of formation is greatly reduced by it. This suggests that cations may act catalytically when the unsaturated fats are associated with tissue components. The mechanism, however, is not clear. Most tissues contain antioxidants which may be chelating agents, from which a cation, possibly iron, could be released by the action of ultraviolet light to catalyze lipide peroxide formation. EDTA would chelate the cation and inhibit its catalytic activity. There is no explanation at present for the effect of EDTA on the antioxidant action of ascorbic acid on pure methyl linolenate.

Since the succinoxidase inhibition both in the presence and absence of EDTA is directly proportional to the lipide peroxide concentration, it appears that lipide peroxides mediate to a great extent the effect of ultraviolet light on this system.

Summary. 1. EDTA inhibits lipide peroxide formation in rat liver homogenates and in rat liver mitochondria exposed to ultraviolet irradiation. 2. Ultraviolet light inhibits succinoxidase in mitochondria and EDTA protects it from inactivation. The protection is proportional to the inhibition of lipide peroxide formation. 3. EDTA does not inhibit lipide peroxide formation in pure methyl linolenate emulsions irradiated with ultraviolet light but does inhibit both the antioxidant effect of ascorbic acid and the iron catalysis of peroxide formation in these emulsions.

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Effect of Dietary Lipids on the Lipids in Rats' Milk.* (23511)

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It is well known (for reviews see 1,2,3) that the amount and type of fat in the diet of ruminants can affect the amount and particularly the composition of the milk fat. With monogastric animals these relationships have received less attention. Gogitidse(4) concluded that the iodine number of the milk fat increased after feeding of hemp seed oil or linseed oil to women. Mueller and Cox(5) reported that the iodine number of the milk fat of rats followed that of the fat fed, and that "this relation can be expressed as a straight line function." Loosli *et al.*(6) reported that feeding of corn oil or ethyl linoleate to lactating rats significantly increased the iodine number of milk fat recovered from the stomachs of the young immediately after

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TABLE I. Unsaturated Fatty Acid Content of Rations and Their Lipid Components.

Ration or lipid	Fatty acids,* g/100 g of diet or lipid		
	Dienoic	Trienoic	Tetraenoic
Ration AA_{20}	.283	none	none
" AA_{21}	1.49	.099	.003
" OC_1	3.60	.217	.015
Corn oil	58.8	1.00	.0
Wheat germ oil	48.2	4.42	.15
Fat from rolled oats	35.7	2.00	.21
Methyl linoleate-urea complex†	22.6	none	none

* Calculated as linoleic, linolenic and arachidonic acids respectively.
† Dried *in vacuo* at 50°C.

suckling.

Experiments in this laboratory(8) afforded an opportunity to determine the polyenoic acids in milk of rats which consumed diets containing either methyl linoleate or 3% of vegetable oil as the only source of fatty acids.

Methods. The milk was collected by the method of Luckey *et al.*(9) from rats towards the end of their second reproduction-lactation cycle. Milk donors and their dams had been fed exclusively ration AA_{20} or AA_{21}, previously described in detail(8), since weaning and throughout pregnancy and lactation. Ration AA_{20} was assembled from compounds of known chemical structure and contained sucrose, 16 amino acids, 10 salts, 14 vitamins, choline chloride, propylene glycol and 1.25% of methyl linoleate urea complex. Ration AA_{21} contained the same ingredients as ration AA_{20} except that 3% of a 2:1 mixture of wheat germ oil and corn oil was substituted for the methyl linoleate complex, alpha-tocopherol and part of the sucrose. The polyenoic acid content of rations and their lipid components are shown in Table I. Two specimens of milk fat were also available which had been collected from rats fed ration OC_1(10) which contained 84% rolled oats, 6.5% casein, 2% wheat germ oil, 1% corn oil, salts and vitamins. The milk was stored under nitrogen at −15°C until analyses were made. In a few instances fat of freshly collected milk was separated by centrifugation, washed with water and then stored as indicated. The milk was extracted 3 times under reflux and a slow stream of nitrogen with about 5 times its volume of a mixture of ethanol-diethyl ether (3:1) for 30 minutes at 70°C. Cooled extracts were filtered through Whatman No. 40 filter paper and the latter washed with more hot solvent. Ether was removed from combined extracts and washings at about 35°C with a stream of purified nitrogen, the alcoholic residue was diluted to a convenient volume and aliquots were used for determination of dry weight and for alkali isomerization. As suggested by Holman(11) the ethanolic solution of lipid was used directly for alkali isomerization which was carried out according to procedures of Herb and Riemenschneider(12). In accord with others(13), who have analyzed milk fat, preliminary experiments showed that saponification prior to alkali isomerization did not affect the results obtained with our specimens and it was not used. Measurements of absorbance at 233, 268, 315 and 346 mμ were made before and after alkali isomerization. Concentration of polyenoic acids was calculated by formula C of Herb and Riemenschneider(12) in terms of linoleic, linolenic and arachidonic acids although the chain lengths and the positions of double bonds of unsaturated fatty acids in rats' milk are admittedly not known. Absorbance before alkali isomerization, presumably due to the presence of conjugated polyenoic compounds, contributed in milk from rats fed ration AA_{20} 12.7 and 5.5% of the values calculated for unconjugated dienoic and trienoic acids respectively; with milk fat from rats fed ration AA_{21} the corresponding values were 3.8% and 3.6% respectively. The lipid components of the rations were also analyzed by alkali isomerization.

Results. Table II presents a summary of analyses for polyenoic acids in milk fat. Rats which were maintained on a diet devoid of trienoic and tetraenoic acids were able to secrete fat which, on the basis of spectrophotometric evidence, contained significant quantities of unconjugated trienoic and tetraenoic acids. When polyenoic acid content of the ration was increased (Table I) by addition of natural oils, total fat content of the milk was decreased by 28% but the milk fat had a greatly increased concentration of dienoic acid and a less marked but significant increase in trienoic

TABLE II. Effect of Diet on Unsaturated Fats in Rats' Milk.

Source of fat	No. or type of specimen	Fatty acids as % of total fat		
		Dienoic	Trienoic	Tetraenoic
Milk of rats fed ration AA_{20} (fat content of milk 12.2 ± .92%*)	22	1.86 ± .09*	.71 ± .01	.35 ± .03
Milk of rats fed ration AA_{21} (fat content of milk 8.8 ± .98%*)	16	6.66 ± .38*	1.02 ± .06	.84 ± .05
Milk of rats fed ration OC_4	Pooled, purified specimen†	17.6	1.37	1.08
Idem	Pooled, centrifuged fat‡	36.8	2.63	2.59

* Stand. error of mean.

† Specimen prepared by R. L. Glass from pooled rats' milk, deproteinized with equal vol of 95% ethanol, extraction of alcohol soluble fraction with 5 volumes of a mixture of diethyl ether and petroleum ether (1:1, v/v), drying over Na_2SO_4, removal of solvent and storage under nitrogen at −15°C.

‡ Specimen prepared by R. L. Glass from pooled rats' milk by centrifugation; used without further purification or extraction.

and tetraenoic acids. A 5.2-fold increase in dienoic acid content of the diet brought about a 3.6-fold increase in dienoic acid of milk fat.

Two specimens of pooled milk fat, from rats fed a ration of natural products which supplied 3.6% of dienoic acid, contained 17.6% and 36.8% of dienoic acid. In another specimen, collected from a single rat fed ration OC_4, extracted and analyzed as described above, concentrations of dienoic, trienoic and tetraenoic acids were 21.1%, 1.41% and 1.03% respectively. In comparison with fat from cows' milk for which a maximum value of 2.7% of dienoic acid has been reported(14) the content of the polyenoic acids in fat of rats' milk is unusually high. In the unpurified, pooled specimen of milk fat, from rats fed ration OC_4, which contained 36.8% of dienoic acid, the absorbance at 233 millimicrons before alkali isomerization was only 2.7% of that determined afterwards; hence, most of the dienoic acid appeared to be of the unconjugated type and of dietary origin.

The concentration of polyenoic acids in dietary fats reported in Table I falls into the range of values reported by others(15,16,17).

The chemical nature and origin of unsaturated fatty acids found in milk fat of rats fed ration AA_{20} cannot be ascertained with certainty from available evidence. The analytical methods used give no clue to maximum chain length of the polyenes. The dienoic acid is no doubt linoleic acid of dietary origin since the rat cannot synthesize this compound at a rapid rate. Holman(18) and Deuel and Reiser(19) have reviewed evidence for conversion of linoleic acid to arachidonic acid which, according to recent work(20) can apparently occur in the liver of the rat. We are not aware of evidence for conversion of linoleic acid to linolenic acid. However, Mead and Slaton(21) isolated recently from pooled tissues of 25 male rats, maintained on a fat-free diet, 118 mg of hexabromide of a compound assigned the structure of 5,8,11-eicosatrienoic acid. They suggest that this was formed by reduction of arachidonic acid and not as an intermediate in conversion of linoleic acid to arachidonic acid. Hence it might arise indirectly from linoleic acid. Dam *et al.* (22) have recently reported that total trienoic acid increased in tissues of rats during 18 weeks maintenance on a nearly fat-free diet and attributed this to conversion of linoleic to trienoic acid. If it is assumed that a litter of rats, while solely dependent on mother's milk, makes a weight increment of 200 g of fresh weight or 50 g dry weight(23) with a 35% efficiency(24) of conversion of milk solids into body solids, and that the fat content of rats' milk(25) is 40% of the milk solids, the total output of trienoic acid, during this period, of a rat fed ration AA_{20} would be about 0.4 g. This is about twice the amount of tetraenoic acid produced from linoleic acid (20,26) and greatly exceeds the quantity of the C_{20}-trienoic acid found in tissues of rats fed a fat-free diet(21). There is no assurance

that polyenoic acids found in milk fat from rats fed rations containing natural oils were the same as those observed when the methyl linoleate-urea complex was the only source of dietary fatty acid.

Microbiological synthesis of trienoic acid in the intestinal tract cannot be excluded as a possible source of polyenoic acid in milk fat although coprophagy has hitherto not been reported as a complicating factor in development of dietary deficiency of polyenoic acids. It appears more likely that trienoic acid was formed in the mammary gland or elsewhere, under stimulus of lactation, in amounts far exceeding those which non-lactating young rats can synthesize, if at all, on a fat-free diet.

These studies demonstrate clearly that concentration of dienoic and, to a smaller extent, of trienoic and tetraenoic acids in milk of a monogastric animal can be markedly elevated by an increased consumption of such compounds. Whether such an increase, over amounts needed to meet requirements for linoleic acid, is of nutritional benefit to the young organism is not known but this question merits attention with respect to possible long-range dietary effects of unsaturated fatty acids(27,28).

Summary. Spectrophotometric determinations of polyenoic acids showed that the milk of rats whose diet contained methyl linoleate as the only source of fatty acid contained significant quantities of unconjugated dienoic, trienoic and tetraenoic acids. Feeding of vegetable oils caused a great increase in concentration of unconjugated dienoic acid and, to a smaller extent, an increase of trienoic and tetraenoic acids in milk fat.

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