Catalysts of Lipid Peroxidation in Meats[1]

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ABSTRACT

Hemoprotein and non-heme iron components are active catalysts of lipid peroxidation. The behavior of these two catalysts under a number of conditions was compared as a basis for a study of their activities in meats. In model systems, MetMb accelerated linoleic acid peroxidation in a pH range from 5.6 to 7.8; it catalyzed especially rapidly at higher pH. A complex of ferrous ion [Fe(II)] and EDTA, a non-heme iron model, in a 1:1 ratio accelerated peroxidation at lower pH; no catalysis took place above pH 6.4. Most chelating agents eliminated Fe(II)-EDTA catalysis, but had no effect on MetMb catalysis. Reducing agents, on the other hand, accelerated Fe(II)-EDTA catalysis but inhibited MetMb catalysis. In model systems in which fresh dilute (1.2%, w/v) meat homogenate was the catalyst, the effect of the heme predominated. An exception was ascorbic acid; it accelerated oxidation at pH 5.6. The pattern of linoleate peroxidation catalyzed by heme-free (H_2O_2-treated) beef homogenate and shrimp homogenate was similar to that in the Fe(II)-EDTA model system. Again, ascorbic acid accelerated the catalysis and the acceleration could be eliminated by adding chelating agents. The presence of a non-heme iron catalyst in meat is thus indicated. Evidence is presented for both types of catalytic activity in meats. In cooked meats, heme was the dominant catalyst, but significant lipid oxidation, apparently catalyzed by a non-heme iron-type catalyst, occurred in cooked meats in which the heme had been destroyed by H_2O_2. In raw meats, lipid oxidation was inhibited at high pH because of removal of oxygen by enzymatic reducing systems. Both heme and non-heme iron were active at lower pH values. EDTA inhibited lipid oxidation during storage, presumably by its demonstrated effect on non-heme iron catalysis. Ascorbic acid also inhibited lipid oxidation, probably indirectly by keeping the heme pigment in the catalytic inactive ferrous state.

INTRODUCTION

Although the catalytic effect of hemoglobin (Hb) and other iron porphyrins on lipid oxidation is a generally accepted phenomenon, it is still not certain that this is the main oxidative catalyst in meat. Hematin-catalyzed lipid peroxidation has been demonstrated as an important deterioration reaction in unsaturated fat (1), precooked meats (2) and dehydrated foods (3). However, inorganic ferrous ion has also been demonstrated to be a catalyst of unsaturated lipid peroxidation in mitochondria (4) and microsome (5) as well as in pure unsaturated lipids (6). The pro-oxidant contribution of metals in meat and meat products has not been widely investigated.

Wills (7) presented evidence that both types of catalysts hemoprotein and inorganic iron, functioned in rat tissue. The two types could be differentiated by their relative activities at different pH and in the presence of chelating agents, ascorbic acid and thiol compounds. Barber (8) demonstrated that the catalytic role of iron and ascorbic acid was an important nonenzymic mechanism for lipid oxidation in tissue. He also indicated that sufficient iron was present in tissue particulates to bring about such catalysis. Lipid peroxidation could be inhibited by EDTA. Robinson (5) tried several different metals and found that only iron was capable of increasing lipid peroxidation with ascorbate or cysteine in microsomal suspension. Both heme or non-heme iron-containing enzymes present in the microsomal fractions could catalyze lipid peroxidation if the iron were made accessible through some change in tertiary structure (9).

It has been suggested that oxidative deterioration of the lipids in meat has been caused by the catalysis of hematin compounds, namely, MetMb. Metals have been investigated as pro-oxidants in meats. Moskovits and Kielsmeier (10) demonstrated that the contaminating iron in sausage exerted a powerful pro-oxidant activity. MacLean and Castell (11) have found that trace amounts of iron or copper ion added to whole or blended cod fillets produced a serious off-odor ranging from "seaweedy" to that of strongly rancid fish oil. However, their work did not try to assess the catalytic activity of the metal originally present in tissue.

Another factor which influences the oxidative rancidity in meat is pH. Keskinel et al. (12) indicated that there was an inverse relationship between the pH of meat samples and the thiobarbituric acid (TBA) number. Stewart et al. (13) also demonstrated that the enzymatic reducing activity of MetMb (MRA) increased with pH from 5.1 to 7.1. There is a highly negative correlation between the MRA and malonaldehyde (MA) formation in raw meats (14). Presumably, at higher pH, the reducing enzymes are in a much more active state. Oxygen is utilized by way of the electron transport system and any MetMb present is reduced.

Emanual' and Lyaskovskaya (15) found that a mixture of tocopherol with ascorbic acid and citric acid was 80 times more effective than ascorbate alone or in combination with citric acid in the tissue fats of fish. Several condensed phosphates were found to have antioxidant activity in cooked meat (16). Ascorbic acid again acted synergistically with phosphates to protect against rancidity in meats. The inhibition mechanisms of the chelating agents citric acid and phosphates and the reducing agent ascorbic acid have not been discussed.

Addition of free amino acids to blended cod muscle affected the subsequent development of rancidity (17). The aliphatic amino acids and cysteine showed strongly pro-oxidant activity. They did not explain the mechanism of the catalysis; however, the rancidity was found to be inhibited by adding the chelating agent EDTA and enhanced by adding ascorbic acid. It is thus probable that non-heme iron present in tissue accelerates the catalysis by complexing with cysteine or ascorbic acid and inhibits by combining with EDTA.

EXPERIMENTAL PROCEDURES

Before undertaking the investigation of lipid peroxidation in meats, the properties of both types of catalysts in model systems and semimodel systems should be studied systematically.

In developing a model system, MetMb (6×10^{-7}M) was used to represent the catalyst for heme-iron component,

[1]One of 28 papers presented at the Symposium, "Metal-Catalyzed Lipid Oxidation," ISF-AOCS World Congress, Chicago, September 1970.

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which would produce moderate oxidation with $7x10^{-3}M$ linoleic acid emulsion in 0.1 M phosphate buffer. The non-heme iron model developed for this study consisted of a mixture of Fe(II) and EDTA. Each, present at a concentration of $1.5x10^{-4}M$, was found to produce a constant catalytic rate in 0.05 M phosphate buffer linoleic acid emulsion. Such iron chelates of EDTA have been used to produce free radicals in various oxidative reaction (18-21). Thiol, ascorbic acid and chelating agents were expected to have different effects on hemoprotein vs. non-heme iron catalysis. Addition of these compounds therefore might help to differentiate the two systems.

Homogenates of rat liver, spleen, heart and kidney can actively catalyze peroxide formation in emulsions of linoleic or linolenic acid (7). Observation on a semimodel system, utilizing tissue as catalyst, was considered desirable. In this study, beef muscle homogenate is assumed to contain both heme and non-heme iron components. To differentiate non-heme iron from hemoprotein, Wills (7) gave the tissue a prior treatment with H_2O_2 to destroy all the catalytic functions of hemoprotein and liberate the inorganic iron, so that non-heme iron would be the only catalyst. Shrimp tissue contains no hemoprotein other than cytochromes. Shrimps and H_2O_2-treated beef muscle were, therefore, selected as heme-poor sources to be compared with the heme-rich sources, i.e., beef. Effects of various treatments on these three types of catalyzed linoleate peroxidation may, therefore, shed light on the catalytic systems in these foods.

Meat is more complex and difficult to study than either model or homogenate systems. In homogenate systems, excess of the linoleate substrate was added to the catalysts, and the rate of oxygen uptake, recorded directly during the reaction, was a measure of lipid oxidation. The conditions of oxidation in meat are quite different. The catalysts may not be able to make complete contact with the intramuscular lipid, the ratio of catalysts to unsaturated fatty acids may be quite different from that in model systems, and the extent of lipid oxidation can not be determined directly, since a number of enzymatic oxygen-consuming reactions occur in meat.

Some lipid oxidation product, rather than oxygen uptake, must be measured. The TBA test which measured the pink color produced by reacting TBA with MA has been widely used for measurement of rancidity in foods. The TBA method may be an unreliable test for MA under certain conditions; Wills (22) pointed out that Fe(III) could readily form a colored complex with TBA reagent under test conditions. However, a UV spectrophotometric method developed by Kwon and Watts (23) can be successfully applied to the assay of MA in distillates from rancid foods. Although its sensitivity is only about 40% of the TBA test, it is more specific.

METHODS

In both model and semimodel systems, emulsions of linoleic acid were used as substrates for peroxidation studies by a slight modification of Surrey's method (24). One gram of linoleic acid was added drop by drop to 20 ml water in which 1 ml Tween 20 was dissolved. The contents were thoroughly mixed to disperse the acid into a fine emulsion. Then, 1 N KOH was added and the mixture once again agitated with a magnetic stirrer until a clear transparent solution was obtained. To this solution, 200 ml of 0.2 M phosphate buffer were added. A few drops of concentrated HCl were used to adjust to the desired pH. The final volume was made up to 400 ml with H_2O. The resulting solution contained approximately $9x10^{-3}M$ linoleic acid in 0.1 M phosphate buffer.

To 20 ml of the emulsion were added the catalysts and test solutions desired and the total volume made up to 25 ml in which the concentration of linoleic acid was $7x10^{-3}M$. Changes in oxygen tension were recorded, using the Beckman Oxygen Analyzer 777 calibrated at 160 mm Hg, the partial pressure of oxygen in air. The rates were linear after the first few minutes. The oxygen utilization was then calculated from the recording by measuring the linear slope over a 3 min period and expressing the values as mm Hg pO_2/min.

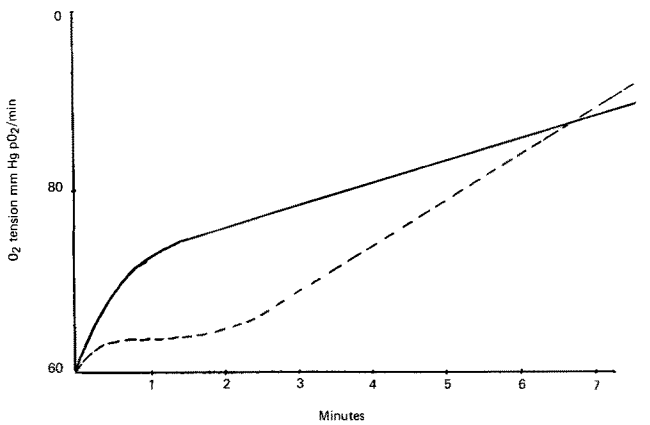

FIG. 1. Change of O_2 tension in linoleate emulsion catalyzed by MetMb or Fe(II)-EDTA at pH 5.6. Linoleic acid 7x10-3M; MetMb 6x10-7M; Fe(II)-EDTA(1.5x10-4M:1.5x10-4M). ——— Fe(II)-EDTA catalysis; – – – – – – – – MetMb catalysis.

In semimodel systems, homogenates from beef and shrimp were used as the catalysts. Eye of the round (semitendenous) beef and shrimp were purchased at local retail markets. Beef was trimmed, ground and mixed thoroughly, to obtain a homogeneous product. Shrimp was shelled and cleaned before homogenizing. Ground beef (50 g) was homogenized for 2 min in a Virtis blender with 100 ml 0.25 M cold sucrose solution, then filtered through four layers of cheese cloth. The filtrate was collected and stored in the refrigerator not longer than 30 min. It was brought to room temperature before using. Shrimp homogenate was prepared in the same way, except that less tissue (10 g) was used to prevent bubble formation.

To separate the non-heme iron, beef homogenate was treated drop by drop with 30% H_2O_2 until the pigment was decolorized. The treated tissue showed no absorption peaks in the range 400-700 mμ when analyzed by reflectance spectrophotometry. One milliliter (1.2%,w/v) of the homogenate was added to the linoleic acid emulsion and additives.

All reagents were of standard quality. The water used for the preparation of all solutions was passed through a deionizing column.

In meat study, beef round was used. The meat was trimmed of visible fat, ground and mixed thoroughly. Three milliliters of 0.1% chlorotetracycline-HCl was added to every 100 g meat in order to prevent spoilage by bacteria during the storage period (13). After the addition of various test solutions or water for the control, an amount equivalent to 50 g of meat was placed in a polyethylene bag and stored in the refrigerator at 3 C until analysis.

The extent of lipid oxidation was determined by distilling MA from the meat sample as described by Tarladgis et al. (22). The distillate was then analyzed for MA by UV spectrophotometry (23). To convert to mg MA per 1000 g meat, the UV absorbance was multiplied by the factor 18. The meat to be analyzed by reflectance spectrophotometry for MetMb formation must be quickly stirred to a uniform color before the determination, and the percentage of the pigment present as MetMb was derived from the assumed linearity of the ratio, K/S 572mμ:K/S 525mμ (25).

TABLE I

Effects of pH and Additives on Lipid Oxidation Catalyzed by Fe(II)-EDTA vs. MetMb

	Fe(II)-EDTA	MetMb
pH	5.6)6.2)7.0=7.8	7.8)7.0)5.6)6.2
Additives		
Chelating agents ($6x10^{-4}$ M)		
EDTA, $Na_3P_5O_{10}$, 8-OH-quinoline	Strong inhibition	No effect
Citric acid, xanthine	Slight inhibition	No effect
Ascorbic acid ($6x10^{-4}$M)	Acceleration	Inhibition
Thiols ($6x10^{-4}$M)	Acceleration	Inhibition

TABLE II

Effect of Chelating Agents, Thiols and Ascorbic Acid on Linoleate Peroxidation Catalyzed by Fe(II)-EDTA

	Rate of O_2 uptake mm Hg pO_2/min, pH			
	5.6	6.2	7.0	7.8
Fe(II)-EDTA	9.6	3.2	0	0
+ $6x10^{-4}$M EDTA	0	0	0	0
+ $6x10^{-4}$M 8-OH-quinoline	0	0	0	0
+ $6x10^{-4}$M $Na_3P_5O_{10}$	0	0	0	0
+ $6x10^{-4}$M Citric Acid	1.6	0	0	0
+ $6x10^{-4}$M Xanthine	2.4	0	0	0
+ $6x10^{-4}$M Ascorbic Acid	64.0	40.0	27.2	16.0
+ $6x10^{-4}$M L-cysteine	32.0	25.6	28.8	24.0
+ $6x10^{-4}$M MEA	25.0	9.6	11.2	51.2
+ $6x10^{-4}$M GSH	16.0	9.6	3.2	1.6

RESULTS AND DISCUSSION

Model System

Comparative Activity of the Two Catalysts. In non-heme iron catalysis, O_2 consumption of 16 recordings from one fatty acid preparation ranged from 8.0-11.2 mmHg pO_2/min with an average of 9.4±7.5% at pH 5.6. MetMb was also compared under the same conditions, except that the phosphate concentration was 0.1 M. The O_2 consumption of 12 recordings from one preparation ranged from 16-19.2 mmHg pO_2/min with an average of 17.5±3.7%. Since the heme iron concentration ($6x10^{-7}$M) used is less than .001 of the non-heme iron, obviously the heme is a much more potent catalyst.

The form of recordings shown in Figure 1 was highly reproducible. A sudden initial drop of O_2 tension with the Fe(II)-EDTA catalysis and a lag period before MetMb catalysis began at pH 5.6 were characteristic of the two types of catalyst.

Effect of Additives on the Fe(II)-EDTA and MetMb-Catalyzed Systems. The affects of pH and various additives on the two types of catalysis in model systems are shown in Table I.

Fe(II)-EDTA was active only at acidic pH; no catalysis took place above pH 6.4 (Table II). The complex neither inhibited nor accelerated linoleate oxidation at neutral or

TABLE III

Effect of Thiols and Ascorbic Acid on Linoleate Peroxidation Catalyzed by MetMb

	Rate of O_2 uptake (mm Hg pO_2/min), pH			
	5.6	6.2	7.0	7.8
$6x10^{-7}$M MetMb	12.8	11.2	20.8	44.8
+ $6x10^{-4}$M Ascorbic acid	12.8	11.2	12.8	9.6
+ $6x10^{-4}$M GSH	8.0	4.0	9.0	5.6
+ $6x10^{-4}$M MEA	6.4	5.3	3.6	6.4
+ $6x10^{-4}$M L-cysteine	8.0	3.2	6.4	4.8

TABLE IV

Effect of Ascorbic Acid and Thiols on Linoleate Peroxidation Catalyzed by Beef Homogenate[a]

	Rate of O_2 uptake (mm Hg pO_2/min), pH			
	5.6	6.2	7.0	7.8
Homogenate (1.2%, w/v)	32.0	28.8	41.6	72.8
+ $6x10^{-4}$M Ascorbic acid	49.6	24.0	8.0	1.6
+ $6x10^{-4}$M L-cysteine	20.8	9.6	11.2	4.8
+ $6x10^{-4}$M MEA	19.2	9.6	8.0	1.6
+ $6x10^{-4}$M GSH	27.2	16.0	25.6	40.0

[a]Linoleate emulsion was prepared in 0.1 M phosphate buffer.

TABLE V

Effect of Chelating Agents on Linoleate Peroxidation Catalyzed by Ascorbate and Homogenate at pH 5.6[a]

	Ratio of O_2 uptake of experiment to control
Control (mm Hg pO_2/min)	16
+ $6x10^{-4}$M Ascorbic acid (I)	3.8
(I) + $6x10^{-4}$M EDTA	0.5
(I) + $6x10^{-4}$M $Na_3P_5O_{10}$	0.6
(I) + $6x10^{-4}$M 8-OH-quinoline	0.8
(I) + $6x10^{-4}$M Xanthine	3.6
(I) + $6x10^{-4}$M Citric acid	2.0

[a]Linoleate emulsion was prepared in 0.1 M phosphate buffer.

alkaline pH. Most chelating agents inhibited Fe(II)-EDTA catalysis at pH 5.6 and 6.2. The inhibition was complete with EDTA, 8-OH-quinoline and $Na_3P_5O_{10}$. Citric acid and xanthine showed less effect. Ascorbic acid and thiols in the concentration range from $6x10^{-5}$M to $6x10^{-3}$M accelerated Fe(II)-EDTA catalysis over the entire pH range investigated. Ascorbic acid has long been recognized as an inorganic iron activator, but the thiols seem not to have been compared in such systems. Data are shown in Table II for both ascorbic acid and thiols at a concentration of $6x10^{-4}$M. In this case, thiols enhanced the Fe(II)-EDTA catalysis, they may be acting as reducing agents to regenerate the ferrous ion from the ferric form during the catalytic oxidative reaction, as has been suggested for ascorbic acid, or, they may act as chelators to render the non-heme iron more active (26).

MetMb catalyzed linoleate peroxidation much faster and without a lag period at more alkaline pH. The oxygen uptake at pH 7.8 was approximately four times greater than at pH 6.2 (Table III). However, at pH 7.8, as the oxidation progressed, the rate of oxygen uptake decreased, probably because of the destruction of the hematin catalyst (27). Ascorbic acid and thiols inhibited MetMb catalysis. The extent of inhibition tended to increase with higher pH. Chelating agents, on the other hand, did not show any effect on MetMb catalysis. When non-heme iron was absent, the inhibition of ascorbic acid and thiols on MetMb catalysis were probably acting as chain breakers during the process of fatty acid oxidation.

Fe(II)-EDTA complex in 1:1 ratio catalyzed linoleate peroxidation at a constant rate. Thus, it can be suggested as a non-heme iron model for free radical formation studied in model systems prior to application in foods. It must be remembered that although Fe(II)-EDTA were used as a model to represent non-heme iron catalyst, this particular iron complex obviously does not occur in food systems.

Tissue Homogenates Systems

It was expected that both heme and non-heme iron would contribute to catalysis by beef homogenate. However, the pattern of homogenate-catalyzed oxidation over a pH range 5.6 to 7.8 (Table IV) is similar to that of MetMb catalysis in the model system. It may be that, since the Mb in the homogenate is dissolved in the supernatant, Mb may make better contact with added substrate than the non-heme iron. Although the pattern remained the same, the extent of catalysis varied in homogenates in the same muscle from different animals. Variable composition of the meat or different amount of peroxides produced during preparation of linoleate emulsions could account for this.

Addition of $6x10^{-4}$M chelating agents did not show any effect on homogenate-catalyzed linoleate peroxidation over pH 5.6-7.8. As if hemoprotein were the only catalyst present, again, thiols and ascorbic acid inhibited the oxidation catalyzed by homogenates at alkaline pH. However, unlike the MetMb model, at pH 5.6, $6x10^{-4}$M ascorbic acid greatly accelerated the homogenate catalysis, indicating that non-heme iron components may play a role in tissue homogenates at this pH.

Further evidence for the presence of non-heme iron in beef homogenates was obtained by adding chelating agents to ascorbate-stimulated homogenate catalysis at pH 5.6 in phosphate buffer emulsion (Table V). Among the chelating agents tested, the best inhibitors with the homogenate catalyst, as with the Fe(II)-EDTA, were EDTA and $Na_3P_5O_{10}$. Variable inhibitions were obtained with the other chelating agents tried.

H_2O_2-treated beef homogenate was prepared to test its non-heme iron catalytic properties. Over a pH range 5.6 to 7.8, linoleate peroxidation catalyzed by H_2O_2-treated homogenate followed a pattern similar to that of the model system Fe(II)-EDTA (Table VI). However, the extent of O_2 uptake was less at pH 5.6 and the decrease with pH was not so rapid. Even at pH 7.8, slight catalytic activity still existed. The reducing agents ascorbic acid, GSH and L-cysteine again accelerated at acidic pH but, unlike the Fe(II-EDTA system, there was almost no effect at pH 7.8.

A comparative study of various chelating agents on linoleate peroxidation catalyzed by H_2O_2-treated homogenate with ascorbic acid or cysteine at pH 5.6 is shown in

TABLE VII

Effect of Chelating Agents on Linoleate Peroxidation Catalyzed by H_2O_2-treated Homogenate With Ascorbic Acid or L-cyteine at pH 5.6[a]

	Ratio of O_2 uptake of experimental to control
Control (mm Hg pO_2/min)	4.8
+ $6x10^{-4}$M Ascorbic acid (I)	5.7
(I) + $6x10^{-4}$M EDTA	0.33
(I) + $6x10^{-4}$M 8-OH-quinoline	0.33
(I) + $6x10^{-4}$M Xanthine	8.6
(I) + $6x10^{-4}$M Citric acid	8.0
+ $6x10^{-4}$M L-cysteine (II)	1.1
(II) + $6x10^{-4}$M EDTA	0.5
(II) + $6x10^{-4}$M 8-OH-quinoline	0.66
(II) + $6x10^{-4}$M Xanthine	1.66
(II) + $6x10^{-4}$M Citric acid	1.66

[a]Linoleate emulsion was prepared in 0.05 M phosphate buffer.

TABLE VI

Effect of Ascorbic Acid, GSH or L-cysteine on Linoleate Peroxidation Catalyzed by H_2O_2-treated Beef Homogenate[a]

	Rate of O_2 uptake (mm Hg pO_2/min), pH			
	5.6	6.2	7.0	7.8
H_2O_2-treated beef homogenate (1.2%, w/v)	4.8	3.2	2.4	1.6
+ $6x10^{-4}$M Ascorbic acid	64.0	22.4	6.4	1.6
+ $6x10^{-4}$M GSH	11.2	4.8	4.0	1.6
+ $6x10^{-4}$M L-cysteine	6.4	4.8	8.0	1.6

[a]Linoleate emulsion was prepared in 0.05 M phosphate buffer.

TABLE VIII

Effect of Ascorbic Acid, Thiols and Chelating Agents on Linoleate Peroxidation Catalyzed by Shrimp Homogenate[a]

	Ratio of O_2 uptake of experimental to control, pH			
	5.6	6.2	7.0	7.8
Control (mm Hg pO_2/min)	8.6	6.4	4.8	3.2
$6x10^{-4}$M Ascorbic acid	2.0	1.2	0.5	0.6
$6x10^{-4}$M L-cysteine	0.66	0.4	0.5	0
$6x10^{-4}$M GSH	0.66	0.6	0.5	0
$6x10^{-4}$M EDTA	0	0	0	0
$6x10^{-4}$M 8-OH-quinoline	0	0	0	0
$6x10^{-4}$M $Na_3P_5O_{10}$	1.0	1.0	1.0	0.5
$6x10^{-4}$M Citric acid	1.0	1.0	1.0	1.0

[a]Linoleate emulsion was prepared in 0.05 M phosphate buffer.

Table VII. Among these chelators, only EDTA and 8-OH-quinoline abolished the acceleration. Xanthine and citric acid accelerated further. It is indicated that chelating agents can act either as metal complex-activator or metal complex-inactivator, depending on the conditions of the reaction.

Shrimp, considered to have no Mb present, was tested for its non-heme iron catalytic reaction on linoleate peroxidation (Table VIII). Shrimp homogenate followed more or less the same pattern as H_2O_2-treated beef homogenate catalysis. The chelating agents $Na_3P_5O_{10}$, xanthine and citric acid did not show any effect on the catalysis. Thiol compounds L-cysteine and GSH and the chelating agents EDTA and 8-OH-quinoline inhibited the catalysis. Again, ascorbic acid accelerated the catalysis at pH 5.6 and 6.2.

In shrimp tissue, non-heme iron components were playing the dominant role. The complete inhibition by EDTA and 8-OH-quinoline and the high acceleration by ascorbate at acidic pH support this. The fact that, unlike their activity in beef, thiols increased inhibition in shrimp as pH increased and showed no acceleration, may indicate that either different types of iron complexes or other metal complexes may be present in shrimp tissue.

Meat System

Based on the results described above, an experiment was designed to identify heme and non-heme iron catalysts in meats. Some important differences in the two types of catalysis, i.e., the effect of pH, EDTA and ascorbic acid are emphasized. Two widely different pH values were compared. The natural pH of the piece of beef round used was pH 5.5. Half of the meat was adjusted to pH 7.8 by adding 5N NH_4OH. This is far outside the normal range of meat but the catalytic activity of Mb is high at this pH according to both model and homogenate systems. Ascorbic acid and EDTA were then added to these two samples. MA formation was determined over a five-day storage period.

At pH 7.8, neither controls nor treated samples increased in MA during storage (Rable IX). All samples were a deep dark red color. This color did not fade until the last day of the experiment. On the other hand, the control samples held at pH 5.5 showed increasingly high rancidity over the storage period. Either ascorbic acid or EDTA inhibited MA formation. The protection was increased when both of them were added together to the meat. As for the pigment, the sample to which ascorbic acid was added maintained a bright red color until the fifth day of storage. Ascorbic acid plus EDTA also gave this desirable appearance. But there was not much difference between EDTA and control samples; both were pale brown.

Superficially, it seems that there is a discrepancy between these results and those found in the previous experiments. At pH 7.8, according to both model and homogenate systems, hemoprotein was the main catalyst

TABLE IX

Effects of Ascorbic Acid, EDTA on MA[a] Formation

	mg MA/1000 g meat					
	pH 5.5, days			pH 7.8, days		
	1	3	5	1	3	5
Control	8.8	10.2	13.6	1.1	1.1	0.8
$4x10^{-4}$moles Ascorbate	4.5	5.5	7.5	0.8	0.6	0.6
$2.5x10^{-4}$moles EDTA	4.5	4.3	4.9	1.0	0.8	1.2
$4x10^{-4}$moles Ascorbate $2.5x10^{-4+}$moles EDTA	3.6	3.7	3.1	1.6	1.9	1.2

[a]MA, malonaldihyde.

TABLE X

Effect of H_2O_2 on MA[a] Formation in Cooked Meat

	mg MA/1000 g meat					
	H_2O_2 treated			untreated		
Days	pH 5	pH 6	pH 7	pH 5	pH 6	pH 7
1	2.3	1.9	1.7	6.0	7.0	6.7
3	5.6	4.0	3.3	9.5	11.2	9.8
5	7.2	4.9	4.2	14.0	14.0	12.0

[a]MA, malonaldihyde.

for linoleate peroxidation. But in meat, no oxidation occurred. Other factors in the complex meat system inhibited the reaction. At this high pH, enzymatic reducing systems in meat are very active, utilizing the available oxygen and maintaining Mb in reduced form which will not catalyze lipid oxidation. At pH 5.5. catalysis by non-heme iron was expected. EDTA inhibited the rancidity, indicating the presence of non-heme iron in meat. The inhibition caused by ascorbic acid is interpreted as an effect on the pigment Mb rather than on the non-heme iron moiety. The effect of ascorbic acid on maintaining a good color in meat has been discussed (28). Thus ascorbic acid does not affect the rancidity directly but indirectly, through inhibiting MetMb formation.

Further evidence comes from cooked meat, since here the picture is not complicated by the presence of interferring enzymes and all the heme iron is converted to the ferric form without any chance to reduce back. The ferric heme has always been considered as the active form in catalyzing lipid oxidation. One experiment was set up to determine the oxidative rancidity developed at various pH values in cooked meat before and after H_2O_2 treatment to destroy heme (Table X). MA values were high and little affected by pH in the untreated cooked meat. In the H_2O_2-treated meat, although less MA formed during storage, the amount was still of practical significance, considering that off-odors are easily detectable in meats when MA values exceed one. As would be expected with a non-heme iron catalyst, oxidation was greater at lower pH values. Once again, it is indicated that non-heme iron as well as hemes have a role in lipid oxidation in meats.

Even though Mb is an active catalyst, the catalytic activity of hemoprotein is limited in raw meat. Oxygen can be removed from the tissues and MetMb reduced back to Mb by the reducing enzymes. This is especially true at higher pH. Furthermore, the reaction between MetMb and polyunsaturated fatty acids is considerably less than would be expected from the same concentrations of reactants in model systems, probably due to the separation of reactants in cellular structures.

Because pH varies over a wide range in meats and variable amounts of reducing agents, i.e., ascorbic acid or cysteine, and because chelators, i.e., citric acid, amino acids, etc., are naturally present in meat tissue, the catalytic activity of non-heme iron might also be expected to show wide variations in different samples of meat. The catalysis is certainly important from a practical standpoint.

The nature of iron complexes responsible for catalyzing lipid oxidation in meat is not known. Among the non-heme iron components of muscle tissue, transferrin, ferritin and a number of enzymatically active compounds of the respiratory chain in mitochondria have been identified. Other non-heme iron components may also be present. The identification and estimation of the activities of possible non-heme iron catalysts in meat might be interesting as a further step in future research on lipid oxidation in meat.

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[Received January 14, 1971]