Biochemical Pharmacology, Vol. 40, No. 7, pp. 1491–1497, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

EARLY MITOCHONDRIAL DISFUNCTION IN BROMOBENZENE TREATED MICE: A POSSIBLE FACTOR OF LIVER INJURY

EMILIA MAELLARO, BARBARA DEL BELLO, ALESSANDRO F. CASINI, MARIO COMPORTI, DANIELA CECCARELLI,* UMBERTO MUSCATELLO* and ALBERTO MASINI*†

Istituto di Patologia Generale dell'Università di Siena, via Laterino 8, 53100 Siena, Italy; and Istituto di Patologia Generale dell'Università di Modena, via Campi 287, 41100 Modena, Italy

(*Received* 22 *February* 1990; *accepted* 30 *April* 1990)

Abstract—The membrane potential of liver mitochondria isolated from bromobenzene treated mice was studied. Specifically, the efficiency of the energy-transducing mitochondrial membrane was measured during the phase between the occurrence of a massive loss of hepatic GSH, after 2–3 hr of bromobenzene intoxication, and the appearance of lipid peroxidation and cell death (12–15 hr after treatment). Partial uncoupling of oxidative phosphorylation was observed in mitochondria during the early period of intoxication (3–9 hr). These anomalies in oxidative metabolism did not result in irreversible damage to the mitochondrial inner membrane. The possibility that phenolic metabolites of bromobenzene are responsible for the uncoupling effects was examined. Orto- and especially para-bromphenol reproduced the alterations of mitochondrial function when added to normal mitochondira at concentrations comparable to those found in the livers of the intoxicated animals. Since the concentration of the bromophenols (especially *p*-bromophenol) largely increases after the intoxidation times as tested here, mitochondrial uncoupling may represent a mechanism of liver damage acting synergistically with or even independently of other factors such as oxidative stress and lipid peroxidation.

It is known that bromobenzene causes centrilobular hepatic necrosis in rats and mice [1–6]. It is also known that bromobenzene metabolism produces electrophilic intermediates which readily conjugate with glutathione (GSH).‡ The result is a marked hepatic GSH depletion. When the GSH stores are decreased, bromobenzene metabolites bind to cellular macromolecules. It has been assumed for a long time that the toxicity of bromobenzene and other benzene substituted compounds dependended mainly on this covalent binding. However, the observation that the treatment of animals [7, 8] or isolated hepatocytes [9] with antioxidants (trolox C, DPPD, desferrioxamine and others) almost completely prevents liver cell death, while not changing at all the extent of the covalent binding of bromobenzene metabolites to cellular macromolecules, cast some doubts on this mechanism. A similar dissociation between cell death and covalent binding was observed for other benzene substituted compounds [10–13].

A number of studies on the toxicity of GSH depleting agents [14, 15] have investigated the possible mechanisms based on oxidative stress. When the GSH stores are depleted, the liver cell is made more susceptible to the development of lipid peroxidation. Indeed, the occurrence of lipid peroxidation in membrane phospholipids in association with liver cell death was detected in primary cultures of hepatocytes exposed to bromobenzene [9] as well as in liver tissue of intoxicated mice [7, 8]. Furthermore, a significant loss of protein thiols was associated with lipid peroxidation [8]. A perturbation in protein sulphydryl groups may lead to alteration in cellular calcium homeostasis [16–18].

Following the *in vivo* intoxication with bromobenzene, as well as with other GSH-depleting agents, a long time period elapses between the GSH depletion (which is already nearly maximal at 3 hr) and the development of lipid peroxidation and necrosis (at 12–15 hr) [7, 8]. It is reasonable to postulate an event during this period of time which could either initiate reactions related to oxidative stress leading to lipid peroxidation or give rise to processes independent of it. Recent studies [19] have shown mitochondrial damage as a mechanism of cell injury, produced by *tert*-butyl hydroperoxide. Such damage consists in the loss of the inner membrane potential of mitochondria and is the result of both, a peroxidation-dependent, and peroxidation-independent mechanism. Under certain conditions (later times of intoxication of both intact animals [8] and cultured hepatocytes [9]) a mechanism independent of lipid peroxidation appears to be operative in bromobenzene toxicity.

In the present study we have therefore investigated the functional integrity of liver mitochondria following bromobenzene intoxication, by estimating the efficiency of the mitochondrial energy transducing membrane, i.e. the transmembrane potential, during the period between the occurrence of the

† To whom correspondence should be sent.

‡ Abbreviations: Trolox C, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid; DPPD, *N,N'*-diphenyl-*p*-phenylendiamine; SGPT, serum glutamate-pyruvate transaminase; GSH, reduced glutathione; MDA, malonic dialdehyde; $\Delta\psi$, mitochondrial transmembrane electrical potential (negative inside); ΔE, electrode potential; TPP^+, tetraphenylphosphonium cation; EDTA, ethylenediaminetetracetate; DNP, 2,4-dinitrophenol; *p*-Br-OH, parabromophenol; *o*-Br-OH, ortobromophenol.

massive GSH depletion and the appearance of cell death. Even more so, as bromobenzene metabolism yields bromophenols, which, as other phenolic compounds, could show an uncoupling effect due to the presence of an acid-dissociable group within their molecule.

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.

Bromobenzene (C. Erba, Milano, 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 16 hr before the intoxication.

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

Hepatic GSH was measured as acid-soluble -SH groups according to Sedlak *et al.* [20].

Lipid peroxidation was measured in whole liver as tissue content of malonic dialdehyde (MDA), as previously reported [21].

Liver mitochondria were prepared in 0.25 M sucrose according to a standard procedure [22].

The respiratory states studied were those originally defined by Chance and Williams [23].

The transmembrane potential ($\Delta\Psi$) was measured at 25° in a final volume of 1.5 mL by monitoring (with a tetraphenylphosphonium (TPP^+)-selective electrode) the movements of tetraphenylphosphonium across the mitochondrial membrane [24]. An inner mitochondrial volume of 1.1 μL/mg protein was assumed. The metabolic medium for assaying electrochemical parameters had the following composition: 100 mM NaCl; 10 mM Tris–HCl (pH 7.4); 10 mM Na,K-phosphate (pH 7.4); 10 mM $MgCl_2$; 2 μM rotenone and 20 μM TPP^+. Mitochondria (3 mg protein/mL) were incubated at 25° and then energized with 2.5 mM Na-succinate.

The determination of *p*- and *o*-bromophenols in liver tissue was performed as follows: 2 mL of a 20% liver homogenate, prepared in ice-cold 0.154 M KCl containing 3 mM EDTA (pH 7.4), were extracted three times with 6 mL of ethyl ether. The ether phases were collected and dried under a nitrogen stream. The residue was dissolved in 0.4 mL of methanol and *p*- and *o*-bromophenols were subsequently measured by HPLC according to Monks *et al.* [25].

Protein determination was performed according to Lowry *et al.* [26].

RESULTS

The time-course of hepatic GSH depletion, liver necrosis (as evaluated by SGPT), lipid peroxidation (as determined by the MDA content of the liver) following bromobenzene intoxication are reported in Table 1. It appears that, in agreement with previous reports [7, 8], and in spite of a massive decrease of

Table 1. Time course of hepatic glutathione (GSH) depletion, liver necrosis (SGPT), lipid peroxidation (hepatic content of malonicdialdehyde, MDA) after bromobenzene intoxication

Time after intoxication (bromobenzene)	0 time	3 hr	9 hr	12 hr	15 hr	18 hr
GSH (nmol/mg protein)	25.3 ± 1.8 (15)	3.9 ± 2.0 (6)	2.4 ± 0.2 (6)	2.2 ± 0.2 (10)	2.1 ± 0.3 (10)	1.9 ± 0.5 (12)
SGPT (units/L)	45 ± 5 (15)	45 ± 4 (6)	41 ± 10 (6)	59 ± 13 (10)	2062 ± 1023 (10)	5637 ± 1611 (12)
MDA (pmol/mg protein)	— —	0 (6)	0 (6)	2 ± 1 (10)	324 ± 109 (10)	1337 ± 299 (12)

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

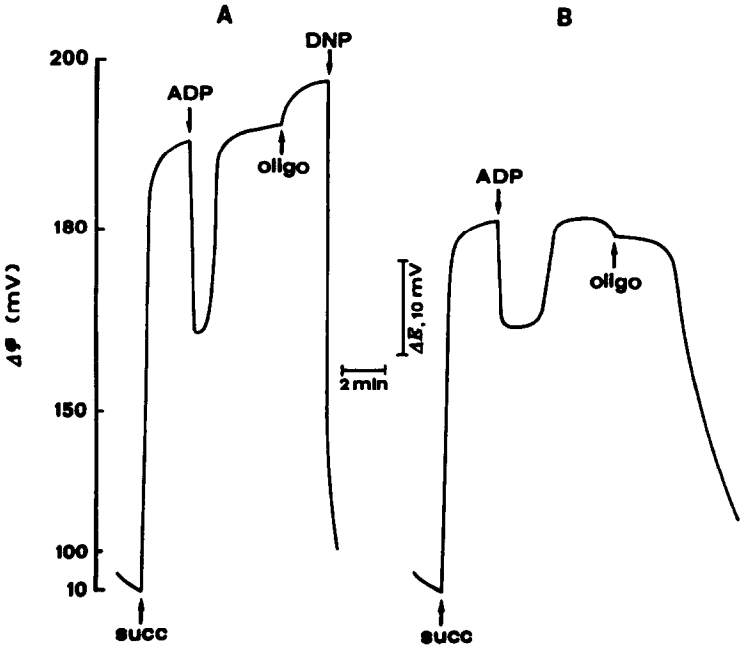

Fig. 1. Effect of mice intoxication with bromobenzene on the transmembrane potential of liver mitochondria. Mitochondria (3 mg/mL) from either control (A) or 9 hr bromobenzene-treated mice (B) were incubated in standard medium described in Materials and Methods. Arrows indicate the following addition: 2.5 mM K-succinate (succ); 0.33 mM ADP; 1 μg/mg oligomycin (oligo); 25 μM 2,4-dinitrophenol (DNP). The transmembrane potential ($\Delta\Psi$) was measured as described in the presence of 20 μM tetraphenylphosphonium chloride. ΔE, electrical potential. The traces presented are representative of at least three different experiments performed on a pool of 15 mice.

the hepatic GSH level in the early phase of intoxication, both lipid peroxidation and liver necrosis are evident only 12 hr after treatment.

The functional efficiency of liver mitochondria was tested during this lag phase. Figure 1 shows the membrane potential of mitochondria from control and bromobenzene intoxicated mice at 9 hr (by changing the metabolic state). As stated above (Table 1), no liver necrosis nor lipid peroxidation was detected in the animals killed at this point. Control mitochondria (Fig. 1A), immediately develop a normal membrane potential, upon addition of substrate, of about 190 mV (negative inside). Addition of ADP, which induces transition to state 3, causes an immediate fall to 167 mV. When the state 3 respiration cycle is completed, the membrane potential returns nearly to its initial value. Addition of oligomycin, a well-known inhibitor of respiration (tightly coupled to phosphorylation) further enhances $\Delta\Psi$ to about 196 mV, due to the fact that also in this respiratory state (i.e. state 4) part of the respiratory energy is utilized for the phosphorylation process [22, 27]. Upon addition of an uncoupler, such as DNP, $\Delta\Psi$ collapses immediately. Mitochondria from bromobenzene treated mice (Fig. 1B) (upon addition of substrate) acquire a membrane potential consistenly lower than that observed in control mitochondria; addition of ADP induces a drop of $\Delta\Psi$, the extent of which is substantially smaller than that of controls. The time length of the membrane potential trace (in the state 3 cycle) is longer than that of the control. As soon as the $\Delta\Psi$ trace has returned to resting conditions, it starts to decrease. The subsequent addition of oligomycin neither induces an increase of $\Delta\Psi$ nor prevents the $\Delta\Psi$ fall, indicating that energy dissipating processes, not coupled to the ATP synthetase complex, are responsible for the drop. It is worth noting that mitochondria from bromobenzene treated animals presented anomalies in $\Delta\Psi$ pattern as early as 3 hr after the treatment (not shown), although the extent of the overall modification was lower than that present at 9 hr.

The effect of albumin, a scavenger of uncoupling agents, on the membrane potential of bromobenzene-treated mitochondria (as compared to control mitochondria) is reported in Fig. 2. It appears that (Fig. 2B) albumin, when added at a concentration of 1 mg/mL, fully restores the energy transducing efficiency of the mitochondrial membrane. Concentrations of albumin lower than 0.4 mg/mL failed to restore the normal membrane potential (not shown).

In order to verify whether phenolic metabolites, endogenously formed by the metabolism of bromobenzene, may account for the uncoupling of the oxidative phosphorylation, the hepatic concentration of *o*- and *p*-bromophenol, well-known bromobenzene metabolites [2], were determined in bromobenzene intoxicated mice. A substantial amount of both bromophenols was observed 3 hr after the intoxication (Fig. 3). The hepatic level of *p*-bromophenol was particularly increased after 9 hr of treatment.

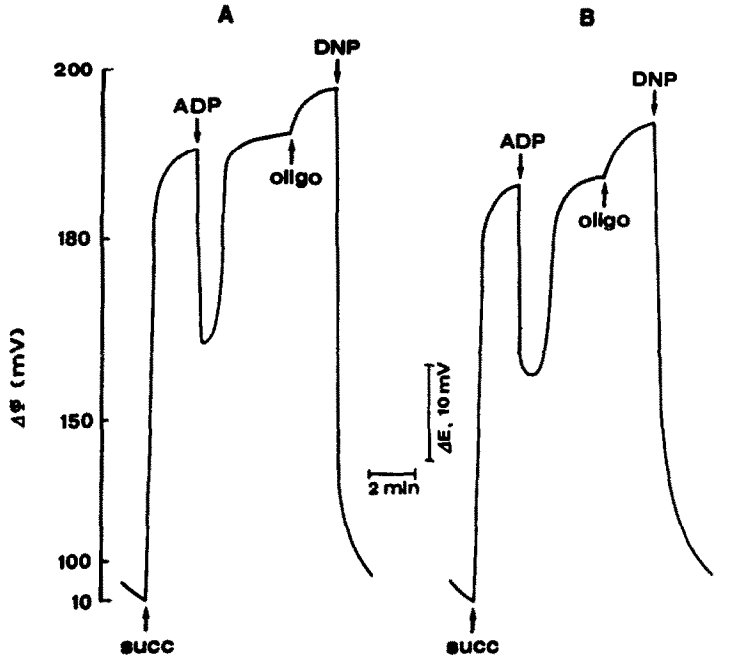

Fig. 2. Effect of albumin on the transmembrane potential on liver mitochondria from bromobenzene-treated mice. Mitochondria from either control (A) or 9 hr treated mice (B) were incubated in standard medium in the presence of 0.1% bovine serum albumin fatty acid free. Experimental conditions as in Fig. 1.

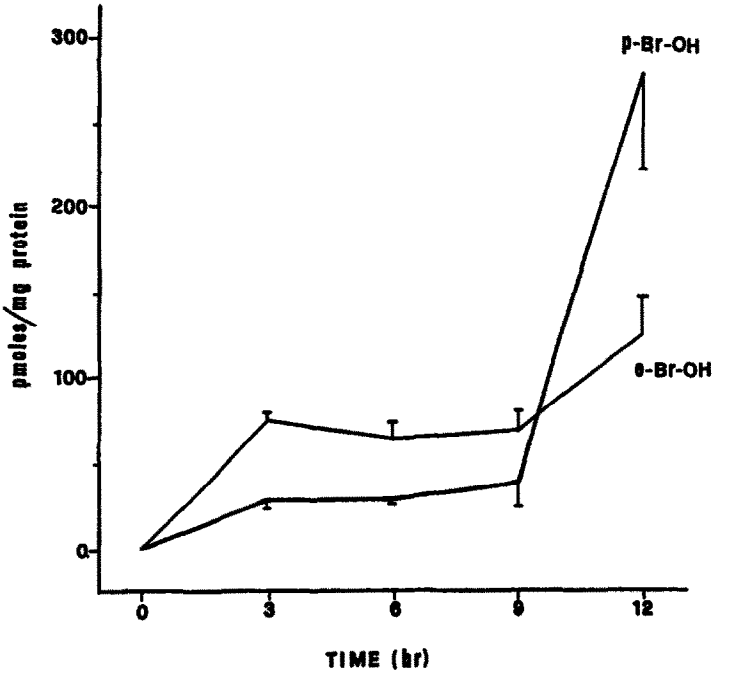

Fig. 3. Hepatic concentration of *o*-bromophenol and *p*-bromophenol at different times following bromobenzene intoxication. The concentration of *p*-bromophenol (p-Br-OH) and *o*-bromophenol (*o*-Br-OH) was measured by HPLC as described in Materials and Methods. Each point represents the mean ± SE of six to eight animals.

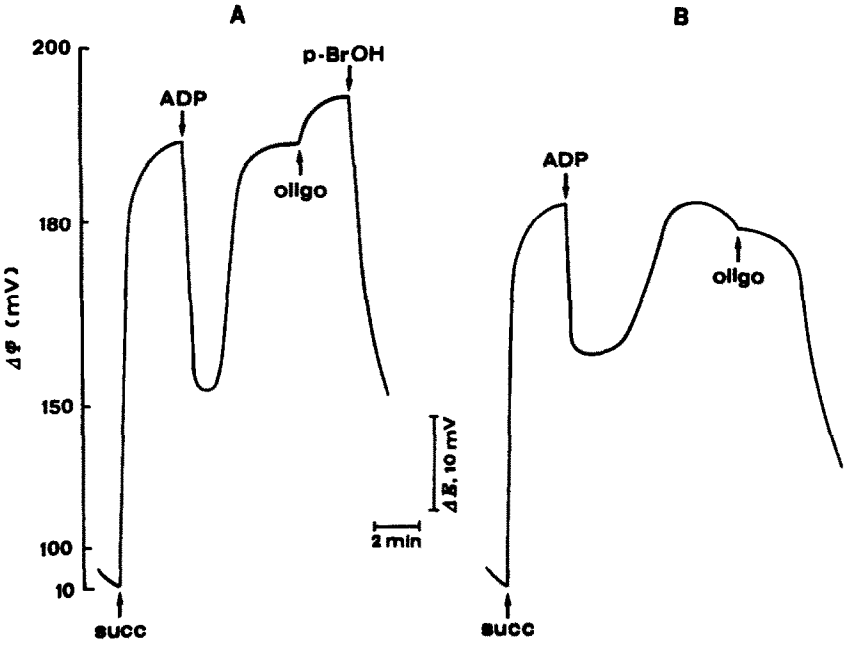

Fig. 4. *In vitro* effect of *p*-bromphenol on the transmembrane potential of mouse liver mitochondria. Mitochondria were incubated in standard medium in the absence (A) and in the presence of 5 μM *p*-bromophenol (B). Experimental conditions as in Fig. 1 except that 50 μM *p*-bromophenol (*p*-Br-OH) was added instead of dinitrophenol (A).

Para-bromophenol, when added to mitochondria from normal animals at micromolar concentration (Fig. 4), is able to dissipate the membrane potential. The complete $\Delta\Psi$ drop is reached at a concentration of 50 μM (Fig. 4A). When *p*-bromophenol is added to control mitochondria at a concentration similar to that found *in vivo*, in intoxicated animals, (Fig. 4B) the features of the membrane potential trace (during different metabolic states) is very similar to that exhibited by mitochondria from 9 hr intoxicated animals. Albumin completely reverses the partial uncoupling by external *p*-bromphenol (not shown).

DISCUSSION

The results presented indicate that liver mitochondria, isolated from bromobenzene-treated mice, exhibit alterations in their oxidative metabolism starting in the early phase of intoxication (i.e. 3 hr). Such alterations consist primarily in partial uncoupling of the mitochondrial oxidative phosphorylation during the intoxidation period investigated (i.e. 9 hr), in which neither cell necrosis nor lipid peroxidation are evident. This mitochondrial disfunction seems to be due to the action of two of the major bromobenzene metabolites, *p*-bromophenol and *o*-bromophenol, which exhibit uncoupling activity. However, under these pathological conditions the structural integrity of the mitochondrial inner membrane does not appear to be irreversibly damaged. The traces of membrane potential of mitochondria from bromozene-treated mice, following the addition of either an oxidizable substrate, ADP, or oligomycin, clearly indicate that these mitochondria are in an uncoupled state. The complete restoration of the inner membrane energy transducing capability by BSA supports this conclusion and indicates that no irreversible damage occurs in the mitochondrial membrane at this phase of intoxication: in fact, the inner membrane does not appear to be irreversibly depolarized.

Fatty acids and bromophenolic metabolites of bromobenzene may account for the uncoupling of oxidative phosphorylation reported here. The observation that low concentrations of BSA, able to "remove fatty acids" but not phenolic compounds [28], fail to restore normal oxidative phosphorylation, strongly suggests that bromophenolic metabolites are the detrimental agents. So far, in spite of a great number of studies aimed at investigating a possible direct involvement of these metabolites in the mechanism of bromobenzene hepatotoxicity [29, 30], no attention has been paid to the possibility that the presence of these compounds could *per se* represent a factor of cell damage: indeed, they could act as protonophoric uncouplers, due to the presence of an acid-dissociable group within their molecule. The dissipation of membrane potential by *p*-bromophenol (Fig. 3A) and *o*-bromophenol clearly indicates that these compounds behave as uncoupling agents according to the chemiosmotic theory [31]. Experimental evidence has been presented, that these metabolites are present in the liver tissue since the early phase of intoxication at a nearly constant concentration up to 9 hr. Their concentration then starts to increase, the extent of this increase being much higher for *p*-bromophenol than *o*-bromophenol (see Fig. 3). Para-bromophenol added to

control mitochondria at a concentration similar to the actual one found in the hepatic tissue after 9 hr of intoxication, makes their membrane potential traces very similar to those of mitochondria from intoxicated mice; a finding giving direct support to the above hypothesis.

In the experimental model of hexachlorobenzene-induced porphyria, it was recently shown that the toxic events caused by hexachlorobenzene at the mitochondrial level are due to the endogenously formed pentachlorophenol, which inhibits ATP synthesis by uncoupling of oxidative phosphorylation [32, 33]. Furthermore, in a study on the toxicity of 4-chlorobiphenyl, it was also demonstrated that its major metabolite, 4-chloro-4'-biphenylol, was responsible for the inhibition of mitochondrial ATP synthesis [34].

The results of the present research suggest a primary involvement of mitochondria in the development of cell damage produced by bromobenzene. Indeed, given the central role played by mitochondria in cellular energy metabolism (they supply about 95% of the total ATP required in eukaryotic cells [35], the disturbance in oxidative phosphorylation observed, renders these organelles unable to fulfil the cell energy requirement. As recently reported for mitochondria isolated from 2,4-dinitrophenol-treated rats [36], a sustained uncoupling of oxidative phosphorylation may trigger an overproduction of reactive oxygen species in mitochondria, an event which may lead to lipid peroxidation and possibly membrane damage in this model of liver injury.

An impairment of Ca^{2+} sequestration, previously observed in mitochondria from bromobenzene-treated mice [8], may be explained too, especially as such inhibition was seen in the absence of detectable lipid peroxidation [8]. Uncouplers not only inhibit the mitochondrial energy-dependent Ca^{2+} uptake, but they also cause the release of the intramitochondrial Ca^{2+} by reversal of the electrogenic uniport pathway [37], bringing about a sustained decrease in the mitochondrial Ca^{2+} concentration and a concomitant increase in the cytosolic Ca^{2+} level. The resulting perturbation in the Ca^{2+}-sensitive matrix enzyme activities [38], as well as in the cellular Ca^{2+} homeostasis [39], may thus play an important role in the pathogenesis of cell injury [40].

In conclusion, the mitochondrial uncoupling induced by bromobenzene phenolic metabolites, may act synergistically with other possible mechanisms of damage, such as oxidative stress and lipid peroxidation. Furthermore, since the concentration of these metabolites increases after the times tested here, mitochondrial uncoupling may represent an important mechanism responsible for the liver damage observed at later times of intoxication, even in cases (about 15%) in which lipid peroxidation was not detectable.

Acknowledgements—This research was supported by a grant from the Italian Ministery of Public Education (Progetto "Patologia da Radicali Liberi e degli Equilibri Redox"). Additional funds were derived from the Association for International Cancer Research (Great Britain).

REFERENCES

1. Brodie BB, Reid W, Cho AK, Sipes IG, Krishna G and Gillette JR, Possible mechanism of liver necrosis caused by aromatic organic compounds. *Proc Natl Acad Sci USA* **68**: 160–164, 1971.
2. Reid WD, Christie B, Krishna G, Mitchell JR, Moskowitz J and Brodie BB, Bromobenzene metabolism and hepatic necrosis. *Pharmacology* **6**: 41–55, 1971.
3. Reid WD, Krishna G, Gillette JR and Brodie BB, Biochemical mechanism of hepatic necrosis induced by aromatic hydrocarbons. *Pharmacology* **10**: 193–214, 1973.
4. Zampaglione N, Jollow DJ, Mitchell JR, Stripp B, Hamrick M and Gillette JR, Role of detoxifying enzymes in bromobenzene-induced liver necrosis. *J Pharmacol Exp Ther* **187**: 218–227, 1973.
5. Jollow DJ, Mitchell JR, Zampaglione N and Gillette JR, Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology* **11**: 151–169, 1974.
6. Mitchell JR and Jollow DJ, Metabolic activation of drugs to toxic substances. *Gastroenterology* **68**: 392–410, 1975.
7. Casini AF, Pompella A and Comporti M, Liver glutathione depletion induced by bromobenzene, iodobenzene, and diethylmaleate poisoning and its relation to lipid peroxidation and necrosis. *Am J Path* **118**: 225–237, 1985.
8. Casini AF, Maellaro E, Pompella A, Ferrali M and Comporti M, Lipid peroxidation, protein thiols and calcium homeosthasis in bromobenzene-induced liver damage. *Biochem Pharmacol* **36**: 3689–3695, 1987.
9. Casini A, Giorli M, Hyland MJ, Serroni A, Gilfor D and Farber JL, Mechanisms of cell injury in the killing of cultured hepatocytes to bromobenzene. *J Biol Chem* **257**: 6721–6728, 1982.
10. Gerber JG, MacDonald JS, Harbison RD, Villeneuve J-P, Wood AJJ and Nies AS, Effect of *N*-acetylcysteine on hepatic covalent binding of paracetamol (acetaminaphen). *Lancet* **1**: 657–658, 1977.
11. Labadarios D, Davis M, Portmann B and Williams R, Paracetamol-induced hepatic necrosis in the mouse: relationship between covalent binding, hepatic glutathione depletion and the protective effect of α-mercaptopropionylglycine. *Biochem Pharmacol* **26**: 31–35, 1977.
12. Devalia JL, Ogilvie RC and McLean AEM, Dissociation of cell death from covalent binding of paracetamol by flavones in a hepatocyte system. *Biochem Pharmacol* **31**: 3745–3749, 1982.
13. Wiley RA, Hanzlik RP and Gillesse T, Effect of substituens on *in vitro* metabolism and covalent binding of substituted bromobenzenes. *Toxicol Appl Pharmacol* **49**: 249–255, 1979.
14. Anundi I, Hogberg J and Stead AH, Glutathione depletion in isolated hepatocytes; its relation to lipid peroxidation and cell damage. *Acta Pharmacol Toxicol* **45**: 45–51, 1979.
15. Younes M and Siegels CP, Mechanistic aspects of enhanced lipid peroxidation following glutathione depletion *in vivo*. *Chem Biol Interact* **34**: 257–266, 1981.
16. Masini A, Botti B, Ceccarelli D, Muscatello U and Vannini V, Induction of calcium efflux from isolated rat liver mitochondria by 1,2-dibromoethane. *Biochim Biophys Acta* **852**: 19–24, 1986.
17. Jewell SA, Bellomo G, Thor H, Orrenius S and Smith MT, bleb formation in hepatocytes during drug metab-

olism is caused by disturbances in thiol and calcium ion homeostasis. *Science* **217**: 1257–1259, 1982.
18. Bellomo G and Orrenius S, Altered thiol homeostasis in oxidative hepatocellular injurs. *Hepathology* **5**: 876–882, 1985.
19. Masaki N, Kyle ME, Serroni A and Farber JL, Mitochondrial damage as a mechanism of cell injury in the killing of cultured hepatocytes by *tert*-butyl hydroperoxide. *Arch Biochem Biophys* **270**: 672–680, 1989.
20. Sedlak J and Lindsay RH, Estimation of total, protein-bound, and nonprotein sulphydril groups in tissue with Ellman's reagent. *Anal Biochem* **25**: 192–205, 1968.
21. Casini AF, Ferrali M, Pompella A, Maellaro E and Comporti M, Lipid peroxidation and cellular damage in extrahepatic tissues of bromobenzene intoxicated mice. *Am J Path* **123**: 520–531, 1986.
22. Masini A, Ceccarelli-Stanzani D and Muscatello U, The effect of oligomycin on rat liver mitochondria respiring in state 4. *FEBS Lett* **160**: 137–140, 1984.
23. Chance B and Williams GR, The respiratory chain and oxidative phosphorylation. *Adv Enzymol* **17**: 65–69, 1956.
24. Lotscher HR, Winterhalter KH, Carafoli E and Richter C, The energy-state of mitochondria during the transport of Ca^{2+}. *Eur J Biochem* **110**: 211–216, 1980.
25. Monks TJ, Pohl LR, Gillette JR, Hong W, Highet RJ, Ferretti JA and Hinson JA, Stereoselective formation of bromobenzene glutathione conjugates. *Chem Biol Interact* **41**: 203–216, 1982.
26. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
27. Masini A, Ceccarelli-Stanzani D and Muscatello U, Phosphorylating efficiency of isolated rat liver mitochondria respiring under the conditions of steady-state 4. *Biochim Biophys Acta* **724**: 251–257, 1983.
28. Bakker EP, Van Den Heuvel EJ and Van Dam K, The binding of uncouplers of oxidative phosphorylation to rat-liver mitochondria. *Biochim Biophys Acta* **333**: 12–21, 1974.
29. Lau SS, Monks TJ and Gillette JR, Multiple reactive metabolites derived from bromobenzene. *Drug Metab. Dispos* **12**: 291–296, 1984.
30. Monks TJ, Lau SS, Pohl LR and Gillette JR, The mechanism of formation of *o*-bromophenol from bromobenzene. *Drug Metab Dispos* **12**: 193–198, 1984.
31. Mitchell P, In: *Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation*, pp. 135–156. Glynn Research Ltd, Bodmin, U.K., 1966.
32. Masini A, Ceccarelli-Stanzani D, Trenti T and Ventura E, Transmembrane potential of liver mitochondria from hexachlorobenzene- and iron-treated rats. *Biochim Biophys Acta* **802**: 253–258, 1984.
33. Masini A, Ceccarelli-Stanzani D, Tomasi A, Trenti T and Ventura E, The role of pentachlorophenol in causing mitochondrial derangement in hexachlorobenzene induced experimental porphyria. *Biochem Pharmacol* **34**: 1171–1174, 1985.
34. Nishihara Y, Comparative toxicity of 4-chlorodiphenyl and its metabolite 4-chloro-4′-biphenylol in isolated rat liver mitochondria. *Biochem Pharmacol* **37**: 2315–2326, 1988.
35. Erecinska M and Wilson BF, Regulation of cellular energy metabolism. *J Membr Biol* **70**: 1–14, 1982.
36. Dryer SE, Dryer RL and Autor AP, Enhancement of mitochondrial, cyanide-resistant superoxide dismutase in livers of rats treated with 2,4-dinitrophenol. *J Biol Chem* **255**: 1054–1057, 1980.
37. Drahota Z, Carafoli E, Rossi CS, Gamble RL and Lehninger AL, The steady-state maintenance of accumulated Ca^{2+} in rat liver mitochondria. *J Biol Chem* **240**: 2712–2720, 1965.
38. McCormack JG, Characterization of the effects of Ca^{2+} on the intramitochondrial Ca^{2+}-sensitive enzymes from rat liver and within intact rat liver mitochondria. *Biochem J* **231**: 581–595, 1985.
39. Nicholls DG, Intracellular calcium homeostasis. *Br Med Bull* **42**: 353–358, 1986.
40. Thomas CE and Reed DJ, Current status of calcium in hepatocellular injury. *Hepatology* **10**: 375–384, 1989.