significantly greater cellular destruction and weight loss in the thymus than occurred after whole-body exposure to the same dose of radiation. Whole-body re-exposure to a massive dose of radiation within one hour after an initial total-body exposure to a small dose was regularly followed by less weight loss and cellular destruction in the thymus than occurred after total-body exposure to the small dose alone. The findings show that exposure of the head during massive whole-body irradiation markedly influences the cellular response in the thymus.

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Effects of Oxidized Fatty Acids on Ascites Tumor Metabolism. (22054)

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The unsaturated fatty acids of cells and mitochondria have been shown to be oxidized by ultraviolet light(1,2) and by aerobic incubation(3). The oxidation products of these fatty acids quite probably alter cellular activities as indicated by the findings that the unsaturated fatty acid esters, methyl linolenate and methyl linoleate, inhibit the activity of certain oxidative enzymes in homogenates and mitochondria(2,3); depolymerize deoxyribonucleic acid (DNA)(4); and inhibit cell division(5,6). The relation of oxidized fatty acids to enzyme action is further examined in the present study of the action of oxidation products of linolenate and linoleate on the respiration and glycolysis of Ehrlich ascites tumor cells.

Methods and materials. Ehrlich ascites tumor growing in A-strain mice was taken from the abdominal cavity 7 days after injection. Blood-free tumor cell suspensions were prepared by centrifuging the ascitic fluid first for 10 minutes at 100 x g. The cells were then resuspended in Ringer solution and centrifuged at 2 x g for 15 minutes. The supernate which contains erythrocytes and platelets was discarded and the tumor cells resuspended in Ringer solution. This procedure was repeated 3 times. For testing the effects of oxidized fatty acids, aqueous extracts containing the oxidation products were made from pure fatty acid esters (Hormel) oxidized by irradiation with ultraviolet light. Samples of 0.5 g of the esters were irradiated for 90 minutes in dishes 3 cm in diameter placed at a distance of 30 cm from a Hanovia Utility Model mercury arc rated at an intensity of more than 250 microwatts/cm^2 at 50.8 cm distance for wavelengths of 3130 Å and shorter. 0.5 g of the irradiated fatty acid ester was dissolved in 10 ml of petroleum ether and extracted against an equal volume of distilled water with shaking for 20 minutes. After separation of the petroleum ether the aqueous phase was centrifuged under fresh petroleum ether to remove any suspended droplets of ether. The aqueous phase was then placed under vacuum for 20 minutes to remove traces of ether. Two volumes of the aqueous phase were diluted with one volume of 3 x isotonic Ringer solution and varying amounts of this mixture were added to Warburg flasks to give the desired amount of oxidized material as determined by the thiobarbituric acid (TBA) color reaction(7). The weight of oxidized ester cannot be calculated but is of the order of micrograms. The respiratory and glycolytic measurements were made by standard Warburg technics. Lactic acid formed aerobically was measured by the method of Barker and Summerson(8). Nitrogen was determined by digestion and nesslerization. Analysis of the unsaturated fatty acids of tumor cells was made on lipid of several grams of packed cells extracted with a 3:1 alcohol-ether mixture for 12 hours in a Soxhlet type extraction appara-

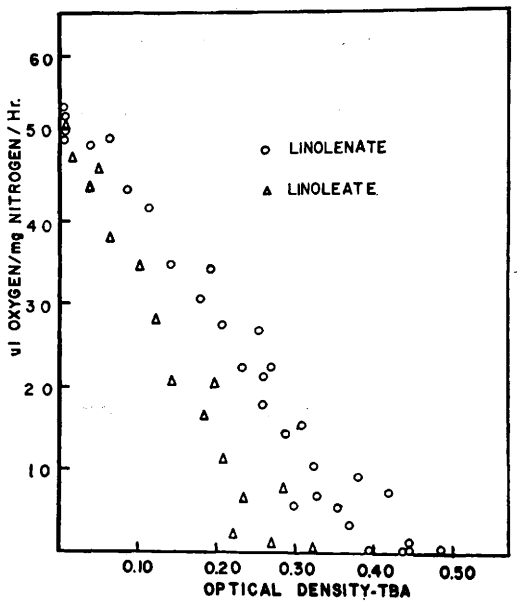

FIG. 1. Effects of aqueous extracts of oxidized methyl linolenate and methyl linoleate on respiratory activity of ascites tumor cells. Flasks contents: 1.0 ml of cell suspension containing about 5×10^7 cells; 2.0 ml of Krebs Ringer phosphate solution. Aqueous extracts of linolenate and linoleate were added with the Ringer solution. TBA values represent total amount of added oxidized esters. Measurements were taken for one hr.

tus. The percentage of each unsaturated fatty acid was measured after alkaline conjugation by the spectrophotometric micromethod of Herb and Riemenschneider(9). The iodine number was determined by the method of Benham and Klee(10).

Results. Respiratory inhibition. Washed tumor cell preparations exhibited a constant rate of oxygen consumption for an hour or longer followed by a gradual decrease in respiration over several hours. $Q_{O_2}(N)$ values during the first hour were between 50.0 and 54.5 as compared with a value of 58 reported by Kun *et al.*(11). The addition of small quantities of aqueous extracts of oxidized methyl linolenate and linoleate caused a decrease in respiratory activity which was a linear function of the concentration of extract (Fig. 1). The inhibition reached a maximum in the first intervals of the respiratory measurements. Inhibition by oxidized methyl linolenate was usually complete at concentrations giving TBA optical densities greater than 0.400. Extracts of oxidized linoleate inhibited oxygen consumption at lower TBA values than oxidized linolenate. However, since the TBA reaction is 30-80 fold more sensitive to oxidized linolenate as compared to oxidized linoleate(7), oxidized linoleate is actually a less potent inhibitor than oxidized linolenate.

Inhibition of glycolysis. Warburg and Hiepler(12) and Kun *et al.*(11) showed that ascites cells exhibited high rates of lactic acid formation in the presence of glucose under both aerobic and anaerobic conditions. The addition of aqueous extracts of oxidized linolenate to aerobic and anaerobic glycolyzing cells decreased the rate of lactic acid formation. The degree of inhibition was proportional to the amount of extract added in excess of a certain threshold concentration which was about the same for both aerobic and anaerobic glycolysis (Fig. 2). The inhibition appears to be slightly greater for aerobic glycolysis at higher concentrations of extract. It is to be noted that the respiration was inhibited completely at the threshold concentration for the inhibition of glycolysis (Fig. 1). The effect of oxidized methyl linoleate extracts followed the same general pattern observed for oxidized methyl linolenate (Fig. 3). The TBA values of oxidized linoleate extracts for any given degree of inhibition are lower than in the case of oxidized linolenate, but because of the difference in sensitivity of the TBA reaction to the 2 esters, higher concentrations of oxidized linoleate are required.

Fatty acid oxidation in the cell. The inhibition of respiration and glycolysis by oxidized unsaturated fatty acid extracts raises the problem of similar effects resulting from the oxidation of unsaturated fatty acids normally present in cells. Accordingly, attempts were made to oxidize the fatty acids within the Ehrlich ascites tumor cells under conditions which bring about fatty acid oxidation in other tissues. Also, analyses of the unsaturated fatty acid content of the cells were carried out. Tumor homogenates in Na-K phosphate buffer at pH 6.7 were shaken in air in a Dubnoff metabolic shaker, and after one hour the extent of lipid oxidation was measured by the TBA reaction. Different concentrations of homogenate were run over a

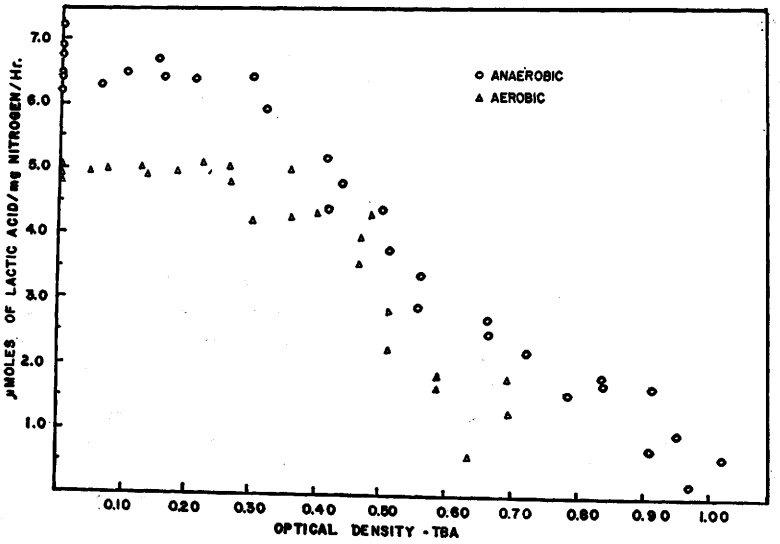

FIG. 2. Effects of aqueous extracts of oxidized methyl linolenate on anaerobic and aerobic glycolysis of ascites tumor cells. In anaerobic measurements each flask contained 1 ml of cell suspension containing approximately 2.5×10^7 cells, 2.0 ml of Krebs Ringer bicarbonate solution, and 0.2 ml of glucose solution (16.6 micromoles). Flasks were flushed with 95% N_2-5% CO_2 mixture, and lactic acid was measured by CO_2 evolution. In aerobic measurements Krebs Ringer phosphate solution was used; the flasks were flushed with 95% O_2-5% CO_2; and lactic acid was measured by chemical determination. TBA values represent total amount of added oxidized methyl linolenate. Measurements were taken for one hr.

range of 25 mg/ml to 250 mg/ml both with and without the addition of 0.3 mg/ml of ascorbic acid as a catalyst for lipid oxidation (13). Tumor homogenates were also irradiated with ultraviolet light and the oxidation products of fatty acids determined by the TBA reaction. 5 ml of homogenate were exposed for intervals up to 2 hours slowly rotating in spherical quartz flasks 6.4 cm in diameter placed 26 cm from the mercury arc used to oxidize the fatty acid esters. The ultraviolet dose was greatly in excess of that required to oxidize lipids of mitochondria(2). In no case could significant fatty acid oxidation be detected. In contrast to tumor tissue, homogenates of mouse brain, liver, and kidney produced high values on incubation under identical conditions. Analyses of lipids of ascites cells are given in Table I. The figures are mean values of 3 replications on each of 2 lots of washed tumor cells, each consisting of the pooled tumor cells from several mice. The proportions of unsaturated fatty acids differ from rat liver(14) and also from rabbit bone marrow(15) analyzed by the same method. The concentration of linolenic acid is higher in the ascites cells than in rat liver and yet the TBA test is negative in the tumor and positive in liver(16). This indicates an increased antoxidant activity in the tumor.

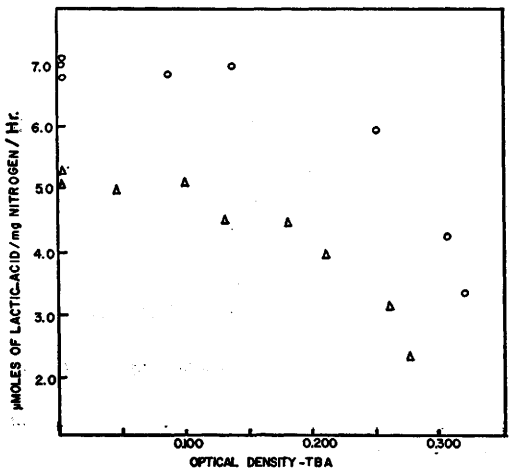

FIG. 3. Effects of aqueous extracts of methyl linoleate on anaerobic and aerobic glycolysis of ascites tumor cells. Experimental conditions as in Fig. 2. ○, anaerobic glycolysis; △, aerobic glycolysis.

Discussion. The inhibition of respiration of intact ascites cells by the oxidation products

TABLE I. Analysis of Unsaturated Fatty Acids of Ascites Tumor Cells.

Fat recovered/mg fat-free dry wt	.128 mg	
" " , wet wt	.019 "	
Iodine No.	72	70
Linoleic acid (%)	14.4	13.7
Linolenic " (%)	5.1	4.9
Arachidonic " (%)	4.0	3.9
Pentaenoic " (%)	3.2	3.3

of methyl linolenate and linoleate extends previous work on the inhibition of certain respiratory enzymes by these compounds(2,3). This study has shown that both aerobic and anaerobic glycolysis are also inhibited by the oxidation products of linolenate and linoleate. The observed effects on both respiration and glycolysis may involve a reaction between the oxidation products of fatty acids and sulfhydryl compounds(17). In view of the effects of oxidized fatty acids on the depolymerization of DNA(4) and on the hemolysis of erythrocytes(18), effects other than those involving sulfhydryl groups may be involved.

Analysis of the unsaturated fatty acids has shown that both linolenic and linoleic acids are present in the ascites cells. The oxidation products of both are inhibitory to cellular activities, and the oxidation products of linolenate (3 double bonds) are considerably more inhibitory than are the oxidation products of linoleate (2 double bonds). A similar difference has been noted in the studies of inhibition of bacterial growth(5) and the depolymerization of DNA(4). Within the cells a mechanism must be operative to hold in check the oxidation of these unsaturated fatty acids and the consequent effects of the oxidation products. Thus there is an absence of fatty acid oxidation products which would be inhibitory to cell division in the ascites tumor. Higher unsaturated fatty acids have also been shown to occur in ascites cells and in other tissues(14,15,19). The action of the oxidation products of these acids is not known.

Summary. 1. Extracts of irradiated methyl linolenate and methyl linoleate inhibited respiratory and glycolytic activities of Ehrlich ascites tumor cells. Respiration showed a greater sensitivity than glycolysis to the oxidation products of both esters. 2. The oxidation products of methyl linolenate inhibited both glycolysis and respiration at lower concentrations than those for methyl linoleate. 3. In contrast to the normal tissues of the mouse, the oxidation of tumor cell lipids by aerobic incubation and ultraviolet light could not be demonstrated by the TBA reaction. 4. Analyses of unsaturated fatty acids of ascites tumor cells are presented.

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