the melting dilation of the corresponding fatty acid.

The melting dilations of the fatty acids and their glyceryl esters, as functions of the chain length, are plotted in Figure 2, which includes both observed values and values calculated by means of the equations.

When calculated by the equations given the melting dilations of those compounds which contain less than 10 carbon atoms deviate from the observed values because of the increased importance of the end-group as the length of the carbon chain decreases. This result was experienced by Garner and associates (3) in their work on heats of fusion. Consequently the equations are not valid for such materials. Also unsaturation in a fatty acid or its glyceryl esters greatly influences melting dilation (2), and the equations herein presented are not valid for unsaturated compounds. The melting dilation of mono-olein was approximately one-half that of monostearin although the number of carbon atoms is the same for each.

Summary

Melting dilations have been determined for the even-numbered, saturated *n*-fatty acids in the series lauric through stearic, and some of their glyceryl esters. These data have been correlated with data obtained previously to determine the relationship between melting dilation and the effective chain length of these compounds.

The melting dilations of the mono-, di-, and triglyceryl esters of a fatty acid were found to be proportional to the acid residue content of each compound. The melting dilation of a monoglyceride was one-half that of the diglyceride and one-third that of the triglyceride of the same fatty acid.

The increment of melting dilation of the fatty acids and glyceryl esters increased regularly with each addition of two methylene groups, with the end group components exerting a contant effect on the volume change.

Equations were developed for calculating the melting dilation of the *n*-fatty acids and their glycerides as a function of chain length of the fatty acid radical.

The melting dilations of those compounds which are unsaturated, or have less than 10 carbon atoms, cannot be calculated by these equations.

REFERENCES

1. Bailey, A. E., and Kraemer, E. A., Oil & Soap, *21*, 251-253 (1944).
2. Bailey, A. E., and Singleton, W. S., Oil & Soap, *22*, 265-271 (1945).
3. Garner, W. E., Madden, F. C., and Rushbrooke, J. E., J. Chem. Soc., *1926*, 2491-2502.
4. Garner, W. E., and Ryder, E. A., J. Chem. Soc., *127*, 720-730 (1925).
5. Handschumaker, H., and Linteris, L., J. Am. Oil Chem. Soc., *24*, 143-145 (1947).
6. Lutton, E. S., and Jackson, F. L., J. Am. Chem. Soc., *70*, 2445-2449 (1948).
7. Malkin, T., el Shurbagy, M. R., and Meara, M. L., J. Chem. Soc., *1937*, 1409-1413.
8. Singleton, W. S., and Vicknair, E. J., J. Am. Oil Chem. Soc., *28*, 342-343 (1951).
9. Singleton, W. S., Ward, T. L., and Dollear, F. G., J. Am. Oil Chem. Soc., *27*, 143-146 (1950).
10. Ward, T. L., and Singleton, W. S., J. Phys. and Coll. Sci. (in press).

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Biochemical Studies on Vitamin A. X. A Nutritional Investigation of Synthetic Vitamin A in Margarine[1,2]

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CERTAIN types of natural vitamin A are currently permitted for use in margarine at a level of at least 9,000 U.S.P. units per pound (5). Actually the level of fortification generally used in the U.S.A. is 15,000 U.S.P. units of vitamin A per pound. Margarine is thus an important food source of vitamin A. Two ounces supply 1,875 units or 47% of the F.D.A. minimum daily requirement for humans (6). With the availability of commercially produced synthetic vitamin A (2), its addition to margarine is of current interest. Synthetic vitamin A concentrates are suitable for use since their blandness and high concentration obviate objectionable flavors and odors in the margarine.

This investigation was designed to study the effect in margarine of vitamin A in the form of synthetic vitamin A palmitate in amounts larger than usual. Three generations of rats were maintained on adequate diets containing 7.5% of margarine oil, one containing natural vitamin A ester at the usual level (18,500 U.S.P. units/gram) and the other fortified with synthetic vitamin A palmitate at a level 100 times that ordinarily used. The criteria of response were growth rate, reproductive performance, and lactation ability. No differences of practical or statistical significance were discovered in the nutritional value of the two margarine oils.

Experimental

Diets. The detailed composition of the diets used in this study is given in Table I. These diets are composed of natural materials and a similar diet with hydrogenated vegetable shortening in place of margarine oil as the source of fat has proven to be adequate for growth, reproduction, and lactation of our stock colony rats over a period of many years. The margarine oil constituted 7.5% of the diet. Fresh diets were prepared weekly, and the prepared diets were stored in the refrigerator until used. All diets were fed *ad libitum*.

Supplements. The margarine oil used to prepare the "regular margarine oil diet" was obtained by the clarification of commercial margarine ("Mayflower" brand made by Armour and Company) labeled as containing 15,000 units of vitamin A per pound of

[1] Presented at the 25th Fall Meeting, American Oil Chemists' Society, Chicago, Ill., Oct. 8-10, 1951.

[2] Communication No. 181.

TABLE I

Diet Composition

		%
1.	Corn (ground)	20.0
2.	Wheat (ground)	30.0
3.	Dry skim milk	21.0
4.	Crude casein	5.0
5.	Calcium carbonate	0.5
6.	Iodized salt	1.0
7.	Alfalfa meal	2.0
8.	Meat-bone scraps	3.0
9.	Liver	1.0
10.	Yeast	2.0
11.	Soybean meal	7.0
12.	Margarine oil [a]	7.5
		100.0

Components 4 through 11 are premixed and then added to components 1 through 3. After thoroughly mixing these dry ingredients, the margarine oil is added and the entire diet again thoroughly mixed.

[a] The "regular margarine oil diet" contained margarine oil prepared from commercial margarine (18,500 U.S.P. units of natural vitamin A per pound). The "high vitamin A margarine oil diet" contained commercial margarine oil fortified with synthetic vitamin A palmitate to a level of 1,850,000 U.S.P. units of vitamin A per pound.

margarine. Actually, 15,000 units/pound is a minimum value since it is common practice for manufacturers to add overages of as much as 20%. On the basis of an 81% fat content of commercial margarine, the regular margarine oil contained 18,500 units of vitamin A per pound of fat (40.75 units/gram). The high vitamin A margarine oil used in the "high vitamin A margarine oil diet" was prepared by fortification of the regular margarine oil to a level of 1,850,000 units of vitamin A per pound of fat (4,075 units/gram) with commercial concentrates of synthetic vitamin A palmitate ("Myvax" brand manufactured by Distillation Products Industries). The fortification

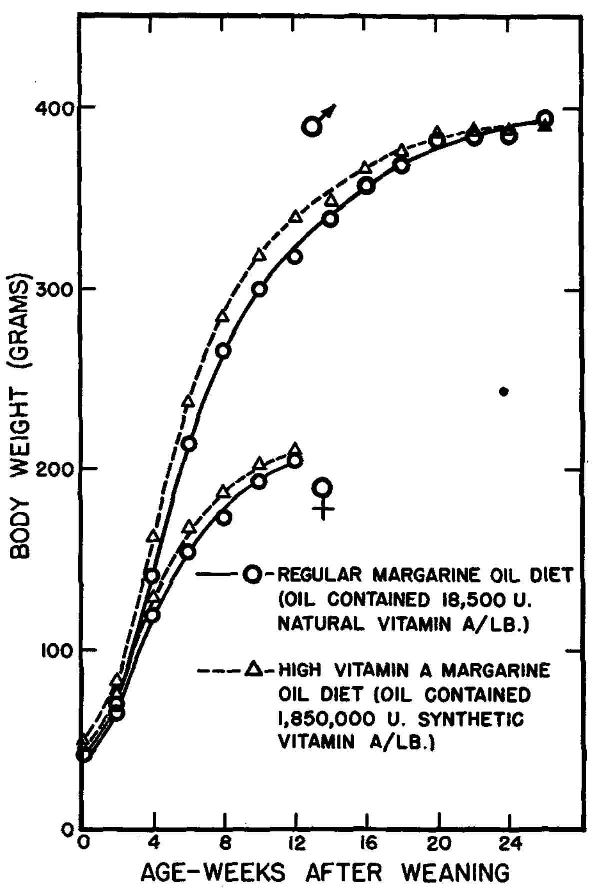

FIG. 1. Growth of F_2 generation rats (15 per group) receiving diets containing 7.5% margarine oil fortified with vitamin A.

was based upon the potency as determined by U. S. Pharmacopeia XIV spectrophotometric procedure (3). Analysis of the "high vitamin A margarine oil diet" indicated that no significant destruction of the vitamin A in the diet occurred in one week under the conditions of storage used.

Treatment of Animals. Sixty albino rats, 30 males and 30 females, were selected at time of weaning from our stock colony. The animals, 15 per group, were placed immediately on the two diets described above. The growth of the male animals was observed over a 26-week period. The growth of the female animals was observed over a 12-week period, at which time reproduction and lactation experiments were started. The females from each dietary group were bred and were maintained on the same diets previously fed. The young of the parental group of animals (F_1 generation) were treated in a similar manner throughout growth, reproduction, and lactation. The young of the F_1 generation were treated in a similar manner.

Growth. The results of the growth studies for the parental group and the F_1 and F_2 generations of rats fed the two margarine oil diets were very similar. The growth curves for the F_2 generation (Figure 1) are representative. There was no significant difference in the rate of growth between animals of the same sex on the two diets. Average weights of all groups of animals at 12 weeks after weaning are given in Table II so that a direct comparison can be made between

TABLE II

Growth Response of Rats on Margarine Oil Diets (12th week after weaning)

Sex	Generation	Regular margarine oil diet [a]		High vitamin A margarine oil diet [b]		Significance of difference between means	
		No. of rats	Av. wt. g. ± S.E.	No. of rats	Av. wt. g. ± S.E.	t	P
Males	P	14	335.9±4.0	15	334.5±5.8	0.20	0.8 -0.9
	F_1	14	292.7±8.5	15	292.2±7.2	0.05	> 0.9
	F_2	12	317.6±9.9	15	339.5±5.8	1.91	0.05-0.1
	Total	40	315.3±5.2	45	322.1±4.8	0.96	0.3 -0.4
Females	P	15	198.7±4.4	15	203.1±3.8	0.76	0.4 -0.5
	F_1	15	202.3±3.6	15	207.7±3.5	1.07	0.3
	F_2	13	205.1±4.5	15	209.4±1.8	0.88	0.4
	Total	43	201.9±2.4	45	206.7±1.7	1.64	0.1

[a] Margarine oil contained 18,500 U.S.P. units natural vitamin A per pound.
[b] Margarine oil contained 1,850,000 U.S.P. units synthetic vitamin A per pound.

the diets with and without the large supplement of synthetic vitamin A. Results of the accompanying statistical analysis indicate that there is no statistical difference in average weight of either male ($p = 0.3$-0.4) or female ($p = 0.10$) rats on the two diets.

Reproduction. The reproductive performance of the three groups of female rats is summarized in Table III. Comparable fertility was observed irrespective of diet or generation. The mean size of the litters was also comparable. No differences in reproductive performance were observed in female rats fed either the regular margarine oil diet or the high vitamin A margarine oil diet.

Lactation. The body weight of the young at weaning was used as the principal criterion of lactation. Lactation performances of the parental group and the F_1 and F_2 generations are summarized in Table III.

TABLE III
Reproduction and Lactation of Rats on Margarine Oil Diets

Genera-ation	Reproduction			Lactation	
	Females, pregnant /mated	Mean litter size	Litters, mean weight (3 days) g.	Weaned /born, %	Mean weaning weight, g.
Regular Margarine Oil Diet (Margarine oil contained 18,500 U.S.P. units natural vitamin A per pound)					
P	10.1	14/15	....	90	35.3
F_1	9.8	13/15	9.0	92	36.3
F_2	7.3	11/13	8.3	89	41.8
High Vitamin A Margarine Oil Diet (Margarine oil contained 1,850,000 U.S.P. units synthetic vitamin A per pound)					
P	9.5	12/15	....	96	35.1
F_1	7.3	15/15	8.7	100	40.3
F_2	8.3	13/15	8.2	88	40.4

The ratios of mean litter size at weaning to mean litter size at birth were very similar, and the mean weaning weights of the young were not significantly different between groups fed either the regular margarine oil or the high vitamin A margarine oil diets.

General Appearance. The animals on both margarine oil-containing diets appear to be normal in every respect. Compared with stock colony animals of the same age and sex, they are identical with respect to growth, fur condition, "feel" and general appearance.

Pathological Examination. Ten rats identified only by number were submitted to Karl E. Mason, University of Rochester School of Medicine and Dentistry, for gross and histological examination. The animals were F_1 generation males, 33 weeks of age. Five were representative of the regular margarine oil group (511, 512, 524, 530, and 536) and five of the high vitamin A margarine oil group (520, 526, 528, 538, and 539). Dr. Mason's detailed report may be summarized as follows. Gross observations of all 10 rats at necropsy showed them to be in good physical condition with no unusual changes. One rat (512) showed some adhesions around the pericardium, and 7 rats had slightly enlarged cecums. Histological examination of skeletal muscle, heart, liver, spleen, large and small intestine, thymus, and adrenals showed them to be normal for adult rats. Some tissues showed abnormalities, but it should be emphasized that such deviations from normal occur with variable frequency in animals of the age represented by those of this group. Some local degeneration of acinar tissue of the pancreas occurred in two animals (512 and 520). Advanced degeneration of the testes was found in one rat (520) and partial degeneration in another (524). Two animals (538 and 539) showed hydronephrosis of the right kidney. Moderate hyperplasia of lymphoid tissue as commonly occurs in older animals was generally present. Peribronchial lymphocytic infiltration, combined with some vascular congestion, was common in most animals of this series. In general, the pathological findings indicated no marked differences in the tissues and organs of animals maintained on the two diets.

Discussion

Results of these nutritional studies on albino rats show that the feeding of margarine oil fortified to 100 times the usual amount of vitamin A with synthetic vitamin A palmitate, compared with regular margarine oil, results in comparable response for growth, reproduction, and lactation. The two groups of rats in these experiments consumed, respectively, about 30 and 3,000 units of vitamin A daily (assuming 10 gms. of diet eaten per day). On the basis of body weight the animals given the highly fortified margarine at the start of the experiment received about 30,000 units/kg. (50 gms. body weight; food intake about 5 gms.), which gradually decreased to about 11,000 units/kg. (400 gms. body weight; food consumption about 15 gms.) at the end of the study. At these levels of synthetic vitamin A no deleterious effects were observed. This is in accord with previous observations on hypervitaminosis A in the rat obtained with both natural and synthetic vitamin A concentrates (1, 4).

Summary

The growth, reproduction, and lactation of three generations of albino rats fed diets containing 7.5% of margarine oil fortified with synthetic vitamin A palmitate to contain 1,850,000 units of vitamin A per pound of fat (1,500,000 units per pound of margarine) was investigated. This level of supplementation gave normal responses comparable to those shown by animals fed regular margarine oil (18,500 units of natural vitamin A per pound of fat or 15,000 units per pound of margarine).

REFERENCES

1. Ames, S. R., and Harris, P. L., unpublished data.
2. Cawley, J., Robeson, C., Weisler, L., Shantz, E., Embree, N., and Baxter, J., Science, 107, 346 (1948).
3. Pharmacopeia of the United States of America, 14th Revision, p. 784 (1950).
4. Rodahl, K., J. Nutrition, *41*, 399 (1950).
5. Standard of Identity for Oleomargarine, Federal Register, *6*, 2761 (June 5, 1941).
6. U. S. Food and Drug Administration, Minimum Daily Requirements of Specific Nutrients, Federal Register, Sec. 403(j) (Nov. 22, 1941).

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Effect of Over-Cooking of Cottonseed Meats on Quality of Meal

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THE cooking of cottonseed meats has received much attention during the past years in an attempt to decrease oil mill maintenance costs, increase mill capacity, and improve oil quality. In pursuing these goals, a cooking process was defined in July, 1950 (1) wherein the necessity of a minimum of 12% moisture during the cooking stage was emphasized. This cooking process consists of maintaining the meats at 185 to 200°F. at the minimum 12% moisture content for a period of time of 15 to 17 minutes. The cooked meats are then dried for expression of oil. Earlier reports (1, 2) showed that this cooking process made it possible to produce a low refining loss oil from cottonseed when the seed were milled by the Exsolex process.