FORMATION OF "LIPID PEROXIDE" UNDER CONDITIONS WHICH LEAD TO SWELLING AND LYSIS OF RAT LIVER MITOCHONDRIA

P.E. Hoffsten, F.E. Hunter, Jr., J.M. Gebicki and J. Weinstein

Pharmacology Department, Washington University
Medical School, Saint Louis, Missouri

Received March 21, 1962

In exploring possible mechanisms for the ascorbate induced lysis of mitochondria reported earlier (Hunter, 1961) we have studied formation of "lipid peroxide" during swelling or lysis of mitochondria by several agents.

"Lipid peroxide" was measured by the thiobarbituric acid (TBA) color reaction (Wilbur, Bernheim and Shapiro, 1949). This reaction is not specific for lipid peroxides, but has been widely used to follow their formation (Tappel and Zalkin, 1959). The actual reactant, according to the absorption spectrum of the product, is malonaldehyde, which may also be derived from 2-deoxy-sugars and certain other compounds after oxidative changes.

Mitochondria were prepared in 0.33 M sucrose, but two washes and the final suspension were in 0.17 M KCl, for sucrose interferes in the TBA method. Most experiments were done at 22-24° with dilute mitochondrial suspensions in 0.17 M KCl + 0.02 M Tris pH 7.4. Time curves were established by incubating reaction mixtures in Erlenmeyer flasks and withdrawing samples for turbidity readings (A_{520}) and the TBA color reaction (A_{535}).

The course of the A_{520} change and the TBA color test with mitochondria + 0.3 mM ascorbate is shown in Figure 1. Swelling or lysis of the mitochondria is associated with the appearance of "lipid peroxide". The lag period is very similar for the two phenomena. GSSG, which accelerates GSH swelling (extensively studied by Neubert and Lehninger, 1962), also hastens ascorbate

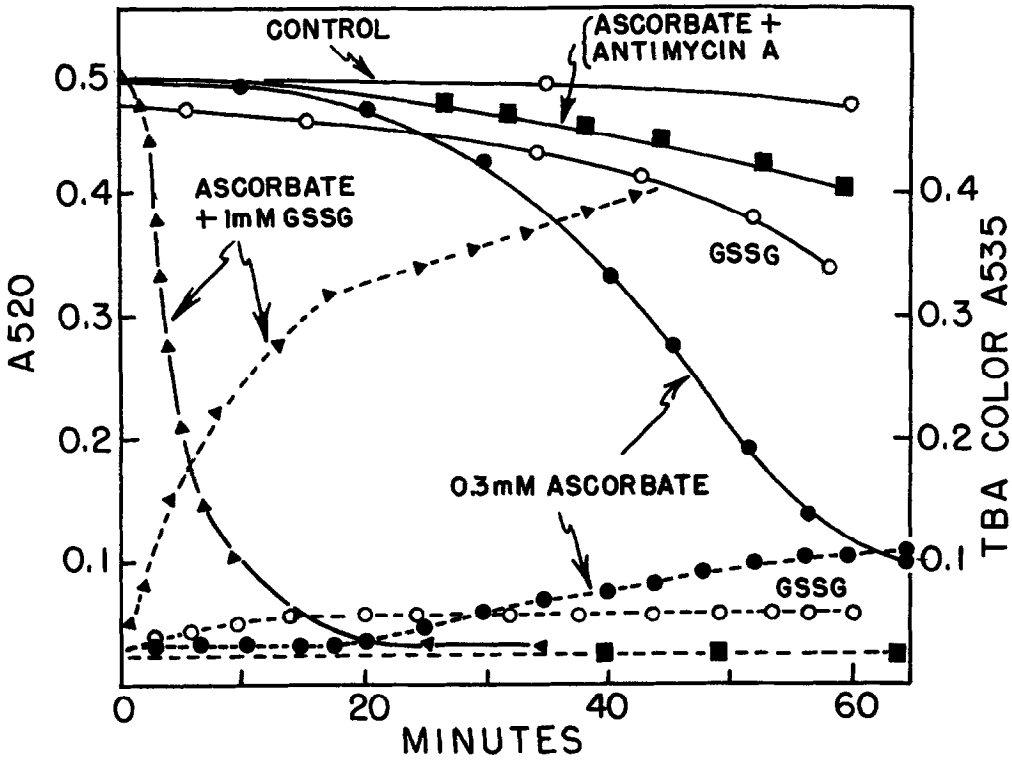

Figure 1. ———— Turbidity, -------- TBA color reaction. 0.3 mM ascorbate, 1 mM GSSG, 4 μM antimycin A.

lysis and greatly increases formation of "lipid peroxide". α-ketoglutarate and D-gulonolactone show somewhat similar effects. GSSG causes a slight swelling.

Every inhibitor of ascorbate lysis which has been studied for its effect on "lipid peroxide" formation shows essentially complete inhibition. The action of Antimycin A is illustrated in Figure 1. Other inhibitors include vitamin E, vitamin A, 5-hydroxytryptamine, 5 mM pyruvate, 20 mM ascorbate, and pH 6.8-7.0. EDTA, Mn^{++} and CN^{-}, which also prevent swelling, had earlier been reported to inhibit "lipid peroxide" formation (Ottolenghi, 1959; Thiele and Huff, 1960). Other inhibitors remain to be studied, particularly oligomycin, which blocks ascorbate lysis, and DNP and gramicidin, which do not.

The correlations between appearance of "lipid peroxide" and the beginning of swelling are so close in the ascorbate studies that it is not possible to say with certainty whether "lipid peroxide" formation begins first and is the cause of lysis. However, TBA color material does not appear when swelling is spontaneous or produced by phosphate + β-OH-butyrate or by fatty acids.

Because of the similarity of GSSG + GSH swelling to ascorbate lysis under our conditions, we followed "lipid peroxide" during glutathione swelling. Figure 2 indicates that pure GSH produces little effect for long periods, while the combination GSSG + GSH causes a steady increase of "lipid peroxide" from zero time and reduces the lag period before swelling to 5'. Formation of appreciable "lipid peroxide" precedes lysis. As in Figure 1, the rate of swelling correlates well with the rate and total amount of "lipid peroxide" formed.

In extending this work we observed that Fe^{++} ion produces a characteristic swelling of mitochondria in KCl medium but not in sucrose. Further investigation has indicated that both Fe^{+++} and Fe^{++} ions are necessary. Fe^{+++} alone gives no swelling, while Fe^{++} solutions carefully prepared to avoid partial oxidation to Fe^{+++} cause relatively little swelling until after a lag period of 40-50'. Fe^{+++} + Fe^{++} mixtures induce swelling after short lag periods.

Figure 3 illustrates that whenever there is rapid swelling or lysis with Fe^{++} or Fe^{+++} + Fe^{++}, a rather rapid increase in "lipid peroxide" begins a minute or two before the turbidity (A_{520}) falls. The lag period is related to the concentration ratio Fe^{+++}/Fe^{++}, while the total yield of TBA color may be dependent on the amount of Fe^{++}.

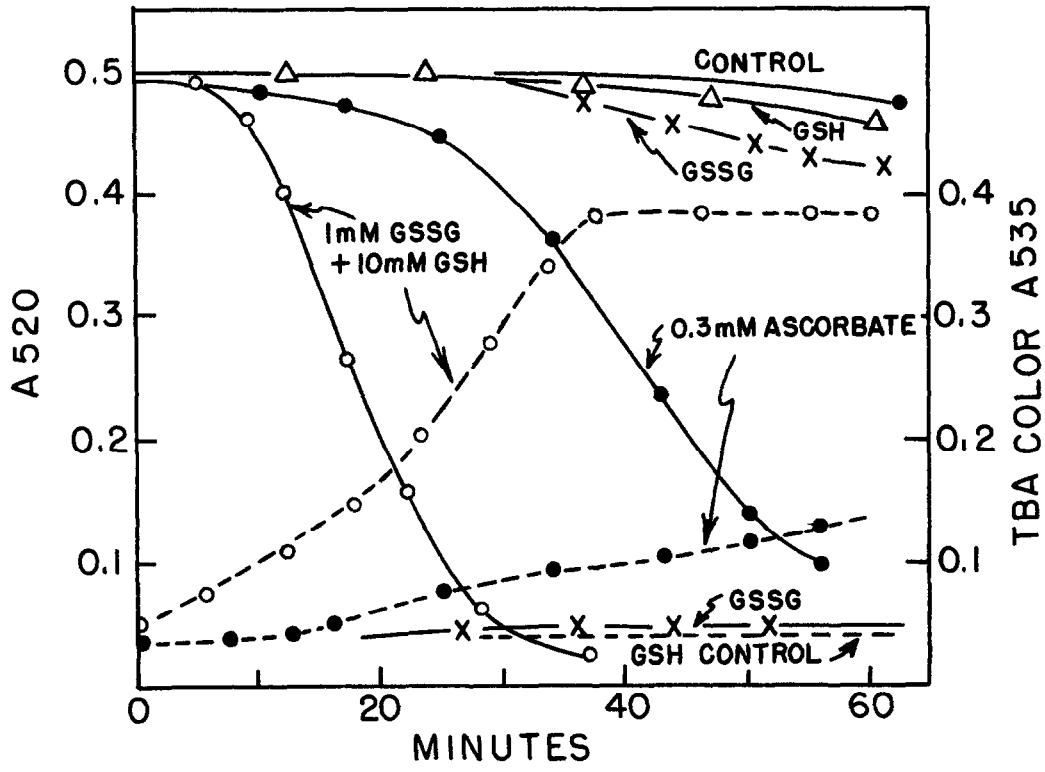

Figure 2. ———— Turbidity, -------- TBA color reaction

Further work will be necessary to establish whether lipid peroxide formation is responsible for this type of mitochondrial swelling. However, it is a reasonable working hypothesis. The changes in double bonds in unsaturated fatty acids of the membranes might in themselves lead to permeability or structural changes. In addition the lipid peroxides behave something like free radicals and could lead to oxidation of thiol, dithiol, or other groups in the membrane.

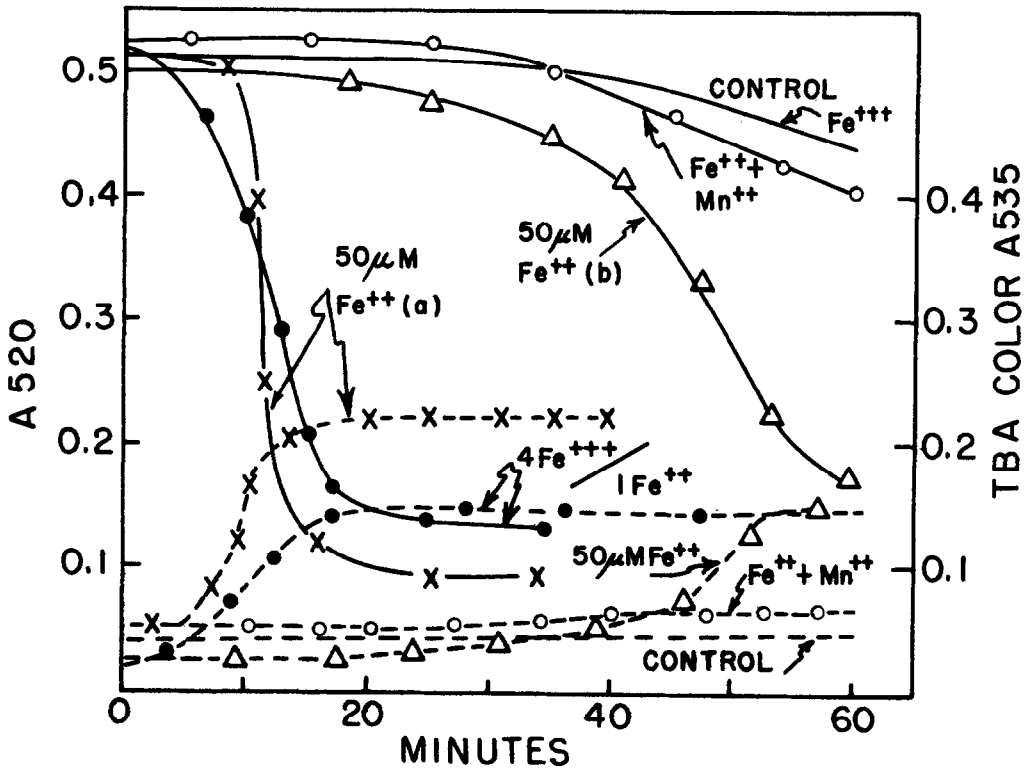

Figure 3. ———— Turbidity, -------- TBA color reaction. Total iron always 50 μM, Mn^{++} 50 μM. Fe^{++}(a) = routine solution, Fe^{++}(b) = special precautions to avoid oxidation.

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