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Vitamin K : ts discovery, development and clinical application

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VITAMIN K; ITS DISCOVERY, DEVELOPMENT AND CLINICAL APPLICATION.

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-Introduction-

Vitamin K is one of the more recent additions to the slowly unraveling vitamin complex, and takes its place in the fat soluble group along with vitamins A and D. The vitamin K complex has not as yet been thoroughly investigated, but already there has been isolated vitamins K1, K3 and K4 and these substances are being used clinically.

The story of vitamin K is interesting because of its accidental discovery almost simultaneously in three independent laboratories, and its rapid development since that time. It is interesting because of its great import to serious cholemic hemorrhage which, though not of high incidence, has been a serious problem confronting surgeons for many years. It is interesting because it means the prevention and cure of hemorrhage of the newborn, a condition which has never been well understood and a serious problem to pediatricians for years.

In presenting this subject, I have considered the experimental work because it is essential to an accurate understanding of vitamin K therapy. Much of the material in the first section of the story has no practical value, but it is essential in arriving at certain conclusions concerning indications for vitamin K therapy.

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Discovery of Vitamin K

In 1929, Henrik Dam (1), working in the laboratories of the University of Copenhagen, began studies of the sterol metabolism of the chicken. During the course of investigation he observed that chicks fed on a fat-free diet for two or three weeks had a marked tendency to large subcutaneous and intramuscular hemorrhages and certain pathological changes in the stomach. He further noted that in all cases the growth was subnormal, and that in one chick the clotting time of the blood was markedly increased. Dam demonstrated at that time that the low content of sterol and fat in the diet was of no importance because the addition of cholesterol and cod liver oil did not prevent the symptoms.

Also in the year 1929, Roderick (2), working for the United States Government, was sent out to discover the cause of "sweet clover disease" in cattle. He found that cattle fed on spoiled sweet clover hay were prone to develop fatal hemorrhage during the dehorning process. The disease is characterized by the development of a gradually increasing prolongation of the time required for the blood of the animals to coagulate when it is drawn into tubes or in the bleeding times of injuries and wounds. Examination of the cattle that died revealed a central liver necrosis. He observed further that

all cattle that obtained alfalfa in addition did not develop these hemorrhages, thereby making it evident that the sweet clover acquires specific disease-producing properties when it spoils in the curing process. In his subsequent analytical work, Roderick showed that the delayed coagulability of the blood in "sweet clover disease" involves a reduction in the plasma prothrombin and that the diminution parallels the delay in the coagulation time.

Apparently independently, other laboratories observed the hemorrhagic chick disease in the three years from 1931 to 1933 (3,4,5). As in the case of Dam's discovery, the finding was an incidental one, noted in the course of experiments in which chicks were fed purified diets low in fats. In 1931, while studying the effect of various proteins on chick growth, McFarlane, Graham and Hall (3) similarly noted spontaneous hemorrhages in their chicks. This hemorrhagic tendency was observed in the same laboratory by McFarlane, Graham and Richardson (4) in their work on the fat-soluble vitamin requirements of the chick. In both instances a prolonged blood clotting time was noted but could not be explained by these workers.

It was considered originally that the hemorrhagic syndrome was due to scurvy, despite earlier reports that

chicks are able to synthesize vitamin C. Subsequent studies, however, served to rule out scurvy as well as other known vitamin deficiencies. Dam's experiments (1) clearly show that ascorbic acid does not prevent the development of the hemorrhagic syndrome and the cause of the disease cannot be a diminished absorption of vitamin C from the intestine or a diminished production of the vitamin in the organism since subcutaneous injections of ascorbic acid were ineffective. It must be considered unlikely that the factor is vitamin A or D because a relatively large supply of cod liver oil was added to the basal diet of the chicks (1,3,4,5) without any noticeable change in the symptoms. Symptoms of polyneuritis or pellagra were never observed in connection with these diets (1,5), thus rendering it unlikely that the syndrome is due to a deficiency of vitamin B1 or B2. The exclusion of vitamin E was made by Dam (6) when he added large quantities of wheat germ and wheat germ oil to the basal diet and found that they did not afford complete protection against the disease. As a result of these experimental findings, it was first suggested by Dam in 1934 (1) that this hemorrhagic condition observed in chicks was a new dietary deficiency disease which he believed was due to an unknown substance lacking in the fat-free diet that was essential for the proper coagula-

tion of the chick's blood. Since the Danish word for coagulation is spelled "Koagulation", Dam named this unknown substance vitamin K, a terminology which has been adopted generally.

It has been mentioned that the basal diets which were employed by Dam (1,6), McFarlane and his coworkers (3,4) and Holst and Halbrook (5) in their respective studies, and which caused the hemorrhagic chick disease, were low in fat content. However, the first definite evidence of the fat-soluble nature of the vitamin is found in the early work of McFarlane, Graham, and Richardson (4). They discovered that chickens fed on a diet containing ether-extracted fish meal suffered from the hemorrhagic tendencies, but that those fed on unextracted fish meal did not suffer the hemorrhages. Later Almquist and Stokstad (7) showed that fish meal and yeast, both of which form large parts of many of the hemorrhage-producing purified diets, contain traces of the vitamin. The ether extraction of these substances before their incorporation in the basal diets is now performed for the successful production of the disease. In the summer of 1935, first Dam (6) and later Almquist and Stokstad (7), obtained concentrates of the vitamin from natural sources by ether extraction, thus establishing the fat-soluble nature of the vitamin. These workers first used purified

fish meal and hog-liver fat as natural sources of the vitamin concentrate, describing it as occurring in the easily soluble non-sterol fraction of the unsaponifiable matter.

Following the recognition of the hemorrhagic chick disease as a nutritional disorder, the relationship of this new dietary factor to blood clotting was soon established. The characteristic prolonged clotting time incident to the disease had been observed by the original workers (1,3,5), but it was Schønheyder in 1936 (8) who first suggested that the hemorrhagic tendency is the result of a hypoprothrombinemia. Shortly thereafter, he, in collaboration with Dam and Tage-Hansen (9) of Copenhagen, published results of their studies on the mode of action of vitamin K and provided evidence which definitely pointed to a lowered prothrombin level in the hemorrhagic chick disease. They first obtained crude prothrombin preparations from normal chick plasma by acetone precipitation and by acetic acid precipitation. Added to a mixture of K-avitaminous chick plasma and thromboplastic muscle extract, the prothrombin preparations corrected the clotting defect. On the other hand, similar preparations from K-avitaminous chick plasma were without effect. Also, addition of a watery emulsion of a vitamin K concentrate to the plasma-thromboplastin

mixtures failed to restore the clotting time to normal. From this they concluded that the clotting abnormality was due to a low plasma prothrombin level, and that vitamin K itself did not possess prothrombic activity.

A more detailed study of the prothrombin in hemorrhagic chick disease was carried out by Quick (10) in 1937. On a series of chicks maintained on a vitamin K-deficient diet, he estimated the plasma prothrombin time at various intervals throughout the course of the disease. The progressive fall of prothrombin as the disease progressed is shown in the following data taken from his experiment:

Days on K-deficient diet.	Prothrombin, per cent of normal chicks.
1	100
4	40
8	22
11	19
15	18
18	10

A distinct hemorrhagic tendency appeared when the low prothrombin levels were reached. The addition of a small amount of alfalfa meal (5%) to the basal diet effectively restored the plasma prothrombin and cured the hemorrhagic tendency.

Following this important study by Quick, rapid advances in our general knowledge of vitamin K were made in various laboratories by further experimental efforts.

-Vitamin K Deficiency in Experimental Animals-

The study of vitamin K deficiency in chicks has served as the source of a great part of our present concept of the vitamin, its chemical constitution and mode of action in the organism. Recent findings of the research laboratories are important because of their practical application to certain disease states in the human, which will be discussed later.

From Quick's study of the plasma prothrombin level in hemorrhagic chick disease (10), and later studies of a similar nature (11), it was determined that hemorrhages do not occur until the prothrombin has declined to approximately ten to fifteen per cent of normal. In the development of the disease, the appearance of hemorrhage is preceded regularly by a prolongation of the clotting time of the whole blood.(12). Tidrick, Joyce and Smith (11) made a detailed study concerning the rate at which the plasma prothrombin level falls when newly hatched chicks are placed on a vitamin K-deficient diet. They found that the whole blood clotting time becomes prolonged when the prothrombin level falls to about 30% of the normal level for chicks of the same age. Thus, early in the course of the disease when the vitamin deficiency is less severe, the plasma prothrombin may be reduced considerably and the clotting time still be within normal limits. It would appear that plasma pro-

thrombin determinations are essential if any but the more severe grades of deficiency are to be detected.

The production of the hemorrhagic chick disease is dependent on the complete removal of vitamin K from the diet, because the presence of minute traces will prevent the development of a severe deficiency. It has been pointed out previously that both fish meal and yeast, which comprise a large part of almost all basal diets, contain traces of the antihemorrhagic factor (7), and that this led to the ether extraction of these substances before their incorporation in the diet. The original diet employed by Dam (1) contained no fish meal and was an artificial diet consisting of vitamin A-free caseinogen, 20 parts; marmite, 10 parts; soluble starch, 65.5 parts; salts, 4.5 parts; plus varying amounts of cod liver oil concentrates. The diet finally used by Almquist and Stokstad (7) and widely adopted by other investigators consists of the following:

Fish meal, extracted with ether	17.5 parts
Brewer's yeast, extracted with ether	7.5 parts
Ground polished rice	73.0 parts
NaCl, plus ferrous and cupric sulfates	1.0 part
Cod liver oil	1.0 part

This diet probably contains a minimal amount of vitamin K, since Almquist (13) noted the hemorrhagic chick disease in chicks on this diet as early as the fifth day after hatching, the average appearance of hemorrhagic

symptoms being about the third week. Nevertheless, it was repeatedly observed that a certain number of chicks failed to develop hemorrhages or prolonged clotting times during a four-week period on the diet (14)

Recognition of the synthesis of the vitamin by bacteria was first reported by Almquist and Stokstad (7) in their earlier work. They later showed that rice bran, fish meal and other foods which had been stored in a moist condition developed vitamin K activity (14), and that the incorporation of these substances in the basal diet prevented the development of the hemorrhagic chick disease. They also demonstrated the curative effect of putrified fish meal on vitamin K-deficient chicks with definite hemorrhagic symptoms (15), indicating that the bacterial growth produced enough vitamin K to restore the chick to normal. Almquist, Pentler and Mecchi (15) in 1938 found several different types of bacteria in the putrified material and isolated one strain in pure culture. They inoculated this organism to wet, sterile, ether-extracted fish meal and found that it had a potent curative action on K-avitaminous chicks after a ten-day period of incubation. Putrefying fish meal was used subsequently by Osterberg and various other workers as a potent source of vitamin K.

In addition to the possibility of production of the

antihemorrhagic factor in the diet by bacterial putrifaction of the food, other factors influencing the incidence of this dietary hemorrhagic disease must be considered. Almquist and Stokstad (16) discovered that the vitamin is present in the fecal matter of the chicks, even though they may be held on a vitamin K-free diet for a long period of time. They believed the vitamin to be produced in the lower portion of the intestine, too low for absorption, since many of the chicks were suffering the disease in the presence of synthesis of the vitamin within the body. Because of the vitamin K content of the feces, these workers found it necessary to prevent coprophagy among the chicks in order to produce the disease successfully.

In the early experiments with vitamin K deficiency, Holst and Halbrook (5) and Almquist and Stokstad (7) noted that the severity of the disease increased with the rapidity of growth of the chick. Another factor influencing the development of the disease, also suggested by Almquist and Stokstad (16), is the transfer of the antihemorrhagic vitamin from the diet of the hen to her chick. This transfer is apparently carried out through the yolk of the egg, and the vitamin is then stored up in the body of the chick in sufficient quantities to protect against a K-free diet for a period of about two

weeks. Experiments were carried out in which two series of chicks were maintained on a K-free diet with supplements of egg yolk and egg albumen, respectively, for two or three weeks. It was found at the end of that period that the yolk supplements to the basal diet had protected against the development of the chick disease, while the egg albumen supplements had not protected. This suggests the possibility that some hens may receive a larger supply of vitamin K in their diet and thereby transfer a greater protection to their chicks.

Seasonal variations in the vitamin reserves of the chicks probably occur, as green plants are the most potent food source of the vitamin (1). This would explain the observations of Thayer and associates (17) that the chick deficiency is most severe in the late winter and early spring months, and of Tidrick, Joyce and Smith (11) that normal chicks have a higher prothrombin level in the summer than in the late fall.

Even if the factor of varying reserves is controlled, individual variations in the susceptibility of chicks to the deficiency have been reported by Almquist and his associates (14). They also demonstrated that although bile acids are important in the absorption of the vitamin, their administration failed to erase these individual differences. The discovery of so many factors

influencing the production of the disease is important in explaining away inconsistencies and failures to produce the disease experimentally, thus definitely establishing the role of the vitamin as a necessary dietary factor for normal chicks.

In regard to the experimental production of hemorrhagic disease in mammals by dietary measures alone, it may be said that, in general, they have not been successful. Dam, Schønheyder and Lewis (18) fed young rats, quinea pigs, rabbits, dogs and pigs a K-deficient diet for a period of two and a half months and obtained no evidence of the disease. They concluded that the fact that certain species of animals can apparently dispense with vitamin K in their diet may be explained in one of three different ways: (1) The animal has no need of the vitamin, (2) the animal can synthesize the vitamin, or (3) the vitamin is synthesized by bacteria in the intestine. The last was accepted as the most likely explanation because of the previous report of Almquist and Stokstad (16) that provided evidence for the view that bacterial synthesis of vitamin K occurs in the gut of the chick, and that it may be the reason why, under certain conditions, the development of the symptoms of the chick may be retarded. It is a known fact that absorption from the lower part of the intestine is much

greater in the mammal than in the chick and, on this basis, Dam and his associates concluded that mammals rarely show vitamin K deficiency except in cases of faulty absorption from the intestine.

It is interesting to note that as early as October of 1935 Quick, Stanley-Brown and Bancroft (19) proved conclusively that plasma prothrombin was definitely decreased in persons suffering from obstructive jaundice. Since he had observed an elevation of plasma prothrombin levels in the hemorrhagic chick disease by administration of vitamin K (10), Quick proposed the use of the vitamin in obstructive jaundice on theoretical grounds, though he had no direct tests to prove the validity of his proposal. His theory was based on the idea that the depletion of prothrombin in obstructive jaundice may be due to the absence of bile acids in the intestinal tract, thus causing a faulty absorption of vitamin K.

Since Quick (19) had already called attention to the low prothrombin level in jaundice, Hawkins and Brinkhous (20) undertook to evaluate the prothrombin concentration in dogs after establishing biliary fistula. They found that the plasma prothrombin was at an extremely low level at the time of spontaneous bleeding, often falling to less than five per cent of a normal control dog. The whole blood clotting time, which was prolonged,

was restored to normal by the addition of a partially purified prothrombin, free of calcium and thromboplastin. Normal values for serum calcium and plasma fibrinogen were obtained, and there was no evidence of an excessive amount of antithrombin or of heparin. These data show definitely that the hemorrhagic tendency was the result of a prothrombin deficiency, and it was demonstrated that bile feeding prevented as well as cured this bleeding tendency. Further studies in 1938 by Smith, Warner, Brinkhous and Seegers (21) showed that in the hemorrhagic disease of biliary fistula dogs the critical prothrombin level at which bleeding is likely to occur is approximately ten per cent of normal.

Though the work just cited definitely proved the existence of prothrombin deficiency in biliary fistula, the first direct evidence in support of Quick's theory concerning the role of bile acids in the absorption of vitamin K was supplied by Greaves and Schmidt in 1937 (22). These workers made observations on a group of operative biliary fistula rats and a group of unoperated rats maintained on a low fat diet for a period of 30-50 days. The bile fistula rats, at the end of this time, showed a marked increase in the coagulation time of the blood, while the unoperated rats were entirely uneffected. Small scratches of the skin or clipping of the tail

of these bile fistula animals led to prolonged bleeding, often resulting in death unless stopped by the application of collodion. Blood studies showed that the prothrombin content of the animals was reduced to 20-30 per cent of normal before coagulation time was markedly increased. In order to rule out a deficiency of any other dietary factor as a possible cause of this condition, vitamins A, B, D, E and G, the essential fatty acids, and lemon juice at levels of two cubic centimeters were added to the basal diet. No alteration of the hemorrhagic condition was observed after these supplements. However, when two to three cubic centimeters of beef bile were fed daily, a marked rise in the prothrombin content of the blood was noted within a period of two to four days after the bile was administered. Also, crude extracts of vitamin K (prepared from alfalfa) which had proven effective in preventing the hemorrhagic syndrome in chicks, when fed to the bile fistula rat, were effective in decreasing the coagulation time of the blood and increasing the prothrombin level. In a similar study of bile fistula and jaundiced rats in 1939, Greaves (23,24) concluded that the marked bleeding tendency in these conditions, associated with an increased bleeding time and a low blood prothrombin value, is the result of vitamin K deficiency and can be cured by the

administration of vitamin K concentrates. They demonstrated that bile salts are essential to the intestinal absorption of vitamin K in the rat; that is, the bile acts as a carrying agent for the vitamin across the intestinal tract.

With attention drawn to the fact that vitamin K appeared to be necessary for the formation of prothrombin, it became necessary to determine the source of prothrombin within the organism. Smith, Warner and Brinkhous (25) and Quick (10) in 1937 proved the source of prothrombin to be in the liver. By using chloroform they caused a liver necrosis in dogs and were able to simulate the hemorrhage that occurred in "sweet clover disease" of cattle, the condition which Roderick (2) investigated several years before and found to have an associated liver damage. These workers found that the bleeding tendency in this toxic liver disease was the result of a prothrombin deficiency. In several experiments of this type, platelets, antithrombin, and calcium studies showed normal or nearly normal values, thus demonstrating in a striking way that prothrombin deficiency can give rise to a bleeding tendency. They concluded from this study that the relation of the liver injury to plasma prothrombin level in this disease indicates that the liver is concerned in the manufacture

of prothrombin. Basing his views on these findings, Quick again advocated the use of vitamin K in treatment of biliary obstruction.

Approximately two months after Hawkins and Brinkhous (20) first published the results of their experiments on biliary fistula dogs, the first successful treatment of human patients with obstructive jaundice was reported from the same laboratory (26). Other clinical evidence lent its support when Butt, Snell and Osterberg at the Mayo clinic successfully demonstrated that postoperative bleeding in gall bladder surgery was minimized by the use of vitamin K. These reports of treatment of human cases will be considered in a later section.

-Sources of Vitamin K-

The first indication of vitamin K occurring in nature was given by Dam in his original investigations of the hemorrhagic chick disease. He demonstrated that the hemorrhagic syndrome could be prevented by the addition of mixed cereals to the basal diet. In later experiments designed to determine the distribution of the vitamin (6), he found that one of its richest sources is hog-liver fat. He further showed that hemp seed and alfalfa are particularly protective against the disease, while unpolished rice, sunflower seed, yellow corn and

rye are nearly valueless. Certain vegetables such as tomatoes, kale, orange peel and spinach appeared to have a fairly good action against the disease.

Almquist and Stokstad (7) reported in 1935 that the addition of 0.5 per cent of dry alfalfa to the deficient diet prevented the appearance of symptoms, and from that time alfalfa has been one of the main sources of vitamin K concentrates. Recognition of a different antihemorrhagic factor resulted from the observation of Almquist and Stokstad (16) that rice bran, fish meal and other foods which had been stored in a moist condition developed vitamin K activity. That this production was due to the action of micro-organisms was definitely concluded from Osterberg's work (27), which showed that large amounts of an antihemorrhagic substance were produced by bacterial putrifaction of fish meal.

Almquist, Pentler and Mecchi (15) continued the study of bacterial synthesis of vitamin K and found that the putrifaction of ether-extracted, K-free fish meal was accompanied by the formation of appreciable amounts of a fat-soluble, antihemorrhagic substance. Several different types of bacteria were present in the putrified material. One organism producing a similar putrifaction was isolated in pure culture and inoculated to beef broth, fish-meal broth, proteose-peptone broth,

gelatin and nutrient agar. Washed bacteria from broth and from nutrient agar were found to be rich sources of the antihemorrhagic factor, while the liquid media from which the bacteria had been removed by filtration, and saline used in washing the bacteria, were negative. They further demonstrated, by administering dried bacteria of several species to K-deficient chicks, that the antihemorrhagic activity of these organisms was from five to eight times that of dried alfalfa. They concluded that this factor is the product of bacterial metabolism, and, though it is also extractable by fat-solvents, its further similarity to the vitamin K from alfalfa was not established.

The most extensive study of the distribution of vitamin K in the vegetable kingdom was carried out by Dam and Glavind (28) in 1938. In accordance with previous results, they found green leaves to be the most potent source of vitamin K among the plants. Among the species examined, chestnut leaves were the most potent. Those lower plants which contained chlorophyll appeared to be somewhat poorer sources than the higher plants. The mushroom, which contains no chlorophyll, was very poor in vitamin K content. Leaves of the chestnut were found to lose only a small portion, if any, of their vitamin K activity when their chlorophyll disappeared

during the withering process. An experiment with germinating peas showed that whereas there was an abundant synthesis of the vitamin in peas grown in the light, synthesis in peas grown in the dark occurred to a very limited extent. Any relationship between vitamin K and chlorophyll was not explained by Dam and Glavind.

However, in more recent studies of substances with vitamin K activity, designated vitamins K1 and K2, Fieser and his associates (29) have pointed out the noticeable presence of large amounts of the vitamin in those parts of the plant containing chlorophyll. This fact has led Fieser to postulate that vitamin K1 contains, like chlorophyll, a phytyl group. He has suggested that K1 arises in nature by the condensation of phytol and 2-alkyl-1,4-naphthohydroquinone, both of which occur in plants. Fieser (29) has further suggested that since both vitamins E1 and K1 contain a phytyl group, it is possible that there may be a close biogenetic relationship between the two.

It is now generally agreed (30) that the substances which contain the antihemorrhagic property in the order of potency are: (1) Putrefied fish meal, (2) alfalfa, (3) hog-liver fat, (4) kale, (5) spinach, (6) dried carrot tops, (7) chestnut leaves, (8) tomatoes, (9) oat sprouts, (10) soy beans, and (11) the naphthoquinones.

-Bioassay-

The methods for assay of vitamin K are based on the prevention or cure of vitamin K deficiency in chicks. The criteria used for the detection of the disease have varied with different investigators and have been changed by the same investigator from time to time. Hemorrhage was first used as the measure of deficiency but as it only occurs when the prothrombin level has fallen to 10-15 per cent that of normal chicks (10,11), only the most severe deficiencies are detected by this symptom. Prolongation of the blood clotting time was later used as a measure of deficiency, but we now know that a moderate deficiency may exist and the clotting time be normal. It is prolonged only if the prothrombin level of the blood is below about 30 to 40 per cent that of the normal chick (11). Dam and Glavind (31) used R-values to indicate the extent of the deficiency. These values depend on the amount of thromboplastin required to give a constant clotting time of three minutes. Almquist and Klose (32) recently adopted a prothrombin time measurement which they believe is a great improvement over the clotting time tests. In view of the findings of Tidrick, Joyce and Smith (11), the use of the two-stage method for prothrombin determination would appear to give an even more accurate index of vitamin K deficiency.

A large number of curative and preventative procedures for the assay of vitamin K have been devised. Each investigator has proposed an individual technique for the standardization of the vitamin, all of which entail considerable detail. For this reason only the results of these assays will be considered here, so that a comparison of the various accepted standards may be made.

The Dam unit of activity (31) refers to a special preparation of dried spinach, to which a value of 500 units per gram has been arbitrarily assigned. Two mg. of this product therefore constitutes one unit. When 2 mg. of this standard preparation per gram of body weight is given daily to a highly K-deficient chick on three successive days, normal blood clotting is obtained.

Dann's method (33,34) is that of giving varying levels of the test material to a group of K-deficient chicks fro three successive days, and at the same time treating another group of deficient chicks with a standard vitamin K concentrate prepared from alfalfa, to which has been assigned arbitrarily a value of 5000 vitamin K units per gram. By comparing the blood clotting times obtained by feeding the unknown and the standard, the vitamin K unitage of the test material is determined. This would indicate that her unit of vitamin K is the amount of material, based on the standard

of known potency, which when administered to chicks daily will bring about a coagulation time of the blood equal to that produced by the reference standard under the conditions of the test.

Ansbacher (12) has defined his unit of vitamin K as "the minimum amount necessary to render the blood clotting time of the vitamin K-deficient chick, weighing 70 to 100 grams, normal within six hours after administration". The material to be tested for vitamin K activity is introduced directly into the crop, and it is fed in different dosage to several groups of five or more chicks to determine this minimum level.

Thayer and his assoviates (35,36) define a unit of vitamin K as "that quantity of vitamin (K) which produces a clotting time of ten minutes or less in 50 per cent of a group of ten or more chicks which has been fed for the fourteen days immediately following receipt from the hatchery on a diet practically devoid of vitamin K".

Almquist and Klose (32) have also presented a method of assay of vitamin K, showing that "the reciprocal of the mean blood clotting time and of the mean prothrombin time bears a linear relation to the logarithm of the vitamin K level in the diet over a practical range of levels".

In a report to the Council of Pharmacy and Chemistry

of the American Medical Association in 1939, Snell and Butt (37) reviewed the work done on assay of vitamin K and pointed out that there is not complete agreement among the investigators working in this field as to the manner of defining a unit or as to the best methods of assay. Since they felt that any attempt to arbitrarily establish a universal unit of vitamin K would be unlikely to suit all investigators, it was suggested at that time that a consideration of units be postponed until exact and final knowledge of the chemical structure of vitamin K be known.

In a more recent report of the status of vitamin K, Brinkhous (30) has attempted to correlate the various units of vitamin K. He tabulated the following comparative values, which he states must be considered only as rough approximations because of discrepancies in the several methods of assay already referred to in the foregoing paragraphs.

1 Ansbacher unit equals 20 Dam-Glavind units.
1 Thayer-Doisy unit equals 30 Dam-Glavind units.
1 Dann unit equals 25 Dam-Glavind units.
100-150 Thayer-Doisy units per kilo of diet are protective by the method of Almquist, Mecchi and Klose (14,32).

The discovery in 1939 (38) of pure naphthoquinone compounds possessing vitamin K activity brought the previous proposal of Snell and Butt (37) nearer to realization. In line with the suggestion of these men, Thayer

and his associates (38) proposed that one of the simplest and most potent of the naphthoquinones, 2-methyl-1,4-naphthoquinone, be adopted as a standard in chick assay work, and all other materials be expressed in terms of the activity of one microgram of this compound. This would simplify considerably the individual unit systems used at present by various investigators, and at the same time it would provide a common standard on which all experimental and clinical study could be based.

-Chemistry of Vitamin K-

1. Properties. In earlier work on the purification of vitamin K, Dam (6) and Almquist and Stokstad (7) observed that the antihemorrhagic factor is extractable from natural sources by fat solvents. Attempts at isolation of the vitamin showed that it occurs in the non-sterol fraction of the lipids. They found that the vitamin is insoluble in water and methyl alcohol but soluble in many of the common organic solvents. Both acetone and petroleum ether proved to be satisfactory for the extraction of the vitamin from natural sources.

Following the discovery in 1935 by Almquist and Stokstad (7) that dried alfalfa leaf meal is an excellent source of vitamin K, they prepared the first crude concentrate of the vitamin by extraction with petroleum ether. Fractionation with solvents proved ineffective

in the separation of the pure vitamin. Likewise, chemical reactions on the crude product accomplished little, except to show that certain common procedures could not be employed. Dam and Schønheyder (39) and Almquist (40) found that saponification of the vitamin concentrates with alkali resulted in destruction of a large part of their activity, proving a lability of the vitamin toward alkali.

In addition to being alkali-labile, Almquist (41) discovered that the vitamin is destructed by ultraviolet light. He reported a destruction of the vitamin activity of alfalfa concentrates within two hours after exposure to sunlight, whereas no alteration was observed in artificial light (500 watt lamp) up to 24 hours. MacCorquodale and his associates (42) confirmed the effect of sunlight reported by Almquist, but in addition found that highly purified preparations of vitamin K dissolved in various solvents rapidly lose activity on exposure to the illumination of ordinary daylight bulbs. They further observed that crude extracts of alfalfa are quite stable, no special precautions regarding light being necessary, but as the potency is increased to 500 and 1000 units (Thayer units) per mgm., the lability is such that decomposition may occur in spite of all precautions regarding light.

One property of vitamin K, namely thermostability, has proved to be quite important, since it led Almquist (43) to introduce molecular distillation for the purpose of purification. He observed that the vitamin in concentrates of alfalfa or hog-liver fat resists heating up to 120 degrees C. for twenty-four hours. Dam and Lewis (44) prepared a concentrate from alfalfa which they found to be resistant to treatment with acetic anhydride at 100 degrees C. for one-half hour. It was also noted that the vitamin is adsorbed on strong adsorption media such as aluminum oxide and magnesium oxide, resulting in destruction of activity.

In a more detailed study in 1938, Klose, Almquist and Mecchi (45) demonstrated that the vitamin is destroyed by oxidizing agents, strong acids and aluminum chloride, and in reactions which eliminate ethylenic linkages. Irreversible loss of activity due to bromination suggested to them that there was more than an ordinary saturation of aliphatic double or triple bonds in the vitamin K molecule. They found that the material is non-nitrogenous and contains an aromatic nucleus; no phosphorous or sulfur is present in the structure. They concluded from their work that the properties of vitamin K are consistent with those of a complex, unsaturated hydrocarbon.

2. Isolation of vitamin K. In the spring of 1935, it became apparent that more than one compound possesses vitamin K activity. McKee and his coworkers (46) reported the isolation of vitamins K1 and K2 from alfalfa and putrified fish meal respectively, and they presented evidence to indicate a quinoid structure for these vitamins. This work was repeated in the same laboratory shortly after by Binkley (47), and the previous findings were substantiated.

Almquist and Klose (48) recently have reported that phthiocol, the chemical formula of which is 2-methyl-3-hydroxy-1,4-naphthoquinone, possesses physical and chemical properties similar to pure vitamin K. It has been shown by these men that phthiocol is effective in preventing the hemorrhagic diathesis in chicks subsisting on a K-deficient diet when given at levels of 20 mgm. per kilogram of diet. They have also suggested that phthiocol is the simplest member of a homologous series of antihemorrhagic substances. Later these investigators (49) reported that the antihemorrhagic activity of phthiocol lay somewhere between that of methyl naphthoquinone and hydroxy naphthoquinone. Their study indicated that the methyl group was functionally important whereas the hydroxyl group seemed to reduce activity. They agreed that the activity of phthiocol is lower than

the more complex form of vitamin K existing in alfalfa. Ansbacher and Fernholz (50), in their study of this compound, were of the same opinion.

Recently Thayer and his associates (51), as well as MacCorquodale and his coworkers (52), working in Doisy's laboratory at St. Louis University Medical School, have found 2-methyl-1,4-naphthoquinone the most active compound studied, but when this compound was compared with the natural vitamins K1 and K2, its activity was relatively insignificant. They believed the structure of the vitamin K1 molecule to be 2-ethyl-3-phytyl-1,4-naphthoquinone. Fieser and his coworkers (53) suggested that the structure of K1 was 2,6(?)-dimethyl-3-phytyl-1,4-naphthoquinone or the 2-mono-methyl compound. From the study of less complex models, Fieser, Campbell and Fry (29) favored the latter structure.

Further study by Binkley and collaborators (54) indicated that vitamin K1 is 2-methyl-3-phytyl-1,4-naphthoquinone. The final step in the proof of this structure of vitamin K1 was the synthesis of the compound in three separate laboratories practically simultaneously, by Almquist and Klose (55), MacCorquodale and associates (56), and Fieser (57). The natural and synthetic products had the same physical and chemical properties and approximately the same antihemorrhagic potency.

This comparison with the synthetic product showed conclusively that the structure of the vitamin isolated from alfalfa was 2-methyl-3-phytyl-1,4-naphthoquinone.

Vitamin K2 was isolated from putrefying fish meal by MacCorquodale (42), McKee (58) and their associates by methods similar to those used for the isolation of vitamin K1. This vitamin compound was obtained as light yellow crystalline plates with a melting point of 53.5-54.5 degrees Centigrade. The potency of vitamin K2 is 60 per cent that of vitamin K1; that is, approximately 600 Thayer-Doisy units per mgm. Although vitamin K2 has been isolated, its structure has not been identified. The data of McKee and coworkers (58) indicate that it also is a 2,3-disubstituted-1,4-naphthoquinone. Fieser and coworkers (29,53), on the basis of the chemical and physical properties reported by McKee and associates (58), postulated the structure 2,3-difarnesyl-1,4-naphthoquinone.

3. Vitamin K activity of simple quinones and related products. Almquist and Klose, in June 1939 (48) reported the first completely identified form of vitamin K. This compound is phthiocol (2-methyl-3-hydroxy-1,4-naphthoquinone) and might be termed vitamin K3. In contrast to many vitamin K preparations, phthiocol is slightly soluble in water. Although the compound does

possess vitamin K potency, its activity is not comparable with that of the vitamin from alfalfa. Experimentally, Almquist (48) has shown that a 0.2 per cent buffered solution of phthiocol is effective in curing K-deficient chicks when given by mouth, intramuscularly or intravenously.

Another synthetic naphthoquinone which has been studied extensively is 2-methyl-1,4-naphthoquinone, or vitamin K4. Amsbacher and Fernholz (50,59) showed that this compound possesses activity of the same order as vitamin K1. All other members of this group which have been tested are less than 1/20 as active as vitamin K1.

Most of the active synthetic compounds are basically 1,4-naphthoquinones or the corresponding hydroquinones; a few, however, are not (30). Many of the 1,4-naphthoquinone compounds are inactive. Ansbacher and Fernholz (59) have shown that often relatively minor changes in the substituent groups of very active compounds of this type may completely eliminate their vitamin activity. Thus, 2-methyl-1,4-naphthoquinone is very active, the 2-ethyl compound show much less activity, and the 2-propyl compound is inactive. Ansbacher (59) states that although the number of naphthoquinones which could be tested for vitamin K activity could be greatly increased, from results of his investigation

it does not appear likely that the preparation of such compounds would lead to the discovery of a substance surpassing in activity 2-methyl-1,4-naphthoquinone.

A complete list of the synthetic compounds, including vitamins K1, K3, and K4, with their respective formulae and activity expressed in units per milligram, has been published recently by Brinkhous (30) in a summary of vitamin K compounds.

-Clinical Causes of Deficiency in Prothrombin-

In order to better understand the development of clinical study of hypoprothrombinemia, it will be well to summarize briefly the sequence of experimental investigation of vitamin K deficiency.

That a vitamin K deficiency exists in cases of obstructive jaundice and biliary fistula was suggested for the first time in 1937 (10) on theoretical grounds. The evidence then available indicated that (1) a hypoprothrombinemia exists in obstructive jaundice (19), biliary fistula (20,21,22), and vitamin K-deficient chicks (8,9,10); (2) that vitamin K is a fat-soluble vitamin, (3) that certain of the other fat-soluble vitamins, A and D, require, as fats do, the presence of bile or bile salts in the intestine for their adequate absorption (60,61), and (4) that the low prothrombin level and the

hemorrhages can be prevented or cured in biliary fistula dogs by feeding bile (20). It was clear that cases of bleeding in biliary fistula or in obstructive jaundice have in common the absence of bile in the intestine and that the bleeding tendency in each is due to a diminished plasma prothrombin. On the basis of these facts, Quick (10) postulated, in addition, that they have a vitamin K deficiency, due to the lack of bile in the intestine.

With the stage thus set by the investigations referred to in the preceding paragraphs, it was only logical to make the next move; namely, the study of the effect of vitamin K in obstructive jaundice and other diseases of the biliary tract.

1. Obstructive jaundice and biliary fistula.

In October, 1937, Greaves and Schmidt experimented with bile fistula and jaundiced rats and presented the first evidence to support the earlier theory of Quick. They produced a hemorrhagic state in these animals by prolonged feeding with a low fat diet and observed a marked increase in the coagulation time of the blood when the prothrombin level had been reduced to 20 to 30 per cent of normal. Bile feeding, as was earlier demonstrated by Hawkins and Brinkhous (20), corrected the low prothrombin levels. They also found that the adminis-

tration of crude extracts of vitamin K, prepared from alfalfa, resulted in prompt decrease of the coagulation time of the blood and increase of the prothrombin level. Further studies in June of 1938 by Smith, Warner, Brinkhous and Seegers (21) showed that bile alone was much less effective in raising the plasma prothrombin level than a preparation containing a combination of vitamin K and bile or bile salts. Vitamin K alone was practically without effect. The equal effectiveness of bile or bile salts in the absorption of vitamin K indicated that the latter was the active material in bile (21,22,23).

The first report of vitamin K therapy for human cases of obstructive jaundice and biliary fistula came from Warner, Brinkhous and Smith in January, 1938 (26). While observing their jaundiced cases over a period of time, they found that many showed no bleeding tendency, and in them the prothrombin level was usually above 50 per cent of normal. When the prothrombin level fell below 35 per cent, as it frequently did in this series of cases, they encountered bleeding from mucous membranes or hemorrhage at operation or from the wound after operation. The restoration of bile to the intestinal tract led to a gradual rise in the prothrombin level, requiring, on an average, a period of two weeks to attain normal level. This was shown in cases where

the obstruction was suddenly released, or in cases where bile was fed by mouth. On the other hand, a vitamin K concentrate prepared from alfalfa, plus human bile or bile salts, led to a rise in prothrombin from 20-40 per cent to 85-105 per cent of normal in six to eight days. It was concluded that these jaundiced patients undoubtedly suffer from disturbances in the digestion and absorption of fats, which in turn causes a vitamin K deficiency characterized by hemorrhagic tendencies.

Further evidence of the beneficial effects of vitamin K in obstructive jaundice came practically simultaneously from Butt, Snell and Osterberg of Mayo Clinic in February of 1938 (27,62) and from Dam and Glavind of Copenhagen in March of 1938 (63).

Snell (62) submitted a report on a patient who had obstructive jaundice due to stricture of the common bile duct, a badly damaged liver and a virtually incoagulable blood. This patient had failed to respond to the usual methods of treatment for the hemorrhagic diathesis of jaundice and was subsequently given a mixture of dried alfalfa and bile salts by mouth. The coagulation time of the patient's blood was thereupon greatly shortened and for a time it was hoped that surgical treatment could be attempted; however, the patient soon failed to tolerate this mixture and the blood returned to its in-

coagulable state. Snell believed this case to be analogous to the experimental dogs of Smith, Warner and Brinkhous (25), in which they caused a liver necrosis by chloroform intoxication and noted a decreased plasma prothrombin with hemorrhagic tendencies. Snell believed that there was some justification for concluding that (1) the hemorrhagic state in jaundice is attributable to a deficiency of prothrombin which in turn is due to failure of absorption or utilization of some substance normally present in the diet which requires bile for its absorption; that (2) this substance may be the coagulation vitamin (vitamin K); and that (3) additional toxic factors may deplete the supply of prothrombin from the liver, as in necrosis of the liver caused by chloroform.

Butt, Snell and Osterberg administered vitamin K and human bile or animal bile salts to 18 patients who had obstructive jaundice, and obtained good results in every case. In most of these 18 cases complete biliary obstruction and subsequent damage to the liver were present. In several cases the prothrombin time was not elevated greatly but vitamin K and bile salts were administered as a prophylactic measure. An example of the prophylactic use was a case of stricture of the common duct and jaundice. The patient had had a severe hemorrhage following a previous operation elsewhere for this

condition. Although her prothrombin time was nearly within normal limits, vitamin K and bile salts were given as a precautionary measure. This patient went through her operative procedure at the clinic without any abnormal bleeding and, although she later died from a hepatic insufficiency, no abnormal bleeding was noted at necropsy.

In the same study, Butt, Snell and Osterberg (27) pointed a case which they believed was typical of the usual course in a case of obstructive jaundice. In spite of repeated transfusions of blood the prothrombin time increased gradually to 140 seconds over a period of two weeks and remained elevated until death. At necropsy a large amount of blood was found in the gastro-intestinal tract and in the peritoneal cavity.

In three cases of obstructive jaundice, these men found a rapid decrease of prothrombin time following the administration of vitamin K and bile salts which was rather striking. In one case a definitely dangerous prothrombin time was reduced to normal limits over a period of three days with the use of vitamin K and bile salts. Vitamin K alone was given to one case for nearly a week without any significant effect of the prothrombin time. However, 24 hours after bile was added to the K intake, there was a marked fall in the prothrombin time.

In one case in which the patient was eating heartily and the prothrombin time was nearly double the normal value, administration of human bile alone resulted in an immediate fall in the prothrombin time.

Butt, Snell and Osterberg (27) calculated the dose of vitamin K required by man by comparing the body weight and the weight of the intake of food to that of a chick for which they knew the dose necessary to prevent hemorrhage. They found that, theoretically, 23 mg. of their material should be an adequate daily dose for man, but they gave orally in capsules 200 mg. daily, which is nearly ten times the calculated dose. In order to facilitate the absorption of this dosage of vitamin K, they gave whole human bile or rather large doses of bile salts (1000 to 4000 mg. of "Bilron Lilly" daily). Human bile was mixed with pineapple juice and administered in doses of 75 to 150 cc. orally before each meal.

Dam and Glavind (63), in their first report of vitamin K therapy, treated five patients with obstructive jaundice. Two of the cases had carcinoma of the pancreas which was causing pressure on the biliary tract and thus obstruction. All of the cases had increased clotting values and increased prothrombin times (high R-values). The parenteral introduction of 15 mg. of vitamin K daily rendered the clotting power of the blood normal in from

six to eight days and the prothrombin times returned to nearly normal values in the same time. It was concluded that the increased clotting time before the introduction of vitamin K was due to reduced absorption of the vitamin from the intestine.

Another report in 1938 by Brinkhous, Smith and Warner (64) confirmed the original finding of a prompt rise in the prothrombin level following vitamin K therapy in jaundiced patients. They made a study of 27 cases of obstructive jaundice, in most cases of which the plasma prothrombin levels were definitely below normal. In this series six cases showed a definite bleeding tendency and in every instance was associated with a marked decrease in the prothrombin level, usually to less than 35 per cent of normal. One case of biliary fistula responded to bile feeding alone with a gradual rise of prothrombin to a nearly normal level over a period of six weeks. In marked contrast to this was the very rapid rise in prothrombin level which occurred in obstructive jaundice cases receiving bile and alfalfa extract together. It was also noted that the amount of lowering of prothrombin is not clearly dependent upon the intensity or duration of the jaundice. In two cases of obstructive jaundice receiving vitamin K and bile therapy, the prothrombin rose rapidly during periods of

increasing jaundice, indicating that the jaundice itself is not the essential factor. Two patients of the entire series, one with a biliary cirrhosis of three years duration and another with a far-advanced malignancy of the liver, did not respond to alfalfa therapy. These men concluded that the failure of these two cases to respond suggests that in addition to certain dietary essentials, a liver of good functional capacity is needed for the manufacture of prothrombin.

Snell, Butt and Osterberg (65) made a second report in November of 1938 on the successful use of vitamin K in obstructive jaundice, in which they used alfalfa as the source of vitamin K rather than putrefying fish meal as in their earlier studies. They found that their original concentrates of vitamin K from putrefied fish meal lost a large part of their potency when allowed to stand at room temperature or in an icebox for 30 to 90 days, and, in an effort to find a more stable concentrate of vitamin K, they selected alfalfa as the most practical source. With the cooperation of the Abbott laboratories, they obtained rather large quantities of concentrate of vitamin K which had been extracted from alfalfa with petroleum ether. This preparation had a potency of 20 mg. or less per kilogram of chick diet as measured by the prophylactic chick method, and 200 mg.

of the concentrate was equivalent to approximately 66 grams of dry alfalfa meal, or approximately 37,500 Dam-Glavind units.

They reported 13 cases of actual bleeding in obstructive jaundice which were controlled by administering this vitamin K concentrate from alfalfa in combination with bile salts. In another large group of jaundiced patients, an elevation of the quantitative level of prothrombin in the circulating blood and a reduction in the elevated prothrombin clotting time was effected by this therapy. When the concentrates were administered orally to 28 patients before and after surgery, none bled seriously and there were no deaths from hemorrhage. They observed this to be in marked contrast to 14 patients who received no concentrate of vitamin K or bile salts preoperatively, 64 per cent of whom bled after surgery. They concluded that although concentrate of vitamin K obtained from alfalfa was at that time still an empiric therapeutic aid, yet with bile salts it had effectively prevented and controlled the hemorrhagic diathesis in cases of obstructive jaundice and deserved, therefore, a more extensive clinical use.

Snell, Butt and Osterberg have reported that the greatest danger of hemorrhage following surgical operation for the relief of obstructive jaundice is between

the third and the ninth day after operation (66); and this usually may be detected by a rise in the prothrombin time, which seems to occur with considerable regularity after operation. They observed that the effect of operation is often sufficient to decrease the concentration of prothrombin in the plasma below the critical level, with a corresponding rise in the prothrombin time. Whether this is a result of injury of the liver resulting from anesthesia or is due to the loss of blood at operation, they were unable to say. In their experience, postoperative bleeding may begin as a slight oozing from the wound or gums, or as hematemesis or melana; it also may become generalized and severe with very little warning. Under such circumstances transfusions of blood are indicated; their effect is to supply a small quantity of prothrombin for emergency use, which exerts its effect for only 6 to 12 hours, if at all. During this interval, the administration of vitamin K and bile may be resumed, preferably by means of the intragastric or duodenal tubes. In addition to these two forms of treatment, the importance of beginning an adequate food intake as soon as possible is obvious since the reestablishment of normal biliary flow into the intestine and the restoration of a normal state of nutrition are the ultimate considerations required

for cure of the hemorrhagic state.

In 1939 there were a large number of reports on the successful use of vitamin K therapy in the bleeding tendency and hypoprothrombinemia in patients with obstructive jaundice. Olson (67) reported a case of obstruction of the common bile duct which was operated and was treated preoperatively with intravenous glucose and a blood transfusion. Unusual bleeding was encountered at the time of operation and oozing occurred for several days after the operation. Six days after operation a severe hemorrhage occurred, was controlled by a blood transfusion, but the ozzing recurred, and four days later another serious hemorrhage requiring blood transfusion again occurred. Prothrombin bleeding time by the method of Quick was 200 seconds, whereas the normal is 20 to 30 seconds. Vitamin K and bile salts were immediately given: 2 capsules orally t.i.d. after meals, accompanied by a 5 gr. tablet of bile salts. Prothrombin bleeding time three days later was 120 seconds, no further hemorrhage had occurred, and oozing from the wound was decreasing. Seven days after the administration of vitamin K was instituted, the prothrombin bleeding time was reduced to 38 seconds, which was comparable to a normal control, and all bleeding had stopped. The convalescence from that time was uneventful.

The case just cited, as reported by Olson (67), is typical of additional reports made in the past two years on the continued successful use of vitamin K in patients with obstructive jaundice. Such reports have been made by Butt, Snell and Osterberg (68,69,70), Smith, Ziffren, Owen and Hoffman (71), Stewart and Rourke (72,73) and Scanlon, Brinkhous, Warner, Smith and Flynn (74).

In addition to the studies of vitamin K deficiency in patients with obstructive jaundice, a number of similar studies have been made of patients with a biliary fistula. One of the first such cases reported, from the laboratory of Brinkhous, Smith and Warner (26,64) at the University of Iowa, had a complete external fistula and a hypoprothrombinemia and a bleeding tendency. With feeding of whole bile, the prothrombin level returned from a low level of 27 per cent of normal to 89 per cent of normal. In this case the vitamin K intake in the food was sufficient, though not determined, and the addition of bile was necessary to permit absorption of the vitamin present. Successful treatment of the hypoprothrombinemia in these patients has been reported also by a number of other investigators including Butt, Snell and Osterberg (65), Stewart (75), Stewart, Rourke and Allen (72) and Smith, Ziffren, Owen, Hoffman and Flynn (74).

It has been reported that the administration of vitamin K in obstructive jaundice is less effective when the prothrombin deficiency is associated with extensive hepatic damage (25,73). That this is true was confirmed by two independent studies conducted in September of 1940. Bollman, Butt and Snell (76) produced hepatic damage in rats by uniform exposure to a known concentration of carbon tetrachloride. Crude concentrates of alfalfa and 2-methyl-1,4-naphthoquinone were administered to some of these animals and in all instances failed to increase the level of prothrombin in the circulating blood. The only factor found was that extensive hepatic injury was associated with depletion of the level of prothrombin and also with failure of response to vitamin K. However, they did observe that in such animals prompt recovery occurs when the hepatotoxic agent is removed and the liver repair is rapid. They concluded that the failure of vitamin K to maintain the prothrombin level of the blood and the rapid recovery to normal after discontinuation of exposure to the hepatotoxic agent indicates that the liver is of fundamental importance in the formation of prothrombin and in the metabolism of vitamin K.

Cullen, Ziffren, Gibson and Smith (77) extended the study of liver intoxication, and its effect on plasma

prothrombin, to human beings. They made determinations of prothrombin levels of patients under chloroform, ether and cyclopropane anesthesia. A group of 6 cases was operated for minor surgical complications under the chloroform anesthetic. Prior to operation the prothrombin levels were within normal range in all cases. Following operation there was a fall in every case with a variation of from 11 to 40 per cent and an average fall of 18 per cent. Neither ether nor cyclopropane produced any such in the uncomplicated cases which they reported. It was concluded that the normal functioning liver is essential to maintenance of prothrombin in the circulating blood, but the fall in the plasma prothrombin level which occurs after operation in patients with obstructive jaundice or biliary fistulas is evidently due to factors other than the anesthetic agent itself.

It had been recognized for years (65) that the bleeding tendency in obstructive jaundice is often observed within the first few days postoperatively. Although often there would be no evidence of excessive bleeding at operation, hemorrhage might be massive and fatal within the next few days after operation. Butt, Snell and Osterberg (65) described eight patients with prolonged plasma prothrombin times and postoperative

hemorrhage. The bleeding occurred in each case between the first and fourth postoperative days. In a few patients, hemorrhage was not observed until later.

The common occurrence of cholemic bleeding in the first few days postoperatively led to a careful study of the plasma prothrombin during this critical period. Butt, Snell and Osterberg (65,69) observed a prolongation of the plasma prothrombin time with considerable regularity in these patients following operation. Those patients in whom considerable liver damage in addition to obstruction of the biliary ducts was noted at the time of operation appeared to be most susceptible to postoperative hemorrhage. Stewart, Rourke and Allen (72) studied the plasma prothrombin level following surgery in a group of 19 patients with obstructive jaundice. Uniformly, they found a fall in prothrombin, despite the fact that a blood transfusion was given routinely at the time of operation. This fall averaged 20-25 per cent, and the minimum level was reached most commonly between the first and fourth days, coinciding, thus, with the time hemorrhage most frequently occurs.

A number of causes for this fall in prothrombin after operation have been suggested. The fact that the fall of prothrombin in obstructive jaundice and biliary fistula cases is partially due to a deficiency of vita-

min K as a result of absence of bile in the intestine has been adequately established by numerous studies (21,23,27,64). The influence of anesthesia in causing postoperative decline in prothrombin level has apparently been minimized through the study of Cullen and his associates (77). Other suggestions (64) which have been made include (1) increased consumption of prothrombin due to hemorrhage and exudate formation, without adequate reserves of prothrombin or vitamin K to restore the prothrombin level promptly; (2) a low bile salt output by the liver and consequent malabsorption of vitamin K for a period after the obstruction has been relieved; and (3) liver damage by infection or operative trauma, and decreased production of prothrombin by the liver. Brinkhous, Smith and Warner (64) believe that because of these variable factors some patients somewhat above the bleeding level before operation must be considered as potential bleeders, and must be treated accordingly.

From the numerous studies on the vitamin K deficiency and hypoprothrombinemia in obstructive jaundice and biliary fistula, it is apparent that the bleeding tendency can be prevented, and in many cases it can be cured by treatment with vitamin K. Butt, Snell and Osterberg (69) have stressed the importance of preoperative and postoperative administration of vitamin K to avoid the

serious bleeding which may occur at the time of operation or soon thereafter. They administer the vitamin for several days prior to operation and continue this therapy for several days after operation, the time depending on the rate of decline of the prothrombin time of the blood. They state that no matter what method of administration is used, or what concentrate of vitamin K is used, the importance of anticipating the danger of prothrombin deficiency and detecting it by laboratory studies will remain a prime consideration in successful treatment.

Many different sources of vitamin K have been used in the treatment of the vitamin deficiency in obstructive, but at present the medicinal products are of two types. One is an extract of alfalfa and contains the fat-soluble natural vitamin K. The other represents a group of synthetic products which are more or less effective, the base being naphthoquinone.

Dam and Glavind (63) reported the treatment of five cases of obstructive jaundice with K-avitaminosis by intramuscular injections of an emulsion of vitamin K in water and found it possible to restore the blood caogulation to the normal value within a week.

Since then Tage-Hansen (78) has studied the effect of vitamin K when given perorally, intramuscularly and

intravenously. For peroral use gelatin or starch capsules containing the concentrate together with desoxycholic acid were prepared. Normal coagulation was obtained within two days with doses of from 100,000 to 200,000 Dam units of vitamin K plus 2 gm. of desoxycholic acid. For injection purposes, concentrates having a strength of more than one million units per gram were used. Intramuscular injection on two successive days of a watery emulsion of such a preparation representing 150,000 units of vitamin K resulted in normal coagulation three days after the first injection. The intramuscular injection of an oil solution of the vitamin in a one day or in a three to five day period led to restoration of normal blood coagulation after one to two weeks, from 150,000 to 400,000 units in all being used for each patient. Intravenous injections of emulsions, from 15,000 to 30,000 units in one dose, yielded normal blood coagulation within eighteen hours, but the effect persisted for only a few days. Tage-Hansen concluded that in urgent cases it is a great advantage that two rapidly acting forms of application are now available: intravenous injection and ingestion together with bile, of which the first is the most efficient.

Butt, Snell and Osterberg (65,69) have also used intramuscular injection of vitamin K in a few cases.

They administered the vitamin in peanut oil intramuscularly, but observed no constant prothrombin response with this therapy as did Tage-Hansen and Dam and Glavind. From their experience with this method, Butt and his associates concluded that the intramuscular administration of vitamin K is not practical when immediate response is desired in serious hemorrhage. However, they believed that this method might be practically employed in cases of obstructive jaundice which require prolonged vitamin K therapy.

The first reported use of intravenous therapy of a known vitamin K preparation was by Smith and coworkers (71) and practically simultaneously by Butt, Snell and Osterberg (68). They demonstrated that phthiocol, one of the synthetic quinone compounds, was very effective in doses of 40-100 mgm. A rise in prothrombin was observed in less than 24 hours in all the cases studied. No toxic actions have been reported by either group of workers. Snell and Butt (37) made similar observations with intravenous injections of 5-10 mgm. of 1,4-dihydroxy-2-methyl-3-naphthaldehyde. This route appears to be ideal, especially in those cases in which there is nausea and vomiting, or in which there is interference with absorption from the intestine.

In the past year there have been several reports of

the continued successful use of the naphthoquinone compounds in the therapy of obstructive jaundice. Butt, Snell and Osterberg (70) reported the oral and intravenous administration of 2-methyl-1,4-naphthoquinone, a compound which has been considered the most potent of the quinone group, practically as active as vitamin K itself. In most instances 1 or 2 mgm. of the substance with 5 or 10 grains of animal bile salts constituted an adequate daily dose. Except in cases of liver damage, the elevated prothrombin clotting time was found to return to a normal value within twelve, twenty-four, or thirty-six hours following the administration of this compound by mouth. Being insoluble in water, the compound was used intravenously by suspending it in a physiologic solution of sodium chloride. It was found that 1 mgm. doses daily by this route was effective in reducing the elevated prothrombin clotting time.

A similar study has been made by Rhoads and Fliegelman (79) in which eight cases of obstructive jaundice and two cases of hemorrhage of the newborn have been treated successfully with 2-methyl-1,4-naphthoquinone. The compound was administered orally in doses of from 1 to 4 mgm. daily. In three cases hemorrhagic phenomena occurred before the first dose was given, but in all three the hemorrhage was controlled.

Recently Andrus and Lord (80) have reported their experience with the intramuscular injection of 2-methyl-1,4-naphthoquinone in oil for the treatment of jaundiced patients. The preparation which they have prepared contains the K-active compound dissolved in corn oil, each cubic centimeter containing 1 mgm. of the active substance. Twenty-eight patients with initial prothrombin levels of from 5 to 70 per cent of normal were treated, the adults receiving 2 mgm. and the infants receiving 0.35 to 1 mgm. of the compound intramuscularly. In many cases single injections of this compound restored the level of plasma prothrombin by as much as 48 per cent, and the effect was evident as early as eight hours after injection. It was also noted that the effect of a single injection may be prolonged for as long as a week, unless adverse factors such as operations on the biliary tract or other liver damage supervened.

Another report of the use of 2-methyl-1,4-naphthoquinone in hypoprothrombinemia was submitted by Norcross and McFarland (81) in December of 1940. They administered this compound intravenously to 22 patients and found it to be efficient in raising the prothrombin level by this route. The minimal effective dose of 2-methyl-1,4-naphthoquinone was found to be 2 mgm., and this dose given intravenously raised the prothrombin level from 50

per cent to 80 per cent in an average of seven and one-half hours and to 100 per cent in an average of twenty hours. Further, a 2 mgm. intravenous dose kept the prothrombin level above 90 per cent of normal for four or more days unless liver damage was present.

It seems apparent from these more recent reports of the successful treatment of hypoprothrombinemia in obstructive jaundice with intramuscular and intravenous naphthoquinones, that the parenteral administration of these substances will eventually be generally adopted as the proper therapy for the hemorrhagic diathesis associated with this disease.

2. Hemorrhagic disease of the newborn.

Low and variable plasma prothrombin levels in the newborn and in early infancy, and the presence of an extreme hypoprothrombinemia in hemorrhagic disease of the newborn were recognized in 1937 by Brinkhous, Smith and Warner (82). Since then, further studies of the low prothrombin level in normal infancy have indicated a possible relation to a vitamin K deficiency.

Brinkhous, Smith and Warner found the prothrombin level of the newly born to vary considerably in different infants and in the same infant at different ages. These workers have further shown that for normal newly born infants the prothrombin values vary from 14 to 39

per cent of the level found for normal adults, while in a case of hemorrhagic disease the prothrombin level was extremely low, in fact less than 5 per cent of the adult norm (82).

With this work in mind, Waddell and Guerry (83) postulated that vitamin K might conceivably be effective in raising the prothrombin content of the newly born infant's blood and thereby prevent both spontaneous hemorrhage or that initiated by the trauma of labor. They made a study of the blood clotting times and the prothrombin clotting times of twenty newly born infants. For ten control infants the prothrombin clotting time and the clotting time of the blood were determined at varying intervals during the first week of life. To ten other infants vitamin K concentrate (Abbott) was administered orally, at the end of 24 hours 1 cc., at the end of 48 hours 0.5 cc. and at the end of 72 hours 0.5 cc. From a study of the prothrombin clotting rate at various intervals in the first week of life for the ten control infants, they concluded (1) that the blood of newly born infants differs markedly as regards the prothrombin clotting time, unusually high rates being common, (2) that the blood of individual infants varies markedly on different days of life as regards the prothrombin clotting time, (3) that the blood of certain infants is

peculiarly deficient in prothrombin as determined by the prothrombin clotting rate and that the period of most marked deficiency is between the ages of 48 hours and 72 hours and (4) that after the fifth day the rate tends to fall to an apparently common level. They believed that the last fact strongly suggests a relationship between hemorrhagic disease of the newly born and the prothrombin content of the blood, in that hemorrhagic disease rarely occurs after the fifth day of life, the time at which the prothrombin clotting rate of the control infants appeared to approach a common level. The most important point in this study was brought out by a comparison of the blood clotting times and prothrombin clotting times of the control group and the vitamin K treated group in the first week of life. The generally lower clotting values and their strikingly common level for the treated infants was very evident. Waddell and Guerry concluded from this study that the prevention and treatment of spontaneous hemorrhage, concealed or apparent, associated with a high prothrombin clotting and blood clotting time and appearing within the first week of life, should be easily effected with vitamin K.

Hellman and Shettles (84) and Shettles, Delfs and Hellman at Johns Hopkins Hospital confirmed the finding of a low prothrombin level at birth. Cord blood, in

their series, contained about 22 per cent as much prothrombin as in the mother's blood. Shettles, Hellman and Delfs (85) also have studied a total of 17 premature infants and have found the average prothrombin level to be only about one-third that of full-term infants; that is, less than 10 per cent of the values of the mothers.

Having established the efficacy of vitamin K therapy in the hypoprothrombinemia of the newborn, Hellman, Moore and Shettles, in June of 1940, made a study to compare the ability of various naphthohydroquinone derivatives in raising the plasma prothrombin levels of newborn infants when fed to their mothers during labor.(86) For this purpose they used the following synthetic compounds: (1) 2-methyl-1,4-naphthoquinone, (2) 2-methyl-3-phytyl-1,4-naphthoquinone and (3) 2-methyl-1,4-naphthohydroquinone dipropionate. In 2000 unit doses, these three synthetic compounds given by mouth, and one given intravenously, were shown to elevate significantly the infant plasma prothrombin level when given to the mothers during labor. They found that the infant plasma prothrombin was raised to the highest level by the administration of the substance having the simplest chemical structure; namely, 2-methyl-1,4-naphthoquinone. This is is in agreement with previous studies in which the hypoprothrombinemia of jaundice was treated with quinones.

More recent studies of vitamin K therapy in the treatment of hemorrhage of the newborn have confirmed the previous reports of successful prevention of dangerously low levels of plasma prothrombin. Reports by Poncher and Kato (87), Waddell and Lawson (88), Maumence, Hellman and Shettles (89), Lawson (90) and Kato and Poncher (91) all indicate that the treatment of hypoprothrombinemia of the newborn, particularly in cases of apparent hemorrhage, is rapidly becoming an accepted measure of therapy.

3. Other conditions.

Recently, Sprue and Hemophilia have been considered as conditions in which vitamin K therapy might be indicated. All that needs to be said in regard to this possibility is that many of the investigators already referred to in previous sections have found no indications for vitamin K therapy for these diseases up to the present time.

-Conclusion-

Vitamin K, a fat-soluble vitamin, is essential for the maintenance of a normal concentration of prothrombin in the blood. Animal experimentation and clinical studies have shown that certain hemorrhagic tendencies are due to a low plasma prothrombin resulting from a vitamin K de-

ficiency . Absorption of this fat-soluble vitamin is dependent on the presence of bile in the intestine rather than on the adequacy of vitamin K in the diet. Lowered prothrombin concentration may occur in conditions in which extensive intestinal lesions interfere with absorption or, more frequently, in conditions in which bile is excluded from the intestine by obstruction of the common bile duct. In such cases the administration of vitamin K will restore the normal prothrombin level. Bile salts, of course, must be given along with the orally administered vitamin in cases in which bile is not present in the intestine; otherwise the vitamin will not be absorbed. Recent work also indicates that newborn infants have a vitamin K deficiency in the first few days of life and that the administration of vitamin K will prevent and cure the hemorrhagic diathesis in at least many cases of hemorrhagic disease of the newborn.

More generally, it is safe to say that vitamin K therapy is only indicated in those conditions of hemorrhage or potential hemorrhage in which there is definite hypoprothrombinemia. Prothrombin metabolism is interfered with when:

(1). Vitamin K is inadequate in the diet.

(2). Bile is not present in the upper intestinal tract.

(3). Fat digestion is interfered with.

(4). Normal mucosa of the upper intestinal tract is destroyed.

(5). Liver function is interfered with.

When the above conditions are responsible for a hypoprothrombinemia, then vitamin K and bile are indicated.

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