P12-26

# PROPAGATION MECHANISM OF REACTION PRODUCTS FORMED DURING THE PROCESS OF IRON(II)-EDTA-INITIATED PEROXIDATION OF RAT LIVER MICROSOMAL LIPIDS

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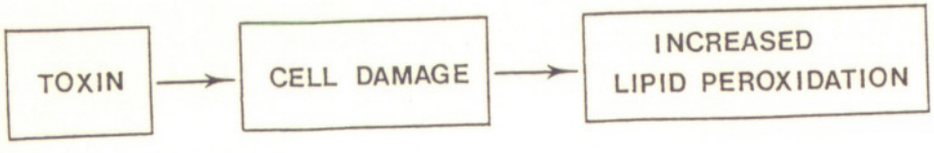
Owing to its high sensitivity and simplicity, the thiobarbituric acid (TBA) test has been extensively employed for the determination of lipid peroxidation (LPO) in biological samples. The experimental procedure involves the treatment of peroxidized lipid with TBA at 20°C, followed by heating for 15 min in a boiling water bath to form a red-coloured complex which can be quantitated colorimetrically at 532 nm. The chromogen is formed through the condensation of TBA with malondialdehyde (MDA) generated from the breakdown of polyunsaturated fatty acid hydroperoxides. Iron(II)-EDTA complex strongly stimulates the propagation of the breakdown process, while t-butyl hydroxy-toluene (BHT) inhibits the lipid autoxidation completely. In the course of our study on the propagation mechanism of LPO in rat liver microsomal liposomes system, we have observed that heating temperature is a critical factor in the TBA test. Immediate addition of hot TBA (95°C) to the sample before the heating step inhibits the colour reaction by 80%. However, there is no difference in colour production when MDA is allowed to react with TBA at either 20° or 95°C. This implies that the gradual warming of the reaction mixture from 20° to 95°C favours the breakdown of hydroperoxides to generate TBA-reactive substances. An optimal incubation temperature at 50-60°C for 5 min has been observed to be a prerequisite step for the induction of formation of TBA-reactive substances, possibly endoperoxide intermediates, during the course of heating. This thermolytic process at 50-60°C is inhibited by BHT suggesting that free radical intermediates are produced at this stage.

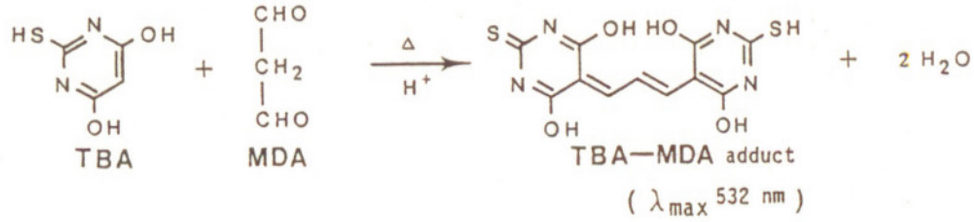
## INTRODUCTION




## THIOBARBITURIC ACID TEST (TBA TEST)

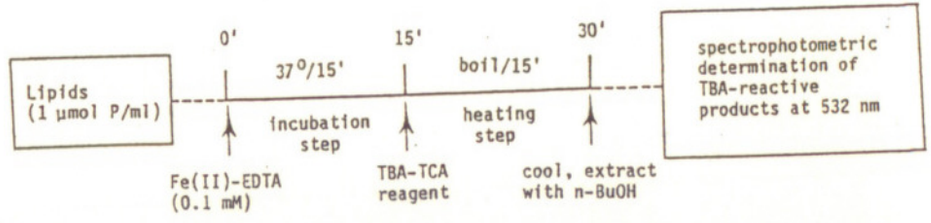
-- extensively employed for the determination of lipid peroxidation in biological samples

-- mechanism:




## EXPERIMENTAL MODEL FOR THE STUDY OF PROPAGATION MECHANISM OF LIPID PEROXIDATION

Standard procedure

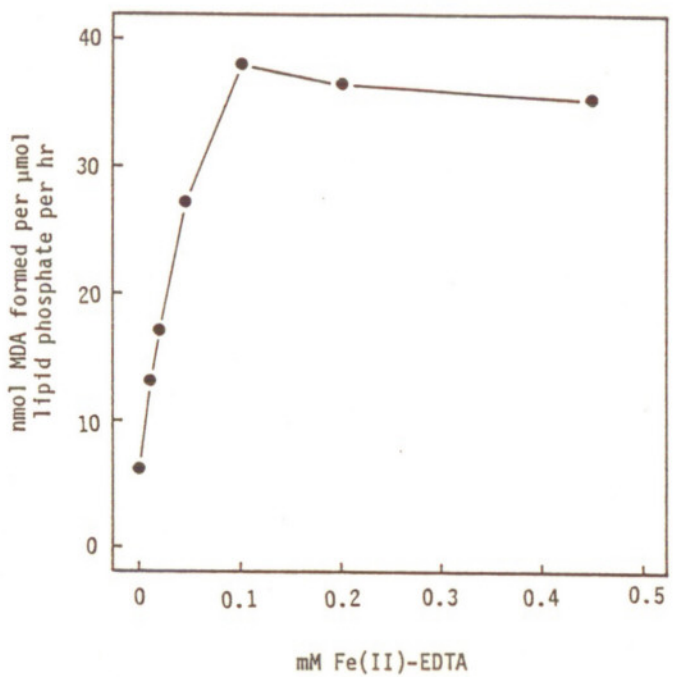



Source of lipids: microsomal lipids extracted from male Sprague-Dawley rat liver by the method of Folch et al. (JBC 174:257, 1958.) and then suspended in 0.02 M Tris-HCl, pH 7.4 containing 0.15 M KCl.

# RESULTS

## I. DEPENDENCY OF Fe(II)-EDTA IN LIPID PEROXIDATION

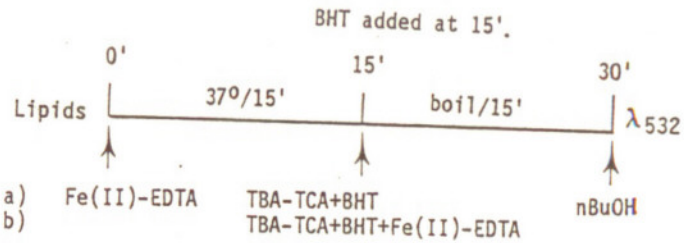
Experimental protocol: varying concentrations of Fe(II)-EDTA.

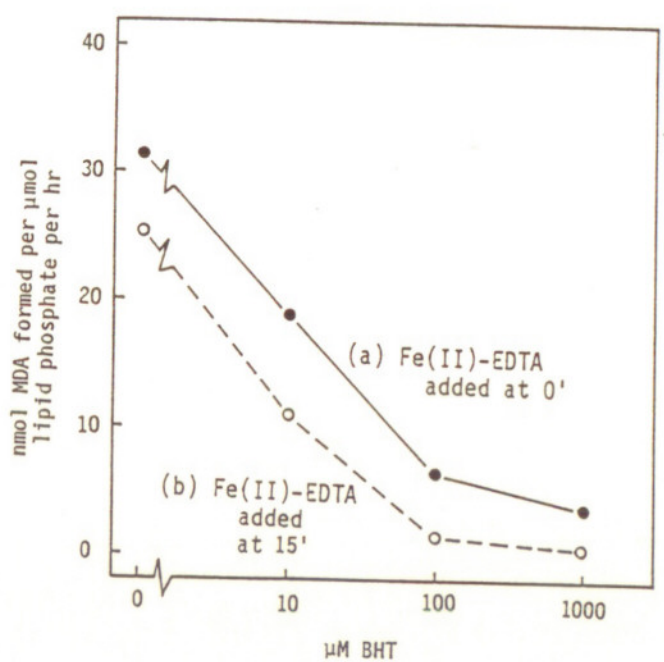



Conclusion: Fe(II)-EDTA at 0.1 mM concentration maximally stimulated formation of TBA-reactive products.

## II. ROLE OF Fe(II)-EDTA IN PROPAGATION OF LIPID HYDROPEROXIDE DECOMPOSITION

Experimental protocol: 0.1 mM Fe(II)-EDTA added at either 0' or 15'.

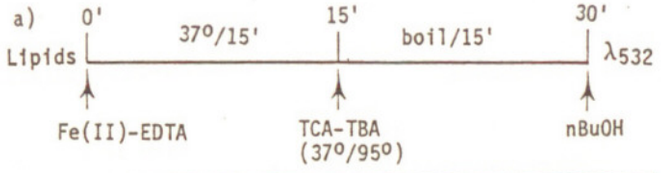


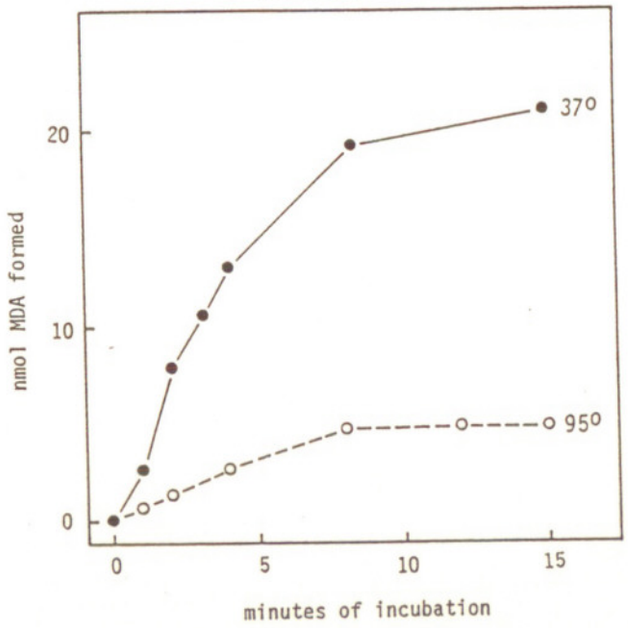



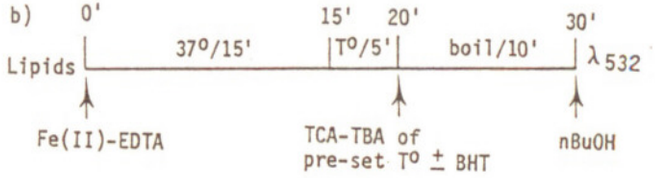

Conclusion: Fe(II)-EDTA catalyses thermolytic breakdown of lipid hydroperoxides to form TBA-reactive products.

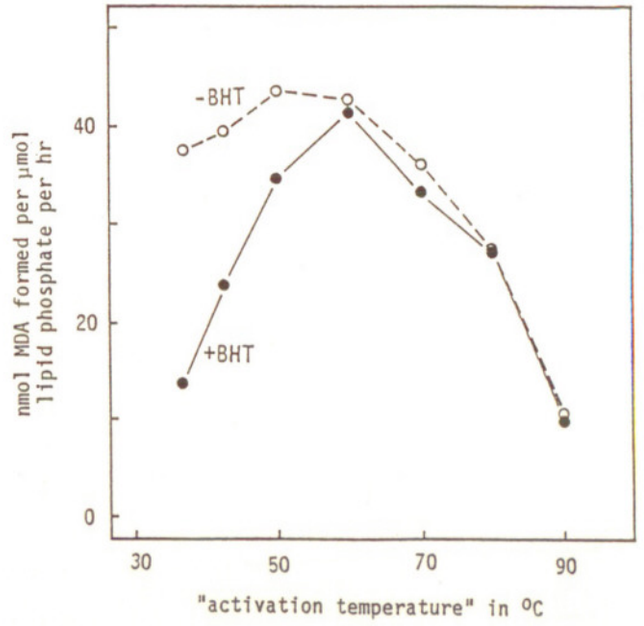
## III. HEATING TEMPERATURE IS A CRITICAL FACTOR IN TBA TEST

Experimental protocol: TBA-TCA reagent preincubated at either 37° or 95° was added at the end of incubation period as indicated.



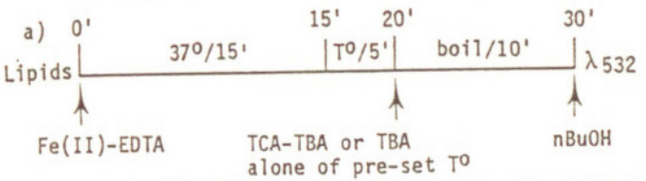



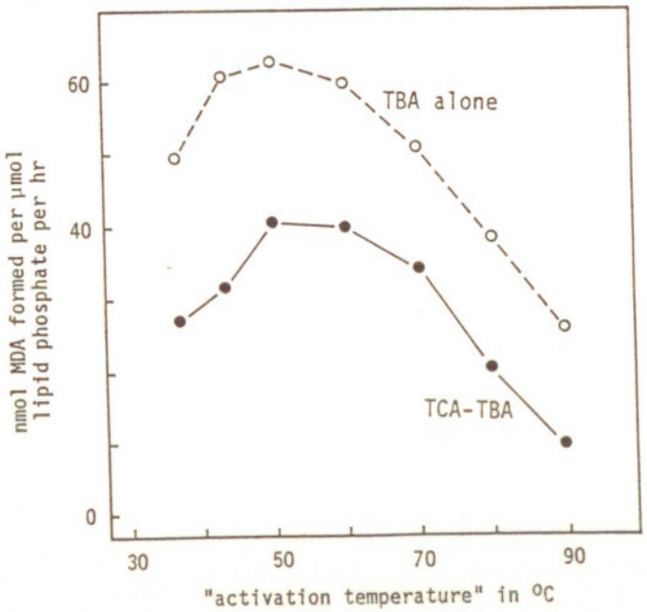




Conclusion: Gradual warming of the incubation mixture from room temperature to boiling favours the breakdown of hydroperoxides to generate TBA-reactive substances. Hot TBA (95°) inhibits breakdown of hydroperoxides to TBA-reactive products.

## IV. INFLUENCE OF REAGENT pH ON TBA-REACTIVE PRODUCT FORMATION FROM THERMOLYTIC DECOMPOSITION OF LIPID HYDROPEROXIDES

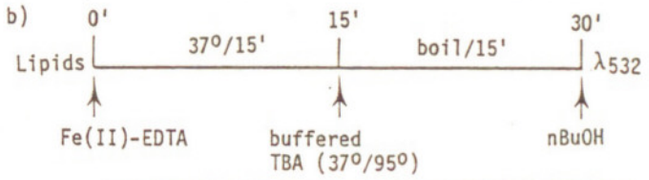
Experimental protocol: (a) TCA effect

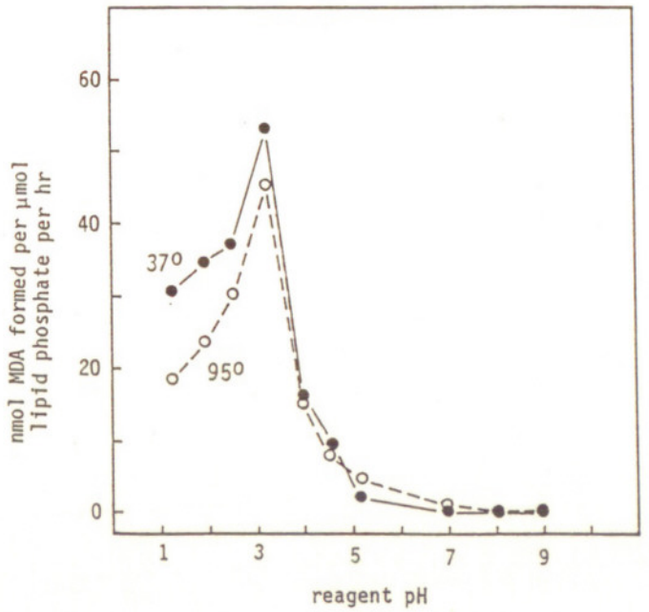



b) buffered TBA reagent effect

(i) 0.1 M Glycine-HCl buffer (pH 1.2 - 3.5)
(ii) 0.1 M Acetate buffer (pH 4.0 - 5.1)
(iii) 0.1 M Tris-HCl buffer (pH 7.0 - 9.1)



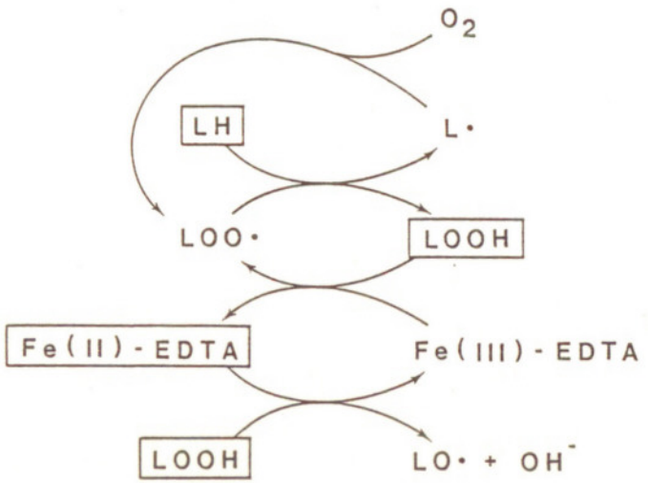




Conclusion: pH 3.5 is optimal for formation of TBA-reactive products from lipid hydroperoxides.

## SUMMARY

A. Fe(II)-EDTA initiates hydrogen abstraction from pre-existing lipid hydroperoxides to generate LO· and LOO· radicals which subsequently catalyses peroxidation of polyunsaturated microsomal lipids to peroxides, by free radical chain reaction.

B. Thermolytic breakdown of hydroperoxides is temperature- and pH- dependent. An optimal incubation temperature at 50 - 60° for 5 min has been observed to be a prerequisite for the induction of formation of TBA-reactive substances, possibly endoperoxide intermediates, during the course of heating. This thermolytic process at 50 - 60° is inhibited by BHT suggesting that free radical intermediates are produced at this stage.




## EXAMPLE OF CHAIN REACTION PATHWAY OF LIPID PEROXIDATION

Thermolytic Decomposition of Lipid Hydroperoxides