Recent Advances in the Chemistry and Nutrition of the Fat-Soluble Vitamins*

NORRIS EMBREE

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A CLUE to some of the special problems in the field of the fat-soluble vitamins can be obtained by examining the label of a multivitamin preparation with a fairly complete formula (Figure 1). It will be noticed that while the content of the water-soluble vitamins is given in milligrams, the potency of the fat-soluble vitamins is expressed in terms of biological units. Further information on the same label will show that the fat-soluble vitamins come from natural sources. If synthetic vitamin E is indicated, a natural occurring substance, phytol, was probably an important raw material in its synthesis.

MULTI-VITAMIN PREPARATION

Each Capsule Contains

VITAMIN A (From Liver Oils)	5000 U.S.P. UNITS
VITAMIN D (Activated Ergosterol)	500 U.S.P. UNITS
VITAMIN E (From Vegetable Oils)	10 INT. UNITS
THIAMIN CHLORIDE	3 MG
RIBOFLAVIN	2 MG
ASCORBIC ACID	50 MG
PYRIDOXINE HYDROCHLORIDE	0.05 MG
PANTOTHENIC ACID	1 MG
NICOTINAMIDE	20 MG

HYPOTHETICAL DRUG CO

FIG. 1. Typical (abbreviated) label for a vitamin supplement. (The dose of vitamin E is often given by mg. of tocopherol.)

Since the manufacture of the fat-soluble vitamins involves raw materials of natural origin and since the final products must be evaluated by biological assay, commercial research laboratories have had to take an important part in the recent development in the chemistry and biochemistry of these materials.

Furthermore, in spite of many years of intense study, there is very little known about the place of the fat-soluble vitamins in enzymic reactions or other simplified biological systems. This seems to be partly due to the fact that the fat-soluble vitamins are involved primarily in building up the structure of the organism rather than in respiration or degradation processes. The problem is complicated by the fact that there are several structurally related compounds occurring in nature that can, with varying efficiencies, exert most or all of the functions attributed to each fat-soluble vitamin.

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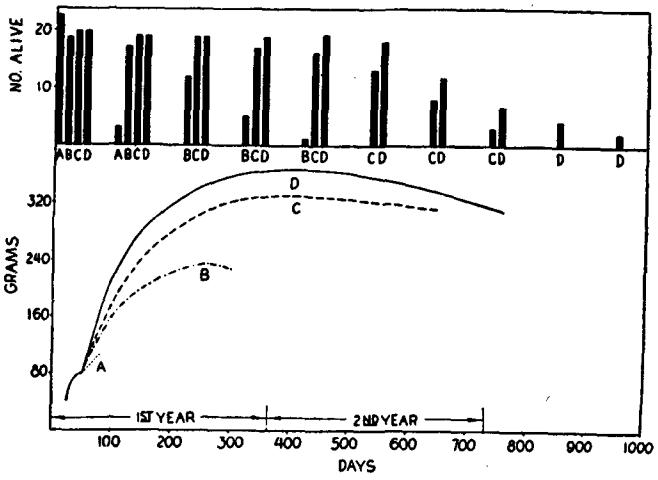


FIG. 2. Body weight curves of rats receiving different amounts of vitamin A per 100 gm. of body weight. The columns at the top show the number alive in each group at 100-day intervals. A—1 U.S.P. unit, B—2 U.S.P. units, C—4 U.S.P. units, D—20 U.S.P. units. Paul and Paul (1946).

Other rough generalizations are that fats, by both quality and quantity, affect the response of vitamins A, D, and E, and that to some extent these vitamins have effects on each other.

Physiology and Nutrition of Vitamin A

Recent publications have established that the vitamin A and carotene in the blood plasma are carried in the form of protein complexes [Dzialoszynski, Mystkowski, and Stewart (1945)] and that carotene, fed to a rat, becomes converted into vitamin A somewhere in the walls of the gastrointestinal tract [Sexton, Mehl, and Deuel (1946)].

A study of the effect of vitamin A dosage on longevity of rats was made by Paul and Paul (1946). Their data, illustrated on Figure 2, showed that while rats could reach maturity on 2 units of vitamin A per day (Curve B), they grew larger and lived longer on 4 units of vitamin A per day (Curve C), and their growth was again greater and life again longer on 20 units of vitamin A per day (Curve D).

Similar results were obtained by Sherman, Campbell, Udiljak, and Yarmolinsky (1945) when they studied a diet upon which rats had apparently thrived for 58 generations. They found that this obviously satisfactory diet, which contained 3 units of vitamin A per gram or about 30 units of vitamin A per day, could be considerably improved by feeding extra vitamin A. Table 1 from their paper shows that doubling and quadrupling the amount of vitamin A appreciably increased the length of the reproductive periods of females and the length of life of both male and female rats. They calculated the comparative human dose on a relative calorie basis and showed that the 6-unit-per-gram diet corresponded to a human intake of 4,800 units per day, approximately the amount recommended by the Na-

TABLE 1

Influence of Vitamin A Intake on Longevity and Reproductive Period of Rats (Sherman, et al., 1945)

	Influence of the Vitamin A Value of the Food		
	On diet with 3 I.U./g.	On diet with 6 I.U./g.	On diet with 12 I.U./g.
Reproductive period of females	265 days	312 days	369 days
Length of life: of females	724 days	801 days	830 days
of males	652 days	685 days	723 days
Comparative human dose	2,400 I.U./day	4,800 I.U./day	9,600 I.U./day

tional Research Council. The rats are, and presumably humans would be, benefited by doubling this amount. Since vitamin A seems to take part in the structure of the organism rather than in the metabolism, perhaps the human dose should be calculated by relative weights, and in that case the comparative human dose would be about three times that indicated by Sherman's laboratory.

A careful study of the vitamin A requirements of calves by Lewis and Wilson (1945) shows about the same results as those just mentioned for rats. They found that, while 32 units of vitamin A per kilogram of body weight just about covered the minimum requirements of calves, for optimum health the daily intake of vitamin A for young calves should be about 250 units per kilogram of body weight or 11,000 units per 100 pounds of live weight.

Wide occurrence of vitamin A deficiency has been shown in China by looking for night blindness [Tang and Wan (1943)] and rough, bumpy skin [Hu (1943)]. Such symptoms are not common in North America and Europe, but the rat and calf experiments just mentioned show that these manifestations of extreme A-deficiency may be cured by one-tenth of the dose required for complete health.

Vitamin A Chemistry and Assay

Vitamin A Chemistry

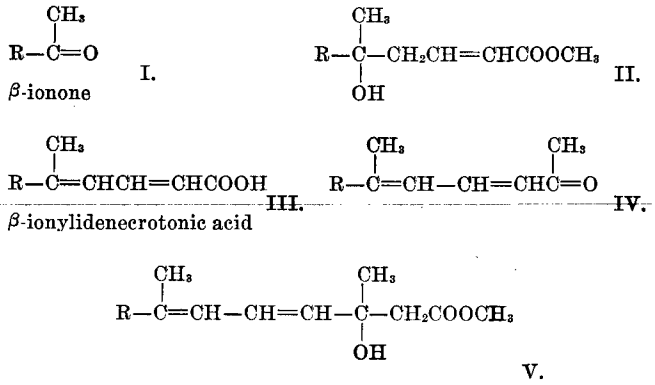
Robeson and Baxter (1945, 1946) have shown that there is a stereo-isomer of vitamin A which occurs naturally. This "neo-vitamin A" has substantially the same biological potency as vitamin A. The difference between the two forms of vitamin A is strikingly shown in the appearance of the crystals of the two forms and their esters. The melting points of vitamin A and neo-vitamin A are 62-64° C. and 59-60° C., respectively, and the values of E(1%,1cm.) at the maximum absorption in the ultraviolet are 1,750 at 325 mμ and 1,645 at 328 mμ, respectively. These and other properties indicate that the neo-vitamin A is a cis-isomer of the better-known form. The ratio of the quantity of neo-vitamin A to that of vitamin A in fish liver oil is about 30:70.

Vitamin A aldehyde has been made by oxidizing vitamin A [Hawkins and Hunter (1944)] and by oxidizing carotene [Hunter and Williams (1945)]. It has ultraviolet absorption maxima at 350 and 368 mμ and its antimony trichloride addition product has a maximum absorption at 657 mμ. The aldehyde can be converted into vitamin A by reduction with aluminum iso-propylate.

The most interesting recent developments in vitamin A chemistry have, of course, been the recent syntheses of compounds possessing vitamin A activity. Arens and van Dorp (1946) produced the acid corresponding to vitamin A by reactions which produce

TABLE 2

Formulas of Intermediates in the Synthesis of Vitamin A Acid (Arens and van Dorp, 1946)



the intermediates illustrated by the formulas in Table 2. β-Ionone (I) was reacted with bromocrotonic ester to give the hydroxy ester (II). After dehydration and saponification, they obtained β-ionylidene-crotonic acid (III). This was reacted with lithium methyl to give the methyl ketone (IV), which then was reacted with bromoacetic ester to give the hydroxy ester (V). This last mentioned compound was dehydrated and saponified to give vitamin A acid.

A preliminary report by Oroshnik (1945) describes the synthesis of vitamin A methyl ether. Milas (1945) has obtained patents on the preparation of vitamin A, vitamin A acid, and vitamin A ethers.

Bioassay

It is generally acknowledged that there is a great need for a new reference standard for bioassay. The International Standard (carotene) and the U. S. P. Cod Liver Oils are both unsuitable. The Vitamin Committee of The American Oil Chemists' Society [Embree (1946)] recommended a solution of crystalline vitamin A acetate in a stable vegetable oil.

The biological assay of vitamin A could stand a great deal of improvement itself although the adoption of a suitable reference standard will help matters a great deal. Part of the difficulties are suggested by Jensen's work (to be mentioned later) which shows that in the absence of considerable quantities of tocopherol many of the rats on an A-deficient diet suffer from gastric ulcers. Gridgeman (1944) has commented that the collaborative bioassays of U. S. P. Reference Oil No. 2 with β-carotene, indicating potencies ranging from 1,000 to 2,500 I. U. per gram, were simply following the variation that this assay method has inherent in it.

Antimony Trichloride Reaction

Although many British workers condemned the antimony trichloride blue color procedure during the early 1930's, during the past ten years it has become a well accepted assay method for vitamin A, especially in food, feed, and physiological extracts. The difficulties with the method used for the first ten years after Carr and Price discovered it in 1926 were due partly to inhibition effects which are now quite well understood and which are especially obnoxious in the case of products such as cod liver oil. The instruments used for measuring the color also contributed to the difficulties. The Lovibond Tintometer, which was given a semi-official standing at first, has been shown

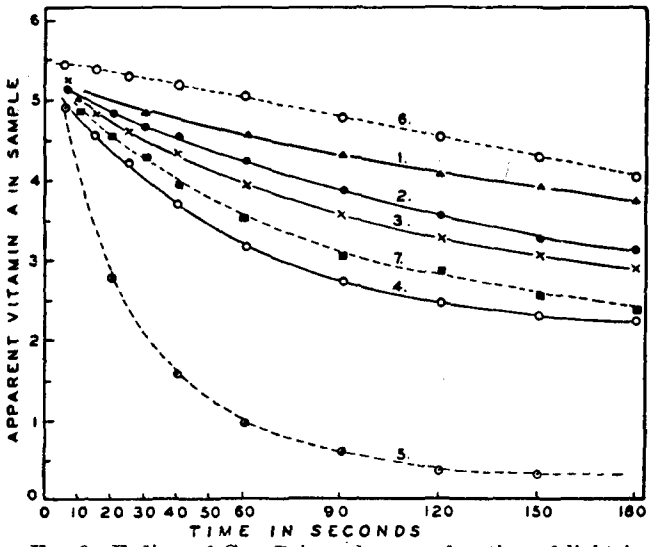


FIG. 3. Fading of Carr-Price color as a function of light intensity. Curves 1, 2, 3, and 4 are obtained with a Coleman photometer, when the reaction mixture is exposed continuously to 0, 13, 30, and 100 per cent of the normal photometer-light intensity. Curve 5 shows the effect of exposure to direct illumination from a 200-watt incandescent lamp. Curves 6 and 7 represent normal fading obtained when the Evelyn and KWSZ photometers, respectively, are used. Caldwell and Parrish (1945).

to be unsuitable for the measurement of this particular shade of blue. The numerous photoelectric spectrophotometers which appeared during the 1930's were expected to relieve the situation, but strangely enough many of them turned out to be unsatisfactory; a notable exception was the Evelyn colorimeter [Dann and Evelyn (1938)] which has been very popular in North America. The explanation of the troubles with most of these instruments is presented in a convincing paper by Caldwell and Parrish (1945) which showed that the antimony trichloride reaction product faded very rapidly in the presence of light. Many colorimeters use a 100-watt bulb as the light source, and this produced an intense enough beam to interfere seriously with the color measurement. Figure 3, taken from this article, compares color values from several instruments.

Ultraviolet Absorption Method

Most chemists specializing in vitamin A believe now that a rigorous physicochemical assay procedure should be set upon an official basis using ultraviolet measurements. Wilkie (1945) recommends in a report to the Association of Official Agricultural Chemists that the value of E(1%,1cm.) (328 mμ) of the unsaponifiable fraction, provided the shape of the curve is suitable, be multiplied by a factor of 2,000 to give an official potency. This is substantially the same recommendation made by Gridgeman (1944) in his booklet on this subject. The Vitamin Committee of the A. O. C. S. recommends further that a confirming test be made by the antimony trichloride method.

Butter and Margarine

During the last few years the Bureau of Dairy Industry of the U. S. Department of Agriculture [Maynard, et al. (1945)] has carried out a nationwide survey of the vitamin A potency of United States market butters. Agricultural Experiment Stations in 20 states cooperated. In general, they found that winter butter had appreciably less vitamin A than has summer butter. The average potency per pound for winter butter was 11,160 units while that of summer butter was 17,955 units.

No wholly satisfactory method has yet been worked out for the assay of vitamin A in margarine. Active work is now going on—studies are being made on purification methods such as saponification and chromatography, as well as method for correcting the "false" values of ultraviolet light absorption and blue color determinations.

Vitamin A_2

Certain fresh-water fish do not contain vitamin A in the common form (vitamin A_1) in their livers but instead have varying quantities of a related substance, vitamin A_2, which has somewhat different chemical and physical properties. Furthermore, the visual purple (porphyropsin) of these fish has a maximum at 530 mμ instead of at 500 mμ, the location of the maximum of the visual purple (rhodopsin) of salt-water fish and mammals. Shantz, Embree, Hodge, and Wills (1946) carried out experiments to see if vitamin A_2 could be substituted for vitamin A_1 in the tissues of the rat. Vitamin A-depleted rats were fed about 100 units of vitamin A_2 per day. The vitamin A stored in the liver was substantially all vitamin A_2 from the first week of the experiment; however, the vitamin found in the blood plasma after six weeks was mainly vitamin A_1. After 12 weeks of feeding substantially all of the vitamin A in the blood was in the form of vitamin A_2, and the visual purple of the eye had properties very nearly like that of porphyropsin (Figure 4). This experiment showed that it is possible almost to replace completely vitamin A_1 by vitamin A_2 in an animal which customarily utilizes vitamin A_1.

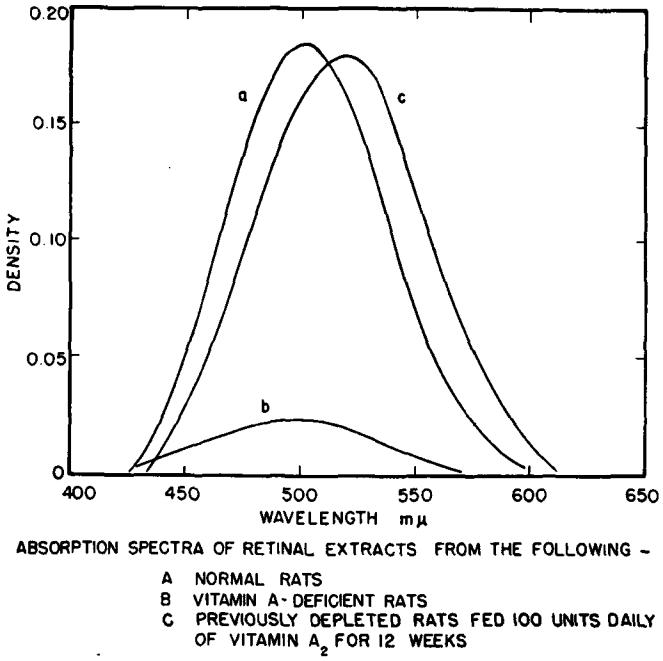


FIG. 4. Absorption spectra of retinal extracts from the following:

A. Normal rats.
B. Vitamin A-deficient rats.
C. Previously depleted rats fed 100 units daily of vitamin A_2 for 12 weeks.

Shantz, et al. (1946).

Carotene

Some of the stereochemical isomers of the biologically active carotene, isolated by Zechmeister and Polgar, have been biologically assayed against β-carotene by Deuel and coworkers (1944, 1945). Adequate supplies of tocopherol were added to the diet so that a deficiency of this material would not complicate the biological assay. The results of their determinations are summarized in Table 3. The information presented here shows that the isomers of β-carotene are appreciably less active than the parent material. This is also true for the isomers of α-carotene. In contrast with these series, the cis-isomer of γ-carotene is almost twice as active as the all-trans γ-carotene.

TABLE 3

Biological Activity of Carotene Stereo-Isomers

Type of Carotene	Relative Biological Activity
All trans-β-carotene	100
Neo-β-carotene U (a)	38
Neo-β-carotene B (b)	53
All trans-α-carotene (a)	53
Neo-α-carotene U (a)	13
Neo-α-carotene B (b)	16
All trans-γ-carotene (c)	28
Pro-γ-carotene (c)	44
Cryptoxanthin (d)	57

(a) Deuel, Sumner, Johnston, Polgar, and Zechmeister (1945).
(b) Deuel, Johnston, Meserve, Polgar, and Zechmeister (1945).
(c) Deuel, Johnston, Sumner, Polgar, Schroeder, and Zechmeister (1944).
(d) Deuel, Meserve, Johnston, Polgar, and Zechmeister (1945).

Fraps and Meinks (1945) reported that the digestibility of carotene in foods averages only 3 to 50% although it was 70% for carotene dissolved in oil. However, Oser and Melnick (1945) found, in a study of heat-processed vegetables and fruits, that good correlation could be obtained between the bioassay estimation of vitamin A potency and the physical methods, provided that in the rat assay sufficient tocopherol were added to give the optimum response by the carotene. In general, they found that in such products 1 μg. of crude carotene (free from xanthophyll and lycopene) is equivalent to 1 U. S. P. unit of vitamin A.

Physiology and Nutrition of Vitamin E

Many of the new findings concerning the biochemistry of vitamin E as well as their possible implications have been reviewed by Hickman and Harris (1946). Consideration of the facts known to apply to human beings lead them to predict that the normal adult requires about 30 mg. of mixed natural tocopherols per day. Studies of American diets indicated that the average industrial worker obtains from 3 to 27 mg. of tocopherol per day.

It is becoming increasingly apparent that many of the biological properties of the tocopherols depend upon their ability to act as antioxidants under certain conditions. Numerous *in vitro* studies of the antioxidant action of tocopherol have been presented at recent Oil Chemists' meetings.

The antioxidant action of tocopherols in the animal was shown by Moore (1940), by Davies and Moore (1941), by Sherman (1941, 1942), and by Quackenbush, Cox, and Steenbock (1942). It was brought into prominence by the work of Hickman and associates [Hickman, Harris, and Woodside (1942)]; [Hickman, Kaley, and Harris (1944)]; [Harris, Kaley, and Hickman (1944)]; [Jensen, Hickman, and Harris (1943)]. They showed that the biological response of vitamin A and carotene was very greatly influenced by the presence of tocopherols and to some extent by other antioxidants.

The difference in biological activity of the tocopherols for the various symptoms of vitamin E deficiency will probably give some clues to the specific biological functions that are characteristic of these substances. Table 4 shows that the racemic tocopherols are appreciably less potent in preventing sterility than are the natural dextro forms. γ-Tocopherol is much less effective than α-tocopherol in preventing sterility but may be more effective when assayed by the weight gain method of Gottlieb and coworkers (1943). All the tocopherols have high activity in protecting vitamin A. β-Tocopherol seems moderately active in preventing sterility in rats but much less active in curing exudative diathesis in chicks.

TABLE 5

The Effect of Vitamin E With and Without Alcohol, on Growth and Ulcer Production of Vitamin A-Depleted Rats Receiving .57 μg. of Vitamin A Daily* (Jensen, 1946.)

Group No.†	Supplement** Alcohol as 95% C_2H_5OH	Supplement** d-α Tocopherol	Gain in body weight after 48 days	Incidence of gastric ulcers
	mg.	*mg.*	*grams*	*pct.*
1	0	0	64	67
2	0	0.5	79	0
3	0	5.0	77	0
4	64	0	41	40
5	64	0.5	63	0
6	64	5.0	67	0
7	128	0	29	30
8	128	5.0	57	10

*The basal diet was fed ad libitum and contained: casein, vitamin-free, 18%; starch, 65%; salt mixture, U.S.P. No. 2, 4%; yeast, 8%; lard, 5%; vitamin D in the lard to furnish 30 units per 10 grams of diet.
†The first group contained 15 rats; the others, 10 each.
**In addition to .57 μg. vitamin A as a natural ester concentrate.

TABLE 4

Comparative Potencies of the Tocopherols

	Relative (Approximate) Biological Potencies					
	d-α	*dl-α*	*d-β*	*dl-β*	*d-γ*	*dl-γ*
Rat						
Antisterility (a)	100	65	33	16	1(f)	<1
Weight Gain (b)		100		25		19
Vitamin A Protection (c)	100		100		100	
Rabbit						
Creatinuria (d)	100				15	
Chick						
Exudative diathesis (e)		100		6		

(a) Harris, Jensen, Joffe, and Mason (1944).
(b) Gottlieb, Quackenbush, and Steenbock (1943).
(c) Hickman, Kaley, and Harris (1944).
(d) Hove, Hickman, and Harris (1946).
(e) Dam, Glavind, Prange, and Ottesen (1941).
(f) Weisler, Baxter, and Ludwig (1945).

In a study of the effect of alcohol and of vitamin E on rats partially deficient in vitamin A Jensen (1946) found that the gastric ulcers typical of vitamin A deficiency were prevented by α-tocopherol and that the harmful effect of alcohol on growth was ameliorated to some extent (Table 5). Other work from this laboratory [see Hickman and Harris (1946)] has shown that the gastric ulcers, which appear in rats partially deficient in essential fatty acids or in pyridoxine, can also be prevented by vitamin E supplementation.

Hove, Hickman, and Harris (1946) have found that tocopherol has a protective effect on rats subjected to anoxia due to low atmospheric pressure (Table 6). In view of the antioxidant action of tocopherol it would not have been unreasonable to have guessed that the animals deficient in tocopherol would have fared better than the others when the concentration of oxygen was low!

TABLE 6

Effect of Tocopherols on Survival Times of Adult Male Rats Subjected to Low Atmospheric Pressure (185 mm. Hg. at 26° C.) (Hove, et al., 1946)

Diet*	Tocopherol supplement	Number of trials	Average survival time
	mg.		*min.*
Low in fat and vitamin E	0	8	34.4
	3.0	8	107.0
5% lard, low in vitamin E	0	10	13.3
	0.5	10	31.4
12% lard, low in vitamin E	0	3	14.0
	0.3	3	24.9

*The rats were given these diets and supplements for 15 to 20 days before decompression. They had previously been receiving commercial dog chow.

More information is accumulating to show that domestic animals are often raised under conditions which give rise to vitamin E deficiency symptoms. Breeding troubles with horses [Zevadovskii and Nesmeyanova-Zavadovskaya (1945)] and stiff lamb disease in sheep [Willman, Loosli, Asdell, Morrison, and Olafson (1945)] have been attributed to insufficient vitamin E. Better growth by foxes and decreased mortality of minks [Masson and Michaud (1941)] and by hens [Masson (1941)] have been found by administration of vitamin E to animals raised on presumably adequate diets.

Clinical reports are continuing to indicate that numerous human troubles are connected with vitamin E. Steinberg (1941, 1942) has established the value of tocopherol treatment for the deterioration of muscle tissue known as primary fibrositis. Milhorat and Bartels (1945) found that progressive muscular dystrophy, while suspected to be caused from a lack of vitamin E, since it resembles the dystrophy of E-deficient animals, did not yield to tocopherol therapy but was considerably improved when treated with a combination of tocopherol and inositol. Hickman and Harris (1946) report that excessive deposition of tartar on teeth of certain dental patients can be greatly ameliorated by tocopherol dosage. Shute (1945) found that damaged kidney functions in his patients could be improved by treatment with α-tocopherol even when the damage was of some duration.

While the connection of vitamin E deficiency with cases of human abortion has not been completely clarified, much evidence has recently been collected to show that there seems to be a barrier to the human placental transfer of tocopherol [Kofler (1945)]; [Varangot, Chailley, and Rieux (1944)]; [Straumfjord and Quaife (1946)]. Table 7, prepared from data by Straumfjord and Quaife (1946A) shows the effect of the placental barrier on tocopherol as well as vitamins A and carotene. The low values of plasma vitamin E in infants is paralleled by the low values of carotene. There seems also to be an appreciable difference in the case of vitamin A.

TABLE 7

Effect of Pregnancy and Parturition on Plasma Vitamin Levels of Humans (Straumfjord and Quaife, 1946, 1946A)

Condition of subjects	Quantities of Vitamin in 100 ml. Blood Plasma			
	Vit. A	Carotene	Vit. C	Vit. E
	units	*μg.*	*mgs.*	*mgs.*
Non-pregnant women (controls)	188	140	0.8	1.05
Pregnant Women (3rd trimester)	198	160	0.7	1.62
At parturition				
Women	130	180	0.5	1.70
Infants	96	20	1.2	.34

Vitamin E Assay

The physicochemical assay of tocopherol has been advanced in several ways. Most workers prefer to use modifications of the original Emmerie-Engel reaction which depended upon the measurement of the red color produced when tocopherol reacts with a mixture of ferric chloride and α,α'-dipyridyl. Kaunitz and Beaver (1944) have shown that many fats inhibit this reaction and they give directions for correcting this effect.

Minot (1944) described a method for the determination of tocopherol in blood serum that involves saponification and chromatography. Quaife and Harris (1944) present an easier method which removes the carotenoids and some other interfering substances by hydrogenation.

A highly important problem is the determination of each tocopherol when it occurs in mixtures. Hove and Hove (1944) have developed a method which will distinguish α-tocopherol from the other tocopherols by carrying out the Emmerie-Engel reaction at two different temperatures. Fisher (1945) has developed a method for determining γ-tocopherol in mixtures of α- and γ-tocopherol; it depends on the reaction of the tocopherols with nitric acid under such conditions that the γ-form gives rise to a red quinone while the α-form produces a yellow product. Quaife (1944) has found that diazotized nitroaniline will react to form a red dye with γ-tocopherol, but not with α- or β-tocopherol.

Vitamin D

Some progress has been made in the understanding of the physiology of vitamin D by the work of Greenberg (1945). He used radioactive calcium and strontium as tracers to follow the effects that vitamin D had on their absorption, deposition, and excretion. In general, the two elements showed about the same mechanisms. Table 8 shows the partition of labelled

TABLE 8

Partition of Labeled Calcium in Body and Excreta of Rachitic and Vitamin D-Treated Rats (Greenberg, 1945)

Mode of Administration	Urine	Feces	Skeleton	Teeth	Residual Carcass
	%	%	%	%	%
Oral, no vitamin D	19.5	60.0	15.0	2.1	3.4
Oral, with vitamin D	29.4	32.5	31.0	3.5	3.6
Injected, no vitamin D	44.0	18.5	28.0	6.5	3.0
Injected, with vitamin D	25.0	18.0	45.0	6.0	6.0

calcium in the body and excreta of rachitic and vitamin D-treated rats.

The fecal excretion of the orally administered calcium confirms the theory that the administration of vitamin D promotes the absorption of calcium from the intestines. However, the fecal excretion of the injected calcium shows that there also seems to be a fairly constant transfer of calcium to the feces. The experiments with the injected calcium show clearly that the vitamin D has a marked direct effect on the deposition of calcium. Thus, vitamin D has at least two favorable effects—first, the promotion of calcium absorption, and second, the promotion of calcium deposition.

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Acid Sodium Stearates*

F. V. RYER

Research Laboratory, Lever Brothers Company
Cambridge, Massachusetts

Introduction

OUR interest in acid soaps developed out of the necessity of obtaining records of their X-ray diffraction patterns so that the latter might not be confused with the patterns of several neutral soap phases recognized for the first time in this laboratory (1). For this purpose the following series of crystallizations from 95% alcohol at 65 and 90°F. were designed to deposit all the crystalline products intermediate between neutral sodium stearate and free stearic acid.

Experimental

Solutions in 250 c.c. volumes of No. 3-A alcohol of constant weights of anhydrous sodium stearate (prepared from stearic acid fractionated to a purity of at least 95%) plus increasing weights of stearic acid, in small increments, were obtained by refluxing. The constant weight of neutral soap was selected for each crystallizing temperature to yield a concentration just under the gel point, a condition found desirable for the growth of satisfactory neutral soap crystals (2). After partial cooling under a condenser each stoppered flask was kept overnight at the crystallizing temperature of 65 or 90° F. in a thermostated room. Each crop of crystals was then suction-filtered and air-dried before examination. Thus, the sole variable in each series was the weight of stearic acid (or ratio of stearic acid to soap) present in the original solution.

Examination consisted of low-power microscopic inspection, titration of the excess stearic acid content in alcohol with N/10 sodium hydroxide and the preparation of X-ray diffraction patterns.**

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** The diffraction patterns were prepared by Miss G. E. Cook under the direction of M. J. Buerger of the Massachusetts Institute of Technology who has already published (5, 6) crystallographic data for several of the neutral and acid soap crystals and is preparing a paper on the diffraction data of the remaining acid soap crystals encountered in this work.