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LIPID PEROXIDATION, PROTEIN THIOLS AND CALCIUM HOMEOSTASIS IN BROMOBENZENE-INDUCED LIVER DAMAGE

ALESSANDRO F. CASINI, EMILIA MAELLARO, ALFONSO POMPELLA, MARCO FERRALI and MARIO COMPORTI*

Istituto di Patologia Generale dell'Università di Siena, Via Laterino 8, 53100 Siena, Italy

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Abstract—The mechanisms of bromobenzene hepatotoxicity *in vivo* were studied in mice. The relationships among glutathione (GSH) depletion, lipid peroxidation, loss of protein thiols, disturbed calcium homeostasis and liver necrosis were investigated. Liver necrosis (as estimated by the serum glutamate–pyruvate transaminase (SGPT) level) appeared between 9 and 12 hr and increased at 18 hr. Lipid peroxidation which was already detectable at 6 hr in some animals, increased thereafter showing a good correlation with the severity of liver necrosis. Despite a quite fast depletion of hepatic GSH, a significant decrease in protein thiols could be observed at 12–18 hr only. Loss of protein thiols in both whole liver and subcellular fractions (microsomes and mitochondria) was correlated with lipid peroxidation. Also a good inverse correlation was seen between lipid peroxidation and the calcium sequestration activity of liver microsomes and mitochondria.

The treatment of mice with desferrioxamine (DFO) after bromobenzene-intoxication completely prevented lipid peroxidation, loss of protein thiols and liver necrosis in the animals sacrificed 15 hr after poisoning. When, however, the animals were examined at 24 hr, although the general correlation between lipid peroxidation and liver necrosis was held, in some animals (about 30% of the survivors) elevation of SGPT was observed in the virtual absence of lipid peroxidation. It seems likely therefore that the liver damage seen during the first phase of bromobenzene-intoxication is strictly related to lipid peroxidation. It is, however, possible that in some animals in which for some reason lipid peroxidation does not develop, another mechanism of liver necrosis unrelated to lipid peroxidation occurs at later times.

Bromobenzene is a prototype halogenated aromatic hydrocarbon which has been used for a long time to produce an experimental model of liver necrosis [1–6]. It is well known [1–6] that bromobenzene metabolites conjugate with cellular glutathione (GSH)† thus producing a marked GSH depletion. While endogenous GSH is largely consumed, bromobenzene metabolites covalently bind to cellular macromolecules and this binding parallels to some extent the GSH depletion [5, 6]. Thus the liver necrosis induced by bromobenzene and other GSH depleting agents has been believed to be mediated by the covalent binding of reactive metabolites to target molecules of the liver cell [1–6].

It has been noted, however, that the depletion of GSH, one of the major defences against oxidative stress, renders the cell more susceptible to the development of peroxidation of membrane lipids. Lipid peroxidation has been in fact observed in isolated hepatocytes treated with a number of GSH-depleting agents [7, 8] as well as in primary cultures of hepatocytes intoxicated with bromobenzene [9]. Also lipid peroxidation, as measured by ethane evolution, was increased in mice acutely intoxicated with paracetamol, provided that the hepatic GSH level was decreased by starvation [10, 11].

Previous studies from our laboratory [12, 13] have shown that bromobenzene or iodobenzene administration to mice results in the development of lipid peroxidation and liver necrosis only when the hepatic GSH depletion reaches critical values. The treatment of the intoxicated animals with Trolox C (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a lower homolog of vitamin E, completely prevented both lipid peroxidation and necrosis, while not changing at all the extent of the covalent binding of bromobenzene metabolites to liver protein. This would suggest that lipid peroxidation is an important factor in the pathogenesis of the bromobenzene-induced liver cell death.

During the last decade evidence has been accumulated [14–17] according to which perturbation of calcium homeostasis is the crucial and irreversible event leading to cell death in the liver injury produced by a number of toxins. As far as the GSH-deleting agents are concerned, a great deal of experimental work carried out with model systems in which an oxidative stress is imposed to isolated hepatocytes, would suggest that the alteration in calcium homeostasis is produced by the loss of protein -SH groups secondary to GSH depletion [17–20]. The loss of protein sulfhydryl groups would be mainly the result of oxidative processes occurring during the oxidative stress imposed by the metabolic redox cycling of some chemicals [17–20].

Therefore in the present study the relationships among GSH depletion, lipid peroxidation, loss of

* To whom correspondence should be sent.

† Abbreviations used: GSH, reduced glutathione; DFO, desferrioxamine; SGPT, serum glutamate–pyruvate transaminase; MDA, malonic dialdehyde.

protein thiols, calcium homeostasis and liver necrosis were studied in bromobenzene-intoxicated animals.

MATERIALS AND METHODS

Male NMRI albino mice (Ivanovas GMBH, F.R.G.) weighing 20–30 g and maintained on a pellet diet (Altromin-Rieper, Bolzano, Italy) were used. As reported in previous studies [13] mice resulted more susceptible to bromobenzene-intoxication than rats, in which the hepatic GSH depletion after bromobenzene-treatment was less pronounced. The animals were starved overnight (16 hr in all). This starvation decreased hepatic GSH by about 50% as compared with that in laboratory chow fed animals and increased the frequency of liver necrosis.

Bromobenzene (C. Erba, Milan, Italy) mixed with two volumes of mineral oil was administered intragastrically under light ether anesthesia at the dose of 13 mmol/kg body wt. Control mice received mineral oil alone. All the animals were starved after the intoxication, unless otherwise stated. In the experiments in which desferrioxamine (DFO) was used, DFO (Ciba-Geigy, Basel) was dissolved in saline (25%, w/v) and given intraperitoneally to the animals 7 and 13 hr after bromobenzene poisoning, at the dose of 250 mg/kg body wt. The respective control animals received saline only.

Liver damage was assessed by measuring the serum glutamate-pyruvate transaminase (SGPT) activity (optimized u.v. enzymatic method, C. Erba, Milan, Italy).

Hepatic GSH was measured as acid-soluble -SH groups according to Sedlak and Lindsay [21]. Protein thiols were determined in whole liver and subcellular fractions (mitochondria and microsomes) essentially according to Di Monte *et al.* [18].

Lipid peroxidation was measured in the whole liver as tissue content of malonic dialdehyde (MDA) as previously reported [22]. It was ascertained in preliminary experiments, that the method measuring the tissue content of MDA correlated with the other methods generally run in this laboratory to detect lipid peroxidation: in particular with the measurement of the diene conjugation absorption in cellular phospholipids, as done by Casini *et al.* [9], and with the evaluation of the amount of carbonyl functions originating from the peroxidative breakdown of unsaturated fatty acids in cellular phospholipids as done by Benedetti *et al.* [23]. The latter method was used to determine lipid peroxidation in the microsomal and mitochondrial fractions of the liver.

The energy-dependent calcium sequestration activity of the microsomal fraction was measured in the presence of oxalate according to Moore *et al.* [24]. The calcium uptake by the mitochondrial fraction was measured in an ATP-supported system according to Bielawski and Lehninger [25].

Protein determination was performed according to Lowry *et al.* [26]. When the mitochondrial Ca^{2+} uptake was studied, protein was determined by the use of "Bio-Rad Protein Assay" (Bio-Rad Laboratories, München, F.R.G.) according to Bradford [27]. This method, which is particularly quick, allowed to incubate equivalent amounts (on the base of protein) of mitochondria in the system for calcium uptake.

Table 1. Time-course of hepatic glutathione (GSH) depletion, liver damage (SGPT), lipid peroxidation (hepatic content of malonic dialdehyde, MDA) and decrease in protein thiol groups (Protein -SH) after bromobenzene poisoning

Time after intoxication	0 time	6 hr	9 hr	12 hr	18 hr
GSH (nmol/mg protein)	30.8 ± 2.1 (13)	2.5 ± 0.4 (4)	1.4 ± 0.2 (4)	1.2 ± 0.2 (7)	2.2 ± 0.3 (24)
SGPT (U/l)	38 ± 9 (14)	22 ± 4 (4)	151 ± 46 (4)	210 ± 93 (7)	2997 ± 879 (24)
MDA (pmol/mg protein)	—	8 ± 3 (4)	33 ± 6.6 (4)	82 ± 35 (7)	918 ± 282 (24)
Protein -SH (nmol/mg protein)	119.8 ± 3.9 (14)	123.5 ± 7.1 (4)	108.4 ± 3.5 (4)	103.7 ± 3.3*[a] (7)	89.0 ± 3.3*[b] (24)

Bromobenzene was given by gastric intubation at the dose of 13 mmol/kg body wt. Results are given as means ± SEM. The number of animals is reported in parentheses.

* Significantly different from the 0 time value: [a] $P < 0.05$, [b] $P < 0.01$.

RESULTS

Table 1 shows the time-course of hepatic GSH depletion, liver necrosis (as assessed by SGPT), lipid peroxidation (as measured by the MDA content of the liver) and protein thiol decrease following bromobenzene-intoxication. Liver necrosis appeared in some animals between 9 and 12 hr and subsequently increased in severity and frequence. Lipid peroxidation which was already detectable in some animals at 6 hr, increased thereafter. Despite a quite fast depletion of hepatic GSH, which was nearly maximal at 6 hr, a significant decrease in protein thiols could be observed at 12–18 hr only. As noticed in the previous studies [12, 13, 22], a large individual variation was observed in the sensitivity of the animals to bromobenzene, as shown by the large dispersion in the SGPT and MDA values (Table 1). There was, however, a readily evident relationship between the different parameters of the response to bromobenzene when the values for the individual animals were graphed together. The plot of the individual values obtained at 18 hr for transaminase or MDA levels against the corresponding hepatic GSH contents (Fig. 1) indicated that, as observed in the previous studies, lipid peroxidation and liver necrosis occurred only when the hepatic GSH levels reached critical values (3.0–1.5 nmol/mg protein). In agreement with the previous studies, a good correlation ($r = 0.837$; $P < 0.001$) was found between the extent of lipid peroxidation and the severity of liver necrosis seen at 18 hr. Also, lipid peroxidation was correlated with the decrease in protein thiols (Fig. 2).

To obtain more precise information at the level of subcellular fractions, lipid peroxidation and protein thiols were measured in both liver microsomes and

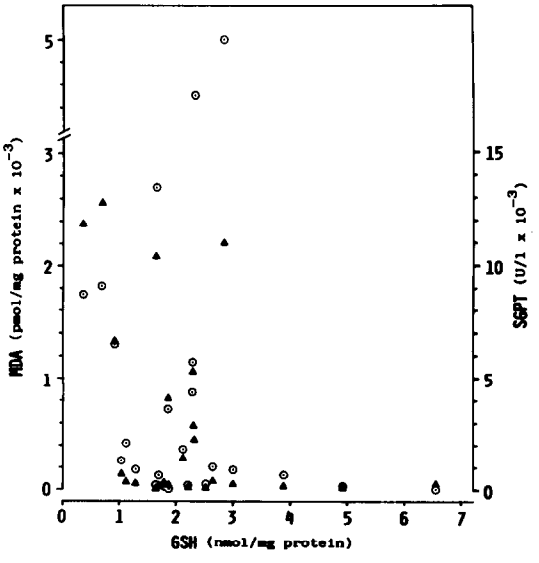

Fig. 1. Plot of serum glutamate–pyruvate transaminase (SGPT, ▲) levels and of malonic dialdehyde contents (MDA, ⊙) of the liver against the corresponding hepatic GSH contents in mice intoxicated with bromobenzene (13 mmol/kg body wt, by mouth). The values were obtained 18 hr after poisoning.

Table 2. Time-course of lipid peroxidation (Carbonyl functions in phospholipids), decrease in protein thiol groups (Protein -SH) and calcium sequestration activity in liver microsomes after bromobenzene poisoning

Time after intoxication	0 time	6 hr	9 hr	12 hr	18 hr
Carbonyl functions (nmol/mg phospholipids)	—	1.5 ± 1.5 (4)†	1.0 ± 0.9 (4)†	2.9 ± 1.4 (6)	31.4 ± 16.1 (10)
Protein -SH (nmol/mg protein)	95.1 ± 4.3 (14)	92.9 ± 2.0 (4)	86.2 ± 2.6 (4)	92.1 ± 1.4 (6)	75.0 ± 6.3* (12)
Calcium uptake (nmol/mg protein/30 min)	122.5 ± 9.2 (11)	N.D.	119.3 ± 7.7 (4)	123.8 ± 8.8 (6)	85.6 ± 23.4 (12)

Bromobenzene was given by gastric intubation at the dose of 13 mmol/kg body wt. Results are given as means ± SEM. The number of animals is reported in parentheses.

* Significantly different from the 0 time value: $P < 0.05$.

N.D. not determined.

† Of 4 animals, only 1 (6 hr) or 2 (9 hr) showed detectable carbonyl functions (5.9, at 6 hr; 0.5 and 3.6 nmol/mg phospholipids, at 9 hr).

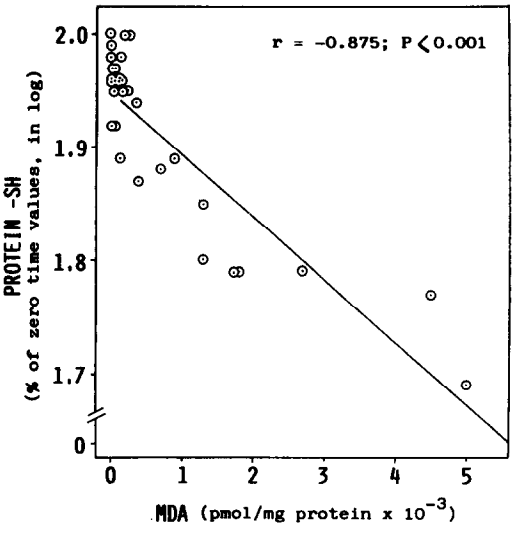

Fig. 2. Plot of the protein -SH groups of the liver against the corresponding lipid peroxidation values (hepatic MDA content) in mice intoxicated with bromobenzene. The values obtained 12–18 hr after poisoning were used. Protein -SH groups are given as per cent values (expressed in log) with respect to the zero time, i.e. control values in each experimental set. The mean of control values was 118.8 ± 3.9 nmol/mg protein (SEM, see Table 1). Bromobenzene intoxication was performed as reported in Table 1.

mitochondria isolated from the intoxicated animals. As can be seen (Tables 2 and 3), lipid peroxidation (as measured by the amount of carbonyl functions in phospholipids) and protein thiols had a behaviour roughly similar to that observed in the whole liver. In fact, a significant correlation was observed between lipid peroxidation in microsomes and mitochondria and the corresponding losses of protein thiols seen at all the times examined ($r = 0.849$, $P < 0.001$ for microsomes; $r = 0.692$, $P < 0.02$ for mitochondria; protein thiols expressed as in Fig. 2).

As far as the evaluation of perturbation of Ca^{2+} homeostasis is concerned, *in vivo* models do not allow the sophisticated approaches (measure of intracellular calcium pools, etc.) as those which are possible in *in vitro* systems (e.g. isolated hepatocytes). However, information can be obtained by evaluating the Ca^{2+} sequestration activity of both the microsomal and the mitochondrial fractions from the intoxicated animals (i.e. the capacity of microsomes and mitochondria to regulate the cytosolic calcium level). As shown in Tables 2 and 3, this activity is impaired in both fractions after bromobenzene intoxication. However, while the mitochondrial calcium uptake was lowered in a significant way at 6 hr already, the microsomal calcium pump was markedly impaired in the animals in which very extensive lipid peroxidation developed, but not in those in which lipid peroxidation was minimal or absent.* Figure 3 shows the plot of the calcium sequestration activity of both the microsomal (Fig. 3A) and the mitochondrial (Fig. 3B) fractions against the corresponding lipid peroxidation values. As can be seen, a good inverse correlation was observed between the two phenomena. A significant correlation was also found between the impairment of calcium pump in microsomes and mitochondria and the corresponding losses of protein thiols ($r = 0.894$, $P < 0.001$ for microsomes; $r = 0.861$, $P < 0.001$ for mitochondria).

As previously stated, lipid peroxidation was correlated with the decrease in protein thiols (Fig. 2), with the impairment of microsomal and mitochondrial calcium sequestration activity as well as with liver cell death. In order to evaluate further the role of lipid peroxidation in the bromobenzene-induced liver damage, experiments were carried out in which DFO, a powerful iron chelator and inhibitor of lipid peroxidation [29, 30], was administered to the animals after bromobenzene-intoxication. The aim of the experiment was to prevent extensive lipid peroxidation and to see whether or not liver necrosis and protein thiol decrease in the whole liver were also prevented. As shown in Table 4, 15 hr after bromobenzene-intoxication, lipid peroxidation, liver necrosis and protein thiol decrease were extremely reduced in the DFO-treated animals as compared to those not given DFO, while the GSH depletion was only minimally affected. These results would suggest a major role of lipid peroxidation in the bromobenzene-induced protein thiol decrease and liver cell death.

However, when some intoxicated animals were sacrificed at times later than 15–18 hr, it was occasionally found that elevation in SGPT was not matched by decrease in protein -SH groups and lipid

* In a recent study [28] it has been reported that bromobenzene-intoxication in rats does not affect the liver microsomal and mitochondrial calcium uptake. Since the measures were performed 4 hr after poisoning this finding is not at variance with ours. Also it must be considered that, as previously noticed, a marked difference in the susceptibility to bromobenzene exists between mice and rats [13].

Table 3. Time-course of lipid peroxidation (Carbonyl functions in phospholipids), decrease in protein thiol groups (Protein -SH) and calcium sequestration activity in liver mitochondria after bromobenzene poisoning

Time after intoxication	0 time	6 hr	18 hr
Carbonyl functions (nmol/mg phospholipids)	—	2.7 ± 2.6 (4)†	38.1 ± 15.6 (8)
Protein -SH (nmol/mg protein)	97.1 ± 1.5 (6)	93.9 ± 1.4 (4)	85.5 ± 4.9 (8)
Calcium uptake (nmol/mg protein/5 min)	70.8 ± 0.6 (6)	38.7 ± 7.0 (4)*	25.8 ± 7.8 (8)*

Bromobenzene was given by gastric intubation at the dose of 13 mmol/kg body wt. Results are given as means ± SEM. The number of animals is reported in parentheses.

* Significantly different from the 0 time value: $P < 0.01$.

† Of 4 animals only 2 showed detectable carbonyl functions (0.5 and 10.4 nmol/mg phospholipids).

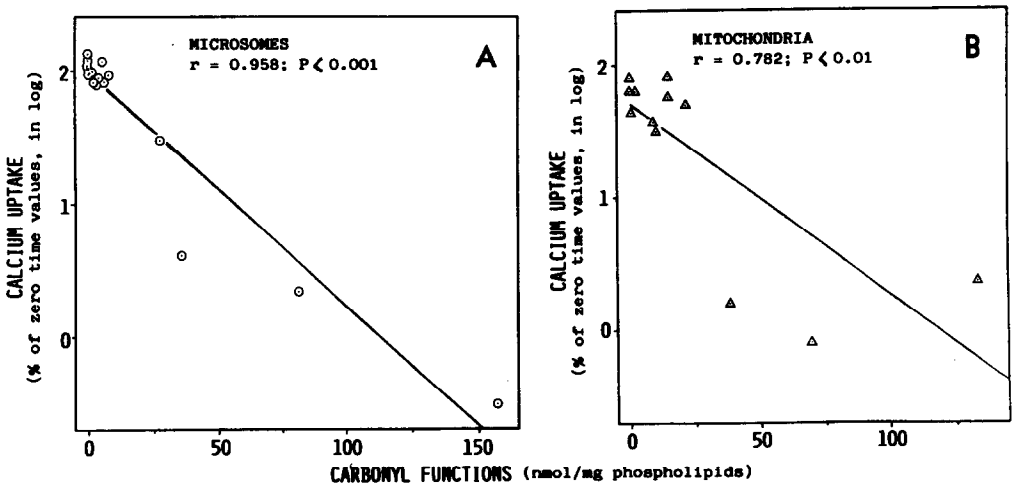

Fig. 3. Plot of the values for calcium uptake against the corresponding lipid peroxidation values (amount of carbonyl functions in phospholipids) in both microsomes (panel A) and mitochondria (panel B) from livers of bromobenzene intoxicated mice. The values obtained 6–18 hr after poisoning were used. Calcium uptake was given as per cent values with respect to the zero time, i.e. control values. The latter values are reported in Tables 2 and 3. Bromobenzene intoxication was performed as reported in Table 1.

peroxidation. Therefore, the effects of bromobenzene-intoxication and of DFO treatment were investigated at 24 hr, and the results are given in Table 5. These results were seemingly similar to those obtained at 15 hr: lipid peroxidation, liver necrosis and loss of protein thiols still were significantly reduced in the DFO-protected animals as compared to the unprotected ones. GSH depletion was not affected at all by DFO treatment. Also, the mortality in the unprotected group was 75%, while in the DFO-treated group it was 12%. However, when the values for each individual animal were considered together, it was observed that out of 34 animals (10 bromobenzene + saline, plus 24 bromobenzene + DFO), 7 showed high SGPT values (1041 ± 255 U/l) in the absence of lipid peroxidation, and 3 showed very high SGPT levels (6608 ± 2195 U/l) in the presence of minimal lipid peroxidation (9.3 ± 4.6 pmol/mg protein). The hepatic GSH level in these cases was 2.6 ± 1.0 nmol/mg protein. Protein thiols were determined in two of these cases only. They were not decreased with respect to controls. Despite the occurrence of such discrepancies, the overall analysis of the data (from 34 animals) still showed a correlation ($r = 0.490$, $P < 0.01$) between MDA values and SGPT levels. Also, a correlation with a high statistical significance was found between MDA values and the loss of protein thiols ($r = 0.940$, $P < 0.001$).

DISCUSSION

The objective of the present and previous [12, 13] studies was to define the mechanisms whereby the metabolism of the model hepatotoxin bromobenzene leads to liver injury in *in vivo* conditions.

The results of the present work clearly show that in bromobenzene-induced liver injury in mice a correlation exists among lipid peroxidation, loss of protein thiols, impairment of the mitochondrial and microsomal Ca^{2+} uptake, and liver necrosis. It is therefore possible that, as suggested by others [15–20, 31, 32], the loss of protein -SH groups is an important factor in the bromobenzene-induced impairment of the calcium sequestration activity of liver mitochondria and microsomes. However, the

Table 4. Hepatic glutathione (GSH) depletion, liver damage (SGPT), lipid peroxidation (hepatic content of malonic dialdehyde, MDA) and decrease in protein thiol groups (Protein -SH) in mice intoxicated with bromobenzene and given either desferrioxamine or saline. The animals were sacrificed 15 hr after poisoning

	GSH (nmol/mg protein)	SGPT (U/l)	MDA (pmol/mg protein)	Protein -SH (nmol/mg)
Controls	27.3 ± 7.7 (3)	15 ± 4 (3)	—	115.9 ± 2.9 (3)
Bromobenzene + saline	1.3 ± 0.2 (16)	9961 ± 2463 (16)	1321 ± 257 (16)	67.3 ± 7.1† (7)
Bromobenzene + desferrioxamine	2.0 ± 0.2* (15)	139 ± 47 (15)	8 ± 3 (15)	97.6 ± 6.0 (7)

Bromobenzene was given by gastric intubation at the dose of 13 mmol/kg body wt. Desferrioxamine was given intraperitoneally, 7 and 13 hr after the intoxication, at the dose of 250 mg/kg body wt. Results are given as means ± SEM. The number of animals is reported in parentheses.

* Significantly different from bromobenzene treated mice: $P < 0.01$.

† Significantly different from either controls or bromobenzene + desferrioxamine treated mice: $P < 0.01$.

Table 5. Hepatic glutathione (GSH) depletion, SGPT levels, lipid peroxidation (hepatic content of malonic dialdehyde, MDA) and decrease in protein thiol groups (Protein -SH) in mice intoxicated with bromobenzene and given either desferrioxamine or saline. The animals were sacrificed 24 hr after poisoning

	GSH (nmol/mg protein)	SGPT (U/l)	MDA (pmol/mg protein)	Protein -SH (nmol/mg)
Controls	50.6 ± 8.2 (8)	99 ± 24 (8)	—	115.2 ± 4.8 (3)
Bromobenzene + saline	2.7 ± 0.9 (10)	6212 ± 2160 (10)	303 ± 176 (10)	85.0 ± 9.0* (5)
Bromobenzene + desferrioxamine	1.6 ± 0.2 (24)	514 ± 197 (24)	8 ± 2 (24)	105.5 ± 2.5 (7)

Bromobenzene was given by gastric intubation at the dose of 13 mmol/kg body wt. Desferrioxamine was given intraperitoneally, 7 and 13 hr after the intoxication, at the dose of 250 mg/kg body wt. Food was reintroduced into the cages 16 hr after the intoxication. Results are given as means ± SEM. The number of animals is reported in parentheses.
* Significantly different from either controls or bromobenzene + desferrioxamine treated mice: $P < 0.05$.

decrease in protein thiols does not appear strictly linked to the hepatic GSH depletion. In fact, first, the kinetics of the two phenomena show a different behaviour (Table 1), and second, the DFO treatment of the intoxicated animals almost completely prevents the loss in protein thiols, while not affecting (or affecting to a minor extent only) the hepatic GSH depletion (Tables 4 and 5).

On the other hand it is likely that the dramatic fall in hepatic GSH overwhelms the cellular defences against oxidative stresses. The result would be the development of lipid peroxidation which in turn would produce loss of protein thiols. Since some protein -SH groups are believed to be essential components of the molecular arrangement responsible for the Ca^{2+} transport across cellular membranes [31, 32], loss of such essential thiols can result in impairment of the calcium sequestration activity of subcellular compartments. Disturbed homeostasis of cellular calcium is generally believed to lead to cell death. This sequence of events is suggested by the results obtained even without the use of DFO. It becomes even clearer in the experiments in which the intoxicated animals were treated with DFO. This iron chelating antioxidant, in fact, prevented lipid peroxidation, loss of protein thiols and liver necrosis (Table 4). Although no direct indication is given in the present work as to whether DFO affects the metabolism of bromobenzene, the fact that the bromobenzene-induced hepatic GSH depletion is essentially not prevented by DFO treatment (Tables 4 and 5) would suggest that DFO does not interfere with bromobenzene metabolism. Also, it has been reported recently [30] that DFO treatment does not affect the microsomal mixed-function oxidase activities responsible for bioactivation of xenobiotics. In short, all the above results are consistent with the hypothesis that at least during the first phase (15–18 hr) of bromobenzene-intoxication lipid peroxidation is responsible for the chain of events leading to liver cell death. In this connection it must be considered that a number of toxic aldehydes derived from lipid peroxidation and in particular 4-hydroxynonenal [33] has been detected in the liver of bromobenzene-poisoned mice [34]. It is known that 4-hydroxynonenal and other 4-hydroxyalkenals specifically bind to -SH groups of proteins and enzymes [35].

However, as previously stated, when the experiments with the use of DFO were repeated at 24 hr, although the general correlation between lipid peroxidation and liver necrosis was held, in some animals (almost 30% of the survivors) elevation of SGPT was observed in the absence of detectable lipid peroxidation. This would suggest the existence of another mechanism of liver necrosis operating at later times of bromobenzene-intoxication and independent of lipid peroxidation and loss of protein thiols. In studies [9] carried out with primary cultures of hepatocytes intoxicated with bromobenzene, two mechanisms of cell killing were proposed: the first which is responsible for the cell killing during the first hours and is based on the peroxidative decomposition of membrane lipids. The second which kills the cells with a slower time-course and is unrelated to lipid peroxidation. Essentially similar results were reported by others [36] who found that the toxicity of bipyridyl on fresh hepatocytes was markedly delayed by various antioxidants. The analogy between the *in vitro* and *in vivo* studies seems therefore evident.

Younes and Siegers recently reported [30] that in mice depleted of GSH and intoxicated with paracetamol, DFO pretreatment, while preventing the increase in lipid peroxidation, does not prevent liver necrosis. Although paracetamol is, like bromobenzene, a GSH depleting agent which promotes lipid peroxidation *in vivo* [10, 11] and *in vitro* [8], it is difficult to compare the data obtained with different toxins. However, an analogy can be seen between the results reported by Younes and Siegers (the elevation of serum enzymes was observed at 24 hr after the intoxication in the animals not induced with phenobarbital) and the cases found in our experiment and reported above (see the Results section) in which elevation of SGPT was found at 24 hr in the virtual absence of lipid peroxidation. Differences in the toxins used and in the pretreatment of the animals (phorone administration in the experiments of Younes and Siegers [30]; starvation in our experiments) may be responsible for the higher incidence of liver necrosis in the absence of lipid peroxidation found by these latter authors [30].

On the basis of studies [37] carried out with freshly isolated hepatocytes it has been suggested that lipid peroxidation in bromobenzene hepatotoxicity is merely a consequence of death of cells depleted of GSH. The experiments with DFO show that when lipid peroxidation is abolished in general liver necrosis is prevented. Furthermore those particular cases

in which elevation of SGPT occurs in the absence of lipid peroxidation clearly show that, if SGPT elevation is due to liver necrosis, the cell death is not necessarily followed by lipid peroxidation even if the hepatocyte is depleted of GSH.

In studies with freshly isolated hepatocytes [15, 16] an impairment of calcium homeostasis has been observed after the addition of bromobenzene. The extramitochondrial calcium pool resulted the most affected by the intoxication, while the mitochondrial calcium uptake is severely impaired in our studies. The difference in experimental model probably accounts for these differences.

The possible mechanisms by which the metabolism of bromobenzene and the consequent GSH depletion results in the onset of lipid peroxidation have been discussed in the previous papers [12, 13]. In addition, the experiments carried out with the use of the iron chelator DFO, in which lipid peroxidation was completely prevented, could suggest that bromobenzene metabolism results in an increased availability of iron in the liver, which then stimulates lipid peroxidation. Iron delocalization from iron stores in the liver cell has been invoked to explain the effects of some hepatotoxins which are prevented by DFO [38, 39]. Thomas and Aust [39] have recently shown that liver microsomes contain ferritin and that O_2^- generated by paraquat via redox cycling releases in some way iron from ferritin. Such iron would then serve to promote lipid peroxidation.

Summing up the results of the present study, a tentative conclusion is that the liver damage seen during the first phase of bromobenzene-intoxication is related to lipid peroxidation, which could affect the protein thiols and consequently impair the Ca^{2+} transport across cellular membranes. It is however possible that in some animals, in which for some reason lipid peroxidation does not develop, another mechanism of liver damage unrelated to lipid peroxidation occurs at later times.

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