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Intestinal Absorption and Lymphatic Transport of Methyl Linoleate Hydroperoxide and Hydroxyoctadecadienoate in the Rat

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Methyl linoleate hydroperoxide and hydroxyoctadecadienoate were given intragastrically to thoracic duct cannulated rats. Triglycerides containing hydroxyoctadecadienoate were present in the lymph lipids, but relatively small recoveries were obtained. The results confirm the claim that lipoperoxides are reduced to hydroxy-compounds in the intestinal mucosa, and they indicate that a preferential metabolism of lipoperoxides and the corresponding reduced compounds takes place in that tissue.

The digestion and absorption of lipoperoxides have been studied previously by Glavind and Tryding.[1] Peroxidized olive oil was split by pancreatic lipase with the formation of peroxide-containing fatty acids. When peroxidized ethyl linoleate or olive oil was given perorally to thoracic duct cannulated rats, no lipoperoxide was found in the thoracic duct lymph. The hypothesis was advanced that ultimate "deperoxidation" takes place in the intestinal mucosa.

Andrews *et al.*,[2] who fed autoxidized soybean oil to rats with thoracic duct fistulae, arrived at similar conclusions. Ethanolic solutions of thoracic duct lymph lipids showed an increased optical density at 232 nm. This suggested that a reduction of the hydroperoxido-acids had taken place.

Nishida and Kummerow[3] observed that feeding of highly purified methyl linoleate hydroperoxide to thoracic duct cannulated rats resulted in an inhibited lipid absorption, but when a 20 % solution in methyl linoleate was administered, an increased optical density at 233 nm of the lymph fat was observed.

Lipoperoxides may occur in adipose tissue of rats, fed a vitamin E-deficient diet high in polyunsaturated fatty acids. This occurrence may hypothetically be explained by antioxidant properties of vitamin E which prevent the autoxidation of unsaturated fatty acids *in vivo*. However, this function of vitamin E is under dispute.

Bunyan *et al.*,[4] for example, claim that lipoperoxides found in adipose tissue are exogenous, and propose that vitamin E prevents their intestinal absorption.

As the intestinal absorption of lipoperoxides seems to be important to an understanding of the biological role of vitamin E, this process has now been re-investigated in the thoracic duct cannulated rat.

MATERIALS AND METHODS

Methyl linoleate was purchased from the Hormel Institute, Austin, Minnesota. Triolein (BDH) was purified on an alumina column. Methyl linoleate hydroperoxide was prepared by autoxidation of the methyl esters from safflor oil at room temperature, followed by partition between petroleum ether and 85 % ethanol. Final purification was obtained by chromatography on highly purified silica (Kieselgel HR, Merck). The peroxide content determined by the iodometric method was 5000 μequiv./g (theoretical value 6135). The content of conjugated diene estimated from the optical density at 232 nm was 78 %. Thin-layer chromatography on silica gel G, solvent system petroleum ether-ether-chloroform (6:3:1) revealed a massive spot of the hydroperoxide (R_F about 0.60) and only insignificant extra bands. Methyl hydroxyoctadecadienoate was prepared from methyl linoleate hydroperoxide by reduction with stannous chloride. The content of conjugated diene was 79 %. Thin-layer chromatography revealed a broad band more polar than the hydroperoxide (R_F about 0.45), a thin band of unreduced hydroperoxide and insignificant extra zones.

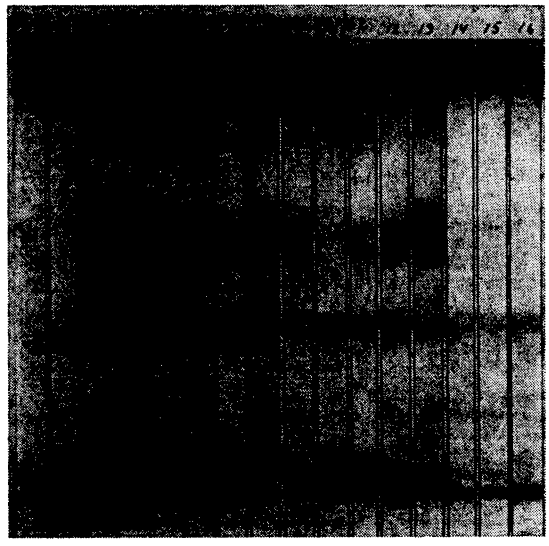

Fig. 1. Thin-layer chromatography of thoracic duct lymph lipids. Designation of samples as in Table 1.

Thoracic duct cannulated rats were prepared and treated as described previously (Sylvén and Borgström [5]). 0.2 ml methyl linoleate or its derivatives together with 0.4 ml triolein were administered intragastrically, followed by 2.0 ml isotonic saline. Lymph was sampled in heparinized bottles under nitrogen. One sample was taken for the first 6 h, and a second for the following 18 h. The samples were immediately frozen until extraction. This was done according to the Folch procedure under nitrogen.

The samples were dissolved in peroxide-free hexane (analytical degree, suitable for UV-spectroscopy). Aliquot samples containing about 0.4 mg lipid were taken for thin-layer chromatography on two plates of silicagel G. The solvent system was petroleum ether-ether-chloroform (6:3:1), and development was carried out twice. One of the plates was sprayed with the leuco-dichlorophenolindophenol-hemin reagent for peroxides.[6] No spot was seen in any of the plates. The second plate was sprayed with 50 % sulfuric acid, followed by charring (Fig. 1).

Aliquot samples containing about 20 mg lipid were evaporated and taken for the determination of peroxide by the colorimetric thiocyanate method.[7] The results are presented in Table 1.

Other aliquot samples were diluted to a suitable concentration with ethanol and examined spectroscopically in the range 224 – 240 nm, and the optical density at 232 nm was measured. The results, calculated as $E(1\ \%,\ 1\ \text{cm})$, are presented in Table 1. The content of conjugated diene ester was calculated from the optical density at 232 nm using $E(1\ \%,\ 1\ \text{cm}) = 813$ for methyl *cis-trans* linoleate hydroperoxide (Cannon *et al.*[8]), which corresponds to $E(1\ \%,\ 1\ \text{cm}) = 855$ for methyl hydroxyoctadecadienoate. The latter figure was used to calculate the amounts of conjugated diene acids recovered in the lymph samples.

In a second experiment, 0.2 ml methyl linoleate hydroperoxide without dilution with triolein was administered followed by saline. Thoracic duct lymph was sampled, and the extract was examined as in the first experiment. When the chromatographic plate was sprayed with the leuco-dichlorophenolindophenol-hemin reagent, weak spots were seen on the point of application of samples 4, 5 (strongest), 6, and 7, indicating the presence of peroxides. A second plate sprayed with 50 % sulfuric acid followed by charring is depicted in Fig. 2. The results of the physico-chemical examination are presented in Table 2.

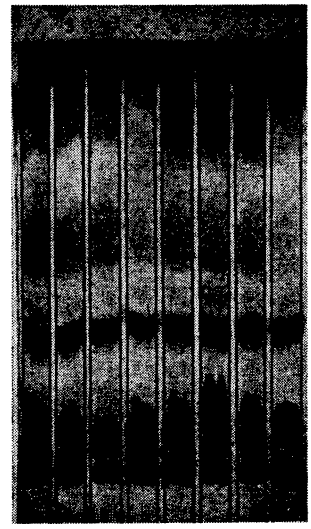

Fig. 2. Thin-layer chromatography of thoracic duct lymph lipids. Designation of samples as in Table 2.

Table 1. Recovery of fatty acid esters in lymph after feeding to rats. Methyl esters, 0.2 ml, diluted with triolein, 0.4 ml, administered.

Chromatogram No.	Rat No.	Substance given	Lymph			Lymph lipids		
			Period h	Volume ml	Weight mg	Peroxide μequiv./g	E(1 %, 1 cm) 232 nm	Conjugated diene, %
1	1	Triolein alone	0— 6	2	238.5	5	(8)	
2			6—24	6	99.3	16	(6)	
3	2	Methyl hydroxy-octadecadienoate	0— 6	4	93.0	9	89	10.4
4			6—24	5	245.0	7	74	8.7
5	2	Methyl linoleate hydroperoxide	0— 6	2	36.7	11	25	2.9
6			6—24	4.6	222.8	4	34	4.0
7			24—30	1.5	30.4	11	(8.5)	
8	3	Methyl hydroxy-octadecadienoate	0— 6	4.5	117.4	15	79	9.2
9			6—24	7.8	245.8	7	69	8.5
10	3	Methyl linoleate hydroperoxide	0— 6	1.5	29.5	41	17.5	2.0
11			6—24	3.8	99.5	10	21	2.5
12	4	Methyl hydroxy-octadecadienoate	0— 6	1.7	24.5	37	46	5.3
13			6—24	5.5	238.8	7	74	8.7
14	4	Methyl linoleate	0— 6	2.7	119.3	8	(10)	
15			6—24	5.5	226.1	3	(9)	
16			24—30	1.5	26.6	22	(20)	

Bracketed figures mean that no absorption maximum about 232 nm was registered, and consequently no reliable estimate of conjugated dienes could be made.

RESULTS AND DISCUSSION

In the following it is assumed that absorbed peroxides, if transported from the intestine, are transported *via* the thoracic duct, and not *via* the portal route. Small amounts of peroxide were found in all the samples of thoracic duct lymph lipids in the first experiment, also those from the rats which had not been given peroxide orally. No peroxide could be detected on the chromatographic plate, and the values found by the thiocyanate method may represent artefacts formed during the sampling and extraction of the lymph. More elevated amounts of peroxide were found in some of the samples from the second experiment, and the corresponding chromatograms indicated the presence of peroxides in the most polar lipids. A preferential incorporation of hydroperoxido-fatty acids in the phospholipids seems unlikely, and the possibility that artefacts have been formed cannot be ruled out.

Table 2. Recovery of methyl linoleate hydroperoxide in lymph after feeding to rats. The hydroperoxide, 0.2 ml, was administered undiluted.

Chromatogram No.	Rat No.	Lymph			Lymph lipids		
		Period h	Volume ml	Weight mg	Peroxide μequiv./g	E(1 %, 1 cm) 232 nm	Conjugated diene, %
1	1	0 – 6	2.5	23.8	58	41	4.8
3	2	0 – 6	19.0	66.0	14	(26)	
4		6 – 24	12.0	110.2	17	(20)	
5	3	0 – 6	1.4	4.5	86	(14)	
6	1	0 – 6	1.8	12.8	20	30	3.1
7		6 – 24	2.3	17.5	115	33	3.4
8	2	0 – 6	15.0	27.9	40	(23)	
9		6 – 24	23.0	112.2	9	(7)	

Bracketed figures mean that no absorption maximum about 232 nm was registered, and consequently no reliable estimate of conjugated dienes could be made.

The spectra from all the lymph samples taken during the first 24 h after the feeding of methyl linoleate hydroperoxide or methyl hydroxyoctadecadienoate showed maxima about 232 nm, indicative of the presence of conjugated double bonds in the lipids. No maxima were present when triolein alone or methyl linoleate was fed. Evidently, the lipids of the thoracic duct lymph contained conjugated fatty acids of dietary origin.

The chromatograms (Figs. 1—2) revealed extra bands with an R_F of 0.5 – 0.6 and less significant bands with a higher mobility in all lymph samples containing conjugated fatty acids. The detection of bands of equal mobilities, irrespective of whether the hydroperoxide or the reduced compound had been administered, is in accordance with the theory that the hydroperoxide group is reduced to a tertiary alcohol group in the intestinal mucosa.

The lipids from the most significant of the extra-zones were isolated from a number of the extracts by quantitative thin-layer chromatography and examined by spectroscopy. The infrared spectrum agreed with a triglyceride (C=O stretching frequencies 1122, 1160, and 1240 cm^{-1}) containing oleate (C=C–H group stretching frequency 3010 cm^{-1}) and hydroxyoctadecadienoate (OH stretching frequencies about 3500 cm^{-1}, conjugated double bonds, band frequency about 990 cm^{-1}). The optical density at 232 nm agreed fairly well with that of a triglyceride containing one conjugated acid.

The extra bands were markedly more significant in thoracic duct lymph extracts from rats fed methyl hydroxyoctadecadienoate than from those fed linoleate hydroperoxide. The same trend emerges when the optical densities at 232 nm are compared. The administered lipoperoxide and the

reduced compound both had a content of conjugated diene of about 78 – 79 %, corresponding to about 26 % after dilution with two parts of triolein. The thoracic duct lymph lipids gave values for the content of conjugated diene of 5.3 to 10.4 % after the administration of methyl hydroxyoctadecadienoate, and only from 2.0 to 4.0 % was found after methyl linoleate hydroperoxide (Table 1). When the hydroperoxide was given alone, both the chromatogram (Fig. 2) and the optical densities at 232 nm indicated the presence of conjugated hydroxyacids in the thoracic duct lymph lipids. The amounts were, however, very small.

We conclude that dietary hydroperoxides in the normal rat most probably are not absorbed into the thoracic duct lymph. The material is too small and inhomogeneous to allow further conclusions. However, the low recovery of methyl linoleate hydroperoxide and methyl hydroxyoctadecadienoate, in comparison with triolein, indicates that the two substances, and especially the hydroperoxide, are less well absorbed or preferentially metabolized by the intestinal mucosa. The latter possibility is substantiated by the fact that a specific hydroperoxide reduction is known to take place in the intestinal mucosa and that this tissue derives its energy to a considerable extent from fatty acid oxidation.[9]

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