Effects of Fresh and Used Hydrogenated Soybean Oil on Reproduction and Teratology in Rats

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ABSTRACT

Groups of 25 pairs of two generations of male and female rats were fed diets containing 15% of either fresh hydrogenated soybean oil (iodine value, 107), a similar fat used 56 hr for deep frying or an unhydrogenated mixture of fats and oils with a fatty acid composition similar to the hydrogenated soybean oil. The first two litters of each generation were permitted to be born naturally. During the third pregnancy of each generation, one-half of the females were sacrificed on day 13 of gestation and inspected for early embryonic death. The remaining females were sacrificed on day 21 of gestation, and the fetuses were examined for either skeletal or soft-tissue abnormalities. There was no evidence of any deleterious effects on the reproductive parameters nor any teratogenic effects due to either hydrogenated soybean oil, a similar oil used for frying foods for 56 hr or an unhydrogenated mixture of fats and oils.

INTRODUCTION

Fats and oils subjected to high temperatures for prolonged periods of time may undergo chemical and physical changes such as oxidation, dehydrogenation, rearrangement, polymerization and pyrolysis (1). Some of these isolated and concentrated reaction products have been shown to be toxic to rats (2-5). However no evidence of toxicity was found in long term feeding studies with fats that were oxidized to a high peroxide value at low temperatures (6), fats polymerized at high temperatures in the absence of air (7), or fats bubbled with air while being heated (8).

More pertinent to human safety, fats used under normal commercial frying conditions were tested in short term feeding studies and found to be nutritious and without ill effects (9,10). Moreover we have previously fed several representative fats, which had been used for frying foods under simulated commercial conditions, to rats for 2 years, and saw no adverse effects (11). In another study (unpublished) soybean oil, hydrogenated to an iodine value of 107 and used 56 hr for frying, was fed to young growing dogs for 1 year, again with no adverse effects.

Although much work has been done on the biological effects of both laboratory heated and commercially used fats, few studies have been reported about the effects of these fats on reproduction and none, to our knowledge, have studied the effects of used fats on the development of the fetus (teratology). Alfin-Slater et al. (7) tested lard, soybean oil and cottonseed oil heated in the laboratory for either 70 or 100 min at 610 F in vacuo for their effects on reproduction in the rat. They found no adverse effects, except that a highly polymerized soybean oil, fed at 15% of the diet, caused some impairment of growth and reproduction. Ramel et al. (12) fed rats with fats that had been heated in air for 8 days at 200 C. The rats were observed through three generations, and no adverse effects on reproduction could be attributed to the inclusion of heated fats in the diet. However we wanted to determine whether a typical fat (soybean oil), used extensively for deep frying under realistic conditions, would affect either reproduction or embryogenesis in the rat.

The hydrogenation of oils to produce plastic shortenings causes some isomerization of the naturally occurring *cis* isomers to the *trans* configuration. Studies have shown that the *trans* isomers are metabolized as readily as the *cis* isomers (13-15), but are incorporated into adipose tissue somewhat differently than the *cis* isomers (16) and have some influence on the integrity of cell membranes (14).

TABLE I

Analyses of Fats

	Storage time, months								
	0			6			18		
	Hydrogenated soybean oil		Unhydrogenated mixture[a]	Hydrogenated soybean oil		Unhydrogenated mixture[a]	Hydrogenated soybean oil		Unhydrogenated mixture[a]
Type of fat	Fresh	Used		Fresh	Used		Fresh	Used	
Acid value	0.1	1.3	---	<0.1	1.3	0.1	0.1	1.3	0.1
Iodine value	107	101	---	107	100	104	105	98	103
Saponification value	193	195	---	191	196	192	192	195	192
Peroxide value	0.6	3.1	---	0.7	5.6	0.7	1.0	3.7	0.5
Hydroxyl value	3.1	2.3	---	<1.0	8.3	2.4	1.0	8.3	2.1
Carbonyl value	0.1	0.1	---	0.4	<0.1	<0.1	---	---	---
Epoxide value	0.2	0.2	---	<0.1	<0.2	0.2	---	---	---
Linoleate-UV	30.6	27.2	---	30.4	28.1	34.1	30.7	26.9	34.0
Linolenate-UV	2.8	2.2	---	2.6	2.2	2.4	2.8	2.2	2.4
Refractive index	47.4	50.3	---	---	50.3	47.4	47.4	50.4	47.3
Polar triglycerides	2.6	14.5	---	---	14.8	2.7	0.9	11.6	1.7
Gas liquid chromatography analysis									
14:0	0.1	0.1	0.01	---	0.1	---	---	---	---
16:0	10.2	11.0	11.8	---	11.3	11.9	10.5	11.1	11.9
18:0	6.6	7.5	5.5	---	7.3	6.3	6.3	7.2	6.0
18:1	48.7	50.0	47.1	---	50.2	47.2	49.5	50.6	48.3
18:2	32.1	29.6	33.3	---	29.1	32.4	32.5	30.6	32.3
18:3	2.1	1.2	2.0	---	1.6	2.1	1.1	0.6	1.3
20:0	0.2	0.2	0.5	---	---	---	---	---	---

[a]See text for description.

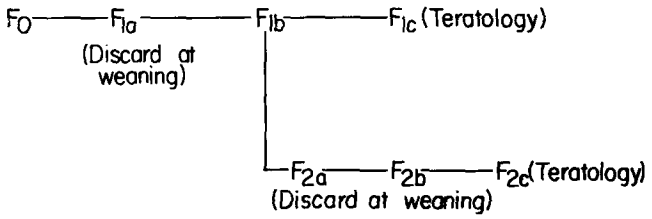

FIG. 1. Design of reproduction and teratology experiment.

Therefore in this study we also compared the effects of hydrogenated soybean oil (iodine value, 107) with a comparable unhydrogenated blend of fats and oils on reproduction and embryogenesis.

EXPERIMENTAL PROCEDURES

Refined and bleached soybean oil, hydrogenated to an iodine value of 107, was used for frying onion rings, scallops and potatoes until the foam reached 75 mm. This took 56 hr. The frying procedures were described previously (11). The unhydrogenated fat was made by blending 9% cocoa butter, 23% soybean oil, 48% olive oil and 20% safflower oil. Its fatty acid composition was similar to the hydrogenated soybean oil (Table I). The three fats were protected from oxidation by the addition of 39 ppm BHT and 31 ppm BHA, by sealing in 1 lb cans under nitrogen, and by storing at 0 C until needed.

The fats were fed at 15% in a semipurified diet. The

TABLE II

Composition of Diet

Ingredient	Per cent
Casein[a]	5.0
Nonfat dry milk[b]	21.0
Ground whole wheat	43.0
Dried egg white[c]	3.0
Dried liver, defatted[d]	3.0
Vitamin mixture in sucrose[e]	3.0
Lysine·HCl	0.5
Calcium phosphate, dibasic[f]	1.0
Salt mixture, Phillips & Hart	3.5
Fat-soluble vitamins in soybean oil[g]	2.0
Experimental fat	15.0
Total	100.0

[a]Labco, Whitson Products, Division of The Borden Co., New York.

[b]Land O' Lakes Creameries, Minneapolis.

[c]Nutritional Biochemicals Corp., Cleveland. Biotin added at a level of 1 mg/100 g egg white.

[d]Wilson Laboratories, Inc., Chicago.

[e]Furnished the following in mg/kg of diet: menadione, 1.8; thiamine, 2.4; riboflavin, 3.0; niacin, 12.0; folic acid, 0.15; Ca pantothenate, 12.0; pyridoxine, 2.4; inositol, 12,000; *p*-aminobenzoic acid, 60.0; biotin, 0.015; cyanocobalamin, 90.0; ascorbic acid, 60.0; and choline chloride, 1800.

[f]Added to maintain calculated Ca and P at 2.0% of diet and Ca/P ratio at 1.1:1.0.

[g]Furnished the following IU/kg of diet: vitamin A, 12,000; vitamin D_2, 3200; and *d*-γ-tocopheryl acetate, 100.

TABLE III

Cumulative 8 Week Post Weaning Growth, Feed Consumption and Feed Efficiency of F_O Rats Fed Fresh or Used Hydrogenated Fat or Fresh, Unhydrogenated Fat

	Sex	Hydrogenated soybean oil		Unhydrogenated mixture[a]	MSD[b] = P ≤ .05
		Fresh	Used		
Gain in body wt, g	M	311	297	302	---
	F	179	180	181	---
Feed consumption, g	M	868	868	850	---
	F	669	677	670	---
Feed efficiency, %[c]	M	35.8	34.2	35.5	1.3
	F	26.8	26.6	26.7	---
Per cent fat absorbed	M	95.8	91.4	95.5	1.2
	F	96.6	93.0	96.3	1.1
Fecal nitrogen, mg/g intake	M	5.1	4.9	5.1	---
	F	6.1	5.2	5.9	0.7
Urine nitrogen, mg/ml	M	21.7	23.1	21.6	---
	F	23.6	23.0	27.0	---

[a]See text for description.

[b]Minimum significant difference.

[c]FE = Body wt gain per feed consumed x 100.

TABLE IV

Cumulative 6 Week Post Weaning Growth, Feed Consumption and Feed Efficiency of F_{1b} Rats Fed Fresh or Used Hydrogenated Fat or Fresh, Unhydrogenated Fat

	Sex	Hydrogenated soybean oil		Unhydrogenated mixture[a]	MSD[b] = P ≤ .05
		Fresh	Used		
Gain in body wt, g	M	251	238	240	---
	F	131	125	126	---
Feed consumption, g	M	703	703	686	---
	F	517	523	525	---
Feed efficiency, %[c]	M	36.0	33.9	35.0	---
	F	25.2	23.7	24.0	---

[a]See text for description.

[b]Minimum significant difference.

[c]FE = Body wt gained per feed consumed x 100.

TABLE V

Effect of Used or Fresh or Hydrogenated Fat on Reproduction of F_O Generation Rats and on Vitality of Their Litters

	Litter no.	Hydrogenated soybean oil		Unhydrogenated mixture
		Fresh fat	Used fat	
No. pregnant	F_{1a}	21	23	24
	F_{1b}	21	23	22
Per cent pregnant	F_{1a}	84	92	96
	F_{1b}	84	92	88
No. of live litters born	F_{1a}	21	23	24
	F_{1b}	21	23	22
No. of fetuses born dead	F_{1a}	2	14[a]	6
	F_{1b}	3	1	1
Average no. born per litter	F_{1a}	11.1±2.4	11.4±2.7	11.7±2.9
	F_{1b}	11.0±2.1	12.8±1.5	12.2±1.7
No. live at 4 days	F_{1a}	10.7±2.3	10.5±3.0	11.2±1.9
	F_{1b}	10.9±2.2	11.8±1.7	12.0±1.5
Average pup wt at 4 days	F_{1a}	9.7±1.8	9.8±1.8	9.4±1.1
	F_{1b}	9.6±1.2	9.7±1.1	9.7±0.9
No. of litters weaned	F_{1a}	20	22	22
	F_{1b}	20	23	22
Lactation index[b]	F_{1a}	100	98.2	95.5
	F_{1b}	99.4	97.8	98.9
Average no. weaned per litter	F_{1a}	7.9±0.3	7.5±1.1	7.6±1.0
	F_{1b}	7.9±0.5	7.8±0.4	8.0±0.2
Average weaning wt of male pups	F_{1a}	50.0±5.7	47.3±6.3	47.1±6.0
	F_{1b}	44.4±6.8	41.3±4.2[c]	46.7±3.9[c]
Average weaning wt of female pups	F_{1a}	47.6±4.7	44.2±7.3	45.3±5.2
	F_{1b}	42.8±8.1	39.0±4.7[d]	43.7±4.4[d]

[a]Significantly high by χ^2 test.

[b]No. of pups weaned per number of pups alive at 4 days x 100.

[c]Significantly different $P \leqslant 0.05$, MSD = 3.8.

[d]Significantly different $P \leqslant 0.05$, MSD = 4.5.

TABLE VI

Effect of Used or Fresh or Hydrogenated Fat on Reproduction of F_{1b} Generation Rats and on Vitality of Their Litters

	Litter no.	Hydrogenated soybean oil		Unhydrogenated mixture
		Fresh fat	Used fat	
No. pregnant	F_{2a}	23	25	20
	F_{2b}	15	16	16
Per cent pregnant	F_{2a}	92	100	80
	F_{2b}	75	80	80
No. of live litters born	F_{2a}	23	25	20
	F_{2b}	15	16	16
No. of fetuses born dead	F_{2a}	4	5	1
	F_{2b}	6	1	5
Average no. born per litter	F_{2a}	11.6±1.6	11.2± 2.1	10.9± 2.6
	F_{2b}	12.3±2.1	10.9± 3.0	9.9± 3.8
No. live at 4 days	F_{2a}	10.5±2.0	10.9± 2.2	10.5± 2.4
	F_{2b}	12.1±2.1	10.7± 2.8	9.7± 3.8
Average pup wt at 4 days	F_{2a}	8.5±1.2	8.6± 1.1	8.7± 1.2
	F_{2b}	9.4±1.1	9.1± 1.2	8.8± 1.5
No. of litters weaned	F_{2a}	22	25	20
	F_{2b}	15	16	16
Lactation index[a]	F_{2a}	98.8	95.4	94.2
	F_{2b}	99.2	99.2	95.4
Average no. weaned per litter	F_{2a}	7.8±0.7	7.4± 1.0	7.3± 1.6
	F_{2b}	7.9±0.4[b]	7.6± 1.3[b]	6.7± 1.8[c]
Average weaning wt of male pups	F_{2a}	45.1±6.2	42.4± 3.8	40.8±14.5
	F_{2b}	54.8±5.2	48.0± 6.4	49.0±15.1
Average weaning wt of female pups	F_{2a}	44.6±5.5[d]	39.8± 3.6[e]	43.5± 5.0[d]
	F_{2b}	53.0±5.1[f]	43.3±12.5[g]	50.0± 8.6

[a]Number of pups weaned per number of pups alive at 4 days x 100.

[b]Significantly different from c.

[c]$P \leqslant 0.05$, MSD = 1.1.

[d]Significantly different from e.

[e]$P \leqslant 0.05$, MSD = 3.6.

[f]Significantly different from g.

[g]$P \leqslant 0.05$, MSD = 8.3.

TABLE VII

Effects of Used, Fresh or Hydrogenated Fat on the Third Litters of F_O and F_{1b} Generation Rats

		Hydrogenated soybean oil					
		Fresh		Used		Unhydrogenated mixture	
		Parent generation		Parent generation		Parent generation	
	Day of sacrifice	F_O	F_{1b}	F_O	F_{1b}	F_O	F_{1b}
No. pregnant	13	8	8	10	8	8	9
	21	8	9	10	7	7	6
Per cent pregnant	13	80	80	100	80	80	90
	21	80	90	100	78	70	67
No. of total resorptions	13	0	0	0	0	0	0
	21	0	0	0	1	0	0
Average no. of corpora lutea	13	13.9±2.6	13.4±3.7	13.9±1.9	13.9±1.7	13.8±2.1	13.3±4.4
	21	13.5±2.5	13.6±2.2	13.3±2.3	14.6±1.7	13.0±1.8	13.7±0.8
Average no. of implantations	13	12.8±2.8	11.9±2.0	13.5±1.4	13.5±1.9	11.9±3.6	10.8±4.9
	21	13.1±2.5	12.8±1.7	12.7±2.1	12.0±1.7	13.0±1.6	10.5±5.2
Average no. of resorptions	13	0.6±0.7	0.5±0.8	0.4±1.0	1.4±1.3	1.8±3.2	0.7±0.9
	21	1.3±1.4	0.3±0.7	0.8±0.6	1.7±3.7	0.4±1.1	0.5±0.8
Average no. of live fetuses	13						
	21	11.9±3.2	12.4±1.8	12.7±2.1	10.3±4.7	12.6±2.2	10.0±5.1
No. of dead fetuses	21	0	0	0	0	0	0
Average no. of males per litter	21	5.6	5.8	6.5	6.0	5.6	7.2
Average wt of male fetuses	21	4.9±0.6	4.6±0.5	5.2±0.8	4.9±0.8	4.5±0.8	4.7±0.8
Average no. of females per litter	21	6.5	6.4	5.1	5.3	7.0	3.8
Average wt of females	21	4.7±0.5	4.4±0.5	4.7±0.8	4.6±0.6	4.2±0.6	4.5±0.4

composition is shown in Table II. The diets were prepared weekly and fed ad libitum. Fresh feed was placed in the feed jars, and old feed was discarded three times weekly. The diet was kept sealed in glass jars and refrigerated between feedings.

Groups of 25 male and 25 female Charles River rats (Sprague-Dawley originated) were fed one of the three diets from weaning in the first generation. During the second generation, the animals were exposed to the diets from conception. After reaching sexual maturity, the parent animals (F_O and F_{1b}) were mated in pairs three times in each generation (Fig. 1).

During the first 8 weeks of the first generation and the first 6 weeks of the second generation, the feed consumed and body weights were recorded weekly. Afterwards in each generation, no feed consumption records were kept, but the same procedure of placing fresh feed in jars and discarding the old three times weekly was continued.

Ten F_O rats of each sex per group were transferred into metabolism cages during the 7th week of the first generation, and their urine was collected while the rats were fasted. The final 12 hr sample in a 24 hr collection was tested for sugar, protein, ketones and bilirubin using Ames clinical test strips. In addition, urine nitrogen was determined by the Kjehldahl method.

Feces were collected with wire screen collectors fastened to the regular cages, during the final week of the prepubertal growth phase in the first generation. The feces were dried in vacuo at 76 C to a constant weight and pulverized. Samples of each were saponified, acidified and extracted with petroleum ether to determine the total fatty acids in the feces. These values and the TFA in the diets were used to calculate the coefficient of absorbability for the three fats. Additional samples of the feces were assayed for nitrogen by the Kjehldahl method.

The first two litters of each generation (F_{1a}, F_{1b}, F_{2a} and F_{2b}) were permitted to be born naturally. The pups were counted at birth and a gross inspection made for abnormal or dead ones. Four days after birth the pups were weighed and sexed. At that time all large litters were reduced to eight pups to equalize the stress of lactation. All F_{1a}, F_{2a} and F_{2b} litters were discarded at weaning (21

TABLE VIII

Effects of Used, Fresh or Hydrogenated Fat on Development of Rat Fetus

	Hydrogenated soybean oil					
	Fresh		Used		Unhydrogenated mixture	
Generation per litter examined	F_{1c}	F_{2c}	F_{1c}	F_{2c}	F_{1c}	F_{2c}
No. of litters	8	9	10	6	7	6
No. of fetuses examined for soft-tissue defects	65	75	82	49	64	41
Fetuses with soft-tissue defects	0	0	0	0	2[a]	1[b]
No. of fetuses examined for skeletal defects	30	37	33	23	24	19
Fetuses with skeletal defects	0	1	0	0	1[c]	0

[a]Folded retina.
[b]Hydrocephalia.
[c]Missing rib.

days after birth).

Thirty F_{1b} rats of each sex per group were selected on the basis of weight and thriftiness at weaning for the second generation. These rats were maintained on a growth study until sexual maturity. It was intended to be 8 weeks after weaning, as in the first generation, but because of their larger size at weaning, this phase was ended at 6 weeks. Consequently, no feces or urine collections were done. Five rats of each sex per group were sacrificed at that time for histopathology, leaving 25 pairs of breeders per diet.

For the teratology phases of the study, each generation parents (F_O and F_{1b}) were mated a third time. Pregnancies were confirmed by examining vaginal smears for cornified cells and spermatozoa, and the day these were found was designated day O of gestation. One-half of each group of females was sacrificed on day 13 and the other half on day 21. All of the females were examined for the number of implantations, resorptions and corpora lutea of pregnancy. In the near term females, the fetuses were removed and their positions in the cornua noted.

The fetuses were dried of amniotic fluid, carefully examined for gross abnormalities, sexed and weighed. One-third of each litter was cleared and stained with Alizarin Red stain (18) and examined for skeletal variations and defects. The remaining two-thirds was examined for soft-tissue abnormalities by the Wilson method (19).

At the time of the laparotomies, the parent rats were examined for gross pathology and the following tissues were removed, fixed in Bouin's solution, stained with hematoxylin-eosin and examined for histopathology: heart, lung, stomach, liver, kidney, adrenals, small and large intestine, spleen, gonads, bladder, pancreas and mesenteric lymph nodes. Tissues from F_{1b} animals sacrificed at the end of the second generation growth phase, mentioned previously, were examined in a similar manner. The data were analyzed statistically by the Analysis of Variance and Chi-square (20).

RESULTS AND DISCUSSION

The growth and feed consumption of rats during the first 8 weeks of the first generation and the first 6 weeks of the second generation following weaning, were not significantly different among the three groups (Tables III and IV). However the feed efficiency of the first generation male rats fed the used soybean fat was significantly lower than that of the other two groups. As was observed in previous studies, the absorbability of the used fat was significantly lower than that of the fresh fats. There was no difference between the coefficients of absorbability of the two fresh fats. The level of fecal nitrogen was significantly lower in first generation female rats fed the used fat, compared to both groups of females fed the fresh fats, but there were no differences in the levels of urinary nitrogen, protein, glucose, ketones or bilirubin. Furthermore there were no significant pathological differences due to the diet observed in rats in either generation after being exposed to these three fats for approximately 11 months. Thus the long term ingestion of a hydrogenated fat, either fresh or used extensively for frying foods, caused no impairment in the growth or general health of rats.

The results from the live born litters are shown in Tables V and VI. The average conception rate was 86% and was not different between groups. The conception rate was somewhat lower for all groups during the second and third litters of the second generation, but is attributed to the increased size and fatness of these parents compared to the first generation.

There were no significant differences in the sizes of the litters at birth, although a greater number of stillborn pups was noted in the first litters of dams fed the used soybean oil in the first generation. This is not considered to be a biologically significant observation, however, because comparable incidence of stillborn pups has occurred in the control groups of other studies of a similar design completed in our laboratory (21). Since this was an isolated event (one time in six different litters) it is not considered to be related to the diet. Similarly, in the second generation, the dams fed the unhydrogenated fresh fat weaned significantly fewer pups than those fed either the fresh or used hydrogenated fat, but again this was an isolated incident and is not considered biologically significant.

At 21 days after birth, the male pups in one of four litters and the female pups in three of four litters on the used fat, weighed significantly less than the pups of dams fed the two fresh fats. Since there were no differences in the pup weights at 4 days, these lower weight gains were probably the result of the reduced absorption of the used fat, as seen in the adult animals, after the pups began eating solid feed.

The numbers of corpora lutea, implantations and resorptions were not significantly different in rats sacrificed on day 13 of pregnancy in either generation (Table VII). Similarly, there were no significant differences in the numbers of live fetuses or their weights and no dead fetuses in the dams sacrificed on day 21 of pregnancy.

No gross malformations were observed in any of the natural born litters, but more important, few were found in those fetuses obtained by laparotomy, where one is assured of seeing all fetuses. Of 87 fetuses examined for soft-tissue defects during the first generation, only two were abnormal. They were in the group fed the fresh, unhydrogenated fat (Table VIII). Of 79 fetuses examined for soft-tissue defects in the second generation, only one was abnormal and was also in the group fed the unhydrogenated fresh fat. Of 166 fetuses examined for skeletal defects in both generations, only two were abnormal. One was in the group fed the unhydrogenated fat, and the other was in the group fed the fresh, hydrogenated fat.

These data indicate that a hydrogenated fat that is typical of what is now widely consumed in the U.S. diet (soybean oil, with an iodine value of 107), when fed either fresh or after having been used extensively for frying foods, does not adversely affect the reproduction proceesses of the rat even when fed at 15% in the diet for long periods of time. Nor was there any evidence of a cumulative or delayed effect on second generation animals. This evidence is consistent with our previous studies in both rats and dogs. Furthermore this study has shown that the development of the embryonic rat was not adversely affected by either a fresh hydrogenated fat or a similar one that had been used for the frying of foods.

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