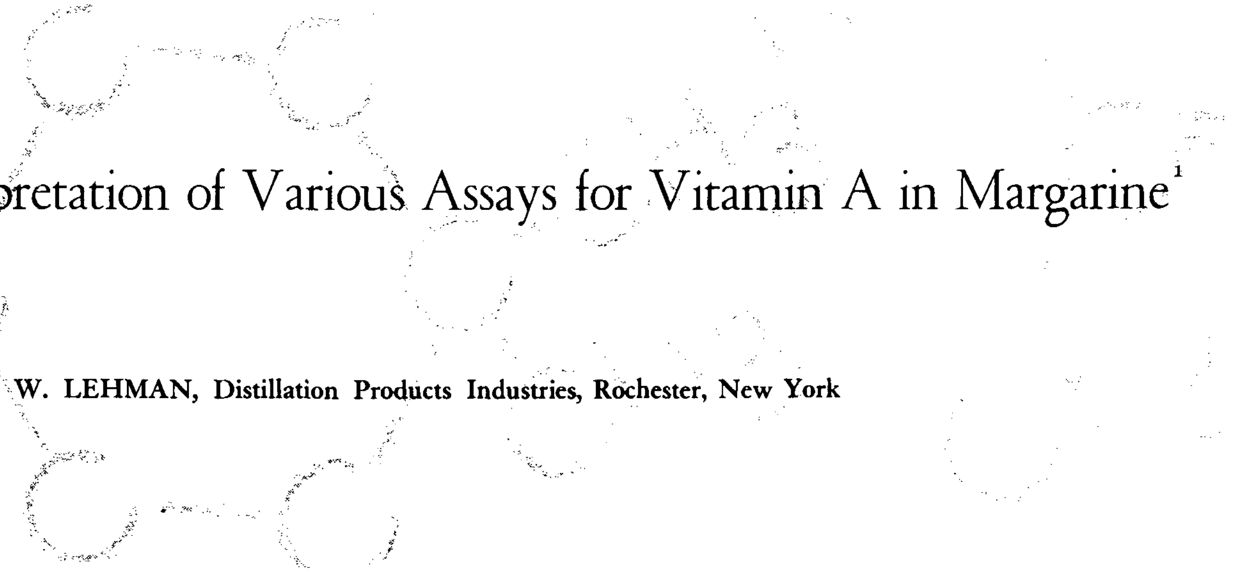

Interpretation of Various Assays for Vitamin A in Margarine[1]

ROBERT W. LEHMAN, Distillation Products Industries, Rochester, New York

Abstract

When margarine is fortified with all-*trans* vitamin A the assay result, whether obtained by colorimetry or spectrophotometry, should be the same as the biological potency. This paper reports the effects to be expected on each method of assay by some commercial sources of vitamin A that contain *cis*-isomers. In most cases the biological potencies are less than the potencies predicted by physicochemical procedures. Methods are presented to calculate the extent of assay differences introduced by these *cis*-isomers. Different commercial sources of vitamin A, in an amount required for 15,000 "biological" units, would give values ranging from 15,200 to 20,800 units by "blue-color" assay, from 15,100 to 19,000 units by uncorrected extinction coefficient, and from 15,000 to 18,000 units by the USP XVI assay.

Introduction

Several different assay methods are in use, or are of interest, for vitamin A in margarine. The USP method (spectrophotometry with Morton-Stubbs correction) is used on the vitamin A added to margarine. The margarine itself can be assayed by: growth rate of rats (Federal Standards of Identity), spectrophotometry with chromatography (AOAC), spectrophotometry with various methods for correcting blank absorption (NAMM, etc.), and antimony trichloride blue-color (Association of Vitamin Chemists). If all-*trans* vitamin A is the only form of vitamin A in the margarine, all methods should give the same "potency" figure. Differences in response will arise when 9-*cis* and 13-*cis* isomers of vitamin A are present. This paper describes the effect on assay results to be expected when various commercial types of vitamin A are used for fortification.

Vitamin A Isomers

The vitamin A molecule (Fig. 1) contains five conjugated double bonds, which give rise to the properties useful for assay.

One property is the formation of an intense blue color (absorption maximum at 620 mμ) when contacted with a chloroform solution of antimony trichloride. The "blue-color" assay has been very useful where vitamin A is present at low concentrations, as in foods. The reaction is reasonably specific, and relatively simple purification steps are required before application of the assay.

Another property of vitamin A that is useful for analysis is the ultraviolet band at 325 mμ. This measurement is usually more precise than that of the transient blue color, but it can be applied only to concentrates of fairly high purity. Often, extensive purification is necessary to remove other materials that absorb in the 325 mμ region. Where only low levels of extraneous absorption are present, a geometric correction may be applied, using absorbance readings at three wave lengths—usually 310, 325, and 334 mμ. This "Morton-Stubbs" correction (1) is part of the USP XVI assay procedure for vitamin A.

Previous publications (2,3,4) have reported the common occurrence of four geometric isomers of vitamin A, all-*trans*, 9-*cis*, 13-*cis*, and 9,13-di-*cis*. [Numbering is by the carotenoid system proposed (8) by the International Union of Pure and Applied Chemistry Commission on the Nomenclature of Biological Chemistry.] The latter two have very low biological activity (5,6,7). The all-*trans* isomer is the only one of the four that can be assayed reliably by a variety of analytical procedures. It is the "standard" for all procedures; a preparation of crystalline all-*trans* vitamin A acetate (in oil, capsulated) is distributed as a Reference Solution by the U.S. Pharmacopeia. Each of the other three isomers gives a different "potency" figure, depending on the assay procedure chosen. This is important to margarine analysts because: (a) several different assay procedures are commonly used, and assay results must often be transferred from one procedure to another; (b) several different commercial sources of vitamin A are in common use for the fortification of margarine, and these differ in their relative contents of the four isomers.

Assay Procedures

Four different procedures are of interest: (a) the antimony trichloride blue-color, (b) the uncorrected extinction coefficient at 325 mμ, (c) the USP XVI procedure (extinction coefficient corrected geometrically), and (d) bioassays. These are associated with margarine in the following ways:

The antimony trichloride blue-color procedure, applied to the unsaponifiable fraction of margarine, was judged in 1946 (9) by the vitamin A subcommittee of the National Association of Margarine Manufacturers (NAMM) to be the most generally applicable procedure. Its use was later supported by Luckmann, *et al.* (10). The four vitamin A isomers give the same "potency" figure per g by this procedure (11). However, since the isomers other than all-*trans* have lower biological potencies per g, the blue-color procedure overestimates the biological potency when these other isomers are present.

The uncorrected extinction coefficient is used in two different ways in the assay of vitamin A in margarine: (a) the vitamin subcommittee of NAMM (9), and Luckmann, *et al.* (10), suggest its use in a precise procedure for control work inside a margarine plant, where unfortified samples of margarine oils are readily available. The fat and aqueous phases of the finished margarine are separated, and a solution of exact concentration (25 g/100 ml for margarine at 16,500 u/g) made of the fat in cyclohexane. Another solution of exactly the same concentration is made

(Continued on page 18)

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[1] Presented at the A.O.C.S. Meeting in Chicago, 1961.
Communication #282 from the laboratories of Distillation Products Industries, Division of Eastman Kodak Company, Rochester, New York.

The Oil Palm . .

(Continued from page 9)

planting should coincide with the commencement of the wet season.

Preparation of Land and Planting

The preparation of land for the final planting of the oil palm is undertaken while the palms are in the nursery, and should be timed to coincide with the wet season, when the palms are 12–18 months old in the nursery. When planting from jungle, this should be felled and burnt during the preceding dry season. Main drains, if necessary, can then be dug. The land is then cleared of unburnt timber and is ready for lining and holing. In case the land to be developed is under lallang, this is destroyed by tractor ploughing and harrowing, or by digging out the roots which should be heaped and burnt. The whole area should be kept free from further growth of lallang by wiping the lallang leaves with lallang oil. After any necessary main drains have been dug, lining and holing is carried out.

A suitable planting distance on flat or undulating land is 30 ft x 30 ft triangular which gives 55 palms/acre. Steep land should not be cleared and planted. A method of lining is shown in Figure 4. The procedure is as follows:

1. Lay down base line AB of any convenient odd number of points, e.g., 11 points 30 ft apart.
2. From A, lay down a line 60 degrees to the right, i.e., AC.
3. From B, lay down a line 60 degrees to the left, i.e., BC.
4. Check that the intersection of these lines at C bears 90 degrees from point D midway between A and B.
5. Mark off points every 30 ft along AC.
6. Mark off points every 30 ft along BC.
7. Join EF, GH, etc., and mark off in 30-ft lengths.
8. Every point should come into alignment in three directions.
9. Produce all lines in three directions to lay out the rest of the field.

After lining, any necessary field drains can be dug between the rows thus lined.

After lining and staking, planting holes are dug out (Fig. 5a) lot less than 2 ft sq and 2 ft deep at each point. Top soil from the holes is heaped near them and the subsoil thrown further away. The holes are left for a week or two, and then refilled. On all soils except peat, top soil is scraped together with 8 oz of rock phosphate (Fig. 5b). The mixture is filled into the hole and the stake replaced. On peat soils, the hole is refilled with burnt top soil mixed with 8 oz of fertilizer (Rubber Research Institute mixture G), plus 8 oz of magnesium limestone. The land is then ready for planting.

When transplanting from nursery to field, it is important to ensure that each palm is lifted with the roots still embedded in soil. Before lifting, the nursery beds are soaked with water and the soil around the roots of the palms is pudded. When lifted, the palm then rests in a separate ball of earth, which should be planted with the palm. In handling, do not hold or carry the palms by the young spear of unopened leaves. If the soil crumbles, the ball of earth should be wrapped with a gunny bag for transplanting. Each palm can then be carried from the nursery to the field in a basket. Great care is necessary to avoid breaking the ball of earth, thereby severing or damaging the roots. A small hole is made in the refilled planting hole (Fig. 5c) to take the palm with the ball of earth surrounding the roots. The collar of the palm should be flush with the surface of the ground. The soil is well pressed (Fig. 5d) round the roots so that the palm is firmly fixed in the ground.

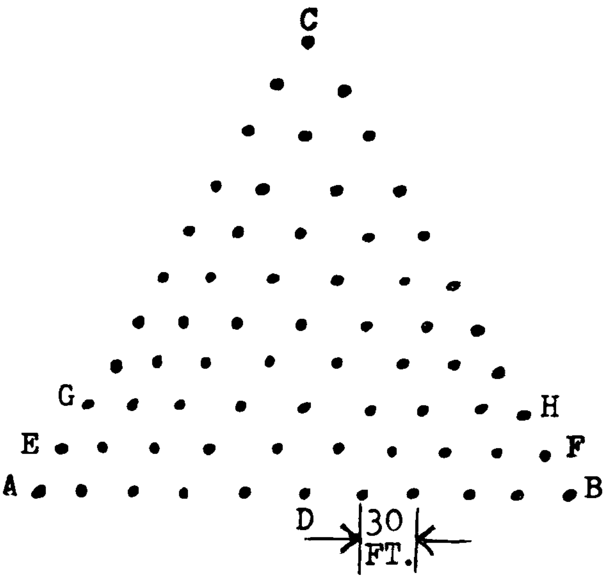


Fig. 4. Method of lining for triangular planting.

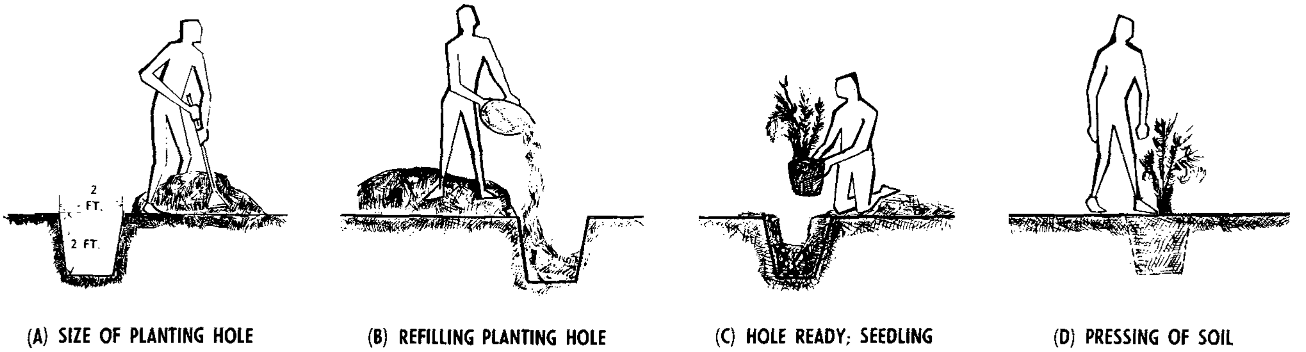
Maintenance and Manuring

Field work includes the maintenance of a suitable ground cover, the pruning of leaves, and manuring.

Ground covers, such as a mixture of half *Calopogonium mucunoides* and half *Centrosema pubescens*, should be established soon after the land is cleared, and maintained by regular weeding and ring weeding around the palms.

Leaf pruning should in no case be undertaken until the lowest fruit bunches are 3½ ft from the ground. From then until the lowest bunches are 5 ft from the ground, the leaf immediately below a bunch is pruned when the bunch is harvested. Thereafter supplementary pruning of a few leaves below those supporting the developing fruit bunches is carried out once a year. In pruning, the leaf is severed close to the trunk. The cut should be made in a sloping direction with a chisel while the leaves can be reached from the ground. Thereafter, harvesting knives attached to bamboo poles are suitable (see Fig. 6). After pruning, the leaves are laid on the ground lengthwise between the rows of palms.

(Continued on page 32)



(A) SIZE OF PLANTING HOLE

(B) REFILLING PLANTING HOLE

(C) HOLE READY; SEEDLING WITH BALL OF EARTH

(D) PRESSING OF SOIL AFTER PLANTING

Fig. 5. Planting seedling in field.

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.

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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.

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• *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 been 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.

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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 non-profit 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.

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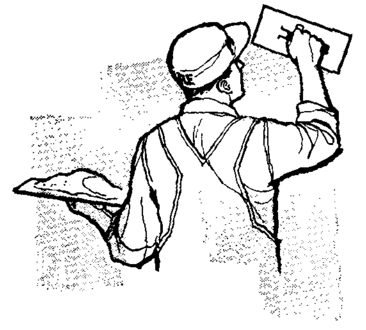

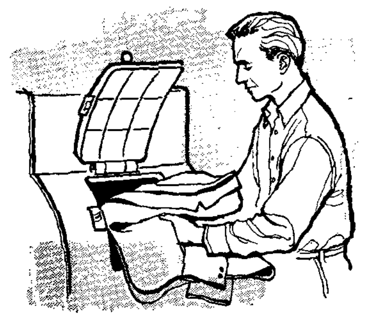

Vitamin A in Margarine . . .

(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

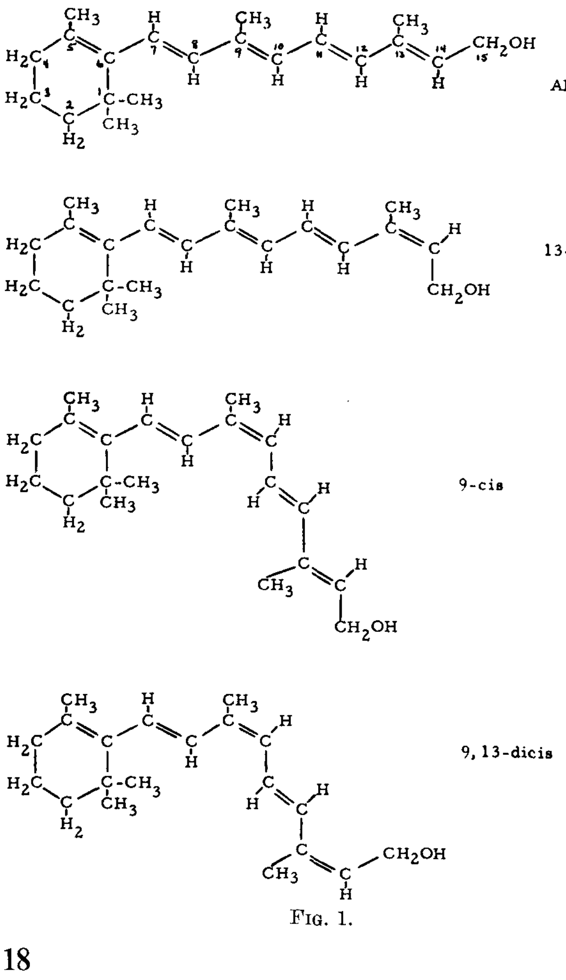

FIG. 1.

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*

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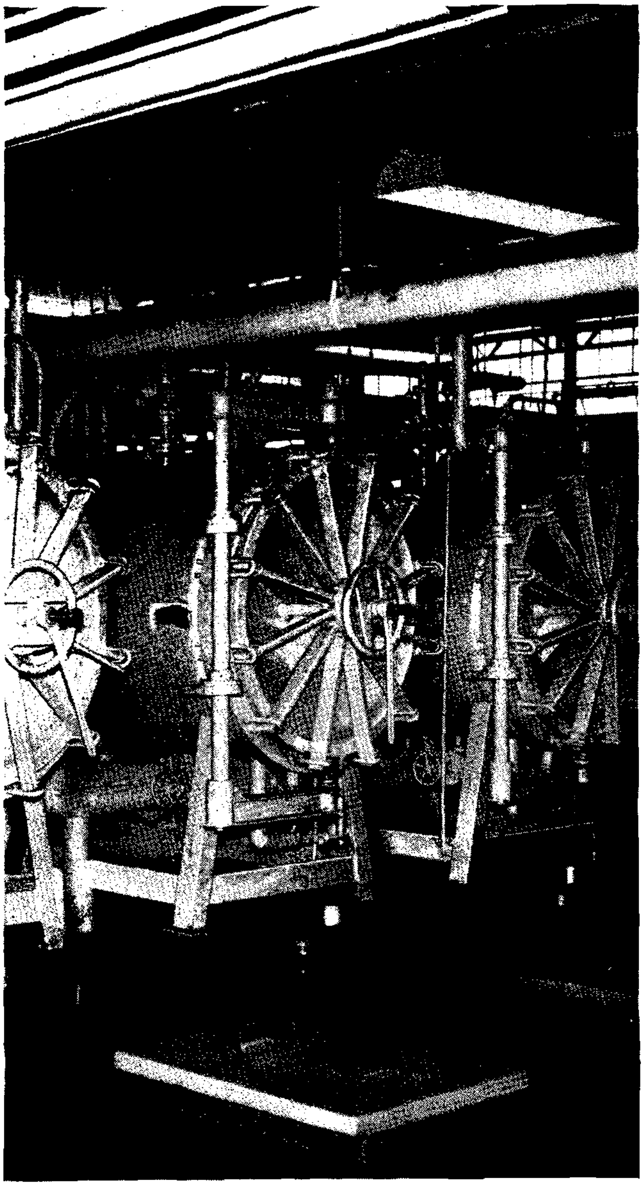

• *New Products*

Blue M Electric Co., Blue Island, Ill., offers a new Power-O-Matic 60 Saturable Reactor Control that achieves temp changes by varying the electrical characteristics of a saturable core reactor.

Mettler Instrument Corp., Princeton, N. J., announces the Vibrio Spatula, a new laboratory device for dispensing powdered substances with 0.1 mg accuracy.

Waters Associates, Inc., *Framingham, Mass., in conjunction* with Procter and Gamble has developed a new Miniature Detector for monitoring the effluent of liquid chromatography columns.

LaPine Scientific Co., Chicago, Ill., has added a new Atom Model, for cyclobutane, to their list of atom models for organic chemistry researchers and instructors. The model is made by E. Leybolds Nachfolger, Cologne, W. Germany.

Trinity Equipment Corp., Cortland, N. Y., developed as a further improvement of the Heat-Les Desiccant Dryer, a steam ejector or a mechanical pump to reduce the purge air required for regeneration.

Applied Science Laboratories, Inc., State College, Pa., now has a number of new Uniformly Tagged Fatty Acid Derivatives available, which should be of considerable interest in biological research.

Thermal Instrument Co., Cheltenham, Pa., has released Model 59 Electric Flowmeter. This instrument will measure the true mass flow rates of liquids and gases over a range of 10 atmospheres.

Andrew Technical Supply Co., Chicago, Ill., announces a new series of motor-driven Tumble Jars, equipped with gear reduction drive and explosion proof motors.

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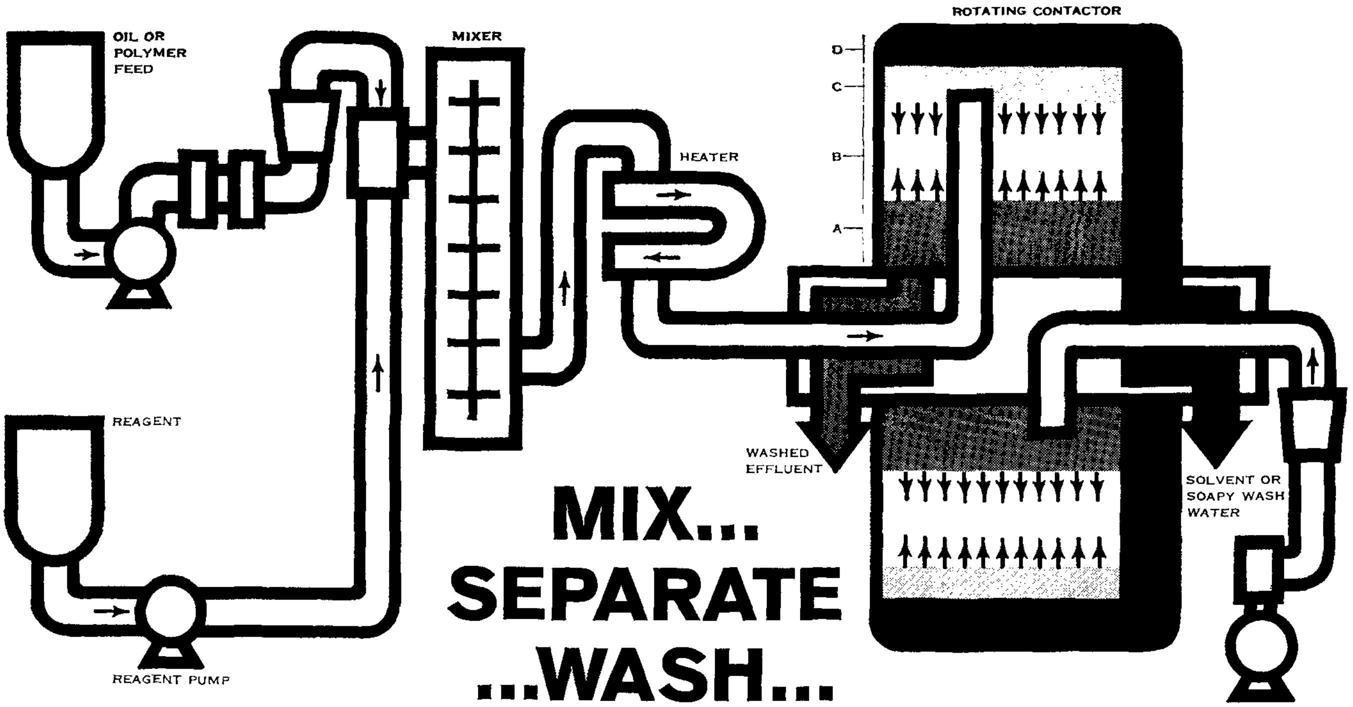
• *New Members*

Active

Mamdouh Hassan Abed, Chemist, Curtis & Tompkins Ltd., San Francisco, Calif.
Murray C. Cooperman, Research Chemist, The Baker Castor Oil Co., Bayonne, N. J.
J. Keith Ferguson, Chief Chemist, Western Canadian Seed Processors, Lethbridge, Alberta, Canada.
Frank M. Ferrell, Commercial Development, Houdry Process and Chemical Co., Philadelphia, Pa.
Donald S. Gardner, Divisional Supervisor, Quality Control, Salada Foods Ltd., Don Mills, Ont., Canada.
DeWitt S. Goodman, M.D., Assistant Professor of Medicine, Columbia University College of Physicians & Surgeons, New York, N. Y.
H. Kenneth Hawley, Manager, Food Products Development, Procter and Gamble of Canada, Ltd., Hamilton, Ont., Canada.
John E. Heilman, Process Engineer, Technical Department Central Soya Co., Inc., Fort Wayne, Ind.
Steven J. Kalich, Chief Chemist, Christie Brown & Co. Ltd., Toronto, Ont., Canada.
Thomas G. Kane, Development and Service Representative, E. I. duPont deNemours, Inc., Wilmington, Del.
Mary Ellen Puthoff, Analytical Chemist, Procter & Gamble Co., Cincinnati, Ohio
Maynard John Rinehart, Chemist, The P. D. George Co., St. Louis, Mo.
Michael R. Schimmenti, Sales Management, Humble Oil & Refining Co., Houston, Tex.

Active Junior

Charles Francis Cook, Research Assistant, University of Wisconsin, Stock Pavilion, Madison, Wis.
Robert Clark MacDonald, Graduate Student, University of California, Department of Physiological Chemistry, Los Angeles, Calif.



Flowsheet showing multiple-step vegetable oil refining or water washing of soluble polymers in a Podbielniak countercurrent centrifugal contactor.

A—CLARIFYING SECTION
B—WASHING SECTION
C—SEPARATING SECTION
D—WITHDRAWING SECTION

MIX... SEPARATE ...WASH... CLARIFY in a single contactor installation!

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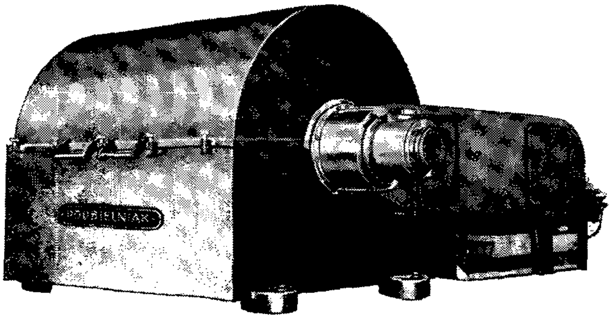

Typical centrifugal contactor with capacities to 600 gpm combined streams.

ABSTRACTS **R. A. REINERS, Editor. ABSTRACTORS:** J. G. Endres, J. Iavicoli, K. Kitsuta, F. A. Kummerow, H. S. Liles, C. C. Litchfield, Louise R. Morrow, E. G. Perkins, and T. H. Smouse

• Fats and Oils

Synthesis and properties of glycerides (a review). F. H. Mattson and R. A. Volpenhein (The Procter and Gamble Co., Miami Valley Lab., P. O. Box 39175, Cincinnati, Ohio). *J. Lipid Research* **3**, 281–96 (1962). In this review is assembled information on the chemistry of glycerides. Information is presented under four headings: synthesis, stability, isolation, and analysis. 103 references.

Determination of cholesterol as the tomatindine using the iron reagent. R. K. Rinehart, Suzanne E. Delaney, and H. Sheppard (Research Dept., CIBA Pharmaceutical Company, Summit, N. J.). *J. Lipid Research* **3**, 383–85 (1962). The purpose of this investigation was to determine the feasibility of using the iron reagent with tomatine for the analysis of cholesterol. The iron reagent may be satisfactorily utilized for the determination of cholesterol in alcohol-acetone extracts of serum and tissue when tomatine is substituted for digitonin as the precipitating agent, provided the following precautions are taken: (1) more time must be allowed for precipitation of samples containing small amounts of cholesterol, and (2) more care must be exercised in handling and washing the precipitates.

A sensitive and specific method for plasmalogens and other enol ethers. J. N. Williams, Jr., C. E. Anderson, and Alice D. Jasik (Lab. of Nutrition and Endocrinology, Nat'l Inst. of Arthritis and Metabolic Diseases, Nat'l Inst. of Health, Bethesda, Md.). *J. Lipid Research* **3**, 378–81 (1962). A spectrophotometric procedure for estimating plasmalogens and other enol ethers, based upon the specific reaction of the enol ether group with iodine, is presented. Optimum conditions for iodination with respect to methanol and KI concentration, pH, and time of incubation have been defined. Reproducibility is ± 3.6% with 0.1 μmole levels of plasmalogen in lipid extracts. The range of enol ether estimated by this procedure is 0.02–0.125 μmole.

The determination of esterified fatty acids in glycerides, cholesterol esters, and phosphatides. W. D. Skidmore and C. Entenman (U. S. Naval Radiological Defense Lab., San Francisco 24, Calif.). *J. Lipid Research* **3**, 356–63 (1962). The conditions for the hydroxamic acid reaction for determining esterified fatty acids have been modified to control the variable factors involved and to obtain molar absorptivities that are equivalent for amounts up to 8 μeq of ester. Control of the amount of water present during the formation of hydroxamates was the most important single factor in obtaining equivalent color values. The accuracy and precision of the method have been well defined by showing that the optical density values for five different ester standards were on the identical straight-line curve. The colored end-products gave identical spectral curves between the wavelengths of 410 mμ and 700 mμ whether they were derived from standard carboxylic acid esters, Folch extracts of rat serum, rat liver, or human serum. A long-chain cholesterol ester must be used as one of the standard esters because of its special solubility characteristics. Cholesteryl acetate *cannot* be used as a reliable representative in place of a long-chain cholesterol ester.

3,7,11,15-Tetramethylhexadecanoic acid, a constituent of butterfat. W. Sonneveld, P. Haverkamp Begemann, G. J. Van Beers, R. Keuning, and J. C. M. Schogt (Unilever Research Lab., Vlaardingen, Netherlands). *J. Lipid Research* **3**, 351–55 (1962). By subjecting the fatty acid methyl esters from butterfat to fractional distillation, urea fractionation, and preparative gas-liquid chromatography, a fatty acid was isolated that was characterized and identified as 3,7,11,15-tetramethylhexadecanoic acid.

Purification of C^{14}-labeled fatty acids by chromatography on acid-treated Florisil. K. K. Carroll (Collip Medical Research Laboratory, Univ. of Western Ontario, London, Ontario, Canada). *J. Lipid Research* **3**, 388–90 (1962). A method for incorporation of radioactive carbon into either the 1- or 2-position of fatty acids was developed and was used to prepare 2-C^{14}-labeled erucic acid and nervonic acid for use in metabolic studies. When the labeled fatty acids were chromatographed on siliconized paper and the paper scanned for radioactivity, it was found that the fatty acids contained appreciable amounts of radioactive impurities. A simple method involving chromatography on acid-treated Florisil was developed, which eliminated most of the impurities and permitted recovery of the fatty acids in a high state of radiochemical purity.

Potential contamination in the analysis of methyl esters of fatty acids by gas-liquid chromatography. F. T. Lindgren, A. V. Nichols, N. K. Freeman, and R. D. Wills (Donner Laboratory, Lawrence Radiation Lab., University of California, Berkeley). *J. Lipid Research* **3**, 390–91 (1961). In gas-liquid chromatography (GLC), as in other microanalytical techniques, it is especially important to guard against the intrusion of contaminants. At the microgram level, contaminants may be introduced at any stage of sample handling or processing. Lipid extraction as well as subsequent lipid-separation techniques by silicic acid chromatography frequently require the use of relatively large volumes of solvents for the extraction or fractionation of relatively small amounts of lipids. It becomes very important to check all solvents used for potential contamination. In certain types of lipid extraction as well as during hydrogenation procedures, it may be necessary to use filter paper. It is absolutely essential to pre-extract such filter paper if used in connection with GLC work.

Paper chromatography of fats L: Qualitative and quantitative determination of hydroxy and keto fatty acids. H. P. Kaufmann and Young Su Ko (Deut. Inst. Fettforsch., Münster). *Fette Seifen Anstrichmittel* **64**, 434–438 (1962). Qualitative and quantitative paper chromatographic analysis of long-chain hydroxy and keto fatty acids on paper impregnated with undecane is described. Acetic acid of various concentrations is employed as the developing phase. The chromatograms are colored and the spots are visualized with copper acetate-verbeanic acid. For quantitation the visualized spots are measured photometrically. The following compounds were chromatographed: 2-hydroxylauric, -palmitic, and -myristic acids; 2-hydroxystearic, -arachidic, and -behenic acids; 2 methyl-4-keto-tridecanoic, -pentadecanoic, -heptadecanoic, -nonadecanoic, -hemicosanoic, and -pentacosanoic acids.

Disproportionation of tall rosin with palladium catalyst. Takeo Wakabayashi, Michio Yoshino, and Tetsuo Ikeda (Nippon Fats and Oils Co., Ltd., Amagasaki). *Yukagaku* **11**, 28–35 (1962). The disproportionation of tall rosin with Pd-catalyst was investigated. The active carbon was the best as carrier and 1 part of Pd to about 100 parts of active carbon was suitable ratio. More catalyst, lower reaction temperature or shorter reaction time, permitted recovery of catalysts of improved activity. In the cyclic reuse of the recovered catalyst, the required amount of the catalysts was about 0.25% (2 cycles with 0.5%) in the average. In the additional use of catalyst, a mixture of new and recovered catalyst was more effective than the use of recovered catalyst first and then new catalyst. The requirement of the catalysts were about 0.13% and 0.2% on the average in the former system and the latter, respectively.

Disproportionation of tall rosin under hydrogen atmosphere. Takeo Wakabayashi, Michio Yoshino, and Tetsuo Ikeda (Nippon Oils and Fats Co., Ltd., Amagasaki). *Yukagaku* **11**, 35–38 (1962). Disproportionation of tall rosin with Pd-active carbon catalyst under hydrogen atmosphere was investigated. The required amounts of the catalyst in disproportionation under hydrogen atmosphere was one-fifth of that in nitrogen, and the extinction coefficient of ultraviolet absorption spectra of the reaction product under hydrogen was reduced to a greater degree than under nitrogen. The reaction was not affected by the amount of hydrogen within the limit of conditions in experiment. The deterioration of activity of catalyst under hydrogen atmosphere was less than that under nitrogen, and therefore cyclic reuse of the catalyst was possible up to five times. When 0.1% of recovered catalyst is used, 0.03% of new catalyst should be added for each cycle. In this case, the consumption of catalyst for each run would become 0.03% at least.

Influence on emulsion polymerization of styrene and butadiene by potassium soaps of disproportionated rosins. Takeo Wakabayashi, Michio Yoshino, Kunishi Hatano, and Mitsuta Shibata (Nippon Oils & Fats Co., Ltd., Amagasaki). *Yukagaku* **11**, 65–8 (1962). The yield of polymer was not affected by the disproportionated rosins having value less than 2.3 in extinction coefficient at 241 mμ (a_{241}) in ultraviolet absorption spectra, but those having values higher than 2.8 in a_{241} had a tendency to decrease the yield. The yield of polymer was not affected by the amount of unsaponifiable matter or free resin acid in the soap except when a large amount of free acid (over 15 in acid number) was present. The stabilities of various latexes were equal.

Utilization of rice bran oil. IV. Solvent extraction of rice bran oil. Yasuhiko Takeshita, Yoshiki Ono, and Seiichi Maruyama (Tokyo Oil & Fat Co., Ltd., Edogawa-ku, Tokyo). *Yukagaku* 11, 5–10 (1962). The effect of drying of rice bran and its solvent extraction was compared with that of corn germ. In the case of corn germ, the area in the constant-drying velocity curve and hygroscopic velocity were smaller than that of rice bran; the curve of solvent extraction rate vs. moisture had a maximum at 3% moisture although the increase in the rate of extraction of the raw material was small. The quantity of miscella remaining in the extracted marc was less than one-third of that of rice bran. Extraction with hot solvent was more effective than the extraction with cold solvent with stirring of rice bran. The differences in properties of extracted oil from different solvents were slight although color differences were great. The color intensities of oils from the solvents were as follows: trichloroethylene : benzene : petroleum ether = 4:2:1. The freezing point of benzene in winter was lowered to −8° by mixing it with 30% of petroleum ether.

V. Qualities of foreign rice bran as the raw material for oil extraction. 2. Yasuhiko Takeshita, Kenzi Urakawa, and Yumi Watanabe. *Ibid.*, 11–14. Qualities of rice bran collected in southeastern Asia were compared with that of domestic product. The oil contents were in the range of 5.2–26.7% and these differences were due to the difference in the extent of polishing of rice and contamination of other substances such as rice hull and crushed rice. There were no striking differences in saponification number, iodine number and percentage of unsaponifiable substance of crude oils obtained from brans of different locations but the color of oil from bran of southeastern Asia was lighter than the domestic oil. Ultraviolet spectra of crude oils were given. The oil from bran of parboiled rice after storage for 1 year showed acid number less than 15.

VI. Molecular distillation of rice bran oil. Yasuhiko Takeshita, Yoichiro Kai, Yoshiki Ono, Hiroshi Nakagawa, and Tatsuo Hamada. *Ibid.*, 52–4 (1962). From the result of estimation of hydroxyl values of distillate and residue, the content of intermediate hydrolyzate in crude rice oils with various acid values is not great. The residue from oils of higher acid value contained higher amounts of unsaponifiable matter of high melting point than those having medium or low acid value. The bottoms showed poor appearance and was unsuitable as the raw material for edible oil. The amount of unsaponifiable matter was less than original oil having medium or low acid value, while it was higher than original oil having higher acid value.

Studies on selective hydrogenation of fatty oils and catalysts. I. Studies on hydrogenation with the KW-type and Adkins catalysts. Kazutomo Maebashi (Asahi Electrochem. Ind., Ltd., Ogumachi, Arakawaku, Tokyo). *Yukagaku* 11, 60–4 (1962). Refined finback whale oil was hydrogenated under normal pressure of hydrogen and three different types of catalysts, namely: KW type (Japan. Patent 200,077), U-Ni-A (Japan. Patent 204,353), and reduced Ni-Cu catalysts. A micropenetration test was used to compare the products hydrogenated to an iodine number of about 70. The KW type catalyst was least sensitive to free fatty acid content of the raw material but lowering of acid number to less than 0.4 was indispensable if the catalyst was to be recycled. A simple method was proposed to regain and accelerate activity of the KW type catalyst by treating spent catalyst with alkaline solution under aeration. By applying the above treatment, the KW type catalyst could be re-used four times in the hydrogenation of whale oil. A refined sperm whale oil was hydrogenated with hydrogen at atmospheric pressure with 2% KW type catalyst and a light colored, almost odorless, stable product was obtained without any conspicuous change in its inherent characteristics. Two per cent Adkins catalyst, used to hydrogenate refined finback whale oil, was almost as active as the KW type and could also be re-activated and re-used by the above treatment.

II. Studies on copper catalysts. Kazutomo Maebashi and Motokazu Yano. *Ibid.*, 54–60. Highly active alkaline co-precipitated copper binary or ternary catalysts were obtained only when acidic metal containing solutions were poured into highly alkaline solutions. Through the above procedure, catalysts of different Cu:Cr molar ratios were prepared and their activities were compared. Co-precipitation of Cr in minor amount (Cu:Cr = 9:1 or less) yielded catalysts of satisfacory activity. Also, a marked increase in catalytic activity was induced by adding such metals as Zn, Al, and Co, whereas Cu alone proved to be almost inactive. These observations showed disagreement with the result reported heretofore, and support the theory of sintering prevention effect by the additives. Studies were made to decrease Ni in the Ni-Cu binary catalyst so as to make Cu predominant over Ni to avoid the inherent catalytic effect of the latter. Though the definite ratio was not determined, less than 1 mole Ni against 9 moles Cu gave an active catalyst. Solid fat index measurements on hydrogenated soybean oil and cottonseed oil indicated that the Cu-Ni catalyst produced hydrogenated products having a lower index than the KW type at the same iodine number down to about 70. The pH during co-precipitation affected catalytic activity. A pH above 9 for Cu:Ni (9:1) and pH above 8 for Cu:Cr (9:1) were suitable to obtain active catalysts. Five kinds of hydrogenated finback whale oil having almost the same iodine number (68–69) were prepared with Adkins, Cu:Cr (9:1), Cu:Al (3:2), Cu:Ni (9:1), and the KW type catalysts. The analytical data showed no significant difference between the three catalysts excepting the Cu-Ni and Adkins. The former made slightly harder products, the latter produced considerable amount of solid and isoöleic acids.

III. Stability comparison of selectively hydrogenated fatty oils. Kazutomo Maebashi and Motokazu Yano. *Ibid.*, 19–24 (1962). In order to prepare samples for stability tests, hydrogenated fatty oils were treated with 1% boiling sulfuric acid, followed by washing with water, alkali refining, activated earth bleaching and deodorization. Such refining was found to be satisfactory for the purpose of eliminating copper and other pro-oxidant metals. A routine test for evaluating stability was proposed, comprising exposure of refined and deodorized specimens under direct sunlight or ultraviolet light and determining the order of their stability by organoleptic evaluation on the slightly reverted specimens. The results indicated that hydrogenated tallow, lard, and finback whale oil developed reversion flavor when their peroxide values were still well under 5 millieq./kg. Also, an evaluation was made on stability of five hydrogenated finback whale oils having almost the same iodine number (68–69) prepared with Adkins (Cu:Cr (9:1), Cu:Al (3:2), Cu:Ni (9:1), and KW (Cu:Cr:Mn) type catalysts.

Effect of the deodorization temperature upon the quality of soybean oil. Kosaku Yasuda, Hisashi Watanabe, and Toshikazu Tokunaga (Research Laboratory, Nissin Oil Mills, Ltd., Yokohama). *Yukagaku* 11, 2–5 (1962). The phenomena of flavor reversion and color reversion of refined soybean oil were investigated by the deodorization at 240C, 270C, and 295C. Increase in deodorization temperature tended to show a progressive decrease in tocopherol content and a higher color stability of deodorized oil, but it was accompanied with a progressive lowering of iodine number. As the deodorization temperature became higher, the linoleic and linolenic acid content of the deodorized oil decreased, while the conjugated diene content increased. Increase in deodorization temperature tended to lower the A.O.M. stability of deodorized oil. The A.O.M. stability was, however, fairly improved by adding citric acid to the deodorized oil. The formation of *trans*-acid became more marked as the deodorization temperature became higher.

Purification of oils and fats by ion-exchange resins. V. Distribution of fatty acids and oryzanol adsorbed by resin in rice bran oil. Hiroshi Inoue and Tatsuo Noguchi (Ind. Research Inst., Hokkaido). *Yukagaku* 11, 45–9 (1962). The distribution of fatty acids and oryzanol absorbed in a bed of strongly basic anion exchange resin (OH type) was determined by passing through the rice bran oil and its methyl ester, respectively, dissolved in a mixture of benzene and methanol. The amount of oryzanol absorbed in the bed decreased remarkably when the ratio of the resin to the oil exceeded a certain limit. From the quantitative relation between oryzanol and fatty acids in the effluents, it is assumed that oryzanol once absorbed on the resin would be replaced by the fatty acids existing in excess.

VI. Effect of free fatty acids of crude rice bran oils on the adsorption of oryzanol. *Ibid.*, 49–51. The adsorption of oryzanol by the resin was less when using crude oil lacking in freshness or having high acid number.

Composition of free fatty acids in natural fats and oils. E. H. Meursing (T. Duyvis N.V., Koog aan de Zaan, Holland). *Rivista Ital. Sost. Grasse* 4, 188–189 (1962). The article discusses differences in composition found between free fatty acids and natural triglycerides from which they were extracted. The lower iodine value of the free acids (in some cases, e.g. whale oil, as much as 35 units lower than in the triglyceride) is explained by the lipase's preferential attack on the triglyceride's *alpha* position, more often occupied by saturated acids.

(Continued on page 30)

• *New Literature*

Hagan Chemicals & Controls, Inc., new 16 page Bulletin MSP-260.1 reviewing Ring Balance Meters. (Controls Division, Hagan Center, P. O. Box 1346, Pittsburgh 30, Pa.)

Roger Gilmont Instruments, Inc., new brochure illustrates recently developed instruments used for precise measurements of vacuum in various ranges. (1 Great Neck Rd., Great Neck, N. Y.)

Walworth-Grove-Aloyco's Bulletin #205 describes and illustrates valves for chemical processing applications. (Sales Division of Walworth, 6529 Hollis St., Oakland 8. Calif.)

Barber-Colman Co., has two new bulletins available upon request. Bulletin F-11413, describes the Series 8000 Two-Pen Recorder and F-11394, Series 8060 Quick Change Multipoint Recorder. Copies of a presentation on gas chromatograph detector systems, by R. E. Johnson of the Barber-Coleman Co. are also available. (Rockford, Ill.)

LaPine Scientific Co., has published their latest "LaPine Apparatus Review" No. 14, 1962, that lists many new products and models of laboratory instruments. (6001 S. Knox Ave., Chicago 29, Ill.)

Research Specialties Co. Review, Vol. 4, No. 5, shows two series of electrically heated, insulated Multi-Block Tube Heaters. (200 S. Garrard Blvd., Richmond, Calif.)

Beckman Scientific and Process Instruments Division, announced the publication of two new brochures. Bulletin SC-4034, describes the Modular Sample Conditioners and No. 779-C, the DB Ultraviolet Spectrophotometer and its accessories. (Technical Information Dept., 2500 Harbor Blvd., Fullerton, Calif.)

Meletron Corp., Engineering Manual available to anyone interested in the problems of sensing hydraulic and pneumatic pressures. (Dept. 374, 950 N. Highland Ave., Los Angeles 38, Calif.)

Wolverine Tube, Division of Calumet & Hecla, Inc., released a 12 page catalog entitled "Wolverine Titanium Tube." (17200 Southfield Rd., Allen Park, Mich.)

The Harshaw Chemical Co., has issued their new catalog "CHEMISCOPE"—The Scope of Harshaw Industrial Chemicals. (1945 East 97th St., Cleveland 6, O.)

Friden, Inc., have announced a 36 page booklet on the Friden Collectadata 30 System. (Promotion Planning Dept., 97 Humboldt St., Rochester 2, N. Y.)

Dorr-Oliver, Inc., have revised their 16 page brochure entitled "Continuous Processing" listing the various types of processing equipment, systems, and services offered. (Bulletin No. 7005, Stamford, Conn.)

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• *Industry Items*

General Aniline & Film Corp's, Calvert City, Ky. Plant, has made plans to expand their facilities for the production of a vinyl ether series of products for commercial use. Plans are expected to be completed by the middle of next year.

Theodore C. Kiesel, Inc., Cincinnati, O., has been appointed to represent the full line of chemical products of Foremost Food and Chemical Co., Oakland, Calif., throughout Ohio and neighboring areas.

Monsanto Chemical Co. announced they will build the world's first plant designed to manufacture synthetic lactic acid. It is scheduled to be in operation by the last quarter of 1963 and will be located at their existing Texas City manufacturing plant.

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The Oil Palm . . .

(Continued from page 15)

TABLE I
Manures for Young Palms

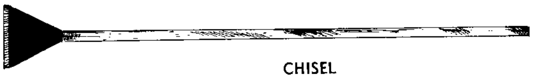
Type of soil	Time of Application	Fertilizer	Quantity
Muck soil	Nil	Nil	Nil
Alluvial clay loam	6 mo after planting	Mix X + Mg	½ lb per palm
	12 mo after planting	Mix X + Mg	½ lb per palm
	18 mo after planting	Mix X + Mg	¾ lb per palm
	24 mo after planting	Mix X + Mg	¾ lb per palm
	30 mo after planting	Mix X + Mg	1 lb per palm
	36 mo after planting	Mix X + Mg	1 lb per palm
	Every six months until palms come into bearing		
All other suitable soils	6 mo after planting	Mix X + Mg	1 lb per palm
	12 mo after planting	Mix X + Mg	1 lb per palm
	18 mo after planting	Mix X + Mg	1 ½ lb per palm
	24 mo after planting	Mix X + Mg	1 ½ lb per palm
	30 mo after planting	Mix X + Mg	2 lb per palm
	36 mo after planting	Mix X + Mg	2 lb per palm
	Every six months until palms come into bearing		

Some form of manuring is necessary on most soils in order to obtain high yields over a long period. The type and quantity of fertilizer mixture required depend on the type of soil in which the palms are grown. Tables I and II will enable the cultivator to calculate the requirements for his land. The recommended manures should be applied to the soil in a broad band around the palms just underneath the spread of the leaves. The manures should be lightly changkolled into the top soil.

Harvesting and Yields

After the third or fourth year in the field, separate bunches of male and female flowers develop in the leaf axils on the same palm. It is rare for male and female flower bunches to reach maturity at the same time on the same palm. Therefore, pollen from one palm usually fertilizes the female on another palm. Bunches of fruit which develop from female flowers usually ripen within a period of about 5½–6 months from the date of pollination (Fig. 7). If a dry spell occurs during development, this period may be extended by another month. An indication of the state of ripeness of fruit bunches is the color of the fruits on the bunch. These fruits are deep violet in color during development; as the bunches ripen, their color changes to an orange-red from the base of each fruit upward. When most of each visible fruit is orange-red in color, the bunch is said to be ripe. It is then possible to remove several fruits easily. During development of fruit bunches, oil is produced in the fleshy pulp and continues to be produced until the bunches are fully ripe. Ripe fruits, in addition to being orange-red in color, also become loose on the bunch. When the bunch starts to shed a few loose fruits it can be considered ripe for harvesting. When the bunches are cut, oil production ceases. If the bunches are left on the palm after they are ripe, the loose fruits fall from the bunches and become bruised. Bunches which have been harvested and stored dry out and the fruits become cracked. Cracking, bruising or over-ripening of fruits cause some of the oil to break down into free fatty acids. The quality and price of oil decrease as the fatty acid content increases. Therefore, in order to produce optimum yields of high quality oil, the bunches must be harvested when they are just ripe and taken at once to the factory for processing. Fatty acid formation is very rapid. In practice, every attempt should be made to process on the same day as the fruit is harvested. Harvesting is carried out at intervals of from 5–10 days. A harvesting knife, attached to a bamboo pole, is used for harvesting fruit bunches when they can no longer be reached with the chisel. The length of the pole varies with the height of the palms. With practice it is possible to use bamboo poles 35–40 ft long. The fruit bunch is harvested by first cutting through the supporting leaf-stalk and then the bunch-stalk. The number of leaves pruned should be restricted to the smallest number necessary to harvest a bunch. Harvested bunches and loose fruits are collected and carried to an appropriate point for transport without delay to the factory.

HARVESTING KNIFE



CHISEL

FIG. 6. Harvesting Implements.

Table III estimates the approx yields under average conditions of soil, drainage and management. Variations in any or all of these three factors may have considerable effect on actual yields.

Transport of Fruit

It has been emphasized already that it is important to transport harvested bunches to the factory quickly. To accomplish this it is essential that all parts of a group of holdings are accessible. A reasonable max carrying distances of 10 chains in the field requires some form of internal transport at 20-chain intervals. If roads can be built to take 3-ton lorries or tractors and trailers, newly harvested ripe fruit bunches could be loaded at collecting points and taken immediately to the factory. The criterion for the correct type of transport is that it must be able to remove all freshly harvested ripe fruit bunches to the factory for immediate extraction of oil.

TABLE II
Manures for Palms in Bearing
Time of Application—March and September Each Year

Type of soil	Fertilizer	Quantity
Muck soils	Nil	Nil
Alluvial clay loam	Rock phosphate Kieserite	1 ½ lb ½ lb } per palm
Shallow peat	Muriate of potash Magnesium limestone	1 ½ lb ½ lb } per palm
Upland loams (poor)	NPK 5	3 lb per palm
Upland loams (good)	RRI Type G Kieserite	2 lb ½ lb } per palm

Pests and Diseases

Diseases of the oil palm are not always easy to identify. Four common diseases are reviewed below.

1. *Stem Rot:* There may be no symptoms until the trunk of the palm breaks. The inside of the trunk will be brown and decayed. The diseased palm should be destroyed to avoid the spread of the disease. If burning is not possible, the palms should be buried. Stem rot can be preventd by careful pruning and harvesting. Avoid wounding the bases of growing leaves unnecessarily.
2. *Bud Rot:* The spear of unopened leaves collapses and can be pulled out from the bud cavity. When the decayed leaves are pulled out the palm usually recovers. New leaves develop.
3. *Crown Disease:* This is not a true disease. It is the effect of mechanical injury by Rhinoceros Beetle. Control of beetles is thereby necessary for prevention. Damage is caused by the beetle to the unopened leaves. When they open they are twisted and shrivelled. The injury is most severe in young

TABLE III
Estimated Annual Yields of Oil Palm Products from Palms of Varying Ages

Age of palm	Fruit bunches per palm per annum	Fruit bunches per acre per annum	Palm oil per acre	Palm kernels per acre
(Years)	(Katis)	(Pikuls)	(Pikuls)	(Katis)
4	45	22	3 – 3½	80
5	90	44	6½– 7	170
6	135	67	10 –10½	255
7	150	75	11 –11½	280
8	180	88	13 –13½	330
9	195	96	14 –14½	355
10 or more	200	98	14½–15	370

FIG. 7. (F) Partially pollinated Female Flower, among fruit bunches of various stages of ripeness.

palms. Damaged palms usually recover after about a year.

4. *Marasmius Disease:* This is a disease of fruit bunches. Normally the disease does not attack the fruits until just before they ripen. Attacked fruits decay. This disease is spread from unpollinated female flower bunches. Control of the disease is achieved by burning or burying the unpollinated female bunches when they begin to decay.

The three most important insect pests that attack oil palms in Malaya are shown in Table IV.

Animal pests are pigs, porcupines, and rats. Shooting is the most effective method of controlling pigs and porcupines. Hunting and trapping rats is advised. When the palms are in bearing, rats may damage the fruit bunches. Regular dusting of the developing fruit bunchs with barium carbonate prevents excessive damage.

TABLE IV
Details of Three Insect Pests of Oil Palm

Pests	Damage	Treatment
Rhinoceros beetle	Adult beetles attack the bases of leaf stalks and young unopened leaves.	The harmless grubs breed in old stumps, decaying timber, and other vegetation. Such breeding places should be destroyed. Hand collection of beetles and grubs may also be necessary.
Red stripe weevil	The grubs tunnel into the trunk. They can kill the palm.	Eggs are laid on wounded surfaces. Avoid excessive wounding of the palm when pruning leaves or harvesting bunches. Never cut the trunk of the palm. Prevent rhinoceros beetle from making wounds.
Nettle caterpillar	The leaves, usually of young palms, are eaten.	Hand collection of caterpillars and cocoons. The spines on the caterpillar are poisonous. Protect the hands when collecting.

Factory

As the fruits bunches come into the factory, they are weighed and then cooked in the sterilizer in order to loosen the fruits from the bunches. From the sterilizer, the bunches go into the thrasher. As the name implies, the individual fruits are thrashed out of the bunches. These loose fruits are then carried by bucket conveyor to the digestor where they are cooked into a mush. This mush is let into the press where the oil is expressed. The oil is pumped into the settling tanks where most of the dirt is settled out. The clean oil is then pumped through a clarifier which separates water and dirt from the clean oil. The pure oil that comes out of the clarifier is pumped to the storage tanks. From the press also comes the cakes which are sent to the fiber screen. In the fiber screen, the fiber is separated from the nuts. The nuts are dried in a silo and then cracked in the nut cracker. The cracked nuts are sent to a mud bath where the kernels are separated from the shell. The kernels are then dried in a kernel drier. When dried, they are sacked ready for export.

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(Continued from page 24)

BLEACHING OF VEGETABLE OILS. P. Fontana and O. Colagrande (Univ. Cattol., Piacenza). *Rivista Ital. Sost. Grasse* 4, 180–187 (1962). The properties of various earths in bleaching vegetable oils have been studied under atmospheric pressure, under vacuum, and under nitrogen atmosphere. The results show that Langmuir's equation is better suited than other mathematical relationships (e.g., Freundlich's) to interpret the adsorption phenomena connected with bleaching, especially in terms of the theoretical maximum color removal to be expected in the operation. In general, bleaching under atmospheric pressure is considerably more effective than bleaching under vacuum; however, bleaching under nitrogen gives slightly better results than bleaching under air at normal pressure.

VAPOR PHASE CHROMATOGRAPHY IN THE PRODUCTION OF COMMERCIAL STEARIC ACID. R. Aeschbacher (Steinfels Labor., Zürich, Switzerland). *Olearia* 2, 65–69 (1962). A gas chromatographic analysis was made of the methyl esters of crude tallow fatty acids as well as the various intermediate and end products of the commercial stearic and oleic acid industry. Results were in very good agreement with those obtained through chemical analysis.

• Fatty Acid Derivatives

ANTISTATIC EFFECT OF SURFACTANTS ON POLYPROPYLENE FIBERS. II. PROLONGED STORAGE AFTER APPLICATION. Koji Onoda and Yugo Saigusa (Miyoshi Oils & Fats Co., Ltd., Tokyo). *Yukagaku* 11, 69–72 (1962). Alkyl phosphates, quaternary ammonium type cationics, and betaine type amphoterics gave good results even after 90-day storage.

PROCESS FOR PREPARING A MIXTURE OF SOAP AND FATTY-ACYLAMINOMETHANE SULFONATE. A. Alsbury, K. A. Phillips, and B. Taylor (Lever Bros. Co.). *U. S. 3,047,509.* A fatty amide having from 10 to 18 carbon atoms is reacted with from 0.8 to 1.2 molecular proportions of sodium or potassium formaldehyde bisulfite in the presence of (a) from 15 to 40% by weight of the amide of a free fatty acid having from 10 to 18 carbon atoms and (b) from 1 to 26% of a water soluble alkali metal soap having from 10 to 18 carbon atoms. The reaction is conducted at a temperature of from 150 to 210C.

WATER-BASED DRILLING FLUID HAVING ENHANCED LUBRICATING PROPERTIES. M. Rosenberg and P. W. Schaub (Gulf Research & Development Co.). *U. S. 3,048,538.* The described composition consists of water and, as an extreme pressure lubricant additive, one of the following: fatty acids having at least 8 carbon atoms, sulfurized fatty acids having at least 8 carbons, alkali metal soap of fatty acids or sulfurized fatty acids. The fluid also contains calcium ions in a concentration above 150 p.p.m. high enough to form insoluble curds of calcium soap and a non-ionic surface active agent (polyoxyethylene derivative of alkyl phenols, alkyl glycols, or anhydroalkitol esters) in a concentration adequate to disperse the calcium soap.

• Biology and Nutrition

FORMATION AND FATE OF ENDOGENOUS TRIGLYCERIDES IN BLOOD PLASMA OF RABBITS. R. J. Havel, J. M. Felts, and C. M. Van Duyne (Cardiovascular Research Inst. and Dept. of Med., Univ. of Calif. School of Med., San Francisco, Calif.). *J. Lipid Research* 3, 297–308 (1962). The formation of hepatic triglyceride fatty acids from palmitate-1-C^{14} and their transport from the liver to the blood and from the blood to peripheral tissues were studied in rabbits. Isotopic equilibration of triglyceride fatty acids (TGFA) in subcellular compartments of the liver required up to 2 hr. Hepatic TGFA appear to be the immediate precursor of TGFA contained in very low-density lipoproteins of plasma. Isotopic transfer between hepatic TGFA and TGFA of very low-density lipoproteins occurred rapidly in relation to the turnover rate of TGFA in liver and plasma. Experiments in which labeled palmitate or labeled TGFA in very low-density lipoproteins were injected intravenously showed that the distribution in tissues of FFA is not affected by the nutritional state, but that of TGFA

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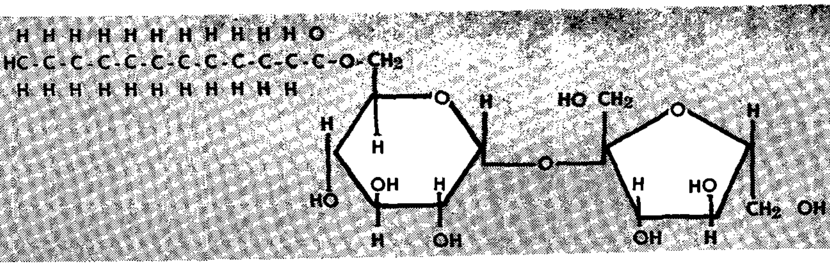

is markedly altered. About 20 times as much TGFA radioactivity was deposited in the adipose tissue of re-fed rabbits as in that of fasted animals, but oxidation was considerably less. The rate of esterification of FFA, however, depended greatly on nutritional state in adipose tissue and, to a lesser degree, in skeletal muscle and lung. These findings are discussed in relation to the roles of lipoprotein lipase activity and esterifying capacity of tissue in the fate of circulating FFA and TGFA.

THE ROLE OF DIETARY FAT IN THE UTILIZATION OF PROTEIN. I. QUALITY AND QUANTITY OF FAT. D. J. Naismith and Rahmat Ullah Quresh (Human Nutrition Research Unit, National Inst. for Med. Research, Mill Hill, London, England). *J. Nutrition* **77**, 373–80 (1962). A study was made of the nutritive properties of a number of fats indigenous to the Pakistani dietary. Ghee, partially hydrogenated cottonseed oil, and mustardseed oil were incorporated in a Pakistani-type diet and fed, *ad libitum,* to weanling rats. The two saturated fats exerted a comparable effect on the rate and efficiency of growth, while the highly unsaturated mustardseed oil was found to inhibit growth and reduce the efficiency of food utilization. The adverse effect on mustardseed oil on growth was attributed to its content of erucic acid. When diets containing ghee, hydrogenated cottonseed oil, mustardseed oil, rapeseed oil, sunflowerseed oil, and lard were pair-fed to young adult rats, no differences in nitrogen retention were noted. The fats were aabsorbed to essentially the same extent. These experiments demonstrate that the protein-sparing property of a fat is independent of its degree of saturation and fatty acid composition. Nitrogen retention was compared under the extreme dietary conditions provided by feeding a fat-free diet and one rich in unsaturated fat (sunflowerseed oil). It was found that, as a source of energy in the diet, fat may be completely replaced with carbohydrate without adversely affecting the utilization of protein. These observations are discussed in relation to the caloric deficiency of the Parkistani diet.

THE ROLE OF DIETARY FAT IN THE UTILIZATION OF PROTEIN. II. THE ESSENTIAL FATTY ACIDS. D. J. Naismith (Human Nutrition Research Unit, Nat'l Inst. for Med. Research, Mill Hill, London, England). *J. Nutrition* **77**, 381–86 (1962). Weanling rats were fed, *ad libitum,* diets containing 1% of hydrogenated coconut oil (essential fatty acid-free diet) or 7% of sunflowerseed oil (control diet). One per cent of cholesterol was incorporated into both diets to increase requirements for essential fatty acids (EFA's). Growth was markedly reduced with the EFA free diet, and characteristic signs of EFA deficiency developed within 5 weeks. Dietary cholesterol, per se, had no influence on protein metabolism.

SODIUM POLYETHYLENE SULFONATE: TISSUE DISTRIBUTION AND EFFECT ON C^{14}-1-PALMITIC ACID OXIDATION. W. L. Miller and J. J. Krake (Dept. of Metabolic Disease, The Upjohn Co., Kalamazoo, Mich.). *Proc. Soc. Exp. Biol. Med.* **110**, 309-311 (1962). Neither injected sodium polyethylene sulfonate (PES) nor heparin affected the rate of oxidation of intravenously administered C^{14}-1-palmitic acid in rats whereas both agents increased the oxidation of C^{14}-1-tripalmitin. These findings are interpreted as indicating that PES and heparin increase the rate of oxidation of triglyceride fatty acids indirectly by increasing the rate of hydrolysis to fatty acids, which in turn are rapidly oxidized. When tritiated PES was given to dogs and rats, only a fraction of the dose was found in the urine. In rats about 50% was excreted in the urine and the remainder could be accounted for in various tissues; liver and skeletal muscle contained the most.

COMPARISON OF LARD, TALLOW, BUTTER, AND HYDROGENATED COTTONSEED OIL IN STARTERS AND OF PELLETED VS. NONPELLETED COASTAL BERMUDAGRASS HAY FOR CALVES. W. J. Miller (Dairy Dept., Univ. of Ga., Athens). *J. Dairy Sci.* **45**, 759–64 (1962). A total of 99 calves was used in three experiments to study: (a) the effect of adding various fats to starters, (b) the influence of calcium level in high fat starters, and (c) the effect of grinding and pelleting vs. grinding Coastal Bermudagrass hay on calf performance. In Experiment 1, 56 baby calves were fed one of seven starters and pelleted or coarsely ground Coastal Bermudagrass hay *ad libitum* in an 8-wk growth trial. The addition of 10% butter, tallow, lard, or hydrogenated cottonseed oil to starters did not significantly ($P = 0.05$) affect weight gains, days of diarrhea, or hay consumption. Level of calcium in starters containing hydrogenated cottonseed oil had little influence on calf performance. Pelleting the hay approximately doubled its consumption and reduced the starter eaten, but did not affect the total amount of feed consumed. In cafeteria trials (Exp. 2) the con-

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Vitamin A in Margarine . . .

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TABLE IV

Calculated Potency of 4.5 mg Vitamin A in Various Commercial Sources by Various Assay Methods

Commercial type	Assay Method				
	Maleic value	Blue-color	$E_{uncorr.}$	USP XVI	Biological
All-Trans	5	15,000	14,900	14,800	14,800
Trans-Neo	33	15,000	14,500	13,600	13,400
Trans-Cis	33	15,000	13,700	12,900	10,800
Fish Liver Oil	33	15,000	13,800	13,100	11,400

have led to this estimate of isomer composition, on the average, for several fish liver oils studied: 52% all-*trans*, 25% 13-*cis*, 15% 9-*cis*, and 8% 9,13-di-*cis*. These are approximate; the actual figures are not significantly different from the 48-24-19-9 proportions mentioned above for the Trans-Cis synthetic vitamin A, and also for the equilibrium proportions estimated for those aqueous systems that induce isomerization.

Table II summarizes these estimates of isomer proportions in the four commercial vitamin A sources.

Assays on Commercial Vitamin A Sources

It is a simple matter to calculate the expected assay potency for a given mixture of isomers using the basic data in Tables I and II. Table III presents an example calculation of the biopotency of 4.5 mg of vitamin A having the isomer composition estimated for fish liver oil.

This type of calculation has been made for the four commercial sources, and for the four assay procedures. Table IV presents the estimates derived in this way.

One interpretation that can be made of Table IV is to consider that margarine samples are fortified at a level to give exactly 15,000 units per lb by blue-color assay, using four commercial vitamin A sources. The three right-hand columns show the assay results that would be expected (neglecting random assay variation, and effects of extraneous materials) by the other assay procedures.

TABLE V

Assay Values for Margarine Containing 15,000 Biological Units per Pound

Commercial source of vitamin A	Assay method		
	Blue-color	$E_{uncorr.}$	USP XVI
All-Trans	15,200	15,100	15,000
Trans-Neo	16,800	16,200	15,200
Cis-Trans	20,800	19,000	18,000
Fish Liver Oil	19,700	18,200	17,200

Table V presents the same information, but on a different basis. Here it is assumed that margarine samples are fortified with that amount of each of the commercial sources necessary to give exactly 15,000 biological units per lb. The three columns present the assay results that would be expected (again neglecting random variation and effects of extraneous materials).

It is apparent that different physicochemical assay procedures give equivalent figures, agreeing within themselves and with biological potency, only when All-Trans vitamin A is used for the fortification of margarine. When other commercial vitamin A sources are used, physicochemical assays overestimate the biological potency by amounts dependent on the isomer composition of the vitamin A source.

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New Findings In Viscose Solutions

Researchers from Buckeye Cellulose Corp. have discovered the presence of a significantly greater number of very small particles in viscose than had previously been known to exist. Their studies show that viscose contains many more millions of gels of 0.2–0.3 μ in diameter than earlier researchers had suspected.

The significance of this discovery lies in the fact that gels may exert major influence on the filterability and spinnability of viscose.

Employing a combination of light scattering measurements, electron microscopy, light microscopy and an electric counter, identification of particles as small as 0.1μ in diameter was obtained. Previously, quantitative determinations on viscose fiber residues were limited to about 15 μ for direct studies on viscose, and limited to about 5 μ when the residues were isolated and redispersed.