Effect of Ethylenediaminetetraacetate on Lipide Peroxide Formation and Succinoxidase Inactivation by Ultraviolet Light.* (23510)

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Exposure of cells, mitochondria and other biological material to ultraviolet light catalyzes the formation of lipide peroxides. The observed inhibition of biological activity (cell division, mitochondrial enzymes) upon irradiation is proportional to the amount of lipide peroxides formed. Similar effects can be produced by lipide peroxides when they are added to the various biological systems(1-5). This suggests that these peroxides may act as mediators of the effects produced by irradiation. If this is the case, then substances which interfere with lipide peroxide formation should also inhibit the biological effects of ultraviolet irradiation. To test this hypothesis, experiments were carried out with ethylenediaminetetraacetate (EDTA) which as the following results indicate, prevents peroxide formation in liver homogenates and mitochondria exposed to ultraviolet light. The results show that it is possible to protect the succinoxidase in mitochondria from inhibition by ultraviolet light if EDTA is present and that the protection is proportional to the inhibition of peroxide formation. Unlike its action in homogenates and mitochondria EDTA does not prevent peroxide formation in pure methyl linolenate emulsions similarly exposed to ultraviolet light.

Materials and methods. Homogenates were prepared from rat liver in cold isotonic KCl buffered at pH 7.0 with Na-K phosphate buffer, to a final dilution 1/8 (w/v). For exposing the homogenates to ultraviolet irradiation 8 ml of the homogenate were placed in a 120 ml round bottom quartz flask. EDTA or other compounds were added to the flask in 1 ml of buffer and 1 ml of buffer was added to the controls. The flasks were rotated at 15 r.p.m. at a distance of 15 cm from the ultraviolet lamp. The ultraviolet source was a Hanovia Utility Model mercury arc lamp rated at an intensity of more than 250 microwatts per sq cm at a distance of 50.8 cm for wavelengths below 3130 Å. A current of air maintained the temperature at 27 ± 1°C. Samples were removed at intervals and peroxide formation determined by the TBA (thiobarbituric acid) reaction(6). Mitochondria from rat liver were prepared by differential centrifugation in isotonic sucrose(7). The fluffy layer was discarded and the mitochondria were washed twice with isotonic KCl and resuspended in isotonic KCl-Na-phosphate buffer, pH 7.4. Succinoxidase activity was measured in the mitochondria by the method of Schneider and Potter(8), using the equivalent of 36 mg of liver in each Warburg vessel. The average oxygen uptake of these preparations was 151 μl/30 min. The irradiation procedure was the same as that for the homogenates. For irradiation of methyl linolenate 0.05 ml of the ester was emulsified with 100 ml of M/10 phosphate buffer, pH 7.0, in a Waring blendor for 4 minutes. Ten ml of this emulsion were placed in the quartz flasks and various substances were added in 1 ml volumes. The final volume in each flask was brought to 12 ml with buffer. Samples were taken at intervals and peroxide formation was measured by the TBA test after extraction with chloroform.

Results. Lipide peroxide formation in liver homogenates is catalyzed by iron or ascorbic acid(9,10). A wide variety of chemical substances will inhibit this oxidation among which chelating agents appear to be the most effective on a molar basis. These substances also inhibit lipide peroxide formation in homogenates exposed to ultraviolet light (Table I). Irradiation of mitochondrial suspensions results in an increased peroxide formation and there is a corresponding decrease in succinoxidase activity(3). In studies on inhibition of peroxide formation in mitochondria, EDTA was chosen as the most suitable inhibitor because of its effect on the homogenate, and its lack of absorption of ultraviolet light. Peroxide formation in mitochondria ex-

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TABLE I. Effect of Various Compounds on Lipide Peroxide Formation in Irradiated Liver Homogenates.

Rat liver homogenates were diluted 1 to 8. Final concentrations of added compounds were 2.3 $\times 10^{-3}$ M glutathione, diethyldithiocarbamate, NaCN, 2×10^{-4} M (1) and 2×10^{-5} M (2) EDTA.

Time of irradiation (min.)	TBA optical density					
	Buffer	Glutathione	Diethyldithiocarbamate	NaCN	EDTA (1)	EDTA (2)
60	.175	.075	.035	.050	.020	.000
120	.530	.140	.085	.105	.000	.145

posed to ultraviolet light for 2 hours was inhibited. Inhibition also occurred in the controls which were rotated in the dark (Table II).

Succinoxidase activity is partially inhibited by 5 x 10^{-3} M EDTA. This concentration will inhibit the oxidation of lipids by ultraviolet light and there is little further loss of enzyme activity due to the action of ultraviolet light. However, at an EDTA concentration of 1.0 x 10^{-3} M there is no inhibition of succinoxidase and a significant protection of enzyme activity occurs when mitochondria are irradiated for different times. The degree of protection is correlated with the inhibition of peroxide formation (Fig. 1).

EDTA does not inhibit peroxide formation in pure methyl linolenate exposed to ultraviolet light. It prevents, however, the initial antioxidant effect of ascorbic acid and the catalytic effect of iron on this system (Table III). In tissue homogenates, ascorbic acid in low concentrations and iron catalyze peroxide formation and EDTA inhibits both (unpublished).

Discussion. The inability of EDTA to inhibit peroxide formation in pure methyl linolenate cannot be ascribed to its presence in the water phase and its consequent inability to come in intimate contact with the fatty acid ester, since ascorbic acid, a highly water soluble substance, acts as an antioxidant under the same conditions. It is therefore necessary to conclude that ultraviolet light produces peroxides in this system without the intervention of cations. In tissues, however, the situation is different. Although some peroxide is formed in the presence of EDTA, the rate

TABLE II. Inhibition by EDTA (1.2×10^{-3} M) of Peroxide Formation in Mitochondria.

Suspension was irradiated for 60 and 120 min. Controls were rotated in the dark.

Time of irradiation (min.)	TBA optical density			
	Not irradiated		Irradiated	
	Buffer	EDTA	Buffer	EDTA
0	.040	.030	.040	.030
60	.050	.035	.080	.040
120	.080	.030	.170	.090

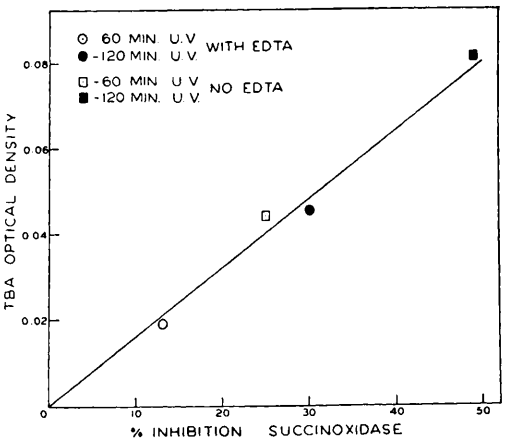

FIG. 1. Effect of EDTA (1.0×10^{-3} M) on peroxide formation and succinoxidase activity in rat liver mitochondria exposed to ultraviolet light.

TABLE III. Effect of Various Compounds on Peroxide Formation in Methyl Linolenate Exposed to Ultraviolet Light.

0.05 ml of methyl linolenate was emulsified in 100 ml of phosphate buffer M/10, pH 7.0, except in the case of iron where 100 ml of demineralized water was used. The final concentrations of added compounds were 1.2×10^{-3} M EDTA, 1.2×10^{-3} M ascorbic acid, and 1.0×10^{-4} M $FeCl_3 \cdot 6H_2O$.

Time of irrad. (min.)	TBA optical density					
	H_2O		Ascorbic acid		$FeCl_3$	
	H_2O	EDTA	H_2O	EDTA	H_2O	EDTA
0	.090	.070	.070	.070	.145	.095
10	.405	.380	.140	.380	.680	.455
20	.700	.700	.470	.670	.900	.620
30	.930	.945	.750	.945	1.155	.800
40	1.060	1.080	.920	1.160	1.300	1.000

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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