The Inhibitory Effect of Reduced Glutathione on the Lipid Peroxidation of the Microsomal Fraction and Mitochondria

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1. GSH efficiently inhibited the ascorbate-stimulated lipid peroxidation of the unsaturated fatty acids in the fresh microsomal fraction and mitochondria of rat liver, whereas the peroxidation in heat-denatured particles was little inhibited. 2. Cysteamine and diethyldithiocarbamate inhibited the peroxidation in both fresh and boiled particles. Thioglycollate and 2-mercaptoethanol had no inhibiting effect. Cysteine and homocysteine both stimulated the lipid peroxidation even in the absence of ascorbate. 3. The added GSH disappeared at nearly the same rate in the presence of fresh and of boiled particles to which ascorbate had been added, although considerably more malonaldehyde was formed in the boiled particles. In the absence of ascorbate little GSH disappeared. 4. It is suggested that the protective effect of GSH against lipid peroxidation depends on the preservation of heat-labile structures in the microsomal fraction and mitochondria.

Peroxidation of the endogenous unsaturated fatty acids in mitochondria and microsomal fractions can be induced by incubation with a variety of redox agents such as ferrous iron (McKnight, Hunter & Oehlert, 1965), haemoproteins (Tappel, 1955), ascorbate (Ottolenghi, 1959), GSSG and especially by mixtures of GSSG and GSH (Hunter *et al.* 1964a) and by cystamine and mixtures of cystamine and cysteamine (Skrede & Christophersen, 1966). Other agents such as carbon tetrachloride (Recknagel & Ghoshal, 1965), ultraviolet light (Wilbur, Bernheim & Shapiro, 1949) and ionizing radiation (Horgan & Philpot, 1954) can also induce lipid peroxidation in subcellular particles.

Hydroperoxides are probably first formed by the peroxidation of unsaturated fatty acids (Bergström, 1945). By further decomposition a number of different reaction products can be formed. Malonaldehyde may thus be formed by peroxidation of fatty acids with three or more unsaturated bonds (Dahle, Hill & Holman, 1962).

In a liver homogenate a rapid lipid peroxidation will start as soon as the supply of endogenous GSH is exhausted by oxidation, and addition of GSH will promptly stop further peroxidation measured by the formation of malonaldehyde (Christophersen, 1966). It therefore seemed likely that GSH might be involved in mechanisms that counteract lipid peroxidation in the intact cell.

In the present work the inhibitory effect of GSH and other thiols on the lipid peroxidation in the microsomal fraction and mitochondria from rat

liver was studied. Ascorbate was used to induce the peroxidation in the particles.

EXPERIMENTAL

Materials. Cysteamine was obtained from Fluka A.-G. (Buchs SG, Switzerland), GSH, GSSG, sodium diethyldithiocarbamate and L(+)-ascorbic acid were from E. Merck A.-G. (Darmstadt, Germany), sodium thioglycollate, DL-homocysteine, L-cysteine and dehydroascorbic acid were from Nutritional Biochemicals Corp. (Cleveland, Ohio, U.S.A.), 2-mercaptoethanol was from Eastman Organic Chemicals (Rochester 3, N.Y., U.S.A.) and catalase from bovine liver was from Sigma Chemical Co. (St Louis, Mo., U.S.A.). Thiolated Sephadex was prepared according to the method of Eldjarn & Jellum (1963) as described by Skrede (1966).

Methods. The microsomal fraction and mitochondria from rat liver were prepared in 0.25 M-sucrose as previously described (Christophersen, 1966). Since sucrose interferes with the assay of lipid peroxides by the thiobarbituric acid method, the particles were washed one additional time with 0.15 M-KCl and resuspended in KCl solution. Heat-denatured particles were prepared by heating the particle suspensions for 10 min. in a boiling-water bath, and then treated ultrasonically to obtain finely dispersed suspensions. Nitrogen gas was run through the particle suspensions during the heating to counteract autooxidation of the unsaturated fatty acids.

The incubation medium contained in some experiments tris-HCl buffer, pH 7.4 (0.05 M) and KCl (0.06–0.08 M); in other experiments the medium consisted of a 'phosphate saline' buffer containing 100 vol. of 0.15 M-KCl, 10 vol. of 0.1 M-KH₂PO₄-Na₂HPO₄ buffer, pH 7.4, and 1 vol. of 0.1 M-MgCl₂ (Pinto, 1961).

The protein content of the subcellular fractions was measured by the biuret method.

To measure lipid peroxidation the formation of malonaldehyde was determined by the thiobarbituric acid method as described by Ottolenghi (1959). The molar extinction coefficient $\epsilon_{535} 1.56 \times 10^5 \text{ cm}^2 \text{ m-mole}^{-1}$ was used (Sinnhuber, Yu & Yu, 1958).

The oxygen uptake was measured with a Clark oxygen electrode.

After preparation the subcellular particles contained small amounts of malonaldehyde. The fresh microsomal fractions contained 0.5–1.0 μmole of malonaldehyde/mg. of protein. The boiled microsomal fractions contained 0.6–1.2 μmole /mg. The fresh mitochondria contained 0.3–0.5 μmole /mg. and the boiled mitochondria contained 0.3–0.6 μmole /mg. of protein. Tables 1–6 give the net amount of malonaldehyde formed during the incubations.

The concentrations of GSH, of cysteine and of homocysteine in the media were measured by the method of Ellman (1959) as modified by Jocelyn (1962). Samples of the incubation media were added to solutions of 5,5'-dithiobis-(2-nitrobenzoic acid) (1 mM) at pH 6.8 and the extinction was read after 1 min. It was tested whether lipid peroxides in the particles interfered with the method. In these control experiments microsomal fractions (9 mg. of protein) were incubated for 120 min. without added thiol but with the other conditions as stated in the legend to Table 1. After incubation the media contained 125–140 μmole of malonaldehyde. Samples of these media were then added to solutions of 5,5'-dithiobis-(2-nitrobenzoic acid) that had previously been reduced by addition of GSH. The reduced form of the reagent was not oxidized to a significant extent by the high amounts of lipid peroxides in the particles under the stated conditions.

RESULTS

Table 1 shows that GSH markedly inhibited the ascorbate-induced peroxidation of the lipids in fresh mitochondria incubated in a tris-potassium chloride medium. In contrast, when heat-denatured mitochondria were used under the same conditions, GSH had no significant effect. Less peroxidation took place in a phosphate medium, but also in this

case GSH was an efficient protector with fresh but not with boiled mitochondria.

Table 2 shows that essentially the same results were obtained when the microsomal fraction was used. However, Table 2 shows that, when boiled microsomal fractions were incubated in a medium that contained phosphate, GSH had a small inhibitory effect on the ascorbate-stimulated peroxidation. In 11 experiments of 60 min. duration in a phosphate saline medium 10% (average) less malonaldehyde was formed in the presence of GSH than without. In a tris-potassium chloride medium GSH had no significant effect on the peroxidation of boiled microsomal fractions.

The inhibitory effect of phosphate on the ascorbate-stimulated peroxidation of mitochondria has been observed by Hunter, Scott, Weinstein & Schneider (1964b) (see also Table 1). When microsomal fractions were used (Table 2), the phosphate inhibition was significantly smaller than with mitochondria.

The results in Tables 1 and 2 suggest that the protective effect of GSH may depend on the presence of heat-labile factors.

To test whether other thiol compounds besides GSH were efficient as inhibitors of lipid peroxidation, the effect of seven different thiols on the ascorbate-induced peroxidation of the microsomal fraction was examined (Table 3). A phosphate saline medium was used since the protective effect of GSH was more efficient in this system (see Table 2). Physiologically occurring thiols as well as synthetic thiols were selected; some were known to have radioprotective effects and some were without this effect (Bacq, 1965). Table 3 shows that the thiols tested affected the lipid peroxidation in three principally different ways. GSH was the only one that was both an efficient protector against ascorbate-stimulated lipid peroxidation of the fresh microsomal fraction and a poor protector with the boiled microsomal fraction. Cysteamine and

Table 1. *Effect of GSH on the ascorbate-stimulated lipid peroxidation of fresh and boiled mitochondria*

Mitochondria with a protein content of 7–9 mg. were suspended in 0.3 ml. of 0.15 M-KCl and made up to a volume of 2 ml. with the stated buffers and incubated in the presence of ascorbate (0.2 mM) for 60 min. at 37°. GSH (1 mM) was added where indicated. A separate mitochondrial preparation was used in each experiment. Mean values $\pm$ S.E.M. are given for the amounts of malonaldehyde formed.

	No. of expts.	Addition of GSH	Malonaldehyde formed (μmole s)
Fresh mitochondria, tris-KCl medium	5	—	58 $\pm$ 6.9
	5	+	20 $\pm$ 5.6
Boiled mitochondria, tris-KCl medium	5	—	53 $\pm$ 8.3
	5	+	52 $\pm$ 5.6
Fresh mitochondria, phosphate saline medium	4	—	17 $\pm$ 2.9
	4	+	5 $\pm$ 1.2
Boiled mitochondria, phosphate saline medium	4	—	9 $\pm$ 0.6
	4	+	10 $\pm$ 0.6

Table 2. *Effect of GSH on the ascorbate-stimulated lipid peroxidation in the fresh and boiled microsomal fraction*

Microsomal fractions with a protein content of 8–10 mg. were incubated as described in the legend to Table 1. A separate microsomal preparation was used in each experiment. Mean values $\pm$ S.E.M. are given for the amounts of malonaldehyde formed.

	No. of expts.	Addition of GSH	Malonaldehyde formed (μ moles)
Fresh microsomal fractions, tris-KCl medium	6	—	88 $\pm$ 13.6
	6	+	38 $\pm$ 3.4
Boiled microsomal fractions, tris-KCl medium	6	—	108 $\pm$ 14.4
	6	+	117 $\pm$ 14.0
Fresh microsomal fractions, phosphate saline medium	11	—	28 $\pm$ 3.1
	11	+	7 $\pm$ 1.1
Boiled microsomal fractions, phosphate saline medium	11	—	71 $\pm$ 3.7
	11	+	64 $\pm$ 3.1

Table 3. *Effect of different thiols on the ascorbate-stimulated peroxidation of fresh and boiled microsomal fractions*

Microsomal fractions with a protein content of 8 mg. were incubated in a phosphate saline buffer as described in the legend to Table 1. To some of the media 40 mg. of SH-Sephadex was added.

Thiol added + SH-Sephadex when stated	Malonaldehyde formed (μ moles)			
	Fresh microsomal fractions		Boiled microsomal fractions	
	With ascorbate (0.2 mM)	Without ascorbate	With ascorbate (0.2 mM)	Without ascorbate
Control without thiol	30	0	58	0
Control without thiol + SH-Sephadex	29	0	63	0
GSH (1 mM)	4	0	51	0
GSH (1 mM) + SH-Sephadex	4	0	61	0
GSH (10 mM)	6	0	73	2
GSH (10 mM) + SH-Sephadex	3	0	76	0
Cysteamine (1 mM)	10	0	2	2
Cysteamine (10 mM)	0	0	8	0
Diethyldithiocarbamate (1 mM)	0	0	0	0
Thioglycollate (1 mM)	36	1	63	1
Mercaptoethanol (1 mM)	33	0	57	1
L-Cysteine (1 mM)	32	16	55	26
L-Cysteine (1 mM) + SH-Sephadex	49	10	63	24
DL-Homocysteine (1 mM)	38	10	57	7
DL-Homocysteine (1 mM) + SH-Sephadex	43	4	78	5

diethyldithiocarbamate strongly counteracted the peroxidation both of fresh and of boiled particles. Thioglycollate, 2-mercaptoethanol, cysteine and homocysteine caused no inhibition of the ascorbate-induced peroxidation either with fresh or with boiled particles.

Since mixtures of GSSG and GSH (Hunter *et al.* 1964a) and of cystamine and cysteamine (Skrede & Christophersen, 1966) are known to stimulate lipid peroxidation, the lack of inhibitory effect of some of the thiols tested could be due to the presence of autoxidation products of the thiols formed during the incubations. An attempt was therefore made to study the effect of the thiols in the absence of their oxidation products. By adding a new material, SH-Sephadex, to the media, low-molecular-weight

thiols can be kept completely or predominantly in the reduced state during incubations, since SH-Sephadex reduces the disulphides formed by oxidation of the thiols and can bind heavy-metal ions that catalyse the oxidation (Jellum, 1964). Thus when SH-Sephadex, GSH (1 mM and 10 mM) and ascorbate were added to microsomal fractions as described in Table 3, the GSH concentration was constant during the incubations. In a typical experiment with fresh microsomal fractions the GSH concentrations measured in the complete incubation medium before incubation were 1.06 mM and 9.7 mM, and after 60 min. of incubation 1.08 mM and 9.9 mM. With boiled microsomal fractions the GSH concentrations were 1.04 mM and 9.9 mM before incubation and 1.05 mM and 10.0 mM after

Table 4. *Effect of different thiols on the ascorbate-stimulated lipid peroxidation of fresh and boiled mitochondria*

Mitochondria with a protein content of 9 mg. were incubated in a tris-KCl medium as described in the legend to Table 1.

Thiol added	Malonaldehyde formed (mμmoles)			
	Fresh mitochondria		Boiled mitochondria	
	With ascorbate (0.2 mm)	Without ascorbate	With ascorbate (0.2 mm)	Without ascorbate
Control without thiol	66	0	56	0
GSH (1 mm)	22	0	56	0
Cysteamine (1 mm)	27	0	0	0
Diethyldithiocarbamate (1 mm)	0	0	0	0
L-Cysteine (1 mm)	68	50	21	23

Table 5. *Effect of GSH on the ascorbate-stimulated lipid peroxidation of fresh microsomal fractions pretreated with N-ethylmaleimide*

Microsomal fractions suspended in a phosphate saline medium (8 mg. of protein/ml.) were incubated at 23°, one sample in the presence of 10 mM-*N*-ethylmaleimide (NEM) for 10 min. and another sample in the presence of 30 mM-*N*-ethylmaleimide for 30 min. The samples and a control suspension not treated with *N*-ethylmaleimide were subjected to gel filtration on a Sephadex G-25 column (35 cm. × 1.5 cm.) to remove unchanged *N*-ethylmaleimide. The gel filtrates contained microsomal fractions with a protein content of 6–7 mg./ml. Portions (2 ml.) were incubated as described in the legend to Table 1.

Additions	Concn. of NEM used in pretreatment	Malonaldehyde (mμmoles)		
		0 mM	10 mM	30 mM
Control (unincubated)		5	5	7
No additions		5	14	22
Ascorbate (0.2 mM)		20	34	43
Ascorbate (0.2 mM) + GSH (1 mM)		8	16	26

incubation. When SH-Sephadex was not used, a substantial part of the GSH added was oxidized after 60 min. of incubation (see also Figs. 1 and 2). However, when ascorbate, SH-Sephadex and either cysteine or homocysteine were added to microsomal fractions as described in Table 3, the presence of SH-Sephadex did not completely prevent a slight decrease of the thiol concentration, probably because cysteine and homocysteine autoxidize more rapidly than GSH does. The concentrations of both cysteine and homocysteine decreased by 10–15% during the 60 min. of incubation in these experiments. Thus small concentrations of the oxidation products of the latter thiols were present.

Table 3 also shows that cysteine and homocysteine stimulated the lipid peroxidation in the media without ascorbate.

Table 4 shows that with mitochondria also GSH was the only one of the thiols tested that was both an efficient protector against ascorbate-stimulated lipid peroxidation in fresh particles and a poor protector with boiled particles. Cysteamine and diethyldithiocarbamate inhibited the peroxidation both of fresh and of boiled mitochondria. In

contrast with the findings with microsomal fractions, cysteine and ascorbate together stimulated the peroxidation of boiled mitochondria less than ascorbate alone did.

It was tested whether protein thiol groups in the microsomal fraction influenced the stimulating effect of ascorbate on lipid peroxidation, and whether the protective effect of GSH against peroxidation depended on the presence of protein thiol groups. In these experiments *N*-ethylmaleimide was used to pretreat the microsomal fractions as described in the legend to Table 5. The excess of the reagent was removed by gel filtration before the peroxidation experiment. When protein thiol groups had been blocked in this way, a considerable peroxidation occurred during the incubations even without ascorbate, but the effect of the latter agent was still evident. Also, in microsomal fractions treated with *N*-ethylmaleimide GSH still counteracted the ascorbate-stimulated peroxidation. The effects of several common enzyme inhibitors were also tested. GSH counteracted the ascorbate-induced peroxidation of microsomal fractions equally well in the presence of azide (10 mM), cyanide (0.1 mM and 1 mM), Amytal

Table 6. *Effect of different thiols on the oxygen uptake stimulated by ascorbate and ferrous iron in fresh and boiled microsomal fractions*

Microsomal fractions with a protein content of 9 mg. were incubated in 2.5 ml. of a tris-KCl medium for 2 min. at 24° in the chamber of a Clark oxygen electrode. The oxygen uptake in controls in the presence of ascorbate (0.2 mM) but without thiol and without added iron was 15 μ moles with fresh microsomal fractions and 20 μ moles with boiled microsomal fractions. Ferrous iron was added as a solution of $\text{FeSO}_4 \cdot (\text{NH}_4)_2\text{SO}_4$.

Thiol added	Oxygen uptake (μ moles) and (in parentheses) malonaldehyde formation (μ moles)					
	Fresh microsomal fractions			Boiled microsomal fractions		
	With ascorbate (0.2 mM) + Fe^{2+} (2 μ M)		No addition	With ascorbate (0.2 mM) + Fe^{2+} (2 μ M)		No addition
		With Fe^{2+} (2 μ M)			With Fe^{2+} (2 μ M)	
Control without thiol	121 (11)	11 (1)	5 (0)	168 (14)	12 (0)	7 (0)
GSH (1 mM)	59 (4)	9 (1)	8 (0)	172 (16)	13 (1)	5 (0)
Diethyldithiocarbamate (1 mM)	3	2	2	3	3	2
Thioglycollate (1 mM)	120	26	9	179	34	10
L-Cysteine (1 mM)	117	28	13	175	72	17

(4 mM) and fluoride (2 mM and 20 mM). Cyanide itself inhibits ascorbate-stimulated lipid peroxidation (Hoffsten, Hunter, Gebicki & Weinstein, 1962), but the peroxidation reaction was not affected by azide, Amytal and fluoride. Catalase (10 μ g. and 100 μ g. in 2 ml. of medium) had no effect on the peroxidation.

Table 6 shows that GSH inhibited both the oxygen uptake and the malonaldehyde formation caused by lipid peroxidation in the fresh microsomal fractions. Only a fraction of the oxygen uptake could be accounted for by the formation of malonaldehyde, probably because a number of other end products than malonaldehyde are also formed by lipid peroxidation (Dahle *et al.* 1962). Since the oxygen uptake was measured with a Clark oxygen electrode, short incubation periods had to be used. Since ascorbate alone only stimulated a slow oxygen uptake, catalytic amounts of ferrous iron were added together with ascorbate to get an oxygen uptake that was sufficiently high to be measured by the oxygen electrode. In the absence of ascorbate, the small amounts of iron used caused little lipid peroxidation. Table 6 also shows that diethyldithiocarbamate completely inhibited the oxygen uptake with both fresh and boiled particles. Thioglycollate and cysteine had no inhibitory effect. The combination of ferrous iron with thioglycollate or cysteine stimulated a small oxygen uptake with both fresh and boiled microsomal fractions.

The GSH concentration in the media was correlated with the rate of ascorbate-stimulated lipid peroxidation in the microsomal fractions. Fig. 1 shows that in the absence of ascorbate little GSH was oxidized and little peroxide was formed when

fresh microsomal fractions were incubated in a phosphate saline medium, and other experiments showed that the same was true when a tris-potassium chloride medium was used. In the presence of ascorbate and GSH the lipids in the boiled microsomal fractions started to peroxidize immediately. When fresh microsomal fractions were used, a delay in the ascorbate-stimulated lipid peroxidation was observed until most of the added GSH had been oxidized. In the presence of ascorbate the added GSH was oxidized at about the same rate regardless of whether boiled or fresh microsomal fractions were used.

When microsomal fractions were not added, control experiments showed that GSH was relatively stable in the media in the presence of ascorbate. Thus, in a phosphate saline medium containing 0.2 mM-ascorbate, the GSH concentration had decreased from 0.55 mM to 0.51 mM after 150 min. of incubation at 37°, and without ascorbate decreased from 0.55 mM to 0.43 mM. In a tris-potassium chloride medium containing 0.2 mM-ascorbate the GSH concentration had decreased from 0.55 mM to 0.54 mM, and without ascorbate decreased from 0.55 mM to 0.50 mM.

Dehydroascorbate was also incubated with GSH. In a tris-potassium chloride medium containing 0.25 mM-dehydroascorbate the GSH concentration had decreased from 0.55 mM to 0.47 mM after 150 min. of incubation at 37°. When fresh microsomal fractions (8 mg. of protein in 2 ml. of medium) were added together with 0.25 mM-dehydroascorbate, the GSH concentration had decreased from 0.55 mM to 0.44 mM after 150 min.

A co-oxidation of ascorbate and the unsaturated fatty acids occurs when ascorbate stimulates

Fig. 1. Oxidation of GSH related to the rate of ascorbate-stimulated lipid peroxidation in fresh and boiled microsomal fractions in a phosphate saline buffer, and GSH oxidation related to the rate of peroxidation in the absence of ascorbate. Microsomal fractions (9mg. of protein) were incubated in 2ml. of a phosphate saline medium as described in the legend to Table 1, except that GSH (0.5mm) was added to all media. Ascorbate (0.2mm) was added where indicated. ▲, GSH oxidation with fresh microsomal fractions without ascorbate; △, malonaldehyde formation in fresh microsomal fractions without ascorbate; ■, GSH oxidation with fresh microsomal fractions with ascorbate; □, malonaldehyde formation in fresh microsomal fractions with ascorbate; ●, GSH oxidation with boiled microsomal fractions with ascorbate; ○, malonaldehyde formation in boiled microsomal fractions with ascorbate.

lipid peroxidation. The amount of dehydroascorbate formed is thus probably related to the degree of peroxidation (Ottolenghi, 1959). Therefore the rate of formation of dehydroascorbate during the ascorbate-stimulated peroxidation of boiled microsomal fractions probably was higher than when fresh microsomal fractions were used (Figs. 1 and 2). Since the GSH oxidation in the media with boiled microsomal fractions was not higher than with fresh microsomal fractions, it seemed likely that only a relatively small part of the GSH oxidation was caused by the dehydroascorbate formed. This interpretation was also supported by the experiments with added dehydroascorbate.

Fig. 2 shows that the presence of glutathione only inhibited the ascorbate-stimulated lipid peroxidation of fresh microsomal fractions as long as a large part of the added GSH was still in the reduced state. When the greater part of the GSH added had

Fig. 2. Effect of glutathione on the rate of lipid peroxidation in fresh and boiled microsomal fractions incubated for long incubation periods with ascorbate in a tris-KCl medium. Microsomal fractions (10mg. of protein) were incubated in 2ml. of a tris-KCl medium as described in the legend to Table 1. Ascorbate (0.2mm) was added to all media. GSH (0.65mm) was added where indicated. ○, Malonaldehyde formation in fresh microsomal fractions without GSH; □, malonaldehyde formation in fresh microsomal fractions with GSH; △, GSH oxidation in media with fresh microsomal fractions and GSH; ●, malonaldehyde formation in boiled microsomal fractions without GSH; ■, malonaldehyde formation in boiled microsomal fractions with GSH; ▲, GSH oxidation in media with boiled microsomal fractions and GSH.

been oxidized, the velocity of lipid peroxide formation became higher in the media with glutathione present than in the media without. When boiled microsomal fractions were used, the presence of glutathione had no significant effect on the ascorbate-stimulated lipid peroxidation during the first part of the incubation. When the GSH was partly oxidized, more peroxide was formed in the presence of both glutathione and ascorbate than with ascorbate alone with boiled microsomal fractions. A mixture of GSH (0.15mm) and GSSG (0.2mm) was added in some experiments where the other conditions were as described in the legend to Fig. 2. When fresh microsomal fractions and ascorbate were incubated for 30min. with GSH-GSSG 21mμmoles of malonaldehyde were formed. In controls without glutathione 44mμmoles of malonaldehyde was formed. After 60min. of incubation 70mμmoles of malonaldehyde was formed with GSH-GSSG and 73mμmoles without glutathione. Addition of the GSH-GSSG mixture thus offered a protection against the ascorbate-

stimulated peroxidation only for a short period compared with the addition of the corresponding amount of GSH (Fig. 2). It is suggested that the GSH/GSSG ratio will determine whether glutathione will protect against lipid peroxidation in fresh subcellular particles.

DISCUSSION

GSH is related to the peroxidation of the unsaturated fatty acids in microsomal fractions and mitochondria in several different ways. Mixtures of GSH and GSSG can stimulate lipid peroxidation (Hunter *et al.* 1964a), GSH can be oxidized by lipid peroxides (Little & O'Brien, 1967) and GSH can inhibit lipid peroxidation.

Reduced metal ions, especially ferrous iron, stimulate lipid peroxidation, probably by a co-oxidation of the unsaturated fatty acids and the metal ions (Ottolenghi, 1959). It is thus possible that some thiols may stimulate lipid peroxidation in subcellular particles by keeping contaminant metal ions in the reduced state. Cash & Gardy (1965) suggested that the enhanced mitochondrial-swelling activity of mixtures of GSH and GSSG, which is known to be accompanied by an enhanced lipid peroxidation (Hunter *et al.* 1964a), is attributable to the ability of GSH to reduce Fe^{3+} in the GSSG samples to Fe^{2+} . However, under the conditions used in the present experiments, the addition of Fe^{2+} to media that contained GSH and microsomal fractions did not significantly increase the lipid peroxidation (Table 6), although the amounts of iron used were high compared with the amounts of contaminant iron in commercial samples of GSH and GSSG (Cash & Gardy, 1965). A second possible explanation of the stimulating effect of mixtures of GSH and GSSG and of cysteamine and cystamine (Skrede & Christophersen, 1966) on lipid peroxidation is that it may be due to the catalytic effect of disulphides on the autoxidation of thiols. The autoxidation intermediates formed by oxidation of the thiols may stimulate the lipid peroxidation. The stimulating effect of cysteine and of homocysteine on lipid peroxidation (Table 3) may be due to the strong tendency of these thiols to autoxidize.

The thiols tested, GSH, diethyldithiocarbamate, thioglycollate and cysteine, had, in the presence of comparatively high concentrations of added iron (Table 6), principally the same effects on the lipid peroxidation as in the absence of added iron (Table 3). This finding seems to suggest that the completely different effects of the thiols on the lipid peroxidation cannot in the main be explained by a difference in contaminant metal concentrations in the thiol preparations used.

Linoleic acid hydroperoxide is known to oxidize low-molecular-weight thiols, and the reaction with

GSH is known to be catalysed by a glutathione peroxidase present in mitochondria and to a smaller extent in microsomal fractions (O'Brien & Little, 1967). A part of the GSH oxidation observed (Figs. 1 and 2) may be due to the lipid peroxides formed. The protective effect of GSH against lipid peroxidation in the fresh subcellular fractions may possibly be explained by a reaction of GSH with the peroxide intermediates formed. Since peroxide intermediates stimulate further lipid peroxidation by autocatalysis (Dahle *et al.* 1962), GSH might thus break the chain of reactions. Since the reaction of GSH with lipid hydroperoxides can be catalysed by enzymes present in the subcellular fractions, this may explain why GSH only protects the fresh subcellular particles efficiently against lipid peroxidation whereas it is ineffective with boiled particles. The protective effect of cysteamine, which is highly reactive, may be due to a non-enzymic reaction with peroxide intermediates.

The unsaturated fatty acids in the intact subcellular membranes may possibly be less exposed to peroxidating agents than the fatty acids in boiled particles are. This may explain why ascorbate stimulated a higher rate of lipid peroxidation in the boiled particles (Tables 1 and 2).

EDTA totally inhibits lipid peroxidation (Ottolenghi, 1959). Since ascorbate-stimulated lipid peroxidation probably is catalysed by iron (Elliot & Libet, 1944) (see also Table 6), chelation of heavy metals by thiols therefore is a second possible explanation of the observed inhibiting effect on lipid peroxidation. The effect of diethyldithiocarbamate may be due to chelation. This compound has a low reactivity and does not autoxidize, but is a potent chelating agent. However, it seems difficult to explain that GSH is a more efficient protector against lipid peroxidation with fresh than with boiled particles on the basis of chelation.

Since ionizing radiation can induce the formation of lipid peroxides (Horgan & Philpot, 1954) that are highly toxic (Desai & Tappel, 1963), it is noteworthy that the thiols that counteract lipid peroxidation most efficiently both in fresh and heat-denatured particles are also good protectors against radiation damage.

Since lipid peroxidation is highly deleterious to membrane structures, there must be an efficient protection against this process in the cell. α -Tocopherol has been considered to be responsible for this protection (Tappel & Zalkin, 1959). The finding that GSH inhibits lipid peroxidation in fresh microsomal fractions and mitochondria efficiently may point to a role of GSH in the protection against lipid peroxidation *in vivo*.

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