FAT-SOLUBLE VITAMINS*

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VITAMIN A

Studies relating to vitamin A₂.—In the last review¹ it was noted that probably there were two forms of vitamin A, now generally referred to as "vitamin A₁" and "vitamin A₂." The compound formed by treatment of vitamin A₁ with antimony trichloride has an absorption maximum at 620 mμ while the antimony trichloride compound of vitamin A₂ obtained from fresh-water fishes has an absorption maximum at 693 mμ. Rather extensive investigations of the distribution of these two forms of vitamin A have led definitely to the conclusion that vitamin A₁ predominates in the tissues of salt-water fishes and vitamin A₂ predominates in the tissue of fresh-water fishes.

Gillam, Heilbron, Jones & Lederer (1) studied the ratio of the substances that have absorption maxima at 693 and 620 mμ and found the ratios to be 2:1 and 0.15:1 respectively in the liver oils from fresh-water and marine fish from Russian waters. These ratios show a considerable range of variation in different tissues, such as eyes, intestines, and pyloric caeca of various species of fish. Edisbury, Morton, Simpkins & Lovern (2) found a high concentration of vitamin A in oils prepared from tissues of the alimentary tract especially from the pyloric caeca which they reported for the cod to be ten to twenty times greater than that of average cod-liver oil. These observations concerning the presence of vitamin A in the intestinal wall have led to speculations concerning a possible rôle of the vitamin in assimilation of fat from the intestinal tract, but there are as yet no definite data supporting these hypotheses. Gillam and his associates (1) came to the conclusion that concentrates of the chromogens having absorption maxima at 693 and 620 mμ have approximately the same biological activity.

Investigations have shown that the livers of mammals, birds, and reptiles do not contain vitamin A₂. However, it has been demon-

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strated that these animals can absorb vitamin A₂ from the intestine and store it in the liver. Lederer & Rathmann (3) concluded that the absence of vitamin A₂ from the liver of mammals and other land animals is explained simply by the absence of vitamin A₂ from their food. They believe that vitamin A₂ is found in certain species of fresh-water fish because it is a specific product of liver metabolism of these species. Gillam (4) found no evidence of the presence of vitamin A₂ in the livers of twenty-one species of mammals and birds except those which had consumed fresh-water fish.

The chemical constitution of vitamin A₂ has been studied by several investigators. Gillam (1) and his associates were unsuccessful in separating vitamins A₁ and A₂ by distillation or by chromatographic adsorption. They were of the opinion that vitamin A₂ was closely related to vitamin A₁, probably containing an additional conjugated ethylenic linkage. When equivalent quantities of concentrates from halibut-liver oil and fresh-water fish-liver oil were ozonized the same quantities of geronic acid were formed, indicating that vitamins A₁ and A₂ both possess the same ring structure. Upon oxidation with aluminum tertiary butoxide in the presence of acetone the vitamin A₂ preparation yielded a ketone having the formula C₂₅H₄₄O₂, and having spectrophotometric properties in conformity with a compound having seven double bonds, or one more double bond than would have been present in the compound obtained by applying the same process to vitamin A₁. It was concluded, therefore, that vitamin A₂ has the following structural formula:

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\text{Vitamin A₂ (1)}
\]

Edisbury, Morton, Simpkins & Lovern (2), and also Wolff (5), have reached the conclusion that vitamin A₂ has a side chain containing one vinylene group more than vitamin A₁.

On the basis of solubility Chevallier & Choron (6) have advanced the view that vitamin A occurs in different forms in the liver and in the blood. Since the differences observed may be due to different esters of vitamin A₁, it is not clear that they were dealing with different forms of vitamin A.
There are suggestions in the literature of a third chromogen (1, 5) having an absorption maximum at 285 μ, and Lederer & Rathmann (7) have observed a second band at 645–650 μ. This suggested to them that the ratio of the absorption of the two bands—E at 693/ E at 620 = 2—in the oil from livers of fresh-water fishes does not correctly represent the ratio of A₂ to A₁ in that the band at 645–650 μ overlaps the band at 620 μ. Further investigations are necessary to substantiate this conclusion.

Dark adaptation and vitamin-A requirement.—Attempts to determine vitamin-A requirements in man by making use of improper dark adaptation of the eye have led to further studies of the instruments and methods used in detecting the physiological response of the eye to different intensities of light and correlating this with vitamin-A deficiency.

The reason that dark adaptation can be used as a measure for vitamin-A deficiency lies in the chemical relationship existing between vitamin A and visual purple in the eye. The rate of regeneration of visual purple which is bleached by bright light is dependent upon the adequacy of vitamin A in the organism. When there is a deficiency of vitamin A, regeneration takes place much more slowly. The instruments used are designed primarily to measure the rate of regeneration of visual purple.

The so-called biophotometer has been used extensively in studying dark adaptation. Palmer & Blumberg (8) have pointed out some definite limitations in the use of this instrument, particularly as applied to determining the vitamin-A requirement of children. They believe there is a learning factor which influences the results obtained and they do not believe that biophotometric tests are adequate for determining the presence of mild degrees of vitamin-A deficiency in school children. Schuck & Miller (9) reported biophotometric tests on ninety-four college women and stated that the variability in their results suggested that further work should be done to determine the response of individuals known to have a satisfactory vitamin-A intake. The investigations of Isaacs, Jung & Ivy (10) throw further doubt on the reliability of the biophotometer in detecting vitamin-A deficiency. They studied medical students whose vitamin-A intake by calculation ranged from 1,650 to 9,725 International units per day. Definite correlation between biophotometric readings, dietary vitamin A, and clinical symptoms of vitamin-A deficiency could not be obtained. Snelling (11) was unable to observe any consistent improve-
merit in dark adaptation in a group of thirty-four school children after receiving large supplies of vitamin A in the form of percomorph oil even though seventeen members of the group were abnormal, and fourteen considered borderline cases before vitamin-A administration was begun. The necessity for careful and frequent calibration of the biophotometer is stressed in a paper by Booher & Williams (12). These investigators fed daily doses of 5,000 to 8,500 International units of vitamin A to thirteen of 106 employees of the United States Department of Agriculture who showed the poorest dark adaptation. Five of these individuals responded to the increased vitamin-A intake and eight (one of whom was known to be afflicted with congenital nightblindness) showed no improvement.

Corlette, Youmans, Frank & Corlette (13) found twenty of fifty ambulatory adults to have poor dark adaptation. When these subjects were treated with vitamin A in the form of halibut-liver oil, all but one showed improvement. Ahmand & Harris (14) observed two of thirty boys receiving 1.5 pints of milk daily and also fish-liver oil to have subnormal dark adaptation. With the administration of additional vitamin A these individuals showed normal response.

Hecht & Shlaer (15) introduced several refinements in the instrument for measuring dark adaptation which they call the “Adaptometer.” They present evidence to show that the cones of the eye are affected by exposure to intense light as well as the rods. The recovery after exposure begins earlier and is more rapid in the cones. These authors (16) found that thirteen out of fourteen patients, suffering from alcoholic cirrhosis, showed disturbance of dark adaptation. Two of these were given 40,000 U.S.P. units of vitamin A daily and one became normal in nineteen days. The other needed a tenfold increase in vitamin intake for complete recovery. Four normal (17) individuals were observed on normal, low and high vitamin-A intakes extending over a period of several months. The most striking aspect of these studies was the almost parallel behavior of the rods and cones during recovery periods. The investigators feel that this parallelism indicates that vitamin A is probably associated with visual violet or iodopsin formation in the cone in much the same manner as it is associated with visual purple formation. The photosensitive pigment “iodopsin” has been isolated from the cones of the retina of chicken eyes (18). There is also evidence of the presence of a photosensitive substance in the eye of the frog (19).

In studying the relation of dark adaptation to vitamin-A deficiency
it must be recognized that as yet no normal threshold of dark adaptation has been established and that wide variations among individuals exist. Light-eyed individuals (20) are more sensitive to bright light and have poorer vision in low illumination than dark-eyed persons. Prolonged or slow dark adaptation is also associated with a number of diseases: congenital nightblindness has long been recognized.

Several investigators (8, 9, 10, 11, 12, 33) have discussed the inconsistencies of the results obtained and the reliability of criteria used in determining vitamin-A deficiency by dark-adaptation methods. These discussions show that the methods are still in the developmental stage and indicate that further studies in this field are necessary in order to determine their reliability.

Friderichsen & Edmunds (21) have studied the minimum amount of light to which infants will respond as a criterion of vitamin-A balance. They found that when infants were receiving daily a certain quantity of vitamin A, an increase in the amount had no effect on the irritability of the eye; but if the amounts were decreased the eye became more sensitive to the same intensity of light. The investigators believe that this procedure lends itself to determining vitamin-A deficiency in infants from one to twenty-four months of age.

An infrared photographic method (22, 23) has been used studying the rate of pupil dilation of the rabbit during dark adaptation. Perhaps this procedure may afford a means of detecting changes due to vitamin-A deficiency.

Through dark-adaptation studies on prisoners in Denmark, Edmund & Clemmesen (24) came to the conclusion that adults receiving daily slightly less than 1,400 International units of vitamin A over a period of nine months show no evidence of vitamin-A deficiency.

Utilization of precursors of vitamin A.—Previous investigations have shown that a difference exists in the capacity of animals of different species and even different breeds of the same species to convert carotene into vitamin A. The extent to which precursors can be utilized has received further study.

Lanzing & Van Veen (25) and Spruyt & Donath (26) studied the distribution of carotenoids in various fruits and vegetables generally used in the native diets of the Dutch East Indies. As is to be expected they found considerable variations in the same fruit depending upon stage of maturity, variety and other factors. The same investigators (27) then studied the distribution of these carotenoids in the blood of seventy-four prisoners who were receiving 9,000 Inter-
national units of vitamin A in their vegetable diet. Blood analyses revealed the presence of 7.9 International units of vitamin A per 10 cc. of serum and also 8.6 µg. of total carotenoids. These values were practically identical with those found for Europeans in Batavia, and native servants (28) receiving a mixed diet containing abundant vitamin A derived from vegetables, animal fat and dairy products. While alpha- and beta-carotene occurred in the highest concentration in the food they were found in low concentration in the blood indicating almost complete conversion of these carotenoids into vitamin A. On the other hand cryptoxanthin, which formed only 10 per cent of the total precursors of vitamin A in the diet, was found in larger quantities in the blood. The authors believe these results indicate that human beings may have difficulty in utilizing cryptoxanthin as a source of vitamin A.

That carotene in spinach and carrots may be poorly utilized is indicated in a report by Van Eekelen & Pannevis (29). It was observed that 94 to 99 per cent of the total carotene ingested was excreted in the feces while only 41 per cent of the carotene dissolved in oil was excreted. Whether this difference is due to absorption or destruction of the carotene is not apparent. Studies on dogs (30) indicate that carotene from carrots is utilized as readily as carotene in oil, or vitamin A in cod-liver oil, when the intake is 20 International units of vitamin A per 100 gm. of body weight.

The blood of eleven infants and sixteen young children suffering from xerophthalmia was studied by Haas & Meulemans (31) to determine vitamin-A and carotenoid content. Six of the eleven infants and thirteen of the sixteen children showed no vitamin A in the blood. The infants had a carotenoid content varying from 12 to 46 µg. per 100 cc. of blood and in the children the range was from 8 to 29 µg. The authors believe that vitamin-A content of the blood is a better criterion for judging vitamin-A deficiency than carotenoid content.

Clausen & McCoord (32) have made extensive studies of the vitamin-A, carotene and xanthophyll content of the blood of different age groups of infants up to two years to determine the normal variations and changes due to disease. The carotene content is lowest at birth and at that time is exceeded by xanthophyll. This ratio is reversed later in life. Vitamin A is also relatively low at birth. Carotene increases in the blood because it is rapidly absorbed from the diet but the rate of absorption is slower than for vitamin A. The vitamin-A, carotene and xanthophyll content of the blood is decreased during
infections due in part to low intake and in part to fever. When body
temperature returns to normal after fever the vitamin-A content of
the blood may temporarily go above normal.

Compounds other than carotene can be converted to vitamin A by
the animal organism (34). Beta-apo-2-carotinal was found to possess
a biological activity at daily doses of 5 µg, and the polyene alcohol
obtained from this compound had biological activity when fed in daily
doses of 10 µg. Von Euler, Günther, Malmberg & Karrer fed beta-
apo-2-carotinal to rats. When they examined the livers of these ani-
mals they found that the unsaponifiable fraction of the liver had an
absorption maximum in the ultraviolet which was approximately the
same as for vitamin A.

Hypervitaminosis A.—Opinions differ as to the toxicity of vita-
min A when fed in massive doses. Weslaw, Wronski, Wroblewski &
Wroblewski (35, 36) observed injury to rats by oral, subcutaneous
and percutaneous administration of “Vogan” and “Cresavit.” Injec-
tions were more than twice as active in impairment of tissue as oral
administration. Typical symptoms of hypervitaminosis A were
cachexia, loss of weight, fragility of the bones, skin changes—such
as loss of hair—and inflammation of the eyes accompanied by xeroph-
thalmia. The exact composition of the products used in these studies
is not known so it is not possible to explain why these results differed
from those obtained by Vedder & Rosenberg (37) and by Ikegaki
(38). Vedder & Rosenberg fed jewfish-liver oils containing approxi-
mately 600,000 International units of vitamin A and approximately
5,000 International units of vitamin D per gram to rats weighing
50 gm. at the beginning of the experiment. The dosage of vitamin A
ranged from 25,000 to 100,000 units daily. Evidence of injury was
not observed and the authors concluded that “If vitamin A is ever
toxic it is in excess of 100,000 International units daily for 50-gram
rats.” Ikegaki came to a similar conclusion after two to three months
of subcutaneous or intramuscular injection of high doses of vitamin A
in the form of “Biostearin.” His studies were made on both rats and
guinea pigs. Vedder & Rosenberg studies provided data which show
that there must be considerable destruction of vitamin A when fed
in massive doses, and the authors believe that this accounts for failure
to observe toxicity. Papke (39) found symptoms of overdosage of
“Vogan” in young rats when fed in dosages of 0.5 to 2 cc. daily. He
observed spontaneous fractures and anemia. He failed to find similar
symptoms in rats over six months of age. It is important to note that
he does not believe that the influence of vitamin D has been eliminated in these studies on "Vogan."

Vitamin D

It is impossible in a brief review to do justice to all of the fields of investigation on vitamin D. It seems advisable therefore to limit this paper to the more important studies on the chemistry of this vitamin.

A number of papers have dealt with the relative efficacy of different forms of vitamin D in the treatment of different species of animals. For the most part the results of earlier investigations have been confirmed.

It is now apparent that vitamin D can be produced artificially or obtained from fish liver oils which has a greater or lesser antirachitic effect on chicks than the vitamin D of cod liver oil if the products are fed at the same unitage as determined by biological assays with rats. Consequently, the results obtained from feeding rats cannot be used for judging the value of vitamin-D products for poultry.

On the contrary there appears to be no convincing evidence that man responds very differently from the rat so that this animal can be used to determine the potency of materials intended for human consumption.

There are reports which may lead to the conclusion that the vitamin D produced from 7-dehydrocholesterol is more efficacious in the treatment of rickets than the vitamin D produced from ergosterol. Since small differences in antirachitic effects are difficult to establish in clinical studies these results need further confirmation.

Evidence is accumulating that vitamin D plays a definite rôle in the prevention of dental caries.

Chemistry.—Hickman and his associates have extended their investigations of the chemical nature of vitamin D by means of molecular distillation of concentrates from fish-liver oils. Hickman & Gray (40) determined first the elimination curve for pure calciferol when dissolved in a cod-liver oil especially treated to remove interfering substances. They recovered 95 per cent of the added vitamin and the curve had a peak at 146°C. By a similar procedure they were unable to demonstrate a smooth distillation curve for vitamin D₂ but found a maximum in the curve at about 150°C. By assaying the different fractions from the distillation of vitamin D from cod-liver oil evidence was obtained for the existence of at least four and possibly six forms of vitamin D. Most of the vitamin D consisted of two
forms which gave maxima in their elimination curves close to the median temperature for distillation of vitamin D. The lowest boiling fraction of vitamin D when assayed by Bills, Massengale, Hickman & Gray (41) was found to be from one-half to one-fourth as effective for chickens as the total vitamin D of cod-liver oil when compared on the basis of rat-units. Liver oils obtained from other fish, such as spearfish and white sea bass, when subjected to analysis by distillation, yielded elimination curves distinctly different from each other and from the one obtained with cod-liver oil. By adding pilot dyes, whose distillation curves had previously been determined, to the distillation mixtures, the authors obtained evidence that a difference of CH₂ in the side chain makes a difference of approximately 4.5° C. in the distillation temperature. This fact enabled them to estimate from the temperature of distillation the size of the vitamin-D molecule in various fractions.

Brockmann & Busse (42, 43) crystallized a vitamin from tunny-liver oil which they identified as the vitamin D produced from 7-dehydrocholesterol. The extinction curves, melting points, and analytical values of the two agreed. Brockmann & Busse disagree with Zucker and coworkers (44) that vitamin D from tunny-liver oil has a lower molecular extinction coefficient than vitamin D₃ and a lower biological assay value than 40,000 International units of vitamin D per milligram. Brockmann & Busse believe that their previous assumption is correct, that the low molecular extinction values for vitamin D from tunny-liver oil were due to partial destruction of the ester during saponification.

In view of the practical importance of the synthesis of the different forms of vitamin D, it is noteworthy that Milas & Heggie (45) have reported (in a brief communication) the production of vitamin D from cholesterol. They treated the acetate with benzoquinone in a sealed tube for two hours at a temperature of 120 to 130° C. After some purification the crude product was exposed to ultraviolet radiation and upon biological assay was found to have a vitamin-D potency of more than 6,500 U.S.P. units of vitamin D per gram. Eckhardt (46) was unable to obtain 7-dehydrocholesterol from 7-aminocholesterol or from some of its derivatives.

As a result of rather extensive chemical and physical studies of the structure of the cyclic portion of the molecule of vitamin D, Auwers (47) has concluded that the position of the double bonds in the formula proposed by Windaus is correct.
In 1922 Evans & Bishop discovered, a dietary factor, subsequently called "vitamin E," which is particularly concerned with reproduction in the rat. A deficiency of vitamin E in the female rat does not interfere with oestrus, ovulation, or impregnation of the ovum, but the deficiency causes death and resorption of the fetus before maturity is reached. If a female rat on a vitamin-E deficient diet is given a single dose of vitamin E a few days after mating, or given sufficient vitamin E during the gestation period, normal young are produced. A deficiency of the vitamin in the male rat causes degeneration of the germinal epithelium which results in sterility. This damage appears to be irreparable.

Now that substances have been found which possess vitamin-E activity much attention has been directed to the elucidation of their chemical nature and structure. At least two naturally occurring substances, alpha- and beta-tocopherol, have been shown to have vitamin-E activity. Evidence has been presented to support the view that cumotocopherol and neotocopherol, described by German and Swiss investigators, are identical with beta-tocopherol (48). Prior to 1938 thermal decomposition indicated that the vitamin may be the monoether of either durohydroquinone or cumohydroquinone.

Chemistry.—Bergel, Todd & Work (49) through the synthesis of cetyl and allyl ethers of durohydroquinone and $\psi$-cumohydroquinone showed that alpha- and beta-tocopherols were not simple alkyl monoothers. They suggested that the properties of tocopherols could be best explained by assuming that in addition to the quinone ring there is a heterocyclic structure and stated that they were investigating the possibility of tocopherols being coumaran or chroman derivatives. John et al. (50) reached the same conclusion with respect to the structural relationship between tocopherols and coumaran or chroman. By studying the products formed by oxidizing alpha-tocopherol with chromic acid, Fernholz (51) concluded that this compound had a chroman structure and suggested the following structure which is based on the empirical formula, $C_{29}H_{60}O_2$. 

![Chemical structure of tocopherol](image-url)
He states: "α-Tocopherol is regarded as a substituted 6-hydroxy-
chromane with a long aliphatic side chain attached to the pyran ring." 
Drummond and coworkers (52) found evidence for the existence of
an oxygen ring in beta-tocopherol and suggested three possible for-
mulas which were in accord with their experimental results. After
further investigation, Moss & Drummond (53) arrived at the formula
suggested by Fernholz. Bergel, Jacob, Todd & Work (54) synthe-
sized two coumaran esters which were similar to beta-tocopherol in
absorption spectrum, reducing properties, thermal decomposition
products, and surface film measurements (117) and proposed the fol-
lowing structural formula for the coumaran portion of the molecule
based on the empirical formula C_{28}H_{48}O_{2}.

They leave the configuration of the side chain to be determined.

Later synthesis by these investigators of coumaran and chroman
derivatives verify the above conclusion (55, 56).

They further state that the formula for beta-tocopherol
(C_{28}H_{48}O_{2}) differs from alpha-tocopherol (C_{29}H_{50}O_{2}) by one less
CH_{2} group and that the compounds are simple homologues.

The synthesis of alpha-tocopherol through condensation of tri-
methyl hydroquinone and phytol bromide in the presence of zinc
chloride has been reported by Karrer, Fritzsche, Ringier & Salomon
(57). The synthetic compound and the naturally-occurring alpha-
tocopherol have the same absorption spectrum and reducing prop-
erties. The allophanates, 2,4-dinitrobenzoates and nitrophenyl ure-
thanes of the substance when mixed with the same derivates of alpha-
tocopherol show no depression of melting points. The optically inac-
tive synthetic compound can be resolved into two optically active
forms by means of bromocamphorsulfonic acid. The sulfonate has
the same optical activity as alpha-tocopherol. A single dose at 6 mg.
was shown to have definite biological activity (58). These investiga-
tors state that according to the method of synthesis the product could
have been either a chroman or coumaran but they believed the com-
ound was a coumaran derivative (57). Later three different labora-
tories (54, 50, 59, 48, 60, 61, 62) on the basis of further syntheses and
by analogy, came to the conclusion that Karrer's synthesis produced
substances with a chroman structure, which supports the view of Fernholz that alpha-tocopherol is a substituted chroman.

John and coworkers (50) prepared synthetic chroman and coumaran derivatives which showed properties similar to alpha- and beta-tocopherol, respectively. The 2,3,5,7,8-pentamethyl-6-hydroxycroman (M.P. 108°C.) had an ultraviolet absorption spectrum similar to alpha-tocopherol. The 2,4,6,7-tetramethyl-5-hydroxycoumaran (M.P. 132°C.)—see Bergold, Jacob, Todd & Work (62)—had previously been found to be similar to beta-tocopherol.

It may be stated that alpha-tocopherol (C₆₃H₉₀O₄) appears to have a chroman nucleus and beta-tocopherol (C₆₃H₈₄O₄) a coumaran nucleus, and the exact nature of the side chain for these compounds has not been determined, but the structure suggested by Fernholz seems to be accepted provisionally.

In attempting to synthesize the tocopherols, a large number of compounds have been prepared which have the biological properties of vitamin E (63, 64, 65). Some of these compounds are much simpler in chemical constitution than the naturally-occurring tocopherols. On the contrary, Karrer & Jensen (67) have demonstrated that 2,5,7,8-tetramethyl-2-[4'-8'-dimethyl-nonyl]-6-hydroxycroman which is very similar in constitution to alpha-tocopherol is wholly inactive when fed in 20 mg. doses. Since the difference between the synthetic compound and alpha-tocopherol is only in the side chain, they conclude that the nature of the side chain influences the vitamin-E activity. They also refer to their previous investigations showing that the presence of methyl groups on the benzene ring have an important bearing on the biological potency of synthetic compounds. John, Günther & Schmeil (68) in experiments on the synthesis of chroman derivatives with the ring system of alpha-tocopherol showed that the ring did not possess biological activity but that the side chain appeared to effect the biological activity. No synthetic compound has been observed to have greater biological activity than either the synthetic or the naturally-occurring tocopherols. The other compounds have been administered only in comparatively large quantities.

Isler (69) reported that the acetyl derivative of dl-alpha-tocopherol is more stable to oxidation than the original compound.

Investigations dealing with the isolation of vitamin E from wheat-germ oil have been reported by Moss & Drummond (53), Karrer & Salomon (71), and Mackenzie, Mackenzie & McCollum (72).

Biological assays.—A critical review of biological assays for vita-
min E was published by Palmer (73) and this subject has been discussed further by Bacharach and his associates (74, 75, 76, 77, 78, 79, 80). Bacharach has suggested an improvement in the method of assay by using virgin rats. He has observed that rats which have gone through a gestation-resorption show a lower fertility than virgin females and also that the requirement of virgin females for vitamin E may be only one-fourth or one-fifth as great as that of females which have had a resorption of a litter due to vitamin-E deficiency. He is of the opinion that resorption may produce toxic products which cause damage to the female that is not entirely reparable. Bacharach advocates the use of a standard in vitamin-E assays, which is a cardinal principle in biological assays, and he also stresses the desirability of plotting results in the form of response curves to provide for more accurate analysis of data. He reports that 1.2 mg. of alpha-tocopherol and 1.9 mg. of beta-tocopherol have approximately the same vitamin-E activity. Mason & Bryan (81), as the result of a rather extensive investigation, have concluded that animals can be obtained which more uniformly show vitamin-E deficiency by putting mother and young on a vitamin-E ration at about the middle of the lactation period.

A colorimetric method (82) for the determination of tocopherol, which depends on the ability of the compound to reduce ferric chloride, has been proposed by Emmerie & Engel. Their results were in good agreement with the determination of alpha-tocopherol by potentiometric titration with gold chloride as proposed by Karrer (83).

Requirement of different species.—Thomas and associates (84, 85, 86) have made an important contribution on the vitamin-E requirements of different species of animals. They destroyed the vitamin E in the rations by treatment with ferric chloride and demonstrated that these rations did not contain sufficient vitamin E to promote reproduction in rats. Goats kept on such rations reproduced normally and the investigators succeeded in producing three filial generations of animals on these rations. The observations led definitely to the conclusion that goats will reproduce normally when fed rations which are deficient in vitamin E. Goats which had been raised on the vitamin-E deficient rations were found to produce adipose and muscle tissue and milk which did not contain demonstrable traces of vitamin E, but the same tissues and the milk from goats raised under ordinary farm conditions were found to contain the vitamin. The authors consider their observations to give definite indications that the goat cannot synthesize
vitamin E. In preliminary studies they found that vitamin-E deficient rations do not interfere with reproduction in sheep or rabbits.

**Vitamin K**

*General.*—The term "vitamin K" was proposed by Dam (87) as an abbreviation of the name "Koagulations-Vitamin" to apply to the substance that was necessary for the prevention of a nutritional-deficiency disease in chicks. Manifestations of the disease are: a delayed clotting time of the blood; and a hemorrhagic condition which could not be cured by the known vitamins, including vitamin C. Schönheyder (88) afterward demonstrated that the delayed clotting time was associated with a low prothrombin content of the blood which could be normalized by feeding preparations containing vitamin K. Other constituents of the blood known to be necessary for clotting were found to be normal. Investigations dealing with the properties of vitamin K (89) and attempts to isolate it have been reviewed previously (90, 91, 92). In the investigations referred to, the vitamin was found in the fat-soluble, unsaponifiable, nonsterol fraction of an ether extract of alfalfa. Lichman & Chambers (93) have recently reported, however, that they obtained from liver a sterol which reduced the clotting time of the blood of jaundiced dogs and rats, and the blood of chicks deficient in vitamin K. The relation of this substance to vitamin K needs further investigation before it can be properly classified.

The chick was used in the early experiments on the discovery, chemical nature, and attempts at isolation of vitamin K, and the pathology of vitamin-K deficiency. The duck, goose and pigeon (94, 95) have also now been shown to be subject to vitamin-K deficiency.

Although the pathological picture of slow blood clotting and hemorrhagic condition can be reproduced by biliary fistulas in the dog and rat, the attempts to produce vitamin-K deficiency in mammals have met with a certain degree of failure. This failure to produce vitamin-K deficiency in mammals (98, 99, 100) may be due to the possible synthesis of vitamin K in the intestinal tract. While this has not been demonstrated it seems to be entirely possible since vitamin K can be produced by bacterial action on such substances as fish meal and casein (96, 97).

*Clinical and experimental observations.*—These observations on vitamin K have led to a very important clinical application in the treatment of patients having hemorrhagic tendencies associated with
a low prothrombin content of the blood. The danger of post-operative hemorrhage in cases of obstructive jaundice has long been recognized. Quick (101, 102) observed that hemorrhage in such cases was frequently associated with a low prothrombin content of the blood and that in severe cases it may be as low as 10 per cent of the normal (103). As long as the prothrombin content of the blood remains about 20 to 30 per cent of the normal, there appears to be no great danger from hemorrhage, but when it falls below these levels the clotting time of the blood may be greatly delayed. The feeding of vitamin-K preparations, together with bile or bile salts, has been found to be effective in raising the prothrombin content of the blood above the danger level.

Brinkhous, Smith & Warner (104) reported that cases of obstructive jaundice and biliary fistula showing a hemorrhagic tendency associated with low prothrombin responded more rapidly to treatment with a combination of bile and vitamin-K preparations than with bile feeding alone. Very shortly thereafter a series of papers from the Mayo clinic reported similar observations (98). Dam & Glavind (100) observed that parenteral administration of vitamin K to jaundice patients is ineffective without concurrent feeding of bile salts. This suggests that obstruction of the bile interferes with the absorption of vitamin K from the intestine. In a later report by Snell, Butt & Osterberg (105) it was stated that the feeding of a vitamin-K preparation, together with bile salts, to thirty patients with obstructive jaundice, brought about normal coagulation time within twenty-four to seventy-two hours.

A tendency to bleed has been observed in dogs (99) with biliary fistulas of long duration. Such dogs are low in prothrombin. The prothrombin content may be raised by the feeding of bile alone, but it responds more rapidly to treatment with bile or bile salts, together with vitamin-K concentrates. Greaves & Schmidt (106) found the blood of rats with bile fistulas low in prothrombin and slow to clot. The feeding of vitamin-K preparations in massive doses relieved these symptoms. The feeding of bile or bile salts also decreased the blood-clotting time. Rats made icteric by ligation of the bile duct and fed a stock diet (free of bile salts) had a low prothrombin content of the blood. When bile salts were fed the prothrombin returned to a normal level. These observations supply proof that the bile salts aid in carrying vitamin K through the intestinal wall.

The literature (98, 107, 105) contains suggestions that vitamin-K
intake may be related to prothrombin deficiency in such conditions as sweet clover disease, liver injury from chloroform and other agents, but further study is necessary to demonstrate such a relation. The fact that the prothrombin content of the blood is low in these cases of liver injury suggests that somehow the liver takes part in the formation of prothrombin.

Assay of vitamin K.—At the present time the methods proposed for the assay of vitamin K involve the use of young growing chicks. Almquist (108) has described a preventive method in which the quantity of substance necessary to prevent the development of abnormal clotting time in day-old chicks is determined. Dam (109) proposed to determine the quantity of material necessary to restore the normal clotting time of the blood of chicks depleted of vitamin K. Later he (110) recommended as a standard the use of a preparation of dried spinach, to which a value of 500 units of vitamin K per gram was ascribed. The activity of the unknown material in curative tests was then compared with this standard, using a prescribed procedure for determining clotting time (111). A modification of the methods of Almquist and Dam have been proposed by Dann (112) and Thayer et al. (113). Ansbacher (114) has published a note which suggests that it may be possible to make curative assays in a period of a few hours.

Occurrence of vitamin K.—Vitamin K occurs rather generally distributed in nature. The following materials have been found to contain the vitamin: pig, dog and beef liver, fish meal, alfalfa, kale, carrot tops, tomato, hemp seed, soybean oil, egg yolk, rice bran, chick feces, dried human feces, dried alcoholic human feces, oat shoots and several bacteria (98, 115, 116).

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