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Cytosolic Factors which Affect Microsomal Lipid Peroxidation in Lung and Liver

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Studies were carried out to determine the effects of lung and liver cytosol on pulmonary and hepatic microsomal lipid peroxidation, to determine the cytosolic concentrations of various substances which affect lipid peroxidation, and to determine which of these substances is responsible for the effects of the cytosol on lipid peroxidation. Lung cytosol inhibits both enzymatic (NADPH-induced) and nonenzymatic (Fe^{2+}-induced) lung microsomal lipid peroxidation. In contrast, liver cytosol stimulates lipid peroxidation in hepatic microsomes during incubation alone, enhances Fe^{2+}-stimulated lipid peroxidation, and has no effect on the NADPH-induced response. Substances which are known to be involved in inhibition of lipid peroxidation, including glutathione, glutathione reductase, glutathione peroxidase, and superoxide dismutase, are found in greater concentrations in liver cytosol than in lung cytosol. However, ascorbate is found in approximately equal concentrations in pulmonary and hepatic cytosol. Most of the effects of the cytosol on lipid peroxidation seem to be due to ascorbate and glutathione. For example, ascorbate, in concentrations found in lung cytosol, inhibits lung microsomal lipid peroxidation to about the same extent as the cytosol. The effects of liver cytosol on hepatic microsomal lipid peroxidation can be duplicated by concentrations of ascorbate and glutathione normally found in the cytosol; i.e., ascorbate stimulates and glutathione inhibits lipid peroxidation with the net effect being similar to that of liver cytosol. The results indicate that ascorbate has opposite effects on pulmonary and hepatic microsomal lipid peroxidation and suggest that ascorbate plays a major role in protecting pulmonary tissue against the harmful effects of lipid peroxidation.

The process of lipid peroxidation has been associated with a wide variety of pathological and degradative conditions in both the lung and the liver. For example, peroxidation of lipids has been associated with oxygen toxicity in the lung (1), lung damage due to exposure to the oxidant gases, ozone, and nitrogen dioxide (2, 3), and lung injury caused by the herbicide, paraquat (4). In addition, it has been proposed that lipid peroxidation is involved in carbon tetrachloride-induced liver injury (5) and in ethanol-induced fatty liver (6).

Lipid peroxidation in lung and liver has been most thoroughly investigated in the microsomal fractions of these organs. It has been known for many years that lipid peroxidation can be initiated in liver microsomes by a variety of substances (7, 8) and, recently, it has been demonstrated that the lipids of lung microsomes will also undergo peroxidation (9, 10). This process can be induced by substances which are normally found in both lung and liver cells. For example, lipid peroxidation may be initiated in lung and liver microsomes by NADPH and this process requires enzymatic activity (7, 9–11). Nonenzymatic lipid peroxidation may be induced in both lung and liver by ferrous iron (7, 9, 12). In addition, ferric iron and ascorbate can also initiate hepatic microsomal lipid peroxidation (7, 12).

Most cells contain a variety of substances which can inhibit lipid peroxidation. Most of these substances are found in the cytosolic fraction of the cells. For example, ascorbic acid can inhibit lipid peroxidation by acting as an antioxidant (13). The enzyme, super-

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oxide dismutase, may also inhibit lipid peroxidation (14, 15) by destroying superoxide anion, a highly reactive free radical that may play some role in the initiation of lipid peroxidation (13, 16). In addition, glutathione and the enzymes glutathione peroxidase and glutathione reductase have been proposed to function as a unit to protect cells from the toxic effects of lipid peroxidation (17). It has been postulated that this unit, known as the glutathione peroxidase system, can protect cells by metabolizing the toxic lipid peroxides (17) and by removing hydrogen peroxide, a potential source of hydroxyl radicals which can initiate lipid peroxidation (18).

Since lipid peroxidation can be a destructive process and since it appears that there are substances in the cytosolic fractions of cells which can inhibit this process, we studied the effects of cytosol on lung and liver microsomal lipid peroxidation. The specific objectives of this investigation were: (i) to study the effects of lung cytosol on pulmonary microsomal lipid peroxidation; (ii) to study the effects of liver cytosol on hepatic microsomal lipid peroxidation; (iii) to determine the cytosolic concentrations of substances which may affect microsomal lipid peroxidation; and (iv) to determine the relative contributions of these substances to the observed effects of the cytosol on lipid peroxidation. A preliminary report of these observations has appeared previously (19).

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing 300 to 450 g were obtained from Zivic-Miller Laboratories, Pittsburgh, Pennsylvania. The animals were killed by decapitation and the livers and lungs were rapidly removed. Both organs were perfused immediately with ice-cold 0.9% NaCl to remove the blood. The livers were homogenized in 0.1 M phosphate buffer (0.081 M K_2HPO_4, and 0.019 M KH_2PO_4; pH 7.4). The lungs were dissected free of the trachea, bronchi, and connective tissue and then finely minced by chopping four times with a McElwain tissue chopper (The Mickle Engineering Co., Gomshall, Surrey) which had been set for a slice thickness of 5 mm. The mince was then homogenized in 0.1 M phosphate buffer using a Teflon-glass Potter–Elvejehem homogenizer (20). After removal of the nuclear and mitochondrial fractions, liver and lung microsomes were obtained by centrifugation at 105,000*g* for 75 min (20). The cytosolic fractions were collected as the supernatants following a second centrifugation at 105,000*g* for 75 min. This was done to minimize contamination of the cytosol with microsomes. For all experiments the microsomes were resuspended in 0.1 M phosphate buffer at final concentrations equivalent to 100 (lung) or 50 (liver) mg tissue/ml. The protein concentrations for the lung and liver microsomes suspended at these concentrations were 0.8 (±0.1) and 1.7 (±0.7) mg microsomal protein/ml, respectively. Various amounts of cytosol at concentrations equivalent to 250 (lung) and 125 (liver) mg tissue/ml were added to the microsomal suspension. The final volume of the incubation medium was 2.5 ml. The protein concentrations for lung and liver cytosols suspended at these concentrations were 6.8 (±0.2) and 8.6 (±0.5) mg protein/ml, respectively.

Oxygen (100%) was bubbled through the microsomal suspensions for 1 min prior to the start of the experiment. The various substances added to the incubation suspensions were NADPH (Type I), L-ascorbic acid, superoxide dismutase, reduced glutathione (Sigma Chemical Co., St. Louis, Mo.), and $FeSO_4$. The samples were incubated at 37°C for 2 h, a time during which maximal amounts of malonaldehyde were formed. The lipid peroxidation which occurred during the incubation period was measured by using the thiobarbituric acid assay of Ottolenghi (21) as modified by Hunter *et al.* (22). Malonaldehyde, a by-product of lipid peroxidation, and other thiobarbituric acid reactants are measured with this assay. After the incubation period each sample was placed in a test tube containing 0.3 ml of 5 N HCl and 0.625 ml of 40% trichloroacetic acid. Thiobarbituric acid (0.625 ml of a 2% solution) was added and the samples were incubated at 90°C for 20 min. After this incubation the samples were placed on ice for 5 min and then centrifuged at 30,000*g* in a Sorvall Model SS-3 centrifuge (Ivan Sorvall Co., Norwalk, Conn.) for 5 min. The amount of malonaldehyde in each sample was determined by measuring the optical density of the supernatants at 532 nm with a Gilford Model 300-N spectrophotometer (Gilford Instrument Co., Oberlin, Ohio). In this study malonaldehyde levels were expressed in optical density units. However, the results may be expressed as amounts of malonaldehyde by using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} (23).

The concentrations of ascorbate, glutathione, glutathione reductase, glutathione peroxidase, superoxide dismutase, and the protein levels in lung and liver cytosol were determined in the following ways. Ascorbic acid was assayed by measuring the reduction of Fe^{3+} to Fe^{2+} according to a modification of the method of Sullivan and Clarke (24) as described by Sikic *et al.* (25). Glutathione peroxidase activity was determined by following the oxidation of NADPH according to the method of Little *et al.* (26). Since cumene hydroperoxide (Polysciences, Inc., Warrington, Pa.) served as the substrate in this assay, glutathione peroxidase was

not distinguished from glutathione *S*-transferase (27). Glutathione reductase activity was determined using a method similar to that of Carlberg and Mannervick (28). With this method the oxidation of NADPH was followed in a reaction mixture containing cytosol (an amount equivalent to 12.5 mg tissue for lung and 5 mg tissue for liver), 0.1 M phosphate buffer (pH 7.4), 0.5 mM EDTA, 2 mM oxidized glutathione, and 0.1 mM NADPH. Both glutathione peroxidase and glutathione reductase measurements were corrected for any contamination by blood. Glutathione was measured according to the 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) assay described by Moron *et al.* (29). These measurements were also corrected for any contamination by blood. The DTNB assay provides a measure of all sulfhydryl groups and, therefore, an estimate of glutathione content. The error involved in this measurement is small since Moron *et al.* demonstrated that the cytosolic fractions of lung and liver cells contain only small amounts of acid-soluble thiols other than glutathione. Superoxide dismutase activity was determined using a modification of the cytochrome *c* reductase assay described by Mustafa *et al.* (30). Superoxide was produced by the xanthine–xanthine oxidase reaction (31). The reaction mixture (1 ml) contained cytosol (an amount equivalent to 5 mg tissue for liver or 2 mg tissue for lung), 0.1 M phosphate buffer (pH 7.4), 0.1 mM EDTA, 0.5 mg cytochrome *c*, 1.5 mM xanthine, and 0.06 U of xanthine oxidase. A standard curve was obtained using known quantities of superoxide dismutase (Sigma Chemical Co.). We determined in a separate set of control experiments that the amount of ascorbate found in the cytosol used in this assay does not interfere with the superoxide dismutase determination. Protein concentrations were determined by the method of Lowry *et al.* (32). All spectrophotometric assays were done on either a Gilford Model 300-N spectrophotometer (Gilford Instrument Co., Oberlin, Ohio) or a Beckman Acta CIII Recording Spectrophotometer (Beckman Instrument Co., Fullerton, Calif.).

RESULTS

Effects of Lung Cytosol on Pulmonary Microsomal Lipid Peroxidation

Nonenzymatic lipid peroxidation occurs in pulmonary microsomes when ferrous iron is added to the incubation medium (9). The effects of lung cytosol on Fe^{2+}-induced lung microsomal lipid peroxidation were studied and the results are shown in Fig. 1. Lung cytosol inhibits this process in a concentration-dependent manner. The lung microsomes used in these experiments were suspended at a concentration equivalent to 100 mg of tissue/ml. A maximal inhibition of approximately 76% occurs when the concentration of cytosol is equivalent to 40 mg of tissue/ml. Therefore, the maximal inhibition caused by lung cytosol occurs when the proportion of cytosol to microsomes is less than that found in the intact cell.

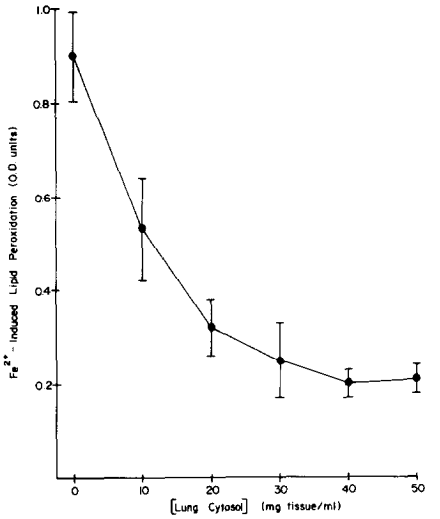

FIG. 1. Dose–response curve for the effect of lung cytosol on Fe^{2+} (2.5 mM)-stimulated lung microsomal lipid peroxidation. Malonaldehyde levels were measured after an incubation period of 2 h at 37°C. In these experiments the microsomes were suspended at a concentration equivalent to 100 mg tissue/ml. The numbers shown on the abscissa represent the final concentration of the cytosol expressed as an equivalent amount of tissue. Each point is the mean value for five experiments and the bars represent the standard errors of the means.

Enzymatic lipid peroxidation may be induced in lung microsomes by NADPH (9, 10). The effects of lung cytosol on this process are shown in Table I. Lung cytosol inhibits NADPH-induced lung microsomal lipid peroxidation and the inhibition is maximal when the concentration of cytosol is equivalent to 50 mg of tissue/ml. In these experiments the microsomes were suspended at a concentration equivalent to 100 mg of tissue/ml. Thus, maximal inhibition is obtained when the proportion of cytosol to microsomes is less than that found in the intact cell. In other experiments (data not presented) we have shown that this inhibition is not due to depletion of NADPH by cytosolic components. Thus, the re-

TABLE I

EFFECTS OF LUNG CYTOSOL ON NADPH-INDUCED PULMONARY MICROSOMAL LIPID PEROXIDATION

Treatment	Lipid peroxidation (OD units)
Incubation alone	0.02 ± 0.01
NADPH (0.4 mM)	0.13 ± 0.02
NADPH + cytosol	0.04 ± 0.02

Note. Malonaldehyde levels were measured after incubation of the microsomal suspensions at 37°C for 2 h. Lung microsomes and cytosol were suspended at concentrations equivalent to 100 mg tissue/ml and 50 mg tissue/ml, respectively. The numbers shown are the mean values for five experiments ± the standard errors of the means.

sults of these experiments indicate that lung cytosol inhibits both nonenzymatic and enzymatic pulmonary microsomal lipid peroxidation.

Effects of Liver Cytosol on Hepatic Microsomal Lipid Peroxidation

The effects of liver cytosol on nonenzymatic and enzymatic liver microsomal lipid peroxidation were studied and the results are shown in Table II. In these experiments liver cytosol and microsomes were suspended at concentrations equivalent to 50 mg of tissue/ml; i.e., the proportion of cytosol to microsomes was the same as that found *in vivo*. When hepatic microsomes are incubated in phosphate buffer at 37°C, very little lipid peroxidation occurs. However, in the presence of liver cytosol there is approximately a 10-fold increase in malonaldehyde formation during incubation. Addition of cytosol to the microsomes also leads to a twofold increase in Fe^{2+}-induced lipid peroxidation. In contrast, liver cytosol has no effect on NADPH-induced lipid peroxidation in liver microsomes although the results were quite variable.

Cytosolic Factors Which May Influence Lipid Peroxidation

Since the cytosolic fractions of cells contain substances which may affect lipid peroxidation, the concentrations of some of these substances in lung and liver cytosol were measured. The substances in which we were most interested were the antioxidants, ascorbate, and glutathione, and the enzyme, superoxide dismutase. We also measured the cytosolic levels of glutathione peroxidase and glutathione reductase. The results are shown in Table III. For these measurements both pulmonary and hepatic cytosol were suspended at final concentrations equivalent to 100 mg of tissue/ml. With the exception of ascorbate, the concentrations of all of the substances measured are greater in liver cytosol than in lung cytosol. The concentrations of ascorbate are approximately equal in lung and liver cytosol. These results indicate that, in general, liver cytosol contains higher concentrations of those substances which are thought to influence lipid peroxidation than does lung cytosol.

Experiments were carried out to determine which of the cytosolic factors contribute to the observed effects of cytosol on microsomal lipid peroxidation. The effects of ascorbate, glutathione, and superoxide dismutase on both lung and liver microsomal lipid peroxidation were studied. These substances were added to lung microsomal suspensions (final concentration equivalent to 100 mg tissue/ml) in concentrations equivalent to those found in lung cytosol suspended at a concentration equivalent to 50 mg tissue/ml; i.e., the amount of cytosol which produces maximal inhibition of microsomal lipid peroxidation. The effects of these substances

TABLE II

EFFECTS OF LIVER CYTOSOL ON HEPATIC MICROSOMAL LIPID PEROXIDATION

Treatment (concn)	Lipid peroxidation (OD units)	
	Microsomes alone	Microsomes + cytosol
Incubation alone	0.02 ± 0.01	0.29 ± 0.08
Fe^{2+} (0.1 mM)	0.60 ± 0.02	1.22 ± 0.06
NADPH (0.1 mM)	1.02 ± 0.06	1.03 ± 0.25

Note. Malonaldehyde levels were measured after incubation of the microsomal suspensions at 37°C for 2 h. The liver microsomes and cytosol were suspended at concentrations equivalent to 50 mg tissue/ml. The numbers shown are the mean values for five experiments ± the standard errors of the means.

TABLE III

CYTOSOLIC CONTENT OF SUBSTANCES WHICH AFFECT LIPID PEROXIDATION

Substance (units)	Cytosolic concentration	
	Lung	Liver
Ascorbic acid (μg/ml cytosol)	13.7 ± 2.1	13.2 ± 1.6
Superoxide dismutase (units/ml cytosol)	42.0 ± 5.0	178.0 ± 20.0
Glutathione (μg/ml cytosol)	6.0 ± 0.5	65.9 ± 7.0
Glutathione peroxidase (nmol NADPH oxidized/min/ml cytosol)	25.0 ± 8.0	198.0 ± 53.0
Glutathione reductase (nmol NADPH oxidized/min/ml cytosol)	27.6 ± 4.5	61.4 ± 12.0

Note. The concentrations of all substances are expressed per ml of cytosol. In these experiments both the lung and liver cytosols were suspended at concentrations equivalent to 100 mg tissue/ml. The numbers shown are the mean values for four to six experiments ± the standard errors of the means.

on Fe^{2+}-stimulated lung microsomal lipid peroxidation are shown in Table IV. Glutathione and superoxide dismutase have no effect on the Fe^{2+}-stimulated response. However, ascorbate inhibits this process by approximately 50%. The inhibition of pulmonary microsomal lipid peroxidation produced by lung cytosol (about 75% inhibition; see Fig. 1) is somewhat greater than that produced by ascorbate. Thus, these results indicate that the ascorbate present in the cytosol probably accounts for most, but not all, of the inhibition of pulmonary microsomal lipid peroxidation caused by lung cytosol.

Studies were also done to determine which of the cytosolic factors is (are) responsible for the effects of liver cytosol on hepatic microsomal lipid peroxidation. In these experiments liver microsomes were suspended at a concentration equivalent to 50 mg tissue/ml. Ascorbate, glutathione, and superoxide dismutase were added in the same amounts as those found in liver cytosol suspended at the same concentration as the microsomes, i.e., a concentration equivalent to 50 mg tissue/ml. The results are shown in Table V. Superoxide dismutase has no effect on hepatic microsomal lipid peroxidation. However, ascorbate alone stimulates lipid peroxidation in hepatic microsomes. In addition, ascorbate enhances the lipid peroxidation which occurs in the presence of Fe^{2+} and that induced by NADPH. In contrast, both Fe^{2+}-induced and NADPH-induced lipid peroxidation are inhibited by glutathione. Furthermore, these observations suggest that the effects of liver cytosol on Fe^{2+}- and NADPH-induced hepatic microsomal lipid peroxidation are due principally to the actions of ascorbate and glutathione. The effects of liver cytosol on NADPH-induced and Fe^{2+}-induced liver microsomal lipid peroxidation can be duplicated by the addition of ascorbate and glutathione to the microsomes (see Table II and Table V). Only the effects of liver cytosol on

TABLE IV

EFFECTS OF INDIVIDUAL COMPONENTS OF LUNG CYTOSOL ON PULMONARY MICROSOMAL LIPID PEROXIDATION

Treatment	Lipid peroxidation (OD units)
Fe^{2+} alone	0.64 ± 0.06
Fe^{2+} + ascorbate	0.34 ± 0.07
Fe^{2+} + glutathione	0.74 ± 0.08
Fe^{2+} + superoxide dismutase	0.66 ± 0.05

Note. Malonaldehyde levels were measured after incubation of the microsomal suspensions with 2.5 mM Fe^{2+} for 2 h. The lung microsomes were suspended at a concentration equivalent to 100 mg tissue/ml. Ascorbate (7.6 μg/ml), glutathione (3 μg/ml), and superoxide dismutase (21.6 U/ml) were added to the suspensions in amounts similar to those found in lung cytosol suspended at a concentration equivalent to 50 mg tissue/ml; i.e., the amount of cytosol which produces maximal inhibition. The numbers shown are the mean values for six experiments ± standard errors of the means.

TABLE V

EFFECTS OF INDIVIDUAL COMPONENTS OF LIVER CYTOSOL ON HEPATIC MICROSOMAL LIPID PEROXIDATION

Treatment	Lipid peroxidation (OD units)
Incubation alone	0.06 ± 0.01
Ascorbate	0.96 ± 0.06
Ascorbate + glutathione	0.76 ± 0.06
Fe^{2+} alone	0.54 ± 0.04
Fe^{2+} + superoxide dismutase	0.66 ± 0.04
Fe^{2+} + ascorbate	1.48 ± 0.06
Fe^{2+} + glutathione	0.24 ± 0.03
Fe^{2+} + ascorbate + glutathione	1.23 ± 0.05
NADPH alone	0.98 ± 0.07
NADPH + superoxide dismutase	0.94 ± 0.06
NADPH + ascorbate	1.45 ± 0.05
NADPH + glutathione	0.40 ± 0.02
NADPH + ascorbate + glutathione	1.00 ± 0.05

Note. Malonaldehyde levels were measured after incubation of the microsomal suspensions either alone, with 0.1 mM Fe^{2+}, or with 0.1 mM NADPH at 37°C for 2 h. The liver microsomes were suspended at a concentration equivalent to 50 mg tissue/ml. Ascorbate (7.6 μg/ml), glutathione (33.2 μg/ml), and superoxide dismutase (89.2 U/ml) were added to the suspensions in amounts similar to those found in liver cytosol suspended at a concentration equivalent to 50 mg tissue/ml. The numbers shown are the mean values for six experiments ± standard errors of the means.

the lipid peroxidation which occurs during incubation of the microsomes alone cannot be entirely accounted for by ascorbate and glutathione. The magnitude of the cytosol-induced response (Table II) is less than that produced by ascorbate and glutathione (Table V). Thus, some additional inhibitory factor(s) may be involved in the cytosol-induced response. The results of these experiments do indicate that almost all of the effects of liver cytosol on hepatic microsomal lipid peroxidation are due to the presence of ascorbate and glutathione.

DISCUSSION

The results of this study indicate that (i) pulmonary and hepatic cytosol have significant, but different, effects on lung and liver microsomal lipid peroxidation, (ii) lung and liver cytosol differ in composition, and (iii) the effects of the cytosolic fractions on microsomal lipid peroxidation are due primarily to ascorbate and glutathione. Lung cytosol inhibits both enzymatic and nonenzymatic lung microsomal lipid peroxidation. On the other hand, liver cytosol stimulates hepatic microsomal lipid peroxidation and also enhances Fe^{2+}-stimulated lipid peroxidation. Liver cytosol has no effect on the NADPH-induced response. The effects of the cytosol are maximal at ratios of cytosol to microsomes which are the same or even less than those found *in vivo*. Therefore, the effects of cytosol on lipid peroxidation may be of significance in the intact cell.

While attempting to identify the cytosolic constituents which affect microsomal lipid peroxidation, we found that lung and liver cytosol differ greatly in composition. The levels of several substances known to influence lipid peroxidation, such as superoxide dismutase, glutathione, glutathione peroxidase, and glutathione reductase, are all far greater in liver cytosol than in lung cytosol. However, ascorbate is found in approximately equal concentrations in pulmonary and hepatic cytosol. Interestingly, it is the ascorbate which seems to be primarily responsible for the effects of the cytosol on lipid peroxidation in both lung and liver microsomes.

A large proportion of the inhibition of lung microsomal lipid peroxidation caused by lung cytosol can be attributed to ascorbate. The maximal inhibition of lung microsomal lipid peroxidation produced by lung cytosol is about 75%. Ascorbate, in a concentration equivalent to that found in the lung cytosol, inhibits lung microsomal lipid peroxidation by about 50%. Therefore, the presence of ascorbate may account for a large part, but not all, of the inhibition. The small amount of inhibition which is not caused by ascorbate may be due to the glutathione peroxidase system or to protein in the cytosol. The glutathione peroxidase system is thought to inhibit peroxidation by metabolizing hydroperoxides (17) or by removing hydrogen peroxide (18), a source of hydroxyl radicals which can initiate lipid peroxidation. In our

experiments we were unable to test the role of this system because glutathione peroxidase was not available to us. It is also possible that protein in the cytosol may be responsible for some of the inhibition of lipid peroxidation. It has been shown previously that certain cytosolic proteins, such as albumin and glutathione *S*-transferase, inhibit liver microsomal lipid peroxidation (33, 34). In other experiments (not reported here) we observed that bovine serum albumin inhibits lung and liver microsomal lipid peroxidation. However, we were unable to quantitatively relate the effects of albumin to those of the cytosolic fractions.

Liver cytosol stimulates lipid peroxidation in hepatic microsomes, enhances Fe^{2+}-induced malonaldehyde formation, and has no effect on NADPH-induced liver microsomal lipid peroxidation. The results indicate that these effects may be due almost entirely to the ascorbate and glutathione in the cytosol. Ascorbate alone, in amounts found in liver cytosol, stimulates liver microsomal lipid peroxidation to a greater extent than does liver cytosol. In contrast, glutathione, in concentrations found in liver cytosol, inhibits liver microsomal lipid peroxidation. The combined effects of ascorbate and glutathione on both Fe^{2+}- and NADPH-induced hepatic microsomal lipid peroxidation are almost identical to those of liver cytosol. Thus, the effects of liver cytosol on Fe^{2+}- and NADPH-induced lipid peroxidation appear to be due to a combination of both stimulatory (ascorbate) and inhibitory (glutathione) factors. However, the effects of liver cytosol alone on lipid peroxidation cannot be entirely accounted for by ascorbate and glutathione; i.e., the magnitude of the lipid peroxidation produced by physiological concentrations of ascorbate and glutathione is greater than that caused by the cytosol. The remainder of the inhibition in this case may be due to the glutathione peroxidase system or to the presence of cytosolic protein.

Some of the effects of liver cytosol on hepatic microsomal lipid peroxidation have been investigated by others. For example, Barber (35) found, as we did, that liver cytosol alone stimulates hepatic microsomal lipid peroxidation. The effects of liver cytosol on NADPH-induced liver microsomal lipid peroxidation have also been studied by others (36–39). The results obtained from all of these studies are different. In some cases it was found that liver cytosol enhances NADPH-induced hepatic microsomal lipid peroxidation (37), while other studies show that cytosol inhibits the NADPH-induced response (37–39). We found that liver cytosol has no significant effect on NADPH-induced peroxidation of lipids in hepatic microsomes. However, both our experiments and those reported by others indicate that the effects of liver cytosol on NADPH-induced hepatic microsomal lipid peroxidation are highly variable. The reasons for the differences in these results are not known, although variability in the age of the rats could be a factor. It has been shown by other investigators that the concentration of ascorbate in the liver varies with age (40). Since ascorbate seems to play a major role in lipid peroxidation, variations in the ascorbate levels with age may account for these conflicting results.

The results of the experiments presented in this paper, together with previous work (9, 41), demonstrate that ascorbate inhibits pulmonary microsomal lipid peroxidation and enhances the same process in liver. Since it is ascorbate which is primarily responsible for the effects of the cytosol and since there are equal amounts of ascorbate in lung and liver cytosol, either of the cytosolic fractions should have the same effect on microsomes from a given organ. Therefore, we tested the effect of lung cytosol on hepatic microsomal lipid peroxidation and the effect of liver cytosol on pulmonary microsomal lipid peroxidation. Lung cytosol stimulates lipid peroxidation in liver microsomes, and liver cytosol inhibits both enzymatic and nonenzymatic lipid peroxidation in lung microsomes. These effects are due almost entirely to the ascorbate in the cytosolic fractions. Thus, these findings further demonstrate that ascorbate has different effects in lung and liver.

There is evidence to indicate that ascorbate plays a major role in protecting the lungs from the harmful effects of lipid peroxidation. For example, ascorbate, when administered to animals prior to exposure to the

oxidant gases, ozone or hyperbaric oxygen, protects against oxidant injury (42–44). Ascorbate may also act in conjunction with vitamin E in protecting against pulmonary lipid peroxidation. It has been suggested that vitamin E and ascorbate act synergistically to inhibit peroxidation of lipids (45) and, indeed, the vitamin E content of lung microsomes has been shown to be high relative to other organs (46, 47). If interactions between ascorbate and vitamin E are important, it is possible that the differing concentrations of vitamin E in lung and liver may account for the different effects of ascorbate on lipid peroxidation in the two tissues.

In summary, pulmonary and hepatic cytosol have important effects on microsomal lipid peroxidation. Lung cytosol inhibits pulmonary microsomal lipid peroxidation and, in general, liver cytosol stimulates hepatic microsomal lipid peroxidation. The presence of ascorbate in the cytosol appears to be primarily responsible for the different effects of lung and liver cytosol. Ascorbate, in physiological concentrations, stimulates lipid peroxidation in liver microsomes and inhibits pulmonary microsomal lipid peroxidation. It is possible that ascorbate plays a major role in protecting pulmonary tissue against the harmful effects of lipid peroxidation.

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