Essential Fatty Acid-Deficient Rats: I. Growth and Testes Development

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ABSTRACT

Partially hydrogenated oils as the sole dietary fat enhances the development of essential fatty acid (EFA) deficiency in young rats. Partially hydrogenated herring oil (HHO) caused total impairment of the spermatogenic tissue after five weeks of experiment, while partially hydrogenated arachis oil (HAO) caused severe degeneration of this tissue in 15 weeks. A fat-free diet caused degeneration in 26 weeks. In the dietary fats, the total content of *trans* acids, calculated as elaidic acid, was 47% and 23% in HAO and HHO, respectively. Further, varying amounts of different positional isomeric fatty acids were also present in the partially hydrogenated oils. Besides the specific tissue changes, poor growth, poor feed efficiency and skin signs characteristic of EFA deficiency were noticed. On the other hand, partially hydrogenated soybean oil (HSO) as the sole dietary fat kept the animals normal in all respects. This oil still contained 32% linoleic acid; the total content of *trans* acids amounted to 11%, calculated as elaidic acid.

INTRODUCTION

Essential fatty acid (EFA) deficiency in weanling male rats causes very severe impairment of the spermatogenic tissue after about 24 weeks on a fat-free diet (1-3). It is our experience that diets containing partially hydrogenated vegetable oils as the sole dietary fat enhance the degeneration of the spermatogenic tissue as compared to fat-free diets (3-5). It should be mentioned that all the effects of feeding partially hydrogenated oils are prevented by the presence of small amounts of essential fatty acids. In the present studies, we are reporting on experiments comparing the effects on growth and testes development of weanling male rats fed on partially hydrogenated vegetable oils or a partially hydrogenated herring oil. Thus, the fatty acid spectrum in the experimental dietary fats is quite different. This fact may be of interest in case the fatty acid pattern as such, or the isomeric unsaturated fatty acids, are of importance for the degenerative changes in the spermatogenic tissue in EFA deficiency. Details on the fatty acid composition of the dietary fats and of the different lipid classes of the testes will be given in forthcoming publications (6,7).

EXPERIMENTAL PROCEDURES

One hundred and fifty weanling male rats were divided into five groups of 30 animals. Each of these groups was further divided into three subgroups of 10 animals. One subgroup from each of the main experimental groups was killed after 5, 15 and 26 weeks, respectively. Group 1 received a fat-free diet of the following composition: Vitamin Test Casein (Genatosan Ltd., Loughborough, England), 20%; sucrose, 74%; vitamin mixture [0.5 g of the vitamin mixture consisted of: biotin, 0.05 mg; folic acid, 0.2 mg; para-aminobenzoic acid, 1 mg; riboflavin, 1.5 mg; pyridoxine, HCl, 1.5 mg; thiamin, HCl, 1.5 mg; inositol, 5 mg; niacinamide, 5 mg; calcium pantothenate, 5 mg; ascorbic acid, 10 mg; tocopherol acetate, 5 mg (as Ephynal acetate, F. Hoffmann-La Roche & Co.); vitamin K, 0.5 mg (as Synkavit, F. Hoffmann-La Roche & Co.); vitamin B_{12}, 0.003 mg (as Bendogen, GEA); vitamin A, 1000 IU, and vitamin D_3, 100 IU (as Rovimix AD_3, 50/5, F. Hoffmann-La Roche & Co.); and sucrose, 500 mg], 0.5%; salt mixture [100 g of the salt mixture contained: NaCl, 6 g; Mg $(HCO_3)_2$, 5 g; HCl, 7 g; K_2HPO_4, 17 g; NaH_2PO_4, 2 H_2O, 10 g; Ca $(H_2PO_4)_2$, 2 H_2O, 17 g; Ca-lactate, 5 H_2O, 36 g; ferric citrate, 2 g. The following trace elements were added per 100 g of salt mixture: $Ca(IO_3)_2$, 6 H_2O, 5.81 mg; zinc carbonate, basic (56% Zn), 72 mg; $MnCO_3$ (47.8% Mn), 42 mg, NaF, 40 mg; $NaMoO_4$, 2 H_2O, 5 mg; $Cr_2(SO_4)_3$, 15 H_2O, 1.3 mg; SeO_2, 0.32 mg. In total: 185.43 mg per 100 g of salt mixture.] 5.0%; and choline chloride, 0.5%. Groups 2, 3, 4 and 5 received the fat-free diet in which 28 wt % of sucrose was replaced by 28 wt % of partially hydrogenated arachis oil (HAO) (Aarhus Oliefabrik Inc., Aarhus, Denmark), partially hydrogenated soybean oil (HSO) (Aarhus Oliefabrik Inc.), normally refined arachis oil (AO) (Aarhus Oliefabrik Inc.) or partially hydrogenated herring oil (HHO) (Dansk Sojakagefabrik Inc., Copenhagen, Denmark), respectively.

Diets and water were provided ad libitum. The animals were weighed and inspected week-

TABLE I

Fatty Acids of Dietary Oils (Wt %)

Fatty acids, per cent of oil	Partially hydrogenated arachis oil (HAO)	Partially hydrogenated soybean oil (HSO)	Arachis oil (AO)	Partially hydrogenated herring oil (HHO)
Saturated	29.3	19.8	19.1	41.9
Monoenoic	67.0[a]	41.4[b]	50.9[c]	44.8[d]
Polyenoic	3.3	39.0	29.9	13.2
$C_{18:2}$, ω6 Linoleic acid	0.8	31.8	28.8	0.6
$C_{18:3}$, ω3 Linolenic acid	—	3.4	1.1	—
Total trans acids (as Elaidic acid)	47	10.7	0	22.9

[a] $C_{16:1}$, 1%; $C_{18:1}$, 64.8%; $C_{20:1}$, 1.2%.
[b] $C_{16:1}$, 0.6%; $C_{18:1}$, 40.8%.
[c] $C_{16:1}$, 0.3%; $C_{18:1}$, 48.5%; $C_{20:1}$, 1.7%.
[d] $C_{16:1}$, 5.6%; $C_{18:1}$, 13.0%; $C_{20:1}$, 11.3%; $C_{22:1}$, 14.1%.

ly. On autopsy, after 5, 15 and 26 weeks, respectively, the left testis was removed, weighed and frozen for lipid analysis (6,7), while the right testis and epididymis were fixed in 4% formaldehyde for histological examinations.

RESULTS AND DISCUSSION

Dietary Fats

The dietary fats were all commercial products. However, the partially hydrogenated soybean oil was hydrogenated by means of a special technique, which primarily eliminated the high content of trienoic acid from the original oil. Gas chromatographic analyses of all the dietary fats are published in detail in a following publication (6).

A summary of the composition of the dietary fats is shown in Table I. The greatest discrepancies in composition are between the HHO and the other three dietary fats. HHO has a large content of different monoenoic fatty acids, especially $C_{20:1}$ and $C_{22:1}$ (6). In the HAO, the unsaturated fatty acid picture is dominated by $C_{18:1}$. The HSO and the AO contain about equal amounts of mono- and dienoic acids.

In the partially hydrogenated oils the contents of *trans* acids are considerable. The amount of these fatty acids in HSO is half that of HHO and one quarter that of HAO. The *trans* acid in HAO and HSO is practically all elaidic acid. In HHO, the *trans* acids comprise about equal amounts of $C_{18:1}$, $C_{20:1}$ and $C_{22:1}$. Further, several other isomers are also present, e.g., *cis*, *trans* $C_{20:2}$ and $C_{22:2}$, and *cis,cis,trans* 20:3 and 22:3. The content of conjugated fatty acids appears negligible in all four experimental fats.

Growth Rates and Skin Signs

On the EFA-deficient diets, growth rates began to level off after five to seven weeks, i.e., Groups 1, 2, and 5 (Fig. 1). The animals on the fat-free diet resumed growth during the 11th through the 14th week, and then again leveled off at a somewhat higher plateau. In contrast, the animals fed HAO or HHO gained very little weight from the 8th through the 25th week. The former of the two fats had twice as much *trans* acids as the latter (Table I), and both were very low in linoleic acid. The HSO contained even more essential fatty acid than the AO (Table I). The animals fed on the HSO diet grew slightly more than the controls fed AO. These results indicate that a possible stressing effect of partially hydrogenated dietary oils was eliminated or counteracted by the presence of EFA.

The scaliness of the feet, tail and skin did not develop to any great extent (Table II). The humidity in the animal quarters varied from 30-40% during the last two months. The yellow-brown pigment on the back of the animals developed normally in the non-EFA-deficient animals, Groups 3 and 4, but did not, or practically not, occur in the EFA-deficient rats (Table II).

Food Consumption

Measurement of food intake in the various groups was carried out through the 9th and 10th week, and again through the 24th and 25th week (Table II). The highest caloric con-

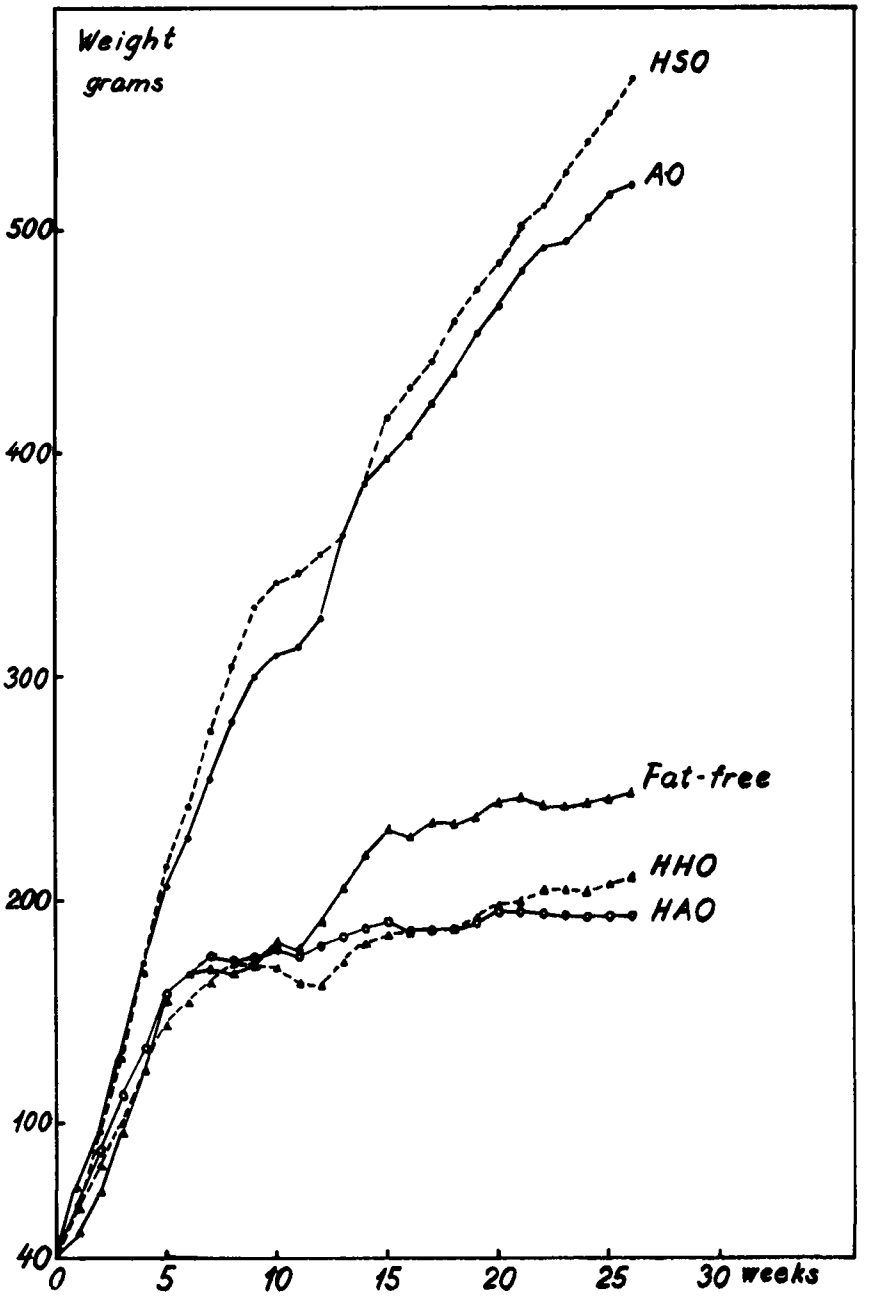

FIG. 1. Average growth rate of male rats fed on a diet with 28% partially hydrogenated soybean oil (HSO), 28% partially hydrogenated herring oil (HHO), 28% partially hydrogenated arachis oil (HAO), 28% arachis oil (AO), or a fat-free diet (Fat-free).

sumption during both periods was observed in the two groups fed AO or HSO (Table II). These animals also grew the most (Fig. 1). The weight increase in these animals was to a great extent due to very large quantities of depot fat.

The caloric intake per square meter surface during both testing periods (Table II) was greatest for the rats fed HAO or HHO, respectively. At the end of the experiment, a somewhat similar picture was obtained with the animals on

TABLE II

Scaliness, Pigmentation and Food Consumption Measurement

Group no.	Diet characteristics	Scaliness[a]		Pigment[b]		Food intake/animal/day					
						Average 8th-9th week			Average 24th-25th week		
		9th week	26th week	9th week	26th week	grams	calories[c]	calories/ sq.m/surface[d]	grams	calories[c]	Calories sq.m/surface[d]
1	Fat-free	1.8	1.2	0	0	11.0	41.4	1186	13.1	49.3	1105
2	Partially hydrogenated arachis oil (HAO)	1.7	1.5	0	0.7	10.0	51.6	1453	9.3	48.0	1270
3	Partially hydrogenated soybean oil (HSO)	0	0	1.7	2.4	12.6	65.0	1195	14.2	73.3	959
4	Arachis oil (AO)	0	0	1.7	2.1	11.2	57.8	1135	11.2	60.9	841
5	Partially hydrogenated herring oil (HHO)	1.5	1.1	0	0.6	9.7	50.1	1446	9.9	51.1	1281

[a]Dermal symptoms were scored with a range of 0 to 3 each for tail, forelegs, hindlegs and dandruff, and the average of these four scores per animal was calculated for the group.

[b]Yellow-brown pigment on the back of the animals was scored 0 to 3.

[c]Calculated by assuming that carbohydrate and protein yield 4 Cal/g, and fat 9 Cal/g.

[d]The surface area is calculated by the formula: surface area (sq.m) = $11.63\sqrt[3]{w^2}$, where w is weight in grams (17,18).

the fat-free diet (Table II). These data compared to those obtained with animals fed AO or HSO illustrate the poor caloric efficiency in the EFA-deficient animals. This corroborates the findings of previous experiments from this laboratory (8,9). The reduced caloric efficiency may possibly be explained, at least to some extent, as an uncoupling of the oxidative phosphorylation process (10-15). It is tempting to speculate that the present results may partially be explained as an effect of the fatty acid pattern of the dietary partially hydrogenated oils. Thus, the affinity for isomeric unsaturated fatty acids, e.g., in the build up of lipoproteins, may be much less than for EFA, and for the isomeric acids synthesized in EFA-deficiency. The incorporation of isomeric unsaturated fatty acids into lipoproteins may result in drastic changes in the physico-chemical characteristics of membranes and enzyme moieties, thereby directly influencing formation of normal cells, e.g., in spermatogenesis, or indirectly by disturbing the normal course of oxidative phosphorylation. However, the rather similar data obtained after long-term experiments with animals fed on the fat-free diet indicate that, apart from a possible specific role of dietary isomeric unsaturated fatty acids, a similar effect eventually results from the disappearance of EFA, which favors the biosynthesis of large quantities of positional isomers of unsaturated fatty acids.

Testes: Weight and Histology

On gross examination, no differences in size were observed between the left and the right testes from the same animal in any of the experimental groups.

After five weeks of experiments, the absolute and relative weights of the left testis from the animals fed HHO were significantly lower than those of any of the other groups (Table III). Histological examination of the right testis from the former animals revealed an almost complete degeneration of the spermatogenic tissue (Table III).

The fat-free diet and that with HAO also caused a remarkably lower absolute weight of the testis after five weeks compared to that of the EFA supplemented animals (Table III).

During the following 10 weeks the absolute and the relative weights of the testis decreased in the animals fed no fat or 28% HAO (Table III). The decrease in relative weight is to some extent explained by a weight increase in the animals on the fat-free diet (Group 1, Fig. 1), whereas the animals fed 28% HAO hardly gained in weight during this period (5th-15th week, Fig. 1). The decrease in testis weight

TABLE III

Testes: Average Weight of the Left Testis and Histological Examinations of the Right Testis and Epididymis of the Rats

Group no.	Diet characteristics	Left testis: weight (g)						Average degree of degeneration of spermatogenic tissue of *right testis and epididymis*[a]		
		5 weeks		15 weeks		26 weeks				
		absolute	relat.[b]	absolute	relat.[b]	absolute	relat.[b]	5 weeks	15 weeks	26 weeks
1	Fat-free	1.08 ± 0.04	0.70	0.94 ± 0.07	0.42	0.69 ± 0.04	0.28	1.0	2.5	3.9
2	Partially hydrogenated arachis oil	0.93 ± 0.04	0.61	0.70 ± 0.07	0.39	0.64 ± 0.05	0.33	1.6	4.0	4.9
3	Partially hydrogenated soybean oil	1.25 ± 0.03	0.63	1.51 ± 0.06	0.39	1.54 ± 0.04	0.28	0	0	0
4	Arachis oil	1.22 ± 0.06	0.62	1.55 ± 0.05	0.43	1.55 ± 0.07	0.30	0	0	0
5	Partially hydrogenated herring oil	0.49 ± 0.04	0.34	0.59 ± 0.03	0.35	0.76 ± 0.04	0.41	4.7	5	5

[a]Assessed from a scale graduated from 0 (no degeneration) to 5 (total degeneration) (3).
[b]Percentage of body weight.

occurred simultaneously with an increasing degree of degeneration of the spermatogenic tissue. These developments are further underlined by the findings that a much more severe degeneration of the spermatogenic tissue occurred in the animals on HAO than in those on the fat-free diet (Table III).

During the following experimental period (15th through 25th week), there was practically no change in body weight of the animals fed the fat-free or the HAO diets. However, the absolute and relative weights of the testis of these animals decreased and the degenerative changes of the spermatogenic tissue increased.

The HHO had only half the amount of *trans* acids observed in HAO (Table I). The former oil, however, contained about equal amounts of *trans* $C_{18:1}$, $C_{20:1}$ and $C_{22:1}$ and smaller amounts of *trans* dienes and trienes, whereas HAO contained practically all of its *trans* acids as elaidic acid. The partially hydrogenated oils also contained varying but rather small amounts of positional isomers (6). Whether the effects observed may, in part be ascribed to either the geometric or the positional isomers, or a combination of the two types of isomeric fatty acids is the subject of further studies.

The present findings raise two questions. First, the appropriateness of talking about degeneration of the spermatogenic tissue after five weeks of feeding 28% HHO to these young animals. Maybe the degeneration is merely a dietary effect resulting in a delay in the development of the spermatogenic tissue during the period of sexual maturation. In this case, it must be concluded from the results obtained during the last 21 weeks of the experiments (Table III) that the spermatogenic tissue never did develop in these animals. Davis et al. (16) have reported that changes in lipid composition occur in the rat testes at the same time as the appearance and maturation of the spermatids. Second, during the experimental period a slight increase in the absolute and relative weights of the testis was observed in these animals (Table III). Whether this weight increase concerns the deposition of lipids or their compositional changes will be shown by analytical work which is in progress.

It should be pointed out that the relative weights of the testes of Groups 1, 2, 3 and 4 (Table III), after 26 weeks of experiment, are almost equal, although the degenerative changes of those from Groups 1 and 2 are very severe, while the testes from Groups 3 and 4 are normal.

The present experiments underline the fact that partially hydrogenated oils devoid of EFA, as the sole dietary fat of rats, stress the EFA deficiency. Further, partially hydrogenated marine oils apparently have a stronger effect in this respect than partially hydrogenated vegetable oils and fat-free diets. The reduced caloric efficiency and the tissue degenerations described can possibly be explained as membrane changes or reduced enzymatic activity, or both, due to changes in lipoprotein structures.

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