change in stearic acid. Palmitic acid rather than stearic acid is associated with the increase in linoleic acid. This might have some implications in connection with fatty acid biosynthesis in cottonseed.

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Nutritional Evaluation of Inter-Esterified Fats

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Abstract

Studies with inter-esterified fats prepared to maintain a high level of linoleic acid content have been undertaken in several series of experiments with rats. These fats are as digestible as the liquid nonhydrogenated oils and the biological value of the linoleic acid is not impaired by the inter-esterification. Investigations involving growth, reproduction and lactation, longevity, tissue cholesterol levels and histological tissue examination have revealed that these inter-esterified fats are utilized by the animal similarly to cottonseed oil. No tissue pathology or interferences with any of the nutritional indices are observed. When the inter-esterified fats are included in atherogenic diets, the atherosclerotic lesions which develop in the coronary arteries and aorta of the animals are similar to, but less marked than, those found when animals are fed cholesterol-containing diets with butter oil or conventional margarine oil of the all-hydrogenated type. It is concluded that these inter-esterified fats are at least nutritionally equal to other similar edible fats of equivalent essential fatty acid content.

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Introduction

IN RECENT YEARS production of solid fats with high linoleic acid content has been achieved through an inter-esterification process. Nonhydrogenated vegetable seed oils are mixed with small amounts of highly hydrogenated fats and the blend is then inter-esterified to rearrange the fatty acid radicals; this imparts the firmness characteristic of margarine fats to the blend (1). Since the nutritional value of inter-esterified fats had not been investigated to the same extent as had been done for hydrogenated fats, an evaluation of inter-esterified fats in rats was undertaken and has now been completed. These investigations include determination of the digestibility and the essential fatty acid activity of these fats. The more extended studies include measurements of growth, reproduction, longevity and tissue cholesterol levels as criteria for nutritional value and safety. Similar long-term studies have been performed with animals fed (a) an unhydrogenated cottonseed oil, (b) a mixture of the inter-esterified fat with hydrogenated coconut oil, and, in some experiments, (c) butter oil and (d) a conventional margarine oil.

Experimental Procedures

The description of the fats fed is shown in Table I. The fatty acid composition was determined by spectrophotometric (2) and the trans isomers by in-

TABLE I

Description of Fats

Fat	Iodine No. (Wijs)	Fatty acid composition (%)					Digestibility[d]
		Saturated[a]	Oleic[a]	Trans[b]	Linoleic		
					Spec.[a]	Bioassay[c]	
MBO 1[e]	89.6	39.2	13.6	1.8	42.8	44.0	not tested
MBO 4[f]	89.6	39.2	13.6	1.8	42.8	41.5	94.3
MBO 45[g]	76.3	47.3	11.7	1.5	36.4	34.5	95.6
Cottonseed oil	112.0	23.6	20.1	....	51.9	.....	.....
Coconut oil (hydrogenated)	1.0	94.5	1.0		0.0	.....	.....
Butter oil	41.0	55.0	37.2	7 (approx)	3.5	.....	.....
Margarine oil (all hydrogenated)	80.0	19.4	69.5	35.0	9.7	6.9	92.9

[a] By spectrophotometric assay (2); as % of triglycerides.
[b] According to infrared absorption (3).
[c] Bioassay, based upon weight gain of male rats following supplementation of the basic fat-free diet (5).
[d] Based upon the amount of dietary fat absorbed from the digestive tract; correction is made for the metabolic fat found in the total fecal fat.
[e] Inter-esterified blend of 82.5% unhydrogenated and 17.5% completely hydrogenated cottonseed oil (1).
[f] Same as MBO 1 but containing added antioxidants, 0.02% of butylated hydroxytoluene (in solution) and 0.002% of ethylenediaminetetracetic acid (in suspension).
[g] Composed of 85% MBO 4 + 15% saturated coconut oil.

TABLE II

Average Gain in Weight and Survival of Rats Fed Inter-esterified Fats

Diet		Gain in weight (g) [c]			Survival of generation I (in weeks)		
		Generation I	Generation II	Generation III	75%	50%	25%
MBO 1	M[a]	305 ± 5.2	287 ± 8.4	263 ± 5.5	59	96	108
	F[b]	188 ± 3.8	175 ± 3.5	171 ± 2.9	54	97	116
MBO 4	M[a]	286 ± 7.1	264 ± 8.1	254 ± 7.9	79	97	115
	F[b]	165 ± 8.8	160 ± 3.9	154 ± 3.2	79	104	119
MBO 45	M[a]	303 ± 6.8	275 ± 8.7	243 ± 5.7	77	107	115
	F[b]	185 ± 3.3	162 ± 3.1	159 ± 3.1	53	97	123
Cottonseed oil	M[a]	274 ± 8.7	282 ± 8.8	315 ± 7.0	57	91	112
	F[b]	176 ± 4.9	170 ± 2.2	163 ± 4.2	77	107	117
Laboratory chow[d]	M[a]	306 ± 8.4	.....	.....	....	....	....
	F[b]	166 ± 7.2	.....	.....	....	....	....

[a] Males—100 days on diet, 12 animals per group.
[b] Females—70 days on diet, 20 animals per group.
[c] Values include standard error of mean.
[d] Rockland diet.

frared absorption methods (3). Also listed are the digestibilities of the inter-esterified fats as determined by a modification of the method of Augur et al. (4), and a comparison of the essential fatty acid activities of the fats as determined by bioassay (5) and spectrophotometric procedures (2). The digestibility is good for all the fat products tested. The agreement between bioassay and spectrophotometric values for linoleic acid in the inter-esterified fat (MBO 1), this fat containing antioxidants (MBO 4) and a blend of MBO 4 with coconut oil (MBO 45) indicates that no isomers of linoleic acid were formed as a result of the inter-esterification reaction.

In a multigeneration-longevity experiment, groups consisting of 20 weanling female and 12 weanling male rats of our former USC strain were fed the three fats at a level of 15% of the diet for 90 days. Other constituents of the diet were casein (24%); sucrose (52.53%), celluflour (4%), salt mixture (4%), choline, and water-soluble and fat-soluble vitamins. Weight was recorded weekly. At 90 days, females were bred to provide a second generation. Twenty females and 12 males of the second generation were also fed for 90 days and then bred to produce the third generation; the growth of the third generation animals was also followed for 90 days. In a longevity experiment, groups of male and female animals were fed the various diets until 75% of the animals had died. Other groups of male and female rats on the various diets were sacrificed after 24 and 96 weeks. Cholesterol determinations were performed on plasma and liver extracts (6) using a modified Sperry-Schoenheimer method (7); liver total lipids were determined gravimetrically.

TABLE III

Summary of Breeding Data

Dietary fat	MBO 1		MBO 4		MBO 45		CSO	
Generations	I	II	I	II	I	II	I	II
No. females bred	20	19	20	20	19	20	19	20
% Successful pregnancies	75	84	70	85	70	90	74	70
No. born	149	137	132	150	124	164	157	113
No. at 3 days[a]	142	120	122	112	111	133	153	111
Wt. at 21 days (Avg. per rat)	35.4	35.4	35.7	34.0	33.9	41.6	35.0	39.6
Mortality %								
0–3 days	4.6	12.4	7.5	25.3	10.4	18.9	2.5	1.7
3–21 days	0	1.2	1.1	7.6	0	1.9	1.0	0

[a] Litters of more than 7 animals cut to 7 after three days.

The effect of dietary cholesterol was evaluated by another series of experiments in which fats were fed to adult male rats (ranging from 250–300 g in weight) without and with cholesterol (and bile acids to facilitate absorption) for periods of 1 and 4 weeks. The diet used (casein, 10%; salt mixture, 5%; celluflour, 5%; fat, 20%; sucrose, 59.45%; and water-soluble and fat-soluble vitamins to 100%; when cholesterol was added, 3% cholesterol and 1% cholic acid replaced 4% of sucrose) is similar to the diet used by Hegsted et al. (8,9). At the end of the experimental periods the animals were killed, and cholesterol and lipid determinations were performed on plasma and liver extracts as described above.

Results and Discussion

The average gain in weight achieved on the experimental diets is shown in Table II. No striking differences in weight gain are noticeable in either the first, second, or third generations of rats fed the inter-esterified fats when compared with animals fed either cottonseed oil or laboratory chow diet indicating that all diets are similar in growth-promoting qualities. The results of the longevity study are also shown in Table II. Survival was good in all three groups and compared well with the results obtained when cottonseed oil was the sole source of fat in the diet.

Summary of the breeding performance is shown in Table III. In general, there were no significant differences in reproduction or lactation among the various groups except possibly for the higher mortality of the very young in the second breeding of the MBO groups.

Total cholesterol levels of plasma and liver, and liver lipid levels are shown in Table IV. After 96 weeks, the animals fed the low-fat laboratory ration had somewhat lower values than those obtained with rats fed the inter-esterified fats and cottonseed oil; the latter two types of products provided values

TABLE IV

Plasma and Liver Cholesterol and Liver Lipid Values of Male and Female Rats Fed Inter-esterified and Other Fats for 24 and 96 Weeks from Weaning

		Plasma cholesterol (mg/%)		Liver cholesterol (mg/g)		Liver total lipids (mg/g)	
		24 weeks	96 weeks	24 weeks	96 weeks	24 weeks	96 weeks
MBO 1	M	85.7 ± 8.0[a]	99.4 ± 3.8	2.25 ± 0.04	2.30 ± 0.09	34.4 ± 0.9	47.2 ± 3.4
	F	76.5 ± 5.4	85.1 ± 7.1	2.13 ± 0.05	2.12 ± 0.09	34.9 ± 1.4	45.2 ± 2.6
MBO 4	M	62.5 ± 5.2	102.4 ± 8.6	2.79 ± 0.16	2.28 ± 0.02	40.7 ± 2.9	43.2 ± 0.7
	F	77.1 ± 4.4	84.4 ± 4.8	2.32 ± 0.08	2.13 ± 0.04	32.6 ± 1.3	46.8 ± 2.8
MBO 45	M	62.5 ± 3.3	92.3 ± 7.2	2.67 ± 0.13	2.37 ± 0.13	41.5 ± 2.3	49.2 ± 3.6
	F	66.9 ± 5.9	99.8 ± 17.0	2.12 ± 0.04	2.30 ± 0.07	32.3 ± 1.5	48.2 ± 2.0
Laboratory chow	M	62.6 ± 4.9	65.7 ± 9.8	2.26 ± 0.07	1.97 ± 0.06	36.2 ± 2.4	40.7 ± 1.6
	F	53.9 ± 2.3	75.9 ± 2.0	2.34 ± 0.04	1.70 ± 0.04	35.0 ± 1.1	42.9 ± 1.7
Cottonseed oil	M	.....	107.5 ± 6.6	.....	2.56 ± 0.12	.....	48.8 ± 6.4
	F	.....	89.9 ± 5.6	.....	2.43 ± 0.06	.....	44.5 ± 1.6

6–10 rats per group.
[a] Values include standard error of the mean.

TABLE V

Plasma and Liver Cholesterol and Liver Lipid Values of Adult Male Rats Fed Interesterified and Other Fats, with and without Cholesterol, for 1 and 4 Weeks

		Plasma cholesterol (mg%)		Liver cholesterol (mg/g)		Liver lipids (mg/g)	
		1 week	4 weeks	1 week	4 weeks	1 week	4 weeks
MBO 1	—Cholesterol	61.9 ± 5.2[a]	56.8 ± 2.2	1.82 ± 0.10	2.33 ± 0.13	42.3 ± 2.3	48.3 ± 2.7
	+Cholesterol	87.7 ± 7.5	90.9 ± 9.1	18.5 ± 0.8	38.6 ± 2.0	98.8 ± 3.5	163 ± 11
MBO 4	—Cholesterol	64.0 ± 2.7	70.7 ± 3.6	2.20 ± 0.08	2.51 ± 0.15	40.3 ± 1.5	40.9 ± 2.2
	+Cholesterol	101 ± 10	100 ± 6	16.1 ± 1.3	32.3 ± 1.2	77.4 ± 4.7	157 ± 12
MBO 45	—Cholesterol	57.3 ± 2.2	66.7 ± 2.7	2.10 ± 0.11	2.14 ± 0.09	39.3 ± 2.4	48.3 ± 3.2
	+Cholesterol	87.2 ± 6.3	104 ± 6	16.1 ± 1.1	29.4 ± 1.5	81.7 ± 3.8	135 ± 6
Butter oil	—Cholesterol	67.5 ± 3.3	68.1 ± 2.6	1.86 ± 0.07	2.30 ± 0.08	43.4 ± 1.7	51.7 ± 1.8
	+Cholesterol	124 ± 12	168 ± 20	13.3 ± 0.9	22.3 ± 1.6	69.9 ± 2.9	112 ± 8
Margarine oil (conventional)	—Cholesterol	69.3 ± 2.0	64.7 ± 2.4	1.87 ± 0.06	2.08 ± 0.06	40.9 ± 1.8	41.7 ± 1.9
	+Cholesterol	130 ± 13	210 ± 6	11.5 ± 0.8	22.0 ± 1.7	62.7 ± 3.3	92.0 ± 4.8

10 rats per group.
[a] Values include standard error of the mean.

which were similar in all cases. There is a tendency for plasma cholesterol and liver total lipid levels to increase and liver cholesterol levels to decrease with time.

Histological examination of liver, kidney, spleen, thyroid, lung and heart of animals on longevity studies and killed after 96 weeks on the respective diets did not reveal any abnormalities in these tissues. Aortas and coronary vessels in both male and female animals were free of lipid infiltration.

The results of the short term experiments where male rats were fed the various fats with and without cholesterol supplements are presented in Table V. There are no significant differences in plasma cholesterol levels among the groups of animals fed the cholesterol-free diets in this short-term experimental period. The administration of cholesterol results in an elevation in plasma cholesterol levels which, in general, becomes more pronounced with time. Plasma cholesterol levels in the butter oil + cholesterol and margarine oil + cholesterol groups are considerably higher than the groups fed the inter-esterified fats + cholesterol.

This is in agreement with the results reported by Hegsted et al. (9) in which it was concluded that fats containing large quantities of nonessential, unsaturated fatty acids (e.g., oleic acid) promoted marked hypercholesterolemia in cholesterol-fed rats, whereas fats containing essential fatty acids and saturated fatty acids yielded much lower serum cholesterol levels and also counteracted the high serum cholesterol levels developed by feeding oleic acid-containing fats. In the present study the hypercholesterolemic effect of the various fats roughly parallels their oleic acid content. Thus butter oil and margarine oil, which yield the highest serum cholesterol levels, contain 37.2% and 69.5% oleic acid, respectively. MBO 1, 4 and 45 which are markedly less hypercholesterolemic in cholesterol-fed rats have oleic acid contents of 13.6, 13.6 and 11.7%, respectively.

In the absence of dietary cholesterol, no significant changes in liver cholesterol levels due to the differences in dietary fat are observed. When cholesterol is included in the diet, the inter-esterified fat-fed groups have a higher liver cholesterol content than do the butter oil-fed and margarine oil-fed groups, which is in direct contrast to the results observed in plasma. In all groups of animals fed cholesterol the cholesterol content of liver increases with time.

This inverse relationship between liver and plasma cholesterol has also been observed in essential fatty acid deficient rats (10), where a low plasma cholesterol value is associated with elevated liver cholesterol concentrations and increased adrenal cholesterol levels as well. Elevated plasma cholesterol levels could conceivably result from transfer of cholesterol from various organs via the plasma to depot areas, or movement through plasma out of depot areas for subsequent metabolism. Elevated liver cholesterol levels may be an attempt of the organism to lower plasma cholesterol levels or may be preliminary to accelerated bile acid formation.

Liver lipid levels in the rats fed diets from which cholesterol is excluded parallel liver cholesterol levels during the entire experimental period. Liver lipid levels increase when cholesterol is included in the diet and the accumulation of lipid becomes more pronounced with time. Liver lipid levels are qualitatively similar to liver cholesterol levels; the highest lipid levels occur in the animals fed the inter-esterified fat with cholesterol; the butter oil + cholesterol fed animals are intermediate, and the margarine oil + cholesterol-fed animals have the lowest lipid accumulation.

Histological examination of frozen sections of hearts and aortas, stained with oil red O, revealed atherosclerotic changes in coronary arteries and early involvement of the aorta (thickened intima) in the animals on all the diets which included cholesterol. The male rats fed the cholesterol + butter oil and cholesterol + margarine oil had more marked coronary involvement than did the animals fed cholesterol with either MBO 1, MBO 4, or MBO 45. The butter oil and margarine oil used in these studies have the lowest concentration of polyunsaturated fatty acids (butter oil, 3.5% linoleic acid and margarine oil, 9.7% linoleic acid). Also, the plasma cholesterol levels observed in the animals on butter oil plus cholesterol and margarine oil plus cholesterol diets (Table V) are considerably higher than those of the animals fed the inter-esterified fats plus cholesterol. In the work reported by Hegsted et al. (8), the animals with the most elevated serum cholesterol levels also had the most marked endocardial sudanophilia and the highest incidence of early aortic atherosclerotic lesions.

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