## TABLE II

Fatty Acid Composition of the Oila Extracted from P. macrophylla and T. africana Seeds (% by wt)

Fatty acid	P. macrophylla	T. africana
Lauric	0.48	
Myristic	trace	trace
Palmitic	5.2	19.2
Palmitoleic	_	0.58
Stearic	1.8	9.8
Oleic	15.6	13.1
Linoleic	73	44
Linolenic	3.9	13.2

<sup>a</sup>The extracted oils accounted for 31 and 36% of the dehulled P. macrophylla and T. africana seeds, respectively.

cysteine and methionine, the proteins were fairly rich in essential amino acids and could in fact be considered as good as, if not superior, to the FAO reference protein (6,7).

Table II shows the results of the fatty acid composition analysis. The oils extracted from these seeds were high in the principal essential fatty acid, linoleic acid. Both oils were, however, low in the other essential fatty acid, linolenic, and P. macrophylla contained substantially less of this acid than T. africana.

Arachidonic acid was not found in the oils. In man, arachidonic acid is synthesized from linoleic acid, so the absence of arachidonic acid would not constitute a nutritional disadvantage. With linoleic and linolenic acids together making up almost 80 and 60% of all the fatty acids in P. macrophylla and T. africana seed oils, respectively, the potential for the use of these oils as drying oils in the manufacture of paints and varnishes should be investigated. Work on the physicochemical properties of these oils is in progress.

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# Biological Evaluation of Hydrogenated Rapeseed Oil

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## ABSTRACT

A 91-day feeding study evaluated soybean oil, rapeseed oil, fully hydrogenated soybean oil, fully hydrogenated rapeseed oil, fully hydrogenated superglycerinated soybean oil and fully hydrogenated superglycerinated rapeseed oil at 7.5% of the diet in rats; a 16-wk feeding study evaluated soybean oil and the three rapeseed oils or fats at 15% of the diet. Each fat was fed to 40 rats as a mixture with soybean oil making up 20% of a semi-synthetic diet. No significant differences in body weight gains or diet-related pathology were seen in the 91-day study although the rats fed liquid rapeseed oil had slightly heavier hearts, kidneys and testes than the others. The rats fed the four fully hydrogenated fats ate more feed and had lower feed efficiencies than those fed oils but no differences were seen among the four hydrogenated fats. In the 16-wk feeding study, no pronounced pathology related to the diet was seen although the rats fed liquid rapeseed oil had a slightly higher incidence of histiocytic infiltration of cardiac muscle than the rats in the other groups. The female rats fed the three rapeseed oil fats gained significantly less weight and the females fed liquid rapeseed oil had enlarged hearts compared to the other groups. The absorbabilities of the six fats were measured in the 91-day study when fed as a mixture with soybean oil and as the sole source of dietary fat in a separate 15-day balance study. The four fully hydrogenated fats were poorly absorbed and the absorption of behenic acid from the two hydrogenated rapeseed oils was found to be 12% and 17% in the balance study and 8-40% in the feeding study. The adverse biological effects of unhydrogenated rapeseed oil containing erucic acid as reported in the literature do not occur with fully hydro-

genated rapeseed oil. In addition, the low absorbability of the fully hydrogenated rapeseed oil is an added factor in its biological inertness.

## INTRODUCTION

Fully hydrogenated rapeseed oil (iv 8) may be used as a stabilizer in peanut butter (1). It normally is used at 1% or less for this purpose. The safety of hydrogenated vegetable oils has been widely recognized. However, the safety of hydrogenated rapeseed oil was questioned because of the recognized hazards associated with the ingestion of unhydrogenated rapeseed oil by laboratory animals.

It has been known for a number of years that rapeseed oil as the sole source of dietary fat will not support a normal rate of growth in the rat. Thomasson and Boldingh (2) demonstrated that this was due to the erucic acid content of the rapeseed oil. In 1960, Roine (3) observed a myocarditis in rats fed 50-70% of their daily calories as rapeseed oil. Subsequently, other workers in Canada and Europe reported similar observations (4,5). The myocarditis, which is characterized by lipidosis in young animals and fibrosis in older animals, was attributed to the erucic acid that comprises 20-55% of the fatty acids of rapeseed oil. The effects of the erucic acid, however, are mitigated by the addition of saturated fatty acids to the diet (6,7).

Because hydrogenation of rapeseed oil converts the

erucic acid to behenic acid, which does not produce the pathology associated with erucic acid, it seemed likely that hydrogenated rapeseed oil would not produce the adverse effects reported for liquid rapeseed oil. Little work with hydrogenated rapeseed oil (and then only partially hydrogenated) has appeared in the literature to validate this assumption (8,9).

Therefore, we undertook a 91-day feeding study and a 16-wk feeding study in rats to determine whether any adverse affects might be associated with the ingestion of completely hydrogenated rapeseed oil. Because hydrogenated rapeseed oil is sometimes used in the form of partial glycerides for emulsifying purposes, the study included superglycerinated hydrogenated rapeseed oil and similar forms of soybean oil. Absorbability studies were carried out to determine the extent to which the fully

## TABLE I

Analyses of Fats Fed in 91-Day Feeding Study

hydrogenated fat was absorbed by animals that ingested it.

## **EXPERIMENTAL PROCEDURES**

## 91-Day Feeding Study

Fats and Diets. Rapeseed and soybean oils were obtained from commercial sources. Portions of each were fully hydrogenated using a commercial nickel-based catalyst. Portions of each of these were superglycerinated by heating with 20% glycerol for approximately 1 hr at 330 F in the presence of sodium hydroxide catalyst. Additional portions of both oils were retained for use in control diets. The analyses of the fats and oils are shown in Table I.

The six fats or oils were incorporated into the diet shown in Table II. In this diet, the total fat provided about

Analysis	Soybean oil (SBO)	Rapeseed oil (RSO)	Hydrogenated soybean oil (H-SBO)	Hydrogenated rapeseed oil (H-RSO)	Superglycerinated hydrogenated soybean oil (GH-SBO)	Superglycerinated hydrogenated rapeseed oil (GH-RSO)
Iodine value	129	105	2.5	2.3	1.6	2.0
Saponification no.	177	150	178	167	170	153
Peroxide value	0	<0,1	0	0	0	0
Hydroxyl value	1.3	4.3	0	0	164	158
% TFA	95.4	93.1	95.6	95.9	86.4	86.2
FFA	0.3	0.3	0.1	0.9	1.6	1.5
Monoglyceride			0.5	0.5	40	38
Diglyceride			4.0	3.5	40	44
Triglyceride			96	96	20	18
GLC, fatty acids (%)						
16:0	10.5	3.8	10.7	5.0	8.4	4.5
16:1	0.4	0.3	0.2			_
18:0	4.3	1.2	84.5	40.6	89.1	41.6
18:1	25.1	14.7	4.3	1.2	1.3	1.4
18:2	49.7	14.5	<0.1	-	_	<0.1
18:3	7.5	18.4	_	-	-	-
20:0	2.1	1.1	<0.1	9.1	0.5	10.4
22:0	_	0.5	_	43.9	_	42.1
22:1	_	44.1		<0.1	_	<0.1
kcal/g	9.4	9.4	9.5	9.5	8.9	9.0

#### TABLE II

Compositions of the Diets (91-Day Feeding Study)

	%	by weight
Ingredient	Feeding study	Absorbability study
Casein, vitamin-free <sup>a</sup>	27.0	27.0
Sucrose	41.0	46.0
Salt mixture, P&H <sup>b</sup>	4.0	_
Salt mixture, U.S.P. XIV	_	4.0
Water-soluble vitamins in sucrose <sup>c</sup>	5.0	5.0
Fat-soluble vitamins in soybean oild	1.0	_
Cellulose <sup>e</sup>	3.0	3.0
Soybean oil	11.5	_
Experimental fat <sup>f</sup>	7.5	15.0
	100.0	100.0

<sup>a</sup>Labco; Whitson Products Division, the Borden Company, NY.

<sup>b</sup>Nutritional Biochemicals Corporation, Cleveland, OH.

<sup>c</sup>Supplies the following in mg/kg of diet: menadione, 3; thiamine, 4; riboflavin, 6.7; pyridoxine, 4; niacin, 20; calcium pantothenate, 20; biotin, .25; folic acid, .25; cyano-cobalamin, .15; inositol, 2000; choline chloride, 3000; para-aminobenzoic acid, 100; and ascortic acid, 100.

dSupplies the following U.S.P. units/kg of diet: vitamin  $D_2$ , 12,000; vitamin A ester, 12,000; and  $\alpha$ -tocopheryl acetate, 100.

<sup>e</sup>The Chicago Dietetic Supply House, Chicago, IL.

<sup>f</sup>See Table I for fats and their analyses.

40% of the dietary energy, where 25% was provided by unhydrogenated soybean oil and 15% by the experimental fats. Fifteen percent liquid rapeseed oil has been reported as being capable of producing cardiac lipidosis.

Animals. Weanling male and female rats of the Sprague-Dawley strain obtained from Charles River Breeding Laboratories, Wilmington, MA, were distributed into six groups of 20 rats per sex so that the litter mates were distributed evenly among the groups and so that the mean body weights did not vary more than 0.5 g.

The animals were housed in individual stainless steel wire-mesh cages and the groups were positioned randomly on the racks. The environment was maintained at a temperature of  $23 \pm 1$  C with a relative humidity of  $50 \pm 5\%$ . Twelve-hour light and dark periods alternated and background music equalized ambient noise. Feed and water were given ad libitum. Fresh diet was placed in the glass feed jars three times weekly and the consumption measured. The rats were weighed and the feed consumption was reported weekly. The duration of the first study was 91 days.

Feces and Urine Collections. During the third and eleventh weeks of this study, feces were collected from 10 males and 10 females per group (randomly selected) using wirescreen collectors fastened under their regular cages. The feces were dried to constant weight in vacuo at 80 C and pulverized. An 0.25 g sample of the feces from each rat was saponified with alcoholic KOH and extracted with 2:1 (v/v) chloroform:methanol. The total fatty acids were determined gravimetrically. Samples of the dietary fats were analyzed similarly. Composite samples of the feces from each dietary group were extracted similarly and the fatty acids were methylated for quantitative gas chromatography. Other samples of the individual rats' feces were analyzed for nitrogen by a micro-Kjehldahl method.

Before the final feces collection, 10 rats of each sex per group were transferred into metabolism cages and their urine was collected for 24 hr while they were being fasted. The 24-hr-volume was measured but the urine collected during the first 12 hr was discarded. The urine from the second 12 hr was analyzed for nitrogen by the micro-Kjehldahl method and for ketones, glucose, bilirubin, albumin and pH by the use of Ames clinical test strips.

Necropsy and Histology. At the end of the study, all animals were sacrificed with excessive ether and necropsied. Ten animals of each sex were selected randomly from each group for the following analyses. Blood was removed from the aorta and standard hemograms done and then the serum was separated by centrifugation. Serum cholesterol was determined by the technicon Auto Analyzer Technique

#### TABLE III

Analyses of Fats Fed in 16-Wk Feeding Study

(N24A method) and phospholipids were determined by the method of Zilversmit and Davis (10).

The heart, liver, kidneys and gonads were removed and weighed. Sections of these organs and of lung, pancreas, stomach, jejunum, adrenals, spleen, mesenteric lymph nodes and gastrocnemius muscle were fixed in Bouin's solution, stained with H&E or Sudan IV and examined by a pathologist.

Statistical Analyses. All of the data were analyzed by the Analysis of Variance (11) and partitioned by the Tukey minimum significant difference method described by Scheffe (12).

#### Fat Absorbability Study

Fats and Diets. The fats used were identical to those described before for the 91-day feeding study. They were incorporated into diets whose composition was similar to the one shown in Table II at the 15% level as the sole source of dietary fats.

Animals. Male Sprague-Dawley (ARS Sprague-Dawley, Madison, WI) rats weighing about 200 g were randomly distributed into six groups of 10 animals. The rats were housed under the conditions described previously. The diets were fed to the rats for 15 days with the first five days an orientation period. For the final 10 days, the feces were collected with wire-screen collectors fastened to the bottom of their cages and the feed consumption was measured and recorded. At the end of this period, the animals were weighed and discarded.

The feces were dried, weighed, pulverized and analyzed as described previously for the 91-day study.

## 16-Wk Feeding Study

Fats and Diets. Rapeseed and soybean oils were obtained from commercial sources and portions of the rapeseed oil were fully hydrogenated and superglycerinated as described previously. The analysis of these fats are shown in Table III. Slightly different diets were used in this study and the composition is shown in Table IV. Again, the total energy in the diet derived from fat was 40% but the experimental fats provided 30% of the energy with the remaining 10% being supplied by soybean oil.

Animals. Weanling male and female Sprague-Dawley rats obtained from Charles River Breeding Laboratories, Wilmington, MA, were distributed into four groups of 20 animals of each sex. The distribution methods, the animal housing and environment were as outlined previously although this study was done at Bio/dynamics, Inc., East Millstone, NJ.

Analysis	Soybean oil (SBO)	Rapeseed oil (RSO)	Hydrogenated rapeseed oil (H-RSO)	Superglycerinated hydrogenated rapeseed oil (GH-RSO1)
Iodine value	127	104	2-4	2-4
GLC, fatty acids (%)				
C16	10.3	3.5	4.0	4.0
18	4.1	1.5	52.5	52.2
18:1	23.2	28,5	0.7	0.02
18:2	53.3	16.7	0.08	0.14
18:3	7.8	17.5	NF	NF
C20	0.5	0.5	11.7	11.9
C22	0.4	0.4	29.8	30.6
22:1	0.06	30.2	<0.4	<0.4

Feed and water were given ad libitum. Fresh diet was added to the animal feed jars three times per week and the consumption measured. The rats were weighed weekly and the feed consumption was reported weekly. No feces or urine collections were done in this study.

#### Necropsy and Histology

All animals were sacrificed with chloroform after 16 wk on the study and necropsied. The heart was removed and weighed. The following tissues were removed and those from 5 rats per sex per group were frozen pending staining with special lipid stains (these tissues were lost in transit), while those from the remaining 15 rats of each sex per group were fixed in 10% formalin: heart, adrenals, lung, kidneys, jejunum, liver, mesenteric lymph nodes, gonads, pancreas, spleen, stomach and gastrocnemius muscle. Sections of all tissues were stained with H&E but heart sections were also stained with Oil Red O for lipids and trichrome for fiber. The tissues were examined by independent pathologists (Experimental Pathology Laboratories, Inc., Hernedon, VA) and some of the heart slides were randomly selected and examined by E.A. Nera of the Canadian Food and Drug Protectorate.

Statistical Analyses. Statistical analyses were done by the F-test and Students T-test with Cochran's approximation (11).

## RESULTS

#### 91-Day Feeding Study

Growth and Caloric Efficiency. The growth and feed data are shown in Table V. There were no significant differences among the six groups in their growth except for the difference between sexes. There were no appreciable differences in feed intake or efficiency during the first four weeks of the study but thereafter the animals receiving the hydrogenated fats increased their feed intake. This led to a significant increase in the kilocalories used per gram of gain in the groups fed the hydrogenated fats. However, there were no significant differences among the four groups fed hydrogenated fats.

The output of urinary nitrogen and the urinary pH, albumin, ketones, glucose and bilirubin were within normal limits and were not significantly different among the groups of either sex. Therefore, these data are not shown. However, all of the rats fed hydrogenated fats excreted significantly more nitrogen in their feces (65-75 mg) than the rats fed unhydrogenated oils (40-52 mg) with no differences between the third and eleventh week collections.

Hematology and Histopathology. There were no significant differences in red or white blood cell counts and

#### TABLE IV

Composition of the Diet (16-Wk Feeding Study)

Ingredient	% by weight
Casein, vitamin-free <sup>a</sup>	27.0
Sucrose	41.0
Water-soluble vitamins in sucrose <sup>b</sup>	5.0
Salt mixture, USP XVII <sup>C</sup>	4,0
Salt mixture, USP XVII <sup>c</sup> Cellu flour <sup>d</sup>	3.0
Fat-soluble vitamins in SBO <sup>e</sup>	1.0
Soybean oil	4.0
Test or control fat <sup>f</sup>	15.0
	100.0

<sup>a</sup>Labco; Whitson Products Division, the Borden Company, NY. <sup>b</sup>Supplies the following in mg/kg of diet; menadione, 3; thiamine, 4; riboflavin, 6.7; niacin, 20; folic acid, 0.25; Ca pantothenate, 20; pyridoxine, 4; inositol, 2000; *p*-amino benzoic acid, 100; biotin, .025; cyanocobalamin, 0.15; ascorbic acid, 100; and choline chloride, 3000.

<sup>c</sup>Nutritional Biochemicals Corporation, Cleveland, OH.

<sup>d</sup>The Chicago Dietetic Supply House, Chicago, IL.

<sup>e</sup>Supplies the following in U.S.P. units/kg of diet: vitamin A, 12,000; vitamin  $D_2$  (ereosterol), 1200; and  $\alpha$ -tocophenyl acetate, 100.

<sup>f</sup>See Table II for fats and their analyses.

hematocrit levels although the male rats fed hydrogenated rapeseed oil had a significantly higher hemoglobin level (14.1 mg/DL) than those fed liquid rapeseed oil (12.0 mg/ DL). No significant differences were seen in the serum cholesterol levels but the female rats fed superglycerinatedhydrogenated rapeseed oil had a significantly lower level of serum phospholipids (65 mg/DL) than those fed liquid rapeseed oil (200 mg/DL).

No gross pathology was noted at the time of sacrifice and examination of the tissues after staining with either H&E or Sudan IV (a lipid stain) revealed no diet-related histopathology or lipid accumulation even in the heart, which is the main target organ in the case of unhydrogenated rapeseed oil. However, there were some significant differences in organ/body weight ratios (Table VI). In both sexes, the heart/body weight ratio was highest in the rats fed liquid rapeseed oil; however, those ratios were significant only when compared to the animals with the lowest ratios for each sex. The only other organ significance was that the weights of the ovaries of the rats fed either hydrogenated rapeseed oil or superglycerinated-hydrogenated soybean oil were higher than those of rats fed either liquid rapeseed oil or soybean oil.

Fat Absorbability. As expected the hydrogenated fats were poorly absorbed, either fed as a mixture with soybean

#### TABLE V

91-Day Cumulative Gain in Body Weight, Feed Consumption and Caloric Efficiency of Rats Fed Hydrogenated Rapeseed and Soybean Oils

	SBO	RSO	H-SBO	H-RSO	GH-SBO	GH-SBO
Males						
Weight gain (g)	523 ± 66	484 ± 46	482 ± 49	501 ± 63	$500 \pm 63$	506 ± 51
Feed consumed (g)	1677 ± 148	1609 ± 119	$1741 \pm 118$	1785 ± 170 <sup>a</sup>	1766 ±131	1794 ± 208
Caloric efficiency						
(kcal/g gain)	16.8 ± 1.1	17.3 ± 0.9	18.9 ± 1.1 <sup>a</sup>	$18.6 \pm 1.0^{a}$	18.5 ± 1.3 <sup>a</sup>	$18.5 \pm 1.3^{a}$
Females						
Weight gain (g)	282 ± 55	246 ± 44	257 ± 34	258 ± 43	269 ± 36	258 ± 42
Feed consumed (g)	1266 ± 160	1205 ± 115	1324 ± 127	1278 ± 145	1311 ± 146	1307 ± 155
Caloric efficiency						
(kcal/g gain)	23.6 ± 1.9	$25.8 \pm 2.5^{a}$	$26.9 \pm 1.8^{a}$	26.1 ± 2.5 <sup>a</sup>	25.4 ± 1.4	25.6 ± 2.2ª

<sup>a</sup>Significantly higher than SBO fed group;  $P \le .05$ .

## TABLE VI

## Organ/Body Weight Ratios (g/kg) of Rats Fed Hydrogenated Rapeseed and Soybean Oils for 91 Days

	SBO	RSO	H-SBO	H-RSO	GH-SBO	GH-RSO
Males						
Heart	$3.0 \pm 0.3$	$3.1 \pm 0.3^{a}$	$3.0 \pm 0.2$	$2.7 \pm 0.3$	2.8 ± 0.4	$2.9 \pm 0.4$
Liver	35.7 ± 6.6	34.4 ± 2.5	33.9 ± 4.6	32.0 ± 2.6	$32.3 \pm 4.1$	$30.1 \pm 2.5$
Kidney	$6.4 \pm 0.8$	$6.6 \pm 0.4^{a}$	$6.5 \pm 0.6$	5.7 ± 0.4	$6.2 \pm 0.9$	$6.3 \pm 0.6$
Gonad	$8.0 \pm 1.6$	$9.0 \pm 2.1^{a}$	$7.4 \pm 2.1$	$6.0 \pm 0.7$	$7.0 \pm 1.6$	6.7 ± 0.6
Females						
Heart	$3.6 \pm 0.8$	$4.3 \pm 0.7^{b}$	$3.9 \pm 0.4$	$3.8 \pm 0.6$	$3.7 \pm 0.5$	3.6 ± 0.4
Liver	$36.0 \pm 1.5$	$34.4 \pm 2.5$	$33.9 \pm 4.7$	$31.8 \pm 2.2$	$32.2 \pm 2.1$	$31.7 \pm 1.7$
Kidney	$7.1 \pm 0.9$	$7.4 \pm 1.0$	$7.6 \pm 1.0$	$7.1 \pm 0.8$	$7.6 \pm 0.5$	$7.2 \pm 0.8$
Kidney Gonad	$1.0 \pm 0.2$	$1.1 \pm 0.4$	$1.2 \pm 0.2$	$1.4 \pm 0.2^{c}$	$1.5 \pm 0.3^{\circ}$	$1.2 \pm 0.2$

<sup>a</sup>Significantly higher than the ratios of rats fed H-RSO;  $P \le .05$ .

<sup>b</sup>Significantly higher than the ratios of rats fed either SBO or GH-RSO;  $P \le .05$ .

<sup>c</sup>Significantly higher than the ratios of rats fed SBO or RSO;  $P \le .05$ .

## TABLE VII

Absorption of Hydrogenated Rapeseed and Soybean Oil When Fed As a Mixture with Liquid Soybean Oil<sup>a</sup>

	Fat <sup>b</sup>					
	SBO	RSO	H-SBO	H-RSO	GH-SBO	GH-RSO
Males	<u> </u>					
Total fat absorbed (%)	95 ± 1	93 ± 2	64 ± 4 <sup>c</sup>	66 ± 2 <sup>c</sup>	72 ± 2c,d	$62 \pm 5^{\circ}$
Exptl. fat absorbed (/)	_	89 ± 4	17 ± 8 <sup>c</sup>	18 ± 4 <sup>c</sup>	35 ± 5c,d	$14 \pm 8^{c}$
Fatty acids absorbed (%)						
C16	77	78	50	62	59	65
C18	56	37	14	27	36	27
18:1	97	98	95	95	96	96
18:2	100	100	100	99	100	100
18:3	100	95	100	100	100	100
C22	_	_	_	9		8
22:1	_	95	-	100	_	100
Females						100
Total fat absorbed (%)	98 ± 1	97 ± 1	68 ± 3 <sup>c</sup>	66 ± 4 <sup>c</sup>	73 ± 2c,d	68 ± 3 <sup>c</sup>
Exptl. fat absorbed (%)	_	95 ± 2	$17 \pm 7^{\circ}$	$16 \pm 10^{\circ}$	$32 \pm 6^{c,d}$	$17 \pm 7^{c}$
Fatty acids absorbed (%)		,				
C16	91	91	62	69	65	79
C18	73	69	29	42	44	53
18:1	99	98	96	95	96	98
18:2	100	100	100	100	100	100
18:3	100	99	100	100	100	100
C22	-	_		31 .		42
11:1	_	95	-	100	_	100

<sup>a</sup>Absorption measured during 11th week of study.

<sup>b</sup>20% total fat; 7.5% exptl. fat and 12.5% SBO.

<sup>c</sup>Significantly lower than SBO or RSO;  $P \le .05$ .

<sup>d</sup>Significantly higher than other hydrogenated fats;  $P \le .05$ .

## TABLE VIII

Absorption of Hydrogenated Rapeseed and Soybean Oil When Fed to Male Rats As Sole Source of Dietary Fat<sup>a</sup>

	SBO	RSO	H-SBO	H-RSO	GH-SBO	GH-RSO
Absorption of fat (%) Fatty acids absorbed (%)	94 ± 2	86 ± 3	6 ± 4 <sup>b</sup>	8 ± 6 <sup>b</sup>	14 ± 5 <sup>b</sup>	12 ± 10 <sup>b</sup>
C16	81	82	10	0	0	3
C18	37	0	3	ŏ	14	11
18:1	95	96	78	100	26	-
18:2	99	100	_	-	-	-
18:3	100	95	_	-	_	
C22	_			17		12
22:1	_	78			-	

<sup>a</sup>Determined in fat-balance study separate from 91-day study.

<sup>b</sup>Significantly different from SBO and RSO;  $P \le .05$ .

oil (Table VII) or as the sole source of dietary fat in a fatbalance type study (Table VIII). Soybean oil was absorbed somewhat better than rapeseed oil but after hydrogenation there were no differences between the absorbability of the two fats. The absorbability of the rapeseed oil was 10-20% higher than the range of 70-82% that has been reported previously (13). However, the absorbability of the hydrogenated or superglycerinated hydrogenated fats was increased from 6-12% to 14-35% by feeding them in a mixture with soybean oil with the absorption of the superglycerinated-hydrogenated soybean oil being improved more than the other three.

The absorbabilities of the individual fatty acids were somewhat as expected; the saturated fatty acids were poorly absorbed and the unsaturated ones were well absorbed. The absorbability of erucic acid ranged from 78% in the fat-balance study to 95% in the feeding study; much higher than the 37-59% range cited in the review by Beare (14). On the other hand, behenic acid in the hydrogenated rapeseed oils was absorbed only 12-17% in the fat-balance study but was absorbed at 30-42% when fed as a mixture with soybean oil, which agrees with the 40% absorption of behenic acid reported by Bernhard and Vischer cited in the review by Beare (14).

### 16-Wk Feeding Study

Growth and Feed Efficiency. Table IX shows the gains in body weight, cumulative feed consumption and feed efficiency of the rats after 16 wk of feeding. In this study, the females fed either liquid rapeseed oil or the two hydrogenated rapeseed fats gained significantly less weight than the controls fed soybean oil. Both males and females fed the hydrogenated rapeseed oil and the superglycerinatedhydrogenated rapeseed oil ate significantly more feed than rats fed either soybean or rapeseed oil which led to lower feed efficiencies.

Histopathology. At the necropsy, no significant gross pathology attributable to the diets was seen, but, as seen in a previous feeding study that we conducted, the hearts of the rats fed liquid rapeseed oil were enlarged (Table X). No fibrosis was seen in any of the hearts nor was there any pathology in any of the other tissues related to dietary treatment. Some myocarditis (histiocytic infiltration) was seen in the hearts of several animals and the incidence was greater in male rats fed liquid rapeseed oil. However, the incidence in the animals fed fully hydrogenated rapeseed oil was the same, or less than, the incidence in the soybeanfed control animals. This condition usually precedes the fibrotic lesion (15).

## DISCUSSION

Liquid rapeseed oil (high in erucic acid) is toxic to several species of laboratory animals and its toxicity has been attributed to its erucic acid content. However, the threshold level for producing lipidosis is generally taken to be about 10-15% of calories or 5% of the diet (16) and is dependent upon the level of erucic acid in the rapeseed oil. Other factors that have been shown to influence the infiltration of fat in the heart muscle include the level of saturated fatty acid, especially palmitic (7), the age of the

#### TABLE IX

Summary of the 16-Wk Gain in Body Weight and Feed Consumption of Rats Fed Liquid or Hydrogenated Rapeseed Oil

	Weight gain (g)	Feed consumed (g)	Feed efficiency <sup>a</sup> (%)
 Males			
SBO	376 ± 60	2323 ± 280	16.2 ± 1.5
RSO	363 ± 27	2372 ± 220	$15.3 \pm 0.8$
H-RSO	349 ± 36	2768 ± 268 <sup>b</sup>	12.6 ± 0.8 <sup>b</sup>
GH-RSO	365 ± 47	2934 ± 326 <sup>b</sup>	12.4 ± 1.1 <sup>b</sup>
Females			
SBO	207 ± 29	2664 ± 203	7.8 ± 0.9
RSO	166 ± 39 <sup>b</sup>	2618 ± 262	$6.3 \pm 1.1^{b}$
H-RSO	$162 \pm 22^{b}$	$3258 \pm 265^{b}$	$5.0 \pm 0.6^{b}$
GH-RSO	$160 \pm 16^{b}$	3049 ± 230 <sup>b</sup>	$5.3 \pm 0.6^{b}$

<sup>a</sup>Feed efficiency = body weight gain/feed consumed <100.

<sup>b</sup>Significantly different from control (SBO) at  $P \le .01$ .

#### TABLE X

Heart/Body Weight Ratios and Number of Rats with Cardiac Lesions

	Heart weights (g/kg)	No. of rats with myocarditis/no. examined <sup>a</sup>
Males	·····	• · · · · • • · · · • · · · · · · · · ·
SBO	2.9/ .4	4/15
RSO	3.1/ .3	8/15
H-RSO	2.0/ .5	1/15
GH-RSO	2.8/ .2	3/15
Females		
SBO	3.1/ .3	2/15
RSO	5.6/1.3 <sup>b</sup>	1/15
H-RSO	3.6/ .6	1/15
GH-RSO	3.7/ .6	0/15

<sup>a</sup>Myocarditis = histocytic infiltration.

<sup>b</sup>Significantly different from SBO controls at  $P \le .01$ .

animal and the level of dietary protein (4). Furthermore, if animals are continuously exposed to high levels of rapeseed oil for long periods, the fat in the heart tissue is replaced by fibrotic tissue (15) although the pathogenesis of this lesion is less well characterized than the earlier lipidosis. Thus, the pathology induced by liquid rapeseed oil is affected by a number of factors in addition to the level of rapeseed oil in the diet although the intake of erucic acid seems to be the primary one.

Partially hydrogenated rapeseed oil has been used in margarines in Europe with no apparent ill-effects (14) and a few studies have been done with the hydrogenated oil (7) which indicate that the hydrogenated oil is less toxic than the unhydrogenated rapeseed oil. This has been considered to be due to the conversion of erucic acid to behenic. Although the rats in our experiments were fed exaggerated levels of fully hydrogenated rapeseed oil, there was no significant diet-related pathology in any of the rats. The histological data show that there was no deposition of lipids in the rats fed the fully hydrogenated rapeseed fats. This confirms the study done in weanling rats by Mattson and Streck (17). The general growth and thriftiness of the animals did not differ whether they were fed the fully hydrogenated soybean oil or the fully hydrogenated rapeseed oil.

The absorbability studies explain that the higher feed consumption of animals receiving hydrogenated rapeseed oil was probably due to the poor absorbability of the saturated fatty acids that make up this fat. Behenic acid in particular is poorly absorbed from both hydrogenated rapeseed oil and superglycerinated-hydrogenated rapeseed oil. However, even where the absorption of this long chain fatty acid was enhanced by feeding liquid soybean oil concomitantly, no adverse health effects or histopathology were seen.

The experiments reported here support the safety of

fully hydrogenated rapeseed fats for human consumption.

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