups of animals (Wagner and Folkers, 1962). This laborious procedure has been simplified in recent years by the discovery that several vitamins are also growth factors for microorganisms that can therefore replace animals for potency testing. By such methods, it is now possible to isolate vitamins and subsequently to identify them chemically. A remarkable achievement has been the direct synthesis by chemists of at least ten vitamins identified in this way. The last vitamin to be discovered was vitamin B_{12} in 1948, which brought the period of vitamin discovery to a close. On the other hand, the possibility that there are still undiscovered vitamins must be recognized (see Chapter 17). More detailed historical considerations for each vitamin are presented in the respective chapters (Chapters 2 through 16).

■ REFERENCES

Carpenter, K.J. (1981). *In* Pellagra, Hutchinson Ross Publishing Company, Stroudsburg, Pennsylvania.

Carpenter, K.J. (1986). *In* The History of Scurvy and Vitamin C. Cambridge University Press, Cambridge, London.

CNAIN. (1981). (Committee on Nomenclature of the American Institute of Nutrition). *J. Nutr. 111,* 8.

Eijkman, C. (1890–1896). Polyneuritis in Chickens, or the Origin of Vitamin Research. Roche, Basel.

Funk, C. (1911). *J. Physiol. 43,* 395.

Funk, C. (1922). The Vitamins, Williams and Wilkins Co., Baltimore.

Guggenheim, K.Y. (1995). *In* Basic Issues of the History of Nutrition, The Magnes Press, Hebrew University, Jerusalem, Israel.

Harris, H.F. (1919). Pellagra, The Macmillan Co., New York.

Hess, A.F. (1920). Scurvy Past and Present, J.B. Lippincott Co., London.

Loosli, J.K. (1991). *In* Handbook of Animal Science (P.A. Putnam, ed.) Academic Press, San Diego.

Maynard, L.A., Loosli, J.K., Hintz, H.F., and Warner, R.G. (1979). Animal Nutrition, 7th Ed., p. 283. McGraw-Hill, New York.

McCollum, E.V. (1957). *In* A History of Nutrition, p. 217. Houghton Mifflin, Boston.

Mickelsen, O. (1956). *Vitam. Horm. 14,* 1.

Scott, M.L., Nesheim, M.C., and Young, R.J. (1982). Nutrition of the Chicken, p. 119. Scott, Ithaca, New York.
Smith, S.E. (1970). *In* Duke's Physiology of Domestic Animals, 8th Ed. p. 634 (M.J. Swenson, ed.), Cornell University Press, Ithaca, New York.
Wagner, A.F., and Folkers, K. (1962). Vitamins and Coenzymes, Wiley (Interscience), New York.
Widdowson, E.M. (1986). *Nutr. Rev. 44,* 221.
Williams, R.R. (1961). Toward the Conquest of Beriberi, Harvard University Press, Cambridge.
Wolf, G., and Carpenter, K.J. (1997). *J. Nutr. 127,* 1255.

chapter two

VITAMIN A

INTRODUCTION

Although all vitamins are equally important in supporting animal life, vitamin A may be considered the most important vitamin from a practical standpoint. It is important as a dietary supplement for all animals, including ruminants. Vitamin A itself does not occur in plants; however, its precursors (carotenoids) are found in plants, and these can be converted to true vitamin A by a specific enzyme located in the intestinal walls of animals. Prior to the discovery of vitamin A, farmers complained that hogs in dry lot or barns did poorly when fed a ration consisting largely of white corn instead of yellow corn. Agricultural chemists would disagree and explain to farmers that chemical analysis showed that white corn and yellow corn were the same with the exception of color. Then came the vitamin era, which explained what the farmers already knew, that white corn has no carotene, the precursor of vitamin A (Ensminger and Olentine, 1978).

In human nutrition, vitamin A is one of the few vitamins of which both deficiency and excess constitute a serious health hazard. Deficiency occurs in endemic proportions in many developing countries and is considered to be the most common cause of blindness in young children throughout the world. McLaren (1986) lists 73 countries and territories that are considered to have potentially serious vitamin A deficiency problems. Vitamin A toxicity usually arises from abuse of vitamin supplementation.

HISTORY

For thousands of years humans and animals have suffered from vitamin A deficiency, typified by night blindness and xerophthalmia (a condition named for the Latin words for dry eye, a manifestation of vitamin A deficiency in which the conjunctiva [covering of the eye] dries out, the cornea becomes inflamed, and the eye becomes ulcerated). The cause was unknown, but it was recognized that consumption of animal and fish livers had curative powers according to records and folklore from early civilizations. One of the earliest known reports was from Eber's Papyrus, an ancient Egyptian medical treatise of about 1500 B.C., which recommended the livers of cattle or poultry as curative agents (Arykroyd, 1958). An early reference to vitamin A deficiency in livestock is the Bible (Jeremiah 14:6): "and the asses did stand in high places, their eyes did fail, because there was no grass." Also from the Bible was the cure of the blind Tobias by means of fish bile.

The observation that experimental animals lose weight and die on purified diets was noted by many investigators toward the end of the nineteenth century. However, it was not until early in the twentieth century that vitamin A was discovered. Its history has been reviewed by a number of authorities (Funk, 1922; McCollum, 1957; Sebrell and Harris, 1967; Loosli, 1991). From 1906 through 1912, Hopkins of Great Britain found that a growth-stimulating principle from milk was present in an alcoholic extract of milk rather than in the ash. In 1909, Hopkins and Stepp found that certain fat-soluble substances were necessary for growth of mice and rats. In the years 1913 through 1915, McCollum and Davis described "fat-soluble A," a factor isolated from animal fats (unsaponifiable fraction of milk fat) or fish oils, which they associated with a growth-promoting activity. In their experiments, the growth of rats ceased prematurely when lard was used as the source of fat in the diet, whereas adequate growth was obtained when the dietary fat was either butter or fat extracted from egg yolk. At the same time, Osborne and Mendel also reported that something in butter appeared to be essential for life and growth in rats. Later, Drummond suggested that the "fat-soluble factor A" should be named vitamin A. In 1919, Steenbock called attention to the fact that among vegetable foods, vitamin A potency was associated with yellow color. He suggested that carotene was the source of the vitamin, but later recognized that the vitamin was not carotene itself because certain potent sources of the vitamin were colorless. Ten years later, Von Euler and associates in

Stockholm obtained a definite growth response when carotene was added to vitamin A-deficient diets. In 1929, Moore produced proof that the animal body transformed carotene into vitamin A. Animals fed carotene had vitamin A in livers, whereas controls did not.

Research in the 1920s and 1930s demonstrated that most animal species need dietary vitamin A. The simultaneous use of chemical methods and experimental rats to test metabolic products resulted in the successful demonstration of vitamin A activity, making it the first confirmed vitamin rather than vitamin B or C, which had received earlier attention. Similar testing methods were used to identify most of the other vitamins.

Only a few years after vitamin A was discovered, it was thought that rickets was also a vitamin A deficiency. Proof that rickets was not caused by vitamin A deficiency was provided by McCollum and associates in 1922. This proof was obtained by oxidizing cod liver oil until vitamin A was destroyed, as shown by the inability of the oil to cure xerophthalmia, and then by demonstrating that the oxidized oil was still effective in curing rickets.

Vitamin A deficiency was shown to be responsible for xeropththalmia and certain forms of night blindness. A link between vitamin A and the visual process was demonstrated in 1935 when Wald, in a series of experiments, obtained a specific form of vitamin A (retinal) from bleached retinas. Wagner and coworkers suggested in 1939 that the conversion of β-carotene into vitamin A occurs within the intestinal mucosa. In 1944, Morton suggested that retinal from bleached visual purple (rhodopsin) might be identical with vitamin A aldehyde; he was able to prove this by synthesis.

The isolation of pure vitamin A became possible when a relationship was found between its growth-promoting activity and the intensity of the Carr-Price antimony trichloride color at 620 nm or the light absorption at 328 nm. Karrer and his group were thus able to obtain a pure oily retinol from vitamin A-rich concentrates. From 1930 to 1931, Karrer and coworkers proposed the exact structural formulas for vitamin A and β-carotene. Six years later, the first crystals of vitamin A were obtained, and still another growth-promoting factor-vitamin A_2 was isolated from freshwater fish liver oils.

In 1942, Baxter and Robeson crystallized pure vitamin A and several of its esters; five years later, they also succeeded in isolation and crystallization of the 13-*cis*-vitamin A isomer. Isler and coworkers synthesized the first pure vitamin A in 1947. In 1950, Karrer and Inhoffen reported the synthesis of β–carotene. In the early 1980s, β–carotene and other carotenoids began to be recognized as important factors (inde-

pendent of provitamin A activity) in potentially reducing the risk of certain cancers and other disease conditions.

CHEMICAL STRUCTURE AND PROPERTIES

Vitamin A itself does not occur in plant products, but its precursor, carotene (Fig. 2.1) occurs in several forms. These compounds are commonly referred to as provitamin A because the body can transform them into the active vitamin. This is how the vitamin A needs of farm animals are met, for the most part, because their rations consist mainly or entirely of foods of plant origin. The combined potency of a feed, represented by its vitamin A and carotene content, is referred to as its vitamin A value. Retinol is the alcohol form of vitamin A (Fig. 2.1). Replacement of the alcohol group by an aldehyde group gives retinal, and replacement by an acid group gives retinoic acid. Esters of retinol are called retinyl esters. Vitamin A in animal products exists in several forms, but principally as long-chain fatty acid esters (e.g., retinyl palmitate).

In addition to retinol, there is another form that is isolated from fish. It was originally distinguished on the basis of a different maximum spectral absorption and named A_2 to differentiate it from the previously isolated form. Vitamin A_2 is closely related to vitamin A_1 but contains an additional double bond in the β–ionone ring (Fig. 2.1). Liver oils of marine fish origin usually average less than 10% vitamin A_2 of the total vitamin A content. The relative biological activity of vitamin A_2 is 40 to 50% that of A_1.

Vitamin A is a nearly colorless, fat-soluble, long-chain, unsaturated alcohol with five double bonds. The vitamin is made up of isoprene units with alternate double bonds, starting with one in the β–ionone ring that is in conjugation with those in the side chain (Fig. 2.1). Since it contains double bonds, vitamin A can exist in different isomeric forms. More common isomeric forms of vitamin A and their relative biological activities are presented in Fig. 2.2.

The most active vitamin A form and that most usually found in mammalian tissues is the all-*trans*-vitamin A. *cis*-Forms can arise from the all-*trans*-forms, and a marked loss of vitamin A potency results. These structural changes in the molecule are promoted by moisture, heat, light, and catalysts. Therefore, conditions present during hay making and ensiling, dehydrating, and storage of crops are detrimental to the biological activity of any carotenoids present.

Precursors of vitamin A, the carotenes, occur as orange-yellow pigments mainly in green leaves and to a lesser extent in corn. Of more than

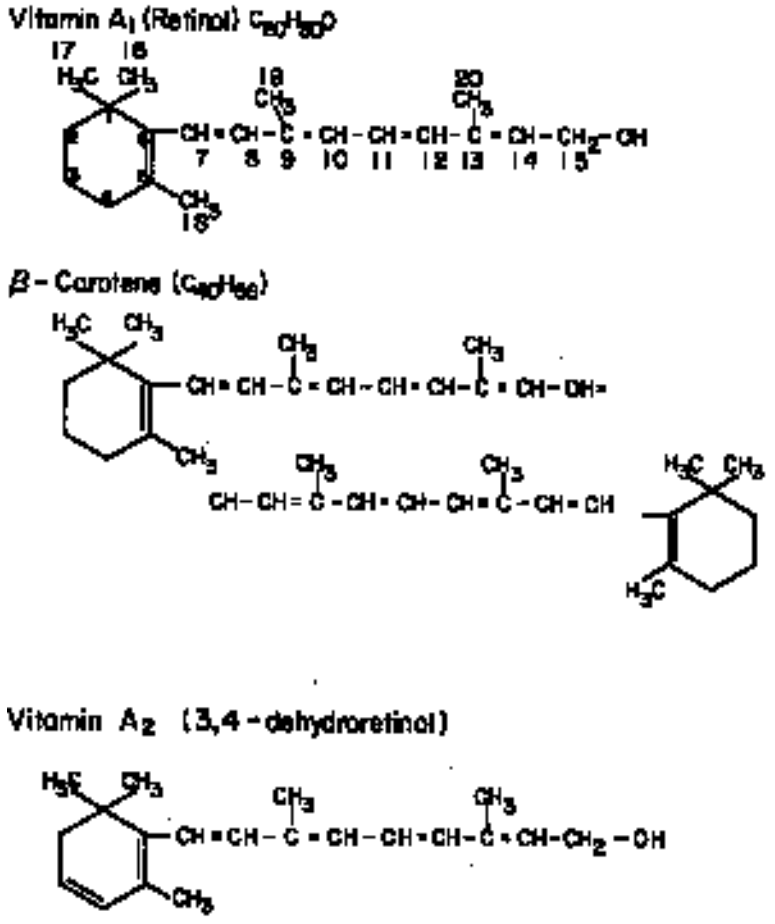

Fig. 2.1 Chemical structure of vitamin A_1, β-carotene, and vitamin A_2.

500 carotenoids that have been isolated from nature, only 50 to 60 possess biological activity. Structures of some of the important carotenoid pigments and their distribution and relative biological activity are presented in Fig. 2.3. Four of these carotenoids—α-carotene, β–carotene, γ-carotene, and cryptoxanthine (the main carotenoid of corn)—are of particular importance because of their provitamin A activity. Vitamin A activity of β–carotene is substantially greater than that of other carotenoids. Lycopene is an important carotenoid for its antioxidant function but does not possess the β–ionone ring structure, and therefore is not a precursor of vitamin A. In humans, β–carotene and lycopene are

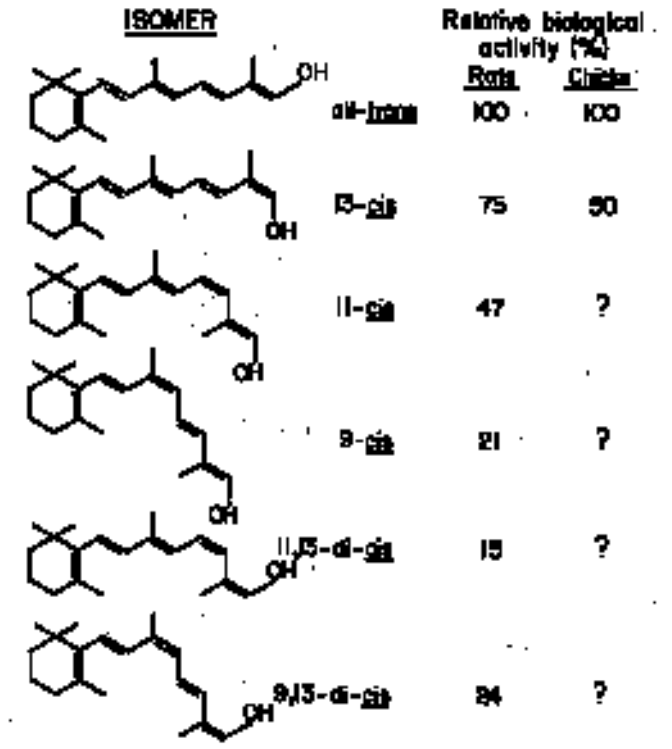

Fig. 2.2 Isomers of vitamin A (retinol). (Adapted from Ullrey, 1972.)

the predominant carotenoids in tissue (Ribaya-Mercado et al., 1995).

Theoretically, 1 mol of β–carotene could be converted (cleavage of the C15=C15´ bond) to yield 2 mol of retinal. However, biological tests have consistently shown that pure vitamin A has twice the potency of β–carotene on a weight-to-weight basis. Thus, only one molecule of vitamin A is formed from one molecule of β–carotene. Loss of potential activity results from inefficient cleavage and intestinal absorption.

Vitamin A activity is expressed in international units (IU) or, less frequently, in United States Pharmacopeia (USP) Units, both of which are of equal value. An IU is defined as the biological activity of 0.300 μg of vitamin A alcohol (retinol) or 0.550 μg of vitamin A palmitate. One IU of provitamin A activity is equal in activity to 0.6 μg of β–carotene, the reference compound. Vitamin A may be expressed as retinol equivalents (RE) instead of IU. By definition, 1 retinol equivalent is equal to 1 μg of

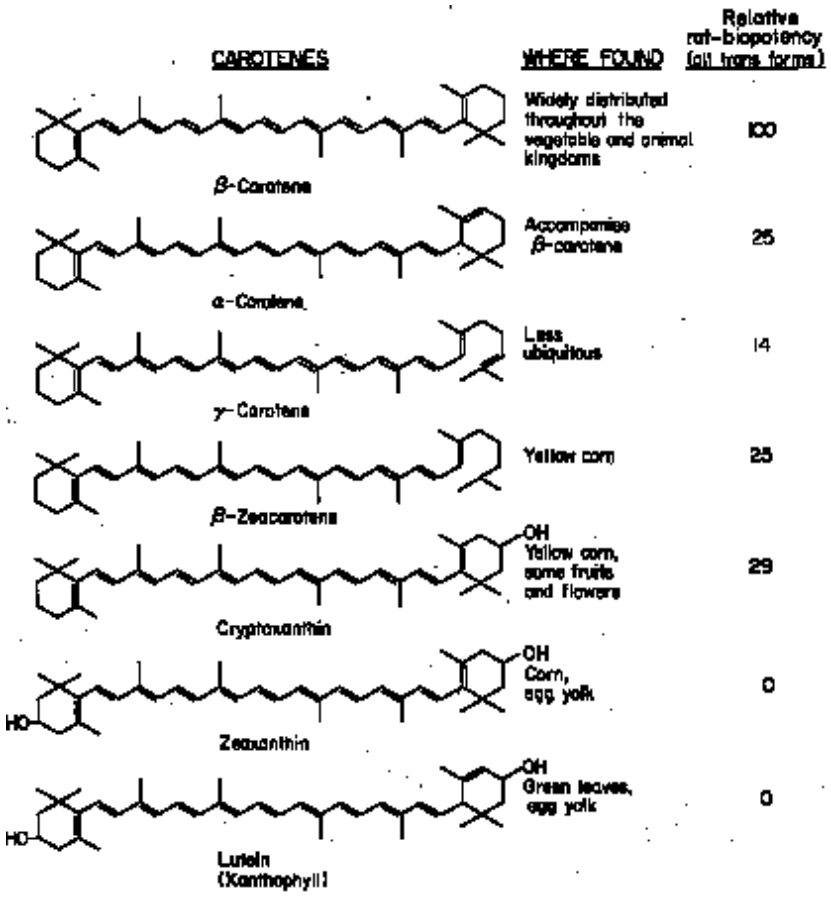

Fig. 2.3 The yellow carotenoids. (Adapted from Ullrey, 1972.)

retinol, 6 μg of β–carotene, or 12 μg of other provitamin A carotenoids. In terms of international units, 1 RE is equal to 3.33 IU of retinol or 10 IU of β–carotene.

ANALYTICAL PROCEDURES

A number of methods are available for carotene and vitamin A determination (Pit, 1985). Biological methods include growth responses of rats or chicks, the storage test (liver), and quantitative evaluations of cell changes in vaginal smears (rats). Physicochemical methods include

color reactions with antimony trichloride (Carr-Price method), gas chromatography, thin-layer chromatography, and spectrophotometric procedures. A number of reports (Grace and Bernhard, 1984; Hidiroglou et al., 1986; Horst et al., 1995) indicate excellent results and high recovery rates from high-pressure liquid chromatography (HPLC). The HPLC procedure is the most common method for analyzing carotenoids, vitamin A and its analogs in pharmaceutical preparations, feedstuffs, and tissues combined with an ultraviolet (UV) detector. A procedure for retinol-binding protein is radioimmunoassay (Vallet, 1994).

METABOLISM

Digestion

Vitamin A in animal products and carotenoids are released from proteins by the action of pepsin in the stomach and proteolytic enzymes in the small intestine (Ong, 1993; Ross, 1993). In the duodenum, bile salts break up fatty globules of carotenoids and retinyl esters to smaller lipid congregates, which can be more easily digested by pancreatic lipase, retinyl ester hydrolase, and cholesteryl ester hydrolase.

A number of factors influence digestibility of carotene and vitamin A. Working with lambs, Donoghue et al. (1983) reported that dietary levels of vitamin A ranging from mildly deficient to toxic levels affect digestion and uptake. Percentage transfer from the digestive tract from supplemental dietary levels of 0, 100, and 12,000 μg of retinol per kilogram were 91, 58, and 14%, respectively. Wing (1969) reported that the apparent digestibility of carotene in various forages fed to dairy cattle averaged about 78%. Variables that influenced carotene digestibility included month of forage harvest, type of forage (hay, silage, greenchop, or pasture), species of plant, and plant dry matter. In general, carotene digestibility was higher than average during warmer months and lower than average during winter.

Several reports indicate that appreciable amounts of carotene or vitamin A may be degraded in the rumen. Various studies with different diets have resulted in preintestinal vitamin A disappearance values ranging from 40 to 70% (Ullrey, 1972). Rode et al. (1990) compared microbial degradation of vitamin A (retinyl acetate) from steers fed concentrate, hay, or straw diets. Estimated effective rumen degradation of biologically active vitamin A was 67% for cattle fed concentrates compared to 16 and 19% for animals fed hay and straw diets, respectively.