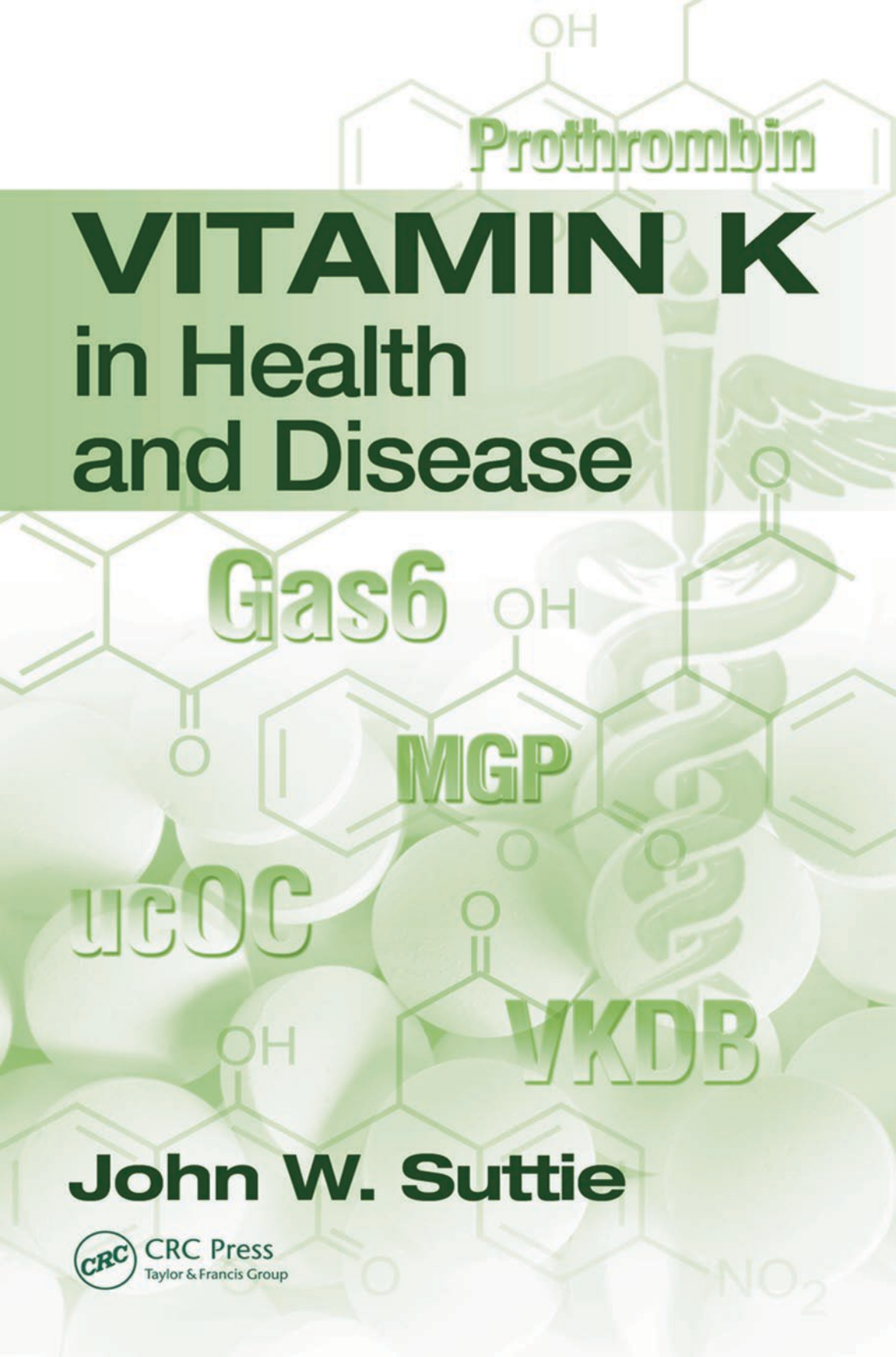


# VITAMIN K in Health and Disease




John W. Suttie

CRC Press
Taylor & Francis Group

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CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742


Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number-13: 978-0-8493-3392-7 (Hardcover)



**Library of Congress Cataloging-in-Publication Data**

Suttie, J. W. (John Weston), 1934-
Vitamin K in health and disease / John W. Suttie.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-0-8493-3392-7 (hardcover : alk. paper)
1. Vitamin K--Health aspects. I. Title.
[DNLM: 1. Vitamin K. W1 OX626 v.26 2009 / QU 181 S967v 2009]

QP772.V55S88 2009
612.3'99--dc22 2009005528

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# Preface

Research efforts directed toward an understanding of the metabolic role of vitamin K have had a somewhat unusual history. The discovery of this fat-soluble vitamin in the early 1930s was based on the observation of a blood clotting disorder in chicks fed a diet designed to lack cholesterol. It was soon shown that the lack of a previously unknown fat-soluble factor was responsible for the hemorrhagic condition, and it was identified and designated "vitamin K." A decrease in the activity of the plasma procoagulant prothrombin was noted early in these studies, and over the next 20 years, three other plasma procoagulants, factors VII, IX, and X, were found to require vitamin K. These four proteins were collectively called the "vitamin K-dependent clotting factors," and it was another 20 years before other vitamin K-dependent proteins were discovered. An understanding of how vitamin K was involved in the synthesis of these clotting factors was not elucidated during the early phase of vitamin K research as scientists did not know how proteins were synthesized. I became interested in vitamin K research in the mid-1960s and was involved in the efforts of a few small research groups to eventually demonstrate that the biological role of vitamin K is to function as one of the substrates of an enzyme that modifies the biological activity of a small number of proteins by the posttranslational conversion of specific Glu residues to γ-carboxyglutamyl (GLA) residues. A substantial amount of this book deals with the vitamin K-dependent carboxylase, with the vitamin K-epoxide reductase, a closely associated enzyme, and with the identification and function of other vitamin K-dependent proteins and their functions. The book focuses more on the history of the development of our current understanding of vitamin K function than would be found in most extensive reviews, but I feel that the retention of some of the history of any field of research is of value. I have also attempted to keep the discussion of recent advances in this research field as current as a publication schedule will allow, as a number of new and exciting venues of research have opened up in the last few years.

The other major focus of this book is directed toward the nutritional and public health aspects of vitamin K research. The vitamin K requirements of animals and humans were historically based on the amount of vitamin needed for the normal coagulation of blood, and in the case of the healthy adult human, it is essentially impossible to produce a prothrombin time that is out of the "normal" range. The low dietary requirement for the vitamin and the low amounts of vitamin K in most foods prevented the publication of accurate databases to calculate dietary intakes for some time, but these are now available. This book provides what I feel will be helpful tables on food sources, population intakes of vitamin K, and the impact of diet on the circulating levels of vitamin K and of other potential markers which have been used to assess vitamin K status. Much of the public health concerns regarding vitamin K intake has been related to the possible role of vitamin K in the promotion of skeletal health. However, more of the recent efforts are directed toward the possible role of vitamin K in preventing vascular calcification, and there are a number of other public

health-related responses that may be associated with vitamin K status that are being currently investigated. These efforts are covered in this book, as well as a discussion of the difficulty in establishing an appropriate dietary reference intake for vitamin K.

As the number of research laboratory leaders involved in vitamin K-related research has always been small, I have had the pleasure of personally knowing most of them as competitors, collaborators, and friends. The open interaction between this group of scientists has aided progress in the field, as have the graduate students, postdocs, and visiting scientists who have worked in my laboratory on vitamin K-related projects: Charles Coven, Dharmishtha Shah, Myrtle Thierry-Palmer, Gary Nelsestuen, David Lorusso, Charles Esmon, Gregory Grant, Johan Stenflo, James Sadowski, Louise Canfield, Jeffrey Finnan, Thomas Carlisle, John McTigue, Reidar Wallin, Donna Whitlon, Megumi Kawai, Lasse Uotila, Ann Larson, Jean Patterson, Takami Ueno, Peter Preusch, Sherwood Lehrman, Tom Brody, Ellen Hildebrandt, Jhan Swanson, Alex Cheung, Richard Pottorf, Angelika Hopfgartner, Kathleen Creedon, Moa Schalin-Karrila, Susumu Funakawa, Gary Wood, Carl Kindberg, James Knobloch, DeAnn Liska, Sharyn Gardill, Yuji Usui, Carol Grossman, Tina Misenheimer, Mark Harbeck, Charlet Keller Reedstrom, Birgit Will-Simmons, Randall Bolger, Pumin Zhang, Cassandra Kight, Margaret Benton, Cynthia Hinck, Alexandra Bach, Richard Moulton, Wei Wu, Robert Davidson, Gwendolyn Alexander, Andrea Garber, Sherri Millis, Anna Sessler, and Zhongjian Lu.

# Author

John Suttie is the Katherine Berns Van Donk Steenbock professor emeritus in the Department of Biochemistry and former chair of the Department of Nutritional Sciences at the University of Wisconsin–Madison. He has broad expertise in biochemistry and human nutrition. Dr. Suttie received his B.S., M.S., and Ph.D. degrees from the University of Wisconsin–Madison. He was a National Institutes of Health (NIH) postdoctoral fellow at the National Institute for Medical Research, Mill Hill, England, before joining the University of Wisconsin faculty. His research activities were directed toward the toxicology of inorganic fluorides and the metabolism, mechanism of action, and nutritional significance of vitamin K. He received the Mead Johnson Award, the Osborne & Mendel Award, and the Conrad Elvehjem Award of the American Society for Nutrition (ASN); the Atwater Lectureship from the Agricultural Research Service; the Bristol Myers-Squibb Award for Distinguished Achievement in Nutrition Research; an award for his contributions to hemostasis from the International Society on Thrombosis and Haemostasis; and in 1996 he was elected to the National Academy of Sciences.

Dr. Suttie served as president of the ASN and as their editor-in-chief of the *Journal of Nutrition*. He served as chairman of the Board of Experimental Biology, president of the Federation of American Societies for Experimental Biology (FASEB), and as a member of the National Academy's Board on Agriculture and Natural Resources and the Food and Nutrition Board of the Institute of Medicine. He also served on the Blood Products Advisory Committee of the Food and Drug Administration and the American Heart Association Nutrition Committee, the Public Policy Committees of the ASN and the American Society for Biochemistry and Molecular Biology, the USDA/NAREEE Advisory Board, the International Life Sciences Institute (ILSI) Food, Nutrition and Safety Committee, and on Dietary Guidelines and Dietary Reference Intakes Committees.

# 1 Historical Background

The dietary essentiality of vitamin K was discovered as the result of a series of experiments carried out by the Danish nutritional biochemist Henrik Dam, working at the University of Copenhagen. Dam was studying the possible essentiality of cholesterol in the diet of the chick and noted [1,2] that chicks ingesting diets that had been extracted with nonpolar solvents to remove the sterols developed subdural or muscular hemorrhages and that blood taken from these animals clotted slowly. As was the case in the discovery of almost all of the vitamins, Dam's observations were not unique. A similar response was subsequently observed by McFarlane and his collaborators [3] at the Ontario Agricultural College in Guelph, Canada, who described a clotting defect seen in experiments designed to assess the nutritive value of different protein sources in purified chick diets. The chicks bled when their wings were banded, and the same response was observed [4] when they fed chicks ether-extracted fish or meat meal in an attempt to establish the vitamin A and D requirements of the chick. Holst and Halbrook [5] in the Division of Poultry Husbandry of the College of Agriculture at the Berkeley campus of the University of California also observed "scurvy-like" symptoms, including internal and external hemorrhages, in chicks fed fish meal and yeast as a protein source. The hemorrhagic condition was cured by the addition of cabbage to the diet, and they concluded from this observation that vitamin C was essential for the chick. It is of interest that, although most North American scientists in the 1930s were capable of reading the German literature, neither the Guelph nor the Berkeley group cited Dam's observations of similar hemorrhagic responses in chicks fed altered diets.

Cribbett and Correll [6] at Kansas State College subsequently failed to reproduce the "scurvy-like" symptoms observed by Holst and Halbrook, and Dam's studies of the hemorrhagic condition as a "new deficiency disease" [7] continued. The observations from Dam's laboratory that lemon juice [2] or ascorbic acid administration [7,8] did not influence the hemorrhagic response provided further evidence against the vitamin C theory, and studies shifted to an attempt to isolate and identify the unknown factor. Progress in elucidating the nature of the hemorrhagic condition was confused to some extent, but not seriously delayed, by the hypothesis of Cook and Scott at the University of California Medical School that the response was caused by the presence of toxic factors in the protein source used [9,10]. Subsequent efforts to identify the new essential factor were initially led by Dam and by Almquist in the Poultry Science Department at Berkeley, who followed up the original observations of Holst and Halbrook.

A series of short, one- to three-page reports by these two groups established the new essential nutrient as being in the nonsterol, nonsaponifiable portion of the lipid extracts of liver and a number of green vegetables [11–13]. In a somewhat more extensive report by Almquist and Stokstad [14] it was noted that Halbrook [15] had found

that when fish meal was used as the protein source in the basal (antihemorrhagic factor deficient) ration, the response was variable; and, if the fish meal was kept moistened for some time, the hemorrhagic disease did not develop. The Berkeley group clearly established (Table 1.1) that drying the fish meal slowly, which would have allowed bacterial action, resulted in a protective response that was not obtained when the meal was dried rapidly. These studies also established that the protective action which was developed in wet fish meal could not be explained on the basis of the removal of some water-soluble prohemorrhagic toxic factor in the fish meal. Although these studies pointed out the possibility that bacterial action in the wet fish meal might be involved in developing the antihemorrhagic properties of the product, the authors did not hypothesize that the bacteria were synthesizing the elusive factor. A subsequent report [16] described the demonstration of the presence of the antihemorrhagic factor in an ether extract of the droppings of chicks fed only the basal diet, with the conclusion that "the vitamin was evidently synthesized by bacterial action either after the droppings were voided or within the lower portions of the digestive tract where absorption could not take place readily, or both." These early advances in establishing the nature of the antihemorrhagic factor were made using only rather insensitive whole blood clotting times and the incidence of hemorrhage or mortality as an end point, and it is of interest to review the types of data that were accepted by leading biochemical journals at that time (Tables 1.2 and 1.3). It is apparent the statisticians were not heavily involved in manuscript review during the 1930s. The difficulty in producing a diet that would induce spontaneous hemorrhagic condition in any species other than the chick was not apparent until later, and the fortuitous

**TABLE 1.1**
**Impact of "Slow Drying" on the Antihemorrhagic Factor in Fish Meal Measured by a Chick Bioassay**

| | % of Chicks | |
|---|---|---|
| **Diet** | **Survived** | **Hemorrhage** |
| Basal diet | 0 | 69 |
| Basal diet with fish meal portion washed with water and rapidly dried at 200°F | 6 | 94 |
| Basal diet with fish meal portion dried by washing with 95% ethanol | 19 | 75 |
| 10% of the 65% polished rice in the basal diet replaced with 10% rice bran | 37 | 50 |
| 10% of the 65% polished rice in the basal diet replaced with moistened rice bran and the diet slowly dried at room temperature | 94 | 0 |

*Note:* Basal diet contained: 20% fish meal (not putrefied), 65% ground polished rice, 12% dried yeast, 1% $CaCO_3$, 1% salt, 1% cod liver oil. White leghorn chicks (15/group) were fed the diets for 6 weeks, while survival rate and incidence of hemorrhagic syndrome were recorded.

*Source:* Adopted from Almquist and Stokstad [14].

**TABLE 1.2**
**Effect of Liver Fat Fractionation on Antihemorrhagic Activity**

| Addition to Basal Diet | Whole Blood Clotting Time (min) |
|---|---|
| None | 10, 20, >30, >60, >1000 |
| 3% hog liver fat | 2, 2, 2, 4, 4 |
| 3% fatty acids from fat | 5, >30, >30, >30, >60 |
| 0.4% nonsaponifiable portion of fat | 3, 4, 12, 12, 13 |
| 0.4% sterol from fat | 15, 60, 75, >60 |

*Note:* Basal diet contained: 20% ether extracted dried hog liver, 15% dried yeast, 2.7% salts, 62.3% sucrose with 4% cod liver oil added. White leghorn chicks (5/group) were fed the diets for 30 days.
*Source:* Adopted from Dam [12].

**TABLE 1.3**
**Effect of Fish Meal and Alfalfa Extracts on Antihemorrhagic Activity**

| Addition to Basal Diet | Mortality (%) | Hemorrhage (%) |
|---|---|---|
| None | 87 | 73 |
| Putrefied fish meal | 20 | 7 |
| Ether extract of putrefied fish meal (2%) | 0 | 0 |
| Dehydrated alfalfa (2%) | 0 | 0 |
| Ether extract of alfalfa, equivalent to 10% alfalfa | 7 | 0 |
| Nonsaponifiable fraction of alfalfa extract | 6 | 0 |
| Saponifiable fraction of alfalfa extract | 60 | 47 |

*Note:* Basal diet contained: 20% fish meal (not putrefied), 65% ground polished rice, 12% dried yeast, 1% $CaCO_3$, 1% salt, 1% cod liver oil. White leghorn chicks (15/group) were fed the diets for 6 weeks, while survival rate and incidence of hemorrhagic syndrome were recorded.
*Source:* Adopted from Almquist and Stokstad [14].

choice of the chick as an experimental animal in the early experiments was essential to the chance discovery of the vitamin.

The term "vitamin K" was first used by Dam in a short note in *Nature* concluding that the factor could not be identical with vitamin A, D, or E; he said "I therefore suggest the term vitamin K for the antihemorrhagic factor" [11]. Although not commented on in the *Nature* article, Dam [17] subsequently pointed out that "the letter K was the first one in the alphabet which had not, with more or less justification, been used to designate other vitamins, and it also happened to be the first letter in the word 'koagulation' according to the Scandinavian and German spellings."

The complex series of enzymatic reactions involved in the coagulation of blood were not well understood in the 1930s, and only prothrombin and fibrinogen were definitely characterized as plasma proteins involved in the formation of a fibrin clot. Dam's group [18] succeeded in preparing a crude plasma prothrombin fraction from chick plasma and demonstrated that its procoagulant activity was decreased when it was obtained from vitamin K-deficient chicks. Quick, at the Marquette University School of Medicine, Milwaukee, who developed the widely used "Quick Prothrombin Time" measurement of plasma procoagulant activity, independently recognized [19] that the clotting defect in chicks fed hemorrhagic diets was due to a decrease in plasma prothrombin. The same report demonstrated that the coagulation defect in sweet clover disease, eventually shown to be due to the presence of Dicumarol™, was also related to a decrease in the prothrombin concentration and that sweet clover disease and the hemorrhagic chick disease were closely related. Although it is now known that the Quick prothrombin time is not specific for prothrombin, at the time of these studies, the other procoagulant vitamin K-dependent proteins had not yet been discovered.

Within a few years of the time that these animal studies were in progress, the hemorrhagic diathesis resulting from obstructive jaundice and the related lack of intestinal bile was shown to be related to a decrease in prothrombin concentration [20–22], and it was realized that this defect was associated with the lack of the same dietary factor responsible for the hemorrhagic disease of chickens that was being studied. Hemorrhagic episodes in patients diagnosed as having hemophilia were, however, not found to have decreased prothrombin concentrations. A short report from the Brinkhous laboratory at the University of Iowa Medical College [20] established that treatment of obstructive jaundice by the administration of bile mixed with a petroleum ether extract of ground alfalfa raised prothrombin levels, as measured by a whole blood clotting time, considerably faster than treatment with bile alone. A very comprehensive review in 1940 by Brinkhous [23] points out the progress made in establishing that hemorrhagic conditions resulting from malabsorption syndromes or starvation and the hemorrhagic disease of the newborn would respond to vitamin K administration. Following these early advances in an understanding of the role of this fat-soluble vitamin, progress in determining the metabolic role of the vitamin was slow. It was not until the early 1950s that the remainder of the classical vitamin K-dependent clotting factors—factors VII, IX, and X—were clearly demonstrated as essential plasma proteins and subsequently shown to require vitamin K for their biosynthesis. It would be over 20 years before the metabolic role of vitamin K as a cofactor in a posttranslational protein modification would be established, and nearly 25 years before any other vitamin K-dependent proteins were discovered.

After the existence of a previously unknown dietary antihemorrhagic factor was demonstrated, an active program to isolate and characterize this factor was begun by a number of research groups. The work was continuously hampered by the tedious nature of the assays used. Early studies by all groups used a "preventative" chick assay where crude fractions of a potentially active compound were added to a hemorrhagic diet that was fed for a number of weeks as the procoagulant ability of blood was monitored. These assays were extremely time consuming, and "curative" assays were subsequently developed. Chicks were first made deficient by feeding a hemorrhagic diet, and the short-term response to the feeding of dietary ingredient, or the administration of a

test compound as a tablet was measured. Either whole blood clotting times [12, 24] or crude plasma clotting factor assays [25, 26] were used as a measure of deficiency.

Early studies of dietary components with high vitamin activity demonstrated [13,14,27] that the active compound was lipid soluble and was present in the nonsaponifiable fraction of a lipid extract. Hexane appeared [28] to be the solvent of choice, and large amounts of other pigments were extracted by this procedure. Fractionation proceeded by the bulk methods in use at that time; adsorption and elution from a wide range of inert materials [29], and differential fractionation into various organic solvents. The isolation was aided by the realization [30] that the active fraction was stable to molecular distillation, and low pressure distillation was used extensively as a method of purification. A detailed description of the progress of the various groups involved in the isolation is available in a review of the field in 1941 by Doisy, Binkley, and Thayer [31].

Dam collaborated with Karrer of the University of Zurich in the isolation of the vitamin, and by 1939 they had succeeded [32] in isolating the vitamin as a yellow oil from alfalfa. Although they described some of its chemical properties, they did not recognize it as a quinone derivative. The group led by Almquist and a group at the Squibb Institute for Medical Research led by Ansbacher [33,34] were also isolating the vitamin from alfalfa, and the St. Louis University group led by Doisy was purifying it from both alfalfa and putrefied fish meal. Pure or nearly pure preparations of the vitamin were obtained by the Doisy [35] and Ansbacher [36] groups at nearly the same time as the Dam and Karrer preparation. It was soon recognized [37,38] that the active preparations were quinones. Almquist and Klose [39] had been studying vitamin K activity in bacterial extracts, and discovered the first known chemical compound with vitamin K activity. They demonstrated that phthiocol (2-methyl-3-hydroxy-1,4-naphthoquinone) (Figure 1.1), which had previously been isolated from *Mycobacterium tuberculosis*, had biological activity [40]. This observation led to a number of studies of the structural requirements of compounds with antihemorrhagic activity and the finding that the 2-methyl group of phthiocol, but not the 3-hydroxyl group, was essential for activity. Reports of the biological activity of menadione (2-Me-1,4-naphthoquinone) (Figure 1.1) were published in the July 1939 issue of the *Journal of the American Chemical Society* by the Almquist [41], Fieser [42], Doisy [43], and Ansbacher [44] laboratories. As it could readily be obtained in a pure form and, on a weight basis, had very high biological activity, much of the subsequent animal work in this area utilized the activity of menadione as a reference. Vitamin $K_1$ (phylloquinone) (Figure 1.1) was characterized as 2-methyl-3-phytyl-1,4-naphthoquinone and synthesized by Doisy's group [45,46], and their identification was confirmed by independent syntheses of this compound by Karrer and collaborators [47], Almquist and Klose [48], and Fieser [49,50].

The Doisy group also isolated a form of the vitamin from putrefied fish meal which in contrast to the oil isolated from alfalfa was a crystalline product. Subsequent studies demonstrated that this compound, called "vitamin $K_2$" contained an unsaturated side chain, and it was characterized [35] as 2-methyl-3-(*all trans*-farnesylfarnesyl)-1,4-naphthoquinone, the compound now known as menaquinone-6. This structure was assumed to be the correct structure of the first menaquinone isolated for many years. However, Isler et al. [51] later demonstrated that a crystalline form of the vitamin isolated by Doisy's method and shown by mixed melting point determination to be identical to Doisy's compound contained seven, not six, isoprenyl units and was in

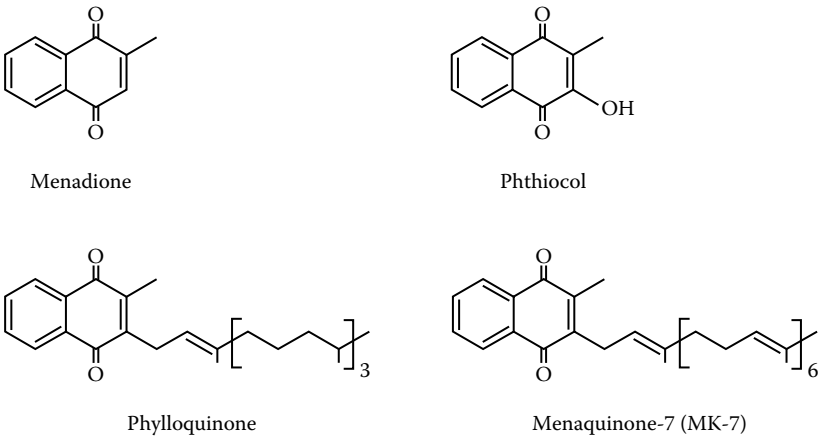


**FIGURE 1.1** Structures of phthiocol, the first chemical compound shown to have antihemorrhagic activity; menadione, the compound used as a standard in most of the early studies involved in isolating the natural antihemorrhagic factor; phylloquinone, the form of the vitamin first isolated from alfalfa; and menaquinone-7, one of a series of compounds with antihemorrhagic properties that were isolated from bacteria.

fact 2-methyl-3-(*all trans*-farnesylgeranylgeranyl)-1,4-naphthoquinone (Figure 1.1) or menaquinone-7, not menaquinone-6. Isler et al. [51] also demonstrated that the crude product obtained from putrefied fish meal did contain some menaquinone-6.

The elucidation of the structure of vitamin K was an extremely competitive research area with a number of large groups involved. Some indication of the effort involved can be gained from an inspection of the large number of papers published in the 1939 issues of the *Journal of the American Chemical Society*, the *Journal of Biological Chemistry*, and *Helvetica Chimica Acta*. Many of these are short notes that appeared within a few weeks of submission, and numerous notes and full papers appeared in various other journals during that year. The result of this concentration of effort in characterization and synthesis was an extremely rapid solution to what was at that time a very difficult problem in organic chemistry. It is not easy at this time to assess the importance of observations made by the different groups involved or to assign priority and credit. A review of the literature from the 1930s does, however, make it difficult to understand why Almquist did not share the Nobel Prize which was awarded to Dam and Doisy in 1943 (Figure 1.2).

Details of the progress in isolation and characterization of vitamin K are available in reviews written by Doisy [31], Almquist [52], and Dam [53] shortly after the characterization of the vitamin. Doisy did not present a lecture upon his receipt of the 1943 Nobel Prize in Physiology and Medicine, but some insights into his progress in the determination of the structure and synthesis of vitamin K are available in an autobiography published in the 1976 issue of the *Annual Review of Biochemistry* [54]. Dam's Nobel lecture [17] contains a number of personal insights into his role in these investigations. Almquist [55,56] has also reviewed his personal involvement in the discovery of vitamin K, and Jukes [57] and Olson [58], both of whom were personally acquainted with the investigators involved, have provided their own insight

In 1943, the Nobel Prize for Physiology or Medicine was awarded to Henrik Carl Peter Dam of the Polytechnic Institute of Copenhagen, Denmark, for his "discovery of vitamin K" and to Edward Adelbert Doisy of the Saint Louis University at St. Louis, Missouri, for his "discovery of the chemical nature of vitamin K." Biographies of each are available at http://nobelprize.org/medicine/laureates/1943/index.html. Herman James Almquist also contributed substantially to the efforts to identify, characterize, and synthesize the natural antihemorrhagic factor but was not included in the award. A biographical sketch of H. Almquist published in 1986 by Jukes (53) indicates that Almquist's publication (13) regarding evidence for the presence of this factor, which followed Dam's publication (11) by 10 weeks, had been held up by the Berkeley administration for a substantial period. The Jukes biography also contains a 1944 letter from Dam to Almquist which suggests that he was not very comfortable with the fact that Almquist had not shared in the prize.

**FIGURE 1.2** The discovery of vitamin K led to the award of a Nobel Prize in 1943.

into the race to identify and characterize the antihemorrhagic factor. A biographical sketch of Almquist prepared by Jukes [59] provides additional information on the early studies of this important fat-soluble vitamin.

Additional extensive reviews of the vitamin K field were published prior to the development of an understanding of the biochemical role of the vitamin. Dam [60] and Isler and Wiss [61] surveyed the vitamin K literature in the *Vitamins and Hormones* series. Both the first and second editions of *The Vitamins* [62,63] contain multi-authored sections dealing with various aspects of chemistry and biological activity of vitamin K, and the proceedings of a 1966 symposium held in honor of Professor Dam have been published [64] in *Vitamins and Hormones*.

Very little research that substantially advanced an understanding of the metabolic and nutritional roles of vitamin K was published from the 1940s to the mid-1960s. Three additional plasma procoagulants, factor VII [65,66], factor X [67], and factor IX [68,69] were identified in the early 1950s; and, along with prothrombin (factor II), they came to be known as the "vitamin K-dependent clotting factors." However, the molecular basis for this distinction was not known. In the mid-1950s Martius and Nitz-Litzow [70] and Martius [71] proposed that vitamin K functioned as an electron carrier in the mammalian respiratory chain and that a deficiency of the vitamin would lead to a decrease in ATP production needed for protein synthesis. The apparent rapid turnover rate of prothrombin was postulated to make it a particularly sensitive protein. Studies from a number of laboratories that failed to support this theory have been reviewed by Johnson [72]. In retrospect it is easy to understand that the task of determining how the amount or the activity of a specific protein such as

prothrombin could be altered by vitamin K status was impossible if researchers did not know how proteins were synthesized. It is also understandable that the nutritional importance of vitamin K and an understanding of the dietary requirements for the vitamin would be difficult to obtain when the analytical capacity to measure microgram and nanogram amounts of the vitamin in foods and tissues was not yet developed.

Research activity directed toward an understanding of the role of the vitamin K-dependent proteins that had been identified and the function of vitamin K in their synthesis increased in the early 1960s as an understanding of mammalian protein synthesis became available. Researchers whose expertise was in hematology and nutritional biochemistry began to accumulate the data that led to the identification in the mid-1970s of vitamin K as a substrate for a γ-glutamyl carboxylase that drove a posttranslational modification of vitamin K-dependent proteins. Although a large number of investigators contributed to the continual advance in an understanding of the metabolic role of vitamin K in the mid- to late-1960s much of the effort was centered in the laboratories of Connor Johnson at the University of Illinois, Bob Olson at St. Louis University, and John Suttie at the University of Wisconsin. During the same period, Caen Hemker's laboratory at the University of Lieden in the Netherlands identified a circulating, inactive form of prothrombin in the plasma of patients treated with oral anticoagulants. Gary Nelsestuen's group at the University of Minnesota and Johan Stenflo's laboratory at the University of Lund in Sweden provided the characterization of γ-carboxyglutamic acid (Gla) as the missing factor in the "abnormal" prothrombin, and this advance led directly to the demonstration of the vitamin K-dependent carboxylase as the metabolic enzyme with a requirement for the vitamin. Efforts to characterize the properties of this enzyme, detail its mechanism of action, and purify it were actively carried out in a number of laboratories, but initially in the Suttie laboratory, in the laboratory of Barbara and Bruce Furie at Harvard, Paul Dowd at the University of Pittsburgh, Kathy Berkner at the Cleveland Clinic, Cees Vermeer at Maastricht University in The Netherlands, and Darrel Stafford at the University of North Carolina. The products of the vitamin K-dependent carboxylase were Gla residues and the 2,3-epoxide of vitamin K which was shown to be recycled to the reduced form of vitamin K by a vitamin K epoxide reductase (VKOR). Studies from the laboratory of John Matschiner at St. Louis University were key to an understanding of this recycling activity, and the properties of the enzyme were investigated by many of the laboratories working on the carboxylase, and also by Reidar Wallin's group at Wake Forest, and Johannes Oldenburg at the University of Bonn in Germany.

The modification of the primary gene product by the formation of Gla residues gave these proteins their unique biological activity, and the presence of Gla residues in these proteins resulted in the identification of additional vitamin K-dependent proteins by biochemists and molecular biologists which continues to this time. These efforts occurred in the same period of time that the structures of the vitamin K-dependent coagulation factors were being described and the complex process involved in the generation of thrombin from prothrombin was being elucidated. Major efforts in this direction were led by Earl Davie at the University of Washington, Harold Roberts at the University of North Carolina, Craig Jackson at

Washington University, Ken Mann at the University of Vermont, and Chuck Esmon at the Oklahoma Medical Research Foundation. The range of vitamin K-dependent proteins was expanded when Paul Price at the University of California at San Diego and a group led by Paul Gallop and Jane Lian and their collaborators at Harvard found Gla proteins in bone, and Baldomero Olivera at the University of Utah found a large number of Gla-containing peptides in the venom of the *Conus* snail. The availability of analytical techniques suitable for measuring the content of vitamin K in foods, plasma, and tissues was driven by efforts in Martin Shearer's laboratory in London and Jim Sadowski's group at Tufts University, and these efforts have enabled a small number of laboratories to develop the databases of the vitamin K content of foods that are now available. Relationships between vitamin K intake and plasma and tissue concentrations have been determined by these laboratories along with Vermeer's group, while efforts to understand the degradative metabolism of vitamin K have been led by Shearer's laboratory. The metabolic functions of two vitamin K-dependent proteins, osteocalcin and Matrix Gla protein (MGP), are still not well defined, but interest in these proteins has led to epidemiological and controlled population studies that are directed toward an understanding of the possible role of the vitamin in the prevention of chronic disease. These efforts are widespread, but much of it has been initiated by the laboratory of Sarah Booth at Tufts University and by Shearer, Suttie, and Vermeer, as well as a large number of studies in Japan, much of it centered in the Sato and Iwamoto laboratories.

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