LIPID PEROXIDATION IN LIVERS AND KIDNEYS FROM YOUNG AND OLD RATS

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SUMMARY: Lipid peroxidation was measured in young (6 months) and old (24 months) rat tissue homogenates and microsomal fractions. Decreases in lipid peroxidation were noted in homogenates and microsomes from old animals. The decreases in liver and kidney appear due to reduced ascorbic acid content and the appearance of an inhibitor in the soluble phase. In addition old kidney microsomal fractions contain a membrane associated lipid soluble inhibitor. The significance of these inhibitors and of lipid-protein interactions in the regulation of lipid peroxidation are also discussed.

In vitro peroxidation of the polyunsaturated lipids found in cellular membranes leads to the formation of a wide variety of toxic products, destruction of lipid requiring functions and deterioration of the membraneous structure(1). The age related accumulation of lipopigments containing peroxidized lipid is considered to be evidence for possible *in vivo* peroxidation and has lead to the postulation of a model of aging involving lipid peroxidation(2). However, peroxidation has not been systematically examined in young and old animals.

The lipid peroxidation reaction in aerobically incubated tissue fractions is dependent on the content of polyunsaturated fatty acids, iron and ascorbic acid(3). This study was carried out to compare the lipid peroxidation potential of liver and kidney homogenates and microsomal fractions prepared from 6 and 24 month old rats, as well as to study the tissue content of each component required for peroxidation.

METHODS: Male Sprague-Dawley rats were maintained on a Purina stock diet. Animals were sacrificed by stunning followed by exsanguination and decapitation. Livers and kidneys were removed and homogenized (1:5, w/v) in buffer (0.01M phosphate-0.15M NaCl, pH 7.0) and microsomal fractions were isolated as previously described(4).

Lipids were extracted according to the method of Folch et al.(5). The non-lipid portion of the extract was dried in air, homogenized in buffer and used as the residue fraction. Fatty acids were prepared using BF_3-methanol esterification kits (Applied Science) and analysed as previously described(6). Separation of neutral and polar lipid fractions was performed on precoated Silica gel H plates (Applied Science) using ethyl ether as solvent. Samples were eluted with chloroform, chloroform:methanol (1:1), and methanol.

Protein was determined according to the method of Lowry et al.(7). Ascorbic acid was measured using 2,6-dichlorophenolindophenol solution according to the method of Roe(8). Iron was determined using a modification of the O-phenanthroline method(9). Phosphorus was determined using a modification of the method of Youngburg and Youngburg(10). Samples used for measuring iron and phosphorus were ashed in H_2SO_4 and $HCLO_4$. Tocopherol was measured by the method of Tsen(11). Lipid peroxidation was measured colorimetrically by the thiobarbituric acid(TBA) procedure following incubations at 37C for 90 minutes(3).

RESULTS: The ascorbic acid, iron and lipid phosphorus contents and the peroxidation values of liver and kidney homogenates from 6 and 24 month old animals are compared in Table I. The iron and phospholipid contents of liver remained essentially unchanged between 6 and 24 months. The iron content of kidney, on the other hand, increased three fold by 24 months whereas the phospholipid content of the kidney was greatly reduced. Both tissues had a 30% reduction in ascorbic acid at 24 months. The kidney contained less ascorbic acid than the liver. Peroxidation was markedly reduced in homogenates of both tissues prepared from old animals.

Homogenate peroxidation could be increased by the addition of ascorbic acid. The amount of peroxidation in the old tissues, however, could not be increased to that of the young tissues(Table II). The decrease in peroxidation with age is therefore only partially due to the decrease in ascorbic acid in the soluble phase of old animals.

TABLE I. ASCORBIC ACID, IRON, LIPID PHOSPHORUS AND PEROXIDATION VALUES OF LIVER AND KIDNEY HOMOGENATES

Values are expressed per gm tissue. Peroxidation expressed as TBA O.D. at 530 mμ. Each incubation beaker contained 20 mg protein in a final volume of 4 ml. Homogenates were prepared from a minimum of three animals. Values represent the average of at least 4 independent determinations. Standard deviations are indicated. The ranges of TBA values are given.

TISSUE (mos)	Ascorbic Acid μg/gm	Iron μg/gm	Lipid Pi mg/gm	TBA O.D.
Liver (6)	245 ±16	145 ±29	2.3 ±.24	.630(.415-.970)
Liver (24)	168 ±20	154 ±23	2.5 ±.45	.170(.080-.380)
Kidney (6)	112 ±20	63 ±13	1.7 ±.23	.360(.320-.410)
Kidney (24)	80 ± 9	180 ±13	1.1 ±.13	.100(.070-.190)

TABLE II. EFFECT OF ADDED ASCORBIC ACID ON HOMOGENATE PEROXIDATION

Each incubation beaker contained 20 mg homogenate and the indicated amount of ascorbic acid in a final volume of 4 ml. Peroxidation is expressed as TBA O.D. at 530 mμ. Values are from a single experiment run in duplicate.

	TBA O.D.			
	Liver		Kidney	
Ascorbic Acid Added	6 mos	24 mos	6 mos	24 mos
None*	.97	.08	.36	.07
35 μg	1.80	.24	.69	.21
70 μg	2.40	.18	1.26	.36

*Endogenous ascorbic acid present in 20 mg homogenate: Liver 13 μg (6 mos), 9 μg (24 mos), Kidney 8 μg (6 mos), 7 μg (24 mos).

Soluble phase inhibitors of lipid peroxidation have been demonstrated in certain rat homogenates(12). The soluble phases of liver and kidney were isolated as post microsomal supernatants and their ability to inhibit the peroxidation of young liver microsomes was measured. The data in Table III show that the soluble phases of old liver and old kidney inhibited peroxidation more than 90%. These soluble phase inhibitors were non-dialysible and were

TABLE III. INHIBITION OF PEROXIDATION BY SOLUBLE PHASES

Each incubation beaker contained 1 mg liver microsomal protein (6 mos), 10 mg of soluble phase protein and 70 µg ascorbic acid in a final volume of 4 ml. Peroxidation is expressed as TBA O.D. at 530 mµ. Values are from a single experiment run in duplicate.

	SOLUBLE PHASE ADDED				
	NONE	LIVER		KIDNEY	
		6 mos	24 mos	6 mos	24 mos
TBA O.D.	.95	.82	.05	1.28	.06

TABLE IV. IRON, LIPID PHOSPHORUS AND PEROXIDATION VALUES OF LIVER AND KIDNEY MICROSOMAL FRACTIONS

Iron and lipid values are expressed as ug/mg microsomal protein. Peroxidation values are expressed as TBA O.D. at 530 mµ. Incubation beakers contained 1 mg protein and 140 µg ascorbic acid in a final volume of 4 ml. Values represent the average of at least 4 separate determinations. Standard deviations are indicated. The ranges of the TBA values are given.

TISSUE (mos)	Iron	Lipid Pi	TBA
	µg/mg	µg/mg	O.D.
Liver (6)	3.8 ±.4	14.7 ±1.0	.610(.500-.770)
Liver (24)	4.3 ±.6	11.6 ±1.1	.480(.460-.500)
Kidney (6)	1.5 ±.1	15.2 ±1.3	.210(.200-.230)
Kidney (24)	4.8 ±.2	8.8 ±1.2	.050(.045-.055)

found to be labile to incubation at 55C for 15 minutes. These inhibitors are under further investigation.

Lipid peroxidation was also examined in microsomal suspensions, since they undergo extensive peroxidation following addition of ascorbic acid(13). Peroxidation was markedly reduced in the microsomal suspensions prepared from kidneys of old animals but only slightly reduced in those prepared from livers of old animals(Table IV). Kidney microsomal fractions, like homogenates,

prepared from old animals contained three times more iron but considerably less phospholipid than those prepared from young animals. Livers from young and old animals contained similar contents of iron whereas the phospholipid content was slightly reduced in the liver microsomes from old animals.

Recombination experiments using lipid extracts and residue fractions of the microsomes prepared from liver and kidney were carried out(Table V). The

TABLE V. LIPID PEROXIDATION IN RECOMBINED LIVER AND KIDNEY MICROSOMAL SUBFRACTIONS

Microsomal lipids were extracted as described in methods. Dried lipid was suspended in buffer at approximately 1:75 (microsomal pellet weight: final volume). Residue was brought to the same final volume. Incubation beakers contained 1 ml each of lipid and residue, and 70 µg ascorbic acid in a final volume of 4 ml. Peroxidation is expressed as TBA O.D. at 530 mµ. Values are from a single representative experiment.

LIPID	RESIDUE			
	LIVER		KIDNEY	
	6 mos	24 mos	6 mos	24 mos
Liver (6 mos)	.480	.540	.485	.490
Liver (24 mos)	.500	-	-	-
Kidney (6 mos)	.660	-	-	-
Kidney (24 mos)	.050	.055	.040	.040

residue fraction contains the catalytic iron needed for the reaction. All residue fractions prepared from young and old animals catalyzed peroxidation to the same extent. The kidney lipid fraction prepared from old animals did not undergo peroxidation regardless of the amount of lipid added or the residue fraction used. The absence of peroxidation in kidney microsomes prepared from old animals, therefore, is due to some component in the lipid fraction.

The fatty acid composition of liver and kidney microsomal fractions prepared from 6 and 24 month old animals were compared. Little age related difference was noted. All tissues contain large amounts (>30%) of peroxidizable fatty acids. The tocopherol contents of the microsomal fractions were also

compared and were found to be unchanged or only slightly reduced with age. The inhibition of peroxidation in old kidney microsomes is therefore not due to lack of peroxidizable fatty acids or to increase in tissue antioxidants such as tocopherol.

The lipid extracts of the young and old kidney were further separated in neutral and polar fractions. These were tested individually as well as recombined. The polar fractions of young and old kidney undergo equal amounts of peroxidation, whereas the neutral fractions, being low in polyunsaturates, do not undergo peroxidation. Recombination of neutral and polar fractions resulted in only very slight inhibition of the peroxidation of the polar fraction. The lipid soluble inhibitor of the old kidney therefore was not present following the separation of the neutral and polar fractions. This lipid soluble inhibitor is still under investigation.

DISCUSSION: Lipid peroxidation is decreased in aerobically incubated homogenates of livers and kidneys isolated from old rats. Although several mechanisms such as reduced ascorbic acid and phospholipid might contribute slightly to the decrease, the most important factor is an increase in inhibitors of peroxidation in tissues isolated from old animals. These inhibitors are in the soluble phases of both tissues and in the microsomal fraction of kidney. The soluble phase inhibitors are non-dialyzable, heat labile and presumably protein. The microsomal membrane bound inhibitor is lipid soluble and is destroyed by the techniques used in this study to separate polar and non-polar lipids. The significance of these inhibitors in the aging process is unknown and they are being further characterized.

The lipid peroxidation reaction is affected by the nature of the lipid-protein interactions in membranes(14). These interactions are altered sufficiently in the aging process to affect the kinetic activity of microsomal glucose-6-phosphatase(4). This altered interaction could contribute to the reduced peroxidation noted in microsomal fractions isolated from old animals. The absence of peroxidation in the isolated lipid fraction of old kidney sug-

gests, however, the importance of a lipid soluble inhibitor. The possible role of lipid peroxidation in aging is still obscure. The reaction, is however, of significance for identifying alterations in subcellular membranes and it is these alterations which may prove to be of greater significance for studies on the mechanism of aging at the subcellular level.

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