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GLUTATHIONE AND THE MITOCHONDRIAL REDUCTION OF HYDROPEROXIDES

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Summary

Various organic hydroperoxides are reduced when added to rat liver mitochondrial suspensions. Succinate increases the rate and duration of the reductions except for linoleic acid hydroperoxide which appears to inhibit its own reduction. 3-Hydroxybutyrate replaces succinate but other reductants used are less effective. The rate of reduction of *tert*-butyl hydroperoxide by succinate is not inhibited by cyanide but is partly inhibited if antimycin or rotenone are also added; ATP reverses the antimycin inhibition. Other inhibitors include the uncoupler, carbonyl cyanide *p*-trifluoromethoxyhydrazone, ADP + P_i, the thiol reagents *N*-ethylmaleimide and *p*-hydroxymercuribenzoate and inhibitors of the mitochondrial transport of carboxylic acids. In some cases, the GSH concentration of the mitochondria during the reductions correlates with the reduction rate (e.g. with succinate and after *N*-ethylmaleimide) but in others it is dissociated. The results suggest that hydroperoxide reduction requires the GSH-glutathione peroxidase pathway but that entry of the oxidants into the mitochondrial matrix is also an energy-dependent step.

Introduction

The hydroperoxy group is recognised or postulated to be formed in many metabolic oxidations (e.g. lipid peroxidation [1], steroid hydroxylation [2], interconversion of prostaglandins [3,4], radiation mutagenesis [5]) and for this reason the biochemical properties of available synthetic hydroperoxides has

Abbreviations: BuOOH, *tert*-butyl hydroperoxide; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

attracted much interest. Stable neutral hydroperoxides induce such effects as perturbing cellular redox equilibria [6,7] with release of GSSG [8], interacting with microsomal hydroxylating systems [9,10] and forming membrane lipid hydroperoxides [11]. At the same time, these model compounds are themselves reduced. Thus they are rapidly lost when perfused into rat liver or incubated with hepatocytes [11]. This loss has been studied with different subcellular fractions [12] and various mechanisms proposed. In the microsomes hydroperoxides are reduced non-specifically utilising either $NADH_2$ [9] or $NADPH_2$ [10] via a cytochrome *P*-450-linked system. In the cytosol, though a specific system reducing a prostaglandin-15-hydroperoxide by means of aromatic reductants has been described [13], the principal route for hydroperoxide reductions is probably via the system comprising GSH and the glutathione peroxidases [14].

Liver mitochondria are also able to reduce hydroperoxides [15] and, as they possess their own supply of glutathione peroxide [16,17] and of GSH [18,19] which can be depleted by a hydroperoxide [20], there is a presumption that the same system may operate.

In this paper the presumption is examined by studying the reduction of hydroperoxides and the mitochondrial GSH concentration under various conditions. Some of this work has been presented in outline [21].

Materials and Methods

The mannitol and Tris-KCl buffer (containing EDTA and NH_4Cl) and the concentrated mitochondrial suspension were each made up as previously described [22]. Neutral hydroperoxides were kindly donated by Akzo Chemie U.K. Ltd., Wandsworth, London. Linoleic acid hydroperoxide was prepared [23] from linoleic acid (Koch-Light, Colnbrook). Solutions of the hydroperoxides in the Tris-KCl buffer were made up just before use by diluting stock solutions (20 mM) in ethanol.

A glutathione peroxidase preparation was obtained by dialysis in 0.01 M phosphate buffer, pH 8 of a rat liver cytosol fraction obtained from a 20% homogenate by centrifugation at 100 000 × *g* for 1 h. Aliquots stored at −15° C retained activity when thawed after 3 months.

Incubations

To 0.8 ml or 0.7 ml of the ice-cold Tris-KCl buffer in Eppendorf centrifuge tubes (capacity 1.5 ml) containing the specified inhibitors and substrates were added either 0.1 ml of mitochondrial suspension (containing 4.3 ± 0.8 mg protein) or (see Figs. 4,6,7, Table I) 0.2 ml suspension previously loaded with substrate at 0° C by adding one volume of mannitol buffer containing the substrate (0.05 M) 6 min before dispensing. After preincubating the mixture for 2 min at 30° C, approx. 0.5 μmol of hydroperoxide in 0.5 ml buffer kept at 30° C was added to each tube simultaneously. After reincubating at 30° C, tubes were loaded into an Eppendorf high speed centrifuge and the mitochondria sedimented by centrifuging for 1.5 min at 7000 × *g*. The stated incubation time is the interval between the time of addition of the oxidant to the time of starting the centrifuge (loading time, 30 s). Supernatants were poured off from

the sedimented pellets and a sample (0.25 ml) used immediately without acidifying to assay the amount of hydroperoxide remaining. To measure the hydroperoxide consumed during the incubation, the precise initial concentration used was obtained by incubating 0.1 ml mitochondria with 0.8 ml buffer then centrifuging and adding the supernatant to 0.5 ml buffer containing the hydroperoxide. Mitochondrial pellets were treated by adding 0.5 ml 2.4% perchloric acid (containing 0.05 M ascorbic acid), shaking vigorously then centrifuging after 1—6 h. The acid extract was used for the assay of GSH.

Assays

GSH was assayed with 5,5′-dithiobis-(2-nitrobenzoic acid) [22] (80—90% of mitochondrial acid-soluble thiol thus assayed has been shown to be GSH [24]).

Hydroperoxides were assayed by incubating supernatants in the presence of glutathione peroxidase and measuring the loss of GSH [25]. 0.25 ml of the sample containing the hydroperoxide was added to 0.5 ml of the Tris-KCl buffer containing 0.3 μmol GSH, 0.03 μmol EDTA and 30 μl of the glutathione peroxidase preparation. After incubation at 30° C for 15 min, the solution was acidified with 12% perchloric acid (0.25 ml) and GSH assayed on 0.5 ml samples of the filtrate as described above. Allowance was made for GSH lost by autoxidation by including in each run a control using the mitochondrial supernatant without added hydroperoxide. Using standards containing varying amounts of each of the hydroperoxides used, a linear response was obtained. Values found for the neutral hydroperoxide concentrations were at least 85% of the values (by iodometric assay) supplied by the manufacturers.

Protein was assayed by a modified biuret method [26].

Results

Various organic hydroperoxides were added at equal concentrations to a mitochondrial suspension in a Tris-KCl buffer at 30° C and the loss of hydroperoxide determined at intervals after sedimenting the mitochondrial pellet. Results obtained using four different hydroperoxides are shown in Fig. 1A. There is in each case an initial loss of oxidant which shortly declines, attributable to reduction and thus exhaustion of endogenous substrates. In the presence of succinate (Fig. 1B) the three neutral hydroperoxides used show an increased rate of loss, approximately equal for them all and linear with time. The rate with linoleic acid hydroperoxide alone is unaffected by succinate perhaps because this substance penetrates faster than the others into the mitochondrial matrix and achieves a concentration there sufficient to inhibit enzymes necessary for its own reduction [27,28]. The detergent properties of this fatty acid may also be a relevant factor. Because of the likely involvement of mitochondrial GSH in the reduction of hydroperoxides (see Introduction) the concentration of this thiol was determined on the mitochondrial pellets sedimented during the incubations. In the presence of each of the hydroperoxides but without oxidisable substrate the GSH falls from its initial level (see caption to Fig. 2). This fall is largely prevented with succinate present except where linoleic acid hydroperoxide was used when the GSH fall remains

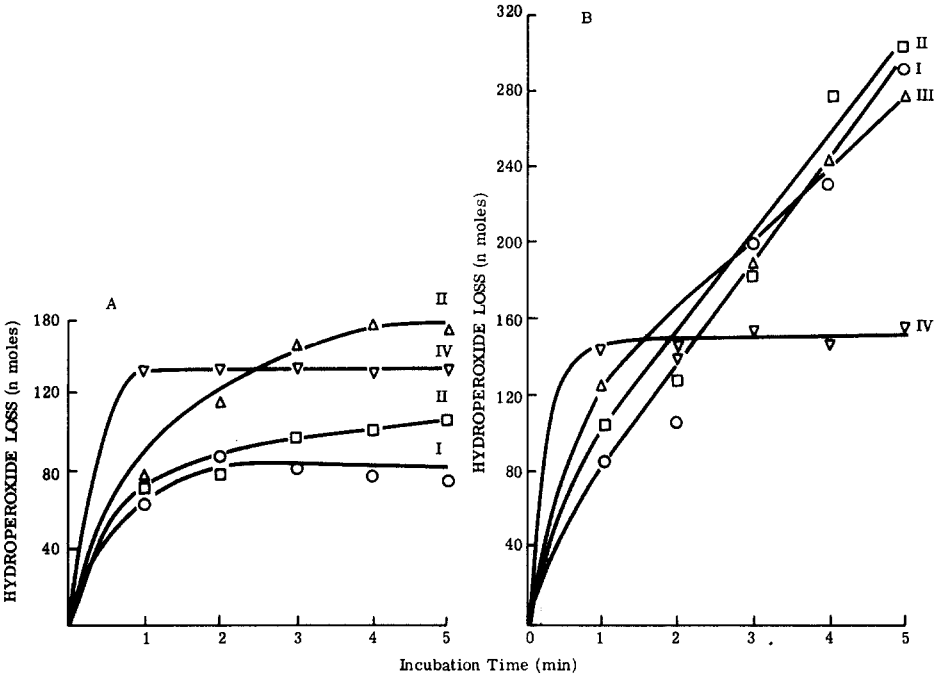


Fig. 1. Time course of the loss of some hydroperoxides by a mitochondrial suspension at 30°C (A) without added substrate, (B) with succinate present. For techniques see Methods. For each mixture a batch of identical tubes was incubated. Equal amounts (about 0.5 μmol) of hydroperoxide I, II, III or IV (see below) were added to a mitochondrial suspension (3.7 mg protein) in buffer with or without succinate (5 μmol). The mitochondrial pellet (see Fig. 2) was sedimented from one of the tubes at each of the times shown and hydroperoxide concentrations determined on the supernatant. I, BuOOH; II, cumene hydroperoxide; III, 2,4,4-trimethyl-pentyl-2-hydroperoxide; IV, linoleic acid hydroperoxide, Mean values for reduction of BuOOH obtained in 9 assays (using 4.3 ± 0.8 mg protein) were (with succinate present) 20 ± 5 nmol · mg^{-1} protein · min^{-1}. Mean values without the succinate were 37 ± 8% of those with succinate.

unchecked (Fig. 2B). Comparison of Fig. 1 and Fig. 2 shows that, though there are discrepancies with the neutral hydroperoxides in the absence of substrate, in general there is a correlation between the GSH concentration found in the pellet and the corresponding rate of hydroperoxide reduction. Other substrates were compared at different concentrations in the presence of cyanide (see below) for their ability to increase both the amount of reduction of one of the neutral hydroperoxides (BuOOH) and also the corresponding mitochondrial concentration of GSH above the amount obtained without substrate (Fig. 3). Maximum effect of the substrates on both parameters was obtained when about 2.5 μmol were added initially. 2-Oxoglutarate and pyruvate (not shown) are ineffective probably as a result of the oxidation of coenzyme A by hydroperoxides [15,20] but the other substrates increase the amount of hydroperoxide reduced in the ascending order citrate, malate, isocitrate, succinate and 3-hydroxybutyrate. Since the oxidation of these substances by phosphorylating mitochondria is little affected by the presence of BuOOH [15], these differences are not due to toxic effects on their dehydrogenases.

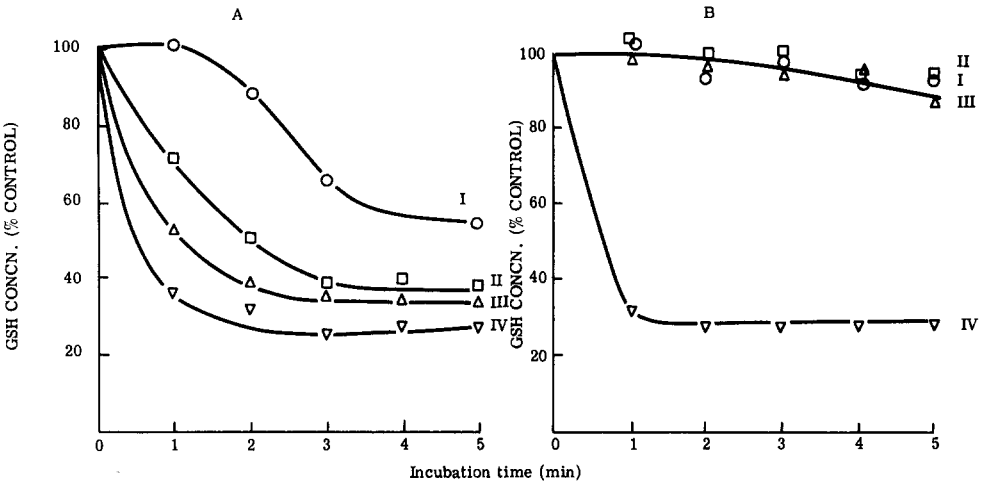


Fig. 2. Time course of mitochondrial GSH concentration during the reduction of some hydroperoxides (A) without added substrates, (B) with succinate present. GSH was assayed (see Methods) on the pellets sedimented at intervals during the experiment described in Fig. 1. 100% GSH corresponds to the amount (27.5 nmol) present in the pellet obtained after adding 0.1 ml mitochondrial suspension to 0.8 ml of the Tris-KCl buffer, incubating 2 min at 30°C then immediately sedimenting the pellet after adding a further 0.5 ml buffer. Mean GSH values (per mg protein) obtained in 9 assays using BuOOH were: 100%, 5.7 ± 0.85 nmol; after 4 min incubation with BuOOH and succinate, 5.1 ± 0.6 nmol; after 4 min incubation with BuOOH but without succinate, 1.9 ± 0.85 nmol.

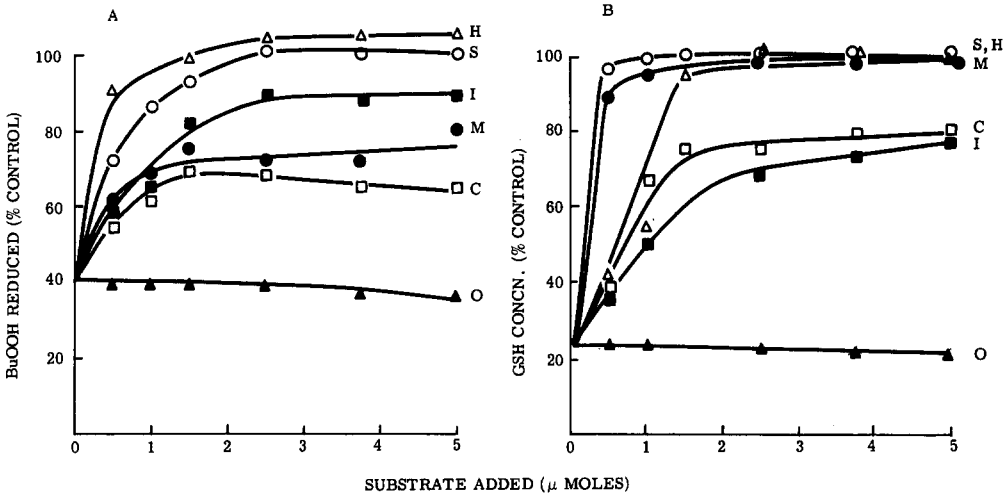


Fig. 3. Effect of varying the concentrations of various oxidisable substrates (A) on the rate of reduction of BuOOH, (B) on the mitochondrial GSH concentration. For techniques see Methods. KCN (0.5 μmol) was added to all tubes (see text). Incubations using 3.6 mg protein were for 4 min at 30°C. Substrates: S, succinate; M, malate; C, citrate; I, isocitrate; H, 3-hydroxybutyrate; O,'2-oxoglutarate. 100% values, obtained using a control containing KCN and incubated for 4 min with succinate (5 μmol) were: BuOOH reduced, 279 nmol; GSH content; 27.7 nmol. Other data obtained for BuOOH reduction and GSH concentration using 2.5 μmol of the above acids were (mean and S.D. from 4 assays; GSH values in brackets): succinate, 100 (100); 2-oxoglutarate, 27 ± 11 (37 ± 12); citrate, 48 ± 20 (90 ± 15); malate, 64 ± 21 (100 ± 11); isocitrate, 69 ± 22 (88 ± 20); 3-hydroxybutyrate, 81 ± 7 (97 ± 12).

Although mitochondrial GSH concentrations are also elevated by these substrates in the presence of BuOOH, there are discrepancies between their effect on the two measurements, most notably with malate which, despite its modest effect in increasing the rate of hydroperoxide reduction, consistently raises the GSH levels to the values found with succinate.

Effect of respiratory inhibitors

The rate of hydroperoxide reduction is not significantly affected by the presence of cyanide in the incubation medium but, using mitochondria preloaded with succinate, other respiratory inhibitors have interesting effects on the reduction of BuOOH. Thus rotenone, though it has little or no action when added alone, inhibits the reduction in the presence of ATP (weakly), cyanide (moderately) or both together (strongly) (Fig. 4A). Antimycin also inhibits more with than without cyanide present (Fig. 4B) but its effect differs from that of rotenone in that (a) the maximum inhibition is obtained at higher concentrations of antimycin than required for rotenone and (b) ATP counteracts to some extent the inhibition obtained with cyanide present. These results suggest that antimycin partly inhibits by its effect on energy generation whereas rotenone does so in a different way, i.e. by blocking reversed electron transport [29]. Thus it is significant that, using mitochondria preloaded with 3-hydroxybutyrate in place of succinate, no inhibitory effect on the reduction of BuOOH is apparent with rotenone plus cyanide and only a small inhibition with antimycin plus cyanide (not shown). Osmotically shocked mitochondria do not significantly reduce BuOOH with added $NADH_2$; sonic particles prepared from them do not do so either with $NADH_2$, succinate, ascorbate or reduced cytochrome *c*. Thus, direct reduction by a component of the electron transport chain is not a feasible route for hydroperoxide reduction.

The concentrations of GSH found in the mitochondrial pellet in all these

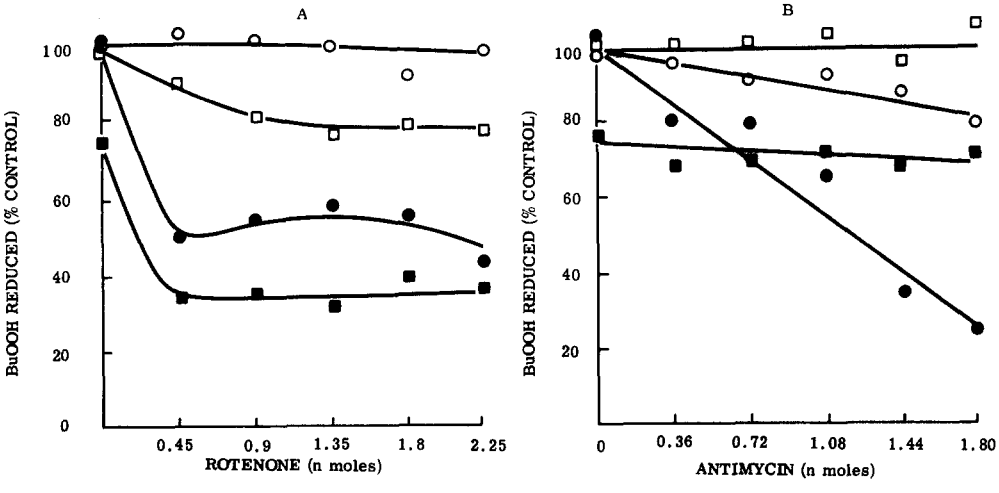


Fig. 4. Reduction of BuOOH by succinate alone (○) and in the presence of KCN (0.5 μmol) (●), ATP (0.5 μmol) (□), ATP with KCN (■) and varying concentrations of (A) rotenone, (B) antimycin. The mitochondria used were preloaded with succinate (see Methods). Results were obtained after 4 min incubation. The control (100%) values (see Fig. 3) was 382 nmol BuOOH reduced.

incubations with KCN plus rotenone or antimycin are not significantly different from those obtained when these inhibitors are omitted.

The effect of some other inhibitors of mitochondrial function on the reduction of BuOOH either by succinate or by 3-hydroxybutyrate loaded mitochondria is shown in Table I. With both substrates, malonate inhibits strongly; this is to be expected with succinate as substrate but it is not easy to explain with 3-hydroxybutyrate. Oligomycin is ineffective but azide, also a phosphorylation inhibitor [30] inhibits strongly. This evidence that reduction of BuOOH may be an energy-requiring process is supplemented by the following observations: (a) almost complete inhibition of reduction is obtained on disrupting the membrane with lubrol (Table I) or ultrasound (not shown), (b) the most effective inhibitor tested is the uncoupler FCCP using any of the substrates shown in Fig. 3. This substance inhibits reduction of BuOOH 80—90% with or without cyanide present, (c) phosphate and ADP (0.5 μmol) inhibit little or not at all when added separately with cyanide present but together they strongly inhibit reduction with succinate though not with 3-hydroxybutyrate. The effect observed in (c) is due to formation of ATP and consequent diversion of high energy intermediates required for reduction of BuOOH with succinate. This is more clearly shown by using less ADP (0.1 μmol). Inhibition with phosphate is then not detectable but after adding glucose and hexokinase (themselves not inhibitory) to regenerate ADP from ATP formed, the inhibition is restored. Subsequent assays of glucose 6-phosphate show that the amount of ATP cycled is only slightly less than the amount formed when the hydroperoxide is omitted so that phosphorylation of ADP must take priority over reduction of hydroperoxides (Table II).

As with the respiratory inhibitors, the GSH content of the pellets obtained during these reductions is dissociated from the reduction rate. Thus although FCCP depresses the reduction of BuOOH to the same extent with both 3-hydroxybutyrate and succinate preloaded mitochondria, the GSH values are

TABLE I

EFFECT OF SOME RESPIRATORY AND PHOSPHORYLATION INHIBITORS ON THE MITOCHONDRIAL REDUCTION OF BuOOH AND THE CORRESPONDING GSH CONCENTRATION

Mitochondria were preloaded at 0°C with either succinate or 3-hydroxybutyrate (BHB). For techniques see Methods. Incubations were for 4 min. KCN (0.5 μmol) added to all tubes.

Inhibitor	Amount	BuOOH reduced ** with		GSH concentration ** with	
		Succinate	BHB	Succinate	BHB
Nil		100 *	81 ± 7	100 *	97 ± 11
Malonate	2.5 μmol	26 ± 19	42 ± 14	88 ± 22	71 ± 19
Azide	0.1 μmol	31 ± 3	52 ± 15	110 ± 6	—
Oligomycin	10 mg	91 ± 8	80 ± 9	106 ± 13	98 ± 9
P_i	2.5 μmol	91 ± 12	92 ± 6	96 ± 7	104 ± 18
ADP	0.5 μmol	88 ± 16	88 ± 15	105 ± 13	97 ± 18
ADP + P_i		51 ± 18	85 ± 14	99 ± 5	109 ± 16
Lubrol PX	2 mg	11 ± 11	—	—	—
FCCP	5 nmol	22 ± 13	14 ± 15	77 ± 14	47 ± 11

* All results are given as percentages of values found with succinate without inhibitor (see Methods).
** Means and S.D. are given for 4 assays each using a different batch of mitochondria.

TABLE II

THE AMOUNT OF ATP FORMED AND BuOOH REDUCED ON INCUBATING MITOCHONDRIA WITH ADP PLUS P_i AND AN ADP REGENERATING SYSTEM

For techniques see Methods. The preincubation medium contained KCN (0.5 μmol) as shown, succinate (5 μmol) and the regenerating system made up of hexokinase (60 μg; 1 I.U.), glucose (150 μmol), $MgCl_2$ (0.02 μmol) and sodium phosphate (3.8 μmol). ADP (0.1 μmol) was added after the preincubation in the 0.5 ml buffer containing (where present) the BuOOH. Incubation was for 3 min. ATP was assayed [41] on supernatants obtained after sedimenting the mitochondrial pellet and acidifying with perchloric acid (0.1 vol of 2.4%).

Addition	BuOOH reduced *,**		ATP formed ** (nmol)		
			With BuOOH		No BuOOH with KCN
	With KCN	No KCN	With KCN	No KCN	
Omit ADP and regenerating system	100 *	—			
Omit ADP only	108 ± 8	102 ± 6			
Omit regenerating system only	104 ± 4	100 ± 5			
Complete system	55 ± 5	59 ± 4	487 ± 3	5000 ***	540 ± 20

* BuOOH is given as a percentage of the control reduction (425 ± 65 nmoles).

** Mean are given for 2 assays.

*** Minimum value calculated from the O_2 consumption in the presence of ADP and P_i and assuming a P/O ratio of 2.

considerably lower for the former (Table I). A more striking illustration of this effect is shown by incubating mitochondria with varying amounts of succinate in the presence of FCCP and cyanide (Fig. 5). Though increasing the succinate concentration does not affect the inhibition of BuOOH reduction, it progressively increases the GSH concentration from a low value to above the control

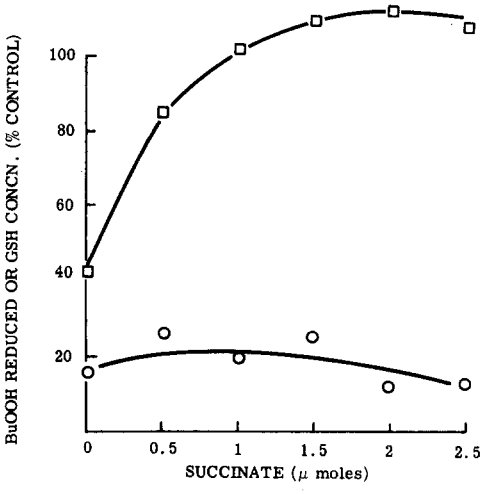


Fig. 5. Effect of FCCP on the extent of reduction of BuOOH (○) and the corresponding GSH concentration (□) in the presence of varying amounts of succinate. See Methods. Mitochondria (5.2 mg protein) were preincubated with FCCP (5 nmol), KCN (0.5 μmol) and the amounts of succinate shown. Results obtained after 4 min incubation with BuOOH. Control (100%) values obtained using 2.5 μmol succinate with cyanide but omitting the FCCP were: BuOOH reduced, 279 nmol; GSH, 27.7 nmol.

level. Similar results are obtained using 3-hydroxybutyrate or isocitrate (not shown).

GSH levels accompanying the inhibition of reduction of BuOOH by ADP plus phosphate as described above also remain at the control value.

Using either succinate or 2-hydroxybutyrate loaded mitochondria, reduction of BuOOH is also inhibited by thiol reagents. Thus both *N*-ethylmaleimide and *p*-hydroxymeruciribenzoate (the latter at higher concentrations) inhibit the reductions. As the concentration of these two inhibitors is raised no inhibition occurs until after a threshold value is reached after which there is a progressive fall with increased inhibitor concentration. *N*-Ethylmaleimide, a known penetrant [31], depletes GSH by titration and hence its concentration in the pellets falls with increasing concentration of inhibitor whereas with *p*-hydroxymercuribenzoate, a non-penetrant [31], the GSH concentration remains throughout near to the control level (Fig. 6).

Substances inhibiting the transport into mitochondria of intermediates of the tricarboxylic acid cycle have previously been shown to affect the reduction of diamide by mitochondria [22]. Using succinate loaded mitochondria, these substances were also found to inhibit the reduction of BuOOH (Fig. 7). The two monocarboxylate carrier inhibitors, phenylpyruvate [32] and 4-hydroxy-α-cyanocinnamate [33] and the tricarboxylate carrier inhibitor, propane-1,2,3-tricarboxylate [34] all inhibit at concentrations at which they are used to inhibit transport. A smaller inhibition was also obtained using the dicarboxylate transport inhibitor, *n*-butylmalonate [35]. GSH concentrations are, though to a lesser extent, lowered by the monocarboxylate and tricarboxylate (but not the dicarboxylate) transport inhibitors.

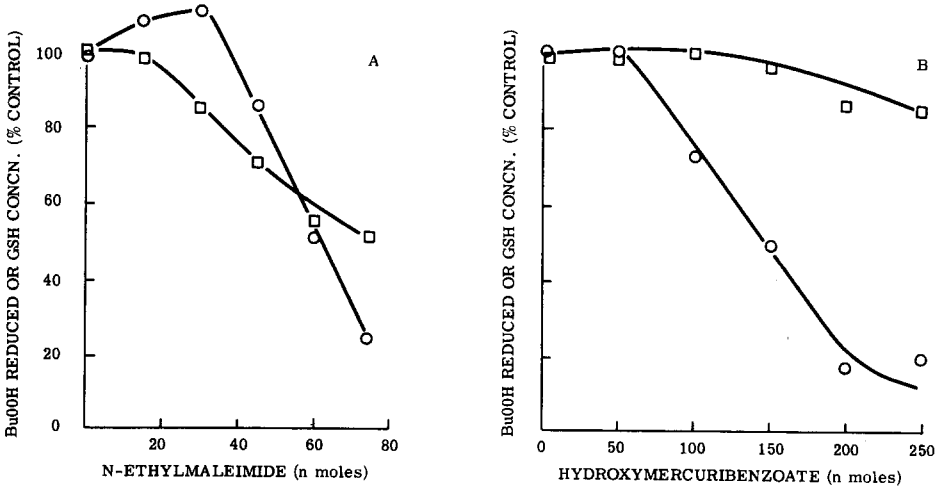


Fig. 6. Effect of (A) *N*-ethylmaleimide, (B) *p*-hydroxymercuribenzoate on the mitochondrial reduction of BuOOH (○) and the corresponding GSH concentration (□). Mitochondria (3.8 mg protein) were preloaded with succinate (see Methods). KCN (0.4 μmol) was present in all tubes. Incubations were for 4 min. Control (100%) values (BuOOH reduced, 380 nmol; GSH, 24 nmol) are those obtained with the inhibitor omitted.

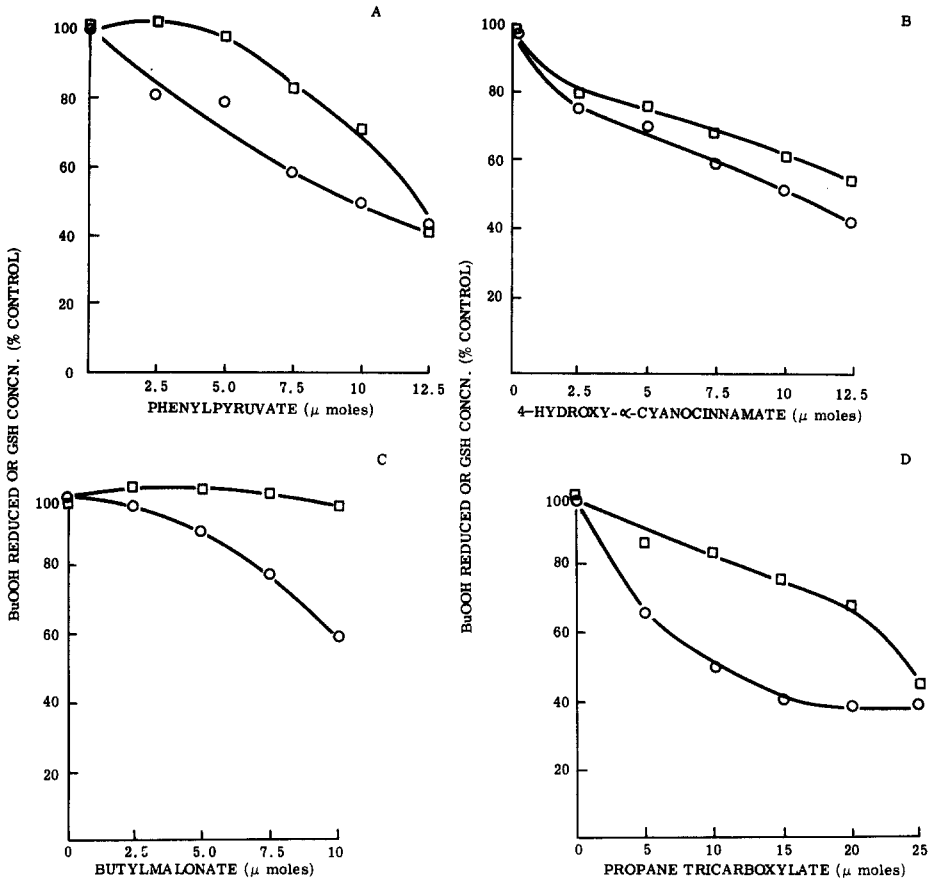
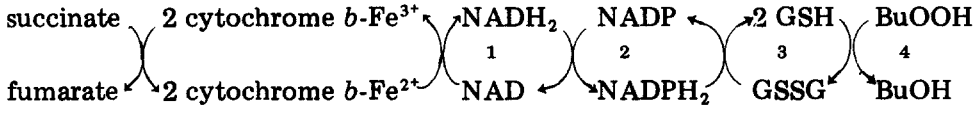


Fig. 7. Mitochondrial reduction of BuOOH (○) and the corresponding GSH concentration (□) in the presence of some inhibitors of the mitochondrial transport of carboxylic acids. A, phenylpyruvate; B, 4-hydroxy-α-cyanocinnamate; C, butylmalonate; D, propane-1,2,3-tricarboxylate. Mitochondria (5.2 mg protein) were preloaded with succinate (see Methods). KCN (0.5 μmol) was present in all tubes. Incubations were for 4 min. Control (100%) values obtained without the inhibitor present were BuOOH reduced, 426 nmol; GSH, 17.6 nmol.

Discussion

The pathway propsed for the reduction of BuOOH and other hydroperoxides by succinate is shown below.



A similar pathway has also been proposed by Oshino and Chance [7]. Evidence for steps 3 and 4 (involving GSH) is as follows: (i) mitochondria contain an active glutathione peroxidase able to utilise organic hydroperoxides for oxidising GSH [16,17], (ii) the GSSG produced can be reconverted to GSH using reducing equivalents from substrate via $NADPH_2$ (step 3) because mitochondria

have an $NADPH_2$-specific glutathione reductase [16,22], (iii) *N*-ethylmaleimide inhibits the reduction over the concentration range at which it removes mitochondrial GSH by direct titration, (iv) no conditions have been observed in which mitochondria with lowered GSH levels show a high rate of hydroperoxide reduction.

The pathway proposed has energy-dependent stages at steps 1, 2 and 4. The source of the necessary high energy equivalents is uncertain since in the presence of cyanide (when 95% of the oxygen consumption is prevented) hydroperoxide reduction is not inhibited.

The occurrence of energy dependent step 1, namely reversed electron transfer to NAD [29], explains the inhibition of reduction by antimycin and its reversal by ATP, the synergism with P_i plus ADP and the fact that when 3-hydroxybutyrate which reduces NAD directly [36] replaces succinate, these inhibitions are not found.

An energy dependence for step 2 lacks direct experimental support but is postulated because the transhydrogenation to generate $NADPH_2$ is catalysed by mitochondrial transhydrogenase and this is well known to be an energy dependent enzyme system [37].

Evidence for the energy dependence for step 4 is that the uncoupler FCCP in the presence of succinate (see Fig. 5) strikingly inhibits reduction of BuOOH and yet maintains a high level of GSH. FCCP does not inhibit glutathione peroxidase in lysates hence, in the presence of this enzyme, a high GSH concentration is not compatible with the accumulation of BuOOH in the matrix. Consequently high energy equivalents dissipated by the uncoupler may be needed for penetration of oxidising equivalents from the hydroperoxide. Some of the other inhibitors which prevent energy generation (antimycin, ADP + P_i, *p*-hydroxymercuribenzoate) also show a high GSH concentration and may therefore partly act at this step. However the interpretation does not directly account for the inhibition of reduction of BuOOH by rotenone using succinate as substrate. This has been attributed to inhibition of the necessary reversed electron transport and a possible explanation is that oxidant entry is prevented by the malate formed via succinate dehydrogenation. Thus malate, added in place of succinate as a reducing substrate, maintains the GSH concentration almost at the level found without BuOOH but only poorly supports BuOOH reduction. One possibility is that malate may act by competing with BuOOH (or oxidising equivalents derived from BuOOH) for entry, implying that mitochondrial carriers of the tricarboxylic acid cycle may be involved. More direct evidence of this is given by the effect of inhibitors of these carriers which in general inhibit BuOOH reduction to a greater extent than they deplete the GSH concentration.

This study reveals a striking similarity between the mitochondrial reduction of BuOOH (and other hydroperoxides) and the reduction of the glutathione oxidant, diamide [22] particularly in that, for both substances, it is possible to inhibit the reductions without affecting the GSH concentration. In the case of diamine a requirement for protonation and hence a maintained Δ pH for entry into the matrix was proposed and this could also be invoked for the hydroperoxides. Thus although $H_3O_2^+$ [38] is probably present in negligible amounts in solutions of H_2O_2 [39], the electron-donating groups present in the

neutral hydroperoxides would increase the contribution of the cationic forms in solutions of ROOH. An alternative explanation notices that both hydroperoxides [11] and diamide [40] can induce lipid peroxidation. A membrane-bound hydroperoxide formed in either way could then be the actual substance to be transported into and reduced by the matrix glutathione peroxidase system.

Acknowledgement

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References

1 Plaa, G.L. and Witschi, H. (1976) Ann. Rev. Pharmac. Toxicol. 16, 125—141
2 Van Lier, J.E. and Kan, G. (1971) Biochem. J. 125, 47P
3 Hamberg, M. and Samuelsson, B. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 899—903
4 Christ-Hazelhof, E., Nugteren, D.H. and Van Dorp, D.A. (1976) Biochim. Biophys. Acta 450, 450—461
5 Thomas, H.F., Herriott, R.M., Hahn, B.S. and Wang, S.Y. (1976) Nature, Lond. 259, 341—342
6 Nishiki, K., Jamieson, D., Oshino, N. and Chance, B. (1976) Biochem. J. 160, 343—355
7 Oshino, N. and Chance, B. (1977) Biochem. J. 162, 509—525
8 Sies, H., Gerstenecker, C., Menzel, H. and Flohé, L. (1972) FEBS Lett. 27, 171—175
9 Hrycay, E.G. and O'Brien, P.J. (1974) Arch. Biochem. Biophys. 160, 230—245
10 Hrycay, E.G. and O'Brien, P.J. (1973) Arch. Biochem. Biophys. 157, 7—22
11 Sies, H. and Summer, K.H. (1975) Eur. J. Biochem. 57, 503—512
12 O'Brien, P.J. and Little, C. (1969) Can. J. Biochem. 47, 493—499
13 Ohki, S., Ogino, N., Yamamoto, S. and Hayaishi, O. (1979) J. Biol. Chem. 254, 829—836
14 Little, C. and O'Brien, P.J. (1968) Biochem. Biophys. Res. Commun. 31, 145—150
15 Sies, H. and Moss, K.M. (1978) Eur. J. Biochem. 84, 377—383
16 Flohé, L. and Schlegel, W. (1971) Hoppe-Seyler's Z. Physiol. Chem. 352, 1401—1410
17 Zakowski, J.J. and Tappel, A.L. (1978) Biochim. Biophys. Acta 526, 65—70
18 Jocelyn, P.C. (1972) Biochemistry of the SH group, pp. 223—224, Academic Press, London
19 Vignais, P.M. and Vignais, P.V. (1973) Biochim. Biophys. Acta 325, 357—374
20 Jocelyn, P.C. (1975) Biochim. Biophys. Acta 396, 427—436
21 Jocelyn, P.C. (1979) in Functions of Glutathione in Liver and Kidney (Sies, H. and Wendel, A., eds.), pp. 127—138, Springer-Verlag, Berlin
22 Jocelyn, P.C. (1978) Biochem. J. 176, 649—664
23 O'Brien, P.J. (1969) Can. J. Biochem. 47, 485—492
24 Jocelyn, P.C. and Kamminga, A. (1974) Biochim. Biophys. Acta 343, 356—362
25 Zakowski, J.J. and Tappel, A.L. (1978) Anal. Biochem. 89, 430—436
26 Jacobs, E.E., Jacob, M., Sanadi, D.R. and Bradley, L.B. (1956) J. Biol. Chem. 223, 147—156
27 McKnight, R.C. and Hunter, F.E. (1966) J. Biol. Chem. 241, 2757—2765
28 Green, R.C., Little, C. and O'Brien, P.J. (1971) Arch. Biochim. Biophys. 142, 598—605
29 Ernster, L. and Lee, C.P. (1967) Methods Enzymol. 10, 729—738
30 Wilson, D.F. and Chance, B. (1963) Biochem. Biophys. Res. Commun. 23, 751—756
31 Gaudemer, Y. and Latruffe, N. (1975) FEBS Lett. 54, 30—33
32 Halestrap, A.P., Brand, M.D. and Denton, R.M. (1974) Biochim. Biophys. Acta 367, 102—108
33 Halestrap, A.P. and Denton, R.M. (1974) Biochem. J. 138, 313—316
34 Robinson, B.H., Williams, G.R., Halpern, M.L. and Leznoff, C.C. (1970) Eur. J. Biochem. 15, 263—272
35 Robinson, B.H. and Chappell, J.B. (1967) Biochem. Biophys. Res. Commun. 28, 249—255
36 Lehninger A.L. (1949) J. Biol. Chem. 178, 625—644
37 Rydström, J. (1977) Biochim. Biophys. Acta 463, 155—184
38 Alder, R.W. and Whiting, M.C. (1964) J. Chem. Soc. 4707—4712
39 Mitchell, A.G. and Wynne-Jones, W.F.K. (1954) Trans. Farad. Soc. 52, 824—828
40 Power, J.A., Harris, J.W. and Bainton, D.F. (1977) Exp. Cell. Res. 105, 455—460
41 Lamprecht, W. and Trautschold, I. (1974) in Methods of Enzymatic Analysis, 2nd edn., Vol. 4, (Bergmeyer, H.U., ed.), pp. 2101—2110, Academic Press, London