Pergamon

Biochemical Pharmacology, Vol. 47, No. 8, pp. 1395–1400, 1994.

0006-2952/94 $6.00 + 0.00

0006-2952(93)E0093-M

ASSESSMENT OF LIPID PEROXIDATION ASSOCIATED WITH LUNG DAMAGE INDUCED BY OXIDATIVE STRESS

IN VIVO AND *IN VITRO* STUDIES

MARIE D. SCHWEICH, DOMINIQUE LISON and ROBERT LAUWERYS*

Industrial Toxicology and Occupational Medicine Unit, School of Medicine, Catholic University of Louvain, 30.54 Clos Chapelle-aux-champs, 1200 Brussels, Belgium

(*Received* 24 *May* 1993; *accepted* 23 *November* 1993)

Abstract—The lung thiobarbituric acid-reactive substances (TBA-RS) content and the amount of ethane exhaled, two potential markers of the lipid peroxidation process, were measured in rats following intratracheal administration of chemicals stimulating the production of free radicals, i.e. paraquat, phorbol myristate acetate and ferrous ions. Five hours after treatment, autopsy revealed gross pulmonary damage but the lung TBA-RS and the ethane exhalation were not different from control animals. On the contrary, a large increase in ethane production was observed 2 hr after intraperitoneal administration of the hepatotoxic carbon tetrachloride. *In vitro*, incubation of lung and liver homogenates from control rats with ferrous iron led to the development of a lipid peroxidation process in both tissues but the accumulation of TBA-RS and ethane was much lower with homogenates from lung as compared to liver tissue. Those results suggest that the lung may be more resistant than the liver to the initiation and/or propagation of a lipid peroxidation process. The possibility that others markers than ethane and TBA-RS are more appropriate to detect this process in the lung must also be considered.

Key words: lung; liver; lipid peroxidation; *in vivo*; *in vitro*

Excessive free radical production overwhelming the tissue defence mechanisms may induce a lipid peroxidation process causing extensive membrane damages. Several markers have been proposed for detecting the occurrence of lipid peroxidation both *in vitro* and *in vivo*. Among these, the measurement of TBA-RS† is probably the most widely used. Another approach which has received considerable attention is the measurement of the exhaled hydrocarbon gases ethane and pentane that allows the detection of lipid peroxidation *in vivo* and eliminates the artifactual formation of lipid peroxides during the preparation of the samples [1].

The present study was undertaken to assess whether these biological markers may be used to detect the damages resulting from the production of free radicals in the lung tissue. Rats were intratracheally administered paraquat, phorbol myristate acetate (PMA) or ferrous ions. Several studies have underlined the role of free radicals in mediating their toxic effects in the lungs [2–4]. For comparative purpose, lipid peroxidation was induced in the liver of other animals by intraperitoneal administration of carbon tetrachloride (CCl_4) [5–7]. *In vitro* experiments were also carried out to compare ethane and TBA-RS production resulting from iron-induced lipid peroxidation in lung and liver homogenates.

* Corresponding author. Tel. +32.2.764.32.20; FAX +32.2.764.32.28

† Abbreviations: TBA-RS, thiobarbituric acid-reactive substances; PMA, phorbol myristate acetate; CCl_4, carbon tetrachloride; MDA, malondialdehyde; and BHT, butylated hydroxytoluene.

MATERIALS AND METHODS

Treatments. Three-month-old male Sprague–Dawley rats were used, the animals were fasted overnight before the experiment. In order to mainly restrict the toxic effects to the lungs, paraquat, PMA and iron were administered intratracheally. Under diethylether anesthesia a tracheostomy was performed and the substances dissolved in a volume of 0.3 mL were injected into the airways via a fine catheter. After removal of the catheter the incision was sutured with silk surgical thread. Paraquat (ICI, U.K.) dissolved in physiological saline was given at the dose of 9.3 mg per animal. PMA (Sigma Chemical Co., St Louis, MO, U.S.A.) was stored dissolved in DMSO (Merck, Darmstadt, Germany) and diluted in phosphate buffered solution (pH 7.4) just before injection. Each animal received 35 μg PMA. Iron was given as ferric iron (3.5 mg $FeCl_3 \cdot 6H_2O$, Merck, Darmstadt, Germany) mixed with L-ascorbic acid (6.4 mg; Merck); the latter was used as reducing agent for the production of ferrous ions. For each toxicant an equivalent number of control animals was submitted to a tracheostomy and given 0.3 mL of the respective vehicle by the same route. Carbon tetrachloride (Aldrich, Beerse, Belgium) was given intraperitoneally at a dose of 1 mL/kg. The control rats received an equivalent volume of physiological saline solution.

Determination of tissue TBA-RS content. Five hours after intratracheal instillation of the pneumotoxicants and 3 hr after intraperitoneal injection of CCl_4 the rats were anaesthetized with diethylether. The lungs were perfused with an isotonic saline solution via the pulmonary artery, the liver via the left ventricle. The lungs were

excised, the parenchyma separated from the visible bronchi and minced with scissors. A sample of lung tissue (0.5 g) was dried at 120° for 48 hr to determine the wet to dry weight ratio. Samples of lung or liver were homogenized in a 1.15% KCl aqueous solution (w/v: 10%) with a Teflon–glass Potter–Elvehjem homogenizer. The method of Okhawa *et al.* [8] was used for determining the tissue TBA-RS content by fluorometric measurement (excitation: 532 nm; emission: 551 nm). The standard curve was made with tetramethoxypropane (Sigma) which under the assay conditions is hydrolysed to MDA. The detection limit was 0.8 nmol MDA-TBA/mL. The levels of TBA-RS were expressed in nmol/g tissue. In some experiments, BHT (Sigma) was added to the homogenates or to standard samples [final concentration of 1% (v/v)].

Monitoring of ethane production in vivo. For breath collection the rats were placed individually into an all glass chamber (1.63 L). The oxygen partial pressure was maintained constant by replacing the amount consumed. At the time of analysis, air was aspirated from the chamber in a gas chromatograph sampling loop with a 20 mL-syringe. Immediately after, a valve was switched for connecting the sampling loop with the carrier gas stream. Ethane analysis was performed with an Intersmat model 131 gas chromatograph equipped with a PLOT (porous layer open tubular) fused silica column (length: 50 m; i.d.: 0.32 mm; o.d.: 0.45 mm; liquid phase: Al_2O_3/KCl; Chrompack, Antwerp, Belgium) and with a flame ionization detector. Nitrogen (purity 99.999%, Air Liquide, Belgium), used as the carrier gas at a pressure of 3.8 bar was passed through a gas-clean oxygen filter (Chrompack, Antwerp, Belgium) before entering the capillary column. Separation of ethane and methane (which is produced by gut flora) was achieved by placing the column in a water bath at 8°. The temperature of the injector and detector was set at 135°. A standard curve was established by diluting pure ethane (Air Liquide, Belgium) in synthetic air. The calibration curve was linear in the range of the measurements and the detection limit was 0.2 nmol/L. Ethane production was expressed in nmol produced/kg body weight. In experiments with the pneumotoxicants, the measurement of ethane production was limited to 4 hr because after that time interval the treated animals generally developed signs of respiratory distress. Five hours after the treatment the rats were weighed and then anaesthetized by diethylether exposure. The lungs were rapidly removed, blotted on a filter paper, inspected for the presence of macroscopic lesions and weighed. Lung weight to body weight ratio was used as a rough quantitative measurement of the presence of lung damage. In CCl_4 experiments, the amount of ethane exhaled was determined during 2 hr.

In vitro *study*. After diethylether anesthesia, lungs and liver were perfused with isotonic saline as described above, removed, rinsed with cold physiological saline, homogenized (10%; w/v) in Tris buffer (0.05 M, pH 7.5) and diluted in the same buffer (final concentration: 1 mg protein/mL). Proteins were determined by the Coomassie blue staining method (Bio-Rad) with bovine gamma globulin as standard. Homogenates (4 mL) were incubated under synthetic air at 37° in 24 mL-glass flasks sealed with a Teflon septum. Before starting the incubation, synthetic air (free of hydrocarbons, Air Liquide, Belgium) was allowed to stream through the homogenates for 2 min. A solution of $FeSO_4 \cdot 7H_2O$ (Merck) prepared freshly was added to the homogenate to stimulate lipid peroxidation (final concentration: 1.5 mM). The control homogenates received an identical volume of water. A sample (500 μL) of the air phase was removed at different times with a gas-tight syringe and directly injected into the gas chromatograph. The conditions used for ethane analysis were as described for the *in vivo* study with a detection limit of 1.5 pmol/mL of gas phase. The results were corrected for previous withdrawals and expressed in pmol ethane produced/mg protein. Experiments were also performed in which the amount of TBA-RS accumulating in the homogenates was measured by removing 0.2 mL of homogenate with the aid of a needle introduced through the septum. The TBA-RS levels were expressed in nmol/mg protein.

Statistical analysis. The results were expressed as means ± SE. If an F test indicated homogeneous variances, a Student's *t*-test was applied; if not, a modified Student's *t*-test was used. For multiple comparisons, Tukey's test was used after ANOVA. If Bartlett's test indicated that the variances were not equal the analysis was performed on the ranks. A probability of $P < 0.05$ was adopted as the criterion of significance.

RESULTS

In vivo *studies*

Preliminary experiments were carried out to identify the doses of the three selected pneumotoxicants which caused evident lung damage but did not lead to fatal outcome. Within 5 hr following intratracheal instillation of paraquat (9.3 mg), PMA (35 μg) or a mixture of ferric ions (3.5 mg $FeCl_3 \cdot 6H_2O$)/ascorbate (6.4 mg), the animals displayed clinical signs of respiratory distress manifested by an increased breathing rate and gasping. Autopsy revealed gross pulmonary damage; the lesions appeared as dark, haemorrhagic areas scattered throughout the lobes. Hyperinflation of the lung was also evident. In agreement with these macroscopic observations the mean lung weight/body weight ratios in the three treated groups were significantly higher than in control animals (Table 1). Five hours after intratracheal administration of paraquat or PMA no increase of TBA-RS was found in the lung homogenates; a large increase of TBA-RS, however, was observed in lung homogenates from Fe^{3+}/ascorbate treated animals (Fig. 1). Similar results were obtained when TBA-RS content was expressed per gram of dry tissue (data not shown). The addition of the antioxidant BHT to lung homogenates from Fe^{3+}/ascorbate treated rats prevented the increase in TBA-RS. No significant difference in the TBA-RS values was found between liver homogenates of control and CCl_4 treated animals (Fig. 1). The addition of BHT to liver homogenates reduced considerably the amount of

Table 1. Lung weight/body weight ratio 5 hr after intratracheal administration of paraquat (Pq), phorbol myristate (PMA), Fe^{3+}/ascorbate (Fe) or their respective vehicles

	Lung weight/body weight (×100)
Pq	0.83 ± 0.081*
Control	0.5 ± 0.028
PMA	0.73 ± 0.031*
Control	0.48 ± 0.005
Fe	1.06 ± 0.064*
Control	0.49 ± 0.021

Values are means ± SE. (N = 4 in each group).
* $P < 0.05$.

TBA-RS detected but again no difference was observed between treated and control animals (Fig. 1). When added to standard MDA samples, BHT does not interfere with the TBA–MDA complex formation. In none of the pneumotoxicant treated animals was an increased ethane exhalation detected during the 4 hr observation period (Fig. 2a). In contrast, a clear increase in ethane was observed following intraperitoneal administration of CCl_4 (Fig. 2b).

In vitro *studies*

After addition of ferrous iron (1.5 mM) to liver homogenates, ethane became detectable after about 90 min. The concentration increased linearly during the next 2.5 hr and then levelled off (Fig. 3). Under similar incubation conditions, much less ethane was released from lung homogenates. A 2 hr incubation period was required to detect ethane and then its production increased only slightly. No ethane could be detected with lung and liver homogenates in the absence of added iron. A similar pattern was noticed for the accumulation of TBA-RS (Fig. 3) which was much lower in lung homogenates than in liver homogenates. Without added iron, TBA-RS was detected neither in lung nor in liver homogenates.

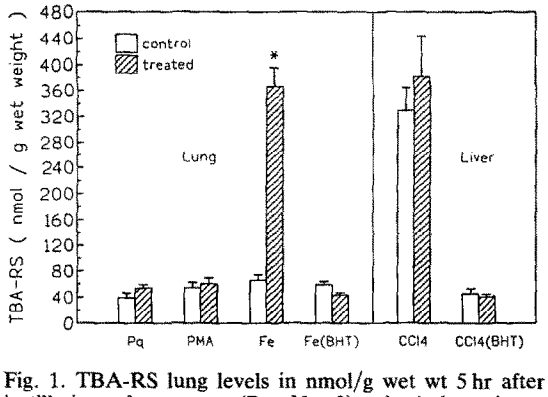

Fig. 1. TBA-RS lung levels in nmol/g wet wt 5 hr after instillation of paraquat (Pq; N = 3), phorbol myristate acetate (PMA; N = 3), Fe^{3+}/ascorbate (Fe; N = 3) or their respective vehicles (control; N = 3) and TBA-RS liver levels in nmol/g wet wt 3 hr following ip injection of CCl_4 (N = 6) or NaCl 0.9% (control; N = 6). (BHT) = Butylated hydroxytoluene added. Values are means ± SE. * $P < 0.05$.

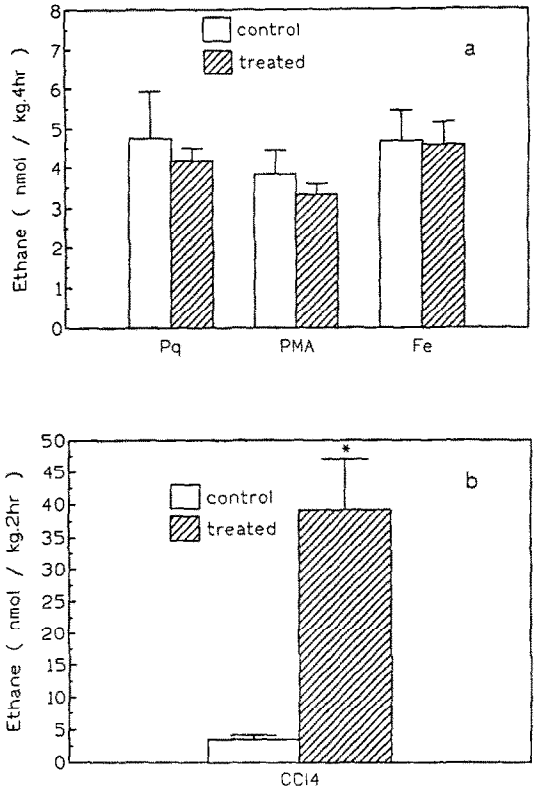

Fig. 2. (a) Ethane production during 4 hr following instillation of paraquat (Pq; N = 4); phorbol myristate acetate (PMA; N = 4); Fe^{3+}/ascorbate (Fe; N = 4) or their respective vehicles (control; N = 4). Values are means ± SE. (b) Ethane production during 2 hr following i.p. injection of CCl_4 (N = 3) or NaCl 0.9% (control; N = 3). Values are means ± SE. * $P < 0.05$.

DISCUSSION

To assess *in vivo* the possible association between free radical production in the lung and development of a lipid peroxidation process we have measured the lung TBA-RS content and the amount of ethane exhaled after intratracheal administration of paraquat, PMA or ferrous ions. The TBA assay has been widely used to detect a lipid peroxidation process. The TBA reactive substances measured with the technique described by Okhawa *et al.* [8], which was used in this study, are likely to be mainly alkenals and alkadienals [9] which under the reaction conditions generate molecules yielding the same chromogen as that produced by the reaction between TBA and MDA [10]. It should be noted that only very small amounts of free MDA are found in oxidized fatty acids [11] and alkenals and alkadienals also arise from the lipid peroxidation process [12].

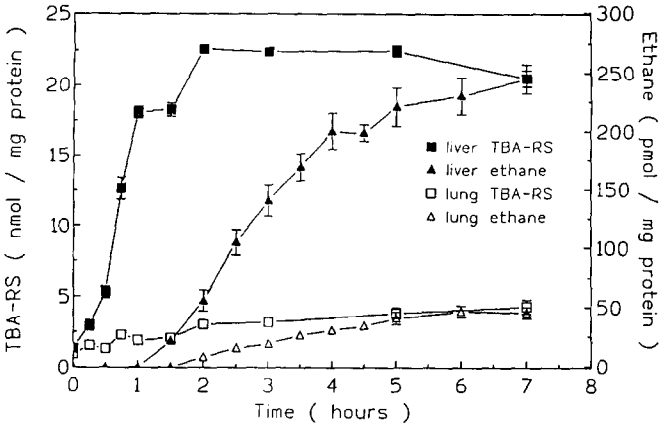

Fig. 3. Time course of ethane released by lung (N = 4) and liver (N = 4) homogenates and of TBA-RS content in lung (N = 3) and liver (N = 3) homogenates incubated in the presence of $FeSO_4$ (1.5 mM final concentration). Values are means ± SE.

However, while clear macroscopic lesions were produced following intratracheal injection of paraquat and PMA, we did not find any increase of TBA-RS content in the lung even if the results were expressed on a dry weight basis to take into account the possible dilution effect of lung oedema in treated animals. The increased concentration of TBA-RS found in lung homogenates from Fe^{3+}/ascorbate treated rats (Fig. 1) likely results from the *in vitro* action of administered iron during the procedure of the test since the addition of BHT, a lipophilic scavenger of free radicals, to the homogenates completely abolished the increase in TBA-RS. The fact that we failed to find any increased TBA-RS concentration in liver homogenate of CCl_4 treated animals is not an argument against the induction of a lipid peroxidative process. A similar observation has also been made by other authors [13, 14]. This is probably due to the fact that following CCl_4 administration lipid peroxidation mainly occurs in circumscribed sites within the cells, which is consistent with activation of CCl_4 by the cytochrome P450 system and, in addition, this activation is restricted to the centrilobular region of the liver. Any localized increase in TBA-RS production may, therefore, be undetected when the liver is assessed as a whole. In contrast, Bacon and Britton [15] have found increased TBA-RS contents in the liver of rats fed a dietary carbonyl iron that was not due to artifactual iron induced lipid peroxidation during the preparation of the samples. This may be due to the fact that contrary to CCl_4 iron overload leads to a widespread lipid peroxidation of the liver. Another factor explaining the poor sensitivity of TBA-RS might be that the high levels of TBA-RS found in liver from control animals may mask the effect of the treatment.

The detection of the hydrocarbon gases ethane and pentane has been proposed as a reliable and sensitive index of lipid peroxidation [16, 17]. They have mainly been used to assess the possible involvement of lipid peroxidation in the pathogenesis of liver damage induced by different xenobiotics [18–22]. In our study, we only focused on ethane detection because pentane is metabolized at a much higher rate than ethane [23]. We have indeed confirmed that following administration of the hepatotoxic CCl_4 a large and rapid increased ethane exhalation occurs (Fig. 2b). In contrast, no increase in exhaled ethane was detected when the animals were intratracheally injected with the three pneumotoxic chemicals. This finding is in line with different reports in the literature. Roberts *et al.* [24] did not find any increase in pentane or ethane content in the expired gases of newborn rats exposed to hyperoxia. Habib *et al.* [25] have reported a 2.5-fold increase in ethane production during 8 hr exposure of rats to 100% oxygen but these authors could not exclude a contribution of liver lipid peroxidation. On the other hand, Dumelin *et al.* [26] have shown that pentane was increased in rats exposed to ozone but only if the animals were fed a vitamin E deficient diet and under these conditions ethane production was decreased. No significant increase in ethane production was observed after lethal intraperitoneal doses of paraquat in rats [27, 28]. It is unlikely that ethane produced in the lung might have been taken up by the animals and further biotransformed since it has previously been demonstrated that when control rats were kept in a metabolic chamber in the presence of a fixed concentration of ethane in the air, the rate of disappearance of the gas was negligible [16, 23]. On the basis of the marker ethane, it can be concluded that the *in vivo* responses of lung and liver tissues to an oxidative stress are different. Several hypotheses may be formulated to explain this difference: (1) ethane may not be an appropriate marker of lipid peroxidation in the lung, (2) the lung may be more resistant to the initiation and/or propagation of a lipid peroxidation process due to better defence mechanisms and/or different structural composition of cellular membranes. Several arguments can be offered in support of both hypotheses.

Although lipid peroxidation is an oxygen-dependent process, oxygen exerts an inhibitory effect

on alkane formation [29, 30]. It has been suggested that oxygen could react with the ethyl radical, the precursor of ethane, more rapidly than hydrogen abstraction could occur leading to the production of oxygen containing products whose structure has not yet been identified. Such a reaction could explain the suppression of ethane production in the presence of a high oxygen tension. Moreover, Smith and Riely [31] have demonstrated the highly effective trapping of the pentyl radical by oxygen. This could explain the increase in ethane exhalation after CCl_4 administration even if peroxidative lesions are limited to the centrilobular region because the lowest hepatic oxygen tension is encountered in this zone. In tissues with higher oxygen tension such as the lung, the reaction of ethyl radical with oxygen may become quantitatively more important and alkane production might be negligible compared to the production of oxygenated products. Additional investigations are needed to determine if oxygen containing compounds such as pentanol or ethanol could serve as *in vivo* indices of lipid peroxidation in well-oxygenated tissues.

This hypothesis, however, can not account for the different susceptibility of lung and liver homogenates to $FeSO_4$ stimulated peroxidation when incubated under identical O_2 partial pressure (Fig. 3). Differences between both tissues in the ability of an oxidative stress to initiate and/or to propagate a lipid peroxidation process might also be involved. Firstly, addition of ferrous ions to homogenates from lung and liver tissues might result in different concentrations of a critical initiator such as hydroxyl radicals [32] or iron–oxygen complexes [33]. More recently, it has been proposed that the extent of lipid peroxidation is dependent on the ratio between ferrous and ferric ions [34, 35]. A different reducing power towards iron ions between both tissues could differently affect this equilibrium and thus the evolution of the lipid peroxidation process. Secondly, homogenates from liver and lung tissues could also contain different amounts of pre-existing lipid peroxides whose breakdown to lipid radicals by iron may be critical for the initiation of the lipid peroxidation process [36]. Thirdly, the possibility that the lung membranes are more resistant to the propagation of the lipid peroxidation than the liver membranes must also be considered. This could be due to different concentrations of scavengers (e.g. vitamin E) and/or differences in polyunsaturated fatty content or composition between lung and liver tissues.

In conclusion, this study suggests that lung is more resistant to lipid peroxidation than the liver. The identification of the factors responsible of this resistance deserves further study. It may also be relevant to examine whether markers other than ethane and TBA-RS (e.g. ethanol, pentanol) may be more appropriate to detect a lipid peroxidation process in the lung.

REFERENCES

1. Hageman JJ, Bast A and Vermeulen NPE, Monitoring of oxidative free radical damage *in vivo*: analytical aspects. *Chem Biol Interact* **82**: 243–293, 1992.
2. Haley TJ, Review of the toxicology of paraquat (1,1′-dimethyl-4,4′-bipyridinium chloride). *Clin Toxicol* **14**: 1–46, 1979.
3. Kuroda M, Murakami K and Ishikawa Y, Role of hydroxyl radicals derived from granulocytes in lung injury induced by phorbol myristate acetate. *Am Rev Resp Dis* **136**: 1435–1444, 1987.
4. Kang JO, Slivka A, Slater G and Cohen G, *In vivo* formation of hydroxyl radicals following intragastric administration of ferrous salt in rats. *J Inorg Biochem* **35**: 55–69, 1989.
5. Kornbrust DJ and Mavis RD, Microsomal lipid peroxidation II: stimulation by CCl_4. *Mol Pharmacol* **17**: 408–414, 1980.
6. Comporti M, Biology of disease: lipid peroxidation and cellular damage in toxic liver injury. *Lab Invest* **53**: 599–623, 1985.
7. Biasi F, Albano E, Chiarpotto E, Corongiu FP, Pronzato MA, Marinari UM, Parola M, Dianzani MU and Poli G, *In vivo* and *in vitro* evidence concerning the role of lipid peroxidation in the mechanism of hepatocyte death due to carbon tetrachloride. *Cell Biochem Funct* **9**: 111–118, 1991.
8. Okhawa H, Ohishi N and Yagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* **95**: 351–358, 1979.
9. Kikugawa K, Kojima T and Kosugi H, Major thiobarbituric acid-reactive substances of liver homogenate are alkadienals. *Free Rad Res Commun* **8**: 107–113, 1990.
10. Bigwood T and Read G, Pseudo-malonaldehyde activity in the thiobarbituric acid test. *Free Rad Res Commun* **6**: 387–392, 1989.
11. Yoden K and Iio T, Determination of thiobarbituric acid-reactive substances in oxidized lipids by high-performance liquid chromatography with a postcolumn reaction system. *Anal Biochem* **182**: 116–120, 1989.
12. Esterbauer H, Cheesman KH, Dianzani MU, Poli G and Slater TF, Separation and characterization of the aldehydic products of lipid peroxidation stimulated by ADP-Fe^{+2} in rat liver microsomes. *Biochem J* **208**: 129–140, 1982.
13. Draper HH, Polensek L, Hadley M and McGirr LG, Urinary malonaldehyde as an indicator of lipid peroxidation in the diet and in the tissues. *Lipids* **19**: 836–843, 1984.
14. Terao J, Asano I and Matsushita S, High-performance liquid chromatographic determination of phospholipid peroxidation products of rat liver after carbon tetrachloride administration. *Arch Biochem Biophys* **235**: 326–333, 1984.
15. Bacon BR and Britton RS, Hepatic mitochondrial malondialdehyde metabolism in rats with chronic iron overload. *Hepatology* **6**: 1209, 1986.
16. Frank H, Hintze T, Bimboes D and Remmer H, Monitoring lipid peroxidation by breath analysis: endogenous hydrocarbons and their metabolic elimination. *Toxicol Appl Pharmacol* **56**: 337–344, 1980.
17. Lawrence GD and Cohen G, Concentrating ethane from breath to monitor lipid peroxidation *in vivo*. *Methods Enzymol* **105**: 305–311, 1984.
18. Riely CA, Cohen G and Lieberman M, Ethane evolution: a new index of lipid peroxidation. *Science* **183**: 208–210, 1974.
19. Hafeman DG and Hoekstra WG, Protection against CCl_4 induced lipid peroxidation in the rat by dietary vitamin E, selenium, methionine as measured by ethane evolution. *J Nutr* **107**: 656–665, 1977.
20. Wendel A, Feuerstein S and Konz K-H, Acute paracetamol intoxication of starved mice leads to lipid peroxidation *in vivo*. *Biochem Pharmacol* **28**: 2051–2055, 1979.
21. Younes M, Albrecht M and Siegers C-P, Inter-

relationship between *in vivo* lipid peroxidation, microsomal sequestration activity and hepatotoxicity in rats treated with carbon tetrachloride, cumene hydroperoxide or thioacetamide. *Res Commun Chem Pathol Pharmacol* **40**: 405–415, 1983.
22. Siegers CP, Steffen B and Younes M, Antidotal effects of deferrioxamine in experimental liver injury—role of lipid peroxidation. *Pharmacol Res Commun* **20**: 337–343, 1988.
23. Daugherty MS, Ludden TM and Burk RF, Metabolism of ethane and pentane to carbon dioxide by the rat. *Drug Metab Dispos* **16**: 666–671, 1988.
24. Roberts RJ, Rendak I and Bucher JR, Lipid peroxidation in the newborn rat: influence of fasting and hyperoxia on ethane and pentane in expired air. *Dev Pharmacol Ther* **6**: 170–178, 1983.
25. Habib MP, Eskelson C and Katz MA, Ethane production rate in rats exposed to high oxygen concentration. *Am Rev Resp Dis* **137**: 341–344, 1988.
26. Dumelin EE, Dillard CJ and Tappel AL, Effect of vitamin E and ozone on pentane and ethane expired by rats. *Arch Environ Health* **33**: 129–135, 1978.
27. Steffen C, Muliawan H and Kappus H, Lack of *in vivo* lipid peroxidation in experimental paraquat poisoning. *Naunyn Schmiedebergs Arch Pharmacol* **310**: 241–243, 1980.
28. Burk RF, Lawrence RA and Lane JM, Liver necrosis and lipid peroxidation in the rat as the result of paraquat and diquat administration. Effect of selenium deficiency. *J Clin Invest* **65**: 1024–1031, 1980.
29. Kostrucha J and Kappus H, Inverse relationship of ethane or n-pentane and malondialdehyde formed during lipid peroxidation in rat liver microsomes with different oxygen concentrations. *Biochim Biophys Acta* **879**: 120–125, 1986.
30. Reiter R and Burk R, Effect of oxygen tension on the generation of alkanes and malondialdehyde by peroxidizing rat liver microsomes. *Biochem Pharmacol* **36**: 925–929, 1987.
31. Smith CV and Reilly MH, Formation of pentane versus 1-pentanol in the ferrous sulfate-initiated decomposition of 15-hydroperoxyeicosatetraenoic acid in hypoxic and hyperoxic conditions. *Biochem Pharmacol* **38**: 1362–1364, 1989.
32. Gutteridge JMC, Richmond R and Halliwell B, Inhibition of the iron catalyzed formation of hydroxyl radicals from superoxide and of lipid peroxidation by deferrioxamine. *Biochem J* **184**: 469–472, 1979.
33. Svingen BA, Buege, JA, O'Neal FO and Aust SD, The mechanism of NADPH-dependent lipid peroxidation. *J Biol Chem* **254**: 5892–5899, 1979.
34. Braughler JM, Duncan LA and Chase RL, The involvement of iron in lipid peroxidation. *J Biol Chem* **261**: 10282–10289, 1986.
35. Minotti G and Aust SD, The role of iron in oxygen radical mediated lipid peroxidation. *Chem Biol Interact* **71**: 1–19, 1989.
36. Janero OR and Burghardt B, Thiobarbituric acid-reactive malondialdehyde formation during superoxide-dependent, iron-catalyzed lipid peroxidation: influence of peroxidative conditions. *Lipids* **24**: 125–131, 1989.