sorption of oxygen occurs in unheated oils without showing the usual induction period even though the presence of antioxidants may be easily demonstrated. It may be that the fatty hydroperoxide-metal complex is part of the mechanism through which oxidation of the fat proceeds. The studies by Martell, Calvin, and others (14) as well as Myers and Zittlemoyer (18) on the reactions and properties of oxygen-carrying metal chelates offer some possibilities for explaining fat-oxidation catalysts.

Summary

Metal-inactivating agents, such as citric acid, sorbitol, lecithin, and carboxymethylmercapto succinic acid, are not active in unheated vegetable oils. Apparently trace metals present in normal glyceride oils are held within a complex of unknown structure. After heating an oil, the metals can be complexed by metal-inactivating agents, such as citric acid. The release of metals appears to be associated closely with the breakdown of the fatty acid hydroperoxides. Formation of some association or complex between the metal and the hydroperoxide group or between the metal and the unsaturated linkage of the fatty hydroperoxide is suggested. The metals are held very tenaciously within this unknown structure. Although the metal is not available as an uncomplexed metallic ion, it does behave as a very strong pro-oxidant catalyst. The application of heat releases the metal so it can be complexed by added metal inactivators.

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The Effect of Mono-enoic Fatty Acid Esters on the Growth and Fecal Lipides of Rats

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THERE IS NOW EVIDENCE that when erucic acid or its ester is fed to rats, their growth is less than when shorter chain fatty acids are fed. Thomasson and Boldingh (9) observed a consistent retardation of growth on feeding rapeseed oil and showed that this was due to the erucate content of the oil. Carroll and Noble (2) also noted growth retardation by erucic acid and methyl erucate as compared to oleic acid or methyl oleate. They further noted that erucic acid and ester increased the fecal excretion of cholesterol.

Less is known concerning eicosenoic acid. Carroll and Noble (2) fed eicosenoic acid to a small number of rats and observed a retardation of growth and an increase in fecal cholesterol of about the same order as that caused by erucic acid.

In the course of an investigation into the deposition of various fatty acids in the body fat of rats by Hopkins *et al.* (5) the opportunity was afforded of comparing the effect of corn oil, methyl oleate, methyl 11-eicosenoate, and methyl erucate on the growth of rats, and on the excretion of fecal lipides. The following is an account of these investigations.

Experimental

The rats were fed a purified diet which contained 5% corn oil, or the pure methyl ester of oleic, eicosenoic, or erucic acids. Four groups of 5 males and 5 females were fed each diet and a fifth group was fed a similar diet in which the fat was replaced by an equal weight of corn starch. The methyl esters were prepared in small amounts as previously described (5) and were used promptly to reduce the danger of oxidation. Details of the diets, method of feeding, and handling of the rats have been published previously (5). At weekly intervals the rats were weighed and examined for symptoms of essential fatty acid deficiency. Feces were collected during the second, third, fourth, fifth, and twelfth weeks of the experiment and were kept frozen until analyzed.

At the end of 12 weeks the males were killed by decapitation, examined for gross pathological changes, and frozen for later analyses. Sections of the lung, heart, arteries, liver, kidney, and bladder were taken for histological study. The females were bred to normal males and were fed the same diets until their litters were weaned, a further eight weeks.

Feces were dried, ground, and allowed to stand in hexane (petroleum ether) containing hydrochloric acid to convert soaps to free fatty acids. Extraction was carried out in Soxhlet extractors with the same hexane-acid mixture. The extracted lipide was weighed and saponified with 10% alcoholic KOH; the unsaponifiable fraction was extracted with hexane. The soaps were acidified, and the acidic material was taken up in ethyl ether. The residue from the evaporation of the ether was dissolved in hexane, and the soluble portion was collected.

Results

Table I shows the final body weights, food consumed, and food efficiencies of both male and female rats. Although it appeared that methyl erucate retarded the growth of the males, statistical analysis showed that this difference was not significant at P = 0.05. The females however gained significantly more weight on the corn oil diet than on any of the ester diets. Food intake and food efficiency were not altered significantly by any of the pure esters. Values from the group fed the low-fat diet were not included in any of the statistical analyses.

TABLE I
Body Weight, Food Consumption, and Food Efficiency of Rats Fed Various Fats for 10 Weeks

Fat in diet	Body weight (g.)		Food eaten (g.)		Food efficiency	
	Male	Female	Male	Female	Male	Female
None	194	146	779	803	.202	.140
Corn oil	195	157	693	632	.238	.192
Oleate	192	134	688	601	.234	.172
Eicosenoate	191	139	701	607	.227	.178
Erucate	170	138	595	590	.245	.186

Moderately severe symptoms of essential fatty acid deficiency, specifically scaliness of the tail, were observed in rats on the pure ester diets as early as the seventh week of the experiment. This did not occur in rats receiving the low-fat diet, which suggested that, on a diet low in linoleic acid, the addition of other fatty acids hastened the depletion of the linoleic acid.

Neither gross examination nor histopathological studies revealed any abnormality that could be attributed to the diet. During the course of the experiment two rats, one from the corn oil and one from the erucate diet, died of undetermined causes.

At the end of 12 weeks the females were bred to normal males. Three of the four females fed corn oil had litters totalling 18 rats, of which 10 were weaned at an average weight of 23 g. Of 12 rats fed the pure esters, six had litters totalling 50 rats, 15 of which reached weaning age with an average weight of 17 g. While the poor performance of the animals fed corn oil indicated that the diet was inadequate for breeding purposes, the even poorer performance of the rats fed the fatty acid esters was probably due to a linoleic acid deficiency. There appeared to be no difference between the ester diets in this respect.

Analysis of the fecal lipides are presented in Table II. To simplify comparison between weeks, these are given in terms of percentage composition; but since the weights of dry feces were relatively constant, these figures reflect the amounts of fat excreted. The lipide content of the feces of rats fed corn oil, oleate, and eicosenoate was similar and somewhat less than that of rats on the erucate diet. Differences in the excretion of hexane-soluble fatty acids did not appear until after the second week but thereafter, as illustrated by the data from the fifth week, was greatest for the erucate and eicosenoate diets. At the second week the excretion of unsaponifiable matter was greatest on the erucate and corn oil diets. The same trend was evident in the third and fourth weeks as well although the figures are not shown in the table. By the fifth week however, there was little difference between any of the treatments.

TABLE II
Total Lipide, Unsaponifiable Fraction, and Hexane-Soluble Fatty Acids Expressed as Percentage of Dried Feces

	Total lipide		Hexane-soluble fatty acids		Unsaponifiable fraction	
Week of test	2	5	2	5	2	5
Dietary fat						
None	3.3	4.0	1.3	1.0	1.4	1.5
Corn oil	4.3	5.5	1.2	1.6	2.2	1.7
Oleate	4.8	4.5	2.1	1.3	1.6	1.9
Eicosenoate	4.8	5.7	1.9	3.0	1.5	1.6
Erucate	6.8	8.7	1.9	4.6	3.5	1.8

As shown in Table III, the iodine value of the mixed fatty acids approached a value of about 50 at the end of the experimental period for all diets except that containing methyl erucate. In the case of the corn oil, oleate, and eicosenoate diets this value represents a decrease from that found earlier in the experiment. The iodine number of the excreted lipides when erucate was fed increased somewhat throughout the experiment and approached that of pure erucic acid.

TABLE III
Iodine Value of Hexane-Soluble Fecal Acids

Dietary fat	Week of test		
	2	3	12
None	41.7	46.4	50.2
Corn oil	65.5	63.5	53.4
Oleate	59.5	50.3	49.8
Eicosenoate	58.2	50.4	50.7
Erucate	64.4	67.6	69.6

The composition of the hexane-soluble acids was determined approximately by ultraviolet absorption analysis and is shown in Table IV. The composition was quite similar for the first three diets, but there was an increase in mono-enoic acid when eicosenoate was fed and a marked increase on the erucate diet with a corresponding reduction in the proportion of saturated acids. The hexane-soluble fecal acids from the animals fed methyl erucate were treated with alkaline permanganate; 13,14-dihydroxybehenic acid was obtained in quantity. After recrystallization it melted at 128–129°, alone and mixed with an authentic sample. The equivalent weight by titration was 373.5 (theory 372.6). This result confirms the presence of a large proportion of erucic acid in the feces fat.

TABLE IV
Composition of Hexane-Soluble Fecal Acids

Dietary fat	Acid, %				
	Diene	Triene	Tetraene	Mono-ene	Saturated (by difference)
None	4.6	0.3	0.1	45.2[a]	49.8
Corn oil	7.2	0.3	0.2	43.2[a]	49.1
Oleate	3.4	0.2	0.1	47.6[a]	48.7
Eicosenoate	1.7	0	0	58.3[b]	40.0
Erucate	2.5	0.2	0.1	85.6[c]	11.6

[a] Calcd. as oleic acid. [b] Calcd. as eicosenoic acid. [c] Calcd. as erucic acid.

While the experiment was not designed to permit a critical study of digestibility, it was possible, from a knowledge of the true fatty acids excreted and the esters ingested during the same period, to estimate the coefficient of digestibility on a group basis. This has been done for methyl eicosenoate and erucate for the second to the fifth week of the experiment. Both appeared to be almost fully digested early in the experiment. At the end of five weeks the coefficient of digestibility of eicosenoate was 96 while that of erucate had fallen to 90.

Discussion

It has been demonstrated by others (9, 2) that erucic acid depresses the growth of rats when fed at a relatively high level. In the experiment described here, methyl erucate, eicosenoate, or oleate, comprising 5% of the diet, did not decrease the weight gain of male rats significantly below that of rats fed corn oil. The growth of females however was depressed significantly by each of the pure esters. Unpublished data from this laboratory indicate that a higher level of erucate results in a significant growth depression in males. Neither the food intake nor food efficiency was reduced significantly by any of the ester diets.

It has been observed (8, 3) that the addition of cholesterol or coconut oil to fat-free diets hastens the appearance of essential fatty acid deficiency symptoms. Each of the three fatty acid esters tested in this experiment exhibited this property and did not differ from each other in this respect. This observation was borne out by an analysis of the body fat (5), which showed that rats fed fatty acid esters had lower reserves of linoleic acid than did rats fed a fat-free diet. There were no gross or histopathological symptoms, other than scaliness of the tail, that could be attributed to an essential fatty acid deficiency.

The amounts of all three components of fecal lipide, unsaponifiable matter, true fatty acids, and acidic matter not soluble in hexane were greater on erucate than on oleate or eicosenoate diets. These observations indicated that feeding erucate caused an alteration in the normal lipide metabolism. As has been reported in a previous paper (5), erucic acid is less readily stored than are linoleic or eicosenoic acid.

The excretion of total lipide by rats fed the eicosenoate diet was not appreciably greater than by rats fed oleate or corn oil. Eicosenoate however increased slightly the excretion of true fatty acids.

The increased excretion of unsaponifiable matter on the corn oil and erucate diets corroborates the observations of Eckstein and Treadwell (4), Lin, Karvinen and Ivy (6), and Carroll and Noble (2).

The approximate analysis of the true fatty acids by ultraviolet absorption confirms the conclusion of Norcia and Lundberg (7) that the composition of the fecal fat is not altered by the variation of diet within fairly wide limits. The results of the analyses agree with the figures given by Norcia and Lundberg for fat-free and oleate diets. It is clear that the feeding of erucate produced marked change in the composition of the excreted fatty acids, which on the erucate diet consisted very largely of erucic acid. Bernhard *et al.* (1) found a similar high content of erucic acid (72%) in the fecal fatty acids of rats fed rapeseed oil. Despite the fact that the composition of the fecal fat was altered by feeding erucate, the amount excreted was small and the coefficient of digestibility was no lower than 90% for erucate. The corresponding figure for eicosenoate was about 95%. These figures are both much higher than those reported by Carroll and Noble (2) for methyl erucate and eicosenoic acid. This may be caused in part by the fact that Carroll calculated the coefficients on the total lipide excreted rather than on the excreted fatty acids as was done in this work.

Summary

The growth rates of rats fed a purified diet containing 5% corn oil or the methyl esters of oleic, eicosenoic, or erucic acid have been compared. At this level none of the esters significantly depressed the growth of male rats, but all three reduced the growth of females below that of those fed corn oil. Food efficiency and food consumption were not significantly reduced by any of the esters. Symptoms of essential fatty acid deficiency occurred only among rats fed the fatty acid esters and not among rats fed the fat-free diet.

Methyl erucate increased the excretion of true fatty acids, acidic material not soluble in hexane and unsaponifiable matter, as compared to methyl oleate. The true fatty acid excreted was largely erucic. Methyl eicosenoate gave results not appreciably different from methyl oleate except for a slight increase in the excretion of mono-unsaturated fatty acid from rats fed eicosenoate. Both corn oil and methyl erucate increased the excretion of unsaponifiable matter. The coefficient of digestibility of methyl eicosenoate and erucate were 96% and 90%, respectively, which are higher values than have been previously reported.

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