IRON-DEPENDENT LOSS OF LIVER CYTOCHROME *P*-450 HAEM *IN VIVO* AND *IN VITRO*

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1. Introduction

It has been reported that the cytochrome *P*-450 content of rat liver microsomes decreases when the microsomes are incubated aerobically in the presence of NADPH [1]. It is now found that the rate of loss of cytochrome *P*-450, which is observed *in vitro* under these conditions, varies according to the nutritional state of the animal and correlates with the formation of lipid peroxides. The loss of the haemoprotein is accompanied by a loss of haem, by a green discolouration of the microsomal pellet and by production of carbon monoxide, indicating that the lost haem undergoes degradation. Evidence is also presented here for the involvement of iron in the loss of cytochrome *P*-450 haem both *in vitro* and *in vivo*.

2. Experimental

Male mice (8–12 weeks old) were kept on Sterolit bedding (Mineral and Chemicals Corporation of America, Menlo Park, New Jersey, USA) for at least 3 weeks before the experiments: they were either of the inbred CFW strain or of the closely related outbred LACA strain. All animals were allowed diet ad lib, or fasted for 24 hr before killing. Liver microsomes were obtained from 10% homogenates in 0.25 M sucrose by spinning the supernatant of a 9000 *g* (20 min) centrifugation at 105,000 *g* for 1 hr. After draining as much cell sap as possible, the microsomes were suspended in either 1.15% KCl or 0.1 M Na phosphate buffer pH 7.4 and used immediately for the incubation experiments, the conditions of which are given in the legends to the tables. Aliquots of the incubation mixtures were taken at the start of the incubation and at various times thereafter for assay of lipid peroxides by the thiobarbituric acid method [2] and of cytochrome *P*-450 [3]. Since carbon monoxide is produced when microsomes are incubated aerobically in the presence of NADPH [4] and the presence of carbon monoxide in the "reference" sample will interfere with the estimation of the cytochrome, a suspension of the same microsomes was kept in an ice-bath under N_2 and without NADPH and used as the "reference" in the estimation of cytochrome *P*-450.

In the *in vivo* experiments male rats of the Porton strain (160–180 g) were injected intraperitoneally with iron-dextran (Imferon; Fisons Limited, Loughborough, Leicestershire, England) at a dose of 100 mg Fe/kg body wt. Control animals were injected with dextran alone (400 mg/kg). All rats were killed 24 hr after treatment, during which time they were kept fasted. The activity of 5-aminolaevulinate synthetase and the concentration of total liver porphryins was measured in liver homogenates as described [5].

3. Results and discussion

Mouse liver microsomes incubated aerobically in the presence of NADPH form lipid peroxides and lose cytochrome *P*-450. The rate of lipid peroxidation as well as the rate of cytochrome *P*-450 loss (both of which showed considerable variations between the individual experiments) are significantly greater in fasted animals (table 1). Fasting also increases the activity

Table 1

The effect of fasting on lipid peroxidation and loss of cytochrome *P*-450 in microsomal suspensions incubated aerobically in the presence of NADPH and on the activity of 5-aminolaevulinate synthetase in total homogenates of livers of mice.

	Nutritional state	
	Fed	Fasted for 24 hr
Rate of lipid peroxidation (E_{535nm} Units/min per mg of microsomal protein)	12.5 ± 4.0 (6)	78.0 ± 19 (6)*
Rate of loss of cytochrome *P*-450 ($\Delta E_{450-490nm}$ Units lost/min per mg of microsomal protein)	0.119 ± 0.029 (5)	0.386 ± 0.048 (5)*
5-Aminolaevulinate synthetase activity of liver homogenates (nmole/min per g wet liver)	1.14 ± 0.17 (11)	3.86 ± 0.59 (8)**

Liver microsomes were obtained from the pooled livers of 8–10 mice and incubated in air at 37° with shaking (100 cycles/min) in 150 ml conical flasks. The incubation mixture contained in a total volume of 30 ml the following components with final conc. in parentheses: microsomes (1.7–2.2 mg of proteins/ml), Na phosphate buffer, pH 7.4 (66.6 mM), KCl (66.6 mM), $MgCl_2$ (4.8 mM), glucose-6-phosphate (4.5 mM) and glucose-6-phosphate dehydrogenase (0.17 U/ml). The reaction was started by adding NADP (0.19 mM). Samples were removed at intervals for the determination of lipid peroxides and of cytochrome *P*-450: initial rates are given above. 5-Aminolaevulinate synthetase was estimated in total homogenates of pooled livers from 3–4 mice. Results given are means ± S.E.M. of the number of observations in parentheses. *$P < 0.01$; **$P < 0.001$, when compared with corresponding "fed" values.

of liver 5-aminolaevulinate synthetase (table 1). A possible relationship between these effects of fasting will be briefly considered below.

Previous work has indicated the involvement of non-haem iron in the NADPH-dependent peroxidation of microsomal lipids, [6, 7]. Table 2 shows that both lipid peroxidation and loss of haemoprotein are increased by the addition of $FeSO_4$ to the incubation mixture, and completely prevented by two iron chelators, EDTA (see also [8]) and desferrioxamine†. It would therefore appear that non-haem iron is also required for the loss of cytochrome *P*-450 observed under these *in vitro* conditions. Table 2 also shows that NAD(H), even though it is without effect when added on its own, significantly increases the effect of NADP(H) on both lipid peroxidation and loss of the haemoprotein, This is reminiscent of the synergism already described between the two pyridine nucleotides in the oxidation of drugs by microsomes *in vitro* [9, 10].

Determination of total microsomal haem as the pyridine haemochrome shows that the loss of haemoprotein is accompanied by a loss of haem; it is also accompanied by a green discolouration of the microsomes and by the production of carbon monoxides [4], indicating that the lost haem undergoes degradation. There are a number of similarities between the iron-dependent degradation of haemoprotein haem described here and the production of bile pigments from exogenous haem by the so-called haem oxygenase of the liver microsomes [11, 12]. Both systems require O_2, NADPH and microsomal activity, and they are both activated by fasting. It remains to be determined whether the green pigments described in this present work are normal degradation products of haem (e.g. biliverdin) or abnormal products like the green pigments produced under the influence of 2-allyl-2-isopropylacetamide [1]. The exact significance of lipid peroxidation in the production of haem degradation also awaits clarification.

The following experiment shows that an iron-dependent loss of cytochrome *P*-450 haem also takes place *in vivo*. In rats the administration of 100 mg/kg of iron (as iron dextran) is followed 24 hr later by a 35% loss of cytochrome *P*-450 haem and by a corre-

† Desferrioxamine (Desferal) was from CIBA Laboratories Ltd. (Horsham, Sussex).

Table 2
Importance of NADPH and non-haem iron for the lipid peroxidation and loss of cytochrome *P*-450 in microsomal suspensions incubated *in vitro*.

Addition (with concentration in parentheses)	Lipid peroxide E_{535nm} Units	Cytochrome *P*-450 $\Delta E_{450-490nm}$ Units
None	0.7	24.7
NAD (0.23 mM)	0.6	24.7
NADP (0.19 mM)	10.6	17.5
NADP (0.19 mM) + NAD (0.23 mM)	14.6	11.0
NADP (0.19 mM) + $FeSO_4$ (0.2 mM)	47.8	7.5
NADP (0.19 mM) + NAD (0.23 mM) + EDTA (1 mM)	0.6	25.8
NADP (0.19 mM) + NAD (0.23 mM) + Desferrioxamine (1 mM)	0.3	24.5

Mouse liver microsomes were incubated *in vitro* under the conditions described in the legend to table 1, except that 3 ml of incubation mixture were incubated in 25 ml conical flasks. A sample was removed at 3.5 min for the determination of lipid peroxide and another at 15 min for estimation of cytochrome *P*-450. Results given are from a typical experiment which was confirmed three times.

Table 3
The effect of a single dose of iron dextran on the cytochrome *P*-450 levels and on the 5-aminolaevulinate synthetase activity of the liver of rats.

Treatment	Cytochrome *P*-450 (nmole/mg of microsomal protein)	5-ALA synthetase (nmole/min/g wet weight)
Control (no treatment)	1.30±0.04 (19)	1.29±0.13 (22)
Dextran (400 mg/kg)	1.25±0.09 (8)	1.25±0.17 (8)
Fe (100 mg/kg) + Dextran (400 mg/kg)	0.85±0.04 (6)*	4.41±0.53 (10)**

Dextran or Fe-dextran were given 24 hr before killing. All rats were fasted for 24 hr. *$P < 0.01$, **$P < 0.001$, when compared with the corresponding value of the Dextran-injected group.

sponding three-fold increase in 5-aminolaevulinate synthetase activity (table 3), without any increase in liver porphyrin concentration. This is similar to the reciprocal behaviour of cytochrome *P*-450 level and 5-aminolaevulinate synthetase activity observed after administration of 2-allyl-2-isopropylacetamide [13], and provides some support for the hypothesis that a decreased concentration and/or an increased turnover of certain pools of haem in the liver may, by reducing the feedback control of haem on 5-aminolaevulinate synthetase, lead to a stimulation of the activity of the enzyme. These effects of iron offer a possible explanation for the potentiation by a large dose of iron of the induction of 5-aminolaevulinate synthetase by drugs [14, 15]. In addition, if the difference observed here *in vitro* in the rate of haem degradation between fed and fasted mice also applied to the intact animals, it might explain, at least in part, both the increased basal activity of 5-aminolaevulinate synthetase (table 1) and the enhanced response of the enzyme to porphyrogenic drugs which are seen in fasted animals.

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