Fiber Foibles . . .

(Continued from page 10)

this, we have ignored the open and the hidden costs of the many price bolstering methods. The costs, including waste of money and resources, are huge. Here and there across the belt, cottonseed mills have been closing. Lower cotton allotments and constant erosion of the position of cotton can only mean more mills closing down.

King Cotton must once again be allowed to compete. Prices must be allowed sufficient freedom to "ration" cotton into consumption. Long term defying of the market mechanism not only ruins the market, but creates economic anomalies that can only result in artificial and disruptive allocation of resources.

JAMES E. MCHALE, Merrill Lynch, Pierce, Fener & Smith, Inc.

New Vitamin E Data Revealed

Through a research program at Distillation Products Industries, Division of Eastman Kodak Co., Rochester, N. Y., some important new information about the biological activity of natural vitamin E and its function in animal reproduction has come to light. Results of the research provide information on the physiological action of vitamin E, knowledge that is expected to open the way to broader understanding of the uses of this vitamin in animal and human nutrition.

Kodak researchers, S. R. Ames and Marion I. Ludwig, of DPI Biochemistry Laboratories, reported their findings at the 142nd meeting of the American Chemical Society in Atlantic City, N. J. This work was described in the Aug. 20th issue of the *Journal of the American Chemical Society*.

The discovery is based on previous chemical research by DPI organic chemists, who recently succeeded in fractionating synthetic vitamin E into a *d*-form and an *l*-form. They found that the *d*-form was identical in its chemical properties with natural vitamin E derived from vegetable oils. The *l*-form had never been obtained in a pure state before.

At first, Dr. Ames and Miss Ludwig studied the preliminary, incompletely separated fraction, and found enough information to encourage the organic chemists to continue their project. The results of the compined pioneering study was the new information about the biological activities of both natural vitamin E and the *l*-form of the synthetic vitamin.

The biochemists followed the classical procedure of feeding graded doses to vitamin E-deficient rats, during the early pregnancy of the rats to determine the amount of vitamin necessary to support normal reproduction. After more than two years of research with the animals, they succeeded in establishing definitely that natural vitamin E (the *d*-form) has much greater potency than the *l*-form prepared synthetically. Repeated tests indicate that the *l*-form has only about 21% as much biological activity as natural vitamin E.

The discovery that the natural *d*-vitamin has far more biological activity in the reproductive test than the *l*-form prepared synthetically is a strong indication that the action of natural vitamin E is connected with specific enzyme functions in the body. The newly isolated *l*-form of the vitamin promises to be of major help to biochemists studying other biological actions of vitamin E.

• *Referee Application*

First Notice. Mr. Claude E. McLean, Jr. of Arizona Testing Laboratories, Box 1888, 817 W. Madison Street, Phoenix, Arizona, has applied for a Referee Certificate on cottonseed, oil cake and meal, fatty oils and protein concentrates. The Chairman of the Examination Board should be contacted by interested parties wishing to comment on this certification. Please write to Mr. N. W. Ziels, Chairman of the Examination Board, Lever Brothers Co., 1200 Calumet Ave., Hammond, Ind.

• *Names in the News*

L. O. Leenerts (1955) was recently promoted Assistant to the Director of Research at the Purex Corp., South Gate, Calif. Mr. Leenerts had previously been Supervisor of the Applications Research Department with the company.

L. O. Leenerts

Distillation Products Industries, Division of Eastman Kodak Co., has announced two reassignments in the Midwest sales areas. R. J. Evans (1956) has been made Assistant Manager under J. F. Hanrahan (1953) in Chicago, and R. W. Carpenter (1956) will now be Sales Representative for the Cleveland-Detroit-Cincinnati territory.

N. H. Nash (1948) was named to the newly created post of Technical Director of Drew Chemical Corp. Food Emulsifier & Specialty Products Dept. Mr. Nash has heen serving as the Director of New Products Development & Marketing for Basic Foods Corp.

Eugene McCauliff was named President of the Chemical Products Division of Chemetron Corporation, Chicago, Ill. Dr. McCauliff was previously President of the Glyco Chemicals Division, and Vice President of the parent Chas. L. Huisking & Co., Inc., New York.

J. N. Shaw, Jr. (1951) has been appointed Assistant Market Development Engineer in the Chemical Division of Johnson's Wax, Racine, Wisc. Mr. Shaw was formerly of Archer-Daniels-Midland of Minneapolis, Minn.

New Research To Emphasize Nutritive Quality In Corn Hybrid Breeding

A new cooperative research program has been announced, the goal of which is to improve nutritional standards in underdeveloped areas without basic changes in food habits or increase in food cost. This effort has been undertaken by Corn Products Co., New Technologicas, A.C., a nonprofit research institute in Mexico City.

The protein fractions of primitive and modern corn samples will be compared with commonly grown varieties to see if they contain better ratios of essential amino acids, particularly lysine and tryptophane.

Success in this project would have far-reaching benefits in areas where widespread protein deficiencies exist because limited meat consumption reduces the availability of certain essential amino acids. Corn is so basic in the food supply of many millions of people throughout the world that improving its ratio of essential amino acids would do much to compensate for the general lack of animal protein in diets in underdeveloped areas.

Dr. A. L. Elder, Director of the Corn Products Institute of Nutrition, stated that this project marks a profound change in emphasis in hybrid corn development. To date the goal in hybrid breeding has been to increase yield of corn per acre. This program is designed to shift some of the emphasis to nutritive quality to meet the special needs of protein-deficient areas.

Vitamin A in Margarine . . .

(Continued from page 12)

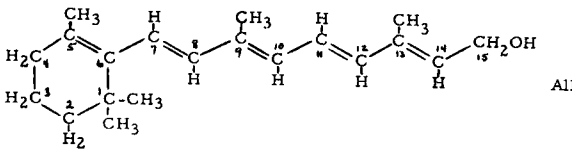
using unfortified margarine oil from the same batch. The two solutions are compared in an U.V. spectrophotometer, reading the absorbance of the fortified oil against that of the unfortified, as a blank. (b) The assay method adopted Official First Action by the Association of Official Agricultural Chemists (12) depends on measurement of the uncorrected extinction coefficient after chromatographic purification of the unsaponifiable fraction of margarine.

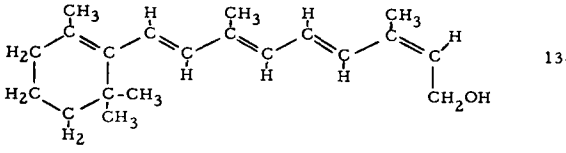
The extinction coefficient of 13-*cis* vitamin A is about 6% lower, and that of the 9-*cis* and 9,13-di-*cis* isomers is about 25% lower than that of all-*trans* vitamin A. Therefore, potency figures by measurement of the U.V. absorption will be lower than those by blue-color when *cis* isomers are present. Yet the biopotencies of the three *cis* isomers are even lower than their indicated values by uncorrected extinction coefficients; thus the uncorrected extinction coefficient overestimates the biological potency for the *cis* isomers.

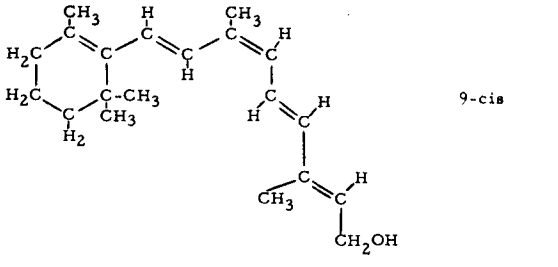
The USP XVI assay procedure (13) is the basis upon which commercial vitamin A sources are customarily purchased and used. This procedure gives potency figures for the three *cis* isomers about 25% lower than for the same weight of all-*trans* vitamin A. Thus, when *cis* isomers are present, the USP XVI potency is lower than that by blue-color, or by uncorrected extinction coefficient, however, the biopotencies of the 9-*cis* and 9,13-di-*cis* isomers are even lower than USP XVI potencies; thus, this procedure also overestimates the biopotency of samples that contain these two isomers.

The biological potency is certainly the measurement of interest to consumers, and this is recognized in the Federal Standards of Identity for Margarine (14) which specifies for vitamin A, when it is included as an optional ingredient: ". . . . the finished oleomargarine contains not less than 15,000 U.S. Pharmacopeia units of vitamin A per lb, as determined by the method prescribed in the Pharmacopeia of the U.S. for total biological vitamin A activity. . . ."

VITAMIN A ISOMERS







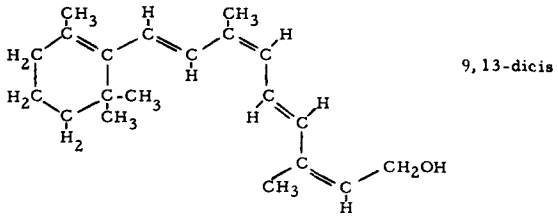


FIG. 1.

The last official USP method (15) for "total biological vitamin A activity" was a rat-growth assay, requiring only four animals at one dosage level on the standard and another four at one level on the sample for assay. Bioassays have been conducted on pure preparations of the four isomers in different laboratories (5,6,7) by more precise rat-growth procedures, rat liver storage, and by vaginal-smear techniques. The relative biological potencies of the four isomers are quite well established as being 100%, 75%, 23%, and 23%, for all-*trans*, 13-*cis*, 9-*cis*, and 9,13-di-*cis*, respectively.

Table I shows the potency figures that would be expected by the four assay methods for hypothetical margarine samples fortified at 4.5 mg per lb with each of the four vitamin A isomers. Except for the USP XVI assays, the data in Table I are derived from published measurements (5,6,7). The USP XVI assay data were obtained in our laboratories using preparations having the purity and the method of preparation as described in the published work.

While the USP XVI assay procedure agrees with bioassays for all-*trans* and (by coincidence) for 13-*cis* vitamin A, all physicochemical procedures grossly overestimate the biopotencies of the 9-*cis* and 9,13-di-*cis* isomers.

Measurement of Isomer Composition

Exact measurement of isomer composition of a vitamin A preparation is extremely difficult. Three procedures are available, but the first gives only partial information, while the other two are more suitable for research studies than for routine analytical control.

The maleic value is a simple determination (16) that will give partial information. The sample for assay is stored overnight in a benzene solution of maleic anhydride; all-*trans* and 9-*cis* vitamin A react in a Diels-Alder condensation, resulting in loss of potency in the blue-color assay. The 13-*cis* and 9,13-di-*cis* isomers are resistant to the reaction, and retain their blue-color potency. This procedure is mainly useful for determining whether a given preparation is substantially all-*trans*, (zero maleic value), or whether *cis* isomers are present. An empirical relationship has been established (17) between maleic value, blue-color, and biopotency, which appears to be useful for fish liver oils and other vitamin A materials that have been subjected to isomerizing conditions. It does underestimate the biopotency of those vitamin A sources (4) that contain mostly *trans* and neovitamin A.

Infrared absorption measurements on a vitamin A aldehyde concentrate, prepared by MnO_2 oxidation of the unsaponifiable fraction of the sample, has been used (2) for quantitative estimation of the proportions of the four isomers. However, this requires a fairly high purity sample, a suitable spectrophotometer, standard preparations of the four isomers in pure form, and considerable experience in interpreting the data. The method cannot be applied to margarine.

Formation of isorhodopsin has also been used (2,18) to estimate the amounts of 9-*cis*, and 9,13-di-*cis* isomers present in an unknown mixture. This is a spectrophotometric measurement, but it requires the isolation of the protein, opsin, by a laborious procedure from the rods of cattle or frog eyes. It cannot be applied to margarine without concentrating the vitamin A considerably.

Commercial Vitamin A Sources

Four distinctly different vitamin A sources are available commercially, and could be used for the fortification of margarine. For purposes of classification, we might designate them as All-Trans, Trans-Neo, Trans-Cis, and Fish Liver Oil, although the last one of the four is the only one commercially identifiable by this system of nomenclature.

TABLE I

Theoretical Potencies of 4.5 mg of Four Vitamin A Isomers

Isomer	Maleic value	Blue color	$E_{uncorr.}$	USP XVI	Biological
	%	*units*	*units*	*units*	*units*
All-*trans*	0	15,000	15,000	15,000	15,000
13-*cis* (neo)	100	15,000	13,800	10,900	11,200
9-*cis*	0	15,000	11,400	11,600	3,400
9,13-di-*cis*	100	15,000	10,900	10,100	3,400

Of course, the isomer composition of each of these types is subjected to some variation and uncertainty. The composition is subject to variations inherent in manufacturing processes, and would be subject to change if the process is changed. The uncertainties arise from the lack of reliable and precise assay procedures for the individual isomers. The data in the following paragraphs were obtained in our laboratories on several preparations of each type, using maleic value, I.R. absorption, reaction with opsin, and relationships between blue color, E uncorrected, USP assay, and rat liver-storage bioassay as criteria of isomer compositions (2,3,4,5,6,7,8,17). The figures given for the composition of each type represent our best estimate, on the average. They are used in Table II mainly for the purpose of illustration.

TABLE II

Percentage Isomer Composition of Commercial Vitamin A Sources

Isomer	Commercial Type			
	All-Trans	Trans-Neo	Trans-Cis	Fish Liver Oil
All-*trans*	95	65	48	52
13-*cis*	5	32	24	25
9-*cis*	0	2	19	15
9,13-di-*cis*	0	1	9	8

At least three basic manufacturers of synthetic vitamin A market an All-Trans product. These commercial products usually contain 3 to 7% of the 13-*cis* isomer, but no detectable quantities of the 9-*cis* or 9,13-di-*cis* isomers. The commercial products of this type respond in all assays substantially like pure all-*trans* vitamin A. When this type of vitamin A source is used for the fortification of margarine, the analytical problems are greatly simplified. For example, the blue-color assay on the unsaponifiable fraction, which is simple and precise, can be used with confidence.

Another type of commercial synthetic vitamin A often used contains much higher quantities of the 13-*cis* isomer [originally called neovitamin A (16)]. We might call this type Trans-Neo. It usually contains about 65% all-*trans*, 32% 13-*cis*, and traces (perhaps 3% total) of 9-*cis*, and 9,13-di-*cis* isomers. During past years, some of the vitamin A used in margarine manufacture has been of this type. More recently, special vitamin A palmitates of the Trans-Neo type have been promoted for use in aqueous multivitamin supplements to reduce the assay changes caused by isomerization in such products. (Vitamin A does not isomerize under ordinary storage in margarine.)

TABLE III

Example Calculation of Potency
(Biopotency of 4.5 mg Vitamin A in Fish Liver Oil)

Isomer	Biopotency[a] of 4.5 mg		Proportion[b] present, %		Biopotency contribution
All-*trans*	15,000	×	52	=	7,800
13-*cis*	11,200	×	25	=	2,800
9-*cis*	3,400	×	15	=	510
9,13-di-*cis*	3,400	×	8	=	272
Total			100		11,382

[a] Data from Table I.
[b] Data from Table II.

A third type of synthetic vitamin A might be designated Trans-Cis. This is isomerized to an equilibrium (3,18a,18b) and is offered specifically to pharmaceutical manufacturers for eliminating the assay changes caused by isomerization. This type has not been recommended for fortification of margarine, and would not ordinarily be supplied to a margarine manufacturer. Trans-Cis vitamin A is estimated to contain about 48% all-*trans*, 24% 13-*cis*, 19% 9-*cis*, and 9% 9,13-di-*cis* isomers.

A fourth commercial source of vitamin A still used for margarine fortification is Fish Liver Oil. There is some question (19) whether *cis* isomers are present in the livers of living fish, or whether they are formed during processing of the liver oil. However, commercial fish oils rarely have low maleic values, and many commercial fish oils contain both 13-*cis* and 9-*cis* isomers. Studies (2) by measurements of amounts of isorhodopsin formed *in vitro*

(Continued on page 36)