Toxicity of Fatty Acid Ester Hydroperoxides. (28809)

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The acute toxicity of fatty acid hydroperoxides is well recognized(1-3). Such toxicity is demonstrable by feeding tests; by injection or oral dosage(4,5); and even by inunction on the skin of rats(6). The mechanism for this toxicity is unknown but it is a reasonable hypothesis that hydroperoxides may be a source of damaging free radicals (7-9). The experiments to be described were done in an attempt to determine whether large doses of tocopherol or ethoxyquin might modify the lethality of hydroperoxides.

Experimental. Peroxide concentrates were prepared by aerating samples of crude methyl linoleate at room temperature (22-24°C). Methyl esters of safflower seed oil fatty acids, furnished by the Pacific Vegetable Oil Co., contained approximately 73% methyl linoleate (by gas chromatography). No antioxidants had been added during its preparation. One sample was aerated at 40-50°C for one day and thereafter at room temperature. On days 3, 4, 5 and 6 the peroxide values were 1990, 2560, 2570, and 2600, respectively (in milliequivalents per kg fat).

Peroxide values were determined by a modification of AOCS standard procedure Cd 8-53 as follows: 20-30 mg samples, 3 ml of chloroform-glacial acetic acid (3:1), 50 ml flask, 1 ml of saturated potassium iodide, 2 minutes reaction at room temperature under nitrogen atmosphere, 10 ml water, titration with 0.01 N $Na_2S_2O_3$.

Hydroperoxide concentrates were prepared by a modification of the procedures described by Banks(10). The reaction mixture was separated between petroleum ether and 85% aqueous methanol. The 85% aqueous methanol-soluble fraction was then recovered after dilution with water, followed by extraction with petroleum ether. Such preparations had peroxide values varying from 3800 to 4700 (theoretical for methyl linoleate hydroperoxide 6135). They were stable for months at —18°C. Preparations made from samples which had been aerated longer than necessary to reach maximum peroxide values contained a fraction characterized by its insolubility in petroleum ether. Peroxide values of these fractions varied from 3300 to 4700. A determination of average molecular weight on one preparation (by osmometry) gave 510. The petroleum-ether-soluble peroxide concentrate had a molecular weight by this method, 340; calculated for methyl linoleate hydroperoxide, 326. The petroleum-ether-insoluble fraction thus appeared to be in part a dimer. It was found to be at least as toxic as the soluble fraction but has not yet been studied in more detail.

Toxicity of these preparations in rats was measured after intraperitoneal injection (Table I). Particularly viscous preparations were diluted with crude methyl linoleate which, by itself, was innocuous by the same route. Rats were kept on a normal stock diet. Death usually occurred within 24 hours but occasionally rats died after 2 or 3 days. Those that survived longer showed no obvious long-term ill-effects. One rat was injected on several occasions with close to lethal doses without evidence of accumulated damage. The only gross symptom in all rats that died was massive ascites. These observations are in accord with those of Horgan *et al*(4) with mice and of Vishida and Kummurow(11) with methyl linoleate hydroperoxide injected into rats.

Intraperitoneal injection of tocopherol suspended in crude methyl linoleate into numerous rats caused no death or obvious discomfort. Results obtained with rats first injected with tocopherol and 24 hours later with hydroperoxide preparations are shown in Table I. If there was a protective effect, it was very slight. Preliminary experiments had shown that simultaneous injections of tocopherol and hydroperoxide also did not change the level of the LD_{50} dose. Similarly in a limited series the antioxidant ethoxyquin ap-

TABLE I. Effect of Tocopherol and Ethoxyquin on Intraperitoneal Toxicity of Hydroperoxide.

Pretreatment*†					
None		Tocopherol		Ethoxyquin	
Hydroperoxide, μM/100 g	Lethality	Hydroperoxide, μM/100 g	Lethality	Hydroperoxide, μM/100 g	Lethality
43–56	0/3	—	—	—	—
61–74	2/2	—	—	—	—
82–109	0/3	93–95	1/2	—	—
113–118	1/2	—	—	—	—
149–150	2/6	150	0/4	150	2/4
155–170	8/8	170	4/5	—	—
180	7/8	180	5/8	185	1/1
185	25/25	188–238	0/4	196	0/1
—	—	240	10/10	238	1/1

* Rats (300-350 g) were injected intraperitoneally with an amount of α-tocopherol or ethoxyquin equivalent to the dose of hydroperoxide injected 24 hr later.

† Rats kept on a stock diet manufactured by Diablo Laboratories in Berkeley, Calif., which contained 4.8% fat, supplemented with Vit. E.

peared not to effect the lethality of the hydroperoxide.

Administration of hydroperoxide concentrates to rats by stomach tube also was fatal if high enough doses were provided (Table II). Symptoms were excessive dehydration suggesting extreme damage to the intestine.

Discussion. The LD_{50} of methyl linoleate hydroperoxide was 150-170 μM per 100 g body weight, whether or not tocopherol in equimolar amounts had been presented to the animal. Horgan *et al* reported that the LD_{50} of the peroxides of autoxidized methyl linoleate was 45-60 μM per mouse. In contrast, the LD_{50} of autoxidized linoleic acid was 6-8 μM peroxide per mouse (about 25 μM *per* 100 g). Holman and Greenberg(5) found that ethyl linoleate hydroperoxide had a LD_{50} of 12 mg (35 μM) in mice. The hydroperoxide of methyl oleate acid was more toxic, LD_{50}, 6 mg (18 μM per mouse).

Introduction of the peroxide by stomach tube killed three rats at the level of 1600 μM per g. Lower levels caused drastic loss of fluid from the intestinal tract but no deaths. In the experiments of Holman and Greenberg (5) mice were not killed by single 200 mg doses of either ethyl linoleate hydroperoxide (588 μM) or methyl oleate hydroperoxide (610 μM) nor by 75 mg doses daily for two weeks of either. These combined observations indicate that hydroperoxides do not easily pass through the intestinal wall (cf. Mead, 12), and also that they are highly irritating. In a separate experiment carried out with the help of F. DeEds and J. O. Thomas at the Western Regional Research Laboratory, USDA, Albany, California, hydroperoxide concentrate (5-10 μM) was injected into the lumen of a tied-off segment of a rat small intestine. In comparison with a control segment the injected segment more than doubled in weight in one hour, mostly due to an increase in mucus excretion into the intestine, clear indication of intense irritation (cf. 13).

TABLE II. Toxicity of Methyl Linoleate Hydroperoxide Concentrate when Administered by Stomach Tube.

Hydroperoxide, μM/100 g	Symptoms*	Loss in wt, %*
400	None	5, 5, 7
800	Diarrhœa	5, 7, 11
1600	Dead	11, 16, 17

* Original weights, 300-350 g. Weighed again at 18 hr. There were no subsequent deaths. Surviving animals recovered and gained weight.

These combined observations suggest that hydroperoxides kill by attack on some vital tissue not yet identified. It is possible that sulfhydryl enzymes(14) or cytochromes(8) are the vulnerable tissue components. Whatever the mechanism, neither tocopherol nor ethoxyquin are effective in reducing the dose required to kill, either when made available simultaneously or 24 hours previous to the administration of the toxic dose.

The lack of effectiveness of tocopherol is parallel to the observed relative ineffectiveness of tocopherol in combating radiation

toxicity which, it has been hypothesized, may be mediated through the formation of hydroperoxides(4).

Summary. Intraperitoneal injection of concentrates of methyl linoleate hydroperoxide into adult rats was lethal at a level of about 150 μM per 100 g. Previous injection of equal amounts of tocopherol or ethoxyquin did not change the LD_{50} level. Sixteen hundred but not 800 μM per 100 g were lethal when administered by stomach tube.

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Inactivation of Streptococcal Bacteriophage by Sulfhydryl Reagents.* (28810)

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Work reported previously indicated that the host range of beta-hemolytic streptococcal bacteriophage is a group-specific phenomenon. Thus, the Group A phage lyses Group A streptococci but not Group C and similarly, Group C phage lyses Group C streptococci but not Group A. Such group specificity of these phages suggests that the cell wall carbohydrate, a significant constituent shared by all streptococcal strains of the same serologic group, may serve as a receptor substance for attachment of the phage to the organism(1). Additional support for this view has been obtained by experiments which demonstrated the inactivation of Group C phage but not Group A phage with the purified cell wall carbohydrate of Group C streptococci. It is clear, however, that factors other than the nature of the specific attachment substance of the bacterial cell wall control the adsorption and penetration of the virus into the host cell. For example, recent studies by Kosloff have emphasized the role of sulfhydryl groups in T2 bacteriophage invasion of *E. coli*(2).

In related experiments with animal viruses, Choppin and Philipson reported inactivation of enteroviruses by treatment with mercaptide forming reagents such as p-chloromercuribenzoate; mercuric ion and Thimerosal; an inactivation which was reversed with sub-

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