Dietary Fat Composition and Tocopherol Requirement: II. Nutritional Status of Heated and Unheated Vegetable Oils of Different Ratios of Unsaturated Fatty Acids and Vitamin E

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Abstract

In studies conducted on male and female rats and involving evaluation of growth, reproductive and lactation performances and of lipid peroxidation, no evidence could be found for the need for added vitamin E (α-tocopherol) over and above that naturally present as tocopherols in the vegetable oils investigated. These oils are in common usage in industry, i.e., liquid nonhydrogenated cottonseed oil, a lightly hydrogenated cottonseed oil and a hydrogenated soybean oil shortening. The ratio of polyunsaturates to total tocopherol in the test oils varied from 640:1 to 9:1. Even those oils obtained from a commercial frying operation after a steady state had been attained contained sufficient vitamin E to meet dietary requirements. Results of in vitro peroxide hemolysis tests conducted on the red blood cells of the test animals did not correlate well with biological performance.

Introduction

An increased requirement for vitamin E (α-tocopherol) as the polyunsaturation of the fat in the diet increases has been proposed by several groups of investigators (1–3). It is well established that polyunsaturates are far more readily oxidized in vitro than are monounsaturates. The possibility that this also prevails in vivo to give rise to metabolites which are handled with difficulty (4) has led some investigators to question the safety of increased quantities of polyunsaturated vegetable oils in the diet. Despite such criticisms, in recent years, considerable emphasis has been directed toward increasing the polyunsaturated vegetable oils at the expense of the more saturated fats in the diet, particularly those of animal origin (5–7). These polyunsaturated vegetable oils are rich in natural tocopherol and processing of the vegetable oils for human consumption spares most of these natural antioxidants (8). The question which has not been adequately answered is whether there is a need for more tocopherols, and particularly for more vitamin E, over and above that native to the vegetable oils and fats in the products as consumed today. An extension of this question is whether the possible deficiency in tocopherols is further aggravated as a result of exposing the oils and fats to heat abuse.

There have been many reports in the literature that polyunsaturated vegetable oils subjected to heat abuse are toxic, either as a result of harmful products produced or as a result of the destruction of the natural tocopherols present or both. Much of the data in this area has been based on tests conducted with heat-abused oils which show little resemblance to the oils heated in commercial operations. For example, Johnson et al. (9) fed corn oil which had been heated under air at 200 C for 24 hr to weanling rats and observed a severe diarrhea, rough fur, a decrease in food intake and early mortality. More saturated fats such as coconut oil or olive oil when heated and fed to rats also produced a diarrhea but slight growth depression. Uksila and Kurkela (10) fed mixtures of fresh and heated fats (20 hr at 200 C in air) to rats and found impairment of growth as the concentration of the heated polyunsaturated safflower oil in the mixture was increased. Kaunitz et al. (11) observed that fresh oils, particularly the more unsaturated oils which are rich in tocopherols, added to diets containing oxidized oils could alleviate any ill effects attributed to the heated oils.

The possibility that some of the deleterious effects are due to an inadequate vitamin supply was proposed by Keane et al. (12) who reported that a more complete vitamin mixture than that used by Johnson et al. (9) when fed to rats given diets containing commercially used frying fats prevented growth depression. It has also been reported that some of the effects of oxidized fats can be counteracted by tocopherols. Stamler (13) was able to alleviate an eclampsia present in pregnant rats fed oxidized cod liver oil by large doses of tocopherol. Alfin-Slater et al. (14) fed fresh and heated soybean oil, cottonseed oil and lard at a level of 15% in the diet to rats for more than two years and studied growth, food consumption, food efficiency, digestibility, reproduction and lactation, and plasma and liver lipids. The only evidence of impaired nutrition was observed in animals fed a polymerized soybean oil where a decrease in iodine value of 10% was associated with a slight depression in growth and an interference with reproductive performance in the female animal. Normal reproduction was restored by supplementing the diets of the animals with α-tocopherol.

In studies of practical value, oils and fats used in actual commercial frying operations have been shown to be free from harmful products. Melnick et al. (15–17) provided physico-chemical data in support of this conclusion, while others [notably Keane et al. (12) and Poling et al. (18)] provided more definitive data based upon feeding studies of heated fats obtained from restaurants and commercial frying plants. More recently, Nolen et al. (19) on the basis of long term feeding studies conducted on spent frying oils and fats, so heat-abused that they could no longer be used in restaurant fryings, concluded that toxic products were present in such low concentrations as to have no practical significance. In these investigations, it is to be noted, the test rations provided adequate supplements of vitamin E so that the only variable under study was the possible presence of harmful products in the heat-abused oils and fats.

In the present investigation to examine the possible need for vitamin E over and above that native to the vegetable oils and fats, these oils were fed at a rather high level (30% by weight or approximately 50% of the total calories) in diets which provided no extra tocopherol in the form of a supplement. Other diets provided additional vitamin E in order to determine whether an improvement in biological response would follow. Sources of essential fatty acids were included when required. The test oils and fats were of vegetable origin and were those commonly used by industry in deep fat frying; these oils and fats varied widely in polyunsaturate content and hence the ratio of polyunsaturate to tocopherol content also varied widely. The fresh and heated oils and fats were evaluated in growth, reproductive and lactation studies. In addition, the possible accumulation of oxidative degradation products in the tissues of the test rats was also investigated, as well as other indices employed in assessing the need for vitamin E in the diet. Hence the present nutritional studies, conducted on vegetable oils of different degrees of unsaturation, before and after extended deep-fat fryings, were designed to provide data of practical value on the relationship of fat composition to vitamin E requirement.

Experimental Procedures

The test oils and fats of vegetable origin used in the present study differed widely in degree of unsaturation but were more or less equivalent in tocopherol content. The most unsaturated of the oils and fats investigated was cottonseed salad oil, a nonhydrogenated winterized product (hereafter referred to as liquid CS oil). The second product included in this study was a lightly hydrogenated cottonseed oil (hereafter referred to as chipper oil) which had a considerable increase in shelf life as a result of a substantial reduction in polyunsaturated fatty acid content (20). The third product in this study was soybean oil hydrogenated to practically eliminate all the polyunsaturates but to increase the stability to oxidative deterioration (hereafter referred to as SBO shortening).

The heated oils were taken from commercial frying fats, in which potato chips had been prepared. These oils had attained a steady state with regard to composition on continuous fryings, with added oil continuously compensating for that absorbed by the potato chips. The oils had been used over a continuous period of about 135 hr at 350 to 385 F (177 to 196 C).

TABLE I

Description of Experimental Groups

Group No.	Diet
1A (CSO)	30% liquid CS oil, fresh state, no added tocopherol.
1B (CSO Δ)	30% liquid CS oil, heated to steady state, no added tocopherol.
1C (CSO Δ + T)	30% liquid CS oil, heated to steady state, supplemented with tocopherol.
2A (chipper oil)	30% chipper oil, fresh state, no added tocopherol.
2B (chipper oil Δ)	30% chipper oil, heated to steady state, no added tocopherol.
2C (chipper oil Δ + T)	30% chipper oil, heated to steady state, supplemented with tocopherol.
3A (SBO shortening)	30% SBO shortening, fresh state, no added tocopherol.
3B (SBO shortening Δ)	30% SBO shortening, heated to steady state, no added tocopherol.
3C (SBO shortening Δ + T)	30% SBO shortening, heated to steady state, supplemented with tocopherol.
3D (SBO shortening Δ + CS oil + T)	28% SBO shortening, heated to steady state + 2% liquid CS oil, supplemented with tocopherol.

TABLE II

Physical Constants of Fresh and Heated Oils

Oil sample	Iodine No. (Wijs)	FFA, %	TBA Test 1% E 1cm 530–535 mμ
Liquid CS oil	115.2	0.05	0.0007
Liquid CS oil, heated	113.9	0.39	0.0006
Chipper oil	84.8	0.04	0.0001
Chipper oil, heated	82.8	0.48	0.0007
SBO shortening	70.0	0.03	0.0003
SBO shortening, heated	70.1	0.09	0.0008

The fresh and heated oils are further described in Tables I, II and III. The thiobarbituric acid (TBA) test (21) provides a measurement of malondialdehyde, believed to be formed during the oxidation of unsaturated fatty acids. The fatty acid composition of the oils was determined by both spectrophotometric (22) and gas liquid chromatographic (23) methods. Tocopherol analyses were conducted by both colorimetric (24) and gas liquid chromatographic (25) methods.

The findings here are in agreement with those previously reported (15–17). The test oils are shown to vary widely in polyunsaturate (linoleate) content and in ratio of polyunsaturate to tocopherol content. According to the results of the physico-chemical tests, little destruction of polyunsaturates (linoleate) and of the tocopherols occurs in these oils.

The fresh and heated oils were fed to male and female rats at a level of 30% of the diet. The composition of the basal diet has previously been reported (26). The heated oils were given alone or supplemented with vitamin E, as *dl-α* tocopherol acetate, at 0.05% of the oil. The least unsaturated of the heated oils, SBO shortening, was also supplemented with 2% liquid CS oil to supply polyunsaturated fatty acids as well as tocopherol. The groups are described in Table I.

Two series of experiments were conducted. Growth and reproduction were measured in both experiments. In the second experiment, a multigeneration study where parents subsisted on the respective test diets from weaning, through gestation and lactation, and thereafter the weaned animals were continued on the test diets, was also included. In addition, in the second series of experiments, studies on tissue peroxidation, erythrocyte hemolysis and fatty acid determinations of erythrocyte lipids were done on selected groups.

Results and Discussion

Growth performance of the rats is shown in Table IV. Among the males or females, growth is the same regardless of whether the fresh or heated oils are fed. Tocopherol (vitamin E) supplementation of even the heated oils results in no improvement in growth response. The lower weight gain observed with the animals of both sexes in Group 3 is due to the decreased content of essential fatty acids in the hydrogenated SBO shortening. The addition of a source of linoleic acid (liquid CS oil) to the diets of the animals on the SBO shortening regimen results in an increased weight gain comparable to that observed in Groups 1 and 2. Growth in selected groups of a second generation is similar to that seen in the first generation except for the females fed the liquid CS oil diet or the chipper oil diet where the

TABLE III

Fatty Acid Composition and Tocopherol Content of Fresh and Heated Oils

Oil	Fatty acid composition,[a] per cent of total FA					Tocopherol content,[c] %					Ratio of polyunsaturated fatty acids to tocopherols	
	Procedure	Linolenic	Linoleic	Oleic	Saturated	Procedure	α	γ	δ	Total Tt	P/Tα	P/Tt
Liquid CS oil	Spec.	0.00	56.3	20.9	22.8	Color.	.....	.....	.....	0.10	.....	563:1
	GLC	0.0	56.2	19.3	24.5	GLC	0.054	0.042	0.010	0.106	1041:1	530:1
Liquid CS oil, heated	Spec.	0.00	53.7	22.4	23.9	Color.	.....	.....	.....	0.08	.....	671:1
	GLC	0.0	53.4	20.8	25.8	GLC	0.030	0.036	0.010	0.076	1780:1	703:1
Lightly hydrogenated CS oil	Spec.	0.00	24.2[b]	48.2	27.6	Color.	.....	.....	.....	0.09	.....	269:1
(Chipper oil)	GLC	0.0	25.1	43.9	28.0	GLC	0.055	0.041	0.009	0.105	456:1	239:1
Chipper oil, heated	Spec.	0.00	23.5[b]	49.4	27.1	Color.	.....	.....	.....	0.08	.....	290:1
	GLC	0.0	24.6	44.2	28.3	GLC	0.034	0.028	0.009	0.071	734:1	346:1
SBO shortening	Spec.	0.00	1.2	75.7	23.1	Color.	.....	.....	.....	0.13	.....	9:1
	GLC	0.5	2.0	74.3	23.2	GLC	0.012	0.083	0.034	0.129	208:1	19:1
SBO shortening, heated	Spec.	0.00	1.1	75.9	23.0	Color.	.....	.....	.....	0.12	.....	9:1
	GLC	0.4	2.0	74.3	23.3	GLC	0.008	0.068	0.034	0.110	300:1	22:1

[a] Spec., spectrophotometric method (22); GLC, gas liquid partition chromatography (23), wherein the methyl esters were prepared by refluxing the oil with sodium methoxide and methanol. Wherever GLC values do not add up to 100%, small quantities of unidentified components are present.
[b] Of which about 2% are conjugated dienes.
[c] Color, colorimetric method (24), GLC, gas liquid chromatography of the tocopherol acetates by a procedure related to that reported (25), using 5 ml of pyridine acetic anhydride (2:1 v/v) to react overnight with the separated unsaponifiables from 4 g of oil. A hydrogen flame detector is used for measuring the effluent from 4 ft × 3/16 in., 2% SE-30 on 80/100 Diatoport S column operated at 230 C and a helium flow of 70 cc/min.

gain in weight in the second generation seems to be somewhat lower than that observed in the first generation (155 g versus 169 g for the liquid CS oil group and 154 g versus 176 g in the chipper oil groups). No reasons other than lack of precise reproductivity of biological response can explain these differences; they cannot be attributed to a low tocopherol to polyunsaturate ratio. The females on the respective heated oils diets fail to show these differences between the first and second generations. If anything, tocopherol lack should have been more apparent in the case of the tests conducted with the heated oils. Furthermore, there is no gradient in response as the ratio of polyunsaturates to tocopherols increases; the animals (males as well as females) do not exhibit a lower gain in weight on the liquid CS oil diet as compared to those on the chipper oil diet.

Reproduction and lactation data are shown in Table V. The animals were fed from weaning the respective diets for 10 weeks, at which time they were bred; they subsisted on the same diets during lactation. The increased mortality during lactation in Group 3 is probably a reflection of the low essential fatty acid composition of SBO shortening since the addition of the liquid CS oil to the animals on the SBO shortening diet reduced the mortality of the young from 14% to 0% in Experiment 1, and from 100% to 28% in Experiment 2. Obviously, however, lack of vitamin E was not responsible for the poorer reproductive performance seen in Experiment 2 since in both Groups 2 and 3, with both the fresh and heated oils, the females had a similar number of successful pregnancies. The addition of vitamin E did not improve reproduction and lactation in any of the groups.

Vitamin E deficiency has been associated with tubule degeneration and the presence of immature spermatogonial cells in the testes (27). Histological studies conducted on the male gonadal tissues of animals after 18 weeks on diet revealed a normal picture in all groups. The tubules of the testes contained active maturing spermatogonial cells undergoing spermatogenesis, with sperm in the lumen. In the epididymis, the lumina of the ducts were filled with mature sperm. These results on reproduction and histological findings indicate that the native tocopherols in the vegetable oils, fresh and heated, are present in adequate amount to supply the vitamin E requirements of both male and female animals despite variable polyunsaturate content.

Several tissues, i.e., liver, brain, depot fat, lung and heart, from animals in each group after 18 weeks on the test diets were examined for evidence of tissue lipid peroxidation using the thiobarbituric acid test (28). Accumulation of the products of lipid peroxidation is regarded by some investigators to be an indication of vitamin E deficiency (29–31). Results are shown in Table VI. In all cases, values were quite low indicating a low order of tissue peroxidation even in the case of the heated oil-fed

TABLE IV

Gain in Weight of Rats Fed Fresh and Heated Oils Over a Ten Week Period

Group No.	Experiment 1		Experiment 2	
	Males g	Females g	Males g	Females g
1A (CSO)	259[a] ± 8(10)[b]	177 ± 5(15)	F1[c] 245 ± 6(15) F2[d] 243 ± 7(6)	F1 169 ± 3(20) F2 155 ± 4(12)
1B (CSOΔ)	264 ± 8(8)	176 ± 5(15)	F1 232 ± 8(15) F2 228 ± 15(6)	F1 160 ± 3(20) F2 165 ± 5(12)
1C (CSO Δ + T)	259 ± 6(10)	182 ± 4(15)	218 ± 11(15)	164 ± 5(20)
2A (Chipper oil)	253 ± 8(10)	171 ± 6(15)	F1 238 ± 6(15) F2 221 ± 10(7)	F1 176 ± 3(20) F2 154 ± 6(12)
2B (Chipper oil Δ)	261 ± 8(10)	166 ± 5(15)	F1 253 ± 8(15) F2 226 ± 9(7)	F1 172 ± 4(20) F2 161 ± 3(11)
2C (Chipper oil Δ + T)	262 ± 8(10)	167 ± 6(13)	228 ± 6(16)	168 ± 3(20)
3A (SBO shortening)	216 ± 6(10)	143 ± 4(15)	179 ± 6(15)	131 ± 8(20)
3B (SBO shortening Δ)	209 ± 7(10)	146 ± 4(15)	182 ± 6(15)	137 ± 4(20)
3C (SBO shortening Δ + T)	221 ± 6(10)	145 ± 4(15)	183 ± 8(14)	139 ± 3(20)
3D (SBO shortening Δ + CSO + T)	230 ± 7(10)	181 ± 6(14)	223 ± 8(15)	162 ± 2(20)

[a] Includes standard error of the mean.
[b] Numbers in parentheses indicate number of animals per group.
[c] F1 indicates first generation.
[d] F2 indicates second generation.

TABLE V

Reproduction and Lactation in Rats Fed Fresh and Heated Oils

Group No.	Identity	Experiment 1 Successful pregnancies %	Experiment 1 Mortality of young during lactation 3–21 days) %	Experiment 2 Successful pregnancies %	Experiment 2 Mortality of young during lactation 3–21 days) %
1A	(CSO)	100(15/15)	1	95(19/20)	18
1B	(CSOΔ)	73(11/15)	18	85(17/20)	17
1C	(CSO Δ + T)	80(12/15)	6	85(17/20)	15
2A	(Chipper oil)	93(14/15)	12	55(11/20)	12
2B	(Chipper oil Δ)	100(15/15)	6	55(11/20)	20
2C	(Chipper oil Δ + T)	92(12/13)	19	80(16/20)	17
3A	(SBO shortening)	87(13/15)	35	50(10/20)	38
3B	(SBO shortening Δ)	80(12/15)	26	63(12/19)	100
3C	(SBO shortening Δ + T)	93(14/15)	14	35(7/20)	100
3D	(SBO shortening Δ + CSO + T)	79(11/14)	0	90(18/20)	28

animals. Apparently, sufficient antioxidant protection from the native tocopherols in the test oils was afforded the tissues of animals in all dietary groups.

The effect of feeding the liquid CSO on erythrocyte hemolysis [method of Rose and György (32) as modified by Horwitt et al. (2)], a test which has been used as an assay method for vitamin E status (33), and on plasma tocopherols levels [method of Quaife et al. (34)] are shown in Table VI. In general, in the animals fed the heated liquid CS oil for a period of 18 weeks, there is an increased susceptibility of their erythrocytes to hemolysis in vitro with hydrogen peroxide. This is usually, but not always, accompanied by a degree in plasma tocopherols levels. The hemolysis observed in the in vitro tests conducted on blood from the females fed the unheated oil is considerably less than that observed in the case of the animals fed the heated product, even though the former group does not exhibit higher plasma tocopherol levels.

TABLE VI

Tissue Peroxide Levels as Measured by a Thiobarbituric Acid Test

Group No.[a]	Sex	Liver	Brain	Depot fat	Lung	Heart
1A (CSO)	M	.039	.037	.003	.021	.022
	F	.078	.020	.002	.050	.030
1B (CSO Δ)	M	.025	.048	.023	.012	.026
	F	.055	.020	.005	.041	.020
1C (CSO Δ + T)	M	.051	.045	.029	.050	.036
	F	.062	.025	.014	.039	.019
2A (Chipper oil)	M	.034	.054	.010	.033	.020
	F	.053	.019	.030	.042	.053
2B (Chipper oil Δ)	M	.022	.030	.015	.013	.020
	F	.062	.030	.009	.038	.036
2C (Chipper oil Δ + T)	M	.017	.036	.010	.022	.033
	F	.053	.022	.009	.034	.039
3A (SBO shortening)	M	.029	.027	.008	.021	.019
	F	.064	.026	.014	.033	.020
3B (SBO shortening Δ)	M	.047	.025	.025	.051	.030
	F	.036	.020	.018	.034	.024
3C (SBO shortening Δ + T)	M	.039	.008	.012	.026	.022
	F	.057	.021	.008	.029	.030
3D (SBO shortening Δ + CSO + T)	M	.026	.027	.035	.060	.036
	F	.075	.058	.018	.052	.040

[a] Groups 1, 2 and 3A (males) show an average value obtained on three animals per group; all other represent averages obtained on five animals per group. Tissues were obtained after the animals had been on the test diets for 18 weeks.
[b] Values are expressed as optical density at 540 mμ produced by extracting 1 g of tissue.

TABLE VII

The Relationship Between Erythrocyte Fragility and Plasma Tocopherol Levels in Rats Fed Fresh and Heated Cottonseed Oil

Group No.	Erythrocyte fragility % Males F_1[a]	Males F_3[b]	Females F_1	Females F_3	Tocopherol in plasma mg/100g Males F_1	Females F_1
1A (CSO)	3(3)[c]	35(6)	12(8)	2(6)	0.47[d]	0.24
1B (CSO Δ)	73(3)	99(6)	100(8)	100(10)	0.14	0.28
1C (CSO Δ + T)	7(7)	4(6)	2(7)	4(6)	0.61	0.76
2A (Chipper oil)	4(9)	13(6)	1(12)	12(7)	.....	.....
2B (Chipper oil Δ)	3(10)	5(5)	1(12)	10(7)	.....	.....
2C (Chipper oil Δ + T)	1(8)	4(6)	2(12)	4(8)	.....	.....

[a] F_1 indicates first generation.
[b] F_3 indicates third generation.
[c] Numbers in parentheses are numbers of animals tested, after having been 18 weeks on the test diets.
[d] Pooled samples of six animals.

The significance of this in vitro peroxidation test as an indication of vitamin E status in these animals is questionable. Leonard and Losowsky (35) have reported that hemolysis occurs when the plasma tocopherol level is below 0.5 mg/100 g and only reliably so, however, when the level falls below 0.3%. Apparently the level of 0.47% seen in the male animals receiving the liquid CS oil diet was sufficient to protect the red blood cells of these animals against the hemolysis in vitro. However, the fact that a low hemolysis value was observed in tests conducted on the red blood cells from the females with a plasma tocopherol level of 0.24 mg/100 g cannot be explained except by the supposition that other factors in the plasma besides tocopherol concentration may affect the results.

Vitamin E deficiency has been reported to affect the composition of polyunsaturated fatty acids in various rat tissues, usually in the direction of decreased concentrations of polyunsaturated fatty acids (36,37). Bunyan et al. (38), however, were unable to show decreases in the concentration of the polyunsaturated fatty acids in rat liver, kidney, heart, spleen, brain, adrenal and adipose tissue during vitamin E deficient experimental periods lasting 13 months. And Bieri and Andrews (39) found increased arachidonate but decreased docosapentaenoate in testes of vitamin E deficient rats. In our experiments, determinations of the fatty acid composition of lipids in the washed red blood cells from animals fed heated and unheated cottonseed oil for a period of 18 weeks were performed using gas chromatography (Table VIII). The lipids were extracted from red blood cells with chloroform-methanol mixture (2:1); methyl esters of fatty acids were prepared by refluxing with a methanol-sulfuric acid-benzene mixture, and after extraction and purification were applied to the gas liquid chromatograph (Barber-Coleman Model 20, 15% diethylene glycol succinate polyester on 80–100 mesh Gaschrom P, with a column temperature of 180 C and an argon gas pressure of 26 lb.). Chromatographic peaks were identified by

TABLE VIII

Major Fatty Acid Composition of Washed Red Cells of Male Rats[a]

Diet	Fatty acid, % 16:0	16:1	18:0	18:1	18:2	20:4
CSO	16.6	9.9	17.9	10.8	17.3	30.3
CSO Δ	27.6	11.3	16.9	12.2	11.6	12.2
CSO Δ + T	23.4	10.5	18.6	10.8	10.4	16.4

[a] Pooled samples of erythrocytes of five animals per group, after 18 weeks on the test diets.

comparison of retention times with those of standards and graphic representation of retention times. In the erythrocyte lipids of the males fed the heated cottonseed oil there was a decrease in both linoleic and arachidonic acid content compensated primarily by an increase in palmitic acid. The addition of vitamin E to the diet of these animals yielded results very similar to those observed in the absence of added vitamin E, which would indicate that there does not appear to be a vitamin E deficiency in the diet containing the heated oils or in the animals ingesting these diets.

On the basis of the results obtained in the present study, we have concluded that the results of the in vitro hemolysis test are invalid as the sole indicator of vitamin E nutriture. The test is an indication of the susceptibility of oxidation in vitro of a lipid substrate in a given environment. If the substrate is more unsaturated, particularly more polyunsaturated, it should be more easily oxidized especially if antioxidants, such as the tocopherols, are present in limited amount. The red blood cell hemolysis test is a measure of the interrelationship of these two factors when the red blood cells are exposed to hydrogen peroxide, a reagent which is not found in in vivo situations. Thus, low levels of hemolysis are noted (Table VII) in the tests conducted with the red blood cells from the animals fed the lightly hydrogenated chipper oil, fresh or heated, the latter with or without the added vitamin E. Chipper oil contains the same level of tocopherols but less than half the linoleate of the liquid cottonseed oil (Table III) and therefore when included in the diet would be expected to provide deposited lipids which are more resistant to oxidation. In the case of the more polyunsaturated cottonseed oil, more vitamin E is required as antioxidant to protect a more labile substrate; this is even more evident in tests conducted on the red blood cells from the animals fed the heated CS oil diet.

In view of all results presented here arising from studies conducted on the intact animal (growth, reproduction, lactation and absence of lipid peroxidation products in the tissues), it would appear that no additional need for added vitamin E has been demonstrated in the fresh or heated vegetable oils used in this investigation other than that which is naturally present as tocopherols.

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