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THE ROLE OF ACROLEIN IN ALLYL ALCOHOL-INDUCED LIPID PEROXIDATION AND LIVER CELL DAMAGE IN MICE

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Abstract—Male NMRI mice were fed a sucrose diet for 48 hr in order to reduce the hepatic glutathione content and to level off its diurnal variation. After administration of allyl alcohol (AA: 1.1 mmol/kg), hepatic glutathione (24.3 ± 7.0 nmol GSH/mg protein) was almost totally lost within the first 15 min (<0.5 nmol GSH/mg protein). Subsequently, a massive lipid perioxidation was observed, i.e. the animals exhaled 414 ± 186 nmol ethane/kg/hr compared to 0.9 ± 0.8 of controls, and the hepatic TBA-reactive compounds had increased from 55 ± 16 pmol/mg protein in controls to 317 ± 163 after 1 hr. Concomitantly, a 40–45% loss of the polyunsaturated fatty acids (arachidonic and docosahexaenoic acid) in the liver lipids was observed. About 80% of the cytosolic alcohol dehydrogenase activity and about 50% of the microsomal P450-content were destroyed.

In vivo-inhibition of alcohol dehydrogenase by pyrazole or induction of aldehyde dehydrogenase by phenobarbital abolished AA-induced liver damage as well as glutathione depletion and lipid peroxidation, while inhibition of aldehyde dehydrogenase by cyanamide made a subtoxic dose of AA (0.60 mmol/kg) highly toxic. These results strongly favour the importance of acrylic acid formation as an additional detoxification pathway. Enhanced hepatic levels of glutathione protected *in vivo* against the damaging effects of AA. Depletion of the liver glutathione content by phorone or diethylmaleate alone caused marginally enhanced lipid peroxidation (phorone) but no liver cell damage. Monooxygenase inhibitors (metyrapone, diethyldithiocarbamate, α-naphthoflavone) or an inducer (benz(*a*)pyrene) did not affect AA-induced toxicity. The ferric iron chelator desferoxamine-methanesulfonate prevented AA-induced lipid peroxidation and liver cell damage *in vivo*. *In vitro*, acrolein alone failed to initiate lipid peroxidation in soy bean phospholipid liposomes or in mouse liver microsomes. Thus, acrolein not only impairs the glutathione defense system but also directly destroys cellular proteins and evokes lipid peroxidation by an indirect iron-depending mechanism.

In a series of papers [1–5], we reported that acute intoxication of mice with certain drugs, such as paracetamol, leads to lipid peroxidation and simultaneously to liver damage. All compounds used were metabolized by the microsomal monooxygenase system, and we discussed reactive oxygen species, derived from the monooxygenase system during the metabolism of these compounds, as possible initiators of lipid peroxidation.

In contrast to this type of compounds, allyl alcohol (AA)* represents an established hepatotoxin [6] which is metabolized via the cytosolic enzyme alcohol dehydrogenase to acrolein [7]. Acrolein is considered as the toxic metabolite [8, 9] which may be responsible for the extensive periportal necrosis observed in the rodent liver after acute allyl alcohol intoxication [6, 9]. Acrolein is of great toxicological importance, not only since AA and acrolein are important synthetic intermediates in chemical industries [10–12] but acrolein occurs also as a toxic metabolite of the widely used anticancer drug cyclophosphamide [13], as a combustion product of petrol, coal, wood and plastics [14] and is a component of tobacco smoke [12]. Acrolein is a powerful electrophile which spontaneously reacts with sulphhydryl groups [15]. Incubation of isolated hepatocytes with acrolein decreased cellular glutathione levels and caused cell death [14, 16]. In this system cytosolic enzymes were released with a similar time course as TBA-reactive material was formed [14]. Therefore, peroxidative damage of lipids might be the cause of the cytotoxicity of acrolein.

The aim of the present study was to investigate the special contribution of glutathione depletion, lipid peroxidation and protein denaturation to allyl alcohol-induced liver cell damage as well as the role of different detoxification pathways *in vivo*. Furthermore, mechanistic aspects of AA-induced lipid peroxidation were addressed.

MATERIALS AND METHODS

Chemicals. Allyl alcohol (AA), pyrazole, phorone, diethylmaleate and α-naphthoflavone were purchased from Fluka AG (Buchs, Switzerland), reduced glutathione was obtained from Boehringer AG (Mannheim, F.R.G.), metyrapone and desferoxaminemethanesulfonate (Desferal®) were obtained from Ciba Geigy AG (Basel, Switzerland) and cyanamide, diethyldithiocarbamate and

* Abbreviations used: AA, allyl alcohol; LPO, lipid peroxidation; TBA, thiobarbituric acid-reactive material; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvate transminase; ADH, alcohol dehydrogenase; AlDH, aldehyde dehydrogenase.

benz(*a*)pyrene were purchased from Sigma Chemie GmbH (Muenchen, F.R.G.).

Animals. Male albino mice (NMRI) with an average body weight of 15 g were fed for at least 4 weeks a low Vitamin E diet, C 1018 (Altromin, Lage, F.R.G.) (0.55 mg vit.E/kg), described in detail elsewhere [2]. Unless otherwise stated the pellet diet was withdrawn from the animals 48 hr prior to the experiment (allyl alcohol administration) to decrease the liver glutathione content and to level off its diurnal variation [17]. In order to avoid complete starvation, the animals received a sucrose solution (10%, w/w) during this period. All experiments were started at 5 p.m. by intraperitoneal application of allyl alcohol (40–100 μl AA/kg corresponding to 0.60–1.5 mmol AA/kg). Various pretreatments of animals were performed as follows: glutathione (0.53 mmol/kg) was injected intravenously 2 hr prior to allyl alcohol; phorone (300 mg/kg) was injected i.p. and the animals were killed 2 hr later; diethylmaleate was injected i.p. in doses of 400 mg/kg 40 min, 20 min and at the start of the ethane measurement, respectively; the mice were killed 1 hr later. The monooxygenase inhibitors metyrapone (150 mg/kg), diethyldithiocarbamate (100 mg/kg) and α-naphthoflavone (100 mg/kg) were injected i.p. 30 min prior to AA; benz(*a*)pyrene was injected i.p. in doses of 20 mg/kg on day 3, 2 and 1 prior to AA; pyrazole (375 mg/kg) or cyanamide (50 mg/kg) were injected i.p. 1 hr prior to AA.

Desferoxaminemethanesulfonate was injected in two daily doses of 0.6 mmol/kg (s.c.) for 3 days. The animals received only sucrose solution in bidestilled water during the pretreatment. The last dose of Desferal® was administered 2 hr prior to AA. Phenobarbital induction was performed by feeding a solution of PB in the drinking water (1 mg/ml) for 5 days. PB was withdrawn 24 hr prior to AA administration.

Methods. Hydrocarbon exhalation of single animals were measured over a period of 1 or 4 hr or until death as described [18]. The animals were killed after 1 or 4 hr. Immediately after they died, blood was taken by heart puncture and the liver was perfused with ice-cold saline, removed and in part homogenized in 3% metaphosphoric acid for determination of total soluble glutathione by the method of Tietze [19]. TBA-reactive material was measured as described [20]. Protein was determined according to Lowry *et al.* [21] using bovine serum albumin as a standard. Another part of the liver was homogenized in 100 mM potassium phosphate buffer (pH 7.4) and the hepatic P-450 content was determined in the homogenate as described by Matsubara *et al.* [22], alcohol dehydrogenase and aldehyde dehydrogenase activities in the postmitochondrial supernatant according to ref. 23. Serum transaminase activities (SGOT, SGPT) were measured in heparinized serum according to ref. 23.

In some experiments a part of the liver was homogenized in saline and extracted with hexane/isopropanol [24] in the presence of heptadecanoic acid methylester as internal standard and 0.05% butylhydroxytoluene as antioxidant. The extract was hydrolyzed in methanolic KOH and esterified in the presence of 14% boron trifluoride Serva AG, Heidelberg, F.R.G.). The methylesters were dried by Na_2SO_4 and 2 μl were injected into a Carlo-Erba 2150 gas chromatograph equipped with an OV 110 capillary column and a flame ionization detector. A temperature program from 70° to 240° was run within 40 min using H_2 as carrier gas. The individual fatty acids were identified with standard mixtures of fatty acids methylesters (Serva AG, Heidelberg, F.R.G.). The total phospholipid content of the livers was determined as described [25].

Liposomes from soy bean lecithin (Nattermann AG; Koeln, F.R.G.) were prepared as described in detail elsewhere [2]. The liposomes (0.75 mg phospholipid/ml) or mouse liver microsomes (1 mg protein/ml) were incubated in potassium phosphate buffer (100 mM; pH 7.4) and in the presence of various concentrations of acrolein (0, 0.3, 1, 3, 10 mM) at 25° and normoxic conditions for 1 hr. The incubations were performed in gas tight syringes as described [4]. After 1 hr, 7 ml of the head space gas were isobarically withdrawn and analyzed by gas chromatography. One milliliter of the suspension was extracted with chloroform/methanol (2:1) and the conjugated dienes were determined as described in detail [26]. Two 500 μl aliquots of each suspension were incubated with thiobarbituric acid at 95° for 15 min and measured at 535 nm. Additionally, two 400 μl aliquots of liposomal suspension were mixed with 400 μl of a GSH solution (30 mM). Since acrolein reacts spontaneously with GSH and forms a 1:1 adduct [27], the amount of disappearing glutathione is equimolar to the amount of acrolein which remained unaltered in the liposomal suspension. Free acrolein, however, reacts also with thiobarbituric acid and gives rise to an additional absorbance at 535 nm [28]. Therefore, the total absorbance was corrected by the absorbance caused by the acrolein-TBA-adduct. The latter absorbance was determined with the free acrolein concentration in the suspension and a standard curve of acrolein with thiobarbituric acid.

Statistics. Results are expressed as mean values ± standard deviation (SD). Data were analyzed by Student's *t*-test. $P < 0.05$ was considered to be significant.

RESULTS

The dose–response relationship of allyl alcohol (AA) intoxication was studied in mice (Table 1). AA administration caused severe liver damage (evaluated by increased SGOT activities) and significant decreases of the liver glutathione content at any dose investigated. Half of the animals died within 2 hr after receiving 0.75 nmole AA/kg, whereas all animals died within 1 hr at higher doses. Furthermore, at any dose investigated significant *in vivo* ethane exhalation and high concentrations of TBA-reactive material in the liver *post mortem* was observed. Both parameters indicate that the metabolism of allyl alcohol in mice led to massive lipid peroxidation in the liver. These conclusions were further corroborated by comparing the hepatic fatty acid composition of AA-intoxicated mice with controls. As shown in Table 2 a significant reduction of the total phospholipid content and significant changes in all fatty acids (except palmitic acid) became evident in severely

Table 1. Dose–response relationship of allyl alcohol-induced *in vivo* lipid peroxidation, liver cell damage and hepatic glutathione depletion in mice

AA (mmol/kg)	Ethane exhalation (nmol/(kg × hr)	TBA (liver) (pmol/mg protein)	SGOT (U/l)	Glutathione (liver) (nmol/mg protein)	N	m	t
0	0.9 ± 0.8	55 ± 16	117 ± 61	24.3 ± 7.0	10	0	—
0.75	85.0 ± 59.7**	191 ± 137**	1650 ± 1590**	2.3 ± 2.2**	10	5	117 ± 53
1.10	414 ± 186**	317 ± 163**	1930 ± 1300**	0.4 ± 0.2**	17	17	56 ± 17
1.50	470 ± 155**	216 ± 93**	1283 ± 739**	0.2 ± 0.1**	10	10	54 ± 28

The animals received different doses of allyl alcohol (AA) intraperitoneally. Ethane exhalation was determined up to 4 hr after AA injection or until death. Serum glutamic oxaloacetic acid transaminase activities (SGOT), hepatic TBA-reactive material and total soluble glutathione content of the liver (GSH-equivalents) were determined post mortem.

N = number of animals used; m = number of animals which died before the end of the experiment (4 hr after AA administration). t = average survival time (min) of the mice in column m. Data are given as means ± SD. ** $P < 0.01$.

damaged livers. The most prominent changes were the 40–45% loss of the polyunsaturated fatty acids arachidonic and docosahexaenoic acid and surprisingly also a 30% loss of the stearic acid. The degree of unsaturation (relative percentage of the fatty acid × number of double bonds) in the hepatic lipids declined from 186.7 ± 12.4% (controls) to 146.9 ± 12.6% ($N = 8$, $P < 0.001$) in AA-intoxicated mice.

In order to elucidate possible cause-and-effect-relationships of AA toxicity, the time dependence of glutathione depletion, LPO and liver damage was studied. As shown in Fig. 1, the rapid loss of hepatic glutathione within the first 15 min after AA administration (1.1 mmol/kg) was accompanied by an immediate increase of hydrocarbon exhalation. As soon as after 30 min, SGOT release was significantly increased. These results indicate that glutathione depletion and LPO are early pathogenic events in AA toxicity, immediately followed by increasing enzyme release from liver indicating severe cell damage.

In the following experiments the oxidation of AA to the respective aldehyde acrolein was blocked *in vivo* by administration of the alcohol dehydrogenase inhibitor pyrazole [29]. Data in Table 3A show that glutathione depletion, LPO as well as liver cell damage was prevented in pyrazole-treated mice. Inhibition of hepatic aldehyde dehydrogenase activities by cyanamide [30] prevents the metabolism of acrolein to acrylic acid. A subtoxic dose of AA (0.60 mmol/kg) became highly toxic in cyanamide-pretreated animals, as indicated by increasing ethane exhalation rates, glutathione depletion and transaminase release as well as a 40% mortality within 1 hr (Table 3B). Since it is known that phenobarbital pretreatment induces aldehyde dehydrogenase activities in mice [31, 32], we used PB to substantiate

Table 2. Phospholipid content and fatty acid composition of livers from control and allyl alcohol-intoxicated mice

		C	AA
Phospholipid content (μg/mg protein)		139 ± 8	117 ± 9*
Fatty acids:			
Palmitic	(16:0)	19.5 ± 1.6	19.8 ± 1.4
Palmitoleic	(16:1)	1.5 ± 0.2	2.6 ± 0.9*
Stearic	(18:0)	17.2 ± 1.3	8.3 ± 1.9*
Oleic	(18:1)	13.8 ± 3.2	20.3 ± 5.5*
Linoleic	(18:2)	21.3 ± 1.6	24.6 ± 3.5*
Arachidonic	(20:4)	14.7 ± 0.9	8.4 ± 2.1*
Docosahexaenoic	(22:6)	11.6 ± 1.6	6.9 ± 2.2*

The animals received allyl alcohol (1.1 mmol AA/kg) or saline (C) intraperitoneally and were killed 1 hr after injection of the compounds. The individual fatty acid is given as per cent of the total identified fatty acids in the liver.

Data are means ± SD of $N = 8$ animals. *$P < 0.05$.

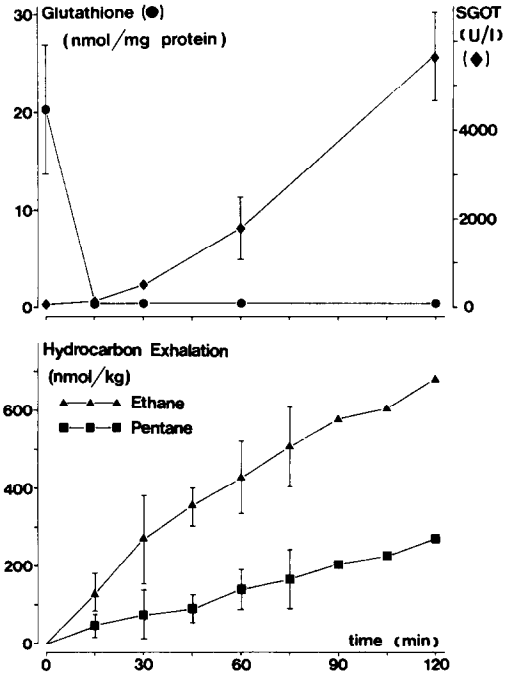

Fig. 1. Time dependence of allyl alcohol-induced toxic effects in mice *in vivo*. The animals received 1.10 mmole AA/kg intraperitoneally and the hydrocarbon exhalation of N = 5–10 animals was monitored. Several animals died within the observation time and only 2 animals survived up to 2 hr. Therefore, the mean values of t = 90–120 min are given without SD. After the times indicated groups of N = 5 animals were killed and hepatic glutathione content (●) and SGOT activities (◆) were determined as described in Materials and Methods. Data are shown as means ±SD. If no SD is shown (glutathine, SGOT), the values are smaller than the symbol.

Table 3. Effects of various enzyme inhibitors, inducers and an iron-chelator on allyl alcohol-induced lipid peroxidation and liver cell damage *in vivo*

	Ethane exhalation (nmol/kg × h)	TBA (liuver) (pmol/mg prot.)	Glutathione (liver) (nmol/mg prot.)	SGOT (U/l)	SGPT (U/l)	N	m
(A) AA (1.1 mmol/kg)	545 ± 125	631 ± 122	0.5 ± 0.2	2098 ± 1387	3210 ± 1765	10	8
AA + Pyrazole	5 ± 2**	39 ± 4**	9.6 ± 2.7**	132 ± 13**	78 ± 15**	5	—
AA + Desferal®	7 ± 1**	82 ± 12**	4.7 ± 2.0**	187 ± 91**	227 ± 70**	6	—
(B) AA + (0.6 mmol/kg)	12 ± 11	68 ± 8	3.4 ± 1.2	175 ± 49	46 ± 14	4	—
AA + Cyanamide	370 ± 74**	491 ± 152**	0.6 ± 0.1**	813 ± 357**	760 ± 530*	8	3
(C) PB + AA (1.1 mmol/kg)	15 ± 4	56 ± 4	8.6 ± 3.9	134 ± 14	109 ± 36	4	—
PB + AA + Cyanamide	416 ± 174**	370 ± 139**	1.3 ± 1.2**	2003 ± 1585**	1489 ± 1083*	4	3
PB + AA + Metyrapone	16 ± 11	123 ± 35**	5.2 ± 4.8	156 ± 96	151 ± 58	7	—

Mice were pretreated with pyrazole (375 mg/kg), cyanamide (50 mg/kg) or metyrapone (150 mg/kg) 1 hr before AA. Phenobarbital was provided in the drinking water (1 mg PB/ml) for 5 days.
Desferoxaminemethanesulfonate (Deferal®) was injected six times (0.6 mmol/kg) in intervals of 12 hr. The last dose was administered 2 hr prior to AA.
The animals received different doses of allyl alcohol (AA) intraperitoneally. The experiments were terminated after 1 hr or with the death of the animals (<1 hr). Parameters were determined as described in legend of Table 1.
Data are given as means ± SD. *$P < 0.05$, **$P < 0.01$.

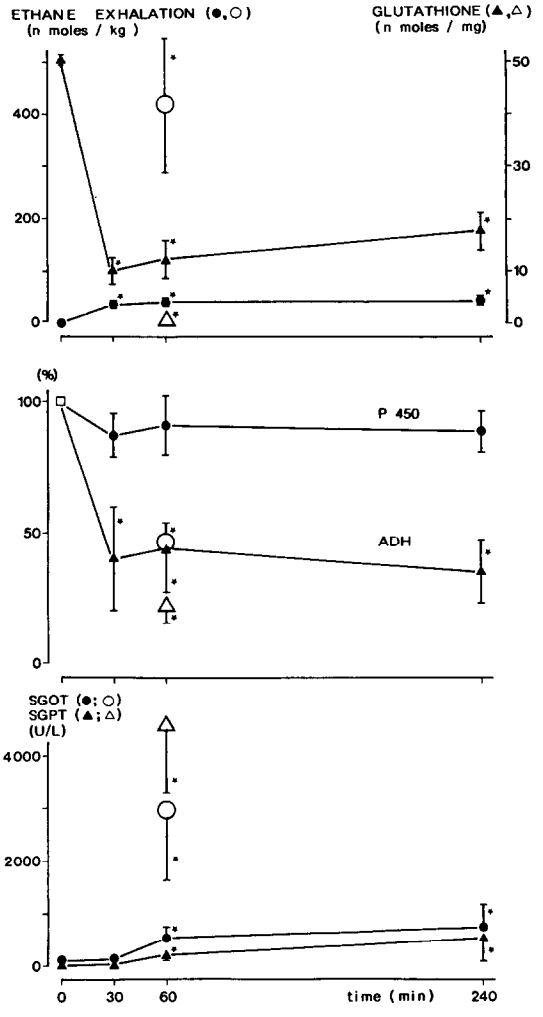

Fig. 2. Time dependence of allyl alcohol-induced toxic effects in glutathione-pretreated mice *in vivo*. Sucrose-fed animals received 0.5 mmol GSH/kg intravenously 2 hr prior to allyl alcohol intoxication (AA: 1.1 mmol/kg). Ethane exhalation from individual animals was measured. Groups of N = 5 mice were killed after various times; serum transaminases (SGOT, SGPT), glutathione concentration and the P-450-content in liver homogenate (100%: 22.7 ± 4.0 nmol/g liver wt.) and hepatic alcohol dehydrogenase (ADH) activities in the postmitochondrial supernatant (100%: 0.86 ± 0.18 U/g liver wt.) were determined as described in Materials and Methods (closed symbols). Additionally, a group of AA-intoxicated animals (N = 5) without glutathione pretreatment were killed after 1 hr (open symbols). Given are the mean values ± SD. *$P < 0.05$ (compared to 0 time values).

further the protective role of AlDH in AA intoxication. PB increased AlDH activities from 1.37 ± 0.42 mU/mg protein (N = 6) in controls to 3.92 ± 1.84 (N = 5; $P < 0.01$) in PB-treated mice. This pretreatment totally inhibited AA-induced toxicity (Table 3C). The protective effects of PB were sensitive to cyanamide (AlDH-inhibitor) but were not affected by the monooxygenase-inhibitor metyrapone.

In order to further elucidate the molecular mechanism of AA toxicity a cytosolic (ADH) and a micro-

Table 4. Effect of hepatic glutathione-depletion on lipid peroxidation and liver cell damage

	Ethane exhalation (nmol/kg × hr)	TBA (liver) (pmol/mg prot.)	Glutathione (liver) (nmol/mg prot.)	SGPT (U/l)
Controls (5)	1 ± 1	55 ± 16	20.4 ± 6.6	84 ± 47
Phorone (5)	11 ± 6**	85 ± 26	0.9 ± 0.2**	69 ± 9
DEM (5)	2 ± 1	54 ± 7	4.2 ± 2.7**	75 ± 29

Phorone was administered as a single dose of 300 mg/kg and diethylmaleate (DEM) was injected as doses of 400 mg/kg three times in intervals of 20 min. The animals were killed 2 hr after phorone and 1 hr after the last dose of DEM. Parameters were determined as described in legend of Table 1. Values are given as means ± SD. **$P < 0.01$.

somal enzyme activity (P-450) was determined 1 hr after AA administration (1.1 mmol/kg). ADH-activities were reduced by 80% in the cytosol of AA-intoxicated mice (Controls: 0.97 ± 0.15 U/g liver wt, N = 8; AA: 0.20 ± 0.07 U/g, N = 8, $P < 0.01$). Hepatic monooxygenase content decreased from 35.2 ± 9.7 nmol/g liver wt. (C, N = 8) to 11.8 ± 2.7 (AA, N = 8; $P < 0.01$). The disappearance of various enzyme activities in the liver was prevented by pretreating the animals with pyrazole.

Since iron ions are thought to play a major role in the initiation and propagation of lipid peroxidation [33], mice were pretreated with desferoxamine-methanesulfonate, a potent chelating agent of Fe^{3+}. As shown in Table 3A, Desferal® protected the animals effectively against AA-intoxication (1.1 mmol/kg), i.e. ethane exhalation, TBA formation and transaminase release were similar to Desferal® treated control animals (without AA). Desferal® also diminished the dramatic decrease of the hepatic glutathione content, but Desferal® did not inhibit ADH activities *in vitro*.

Since the primary metabolite acrolein is likely to deplete the organ glutathione content, enhancement of the organ glutathione level should protect the animals from the deleterious effects of AA treatment. We showed previously that a single intravenous load of glutathione [3, 17, 34] increased the liver glutathione content of sucrose-fed mice from 20 to a maximum of 40–45 nmol GSH/mg protein within 2 hr. Therefore, we applied this regimen of pretreatment also to AA-intoxicated mice. As shown in Fig. 2 the hepatic gluthatione level rapidly declined in the first 30 min after AA administration and slowly increased afterwards. At the nadir of the hepatic glutathione content a slight ethane exhalation was observed, which did not continue after 60 min. At that time the serum transaminase activities started to increase significantly, indicating a moderate liver cell damage. Ethane exhalation rates and serum enzyme levels of animals not pretreated with GSH showed much higher values. Glutathione treatment did not prevent a decline of the ADH activities but totally protected the microsomal P-450-content against acrolein-induced denaturation (Fig. 2).

The question arose whether lipid peroxidation in AA toxicity was caused solely by the total depletion of the hepatic glutathione content. Administration of the glutathione depleting agents DEM [35] or phorone [36] reduced the hepatic glutathione content without affecting the transaminase release of the hepatocytes (Table 4). Phorone, which caused glutathione depletion in the liver comparable to AA, induced moderate lipid peroxidation as indicated by an increased ethane exhalation. However, these animals did not show acute liver lesions either 2 hr (Table 4) or 5 hr (data not shown) after phorone application.

Peroxidative destruction of phospholipids is one pathological mechanism of acrolein-induced toxicity. In order to characterize further the conditions by which acrolein was able to cause lipid peroxidation, lecithin liposomes or mouse liver microsomes were incubated with various concentrations of acrolein (0–10 mM) for 1 hr. Acrolein neither increased the concentration of TBA-reactive material nor increased conjugated dienes in both systems (data not shown). Concomitantly, no ethane or pentane was detected in the head space gas of the incubations. During the incubation period the initial acrolein concentration in the liposomal suspension was maintained, i.e. the reactant was not consumed. However, no free acrolein was detected after incubation with microsomes. Thus, acrolein alone was not able to initiate lipid peroxidation. These results indicate that oxygen radicals were necessary to start the lipid peroxidation process. A potential source of oxygen radicals *in vivo* may be the NADPH-P450-reductase [37] and the P-450-monooxygenase [38].

Epoxide formation of AA and acrolein via P-450-monooxygenase was suggested recently as an alternative metabolic pathway *in vitro* [11]. Therefore, animals were pretreated with different monooxygenase inhibitors or inducers in order to elucidate a potential participation of the P-450-system in the metabolism of AA. However, neither inhibition by metyrapone (150 mg/kg), diethyldithiocarbamate (100 mg/kg) or α-naphthoflavone (100 mg/kg) nor induction by benz(*a*)pyrene (3 × 20 mg/kg) had any significant effect on AA-induced toxicity (LPO, glutathione depletion, transaminase release) *in vivo* (data not shown). As shown earlier, the inhibitor concentrations used were sufficient to protect effectively against acetaminophen-induced toxicity [1].

DISCUSSION

In this study AA-induced hepatotoxicity was studied in male mice. Metabolism is obviously a necessary condition in order to evoke liver damage following administration of AA, accompanied by LPO. Inhibition of the cytosolic enzyme alcohol dehydrogenase

by pyrazole, as well as induction of aldehyde dehydrogenase activities by phenobarbital suppressed lipid peroxidation and liver cell damage and prevented the nearly total loss of the hepatic glutathione content. Inhibition of aldehyde dehydrogenase activities by cyanamide potentiated AA-induced toxicity. These results support previous findings [8, 9, 27] that the reactive metabolite acrolein caused the deleterious effects of AA intoxication. Many toxicological effects of acrolein had been ascribed to its reactions with proteins, which was monitored in the form of covalent binding [9, 39] resulting in a denaturation of various enzymes like microsomal monooxygenase [39–42], P-450-reductase [43] and ADH [42] *in vivo* and *in vitro*. This study confirms these observations and provides an additional mechanism, i.e. lipid peroxidation (LPO). Since it is known that lipid peroxidation also inactivates cytochrom P-450 [44, 45], we cannot decide from our results whether the decline of the monooxygenase activity is due to covalent binding of acrolein or due to the peroxidation of the microsomal membrane.

Liver glutathione content seemed to be a critical parameter in the manifestation of acrolein toxicity. Animals which lost most of their hepatic glutathione content exhibited lipid peroxidation and liver cell damage. The time dependence of these effects (Fig. 1) indicated that the dramatic decrease of the liver glutathione levels represented an early biochemical reponse to acrolein formation and suggest that it is a prerequisite to its toxic effects. However, the total loss of the hepatic glutathione content alone (phorone treatment) did not cause measurable cell damage but gave rise to marginal lipid peroxidation. This kind of LPO has been shown to originate at least *in vitro* from an unproductive cytochrome P-450-complex [46]. It is of particular interest to compare different ethane exhalation rates of mice with their respective glutathione levels in the liver. Diethylmaleate (4.2 nmol GSH/mg protein) and phorone (0.9 nmol/mg) pretreated animals exhaled 2 and 11 nmol ethane/kg/h (Table 4), but allyl alcohol caused ethane exhalation rates of 70 nmol/kg/hr in glutathione-pretreated mice with hepatic glutathione levels of 10 nmol/mg (Fig. 2). This further substantiates that the glutathione depletion alone is not likely to account for AA-induced lipid peroxidation and liver cell damage.

Supplying different precursors of intracellular glutathione protected against acrolein toxicity *in vitro* [16, 27, 40] and *in vivo* [39, this study]. Acrolein reacts spontaneously, rapidly and irreversibly with glutathione [15]. A recent study showed the formation of an acrolein–glutathione adduct (1:1) in isolated hepatocytes [27]. This metabolite was rapidly oxidized via an NAD^+-dependent reaction. It was suggested that the aldehyde moiety of the glutathione adduct was converted to the respective acid by cytosolic aldehyde dehydrogenase activities [27]. These findings were supported by a former study [47], where only *S*-carboxyethylmercapturic acid and the respective methylester had been identified in the urine of acrolein-treated rats. However, Draminski *et al.* [47] concluded from their results, that acrolein was initially oxidized to acrylic acid with the subsequent formation of a methyl ester which was then conjugated with glutathione and metabolized to the respective mercapturic acid. Our study also shows that the direct oxidation of acrolein by aldehyde dehydrogenase (AlDH) activities plays an important role in the detoxification of acrolein. Inhibition of AlDH by cyanamide further decreased the hepatic glutathione level and enhanced lipid peroxidation and liver damage. Furthermore, phenobarbital protected the animals against AA-intoxication. The protective effect of phenobarbital was cancelled by cyanamide, but was insensitive to inhibition of monooxygenase activities. These results suggest that induction of the hepatic AlDH-activities by phenobarbital treatment caused the protection against the reactive metabolite acrolein. Thus, the direct oxidation of acrolein represents an essential detoxification pathway in mouse liver with growing importance when glutathione levels are declining.

This study shows that *in vivo* cytosolically-metabolized allyl alcohol leads to lipid peroxidation. Quantitatively, the ethane exhalation rates observed with AA are about four times as much as with the highest dose of paracetamol in mice [1, 2]. Lipid peroxidation and liver cell damage paralleled in all experiments and the time course of hydrocarbon evolution compared to transaminase release seem to be fast enough to consider LPO as one of the important lesion mechanisms of AA toxicity. It is generally agreed that reactive oxygen species initiate LPO (for review see ref. 48). Redox cycling agents, i.e. compounds which can be reduced by flavin-containing enzymes to semiquinone radicals, are able to transfer their electron to oxygen and form superoxide anion radicals [49] and it was discussed that O_2^- and H_2O_2 are also formed during the microsomal metabolism of paracetamol [1]. However, neither allyl alcohol nor acrolein were metabolized via a P-450-dependent reaction and both compounds did not show the structural features of redox cyclers. The data presented here indicate that *in vivo* acrolein formation *per se* caused LPO. Acrolein may destroy the cellular defense mechanisms against LPO by consumption of glutathione and thus cause shortage of substrate for the glutathione peroxidase reaction. Secondly, possible denaturation of various cytosolic enzymes like glutathione peroxidase, superoxide dismutase or aldehyde dehydrogenase may be involved. Acrolein alone was not able to initiate LPO either in lecithin liposomes or in mouse liver microsomes. Therefore, other compounds must be involved in the initiation of LPO. The total protection of mice against AA-induced LPO and liver cell damage by desferoxaminemethanesulfonate demonstrates the essential participation of iron in this deleterious process. In a recent study, a protective effect of iron depletion by desferrioxamine against CCl_4 intoxication was shown, while this pretreatment did not protect against paracetamol [50]. Desferrioxamine is an effective ferric iron-chelator [51] and inhibits redox cycling of the bound iron [52]. The central role of iron in LPO is well known [33], although it is still a matter of controversy which oxygen species does originally start LPO [48, 53]. However, the intracellular concentration of free iron is extremely low [54]. Further investigations are necessary to clarify

the possible involvement of acrolein in the liberation of bound iron (ferritin, hemo-proteins) within the cell.

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