Relative Susceptibility of Microsomes from Lung, Heart, Liver, Kidney, Brain and Testes to Lipid Peroxidation: Correlation with Vitamin E Content

DOUGLAS J. KORNBRUST and **RICHARD D. MAVIS**, Department of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642

ABSTRACT

Rates of in vitro lipid peroxidation of microsomes and homogenates were found to vary widely among different tissues and species. In rats and rabbits, lung microsomes peroxidized at a 25- to 50-fold lower rate than liver, kidney, testes and brain microsomes. Heart microsomes peroxidized at a rate slightly greater than, but most similar to, lung microsomes. Comparison of tissue homogenates also revealed the unique resistance of lung and heart to lipid peroxidation. The ratio of vitamin E to peroxidizable polyunsaturated fatty acids in lung and heart microsomes was several-fold higher than in microsomes from the other tissues studied, which accounted for the relative resistance of lung and heart to lipid peroxidation. Liposomes of extracted rat lung microsomal lipid were also resistant to peroxidation and the amount of vitamin E contained in the lung lipid extract was sufficient to confer the same degree of resistance when incorporated into an equivalent amount of rat liver lipid. Higher rates of peroxidation in mouse lung microsomes relative to rabbit, rat and human lung microsomes were similarly correlated with a lower ratio of vitamin E to peroxidizable fatty acids in mouse lung microsomes. These data provide strong support for the role of vitamin E as the major cellular antioxidant, especially in the highly oxygenated tissues of heart and lung, and demonstrate the utility of the microsomal system in characterizing tissue differences in susceptibility to peroxidative membrane decomposition.

INTRODUCTION

Intracellular lipid peroxidation has been often hypothesized as a mechanism of action of toxic agents (1) and has been implicated as a degenerative mechanism underlying cellular aging (2) and certain disease states (3). Liver microsomes or reconstituted systems containing extracted liver lipid plus purified liver microsomal NADPH-cytochrome P-450 reductase have been most frequently employed in investigations of the mechanism of peroxidation of the fatty acyl moieties of membrane phospholipids (4,5). Little information is available, however, regarding the relative susceptibility of membranes from various tissues to undergo lipid peroxidation. The peroxidizability of lung is of particular interest since it is the point of entry for oxidant gases contaminating the atmosphere. Roback observed that over 50 times more thiobarbituric acid-reactive material was produced during incubation of liver homogenate than during incubation of lung or spleen homogenates (6). Willis and Recknagel recently reported lung microsomes were only 4% as active as liver microsomes in producing malondialdehyde, although the basis for the low activity of lung microsomes was not identified in that study (7).

The biological antioxidant, vitamin E, has been shown to afford protection against the injurious effects of nitrogen dioxide and ozone on experimental animals (8). In addition, numerous studies have indicated susceptibility to lipid peroxidation is greatly influenced by tissue levels of vitamin E (9). Bieri and Anderson (10) demonstrated that the ability of tissue homogenates to undergo lipid peroxidation in vitro was inversely related to the dietary vitamin E status of the animal. Similarly, other investigators have shown that liver microsomes (11-13) or mitochondria (14) isolated from vitamin E-deficient animals peroxidize at a faster rate in vitro than fractions from control animals. Peroxidation of liver microsomes was inhibited by dietary supplement of vitamin E (12,13) or when vitamin E was added directly to liver microsomal suspensions (11,15), which support the role of vitamin E as a membranous antioxidant.

Taylor et al. (16) have shown there is a high degree of variation in the content of vitamin E in subcellular fractions from different tissues, which indicates there may be considerable differences among tissues with respect to protection against peroxidative reactions. In these investigations, we have measured rates of in vitro lipid peroxidation in microsomes and homogenates from several different tissues and have compared the variation in these rates with the microsomal vitamin E content. Microsomes and homogenates from lung and heart showed a low rate of in vitro peroxidation compared to

the other tissues studied. This resistance of lung and heart to lipid peroxidation can be explained by relatively high levels of microsomal vitamin E in these tissues.

EXPERIMENTAL PROCEDURE

Materials

Distilled water was filtered and deionized in a system custom designed by W.E. Chaffee Co., Inc., an affiliate of Continental Water of Buffalo, NY. NADPH, ascorbic acid, $FeSO_4$, EDTA, cytochrome c and *D,L*-α-tocopherol were obtained from Sigma Chemical Company, St. Louis, MO. Thiobarbituric acid was a product of Eastman Chemicals, Rochester, NY. Fatty acid methyl ester standards were purchased from Nu-Chek-Prep, Elysian, MN. Rats were male Long Evans (200-300 g), mice were female BALB/c (20-30 g) and rabbits were male New Zealand albino (2-3 kg).

Methods

All animals received Agway laboratory chow (Rochester, NY) ad libitum until the time of sacrifice. Animals were sacrificed by intraperitoneal (ip) injection of sodium pentobarbital, the chest was opened and a cannula was inserted into the trachea. Heart, lungs and liver were perfused by injecting ice-cold 0.15 M NaCl into the right ventricle of the heart while simultaneously ventilating the lungs through the tracheal cannula. Lungs were excised and the parenchyma was separated from the visible bronchi and blood vessels and minced thoroughly with scissors. Hearts, livers, kidneys, brains and testes were removed and similarly minced. The minced tissues were washed several times in ice-cold buffered potassium chloride (0.15 M KCl, 5 mM Tris-maleate, pH 7.4) containing 1 mM EDTA and homogenized in the same, using a teflon-glass homogenizer. The homogenate was centrifuged successively at 300, 1600, 8000 and 30,000 x g for 10 min at each speed and the pellets discarded. The "cell-free homogenate" refers to the supernatant after centrifugation at 300 x g. Microsomes were obtained from the 30,000 x g supernatant by centrifugation at 100,000 x g for 1 hr. The microsomal pellet was washed by suspension in and resedimentation from buffered potassium chloride with no EDTA, resuspended in the same type of solution and stored in liquid nitrogen. Lipid peroxidation in these EDTA-treated microsomes has been shown to be absolutely dependent on the concentration of added free ferrous iron (17). Protein concentrations were determined by the Lowry method (18); NADPH-cytochrome c reductase was assayed spectrophotometrically, as described by Williams and Kamin (19) and the initial rates measured were linearly proportional to time of incubation and amount of protein added over the ranges used.

Lipid peroxidation was measured by quantitation of malondialdehyde formed during the incubations. Microsomes (50-80 μg of protein) were incubated at 37 C for the specified lengths of time with 40 mM Tris-maleate buffer (pH 7.4) and either 3.0 μM $FeSO_4$ plus 250 μM NADPH or 1.0 μM $FeSO_4$ plus 500 μM ascorbate in a total volume of 0.5 ml. Peroxidation was terminated by rapid addition of 20% trichloroacetic acid (0.15 ml), 0.05 M thiobarbituric acid (0.3 ml) and 0.2% butylated hydroxytoluene (50 μl). Bovine serum albumin (0.5 mg) was added to facilitate precipitation of protein during a 10 min centrifugation; the resulting clear supernatant was removed and delivered to glass test tubes which were then tightly capped and boiled for 8 min. The amount of colored product was measured spectrophotometrically as described by Buege and Aust (4).

Lipid peroxidation was expressed in terms of nmol malondialdehyde (MDA)/mg protein or as "percent peroxidation" which is simply the percentage of maximal MDA which would be produced by complete peroxidation of the peroxidizable polyunsaturated fatty acids (PPUFA) present in the tissue fractions. PPUFA includes all of the detectable polyunsaturated fatty acids except linoleic acid (18:2), which we have demonstrated, in agreement with others (20,21), to be relatively resistant to peroxidation and is not believed to evolve malondialdehyde (22). Maximal MDA formation per mg of protein was routinely determined for each liver microsomal preparation by incubating with a sufficient amount of $FeSO_4$ and ascorbate over time until MDA formation reached a maximal value. We have previously shown that all of the liver microsomal PPUFA has reacted at this point and that the percentage of the maximal MDA formation obtained during a given incubation closely correlates with the percentage depletion of peroxidizable lipid substrate (17).

In separate experiments, the yield of MDA/mol PPUFA was found to be similar for all tissues studied. Thus, in these studies, the determination of the maximal MDA formation in lung, heart, kidney, brain and testes microsomes was obtained simply by multiplying the value for the yield of MDA/mol PPUFA from liver microsomes by the PPUFA content of the other tissues as determined by phospholipid (23) and fatty acid analysis by gas liquid chromatography (GLC) (24). The amount of

MDA produced during the experimental incubations was then expressed in terms of the percentage of the maximal MDA value and referred to as "percent peroxidation." Thus, "percent peroxidation" is equivalent to the percentage of peroxidizable polyunsaturated fatty acids reacted. Values for 100% peroxidation in terms of nmol MDA/mg protein and microsomal PPUFA contents as nmol PPUFA/mg protein are given in each figure legend (means and standard deviations). Vitamin E was measured using the spectrofluorometric technique of Taylor et. al. (16). Using this method, tissue samples are saponified and extracted yielding a hexane phase which contains partially purified vitamin E. Recovery of an internal α-tocopherol standard was 95%.

Liposomes were prepared by extracting lipids from microsomes by the Bligh and Dyer procedure (25). The chloroform solution was evaporated to dryness under N_2 gas in a glass vessel. The resulting lipid film was hydrated and ultrasonically dispersed into an aqueous suspension in a sonicating bath under N_2 gas. Where indicated, vitamin E extracted from microsomes by the Taylor et. al. procedure (16) or authentic α-tocopherol subjected to the same procedure was incorporated into liver liposomes by mixing the hexane extract with the chloroform solution of liver microsomal lipids prior to drying and dispersing.

RESULTS

Figure 1 shows the time course of lipid peroxidation in rat liver, brain, kidney, testes, heart and lung microsomes as induced by NADPH and ferrous iron. The rate of peroxidation of lung microsomes was extremely low, relative to liver, brain, kidney and testes microsomes. Heart microsomes exhibited a rate of peroxidation slightly higher than lung microsomes but still 20-fold lower than liver microsomes at the early time points. These differences were not caused by differences in peroxidizable fatty acid content since the susceptibility of the various microsomes to peroxidation did not correlate with their PPUFA content (see Fig. 1) and the data are expressed in terms of the percentage of peroxidizable lipids that had reacted.

Although the NADPH-dependent peroxidation is known to be mediated by the microsomal NADPH-cytochrome P-450 reductase (26), differences in peroxidizability could be only partially explained by tissue differences in the activity of this enzyme. Using cytochrome c as an electron acceptor, specific activities (sp act) were found to be 205, 46, 37, 25, 30 and 91 nmol cytochrome c reduced/min/mg protein for liver, brain, kidney, testes, heart and lung microsomes, respectively. These values obviously do not correlate with the pattern of microsomal peroxidation observed in Figure 1, suggesting the reductase activity is not limiting the rate of peroxidation under these conditions.

In order to more accurately assess the relative peroxidizability of the microsomes, rates of peroxidation were determined using ascorbate instead of NADPH. Ascorbate is believed to stimulate peroxidation in a manner similar to NADPH, i.e., by promoting reduction of iron (17), although the ascorbate/iron system involves a direct, rather than enzyme-catalyzed reduction of iron (27) and is therefore independent of the microsomal NADPH-cytochrome P-450 reductase activity. Results of these experiments are shown in Figure 2. Rates of ascorbate/iron-induced peroxidation were similar to those obtained with NADPH/iron (Fig. 1), except that liver microsomes peroxidized less rapidly in the ascorbate-stimulated system. This difference probably results from the much higher reductase activity of liver microsomes which may enhance the rate of NADPH-dependent peroxidation in this fraction relative to the microsomes from other

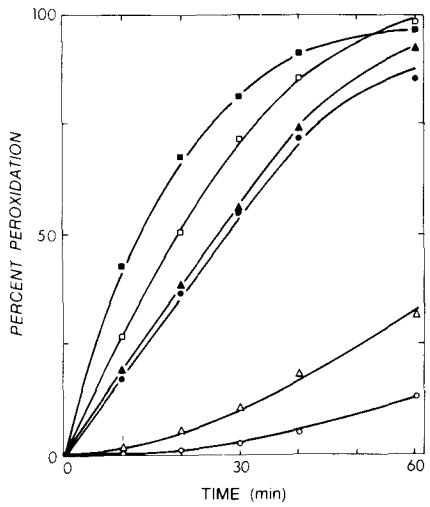

FIG. 1. Rate of NADPH/Fe^{2+}-induced lipid peroxidation in microsomes from various tissues of rat. (●) liver, (■) brain, (▲) kidney, (□) testes, (△) heart, (○) lung microsomes. Each point represents the mean value from 4 determinations. One hundred percent peroxidation is equivalent to 108 ± 11, 67 ± 15, 54 ± 9, 36 ± 4, 62 ± 14, and 92 ± 8 nmol of MDA per mg of protein for liver, brain, kidney, testes, heart, and lung microsomes, respectively. The PPUFA contents of these same microsomes were 803 ± 92, 495 ± 145, 403 ± 62, 271 ± 31, 458 ± 82, and 677 ± 62 nmol of PPUFA per mg of protein, respectively.

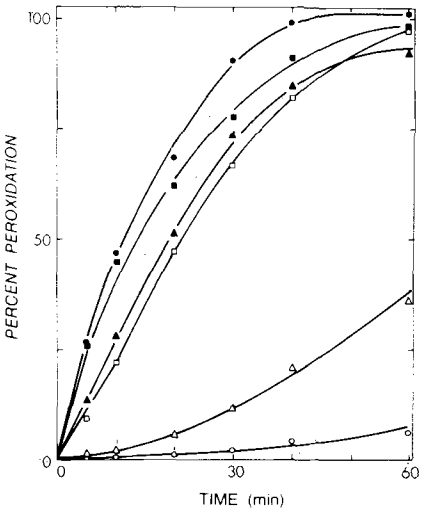

FIG. 2. Rate of ascorbate/Fe^{2+}-induced lipid peroxidation in microsomes from various tissues of rat. Symbols, values for 100 percent peroxidation, and PPUFA contents are the same as in Figure 1. Each point represents the mean value from 4 determinations.

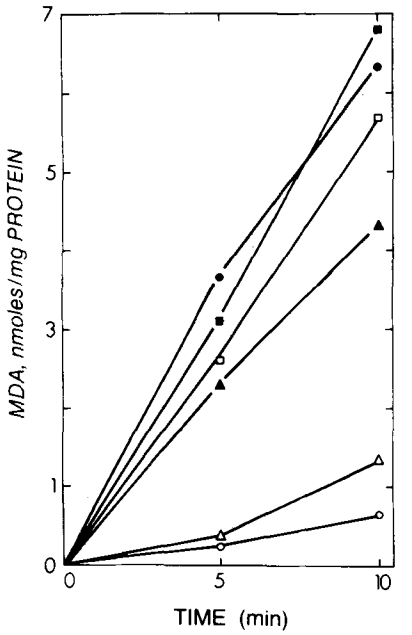

FIG. 3. Rate of Fe^{2+}-induced lipid peroxidation in cell-free homogenates of various rat tissues. Homogenates were prepared as described in Methods and incubated with 40 mM Tris-maleate (pH 7.4) and 40 μM $FeSO_4$ (Note: The homogenates contained EDTA which was present as a 30 μM concentration such that the concentration of free iron, which is effective in inducing peroxidation, was around 10 μM). Symbols are the same as in Figure 1. Points are the mean values from 2 determinations.

tissues.

In order to determine whether the rates of peroxidation in microsomes were representative of the whole tissue or organ, we performed similar measurements using tissue homogenates. As shown in Figure 3, heart and lung homogenates were peroxidized to a much lesser extent than homogenates of the other tissues studied, a result which demonstrates the uniqueness of these tissues as well as the validity of using microsomes as an indicator of whole tissue peroxidizability.

Attempts to elucidate the nature of these apparent tissue differences in susceptibility to peroxidation focused on the differences between lung and liver microsomes. Fatty acid analysis of lung and liver microsomes following incubation revealed that the polyunsaturated fatty acids of the lung microsomes were only slightly decreased under conditions which resulted in total reaction of the peroxidizable lipids of the liver microsomes (data not shown). Increasing concentrations of NADPH or ascorbate did not increase the rate of lung microsomal peroxidation. As shown in Figure 4, peroxidation of lung microsomes changed only slightly as a function of increasing iron concentration, whereas liver microsomal peroxidation increased dramatically. In addition, varying the incubation pH did not augment lung microsomal peroxidation relative to liver microsomal peroxidation (data not shown). Thus, the lack of peroxidation of lung microsomes apparently is not attributable to limiting concentrations of iron, NADPH or ascorbate, or to suboptimal

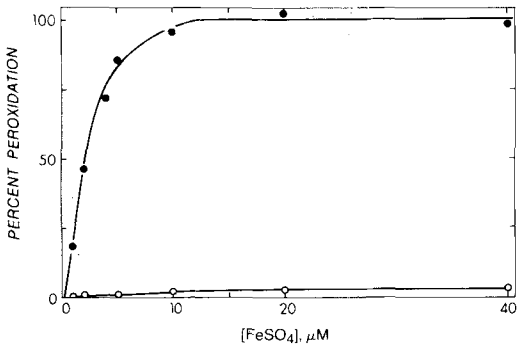

FIG. 4. Lipid peroxidation in rat liver and lung microsomes as a function of ferrous iron concentration. Liver (●) and lung (○) microsomes were incubated for 15 min with 250 μM NADPH and $FeSO_4$ as indicated. Points are the mean values from 4 determinations. 100% peroxidation is equivalent to 85 ± 8 and 70 ± 2 nmol MDA/mg protein for liver and lung microsomes, respectively. The PPUFA contents of these same microsomes were 633 ± 4 and 520 ± 59 nmol PPUFA/mg protein, respectively.

TABLE I

Vitamin E Content of Microsomes from Various Tissues of Rat[a]

Source of microsomes	μg Vitamin E/mg protein	μg Vitamin E/mg PPUFA[b]
Lung	0.77 ± 0.01	4.3 ± 0.4
Heart	0.26 ± 0.05	1.9 ± 0.6
Liver	0.16 ± 0.03	0.73 ± 0.01
Kidney	0.09 ± 0.02	0.69 ± 0.14
Testes	0.10 ± 0.04	0.67 ± 0.07
Brain	0.09 ± 0.04	0.50 ± 0.15

[a]Values are the means ± standard deviations from 4 experiments. The values for μg vitamin E/mg PPUFA can be converted to mmol vitamin E/mol PPUFA by multiplying by 0.76.

[b]PPUFA refers to peroxidizable polyunsaturated fatty acids (18:3, 20:3, 20:4, 20:5, 22:4, 22:5, and 22:6).

pH.

One possible explanation for the different rates of peroxidation in the different tissues is that the microsomes contain different amounts of the biological antioxidant, vitamin E. In order to test this hypothesis, we measured levels of vitamin E in the different microsomes: the results are shown in Table I. Vitamin E was quantitated on a per mg protein basis as well as per mg PPUFA; the PPUFA ratio probably more accurately represents the antioxidant capacity of the tissue. For lung microsomes, the ratio of vitamin E-to-PPUFA was 6-8 times higher than in liver, kidney, testes and brain microsomes, which is consistent with the much slower rate of peroxidation in lung microsomes (Fig. 3). Heart microsomes, which peroxidized faster than lung but slower than liver, kidney, testes or brain microsomes contained an intermediate amount of vitamin E/mg PPUFA.

We also studied rates of peroxidation and vitamin E content in microsomes from different species. As shown in Figure 5, peroxidizability of rabbit microsomes from various tissues was similar to rat microsomes (Fig. 2) and again there was an inverse correlation with the ratio of vitamin E-to-PPUFA, shown in Figure 6. Some variation among species in these parameters was observed, however. Figure 7 summarizes the data on lung microsomes from rats, rabbits, mice and humans. Rabbit lung microsomes exhibited the highest vitamin E/PPUFA ratio, and practically no lipid peroxidation was detectable after a 30 min incubation. In contrast, peroxidation was by far the highest in mouse lung microsomes and the vitamin E/PPUFA ratio was nearly 5 times less than in rabbit lung microsomes. Peroxidizability and vitamin E content of human lung microsomes was most similar to rat lung microsomes. These correlations suggest microsomal susceptibility to lipid peroxidation is determined primarily by the ratio of vitamin E-to-PPUFA present in the microsomal membranes. The relative resistance of lung microsomes to peroxidation, then, appears to result from the high levels of vitamin E.

In order to further substantiate this hypothesis, we measured the rate of ascorbate/iron-induced peroxidation in liposomes prepared from extracted microsomal lipid. Figure 8a shows that liver liposomes were over 50%

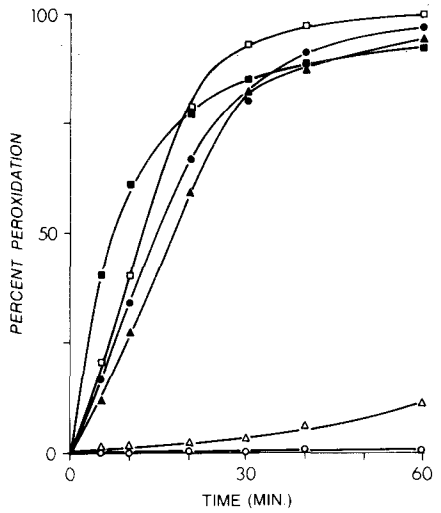

FIG. 5. Rate of ascorbate/Fe^{2+}-induced lipid peroxidation in microsomes from various tissues of rabbit. Symbols as in Figure 1. Points are the mean values from 4 determinations. 100% peroxidation is equivalent to 81 ± 11, 109 ± 14, 62 ± 22, 57 ± 5, 37 ± 4, and 82 ± 9 nmol MDA/mg protein for liver, brain, kidney, testes, heart and lung microsomes, respectively. The PPUFA contents of these same microsomes were 561 ± 25, 752 ± 105, 423 ± 88, 394 ± 12, 251 ± 49, and 562 ± 37 nmol PPUFA/mg protein, respectively.

peroxidized in 30 min whereas lung liposomes under the same conditions were almost completely resistant to peroxidation. As shown in Figure 8b, incorporation of the vitamin E extracted from an equivalent amount of lung microsomes (or an amount of authentic vitamin E equal to that found in the lung microsomes) into liver lipid produced liver liposomes which were also resistant to peroxidation. In contrast, vitamin E extracted from an equivalent amount of liver microsomes and added to liver lipid (essentially doubling the vitamin E content of the liver liposomes) had only a modest effect on the rate of peroxidation relative to control liver liposomes. These findings demonstrate that the 6-fold greater amount of vitamin E in lung compared to liver (Table I) is sufficient to account for the observed resistance of lung liposomes and microsomes to peroxidation.

DISCUSSION

The vitamin E content of various tissues is a somewhat ambiguous concept depending upon the choice of denominators. Previous studies of tissue vitamin E content expressed as μg/g tissue (28,29) did not reveal some of the striking differences among the tissues studied here. Since vitamin E is probably located in cell membranes, it seems most useful to quantitate its presence in tissues as a function of some parameter of the tissue membrane content. Thus, we have chosen to express vitamin E

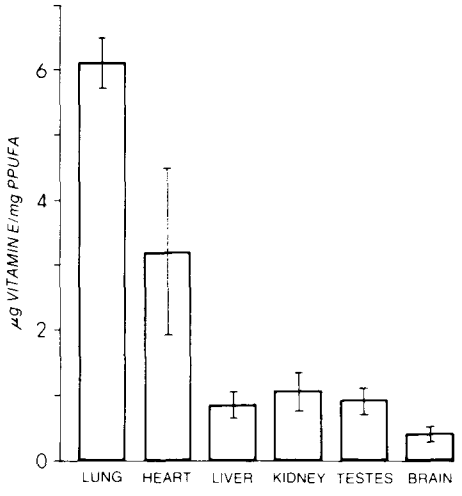

FIG. 6. Vitamin E content of microsomes from various tissues of rabbit. Values shown are means and standard deviations from 3 determinations. Microsomal PPUFA contents were the same as in Figure 5. The values for μg vitamin E/mg PPUFA can be converted to mmol vitamin E/mol PPUFA by multiplying by 0.76.

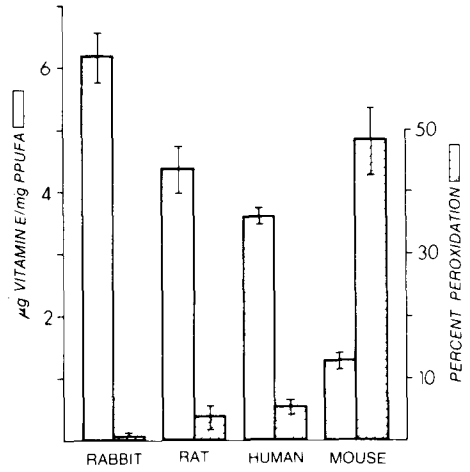

FIG. 7. Lipid peroxidation and vitamin E content of lung microsomes from different species. Lipid peroxidation was determined following a 30 min incubation of microsomes with the ascorbate/Fe^{2+} system. Other procedures are described in Methods. Values shown are means and standard deviations from 2-4 determinations. 100% peroxidation is equivalent to 82 ± 9, 92 ± 8, 82, and 81 ± 13 nmol MDA/mg protein for rabbit, rat, human and mouse lung microsomes, respectively. The PPUFA contents of these same microsomes were 562 ± 37, 677 ± 62, 533, and 536 ± 64 nmol PPUFA/mg protein, respectively. The values of μg vitamin E/mg PPUFA can be converted to mmol vitamin E/mol PPUFA by multiplying by 0.76.

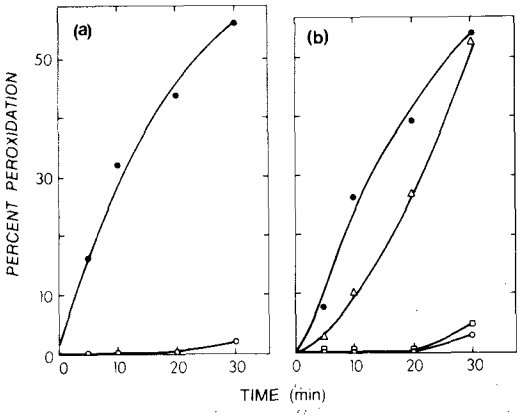

FIG. 8. Peroxidation of liver and lung liposomes by ascorbate/iron. Liposomes were prepared or vitamin E extracted from aliquots of lung or liver microsomes containing equal amounts of PPUFA. (a) Liver liposomes (●), lung liposomes (○); (b) liver liposomes with incorporated vitamin E from lung microsomes (○), liver liposomes with incorporated authentic vitamin E equal to that in lung microsomes (□), liver liposomes with incorporated vitamin E from an equal additional sample of liver microsomes (△), liver liposomes plus vitamin E extract of a water blank (●). Each point is the mean value from 3 determinations.

content on the basis of PPUFA of the microsomes as well as per mg microsomal protein. The values obtained by this method correlate with microsomal susceptibility to lipid peroxidation and can account for the high resistance of lung and heart microsomes to peroxidation. Consistent with our findings, Taylor et al. (16) observed 7-fold and 5-fold higher amounts of vitamin E/mg microsomal protein in lung and heart, respectively, compared to liver microsomes. The Evarts and Bieri data (30) for whole tissue of rats fed soybean oil agree well with our microsomal ratios of vitamin E-to-PPUFA in heart liver and kidney. Whole lungs showed a 30% lower ratio compared to our microsomal data, suggesting that, compared to whole tissue, lung microsomes are enriched in vitamin E relative to PPUFA. Whole testes, in contrast, showed a 73% higher vitamin E/PPUFA ratio than our microsomal data, suggesting a nonmicrosomal enrichment of vitamin E in that tissue.

The presence of vitamin E in lung and heart microsomes at levels sufficient to almost totally prevent peroxidation under conditions which resulted in complete peroxidation of microsomes from other tissues raises serious questions regarding lipid peroxidation as a mechanism of pathological or toxocological damage in lung or heart. Apparently, peroxidation of lung or heart microsomes could only occur after depletion of their relatively higher content of vitamin E.

Vitamin E has long been recognized as a membrane-soluble antioxidant (9). Its importance as a biological protective agent relative to soluble antioxidant factors such as glutathione peroxidase or superoxide dismutase has not been established. Our results show that, in the absence of soluble factors, the susceptibility to peroxidation of microsomes from several tissues and species correlates with the microsomal vitamin E content. Since lung and heart microsomes are highly resistant to lipid peroxidation in the absence of soluble factors, the need for soluble antioxidants in these tissues is not apparent with respect to protection of microsomal membrane lipids. The interesting peroxidizability of whole tissue homogenates (Fig. 3) also reflected microsomal vitamin E content. Lung and heart homogenates underwent very little peroxidation under conditions which produced several-fold greater peroxidation in homogenates of brain, kidney, liver and testes. Thus, it is clear that in the tissues containing lesser amounts of vitamin E, there are no soluble factors which are effective under these conditions in protecting against lipid peroxidation to the same extent that vitamin E protects lung and heart. It is possible that glutathione peroxidase, which is about twice as active in liver as in lung or heart (31), may not function effectively under these conditions because of possible depletion of reducing potential in the homogenate. Soluble superoxide dismutase, however, is over 6 times more active in liver than in lung (32) and might be expected to function in the homogenates, since no depletable cofactors are required. Thus, the amount of protection against lipid peroxidation afforded by these 2 enzymes is not apparent from our data.

The protection of lung and heart against peroxidation by a high content of membranous vitamin E is teleologically sound considering the relatively high oxygen tension in the lung and the exposure of heart to freshly oxygenated blood from the lungs. The mechanism of vitamin E uptake and retention by tissues is unknown and, thus, the biochemical basis for high levels of membranous vitamin E in lung and heart remain to be elucidated. The cellular distribution of the lung vitamin E also remains an intriguing area for future investigations, since the lung contains a number of different cell types (33).

ACKNOWLEDGMENTS

This paper is based on work performed under contract with the U.S. Department of Energy at the University of Rochester Department of Radiation Biology and Biophysics and has been assigned Report No. UR-3490-1688. Douglas J. Kornbrust was supported by NIH training Grant 5 T32 HL07216-02.

REFERENCES

1. Plaa, G.L., and H. Witschi, Ann. Rev. Pharm. 16:125 (1976).
2. Gordon, P., in "Theoretical Aspects of Aging," edited by M. Rockstein, Academic Press, New York, 1974, p. 61.
3. Slater, R.F., "Free Radical Mechanisms of Tissue Injury," Pion Ltd., London, 1972.
4. Buege, J.A., and S.D. Aust, in "Methods in Enzymology," Vol. 52, edited by S. Fleischer, and L. Packer, Academic Press, New York, 1978, pp. 302-310.
5. Svingen, B.A., F.O. O'Neal and S.D. Aust, Photochem. Photobiol. 28:803 (1978).
6. Robak, J., Biochem. Pharmacol. 27:531 (1978).
7. Willis, R.J., and R.O. Recknagel, Toxicol. Appl. Pharmacol. 47:89 (1979).
8. Menzel, D.B., Environ. Hlth. Perspect. 29:105 (1979).
9. Combs, G.F., T. Noguchi and M.L. Scott, Fed. Proc. 34:2090 (1975).
10. Bieri, J.G., and A.A. Anderson, Arch. Biochem. Biophys. 90:105 (1960).
11. Tappel, A.L., and H. Zalkin, Nature 35:4705 (1960).
12. McCay, P.B., J.L. Poyer, P.M. Pfeifer, H.E. May and J.M. Gilliam, Lipids 6:297 (1971).

13. Rowe, L., and E.D. Wills, Biochem. Pharmacol. 25:175 (1976).
14. Tappel, A.L., and H. Zalkin, Arch. Biochem. Biophys. 80:326 (1959).
15. Tinberg, H.M., and A. A. Barber, J. Nutr. 100: 413 (1970).
16. Taylor, S.L., M.P. Lamden, and A.L. Tappel, Lipids 11:530 (1976).
17. Kornbrust, D.J., and R.D. Mavis, Mol. Pharmacol. (In press).
18. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem. 193:265 (1951).
19. Williams, C.H., and H. Kamin, J. Biol. Chem. 237:587 (1962).
20. May, H.E., and P.B. McCay, J. Biol. Chem. 243:2296 (1968).
21. Hogberg, J., A. Bergstrand and S.V. Jakobsson, Eur. J. Biochem. 37:51 (1973).
22. Dahle, L.K., E.G. Hill and R.T. Holman, Arch. Biochem. Biophys. 98:253 (1962).
23. Chen, P.S., T.Y. Toribara and H. Warner, Anal. Chem. 28:1756 (1956).
24. Mavis, R.D., and P.R. Vagelos, J. Biol. Chem. 247:652 (1972).
25. Bligh, E.G., and W.J. Dyer, Can. J. Biochem. Physiol. 37:911 (1959).
26. Pederson, T.C., J.A. Buege and S.D. Aust, J. Biol. Chem. 248:7134 (1973).
27. Ottolenghi, A., Arch. Biochem. Biophys. 79:355 (1959).
28. Aftergood, L., and R.B. Alfin-Slater, Int. J. Vit. Nutr. Res. 48:32 (1978).
29. Edwin, E.E., A.T. Diplock, J. Bunyan and J. Green, Biochem. J. 79:91 (1969).
30. Evarts, R.P., and J.G. Bieri, Lipids 9:860 (1974).
31. Lawrence, R.A., and R.F. Burk, J. Nutr. 108:211 (1978).
32. Paynter, D.I., R.J. Moir and E.J. Underwood, J. Nutr. 109:1570 (1979).
33. Weibel, E.R., P. Gehr, D. Haies, J. Giland and M. Bachofen, in "Lung Cells in Disease," edited by A. Bouhuys, Amsterdam, North Holland, 1976, pp. 3-16.

[Received July 29, 1979]