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DRUG-INDUCED LIPID PEROXIDATION IN MICE—I

MODULATION BY MONOOXYGENASE ACTIVITY, GLUTATHIONE AND SELENIUM STATUS

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Abstract—Feeding male mice for 2 days with sucrose leads to a decrease of total liver glutathione by more than 50 per cent when compared to controls. Such animals were intoxicated with 300 mg/kg paracetamol and upon administration of inducers of the drug-metabolizing system, *in vivo* and *in vitro* lipid peroxidation in these animals was largely increased as well as the susceptibility to the drug. Pretreatment of the mice with methylcholanthrene led to a 28-fold, with benzo(α)pyrene to a 22-fold, and with phenobarbital to a tenfold increase in ethane exhalation. *In vivo* administration of various monooxygenase inhibitors showed that all agents effectively inhibit paracetamol-induced lipid peroxidation. It is concluded that phase I metabolism of paracetamol is a prerequisite for the manifestation of drug-induced lipid peroxidation.

Selenium deficiency in mice neither affected hepatic levels of glutathione nor its decrease following sucrose feeding, nor glutathione transferase, superoxide dismutase, catalase and glutathione reductase activity. Selenium-dependent glutathione peroxidase activity of selenium-deficient mice, reactive with H_2O_2 as well as with *t*-butylhydroperoxide, decreased to 5 per cent of the supplemented controls. A glutathione peroxidase activity, which utilized cumenehydroperoxide as a substrate but insensitive to selenium deficiency, was found. Selenium-deficient diethylmaleate-pretreated animals were much more susceptible to paracetamol-induced lipid peroxidation than controls. Supplemented diethylmaleate-pretreated animals showed no signs of lipid peroxidation if treated with 100 mg/kg aminopyrine or ethylmorphine. However, deficient animals exhibited high ethane exhalation rates, drastically increased serum transaminases, loss of hepatic glutathione and mortality upon administration of these drugs. Qualitatively similar results with lower ethane exhalation rates were observed when 125 mg/kg furosemide was administered to diethylmaleate-pretreated selenium-deficient or -adequate mice. Even administration of 200 mg/kg ethoxycoumarin in combination with diethylmaleate lead to significant lipid peroxidation in phenobarbital-induced mice.

The results demonstrate that *in vivo* selenium-dependent glutathione peroxidase plays a predominant role within the glutathione redox couple system. The enzyme protects the liver from peroxidative damage evoked by phase I metabolism of various drug types, as long as sufficient glutathione is available. It is suggested that activated oxygen released from the microsomal monooxygenase is the species responsible for the observed lipid peroxidation accompanied by severe acute liver lesions.

There is steadily increasing evidence that the complex events summarized under the term lipid peroxidation are involved in basic deteriorative mechanisms e.g. membrane damage, cell lysis, organ necrosis and certain aspects of aging (reviewed in [1–3]). In a previous paper [4] we reported that high doses of the analgesic drug paracetamol lead to LPO *in vivo* in starved mice. According to the established concept, paracetamol hepatotoxicity is due to its metabolism by hepatic microsomal enzymes to a reactive arylating metabolite which covalently binds to essential macromolecules [5–7, review in 8].

Here, we investigated which metabolic parameters modulate LPO induced by paracetamol, and particularly whether other drugs evoke a similar effect. Furthermore, the potential of the predominantly cytosolic and mitochondrial glutathione redox cycle protection system [9] to act against LPO initiated in the membranes of the smooth endoplasmic reticulum has to be estimated *in vivo*. We have tentatively considered the possibility [4] that activated oxygen species released from the microsomal monooxygenase system may have initiated radical chain reactions leading to membrane LPO. To pursue this idea further, we initially investigated whether the monooxygenase turnover parallels the degree of LPO *in vivo*. If this is the case, other drugs metabolized similarly in biotransformation phase I but differently in phase II should also lead to *in vivo* LPO.

Abbreviations used: LPO, lipid peroxidation; Se-GSH-Px, selenium-dependent glutathione peroxidase (E.C.1.11.1.9); Non-Se-GSH-Px, non-selenium-dependent glutathione peroxidase; Se^-/Se^+, selenium deficient/supplemented animals; GSH, reduced glutathione; GSSG, oxidized glutathione.

MATERIALS AND METHODS

Male albino mice of 34 g average wt were maintained for at least 50 days on a selenium-deficient, "low" vitamin E diet supplied by ICN Nutritional Biochemicals (Cleveland, OH). The diet was com-

posed of 30 per cent torula yeast, 61 per cent sucrose, 3 per cent soybean oil, 5 per cent salt mixture, 1 per cent vitamin mix. The control diet was supplemented with 0.5 ppm selenium in the form of Na_2SeO_3. Another group of animals was fed a standard chow diet supplied by Altromin (Lage, West Germany) which had the following composition: 53 per cent starch, 10 per cent sucrose, 22 per cent casein, 3 per cent soybean oil, 6 per cent salt mixture, 2 per cent vitamin mixture. The animals were fed a liquid sucrose diet for 2 days prior to the experiments in order to deplete hepatic glutathione. They consumed an average of 3.5 g sucrose/day/animal.

The following compounds were used for *in vivo* inhibition of the P-450 system: SKF 525 A, a gift from Smith, Kline and French (Bruxelles), was dissolved in 0.9 per cent NaCl and intraperitoneally injected, as were metyrapone (Ciba-Geigy) pyrazole (Sigma), and diethyldithiocarbamate (Sigma). α-Napthoflavone was dissolved in sesame oil before i.p. injection. All compounds were injected with 5 ml solution/kg body wt. Pretreatment with phenobarbital (Merck) was as follows: a single injection of 80 mg/kg was followed by 4 days 0.1 per cent supplementation in the drinking water. This was discontinued 48 hr before the experiment. Benzo(α)pyrene and methylcholanthrene were dissolved in sesame oil. The animals received an injection of 20 mg/kg each day for 3 days, until one day before the experiment. Aminopyrine (Sigma) and paracetamol (Fluka) were dissolved and injected in dimethylsulfoxide (2 ml/kg), ethylmorphine (Merck) and furosemide (Bayer) the same way in isotonic saline.

Breath ethane was determined as described [4] with some modifications: the animals were maintained for 4–6 hr in a closed system of 500 ml volume. CO_2 was absorbed by 120 g of Sodasorb supplied by Rhein-Pharma-Arzneimittelwerk (Planckstadt, West Germany) and O_2 was continuously replaced at constant pressure. After mixing the closed atmosphere with a 50 ml syringe, 10 ml samples of air were withdrawn and transferred to a Carlo-Erba Model 2151 gas chromatograph via a 3 ml gas sampling valve. Ethane was determined by a flame ionization detector using a 5 m Porasil C column (i.d. 4 mm). The following instrumental settings were used: electrometer at 0.1 pA/mV, isothermal mode at 70°, 30 ml/min N_2, 570 ml/min synthetic air, 23 ml/min H_2, calibration by 0.73 ppm ethane in N_2 supplied by Messer Grießheim (Duisburg, West Germany).

After each experiment, the animals were sacrificed. Their livers were perfused until hemaglobin-free, immediately removed and homogenized in 30 g/l metaphosphoric acid solution for determination of malondialdehyde and total glutathione using the procedure described in ref. [4]. Protein was determined according to a modification of Lowry's procedure, using bovine serum albumin as standard. For enzyme determinations, 1 g of mouse liver was homogenized in 5 ml 50 mmol/l morpholinopropane sulfonic acid pH = 7.0. Glutathione peroxidases were determined according to [10] with separate assays using the substrates H_2O_2, *t*-butyl- or cumenehydroperoxide.

Superoxide dismutase was measured as described in ref. [12] and internally calibrated by commercially available superoxide dismutase purchased from Miles. Catalase activity was assayed by determination of H_2O_2 after fixed times using the titanyl sulfate method [13]: 10 μl homogenate (1:100 diluted in buffer, 25 mmol/l morpholinopropane-sulfonic acid buffer with 0.1 per cent Triton X-100, pH = 7.0) was incubated in a total volume of 0.8 ml for 3 min at 25° in the presence of 0.5 mmol/l ethylenediaminetetraacetic acid and 1.5 mmol/l H_2O_2. The reaction was stopped by addition of 0.4 ml titanyl sulfate/H_2SO_4 reagent. The absorption was measured at 410 nm. Enzyme activity is given as the first order rate constant K. Serum vitamin E was determined using the technique described in ref. [14], using a modification provided by Dr. R. F. Burk, San Antonio, Texas.

GSH-*S*-transferase activities were determined as described in ref. [15] with the modification that 5 mmol/l GSH was used when 1-chloro-2,4-dinitrobenzene was the substrate. Alternatively 0.375 mmol/l GSH and 0.3 mmol/l ethacrynic acid were used. Cytochrome P-450 was spectroscopically assayed according to the method of Omura and Sato [16] in isolated microsomes prepared according to [17].

RESULTS

In a previous communication we reported that paracetamol-induced LPO in mice becomes manifest only after decreasing the hepatic glutathione content to about one half by starvation [4]. Since the availability of dietary cysteine has been discussed in affecting the hepatic glutathione concentration [22], we fed the animals a pure sucrose diet for 1 or 2 days instead of starving them. Figure 1 (left panel) shows that under these conditions, the liver glutathione content decreased to below 50 per cent after the first day in a similar way as upon starvation. The experiments described here were therefore performed with animals on a 48 hr sucrose diet.

In measuring the *in vivo* ethane exhalation of sucrose-fed mice, we first assessed the effects of inducers and inhibitors of the drug-metabolizing system using paracetamol as a promoter of LPO. Table 1 illustrates that pretreatment of the animals with phenobarbital increased the amount of *in vivo* LPO tenfold. Benzo(α)pyrene led to a 22-fold, and methylcholanthrene to a 28-fold increase in ethane exhalation. *In vitro* isolated microsomes from these animals exhibited a cytochrome P-450 content which was 2.5–3 fold higher than in untreated controls. Remarkably, the non-pretreated animals, which were given 500 mg/kg paracetamol, showed less LPO and higher mortality than benzo(α)pyrene- or methylcholanthrene-treated mice, which were given a dose of 300 mg/kg.

Table 2 reports the influence of different monooxygenase inhibitors on paracetamol-induced ethane exhalation in induced animals. Four of the five inhibitors depressed the lipid peroxidation by more than 90 per cent whereas perfluorohexane showed a less pronounced effect. Also, α-naphthoflavone, which is believed to be directed to P-448 [18], decreased the ethane exhalation in phenobarbital-induced animals very strongly. If the mice were

Table 1. Enhancement of *in vivo* lipid peroxidation in paracetamol-intoxicated mice after pretreatment with different monooxygenase-inducing agents

Pretreatment (dose)	Dose of paracetamol (mg/kg)	Ethane exhalation (nmol/kg · hr)	N*	M†
None (control)	300	7.5 ± 1.2	5	0
Phenobarbital‡ (80 mg/kg)	300	75 ± 9.3	6	0
Benzo(α)pyrene (3 × 20 mg/kg)	300	168 ± 13.3	4	1
Methylcholanthrene (3 × 20 mg/kg)	300	213 ± 3.5	4	1
None	500	150 ± 19.5	8	6

* N, number of animals.
† M, mortality (number of animals that died within 4 hr).
‡ Single initial i.p. injection followed by 4 days of 0.1 per cent supplementation in the drinking water; withdrawal 48 hr before the beginning of the experiments.

pretreated by benzo(α)pyrene, α-naphthoflavone inhibition was very strong, whereas SKF 525 A was practically without effect. In the same type of experiment using the potent inducer methylcholanthrene (cf. Table 1), higher doses of α-naphthoflavone were needed to observe an inhibitory effect.

So far, we observe that paracetamol metabolism via hydroxylation/conjugation coincides with LPO. Apparently, the substrate turnover of the cytochrome P-450 system in phase I is an obligatory requirement for the manifestation of LPO under conditions where GSH is lowered, e.g. if a conjugation reaction in phase II takes place. An additional pathway consuming GSH is the GSH-Px reaction which reduces e.g. hydroperoxy fatty acids or H_2O_2 to hydroxy fatty acids or water. Only under conditions where the capacity of this reaction is exhausted, LPO should become manifest.

In order to elucidate further the multiple involvement of glutathione-dependent pathways in drug-induced LPO, the following experiments were devised: (a) drugs that are activated by phase I but not conjugated to GSH in phase II were studied, and phase II GSH consumption was simulated by administration of diethylmaleate. This should allow discrimination between the two phases; (b) the contribution of the Se-GSH-Px reaction was estimated by modulating the enzyme activity via a selenium-deficient diet.

In a first series of experiments, 100 mg/kg paracetamol, aminopyrine or ethylmorphine, were administered to sucrose-fed mice not pretreated with diethylmaleate. No significantly higher ethane exhalation or malondialdehyde formation was measured in Se^- or Se^+ animals when compared to untreated controls. In diethylmaleate-pretreated animals, a totally different picture was obtained: data in Table 3 demonstrate that diethylmaleate treatment alone results in a further decrease of liver glutathione in Se^+ mice from 10.5 ± 2.7 nmol/mg protein (N = 3, Fig. 1) to 7.3 ± 1.6 nmol/mg protein (N = 6). This does not take place in Se^- animals: 6.9 ± 0.8 nmol/mg protein, (N = 3, Fig. 1) compared to 6.5 ± 1.7 nmol/mg protein (N = 4, Table 3). None

Table 2. Decrease of *in vivo* lipid peroxidation by monooxygenase inhibitors in pretreated mice intoxicated with 300 mg/kg paracetamol

Inducer	Inhibitor	Inhibitor dose (mg/kg)	Ethane exhalation (nmol/kg · hr)	Per cent of control	N*	M†
Phenobarbital	None	–	75 ± 9.3	(100)	6	0
	Diethyldithiocarbamate	100	4.1 ; 3.4	5	2	0
	SKF-525 A	50	5.3 ± 0.6	7	5	0
	Metyrapone	150	6.5 ; 7.0	9	2	1
	Pyrazole	100	6.75 ; 6.75	9	2	1
	Perfluorohexane	200	47 ; 44.5	61	2	2
	α-Naphthoflavone	100	5.25 ± 0.5	7	7	0
Benzo(α)pyrene	None	–	168 ± 13.3	(100)	4	1
	α-Naphthoflavone	100	5 ± 1	3	4	2
	SKF-525 A	50	199 ; 191	116	2	1
Methylcholanthrene	None	–	213 ± 3.5	(100)	4	1
	α-Naphthoflavone	100	120 ± 35	57	4	2
	α-Naphthoflavone	150	40 ; 40	19	2	2

* N, number of animals.
† M, mortality (number of animals that died within 4 hr).

Table 3. *In vivo* and *in vitro* lipid peroxidation and hepatic glutathione content of selenium-deficient and selenium-supplemented mice pretreated with 400 mg/kg diethylmaleate and intoxicated with paracetamol, aminopyrine, or ethylmorphine

Additional treatment	Ethane exhalation (nmol/kg · hr)				Malondialdehyde (pmol/mg protein)				GSH + 2 GSSG (nmol/mg protein)			
	Se^-	N	Se^+	N	Se^-	N	Se^+	N	Se^-	N	Se^+	N
None*	4.9 ± 4.2	4	1.9 ± 1.4	6	164 ± 22	4	154 ± 20	6	6.5 ± 1.7	4	7.3 ± 1.6	6
100 mg/kg paracetamol	305 ± 93	5	67 ± 41	4	426 ± 160	5	354 ± 81	4	1.2 ± 0.5	5	2.6 ± 2	4
100 mg/kg aminopyrine	301 ± 55	3	4.8 ± 0.7	3	436 ± 25	3	130 ± 28	3	0.4 ± 0.2	3	4.9 ± 0.9	3
100 mg/kg ethylmorphine	285 ± 87	4	1.7 ± 1.3	7	513 ± 44	3	145 ± 30	7	0.4 ± 0.1	3	8.3 ± 1	7

* Solvent only: 2 ml/kg dimethylsulfoxide (paracetamol and aminopyrine were given in DMSO) or 5 ml/kg isotonic saline (ethylmorphine was given in 0.9 per cent NaCl).

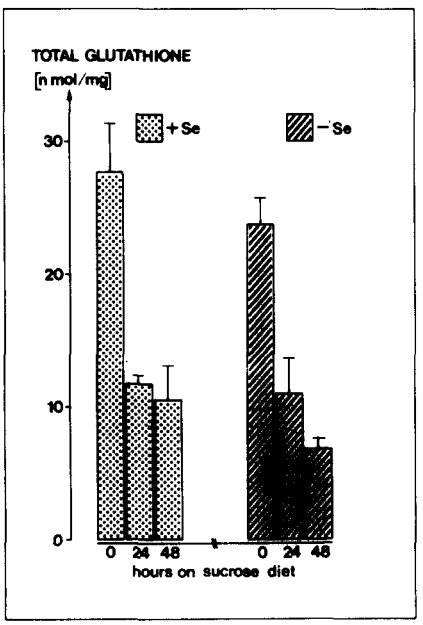

Fig. 1. Glutathione content (GSH + 2 GSSG in nmol/mg protein) of mice livers in selenium deficient (Se^-) and 0.5 ppm selenium-supplemented (Se^+) animals and its decrease after feeding a sucrose-diet for 1 or 2 days.

of these animals treated with diethylmaleate alone exhaled significant amounts of ethane and none of them died within four hours. However, there are striking differences with regard to the susceptibility to drugs between Se^- and Se^+ animals. With all three drugs used, the Se^- animals exhaled large amounts of ethane, in quantitative terms much more than with lethal doses of paracetamol in normal mice or with sublethal doses in induced mice (cf. Table 1). All animals in the Se^- groups treated with any of the three drugs died within 2 hr. The corresponding Se^+ animals in the ethylmorphine and aminopyrine group survived, whereas 2 of the 4 mice of the paracetamol group died after 3.5 hr. Serum transaminases were determined *post mortem* or after 4 hr in the surviving animals. A basal level of 73 ± 33 mU/ml serum (N = 32) for glutamate–pyruvate-transaminase was measured. This was not significantly influenced by starvation, sucrose feeding, or by the selenium status on the animals. The Se^+ animals treated with ethylmorphine or aminopyrine did not exhibit significantly elevated transaminase levels, whereas the transaminases in the similarly treated Se^- groups were very high (SGPT: 4 999 ± 2 172 mU/ml (N = 12); SGOT: 3 083 ± 1 334 mU/ml (N = 12).

No decrease or only a minor decrease in hepatic glutathione was observed in Se^+ animals treated with

Table 4. Effect of phenobarbital pretreatment, glutathione depletion by diethylmaleate (DEM) and selenium status on lipid peroxidation in mice treated with furosemide or ethoxycoumarin

	Dose (mg/kg)	Ethane exhalation (nmol/kg · hr)	N
Selenium-supplemented			
Furosemide	125	1.3 ± 0.6	3
Furosemide + DEM	125 + 400	4 ± 1	4
Chow-fed, phenobarbital-induced			
Furosemide	125	1.7 ± 2.5	2
Furosemide + DEM	125 + 400	234 ± 95	3
Ethoxycoumarin	200	4.0 ± 3.5	2
Ethoxycoumarin + DEM	200 + 400	23 ± 17	3
Selenium-deficient			
Furosemide	125	1.0 ± 0.4	5
Flurosemide + DEM	125 + 400	13.3 ± 4.8	3

ethylmorphine or aminopyrine. Paracetamol administration led glutathione levels to decrease further in these mice, as expected for a drug which consumes GSH in Phase II. In contrast, in Se^- mice which exhibited LPO, a very low liver glutathione content remained. No GSSG was detectable. Malondialdehyde, a frequently used indirect *in vitro* LPO indicator, was higher in the drug-treated Se^- groups, but this did not correlate with the *in vivo* ethane exhalation of the mice. Also, animals treated with diethylmaleate alone exhibited considerable liver malondialdehyde levels and exhaled only very small amounts of ethane.

Table 4 shows some additional experiments with furosemide, a drug which also causes liver necrosis at high doses, and ethoxycoumarin, a common P-450 substrate. In Se^+ animals, furosemide administration alone did not lead to LPO or a significant glutathione decrease. Combination of diethylmaleate and furosemide caused a high LPO in animals pretreated with phenobarbital, as expected for a drug metabolized by the phenobarbital-inducible form or cytochrome P-450 [19]. A qualitatively similar pattern was obtained using ethoxycoumarin. In selenium deficiency, an enhanced LPO was measured following furosemide plus diethylmaleate administration, whereas furosemide alone had the same effect as in Se^+ animals. Regardless of the selenium status, furosemide or ethoxycoumarin treatment without diethylmaleate changed neither the hepatic glutathione nor its redox state (not shown).

Table 5 compiles various enzymatic and other parameters related to the glutathione-dependent hydroperoxide metabolism under different dietary conditions. Apparently, the prominent attribute of selenium deficiency in mice was a large drop in glutathione peroxidase activity, whereas all other parameters are little, if at all, affected. Se-GSH-Px, usually assayed with H_2O_2, exhibits a specific decrease of below 5 per cent in Se^- animals compared to Se-supplemented controls. Surprisingly, the same result was found using *t*-butylhydroperoxide as substrate, which until now was supposed to react with the non-Se-dependent enzyme [37, 38]. Using cumenehydroperoxide as substrate, a 50 per cent higher specific activity was found in Se^+ animals (which dropped in selenium deficiency to a value that corresponds to the surplus over H_2O_2/*t*-butylhydroperoxide reactivity). This means that the surplus was not sensitive to selenium deficiency and that with cumenehydroperoxide the sum of Se- and non-Se-GSH-Px was measured. The results show also that the increased drug sensitivity of Se^- mice is not a vitamin E effect. A comparison between the Se^-/Se^+ diet and the normal diet indicated that nearly all parameters are prone to dietary influences. The proportion of glutathione peroxidases or glutathione transferases within the two selenium-adequate dietary groups are similar, although the absolute values differ considerably.

DISCUSSION

This study shows that drugs which are chemically very different lead *in vivo* to LPO if certain metabolic conditions are fulfilled. The first of these conditions is that the hepatic glutathione level is lowered. In our previous paper using paracetamol [4] we brought about this situation by starving the animals for 2 days. Here we observe that feeding a sucrose diet for the same time leads to the same type of drug sensitivity and we interpret this to be a specific glutathione effect not accompanied by other manyfold consequences of starvation. Enzymological work [21] was well as *in vivo* studies [22, 23] suggest that the availability of sulfur amino acids is one of the major factors regulating the biosynthesis of glutathione. It seems that depletion of this precursor pool for glutathione, which is probably related to one of the

Table 5. Relation of glutathione, glutathione reductase, glutathione peroxidases, glutathione-*S*-transferases, catalase, superoxide dismutase in mouse liver, and serum vitamin E during selenium deficiency

	Se-deficient	N	Se-supplemented	N	Chow-fed	N
GSH + 2 GSSG*	23.8 ± 2	3	28 ± 4	3	61 ± 2	5
GSSG*	0.4 ± 0.2	3	0.6 ± 0.3	3	1.5 ± 0.5	5
glutathione reductase‡	86.5 ± 4	6	68 ± 7	6	64 ± 10	6
Glutathione peroxidases						
H_2O_2 as substrate‡	14 ± 6	10	317 ± 53	8	180 ± 40	16
BuOOH as substrate‡	14 ± 5	10	319 ± 61	8	170 ± 49	16
CuOOH as substrate‡	139 ± 20	10	478 ± 71	8	280 ± 51	16
GSH-*S*-transferase activity§						
1-chloro-2.4-dinitrobenzene§	1.43 ± 0.3	7	1.46 ± 0.37	7	1.84 ± 0.4	17
Ethacrynic acid‡	87 ± 15	9	63 ± 24	9	120 ± 10	17
Serum vitamin E†	2.0	4**	0.7	4**	0.3	6**
Catalase‖	531 ± 180	10	385 ± 81	10	962 ± 79	5
Superoxide dismutase¶	7.0 ± 1.0	8	6.1 ± 1.4	14	6.0 ± 1.3	15

* nmol/mg protein.
† μg/ml serum.
‡ mU/mg.
§ U/mg.
‖ K/mg protein.
¶ μg/mg.
** pooled.

glutathione pools discussed for rat liver [24], renders the organ susceptible to drug-induced LPO. Also, depletion of hepatic glutathione by diethylmaleate exacerbated drug-induced LPO. Although widely used as a glutathione depleting agent, a variety of side effects have been reported when diethylmaleate is given to rats: inhibition of drug metabolism by the agent [25, 26] was expected to decrease the ethane exhalation on our system (cf. Table 2). The opposite holds true. Also, there is no indication that increased heme catabolism [27] or toxic side effects observed in rats at much higher doses [25] might have greatly influenced malondialdehyde formation as well as ethane exhalation. This indicates that in our system the main effect of diethylmaleate was a conjugation to glutathione and excretion of the conjugate, i.e. depletion of intracellular glutathione.

The second factor, which predominantly modulates the paracetamol-induced LPO, is the activity of the drug-metabolizing system. The experiments show that induction of the monooxygenases in general results in a greatly enhanced LPO at doses where paracetamol and other drugs cause neither LPO [4] nor protein binding [5–7] in non-induced animals. Furthermore, the enhancement of LPO was much higher than the increase in enzymatic activity *per se*. This suggests that the observed LPO which is linked to paracetamol metabolism may be mediated by a non-stoichiometric secondary process, probably involving radical species. Assuming that the monooxygenase turnover promotes the manifestation of LPO mediated by phase I of biotransformation if glutathione is simultaneously consumed in phase II, it is expected that other drug substrates metabolized in phase I cause LPO, if phase II is mimicked by a different agent. This is observed in selenium deficiency and leads to the conclusion that it is indeed a phase I-dependent process that initiates LPO.

Here, the question arises as to the chemical nature of the LPO-inducing species. There is unequivocal proof that *in vitro* part of the O_2 consumed by the microsomal drug oxidation is released in the form of H_2O_2 [28, 30]. The same observation was made in reconstituted systems [31]. Again the *in vitro* evidence, which is accumulating, strongly supports the hypothesis that O_2^- released from the oxy-complex of cytochrome P-450 is the hydrogen peroxide precursor that yields H_2O_2 [31–33] by dismutation, either spontaneously or catalyzed by superoxide dismutase. Both agents are reactive enough to attack poly-unsaturated lipid yielding hydroperoxy acids. These compounds react further propagating radical chain reactions and lead ultimately to malondialdehyde (derived from (endo)peroxides) or hydrocarbons: ethane from ω-3 and pentane from ω-6 unsaturated fatty acids [34, 35]. Therefore, we believe that *in vivo* O_2^- release from a highly reduced monooxygenase intermediate causes the observed lipid peroxidation. This view is corroborated by the finding that lipophilic superoxide dismutase-mimicking copper complexes quench *in vivo* paracetamol-induced LPO [36].

The third factor which modulates drug toxicity as well as LPO is the activity of glutathione peroxidases. The data in Table 3 shows that phase II-related parameters and other potential detoxication mechanisms do not respond to selenium deficiency in a way that would allow explanation of the greatly

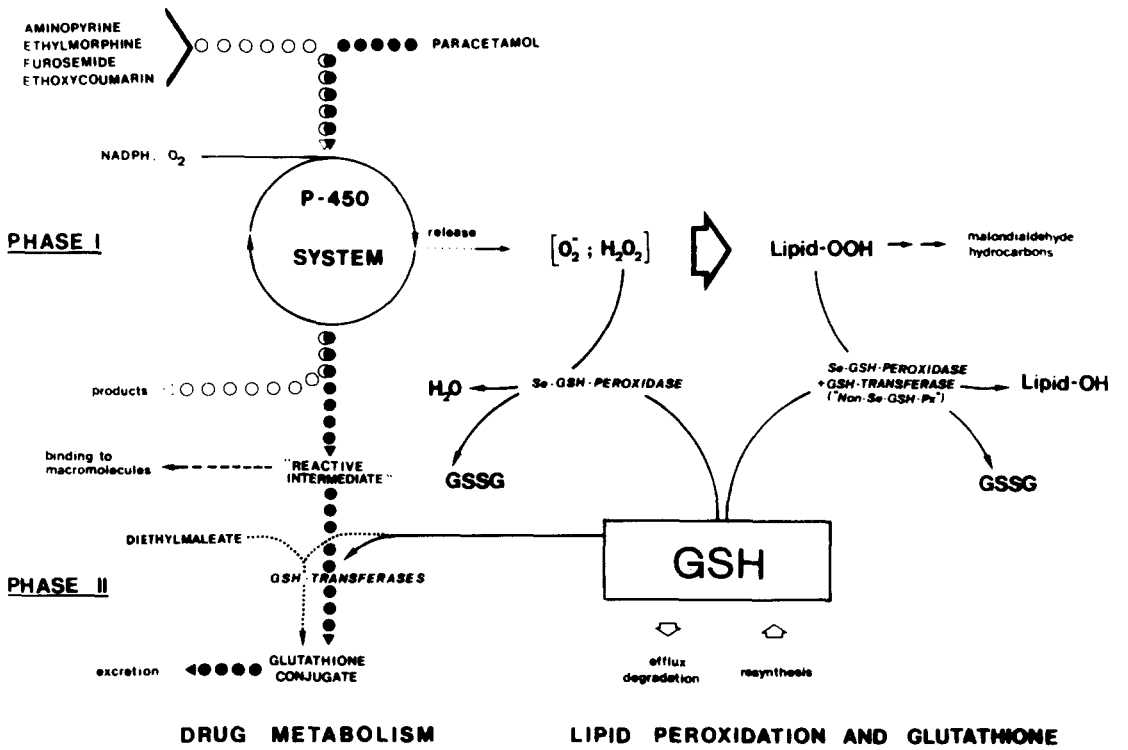

Fig. 2. Possible interrelationships between P-450 mediated drug biotransformation, lipid peroxidation and glutathione metabolism.

enhanced drug sensitivity of Se^- mice. Unlike in other animal species [37], however, a GSH-Px reactive with H_2O_2 as well as with *t*-butylhydroperoxide becomes vanishingly small in the Se^- state and has to be characterized as a Se-dependent GSH-Px. The absolute increase due to cumenehydroperoxide reactivity is practically identical in all dietary groups and represents a non-Se-GSH-Px. In contrast to rats [38], this activity is not increased in Se^- mice. This makes the situation less complex than in rat liver and allows the interpretation that it is Se-GSH-Px, which offers protection against drug-induced LPO.

The scheme in Fig. 2 provides a synopsis of the complex metabolic network related to monooxygenase-dependent LPO. It illustrates that acute drug effects are not manifest unless at least four cellular metabolic events coincide: two types of GSH depletion, a high P-450 turnover and a weakened Se-GSH-Px activity. We do not know at present whether this situation is of significance in organisms which are chronically exposed to drugs or xenobiotics. The view of a central role of the GSH/GSH-Px couple in protection against LPO is corroborated by important *in vitro* studies of others [39–41]; still it remains a challenge to elucidate how a soluble enzyme with a strongly hydrophilic substrate counteracts a process localized within the membranes of the smooth endoplasmic reticulum. It is tempting to assume the idea of a causal relation between LPO and liver necrosis for certain drugs. Two recent studies on this subject report a poor correlation of liver necrosis and LPO in rats upon intoxication with halogenated hydrocarbons, thioacetamide and paracetamol [42], while upon paraquat or diquat administration a coincidence of these two events is observed [43].

There are several observations, which in the case of paracetamol, raise doubts as to the significance of protein binding of metabolites in causing cell injury: *N*-hydroxyacetaminophen seems not to be the primary phase I metabolite of paracetamol [44, 45]. α-Mercaptopropionylglycine offers significant protection of the mouse liver against paracetamol-induced injury, while the extent of protein binding is not affected [46]. Finally, it is not known to which type of macromolecules drug metabolites are bound and whether those target proteins are really essential for the cell: at this stage, the cause-and-effect relation is not established.

Therefore, we believe that the concept of drug toxicity has to be extended to mechanisms other than protein binding. If one of the mechanisms would consist of an oxygen radical-induced LPO, the term reactive metabolite hypothesis seems to be still perfectly appropriate.

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REFERENCES

1. M. Wolman, *Isr. J. Med. Sci.* **11** (Suppl.)1(1975).
2. G. L. Plaa and H. Witschi, *Ann. Rev. Pharmacol. Toxicol.* **16**, 125 (1976).
3. T. F. Slater, Oxygen free radicals and tissue damage, *Ciba Found. Symp.* (new series) **65**, 143 (1979).
4. A. Wendel, S. Feuerstein and K. -H. Konz, *Biochem. Pharmacol.* **28**, 2051 (1979).
5. W. Z. Potter, D. C. Davis, J. R. Mitchell, D. J. Jollow, J. R. Gillette and B. B. Brodie, *J. Pharmacol. exp. Ther.* **187**, 203 (1973).
6. D. J. Jollow, J. R. Mitchell, W. Z. Potter, D. C. Davis, J. R. Gillette and B. B. Brodie, *J. Pharmacol. exp. Ther.* **187**, 203 (1973).
7. J. R. Mitchell, D. J. Jollow, W. Z. Potter, J. R. Gillette and B. B. Brodie, *J. Pharmacol. exp. Ther.* **187**, 211 (1973).
8. J. R. Gillette, in *The Induction of Drug Metabolism* (Eds. R. W. Estabrook and E. Lindenlaub), p. 309. Symp. Med. Hoechst, Schattauer-Verlag, Stuttgart, New York (1979).
9. A. Wendel, in *Enzymatic Basis of Detoxication* (Ed. W. B. Jakoby), Vol. 1, p. 333. Academic Press, New York (1980).
10. W. A. Günzler, H. Kremers and L. Flohé, *Z. Klin. Chem. Klin. Biochem.* **12**, 444 (1974).
11. K. -H. Konz, *J. clin. Chem. clin. Biochem.* **17**, 353 (1979).
12. H. P. Misra and I. Fridovich, *Analyt. Biochem.* **79**, 553 (1977).
13. P. Hübl and R. Bretschneider, *Hoppe-Seyler's Z. Physiol. Chem.* **335**, 146 (1964).
14. J. G. Bieri and E. L. Pival, *Proc. Soc. exp. biol. Med.* **120**, 554 (1965).
15. W. H. Habig, M. J. Papst and W. B. Jakoby, *J. biol. Chem.* **249**, 7130 (1974).
16. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
17. A. Boveris, B. Chance, M. Filimowski, Y. Nakase and K. G. Paul, in *Electrons to Tissues, Frontiers of Biological Energetics* (Eds. A. Scarpa, P. L. Dutton, J. S. High, Jr.), Vol. 2, p. 975. Academic Press, New York (1978).
18. F. J. Wiebel, J. C. Leutz, L. Diamond and H. V. Gelboin, *Arch. Biochem. Biophys.* **144**, 78 (1971).
19. J. R. Mitchell, W. L. Nelson, W. Z. Potter, H. A. Sasame and D. J. Jollow, *J. Pharmacol. exp. Ther.* **199**, 41 (1976).
20. H. U. Bergmeyer (Ed.) in *Methoden der enzymatischen Analyse*, Vol. 1 p. 421. Verlag Chemie, Weinheim (1970).
21. H. Heinle, G. Sawatzki and A. Wendel, *Hoppe-Seyler's Z. Physiol. Chem.* **357**, 1451 (1976).
22. N. Tateishi, T. Higashi, S. Shinya, A. Naruse and Y. Sakamoto, *J. Biochem.* **75**, 93 (1974).
23. D. J. Reed and P. W. Beatty, in *Functions of Glutathione in Liver and Kidney* (Eds. H. Sies and A. Wendel) p. 13. Springer, Heidelberg, New York (1978).
24. T. Higashi, N. Tateishi, A. Naruse and Y. Sakamoto, *J. Biochem.* **82**, 117 (1977).
25. M. E. Andersen, O. E. Thomas, M. L. Gargas, R. A. Jones and L. J. Jenkins, *Toxicol. appl. Pharmacol.* **52**, 422 (1980).
26. M. W. Anders, *Biochem. Pharmacol.* **27**, 1098 (1978).
27. R. F. Burk and M. A. Correia, *Res. Commun. Chem. Pathol. Pharmacol.* **24**, 205 (1979).
28. J. R. Gillette, B. B. Brodie and B. N. La Du, *J. Pharmacol. exp. Ther.* **119**, 532 (1957).
29. A. G. Hildebrandt and I. Roots, *Arch. Biochem. Biophys.* **171**, 385 (1975).
30. G. D. Nordblom and M. J. Coon, *Arch Biochem. Biophys.* **180**, 343 (1977).
31. H. W. Strobel and M. J. Coon, *J. biol. Chem.* **246**, 7826 (1971).
32. C. Richter, A. Azzi, U. Weser and A. Wendel, *J. biol. Chem.* **252**, 5061 (1977).

33. H. Kuthan, H. Tsuji, H. Graf, V. Ullrich, J. Werringloer and R. W. Estabrook, *FEBS Lett.* **91**, 343 (1978).
34. E. E. Dumelin and A. L. Tappel, *Lipids* **12**, 894 (1977).
35. D. H. Donovan and D. B. Menzel, *Experientia* **34**, 775 (1978).
36. A. Wendel and S. Heidinger, *Res. Commun. Chem. Pathol.* **28**, 473 (1980).
37. R. A. Lawrence and R. F. Burk, *J. Nutr.* **108**, 211 (1978).
38. R. A. Lawrence and R. F. Burk, *Biochem. biophys. Res. Commun.* **71**, 952 (1976).
39. H. Sies, G. M. Bartoli, R. F. Burk and C. Waydhas, *Eur. J. Biochem.* **89**, 113 (1978).
40. D. P. Jones, H. Thor, B. Andersson and S. Orrenius, *J. biol. Chem.* **253**, 6031 (1978).
41. P. Moldeus, in *Conjugation Reactions in Drug Biotransformation* (Ed. A. Aito) p. 293. Elsevier Biomedical Press, Amsterdam (1978).
42. R. F. Burk and J. M. Lane, *Toxicol. appl. Pharmacol.* **50**, 467 (1979).
43. R. F. Burk, R. A. Lawrence and J. M. Lane, *J. clin. Invest.* **65**, 1024 (1980).
44. J. A. Hinson, L. R. Pohl and J. R. Gillette, *Life Sci.* **24**, 2133 (1979).
45. S. D. Nelson, A. J. Forte and D. C. Dahlin, *Biochem. Pharmacol.* **29**, 1617 (1980).
46. D. Labadorios, M. Davis and B. Portman, *Biochem. Pharmacol.* **26**, 31 (1977).