Staff Meeting Bulletin
Hospitals of the . . .
University of Minnesota

Vitamins

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STAFF MEETING BULLETIN
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William A. O'Brien, M.D.

I. LAST WEEK

Date: May 6, 1937

Place: Nurses' Hall
Recreation Room

Time: 12:15 to 1:20 P.M.

Program: Movie: Philippine Fantasy

Abstract: Autopsies

Discussion: W. A. O'Brien
R. W. Koucky
R. M. Amberg
R. R. Sullivan
C. J. Lind
Herman Kesting

II. MOVIE

Title: Sky Harbor

Released by: N. W. Bell Tel. Co.
Film.

II. ABSTRACT

THE PRESENT DAY STATUS
OF THE VITAMINS

Marguerite Booth
Arild E. Hansen

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Introduction

Although clinical conditions due to avitaminosis were apparently known as long ago as 2600 B.C., and in spite of the fact that deficiency diseases have been of tremendous economic importance throughout the ages, the tardiness in gaining an understanding of these conditions is remarkable. With a gradual acceleration of knowledge beginning less than a half century ago, the whole subject with its vast ramifications has been built up by means of chemical, biological and clinical studies, until at last it has practically attained the status of an exact science. This has been brought about by individual and organized efforts of a multitude of investigators in all parts of the world. Thus, the fact that we today can actually see the vitamins themselves and know or very nearly know the chemical structure of many of those which are important in nutrition, is not the result of pure coincidence or accident. This is strikingly apparent when one considers that a semi-thorough review during the past several years calls for the consideration of some 200 articles on a single vitamin, or a rough total of about 2000 for any given year.

Not only is the chemical structure of many vitamins known exactly, but recent investigators in various chemical laboratories have also developed chemical tests for determining quantitatively, or approximately so, the amounts of various vitamins in the different tissues. Further developments along these lines may prove to be of far-reaching importance. Many of the ramifications of vitamin experimentation have proved to be surprising. One of the most interesting disclosures is that vitamins have been found to be definitely linked up with the hormones. By the mere removal of a simple methyl group or by the change in a double bond, the vitamin may become hormonal or may take on carcinogenic activity.

One of the popular conditions associated with avitaminosis in the knowledge of the average American is night blindness, because of the fact that this disability corresponds with the alleged time incidence of the greatest number of automobile accidents. The relationship of the vitamins to infections is overemphasized no doubt in a popular way. Nevertheless, there are certain pertinent facts which clearly indicate that such relationships actually do exist. Not only are we interested in the conditions caused by a lack of vitamins but also by

the possible importance of the administration of too much of these substances. As regards the human subject there needs to be little fear of hypervitaminosis.

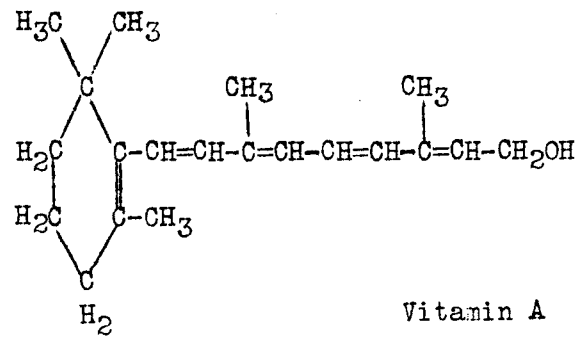
Recent investigations have disclosed that subclinical states of avitaminosis exist in many individuals. This is particularly significant because of the interrelationships between vitamins and certain clinical disorders, e.g. the favorable effects produced by the administration of vitamin B extracts in cardiac dysfunction. There are relatively few frank cases of avitaminosis judging from a survey of the University of Minnesota Hospital admissions. The following review is an attempt to summarize both newer and older knowledge regarding the vitamins, as well as a study of florid avitaminosis in a large hospital practice.

Vitamin A

History

In 1913, McCollum and Davis, and Osborne and Mendel simultaneously described experiments which showed that certain fats were essential for normal growth. Three years later, McCollum suggested the term "fat-soluble A" to distinguish it from the "water-soluble B". Steenbock, in 1919, noted some correlation between the vitamin A effect of certain vegetables and the amount of the yellow pigment carotene present in these foods. Euler demonstrated that carotene could replace vitamin A in the diet (1928). In 1930, Moore showed that carotene is converted into vitamin A in the liver and is stored there as the vitamin. Karrer (1931) and Drummond (1932) isolated the almost pure unsaturated alcohol from fish livers. In 1935, Lasch showed that liver storage of vitamin A is for the most part in the Kupffer cells.

Chemistry $C_{20}H_{29}OH$



Vitamin A

An unsaturated alcohol with four double bonds in the side chain and one in the ring.

Precursors of vitamin A:

1. Alpha carotene
2. Beta carotene
3. Gamma carotene
4. Cryptoxanthin

These precursors of the vitamin are vegetable pigments, which when ingested by most animals, are converted by the liver into the compound vitamin A itself. Carotene occurs in nature usually as a mixture of two or more isomeric forms. The chemical composition of these isomers differs slightly, but all have at least one beta-ionone ring, a grouping which seems necessary for vitamin activity. Vicamin A has the structure of one-half the carotene molecule with an alcohol group at the end of the chain. Since beta-carotene is symmetrical and contains two beta-ionone rings, two molecules of vitamin A could be formed from it by breaking it down at the middle double bond with the formation of a primary alcohol at the terminal carbon atom. Alpha and gamma carotene are not symmetrical and contain only one beta-ionone ring, hence forming only one molecule of vitamin A when broken down. The vitamin activity of beta carotene in small concentrations is double that of alpha carotene. Experimental evidence supports this theory.

Carotene is intensely yellow, while vitamin A is colorless. The vitamin is very soluble in fat and occurs as an ester in fish liver-oils. It gives a characteristic though not entirely spe-

cific blue color with antimony trichloride in the presence of chloroform. It has a highly characteristic strong absorption band at 328 mu in ultraviolet light. Very little vitamin A is lost during processes of commercial canning or home-cooking. Vitamin A has been isolated in nearly pure form, but has not been synthesized.

Standardization:

The U.S.P. XI unit for vitamin A (equivalent to the International unit) is the amount in milligrams producing the growth-promoting and antixerophthalmic activities in vitamin A-depleted rats equal to that of 0.6 gamma of the International Standard beta carotene, or the equivalent amount of U.S.P. Standard Reference cod-liver oil.

The standards of pure beta carotene adopted by the International Conference is dissolved in coconut oil to which hydroquinone has been added. The subsidiary international standard for vitamin A is the U.S.P. Reference cod-liver oil which has a potency of 3000 units per gram.

The U.S.P. XI requires that 1 gram (15 grains) of cod liver oil shall contain at least 600 U.S.P. units of vitamin A.

Pathology

The primary effect of vitamin A deficiency is on epithelial structures - a keratinizing metaplasia of the greater part of the ectodermal covering of the body. There is a substitution of stratified keratinizing epithelium for normal epithelium in various parts of the respiratory, alimentary and genito-urinary tract, in the eyes and in the para-ocular glands. This replacement epithelium is identical in all locations and comparable in all its layers with epidermis. Superficially, it is continuously casting off keratinized cells. The accumulation of these epithelial cells in many glands and their ducts and in other organs is a striking gross pathologic feature of avitaminosis A. Cysts may be formed in the glandular organs. In the lungs, these cysts were at first thought to be abscesses, but there is rarely invasion of the tissues. The pulmonary keratinization leads also to bronchial occlusion, bronchiectasis and atelectasis. This metaplasia in human infants and in a variety of laboratory animals has been found in the conjunctive, mucosa of the nares, accessory sinuses, trachea, bronchi, pancreas, renal pelves, ureters, salivary glands, uterus and peri-urethral glands. It occurs earliest in the trachea and bronchi, then in the kidney pelvis, and as late involvement in the eye. Metaplasia of the epithelium of the cornea and of the conjunctival sac is followed by vascularization, edema, and leukocytic infiltration of the cornea. Infection of the cornea may lead to ulceration and hypopyon.

Secondary effects of vitamin A deficiency are loss of weight due to loss of fat in all storage depots and muscular atrophy, anemia, cessation of growth of bones, degenerative lesions of skeletal muscle, and lymphoid hypoplasia of the spleen. Degeneration of the myelin sheath is a late secondary result.

Restoration of the diet rapidly dispels the lesions of avitaminosis A, unless complicated by destruction of tissue. The change back to the normal epithelium is an abrupt one and affords further evidence that the primary consequence of lack of vitamin A is epithelial, and not of nervous origin.

Chief Symptoms of Avitaminosis-A:

A. In Man

1. Night blindness (nyctalopia or hemeralopia), and xerophthalmia (keratomalacia) eventually leading to partial or complete blindness. Bitot's spots, opaque whitish deposits in the scleral conjunctiva, are the most characteristic sign.

2. Keratinization of epithelial cells in various parts of the body frequently associated with respiratory, gastro-intestinal and genito-urinary disturbances.

3. Cornification and eruption of

the skin with papular and pustular lesions.

4. Retarded growth, weakness, and loss of weight.

5. Increased susceptibility to infections of mucous membranes (claimed by some, denied by others). Only true where supply of vitamin A has been inadequate or its storage in the body depleted.

B. In Animals (rat)

1. Cessation of growth and loss of weight.

2. Xerophthalmia; impaired regeneration of visual purple.

3. Keratinization of epithelium in respiratory, gastro-intestinal and genito-urinary tracts.

4. Formation of urinary calculi.

5. Cutaneous lesions; glandular abscesses.

6. Defective formation of teeth and gums.

7. Impaired reproduction: prolonged gestation, fetal death and dystocia.

8. Loss of vigor.

Laboratory Diagnosis:

Test for subnormal dark adaptation - based on the ability of the patient to regenerate rhodopsin (visual purple) after exposure to a calibrated source of light - elaborated by Jeans and Zentmire (1934). This is particularly valuable in mild deficiency.

Clinical Applications of Vitamin A

1. Promotion of normal growth in children.

2. Prevention and cure of night blindness and xerophthalmia due to lack of vitamin A.

3. Prevention of renal calculi claimed by Higgins - but discredited by the A.M.A., Council on Pharmacology and Chemistry.

4. Maintenance of normal epithelium of the body.

5. Normal tooth formation.

6. Cure of senile vaginitis - by large doses of cod liver oil or haliver oil (Simpson and Mason).

7. Treatment of epithelial lesions and healing of wounds by the local application of vitamin A in an ointment medium.

Vitamin A can be given in many foods containing the factors in the form of the vitamin or as its precursor, carotene. Carotene is not as well absorbed as vitamin A, hence the vitamin is the more satisfactory preparation to use by mouth. The absorption of vitamin A or of carotene may be impaired by infections, pregnancy, absence of bile, and other pathological processes, such as damage to the liver which interferes with its ability to convert carotene to vitamin A. Crystalline carotene is better than vitamin A for parenteral use. At present, there are no pure or injectable preparations of vitamin A available.

Daily Requirements:

The quantitative requirement is as yet unknown.

Children require more per kilogram of body weight because of the demands of growth.

1934 Salter	as minimum	0.3 mg. carotene
1935 Harris	as minimum for adults	1,000 U.S.P. I.U.*
1936 A.M.A.	for children	6,250-10,000 I.U.
1936 League of Nations	for pregnancy and lactation	9,000 U.S.P. units

Larger doses may be required in severe

avitaminosis.

*International units.

Natural Sources - in order of potency:

Vitamin A:

Halibut liver oil is the richest source.
Burbot liver oil ranks next (4 to 10 times as potent as cod liver oil).
Cod liver oil.
Liver.
Whole milk supplies more than any other single food.
Large amounts: butter, egg yolk, animal fats (beef and mutton).

Provitamins:

Apricots are the richest plant source.
Large amounts - spinach, carrots, chard.
Smaller amounts (1/6 as much as butter) - green beans, green peas, Brussels sprouts, lettuce, tomato, yellow squash, sweet potato, pumpkin.

Some Commercial Products:

Abbott -	Haliver oil, plain Cod liver oil, N.N.R. Vita-Kaps
Lederle -	VI - Delta emulsion (A and D)
Mead-Johnson-	Cod liver oil (plain) Cod liver oil with viosterol Oleum percomorphum with cod liver oil Viosterol Halibut liver oil Halibut liver oil with viosterol
Parke Davis -	Natola Irradola A (A, B, D, and iron)
SMA Co. -	Carotene in oil.
Squibb -	Adex

Vitamin B Complex

History

In 1884, Takaki of the Japanese Navy demonstrated that kakke (beriberi) was of dietary origin. Eijkman believed that it was due to a poison in polished rice (1897). Funk in 1912 proposed the name "vitamin" for the substance derived from rice polishings which cured beriberi. Mendel suggested that another factor than certain fats was necessary for normal growth (1914). McCollum found this substance was water soluble and in 1916 proposed the terms "fat-soluble A" and "water-soluble B". The multiple nature of vitamin B was proved by Smith Hendrick, and confirmed by Goldberger, separating the pellagra-preventing factor from the antineuritic factor. Four other elements have been partitioned off, and since 1927 the vitamin has been known as Vitamin B complex.

Constituents

Vitamin B_1 - - - antineuritic factor

Vitamin B_2 (G) complex
1. Vitamin B_2 or lactoflavin - growth-producing factor
2. Vitamin B_6 - rat antidermatitis factor
3. P.P. factor (pellagra-preventing in man) - or Vitamin H of György

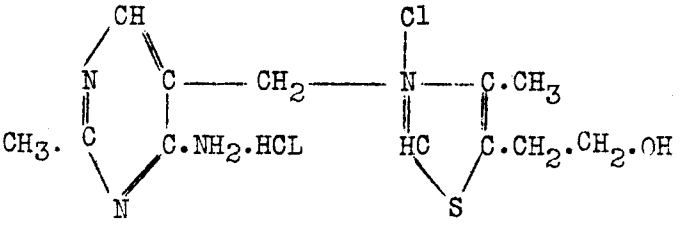
Vitamin B_3 - - - chicken antipellagra factor
? growth-producing factor

Vitamin B_4 - - - antiparalysis and antiencephalomalacia factor. Perhaps a variation of Vitamin B_1.

Vitamin B_5 - - - including chicken antipellagra factor.

Vitamin B_1 (B)

Chemistry: $C_{12}H_{17}N_4$ OS Cl.HCL



Aneurin of Jansen, or Torulin

The hydrochloride of a pyridimine-thiazole compound. Windaus first proposed the formula of $C_{12}H_{16}N_4OS$ - when he isolated the crystalline Vitamin B_1 in 1931. The vitamin is a base and reacts with acids to form salts. The formula usually given at present is that obtained by the action of hydrochloric acid on the free base. There is still some doubt about the positions of certain groups and double bonds. The sulphur linkage is not that of cystine. Vitamin B_1 has also been isolated in crystalline form from baker's yeast or rice polishings by Jansen and Donath, by Peters, Opdake and by Van Veen, some with slightly different formulae. It has been sunthesized by Williams and Cline (1936).

Crystalline Vitamin B_1 - hydrochloride is water-soluble. It is stable to heat in the dry state, but is rapidly destroyed by moist heat at 100°C especially in alkaline medium. Its melting-point is 245°C. Its ultraviolet absorption band is at 250-260 mm. (Windaus) or 245-249 mm. (Peters).

Standardization

The Sherman unit is that amount which when fed as a daily allowance to a standard test animal (rat) previously depleted of vitamin B_1 will suffice to cause a gain in weight of three grams per week during an experimental period of four weeks.

The International Unit is the vitamin B_1 activity of 10 milligrams of the International Vitamin B_1 Reference Standard which is an adsorbate prepared from rice polishings by the method of Seidell as described by Jansen and Donath.

Ten to twenty milligrams per day of this Reference Standard are necessary to maintain normal growth in young rats, or 20 to 30 milligrams for a cure of pigeon polyneuritis.

N.N.R. Requirements - 1936

Foods claiming vitamin B_1 content as a medicinal source must provide at least 200 International units in the quantity of food consumed daily.

Concentrates of vitamin B_1 or a dehydrated natural product must exceed a potency of 25 International units per gram or per cubic centimeter.

Pathology

Human beriberi and pigeon polyneuritis show the same pathologic changes, some enlargement of the heart, particularly the right ventricle, edema, atrophy of muscles, and degeneration of the nervous system. Wolbach believes that it is best to regard all the abnormal findings thus far recorded as secondary effects, and to consider the primary pathologic changes due to vitamin B_1 deficiency as not demonstrable at present.

The striking lesion is Marchi degeneration of the myelin sheath of peripheral nerves - which appears late in avitaminosis B_1. Further work is necessary to prove that this is due to specific lack of vitamin B_1 or to some other factor, such as starvation. Other secondary features are chronic passive congestion, and enlargement of the islands of Langerhans in the pancreas.

Chief Symptoms of Avitaminosis B1

A. In Man

1. Beriberi
   (a) Peripheral neuritis with paralysis of extremities and muscular atrophy or edema.

(b) Vasomotor symptoms: heart palpitation, dyspnea, enlargement of right side of heart.

2. Retarded growth and development.

3. Polyneuritis, especially of alcoholic origin, in pregnancy, in diabetes, or in malnutrition either in children, chronic diseases or some primary alimentary disease.

4. Gastro-intestinal disturbances: atrophy of lingual papillae, achlorhydria, intestinal hypotonicity.

5. Ocular disorders: retinal hemorrhages, optic neuritis.

6. Anorexia.

7. Impaired carbohydrate metabolism.

8. Failure of lactation.

B. In Animals (rat and pigeon)

1. Retarded growth and loss of weight.
2. Polyneuritis (pigeon).
3. Anorexia.
4. Paralysis and convulsions (rat).
5. Impaired oxidation of lactic acid and pyruvic acid in carbohydrate metabolism, resulting in injury to the central nervous system.
6. Bradycardia.
7. Disturbance of intestinal function; gastric atony.
8. Impaired reproduction:
   (a) Atrophy of the testes.
   (b) Atrophy of the ovaries.
9. Failure of lactation.

Laboratory Diagnosis

1. Urinary Excretion Test

The amount of vitamin B_1 excreted in the urine (demonstrated by biological assay of the urine) may be used as an index of the dietary intake.

A daily excretion of less than 12 International units per day (for a 140 lb. man) and failure to show a response to a test dose of 500 International units per day are presumptive evidence that the diet is below normal in vitamin B_1 content. The normal output is from 12 to 35 International units.

2. Arakawa Test

The maternal milk is tested for vitamin B_1 content. The Arakawa reaction is based on the close relationship between the peroxidase reaction of the milk and the state of deficiency in vitamin B_1. If a blue color develops when the milk is mixed with three reagent solutions, a positive test for the presence of the vitamin is obtained. If no blue color appears, the Arakawa test is negative - indicating a lack of the vitamin in the milk.

3. Estimation of previous vitamin B_1 intake and of the requirements of the vitamin by Cowgill's formula.

4. Therapeutic test with the purified vitamin.

Clinical Applications of Vitamin B_1

1. Prevention and cure of beriberi.
2. Promotion of normal growth in children.
3. Anorexia due to avitaminosis-B.
4. In chronic alcoholism with vitamin B-deficiency polyneuritis.
5. In pernicious vomiting and polyneuritis of pregnancy.
6. For nutrition in lactating women.
7. Valuable in concentrated form in conditions where ordinary foods are poorly utilized.
8. In diabetic neuritis.
9. In cardiovascular disease (Weiss and Wilkins - 1936). Sure and Jones, 1937).

Daily Requirements

The requirement is related to the fuel value of the food consumed and proportional to the metabolism (Cowgill).

1934 Cowgill		about 300 I.U.
1934	Jansen	about 200 I.U.

1934 Salter	as minimum	150 I.U.
1934-35 Jones	for infants from for adults to	50 I.U. 200 I.U.
1935 Vorhaus	as minimum therapeutic dose	4000 Sherman units. 10 mg. crystalline vitamin
1936 Van Veen		200 I.U.
1936 Harris		250 to 500 I.U. about 1 mg. crystalline vitamin
1936 A.M.A., Council on Pharmacology and Chemistry	for infants for adults to	50 I.U. 200 I.U.
1937 Wilder and Wilbur		10-20 mg. crystalline vitamin

Natural Sources - in order of potency

Brewer's yeast and wheat germ are excellent concentrated sources.

Whole grain cereals and bread.

Liver and kidney.

Leafy vegetables have one-fourth the content of vitamin B_1 as in yeast.

Egg yolk.

Orange, the highest of the fruits, has one-fifth as much as yeast. The concentration of vitamin B_1 in most raw foods is low and it may further be reduced by heat and loss in solution in the discarded cooking water. Milk, white flour and meat are very poor sources. Vegetables and fruits have but a small amount. Special care should be taken to insure an adequate supply of the vitamin.

Some Commercial Products - Vitamin B Complex

Abbott - Brewer's yeast tablets
Be-Tabs
Vita-Kaps
Vitamin B capsules

Harris - Brewer's yeast powder
Vitamin B complex

Lilly - Betalin

Mead-Johnson - Brewer's yeast powder
Brewer's yeast tablets

Squibb - Vitavose
Yeast tablets
Vitamin B and G capsules or syrup

Some Commercial Products - Vitamin B_1

Harris - Tiki-Tiki Syrup

Merck - Betabion

Parke-Davis - Vitamin B_1, crystalline

Winthrop - Betaxin

Vitamin B_2 (G) Complex

The antipellagra vitamin is now known to have at least two and probably three factors. It was called vitamin G by Goldberger, but is now generally known as vitamin B_2.

1. Vitamin B_2 or lactoflavin - growth-producing, of no therapeutic value in human pellagra.

2. Vitamin B_6 - the rat antidermatitis factor.

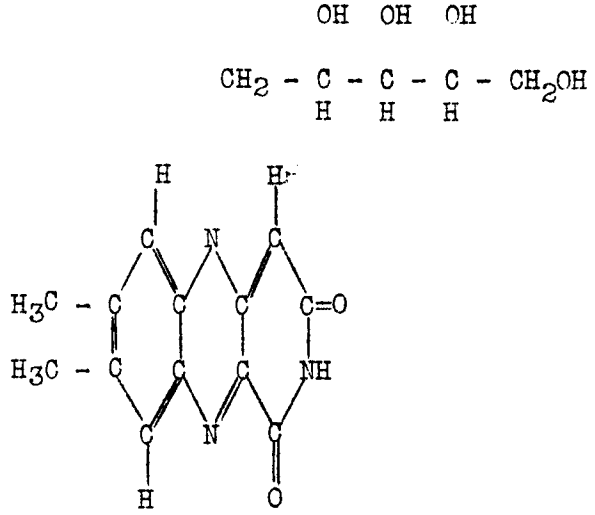
3. P.P. factor (pellagra-preventing in man.)

Rhe relation of vitamin B_6 is not clear Investigators disagree as to its value in pellagra and in black tongue of dogs which is generally accepted as identical with pellagra in man. There is now

thought to be a separate P.P. factor in the vitamin B_2 complex. György calls this fraction vitamin H.

Chemistry: Vitamin B_2 (G)

The chemical formula was at first thought to be $C_{17}H_{20}N_4O_6$ (Kuhn), but was later (1935) proved to be $C_{18}H_{21}N_3O_6$ - 6.7 dimethyl - 9 isoalloxagin.



Vitamin B_2 is the water soluble and heat stable naturally occurring yellow pigment, lactoflavin. It is bleached and destroyed by exposure to visible light, especially in blue-violet portion, and by alkaline media. It is relatively insoluble in alcohol. It is adsorbed by Fuller's earth from acid solution and is precipitated by lead acetate. The melting-point of the best natural and synthetic preparations is 282°C. The specific rotation is: 96.6° for a 0.15% solution in 0.05 N NaOH, and 90.0° for a 0.1% solution. In the presence of boric acid, lactoflavin is dextrorotatory. It possesses an ultraviolet absorption band at 260 mu. and also in the visible range. Flavin dissolves in water giving a bright yellow solution with a characteristic green fluorescence. Strong reducing agents convert it into the colorless form, but it is easily oxidized again by shaking it with air. Lactoflavin has been isolated from milk by Kuhn, Booher, and Karrer, and has been synthesized by Stern and by Kuhn (1935). Ovoflavin and hepaflavin are also growth-producing and are similar chemically. Vitamin B_4 appears to increase the action of lactoflavin in promotion of growth.

György, Kuhn, and Wagner-Jauregg believe lactoflavin is closely related to the "yellow oxidation enzyme" of Warburg. This enzyme seems to consist of flavin in combination with a colloidal carrier, and acts as a carrier catalyst taking up hydrogen from the substrate, later being oxidized to the original enzyme. Since this enzyme is probably necessary for the animal body, and since flavin is not able to be synthesized in the body, it is necessary to include vitamin B_2 in the diet. In the flavin enzyme is the best example hitherto known of the relation between an enzyme and its active group of vitamin or hormone character.

Standardization

The Sherman unit for vitamin B_2 is that amount which when fed daily to a standard test rat that has been previously depleted of vitamin B_2 according to the prescribed technique, will promote a gain in weight of three grams per week over a period of from four to five weeks.

Pathology

As in avitaminosis B_1, the pathologic effects seen in vitamin B_2 deficiency are probably only secondary. The histology of human pellagra, black tongue in dogs, and rat dermatitis throws little light on the subject. Degenerative lesions in nerve-cells and myelin sheaths are characteristic of the deficiency - but may not be specific. Lesions of the skin and mucous membranes are consistently present. At autopsy, ulcerative lesions are found in the intestines, similar to those in colitis.

Chief Symptoms of Avitaminosis B_2 (G) Complex

A. In Man

1. Pellagra - due probably to avitaminosis P.P. of the vitamin B_2 complex.

Brown, scaly, symmetrical dermatitis in exposed areas, glossitis, soreness of mouth, indigestion, diarrhea, and disturbances of the nervous system - at times leading to dementia.

2. Acrodynia may be caused by the lack of one or more of the factors in vitamin B complex.

Symptoms of Acrodynia - irritability, insomnia, appearance of misery, anorexia, acrocyanosis, itching and burning of hands and feet, desquamation of palms and soles, marked perspiration, photophobia, muscular hypotonicity, increased blood pressure, and loss of teeth.

Little is known of deficiency in lactoflavin.

B. In Animals (rat)

1. Retarded growth and loss of weight (deficiency in lactoflavin)
2. Cataract formation.
3. Dermatitis with loss of fur and ulceration of the skin - due to lack of vitamin B_6. (Acrodynia of rats).
4. Keratitis.
5. Black tongue (in dogs) - due probably to deficiency in P.P. factor.

Laboratory Diagnosis - no tests are known.

Clinical Applications of Vitamin B_2.

1. Prevention and cure of pellagra.
2. Promotion of growth and well-being (due to lactoflavin).
3. Possible prevention of cataract formation.
4. Increase of vitamin B_2 content of milk in lactation.
5. Cure of stomatitis and glossitis of chronic alcoholism and of alcoholic pellagra - by early treatment with a high caloric diet and 75 grams of yeast or of liver extract daily. (Blankenhorn and Spies).
6. Treatment of acrodynia.

Pellagra is seen particularly in the southern part of the United States, but one should watch for secondary pellagra in the northern sections - due to organic diseases of the digestive tract - as obstructing and malignant diseases, or to other gastro-intestinal disturbances with faulty absorption: alcoholism, colitis, tuberculous enteritis, celiac disease, etc.

The supply of protein may also have a significant bearing upon the pellagra problem, and the vitamin B_2 complex may not be the only deficiency factor. This has been demonstrated by Sherman, rats on high protein diet being less severely affected by the lack of vitamin B_2 than animals on diets with lower amounts of the same protein.

The relation of vitamin B_2 complex to pernicious anemia has been stressed by Castle and others - claiming that macrocytic anemias of several types are dependent upon vitamin B_2 complex deficiency. However, it has been shown that this vitamin is neither the liver anti-pernicious anemia principle nor the "extrinsic" factor concerned in hemopoiesis.

Daily Requirements - not yet determined.

Natural Sources:

Brewer's yeast and wheat germ - as for vitamin B_1.
Liver and kidney are the richest source of flavin.
Egg white has high content of flavin but no P.P. factor.
Milk and meat (one-fifth as much as yeast).
Leafy vegetables, tomato and banana (one-tenth as much as yeast).
Fish muscle rich in P.P. factor, but lacking in flavin.

Vitamin B_3

Williams and Waterman claim that there is a pigeon vitamin B_3 necessary for supplementing a diet of polished rice to which vitamin B_1 has been added. It is a growth principle and seems to be a stored vitamin factor. Musser reports that more recent work indicates that vitamin B_3 appears to be a more abundant supply of vitamin B_1, and therefore doubts the existence of vitamin B_3. Another worker has found a "filtrate factor" in vitamin B complex - a dietary essential for the chick - which promotes growth and is probably not identical with the antipellagra factor in chicks. This chick antipellagra factor has been believed by some to be in vitamin B_3 and vitamin B_5. Further investigations are necessary to establish any relationship of vitamin B_3 to human nutrition.

Vitamin B_4

Tentative formula . . $C_4N_4H_5Cl$ or $C_4H_4N_4HCl.\frac{1}{2}H_2O$. Barnes in 1932 isolated a heat-labile crystalline preparation of vitamin B_4. The crystals consist essentially of adenine hydrochloride, but probably contain some impurity which causes activation. The vitamin is alkali-labile and is easily destroyed. It is closely associated with vitamin B_1 and some workers suggest that both vitamin B_1 and vitamin B_4 are necessary for the prevention of beriberi, while vitamin B_2 and vitamin B_4 are necessary for the prevention of pellagra. Reader thinks a third factor is necessary in the treatment of pellagra and proposes two vitamin B_4 factors - vitamin B_{4a} and vitamin B_{4b}. It is not abundant in foods; whole wheat is a source of vitamin B_4 needed by the rat in addition to vitamin B_1 and vitamin B_2.

This intimate association between vitamin B_1 and vitamin B_4 is not yet understood. Vitamin B_4 seems to be a variation of vitamin B_1 since vitamin B_1 cannot be obtained free from vitamin B_4 activity. The apparently pure crystalline preparation of vitamin B_1 as isolated independently in different laboratories, is one of the richest sources of vitamin B_4 activity. The standard procedure for producing avitaminosis B_4 actually consists in first subjecting the experimental animals to vitamin B_1 deficiency. Vitamin B_4 deficiency seems to resemble a state of chronic or persistent deficiency of vitamin B_1, since it can always be cured by the administration of a sufficiently large dose of vitamin B_1. Specimens of supposedly pure crystalline vitamin B_1, prepared in different parts of the world, having identical properties, and giving no evidence of admixture with impurity, when examined by x-ray analysis or other means, all possess their characteristic vitamin B_4 activity.

György (1935) claims that in the absence of the vitamin B_4 fraction there occur lesions of the nervous system with disturbances in coordination and ataxia, hence the name, anti-paralytic vitamin. Elvehjem thinks that it may prove to be important in nutrition in man and in the treatment of certain disorders of the brain. He believes that the encephalomalacia of chicks prevented by the addition of certain vegetable oils to the diet is due to lack of vitamin B_4 - and claims that the factor preventing paralysis in chicks is identical with vitamin B_4. Others disagree with this on the basis that vitamin B_4 is water soluble, while soy bean oil, which contains the antiparalytic factor, is a fat.

Vitamin B_5

This fraction of vitamin B complex in conjunction with vitamin B_3 has been thought to be the chick antipellagra factor. At present our knowledge of vitamin B_5 is quite nebulous.

Vitamin B_6

The chemical composition and structure is unknown. With lactoflavin it is one of the principal components of vitamin B_2 complex. Termed the rat antidermatitis factor by György (1934), it is identical with the Y factor of Chick. The P.P. factor (pellagra-preventing) is now thought by György to be a third factor in the complex - probably vitamin H.

Vitamin B_6 is in a filtrate which remains after removal of the flavins from vitamin B_2 complex, and is responsible for the cure of the specific "acrodynia-like" dermatitis developed by young rats fed on a vitamin B-free diet supplemented with purified vitamin B_1 and lactoflavin. Vitamin B_6 is not a true water soluble vitamin, being only partially soluble in that medium, but it is soluble in ethyl alcohol. It is heat-stable, is inactivated by visible light, is adsorbed on fuller's earth from acid solution, is precipitated by phosphotungstic acid, and migrates toward the cathode on electrodialysis. Autolysis, which yields 80 - 100% extraction from wheat germ, is the method adopted as the standard procedure for the preparation of active extracts of the vitamin. It is suggested that the vitamin does not contain a primary amino-group, but is of a basic nature and possibly contains a hydroxyl group. Vitamin B_6 has some similarity to choline, though pure choline chloride does not cure rat dermatitis.

This essential factor must be largely combined in some way with the tissue in which it occurs, since the greater part is not easily extracted by ordinary solvents. No knowledge has been obtained concerning the nature of the union between vitamin B_6 and the tissue, but possibly the vitamin is attached to the protein as an active group which is not easily split off. Fat has a sparing action on the vitamin. In rat dermatitis produced by vitamin B_6 deficiency, vitamin B_6 alone does not cure it, but extra fat (linseed oil) with vitamin B_6 will cure it. This curative factor in fats is probably linoleic acid, and closely associated with vitamin F which is necessary for the normal growth of the young rat. The scaly tail and scurfy appearance of the skin in vitamin F deficiency has often been noted in vitamin B_6 deficiency animals. The relation of vitamin B_6 to man is uncertain, as the "rat pellagra," "chick pellagra" and "human pellagra" are apparently not identical.

Standardization

The unit is the minimum daily dose necessary to cure the rat of this specific "acrodynia-like" dermatitis.

Natural Sources - in order of potency

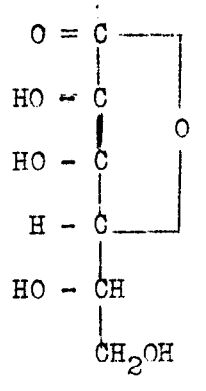
1. Wheat germ exceedingly rich in it - about 5 units per gram.
2. Fresh fish muscle is a rich source (salmon, haddock, herring). Fish muscle contains no vitamin B_2 (lactoflavin).
3. Rice polishings.

Vitamin C

History

Scurvy has long been known in history. In 1535 Jacques Cartier during a winter on the St. Lawrence reported the cure of a disease, obviously scurvy, by a dedoction made from the bark and needles of the spruce tree. A British naval surgeon in 1747 demonstrated the striking effect of fresh lime-juice as an antiscorbutic agent. Lime-juice later became a compulsory supplementary food on all ships in the British navy. Barlow differentiated infantile scurvy from rickets (1883). In 1907 Holst and Fröhlich produced the disease in experimental animals (guinea pigs). The antiscorbutic factor was called vitamin C in 1918 to distinguish it from vitamin B complex, the other water-soluble factor. Isolation in crystalline form as hexuronic acid was made from bovine adrenal glands in 1928 by Szent-Györgyi. This later (1932) proved to be identical with King and Waugh's crystalline active factor derived from lemon-juice. Vitamin C was sunthesized by Reichstein in 1932 starting with l-xylose.

Chemistry



$C_6H_8O_6$

L-ascorbic or cevitamic acid.

Vitamin C is the lactone of threo-3-keto hexonic acid. The properties of crystalline acid are identical with those of hexuronic acid: solubility in water, insolubility in fat solvents, marked sensitivity to exposure to visible light and to heat and oxygen, especially in alkaline solution. Its melting point is 183° - 185° C, and the optical rotation $(a)_D^{20} = 25^{\circ}$ ($\pm 1^{\circ}$). It has a single broad absorption band at 263 mu. The essential condition for the antiscorbutic activity in the ascorbic acid group is the d-configuration of the fourth carbon atom.

Vitamin C has a very characteristic power of reduction, by oxidation losing two hydrogens in acid solution, but retaining its vitamin activity. The chemical mechanism of vitamin C activity in the body is not known. Its biological significance is based on the fact that this reaction is reversible. The oxidized vitamin can be reduced with relative ease by the tissues to its original substance, and may thus act as an oxygen carrier. There is more than a probability that vitamin C does not play a specific organic functional rôle in the animal body, but fulfills a general function in the life of protoplasm. In the absence of this vitamin all cellular functions seem to be injured to the same extent. Besides its activity in the respiratory function vitamin C is fundamentally important in the formation of normal intracellular substance. In avitaminosis-C there is a failure to form this substance with normal properties - possibly as a result of reduced cellular oxidation. The mechanism of its activity in the prevention of hemorrhages is uncertain, although it is thought to cause changes in the intercellular substance of the capillaries. However, clinical results with vitamin C therapy have been disappointing in the hemorrhagic diseases, particularly in thrombocytopenic purpura, leukemia, Schönlein's purpura and hemophilia.

Rats, rabbits, calves and birds can synthesize vitamin C in the body, but guinea pigs, swine, dogs, monkeys and man require it in the diet.

Standardization

The International unit, which was formerly defined as the vitamin C activity of 0.1 cc. of lemon-juice, has now been defined as the vitamin C activity of 0.05 mg. of l-cevitamic (ascorbic) acid. This is the quantity of l-cevitamic acid usually found in 0.1 cc. of lemon juice. An ounce of lemon-juice has a potency of 15 mg. of cevitamic acid, while an ounce of orange juice has a value of 20 mg. of the vitamin.

The claim that a food is valuable because of its vitamin C content should be permitted only if it provides a daily intake of at least 250 units of vitamin C. (N.N.R.)

Pathology

The gross and microscopic pathologic changes in infantile scurvy and experimental scurvy in guinea pigs is practically identical. There is a striking inability of the supporting tissue to produce and maintain intercellular substances, hence the effect is on the cells of mesenchymal origin. The intracellular substances concerned are the collagen of all fibrous tissue structures, the matrices of bone, dentin and cartilage, and all non-epithelial cement substance, including that of the vascular endothelium. Bone pathology is explained as due to failure of osteoblasts to form osteoid tissue, and the hemorrhage of scurvy as due to a failure of cement substance in blood vessels.

Soft tissue changes are hemorrhages in regions determined by mechanical stresses and trauma, as well as anasarca and degenerations of skeletal and cardiac muscle. Secondary changes are hypertrophy of the heart, degeneration of muscles, and anemia with bone marrow destruction.

Gross pathologic changes are hemorrhages and bone lesions: sub-periosteal hemorrhages and those in the epidiaphyseal junctions of growing bones, resorption of bone matrix, inactivity of the osteoblasts, osteoporosis, the trümmerfeld zone of disorganization at the epiphysis, and separation and dis-

placement of the epiphysis. In growing teeth formation of dentin ceases, enamel and cementum fail to develop, and the pulp becomes separated from the dentin by liquid produced by the odontoblasts.

Repair following vitamin C therapy is dramatic in character and rapidity - all pathologic lesions soon changing to normal processes and normal tissues.

Chief Symptoms of Avitaminosis - C

In Man and Animals (guinea-pig)

1. Scurvy - increasing pallor, irritability, spongy and bleeding gums, loosened teeth, sore and swollen joints, petechiae and large superficial hemorrhages, epistaxis, sore mouth, dyspnea, loss of energy, anorexia, loss of weight, anemia, edema, fragility of bones and pseudo paralysis.

2. Less Extreme Deficiency

1. Hemorrhagic tendencies.
2. Dental caries, pyorrhea.
3. Vague aches and pains.
4. Fatigue, pallor, anemia.
5. Abnormal cutaneous pigmentation.
6. Increased susceptibility to infection in general, and to specific cases of diphtheria poliomyelitis, and tuberculosis.
7. Joint disease strikingly similar to rheumatic fever.
8. Vagus nerve disturbance: increased pulse and respiration.
9. Sensory nerve disorders (paresthesias).
10. Increased capillary fragility.

Total absence of vitamin C from the dietary is extremely rare in America and frank scurvy is not common in adults, though somewhat more frequent in children. Infantile scurvy occurs mostly between 6 and 18 months of age, and particularly in the winter and spring following a low intake of vitamin C. Subclinical avitaminosis, that is, a mild or partial deficiency causing ill-defined symptoms, is rather widely accepted and is probably very common.

Laboratory Diagnosis

1. Blood Plasma Test

Estimation of reduced vitamin C in blood by chemical test. Blood plasma values of less than 0.75 to 0.80 milligram per cent of reduced vitamin C indicate subnormal vitamin C intake.

2. Urinary Excretion Test

This test is based on determination by chemical titration with 2.6 - dichlorophenol - indophenol of the amount of vitamin C normally excreted in the urine; and the response to a large test dose or doses of pure cevitamic acid (saturation or retention test).

An excretion of 20 mg. a day is the lower limit of normal excretion (Youmans).

3. Capillary Resistance Test

This method consists essentially in creating a pressure on the arm of the patient and observing, in a small area, the number of petechiae which appear in a certain length of time. This test is not specific for avitaminosis - C.

4. X-ray of Long Bones

Clinical Applications of Vitamin C

1. Prevention and cure of scurvy.

2. In dental caries, pyorrhea, certain gum infections (Hanke), anorexia, anemia, and undernutrition - which may be concomitant signs of vitamin C deficiency.

3. Maintenance of strength of capillaries.

4. Parenterally as sodium cevitamate in conditions interfering with oral ingestion of vitamin C or its absorption in optimal amounts (persistent vomiting, diarrhea, etc.).

5. In infant feeding, routinely.

6. In cases of lowered intake of

vitamin C due to a restricted diet, either voluntary or imposed (Sippy diet).

7. In certain infections which demand an increased supply of vitamin C - as tuberculosis, rheumatic fever, diphtheria, poliomyelitis, and pneumonia.

8. Prevention of peptic ulcer (Smith and McKonley).

9. Demands of pregnancy.

10. Decrease in certain cutaneous pigmentation.

11. Acceleration of coagulation of blood in hemorrhagic diseases (value controversial).

12. Promotion of union of fractures - in conjunction with vitamins D and B.

Vitamins A and C are anti-infectious only in the limited sense that in their absence pathologic changes occur which may open the way to secondary infection. Rinehart in 1935 produced in guinea pigs typical heart lesions of rheumatic fever - the Aschoff bodies, by infection in addition to a partial vitamin C deficiency.

Daily Requirements

1933 Schultzer	in adults	40 mg.
1934 Göthlin	in adults	19-27 mg.
	in children	38-54 mg.
1934 Salter	as minimum	35 mg.
1934 Stepp		10-20 mg.
1934 Svensgaard	in infants	30 mg.
1935 Szent-Györgyi	in infants	25 mg.
	in adults	50 mg.
1935 Turner	in children	40 mg.
1936 King	in infants	25 mg.
	in adults	to 40 mg.
1937 Youmans	as minimum	25-40 mg.

Natural Sources - in order of potency

Oranges and lemons, particularly.

Excellent sources: grape-fruit, tomato juice, limes, tangerines, lettuce, fresh strawberries, raw cabbage, water cress, apples, bananas, paprika, spinach, carrots, fresh pineapple, and grapes.

Good sources: potatoes, peas and string beans, if not cooked too long.

Vitamin C has been called the vitamin of uncooked foods. Nearly all fresh fruits and vegetables have anti-scorbutic value - especially the citrous fruits. These articles must be prepared with care, however, as vitamin C is the most easily destroyed of any of the vitamins. In foods this vitamin deteriorates rapidly on standing. It is completely destroyed by boiling for thirty minutes in the presence of air and a moderately alkaline solution, as when the cook adds soda to the water in which vegetables are boiled to preserve their green color. Oranges from trees sprayed with certain chemicals, and tomatoes artificially ripened by ethylene gas contain little of the vitamin. Fruits or vegetables which have been cooked at high temperatures with full exposure to air may have had their vitamin C oxidized. The vitamin is more stable in fruit than in vegetable juices. Certain metal containers also impair its potency, especially copper and tin, while nickel, chromium, aluminum and glass are harmless. Canning of cruits, and vegetables can now be done with little loss of vitamin C by exclusion of air. Breast milk has four times as much vitamin C as milk from cows on a summer diet.

Vitamin C is widely distributed in relatively high concentrations both in

plants and in the tissues and secretions of animals. Its content is highest in glandular tissues and lowest in muscle and stored fat. The richest tissue in vitamin C is the pars intermedia of the pituitary gland, the adrenal comes next - and then the liver. It is also found in the corpus luteum, pancreas, brain, lens, aqueous humor and intestinal wall. Its storage in the adrenal has been a subject of controversy. It is now believed that a liberal amount of vitamin C is necessary for the normal working of this organ rather than that it is stored there for usage of the rest of the tissues, as the liver stores up vitamin A.

Some Commercial Products

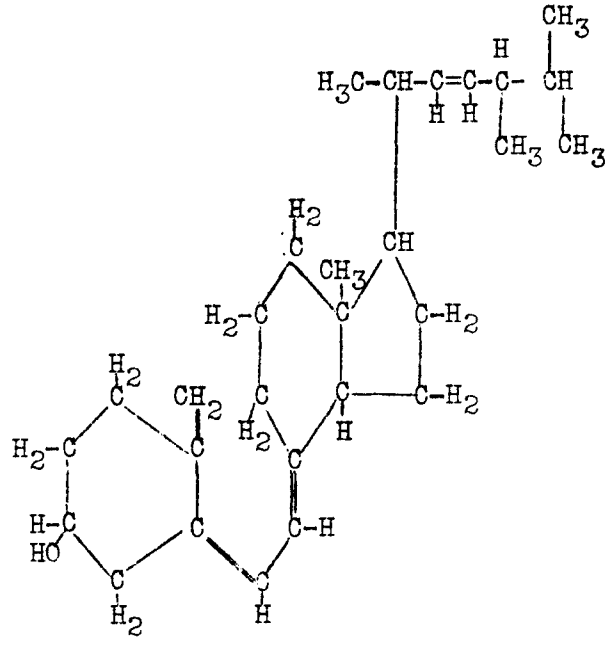
Abbott	- Cevitamic acid Vita-Kaps
Harris	- Ascorbic Acid
Hoffmann-LaRoche	- Cevitamic acid Cal-c-malt
Lederle	- Cevitamic acid
Lilly	- Tablets cevalin
Merck	- Cebione
Parke-Davis	- Cevitamic acid
Squibb	- Cevitamic acid

Vitamin D

History

Mellanby in 1918 gave substantial evidence that rickets was a deficiency disease, due to the lack of a vitamin contained in cod liver oil, which was either vitamin A or one of similar distribution. Four years later, McCollum demonstrated the separate entity of the antirachitic factor - vitamin D. Huldschnisky, in 1919, found that the short ultraviolet rays of a quartz mercury vapor lamp cured rickets. Hess and Steenbock (1924) made certain foods antirachitic by irradiation, activating their cholesterol fraction, and later they and others proved that this activatable impurity in cholesterol was ergosterol. In 1927, it was believed that vitamin D was irradiated ergosterol, and that ergosterol was the only provitamin D. Recently, other precursors have been recognized, and vitamin D has been found to consist of a number of fractions.

Chemistry



$C_{28}H_{43}OH$

Vitamin D is identical with calciferol, the vitamin active substance produced by the action of ultraviolet light on ergosterol. Calciferol is the most powerful antirachitic agent known and is 400,000 times as effective as cod liver oil in curing rickets in the rat. Calciferol is the most important form of vitamin D from a practical standpoint.

Ten forms of vitamin D have been artificially prepared - all sterols:

1. Cholesterilene sulphonic acid, isolated by Bills in 1925 through treatment of cholesterol with fuller's earth. It is not in fish oils and is only of theoretical importance.

2. Irradiated cholesterol by Bills in 1928.

3. Heated irradiated cholesterol - by

Koch and Hathaway (1929).

4. Irradiated ergosterol - whose active principle is calciferol, isolated in crystalline form by Bourdillon and by Windaus in 1932.

5. Non-irradiated ergosterol treated with alkyl nitrites by Bills and MacDonald in 1931. It is not in fish oils and is of no practical significance.

6. Irradiated ergosterol treated chemically by Windaus and Langer in 1933. It has an active substance, 22-dehydrocalciferol.

7. Irradiated 7-dehydro-cholesterol synthesized by Windaus and by Bills in 1935, more potent than 22-dehydro-calciferol for chickens.

8. Irradiated 7-hydroxy-cholesterol subthesized by MacDonald in 1936.

9. Irradiated provitamin derived from sitosterol, the sterol of the higher plants corresponding to cholesterol of animals, - by Bills in 1937.

10. Ergosterol activated by low velocity electrons has been shown by McQuarrie et al. to be effective in rickets in human subjects (1937).

Vitamin D has been called the anti-rachitic vitamin, the sunshine vitamin, or the calcium-phosphate metabolizing vitamin. It is an isomer of ergosterol, the sterol or higher alcohol found in ergot and yeast. The vitamin is fat-soluble, and is very stable to heat and oxygen, although it will be destroyed at temperatures of 180°C or higher. It is not injured by slightly acid or alkaline media. This vitamin is stored in the body. It is the most important calcifying agent, promoting bony growth by facilitating assimilation of calcium and phosphorus. It is of interest that the vitamin has a phenanthrene nucleus, a structure common with several other physiologically highly active substances such as the sex hormone and the carcinogenic hydrocarbon. Furthermore, ergosterol, calciferol and especially neo-ergosterol possess estrogenic activity; also some actively estrogenic substances are definitely carcinogenic.

Standardization

The U.S.P. XI unit for vitamin D (equivalent to the International unit) is the vitamin D activity of 1 mg. of the International Standard Solution of irradiated ergosterol (equal to 0.025 gamma of crystalline vitamin D) or the equivalent amount of U.S.P. Standard Reference cod liver oil. The U.S.P. XI requires that 1 gram (15 grains) of cod liver oil shall contain at least 85 U.S.P. units of vitamin D.

The Steenbock unit is that amount of vitamin D which, when uniformly distributed into the Standard vitamin D deficient diet, will produce a narrow and continuous line of calcium deposits on the metaphysis of the distal end of the radii and ulnae of standard rachitic rats. To convert this unit to the International unit, the multiplying factor is 2.7.

The vitamin D content of average cod liver oil is 100 International units or 37 Steenbock units per gram.

Pathology

In rickets, calcium salts are incompletely deposited, or even not at all, both in the maturing proliferative cartilage and in bone which is in process of formation. This failure in lime-salt deposition is the most striking feature in the pathology of rickets and is the essential cause of the gross changes in the skeleton. The only change outside the skeleton is hypertrophy of the parathyroid glands.

The characteristic bone changes are due to the softening of the bones from loss of inorganic matter and to the subsequent stress on the soft bones, which causes marked deformities. Normally, there is about two-thirds mineral matter in bone, and one-third organic matter. This ratio is reversed in severe rickets. Most of the loss is in calcium phosphate which ordinarily constitutes 85% of the mineral content.

Both long bones and flat bones may be affected. Enlargement of the epiphyses of long bones is most noticeable in the regions of most rapid growth, at the wrists, knee and ankle, as well as at the costochondral junctions. The metaphysis is greatly enlarged in width and thickness. Osteoporosis causes curvatures and fractures. Compensatory thickening of the cortex is often visible grossly. Large frontal and parietal bosses and areas of rarefaction (craniotabes) are characteristic in the skull.

In the microscopic picture, as in the gross, experimental rickets in the rat resembles human rickets. The pathologic conditions arise from retardation and suppression of the usual sequences in normal ossification. There is failure of provisional calcification of the intercellular matrix, the transitional zone between cartilage and bone becomes irregular and uneven, and the metaphysics presents a disorganized appearance. The uncalcified bone or osteoid tissue is particularly characteristic in rickets. Following vitamin D therapy repair rapidly takes place, the first effects being demonstrable in 24 hours.

The teeth also show pathologic changes, evidenced by dental caries and irregularity in size, shape and position. Marked disturbance of the blood calcium and phosphorus occurs. In infantile rickets, the serum calcium is about normal, 10 to 11 mg. %, but the inorganic phosphorus may be reduced as low as 1.2 mg. %. When tetany accompanies the rickets, the serum calcium is diminished to between 5 and 7 mg. %, sometimes as low as 4 mg. %.

Chief Symptoms of Avitaminosis D

1. Rickets - irritability, craniotabes, prominent frontal bosses, delayed closing of fontanelles, pigeon breast, rachitic rosary, flaring ribs, epiphyseal enlargement at wrists and elbows, marked perspiration, delayed eruption of teeth, muscular weakness, protruding abdomen, and bowing of legs.

2. Spasmophilia or infantile tetany- carpopedal spasm, laryngospasm and convulsions, and spasticity.

3. Osteomalacia - extreme softening of bones, especially in pregnancy.

4. Osteoporosis - failure of normal deposition of calcium phosphate leading to impaired calcification of bone.

5. Cessation of growth.

6. Abnormal ratio of calcium and phosphorus in the blood.

7. Dental malformation and caries.

Laboratory Diagnosis

1. X-ray examinations of bones.
2. Determination of calcium and phosphorus in blood serum.
3. Phosphotemic curve of Warkany.
4. Blood phosphatases test for active rickets. Phosphotase of blood increased (Smith 1933). This method has not been extensively used, but should be made the subject of surveys on a large scale. The test may be indicative of disturbances in calcium and phosphorus metabolism other than rickets.
5. Erb's sign for tetany.

Clinical Applications of Vitamin D

1. Prevention and cure of infantile rickets and tetany.
2. Prevention and cure of osteomalacia.
3. Formation and maintenance of normal tooth structure.
4. In defective calcium and phosphorus metabolism.
5. Routinely during infancy and periods of rapid growth, in pregnancy and lactation.

An adequate intake of calcium and phosphorus is also necessary in all cases.

Daily Requirements

1935 Harris	for normal infant	1500 I.U.
	curative	3000 I.U.
1936 Shelling & Hopper	for normal infant	1125 I.U.
	for premature infant	4500 I.U.
1936 Eliot	prophylactic	800 -1600 I.U.
	curative	1200 I.U.
1937 McQuarrie	for normal infant -not above	300 I.U.
	for premature infant -not above	540 I.U.

Vitamin D is required especially during the period of growth, during pregnancy and lactation, as well as in acute and chronic infections, and wasting diseases. There are as yet no controlled clinical reports on the subject.

Natural Sources

1. Fish liver oils: halibut, cod, Burbot, percomorph, salmon, haddock, herring, sardine, puffer fish, shark.

2. Egg yolk.

Foods are inadequate sources of vitamin D and cannot furnish the daily requirement. Cereals have a definite inhibiting effect on the vitamin. Sunshine is not dependable because of the lack of exposure to it, due to clothing, window glass, smoke, dust and fog which destroy the effect of sunshine.

Antirachitics

1. Cod liver oil was the first reliable agent to be established.
2. Direct irradiation of the body by means of ultraviolet energy was next.
3. Irradiated food, particularly milk, was third.
4. Activated ergosterol from yeast.
5. "Yeast milk," produced by feeding cows irradiated yeast, came next.

Since then, other antirachitics have also been used, such as viosterol, haliver oil, percomorph oil, and crystalline vitamin D.

Hypervitaminosis

It is thought that vitamin D is the only vitamin which can cause hypervitaminosis. However, the toxic dose is so large, that this danger is rare. There is little need of anxiety about the administration of viosterol in amounts up to 150,000 International units daily. Except in cases of hypersensitivity, one can give fifty to one hundred times the minimum dose with safety. Vitamin D is made more toxic when a large amount of calcium is given with it. Experimentally, an excess of vitamin D produces increased calcification of tissues, particularly of the cardiovascular system. It increases calcium excretion in the urine and causes loss of appetite and of weight, diarrhea, cachexia and a disturbance in fat and calcium metabolism. The cement substance of the teeth becomes overgrown so that the teeth become ankylosed in the jaw bone. There is over-calcification of the growing bones, and at times children may have a severe eczematous eruption.

Some Commercial Products

Abbott	- Cod liver oil Haliver oil Viosterol
General Mills	- Activated ergosterol
Mead-Johnson	- Cod liver oil (plain) Cod liver oil (with viosterol) Oleum percomorphum 50%. Cod liver oil with percomorph oil Viosterol Halibut liver oil Halibut liver oil with viosterol

Lederle - Cod liver oil liquid concentrate.
Viosterol
VI - Delta Emulsion (A and D)

Parke-Davis - Natola
Irradiola (A, B, D and iron)

SMA Co. - Vitamin D concentrate

Squibb - Cod liver oil
Halibut liver oil
Adex

Winthrop - Drisdol (crystalline vitamin D)
Viosterol

Vitamin E

History

Evans and Bishop in 1922 announced the discovery of a new fat-soluble substance essential in the diet for reproduction, which they designated vitamin E. Evans successfully isolated (1936) from wheat-germ oil a pure crystalline substance possessing vitamin E activity.

Chemistry

Vitamin E is alpha-tocopherol, a higher alcohol containing one or more hydroxyl groups, with a provisional formula of $C_{29}H_{50}O_2$ and a molecular weight of about 440. Reactions with iodine and hydrogen suggest the presence of three reactive double bonds. The active fraction is fat-soluble, extremely stable with regard to high temperatures, ultraviolet ray, atmospheric oxygen, strong alkali, acids, and hydration. It is not inactivated by hydrogenation or saponification process, but is destroyed by bromination, treatment with potassium permanganate, and long exposure to ultraviolet light. It forms biologically active esters with acetic acid and benzoic acid. The activity is correlated with an absorption band at 294 mu. Nothing definite is known regarding the mechanism through which this vitamin brings about its physiological action. This anti-sterility vitamin is thought to be not only biologically but also chemically a female sex hormone. It is stored in the body to a considerable extent. Hill and Burdett, noticing that consumption of "royal jelly" will convert the larva of a working-bee into a queen-bee, suggest that this property is due to vitamin E content.

Standardization

No standard unit has been established.

Pathology

The effect of vitamin E deficiency is on the reproductive system. In female animals fed a diet lacking in this vitamin, the fertilized ova are implanted in the uterus apparently in the normal manner. However, the feti die in the uterus and are resorbed. In the male animal there is a gradual degeneration of the germinal epithelium.

Chief Symptoms of Avitaminosis E

A. In Man

1. Habitual and threatened abortion.
2. Uterine hypoplasia, amenorrhea, sterility.

B. In Animals (rat and chicken)

1. Failure of reproduction.
   (a) Female - resorption of young during gestation.
   (b) Male - sterility with irreversible, incurable lesions in the testes which do not respond to a high vitamin E diet.
      (1) Loss of fertilizing power.
      (2) Absence of motility of spermatozoa.
      (3) Loss of sperm.
      (4) Loss of sex interest.
2. Paresis in young rats from maternal deficiency.
3. Muscular weakness, atrophy

of voluntary muscles in young animals.

The vitamin is held so tenaciously by the tissues, the source is so varied, and the supply so abundant that deficiencies are probably rare in man.

Laboratory Diagnosis:

No test available for avitaminosis E.

Clinical Applications of Vitamin E

1. Treatment of sterility, habitual, and spontaneous abortion in man. Vogt-Møller successfully treated 17 out of 20 cases of habitual abortion with wheat-germ oil, after noting favorable results in sheep and cows.

2. Possibly in hypoplasia and hypofunction of the gonads.

Daily Requirements

Human requirement unknown.

Animal requirement - 0.1 mg. per rat per day as minimal dose (Drummond 1936)

Natural Sources - in order of potency

Wheat- germ oil.
Vegetable oils: cottonseed oil, corn oil, olive oil.
Lettuce.
Whole grain cereals.
Legumes and soy beans.

Some Commercial Products

Abbott - wheat germ oil

Squibb - Zygon

N.N.R. 1936 - states that there is no evidence of therapeutic merit for vitamin E and does not accept vitamin E preparations.

Vitamin F

Vitamin F has become of practical importance because of the great amount of propaganda in cosmetic literature dealing with dermatological conditions. There has been considerable question among investigators as to whether the expression vitamin F should actually be used in this connection.

In 1927, Burr working with Evans on vitamin E found that animals reared on highly purified low-fat diets still failed to attain normal development and nutrition. Subsequent investigations by Burr and Burr revealed that rats on fat deficient diets have early cessation of growth, scaliness of feet and hands, scaliness of the tail so marked that the tip frequently becomes necrotic and falls off, hematuria, and early death. McAmis, Mendel, and Anderson reported somewhat similar findings in animals on a fat-free regimen. Burr and Burr were the first to find that fats of high degree of unsaturation given in relatively small amounts caused complete disappearance of symptoms. Later, they definitely established that esters of linoleic and linolenic acids were essential for the normal nutrition of the rat; hence, the expression, "the essential unsaturated fatty acids." There has been much controversy as to whether this type of deficiency should be considered a type of avitaminosis. Most reports in the literature term this disorder a fat deficiency disease. Evans and his co-workers as well as others have been referring to this essential factor as vitamin F.

These unsaturated fatty acids have been known to cause disturbances in gestation and lactation. As regards the human subject little is known. Relatively recently at the University of Minnesota, one of the workers in this field maintained himself on a strictly fat-free diet for a period of over six months - resulting in some rather interesting but not entirely conclusive findings. In infants maintained on a diet otherwise complete but strictly devoid of fat, it has been shown that eczema developed. Several investigators have found that certain infants suffering from outspoken eczema of long duration have been found to be benefitted by internal administration of oils rich in unsaturated fatty acids over a variable length of time.

Commercial Product

Cole - Vitamin F Perles.

Vitamin H

Györgyi in 1931 found a factor, insoluble in its natural state, which is necessary for neutralizing the toxic action of dried egg white. He called this principle vitamin H, and now identifies it with the P.P. factor which was later extracted from vitamin B_2 complex.

However, the term vitamin H has been ascribed by others to different essential constituents of the diet. The vitamin H of Mackay (1934) in the form of raw liver or preserved raw meat cured trout who failed to thrive on diets with all the known vitamins. Recently, Richardson and Hogan discovered a new vitamin (vitamin H) not identical with vitamin B_6, but which also cures rat dermatitis. It is present in wheat-germ oil, yeast or alcoholic extract of corn starch.

Vitamin K

Chemistry - formula is unknown.

The antihemorrhagic vitamin (clotting or coagulation factor) is fat-soluble, relatively stable to heat and light, destroyed by alkaline medium, and not readily adsorbed by activated magnesium oxide or activated carbon.

Dam in 1935 noted a hemorrhagic tendency similar to scurvy in chicks, not prevented by cevitamic acid but by this new fraction which he called vitamin K. It is neither vitamin A or vitamin D. Avitaminosis K produces a reduced prothrombin content in the blood of chicks. The administration of vitamin K can restore the clotting time to normal in three days. It is probably synthesized in the lower intestinal tract - since it is found in the feces of chicks not receiving this factor in the diet.

Natural Sources

Pig liver, hemp seed and alfalfa are the most potent sources. Green vegetables are a fair source. Cod liver oil is devoid of vitamin K.

Isolation

Almquist (1936) reports progress in its isolation and a rapid method of obtaining it in highly concentrated form from alfalfa. A sterol-free oil is produced which is adequate as a source of vitamin K at a level as low as 3 mg. of oil per kilogram of diet.

A new accessory factor closely related to but not identical with vitamin K has most recently (1937) been reported by Quick. He believes that this principle extracted from alfalfa can cure the hemorrhagic tendency in rabbits produced by feeding them spoiled sweet clover hay. Some toxic substance appears to destroy prothrombin or to inhibit the mechanism by which the body produces this clotting factor. The significance of vitamin K or of this related factor of Quick in the hemorrhagic tendencies of man has not been established.

Vitamin P

Szent-Györgyi, Ruszmyak and Armentano in Germany report a permeability vitamin which they temporarily call vitamin P or citrin.

Chemistry - a diglucoside of a substance of the flavone group.

Formula: $C_{28}\ H_{38-38}\ O_{17}$

It is hardly soluble in water or alcohol, but dissolves in alkali.

Vitamin P - has been isolated from orange juice, but it is not cevitamic acid.

Action

This new principle seems to improve the symptoms of guinea pigs on a scorbutogenic diet, but more studies must be made on the vitamin character of the flavone. If the vitamin character

can be proved, it would indicate that the flavones, so important for the cellular metabolism of plants, have also a definite function in the human cell. The effects of the flavone on human capillaries were studied, showing that it cures vascular purpura. It is practically ineffective, however, in the thrombocytopenic forms of purpura. The citrin inhibits the capillary permeability to proteins in many of the cases.

Natural Sources

In fruit juices and vegetables in association with cevitamic acid.

CASES IN UNIVERSITY OF MINNESOTA HOSPITALS -- 1928 - 1937

Avitaminosis	Hosp.No.	Initials	Age	Sex	Home	Adm. & Disch.	Remarks
A Keratomalacia			14 wks.	F	Town	12- 5-29 4-24-30	Formula of whole milk No cod liver oil. Results: healed eyes but cornea markedly scarred.
B Acrodynia			2 yrs.	F	Rural	7-30-34 12- 8-34	R_x: high vitamin B diet with high protein. Improved.
B Acrodynia			3 yrs.	M	Rural	1- 7-35 3-20-35	R_x: high vitamin B diet. Improved.
B Acrodynia			2 yrs.	F	Rural	3-13-35 4-17-35	R_x: nigh vitamin B diet. Improved.
B Acrodynia			14 yrs.	F	City	4-29-35 5-18-35	Symptoms quite classical but age unusual.
B Acrodynia			26 mos.	M	Town	4-21-37 5-5-37	- - - - - - - -
B_2 Pellagra			54 yrs.	M	Rural	4-24-34 12-14-34	- - - - - - - - -
B_2 ? Pellagra			30 yrs.	F	Town	7-20-31 7-26-31	Severe diarrhea

Avitaminosis D	Hosp. No.	Initials	Age	Sex	Home	Adm. & Disch.	Remarks
Rickets ? Infantile Tetany			1 yr.	M	Rural	6-19-29	Premature Convulsions at 4 months Active rickets
Rickets Infantile Tetany			10 mos.	F	City	5-22-34 6- 8-34	Duration 3 months Cod liver oil until past month R_x: *SAVD, 6,000 units. Good results.
Rickets Infantile Tetany			11 wks.	M	City	9-28-34 1- 3-35	In foster home. Had had some vitamin D. Tetany for several days R_x: calcium and parathormone
Infantile Tetany			7 mos.	F	Rural	6- 8-31 8-16-31	Healing rickets
Infantile Tetany			1 yr.	F	Rural	7-21-31 8-21-31	Healing rickets
Infantile Tetany			13 mos.	M	Rural	8-14-31 9- 3-31	Healing rickets
Rickets			2 yrs.	F	City	9- 7-28 9-28-28	Had bow legs
Rickets			6 mos.	F	Town	11- 6-28 12-19-28	Feeding problem due to cleft palate
Rickets			8 mos.	F	Town	4- 6-29 5-20-29	Active rickets, also bronchopneumonia and otitis media R_x: viosterol
Rickets			1 yr.	M	City	6-28-29 8- 1-29	No cod liver oil
Rickets			18 mos.	F	Rural	7- 2-29 7-28-29 Died	Breast fed for 1 year. Cod liver oil. Malnutrition. ? Celiac disease.

*Sun-Assured Vitamin D

(Cont.)

Avitaminosis D	Hosp. No.	Initials	Age	Sex	Home	Adm. & Disch.	Remarks
Rickets			5 mos.	F	City	7-15-29 7-27-29	No breast milk or diet Cod liver oil Had Little's disease
Rickets			4 mos.	M	Town	8-29-29 9-26-29	Admitted for hernia repair
Rickets			12 mos.	F	City	3-24-30 4-19-30	Premature Cured with viosterol
Rickets			3 yrs.	M	Rural	6- 6-30 7-21-30	Indian Had bilateral osteotomy
Rickets			5 mos.	M	City	7-15-31 7-25-31	Rickets severe R_x: viosterol gtts. vii t.i.d.
Rickets			4 mos.	M	Town	11- 4-31 2- 4-32	Also had pylorospasm
Rickets			11 mos.	F F	Rural	2-25-32 4-17-32	Premature twins R_x: haliver oil and viosterol
Rickets			2 yrs.	M	Town	7-14-32 10- 6-32	Inadequate milk Bowing of legs Bilateral osteotomy
Rickets			6 mos.	M	Town	1-31-34 3-19-34	Breast fed, 6 weeks No cod liver oil R_x: SAVD. Results good
Rickets			7 mos.	F	City	2-12-34 6-16-34	On milk formula. Cod liver oil inadequate; also had pyelitis and otitis media. R_x: SAVD. Healed.
Rickets			5 mos.	M	City	2-14-34 3-19-34	Premature Regurgitated cod liver oil. R_x: SAVD, 3000 units. Healed.

(Cont.)

Avitaminosis D	Hosp.No.	Initials	Age	Sex	Home	Adm. & Disch.	Remarks
Rickets		.	11 mos.	F	Rural	3-12-34 6-13-34	Premature No cod liver oil Admitted for birth mark R_x: SAVD. Healed
Rickets			18 mos.	M	City	4-14-34 6- 8-34	Breast fed 6 weeks. No cod liver oil. Also cervical adenitis and anemia R_x: SAVD. Good results.
Rickets			2 yrs.	M	Town	5-23-34 5-25-34	Duration 8 months No cod liver oil or vitamin D R_x: SAVD. Healed in 1 month.
Rickets		.	9 mos.	M	Rural	3-30-35 4-30-35	Cod liver oil inadequate Admitted for pneumonia R_x: SAVD. Healed.
Rickets			8 wks.	F	Town	8-19-35 10-31-35	Premature. Had viosterol gtts. x daily R_x: Increased viosterol. Healed.

There have been two admissions with slight x-ray evidence of scurvy but without a clinical picture. These were seen before cevitamic acid determinations were used. The listed cases of rickets are those where rickets occupied a prominent part clinically. Many other cases were seen who showed signs of previous rickets. In presenting the cases of acrodynia, the fundamental relationships are not definitely established, but it is felt by many to be related to deficiency in vitamin B complex.

We are presenting all the cases of polyneuritis encountered during this period. Careful study of the charts as regard the dietary regimen does not disclose evidence of outstanding deficiency in vitamin B. Williams states that the dietary of the average American is more deficient in vitamin B than in any of the nutritional essentials. Recent investigations reveal that many of the clinical types of polyneuritis are associated with a quantitative deficiency of vitamin B complex. Of the 30 cases in our series, there are only 6 which were of unknown etiology, and in none of these was avitaminosis B suspected. It is generally agreed that those due to alcohol, diabetes, pregnancy, or possibly toxins are associated with a partial vitamin B deficiency.

Peripheral Neuritis	Hosp. No.	Initials	Age	Sex	Home	Adm. & Disch.	Remarks
Alcoholic			50 yrs.	M	Rural	4- 8-31 6- 3-31	Polyneuritis Cirrhosis of liver
Alcoholic			46 yrs.	M	Rural	8-26-31 9-18-31	Polyneuritis
Alcoholic			25 yrs.	M	Town	7-19-33 10-19-33	Polyneuritis Psycnosis with Korsakoff's Syndrome
Alcoholic			47 yrs.	M	Town	5-21-34 6- 5-34	Polyneuritis Cirrhosis of liver
Arsenic			40 yrs.	F	City	1-29-31	Syphilitic Polyneuritis on arsenical basis
Arsenic			50 yrs.	F	Rural	5- 8-35 10- 4-35	Poison in coffee Husband died next day
Arsenic			59 yrs.	M	Rural	5-25-35 7- 3-35	Ingestion of rat poison
Diabetic			46 yrs.	M	City	7- 6-31 8-19-31	- - - - - - - -
Diabetic			58 yrs.	M	Town	7-24-32 9-30-33	- - - - - - - -
Diabetic			63 yrs.	F	Town	5-30-33 6-13-33	Neuritis for 2 months
Diabetic			51 yrs.	M	Rural	2- 1-34 9-19-34	Diabetes for 8 months
Diabetic			45 yrs.	F	City	3- 5-34 8-12-34	Diabetes for 3 years
Lead			10 yrs.	M	Town	8-17-31	Poisoning from collection of ball of lead - foil

(Cont.)

Peripheral Neuritis	Hosp.No.	Initials	Age	Sex	Home	Adm. & Disch.	Remarks
Lead			54 yrs.	F	Town	11-18-32 3-13-33	- - - - - - - - -
Post-diphtheritic			21 yrs.	M	Rural	9-15-31 2-19-32	Improved
Post-diphtheritic			38 yrs.	F	City	5-12-34 6-22-34	Sudden onset of neuritis
Pregnancy			28 yrs.	F	Rural	9-16-29 9-19-29	Died
Pregnancy			23 yrs.	F	Town	1-27-30 5-14-30	Pernicious vomiting Aborted
Pregnancy			38 yrs.	F	Town	2- 3-34 4-12-34	Two previous attacks with pregnancy
Toxic			42 yrs.	M	Rural	10-20-31 7-19-32	Followed upper respiratory infection - lasted 2 years
Toxic			29 yrs.	F	Town	4- 6-33 5- 3-33	Associated with tuberculosis Died
Toxic			44 yrs.	F	Rural	11- 6-33 12-11-33	Had tertiary syphilis Considered as toxic neuritis
Toxic			15 yrs.	M	Rural	11-20-34 12-16-34	Probably due to infection Improved
Toxic			33 yrs.	M	Town	5-14-35 6- 7-35	Followed food poisoning
? Etiology			56 yrs.	F	Town	2-10-29 4-19-29	- - - - - - - - -
? Etiology			52 yrs.	M	Town	7-16-29 9- 2-29	- - - - - - - - -

(Cont.)

Peripheral Neuritis	Hosp.No.	Initials	Age	Sex	Home	Adm. & Disch.	Remarks
? Etiology		.	13 yrs.	M	Town	5- 2-34 12- 8-34	- - - - - - - - -
? Etiology		.	27 yrs.	M	City	1- 3-35 2-18-35	Improved
? Etiology		.	60 yrs.	F	City	7- 2-35 7-25-35	- - - - - - - - -
? Etiology		.	54 yrs.	F	Town	9-29-35 2-20-36	Duration of six months Died

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