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Effects of repeated intravenous administration of haem arginate upon hepatic metabolism of foreign compounds in rats and dogs

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1 Haem arginate is a new haem compound, recently introduced for the treatment of acute hepatic porphyrias. Porphyrias are characterized biochemically by decreased formation of haem due to defects in certain enzyme activities involved in the haem biosynthesis.

2 Haem is essential for cell respiration and oxidative biotransformation. Hepatic drug metabolism, haem biosynthesis and catabolism were investigated after repeated intravenous administration of haem arginate in connection with toxicity studies.

3 The daily doses of haem for rats were 4, 12 and 40 mg kg^{-1} and for dogs 3 and 9 mg kg^{-1} for 30 days and for 28 days, respectively.

4 Hepatic microsomes were used in the assay of the following drug metabolizing enzymes: cytochrome P-450 and b_5, aminopyrine N-demethylase, ethoxyresorufin *O*-deethylase and UDP-glucuronyl transferase. The assay of NADPH-cytochrome C-reductase and the enzymes reflecting synthesis and metabolism of haem in the liver (δ-aminolaevulinic acid synthase, δ-aminolaevulinic acid dehydratase, uroporphyrinogen I-synthase, uroporphyrinogen decarboxylase, haem synthase, haem oxygenase and biliverdin reductase) were performed from 20,000 *g* supernatants.

5 The lowest dose administered to rats and dogs did not cause any significant changes compared to controls in the parameters measured.

6 The highest doses significantly increased the activities of haem oxygenase and uroporphyrinogen I-synthase but decreased concentrations or activities of other enzymes, e.g. cytochrome P-450 and ethoxyresorufin *O*-deethylase.

7 The results show that it is important to avoid overdosage of haem when restoration of mixed function oxygenase activity is needed.

Introduction

Two haem preparations for treatment of acute porphyric attacks have recently been introduced (haem arginate and lyophilized haematin). (The term haem is used here to indicate an iron-protoporphyrin IX compound irrespective of the oxidation state of iron, while haem arginate means the reaction product of haemin and L-arginine in the solution mixture of propyleneglycol, ethanol and water). The biosynthesis of haem is enzymically regulated, and functional defects of the enzymes involved may be either hereditary or induced by external factors (Goldberg *et al.*, 1984). Haem is the prosthetic group of haemoglobin, myoglobin, cytochromes and many enzymes, and as such is one of the most essential compounds in the organism. In porphyric patients many drugs stimulating the microsomal metabolism of foreign substances, e.g. barbiturates, sulphonamides and oestrogens, may provoke a life-threatening porphyric attack by depleting free haem concentrations, which removes the feedback inhibition of the end product and induces the activity of δ-aminolaevulinic acid synthase (Watson *et al.*, 1977; Moore *et al.*, 1981). Administration of haem to porphyric patients prevents induction of the activity of δ-aminolaevulinic acid synthase, reduces the synthesis of porphyrins and potentially toxic intermediates, and corrects haem

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deficiency (Bonkowsky *et al.*, 1971; Watson *et al.*, 1973; McColl *et al.*, 1981; Pierach, 1982). The prolonged half-life of antipyrine in porphyric patients is a sign of impaired drug metabolism (Anderson *et al.*, 1976). In the phase I metabolism of xenobiotics, quantitatively the most important enzymes are the hepatic microsomal P-450 mixed function oxygenases, which play a key role in determining drug toxicity. Many porphyrogenic chemical compounds cause the destruction of hepatic cytochrome P-450 haem and depletion of haem depots, which again leads to the induction of δ-aminolaevulinic acid synthase (Marks, 1981). Reconstitution of cytochromes and restoration of mixed function oxygenase activity by administration of haem has been suggested as a treatment for the poisonous effects of substrates that destroy cytochrome P-450 by prosthetic haem alkylation (Ortiz de Montellano & Correia, 1983). On the other hand, repeated administration of haem alone considerably retards the drug metabolism in monkeys by decreasing cytochrome P-450 concentrations and by reducing benzo[a]pyrene hydroxylase and ethylmorphine N-demethylase activities in the liver (Muller-Eberhard *et al.*, 1983). In order to shed light on this tangled question and explain the confusing and controversial observations, in the preclinical safety evaluation of haem arginate special heed was paid to parameters reflecting drug metabolism and the enzymes regulating haem biosynthesis and catabolism.

Methods

Rats

Twenty-four male Sprague-Dawley albino rats, aged 4–5 weeks at the start of the study, were used. They were housed in groups of three, in cages with alder-shaving bedding, under artificial lighting (60–200 Lux, 12 h light/dark cycle) at 21–22°C and a relative air humidity of 50–70%. The animals were allowed food and water *ad libitum*. The predosing bodyweight range was 134–168 g. The *treatment* was: (1) control, vehicle dilution (1:1, vehicle: saline), volume injected 0.32 ml 100 g^{-1}; (2) dose 1, 4 mg haem kg^{-1} (1:5, test compound: saline), volume injected 0.096 ml 100 g^{-1}; (3) dose 2, 12 mg haem kg^{-1} (1:1, test compound: saline), volume injected 0.096 ml 100 g^{-1}; (4) dose 3, 40 mg haem kg^{-1} (1:1, test compound: saline), volume injected 0.32 ml 100 g^{-1}. The drugs were administered by intravenous injection into the tail vein daily for 30 days. Each injection was followed by 0.2 ml of sterile saline for rinsing the drug through the vein. After 18 h of fasting and 24 h after the last dose the animals were weighed and killed by exsanguination under ether anaesthesia. The body weight range was 269–362 g. Some minor changes in food and water consumption were observed but the changes were occasional and showed a lack of dose-dependency. Haem arginate treatment had no effect on the weight gain of the rats during the study period. The livers were excised free from connective tissue, rinsed in ice-cold buffer and the liver samples processed in a cold room.

Dogs

Eighteen pure-bred beagle dogs, 3 groups, each of 3 males and 3 females were used. The dogs were housed individually in kennels heated by an underfloor hot-water system. Natural light was supplemented by fluorescent lighting during normal working hours if necessary.

Graded white wood saw dust was used as litter and changed daily. The bodyweight range was 8.8–11.9 kg and age range 22–26 weeks at commencement of dosing. The range of weight increase after 4 weeks of dosing was 0.3–1.4 kg. All dogs gained weight satisfactorily, and all groups similarly during the study period. Administration of haem arginate to dogs had no effect on food consumption or bodyweight gain. The dosage levels were: control (vehicle), 3 mg haem kg^{-1} per day and 9 mg haem kg^{-1} per day. The drugs were given by intravenous infusion at 1.5 ml min^{-1} and test compound at a fixed concentration of 2.5 mg ml^{-1} (1:10, test compound: saline). All animals were killed by exsanguination under pentobarbitone anaesthesia. Liver samples were weighed and placed in a polythene bag with 0.9% sodium chloride solution after routine sectioning. The samples were frozen on dry ice and transferred in a container cooled with dry ice to a freezer (−80°C).

Preparation of the liver samples

The liver parts were excised free from connective tissue, rinsed in ice-cold buffer, blotted dry, weighed, and homogenized in 2 mM HEPES buffer containing 0.25 M sucrose. Hepatic microsomal fractions were obtained from the rats by the method of Guengerich (1982). The calcium-sedimented microsomes from dog livers were obtained by the procedure of Cinti *et al.* (1972). The rat livers were processed in a cold room immediately after decapitation and microsomes were stored as microsomal suspensions (−80°C) until analysed. Dog liver samples were stored at −80°C for six months before separation of the fractions. Frozen liver cubes have been shown to be a good storage form for metabolic studies and most activities were preserved during storage for at least six months (von Bahr *et al.*, 1980; Pelkonen *et al.*, 1986). Microsomal suspensions (rat study) and the suspended microsomal pellets were used for the following assays: protein, cytochrome P-450, cytochrome b_5, aminopyrine N-

demethylase, ethoxyresorufin O-deethylase and UDP-glucuronyl transferase. All the other determinations were done on 20,000 *g* supernatants prepared by the method of Schneider, 1948.

Protein concentrations

These were measured by the modified method of Lowry *et al.* (1951), as described by Hartree, 1972.

Enzyme assays

The composition of cofactor mixture and the conditions of incubation have been given previously (Pelkonen *et al.*, 1973). The following methods were used: cytochrome P-450 and b_5 (Omura & Sato, 1964); NADPH-cytochrome C-reductase (Masters *et al.*, 1967); aminopyrine N-demethylase (Kitchin, 1983); ethoxyresorufin *O*-deethylase (Burke & Mayer, 1974); UDP-glucuronyl transferase (Kitchin, 1983); δ-aminolaevulinic acid synthase activity (Ebert *et al.*, 1971, as modified by Tenhunen *et al.*, 1984); δ-aminolaevulinic acid dehydratase, European Standardized Methods (see Berlin & Schaller, 1974); uroporphyrinogen I-synthase activity (Strand *et al.*, 1972, as modified by Mustajoki, 1976); uroporphyrinogen decarboxylase (Romeo & Levin, 1971); haem synthase activity (Bonkowsky *et al.*, 1975, modified by Tenhunen *et al.*, 1983); haem oxygenase activity (Tenhunen *et al.*, 1969) and biliverdin reductase (Tenhunen *et al.*, 1970).

Drugs

The test compound (Normosang, 25 mg ml^{-1} infusion concentrate, Huhtamäki Oy Pharmaceuticals Medica, Helsinki, Finland) contained haem arginate corresponding to haemin 25 mg ml^{-1}. The vehicle composition was L-arginine 26.7 mg, ethanol 96% 100.0 mg, propyleneglycol for injections 400.0 mg and water for injections to make 1 ml. The ampoules with the test compound were stored at +4°C. The dilutions with 0.9% saline for infusion were made daily immediately before administration. Lyophilized haematin (Panhematin) was obtained from Abbot Laboratories, North Chicago, U.S.A.

Statistics

Student's *t* test for grouped data was used to compare the mean of the control group with that of the treated group.

Results

Microsomal protein in the livers of rats treated with haem arginate did not differ from the corresponding value of the controls (Table 1). The lowest dose, 4 mg haem kg^{-1} daily for one month, did not significantly change any of the metabolic parameters measured. The dose of 12 mg haem kg^{-1} caused a significant decrease in the concentration or activity of the following enzymes: cytochrome P-450, and aminopyrine N-demethylase. The highest dose, 40 mg haem kg^{-1}, caused a significant decrease of many enzymic parameters: cytochrome P-450, cytochrome b_5, aminopyrine N-demethylase, ethoxyresorufin *O*-deethylase, UDP-glucuronyl transferase, and a significant increase of uroporphyrinogen I-synthase and haem oxygenase activities.

In the dogs, 3 mg haem kg^{-1} caused no changes in the measured parameters of foreign substance metabolism and haem synthesis and degradation (Table 2). The highest dose given to dogs, 9 mg haem kg^{-1}, decreased significantly the concentrations of cytochrome P-450 and ethoxyresorufin O-deethylase, while uroporphyrinogen I-synthase, δ-aminolaevulinic acid synthase and haem oxygenase activities increased.

Discussion

Little is known about the effects of prolonged daily administration of haem on the metabolism of foreign substances. In an experimental model of porphyria induced by 2-allyl-2-isopropylacetamide (AIA) the impairment of the oxidative metabolism of foreign substances was not corrected by haem, although the urinary porphyrin excretion was partially reversed; Muller-Eberhard *et al.* (1983) demonstrated that haem impaired the drug metabolism in monkeys and aggravated the AIA-induced decrease in cytochrome P-450. Observations on drug metabolism in rodents are not necessarily applicable to primates; in the study with monkeys haem administration (4 mg kg^{-1} intravenously for 4 days) did not increase the activity of haem oxygenase. This may indicate that the amount of active haem given to the monkeys was possibly quite small due to the extreme instability of conventional haematin solutions at room temperature (Mendenhall, 1984; Bauer & Fornnarino, 1984). In many studies the preparation and storage of haematin solutions are not stated or evaluated in detail. The 'haematin resistance' in certain clinical trials, ineffectiveness in animal models of porphyria and some of the side-effects (coagulation disturbances, thrombophlebitis) may possibly be due to degradation products of haematin (Pierach, 1986; Goetsch & Bissell, 1986). Haem arginate is stable (Tenhunen *et al.*, 1985); its antiporphyrogenic effect even after storage for two years (in stock solutions in ampoules at 6°C) in the 2-allyl-2-isopropylacetamide- induced

Table 1 Effects of intravenous haem arginate on drug and haem metabolism in rats after 30 days of treatment.

Assay	*Control* (vehicle)	*Dose 1* (4 mg haem kg^{-1})	*Dose 2* (12 mg haem kg^{-1})	*Dose 3* (40 mg haem kg^{-1})
Microsomal protein ($mg\ g^{-1}$ liver)	16.62 ± 1.23	18.90 ± 0.65	17.50 ± 0.58	18.00 ± 1.14
Cytochrome P-450 ($nmol\ mg^{-1}$ protein)	0.51 ± 0.04	0.43 ± 0.02	0.35 ± 0.03**	0.21 ± 0.04***
Cytochrome b_5 ($nmol\ mg^{-1}$ protein)	0.25 ± 0.01	0.23 ± 0.01	0.34 ± 0.02	0.16 ± 0.02**
NADPH-cytochrome C-reductase ($u\ mg^{-1}$ protein)	0.036 ± 0.002	0.031 ± 0.002	0.049 ± 0.007	0.029 ± 0.003
Aminopyrine N-demethylase ($nmol\ min^{-1}\ mg^{-1}$ protein)	4.96 ± 0.40	4.11 ± 0.17	3.92 ± 0.19*	2.60 ± 0.19***
Ethoxyresorufin *O*-deethylase $pmol\ min^{-1}\ mg^{-1}$ protein)	36.12 ± 4.62	33.85 ± 1.34	29.23 ± 0.75	23.09 ± 2.01*
UDP-glucuronyl transferase ($nmol\ min^{-1}\ mg^{-1}$ protein)	47.59 ± 2.51	49.70 ± 3.07	47.65 ± 1.70	39.69 ± 1.96*
δ-Aminolaevulinic acid synthase ($pmol\ ALA\ h^{-1}\ mg^{-1}$ protein)	10.53 ± 0.80	13.93 ± 3.22	11.23 ± 0.53	12.73 ± 1.83
Uroporphyrinogen I synthase ($pmol\ uroporphyrin\ h^{-1}\ mg^{-1}$ protein)	43.8 ± 0.8	46.3 ± 3.5	47.0 ± 5.5	57.0 ± 2.7**
Haem synthase ($nmol\ haem\ h^{-1}\ mg^{-1}$ protein)	10.09 ± 1.30	11.47 ± 2.05	14.93 ± 2.53	10.91 ± 1.14
Haem oxygenase ($pmol\ bilirubin\ min^{-1}\ mg^{-1}$ protein)	16.9 ± 2.6	21.6 ± 3.9	37.3 ± 98	130.4 ± 13.1***
Biliverdin reductase ($pmol\ bilirubin\ min^{-1}\ mg^{-1}$ protein)	167 ± 17	178 ± 9	188 ± 15	240 ± 30

The results are presented as mean ± s.e.mean.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; significantly different from control value.

Table 2 Effects of haem arginate, administered by intravenous infusion, on drug and haem metabolism in dogs after 28 days of treatment.

Assay	*Control* (vehicle)	*Dose 1* (3 mg haem kg^{-1})	*Dose 2* (9 mg haem kg^{-1})
Microsomal protein (mg g^{-1} liver)	4.5 ± 0.4	4.1 ± 0.7	4.5 ± 0.8
Cytochrome P-450 (nmol mg^{-1} protein)	0.25 ± 0.03	0.19 ± 0.02	0.13 ± 0.02**
Cytochrome b_5 (nmol mg^{-1} protein)	0.25 ± 0.03	0.25 ± 0.03	0.23 ± 0.02
NADPH-cytochrome C-reductase (u mg^{-1} protein)	0.014 ± 0.003	0.014 ± 0.001	0.014 ± 0.001
Aminopyrine N-demethylase (nmol min^{-1} mg^{-1} protein)	3.78 ± 0.32	4.06 ± 0.46	3.22 ± 0.43
Ethoxyresorufin *O*-deethylase (pmol min^{-1} mg^{-1} protein)	86 ± 9	88 ± 15	44 ± 12*
UDP-glucuronyl transferase (nmol min^{-1} mg^{-1} protein)	68.0 ± 11.6	67.2 ± 6.0	67.4 ± 7.5
δ-Aminolaevulinic acid synthase (pmol ALA h^{-1} mg^{-1} protein)	16.6 ± 1.3	18.5 ± 2.5	22.5 ± 1.0**
δ-Aminolaevulinic acid dehydratase (ul^{-1})	61.0 ± 3.3	68.7 ± 5.3	68.3 ± 4.3
Uroporphyrinogen I-synthase (pmol uroporphyrin h^{-1} mg^{-1} protein)	32.2 ± 0.7	33.8 ± 1.2	35.7 ± 0.8*
Uroporphyrinogen decarboxylase (pmol coproporphyrin h^{-1} mg^{-1} protein)	251.2 ± 11.3	269.0 ± 5.2	254.8 ± 6.3
Haem synthase (nmol haem h^{-1} mg^{-1} protein)	2.09 ± 0.28	2.11 ± 0.21	2.33 ± 0.20
Haem oxygenase (pmol bilirubin min^{-1} mg^{-1} protein)	9.7 ± 1.6	14.7 ± 2.7	23.9 ± 4.0**
Biliverdin reductase (pmol bilirubin min^{-1} mg^{-1} protein)	73.6 ± 94	63.2 ± 10.5	56.7 ± 10.2

The results are presented as mean ± s.e.mean.
*$P < 0.05$, **$P < 0.01$; significantly different from control value.

animal model of porphyria was equal to that of freshly prepared haematin (Tenhunen, Tokola, Lindén, unpublished results).

In the present study the haem dose recommended for treatment of porphyria, 3–4 mg kg^{-1}, given in the form of stable haem arginate to rats and dogs over a period of one month, did not cause any changes in the measured parameters. With higher doses the haem oxygenase activity increased considerably, with consequential release of iron and partial destruction of the enzymes bound in the membranes possibly through lipid peroxidation (Maines & Kappas, 1976; 1978). Possibly those enzymes which are located deeper in the microsomes (e.g. UDP-glucuronyl transferases) were affected to a lesser degree.

This might, but for the increased activity of uroporphyrinogen I-synthase, explain the changes caused by high doses of haem.

The amount of microsomal protein in dogs was only a quarter of that in rats. The different procedures for obtaining microsomes from the rats and dogs may have affected the results, but due to species differences it is difficult to extrapolate the results from one species to another. However, the changes occurring in rats and dogs were parallel in the present study and our results with control animals agree with the comparative study between rats and dogs done by McKillop (1985), where cytochrome P-450 content was higher in the rats than in the dogs, but ethoxyresorufin-*O*-deethylase had a much higher activity in the dogs than in the rats.

It has been suggested (Farrell & Correia, 1980; Ortiz de Montellano & Correia, 1983) that after the ingestion of some poisons, haem might reconstitute the destroyed cytochromes and thus correct the mixed function oxidase activity. Farrell & Correia (1980) showed that exogenous haem was incorporated into residual apoprotein after AIA-induced destruction of hepatic cytochrome P-450 in rats and increased the cytochrome P-450 content and mixed function oxidase activity in liver microsomes, but haem administration failed to elicit these effects in the absence of AIA treatment. In the rat model of experimental cirrhosis (produced by repeated carbon tetrachloride inhalation) hepatic microsomal cytochrome P-450 content was significantly decreased, but haem administration did not enhance hepatic clearance of the cytochrome P-450 substrate antipyrine (Farrell & Zaluzny, 1985). In this study the lowest dose of haem arginate did not increase the oxidative metabolic capacity after one month of daily administration, which is to be expected because in addition to haem the apoprotein is also needed. Perhaps in extrahepatic tissues there is an excess of free apoproteins, which might explain why haematin could prove significant activation of mono-oxygenase activity in maternal or foetal brain tissues of mice (Namkung *et al.*, 1983). With the higher doses of haem arginate, on the other hand, a reduction was seen in many parameters of foreign substance metabolism and this agrees with the findings of Namkung *et al.* (1983) that the mono-oxygenase activities in maternal and foetal hepatic preparations of mice were decreased by haematin.

Further studies are needed to find out whether the effect of haem on hepatic xenobiotic metabolism, which is in some conditions impaired, could be changed favourably by adjustment of the haem dose. Further work is also required to resolve if haem arginate is safer than haematin in respect of drug interactions. Haem arginate in doses of 10 mg haem kg^{-1} did not inhibit drug metabolism in mice (Tokola, 1987) whereas haematin has been found to inhibit metabolism in monkeys in much smaller doses; this may be because primates are more sensitive to depression of drug metabolism than either mice, rats or dogs.

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References

ANDERSON, K.E., ALVARES, A.P., SASSA, S. & KAPPAS, A. (1976). Studies in porphyria. V. Drug oxidation rates in hereditary hepatic porphyria. *Clin. Pharmac. Ther.*, **19**, 47–74.

VON BAHR, C., GROTH, C.-G., JANSSON, H., LUNDGREN, G., LIND, M. & GLAUMAN, H. (1980). Drug metabolism in human liver in vitro: Establishment of a human liver bank. *Clin. Pharmac Ther.*, **27**, 711–725.

BAUER, J. & FORNNARINO, J. (1984). High-performance liquid chromatographic analysis of "available" hemin in hematin solutions. *J. Chrom.*, **283**, 378–382.

BERLIN, A. & SCHALLER, K.H. (1974). European standardized method for the determination of δ-aminolevulinic acid dehydratase in blood. *J. clin. Chem. clin. Biochem.*, **8**, 389–390.

BONKOWSKY, H.L., BLOOMER, J.R., EBERT, P.S. & MAHONEY, M.J. (1975). Heme synthetase deficiency in human protoporphyria. *J. clin. Invest.*, **56**, 1139–1148.

BONKOWSKY, H.L., TSCHUDY, D.P., COLLINS, A., DOHERTY, J., BOSSENMAIER, I., CARDINAL, R. & WATSON, C.J. (1971). Repression of the overproduction of porphyrin precursors in acute intermittent porphyria by intravenous infusion of hematin. *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2725–2729.

BURKE, M.D. & MAYER, R.T. (1974). Ethoxyresorufin: Direct fluorometric assay of a microsomal O-dealkyla-

tion which is preferentially inducible by 3-methylcholanthrene. *Drug. Metab. Disposit.*, **2**, 583–588.

CINTI, D.L., MOLDEUS, P. & SCHENKMAN, J.B. (1972). Kinetic parameters of drug-metabolizing enzymes in Ca^{2+}-sedimented microsomes from rat liver. *Biochem. Pharmac.*, **21**, 3249–3256.

EBERT, P.S., TSCHUDY, D.P., CHOUDHRY, J.N. & CHIRIGOS, M.A. (1971). A simple micromethod for the direct determination of δ-amino [^{14}C] levulinic acid production in murine spleen and liver homogenates. *Biochim. biophys. Acta*, **208**, 236–250.

FARRELL, G.C. & CORREIA, M.A. (1980). Structural and functional reconstitution of hepatic cytochrome P-450 in vivo. Reversal of allylisopropylacetamidemediated destruction of the hemoprotein by exogenous heme. *J. biol. Chem.*, **255**, 10128–10133.

FARRELL, G.C. & ZALUZNY, L. (1985). Hepatic heme metabolism and cytochrome P-450 in cirrhotic rat liver. *Gastroenterology*, **89**, 172–179.

GOETSCH, C.A. & BISSELL, D.M. (1986). Instability of hematin used in the treatment of acute hepatic porphyria. *N. Engl. J. Med.*, **315**, 235–238.

GOLDBERG, A., MOORE, M.R., McCOLL, K.E.L. & BRODIE, M.J. (1984). Porphyrin metabolism and the porphyrias. In *The Oxford Textbook of Medicine*, ed. Weatherall, D.J., Ledingham, J.G.G. & Warrell, D.A., pp. 9.81–9.89. Oxford: Oxford University Press.

GUENGERICH, F.P. (1982). Microsomal enzymes involved in toxicology-analysis and separation. In *Principles and Methods of Toxicology*, ed. Hayes, A.W., pp. 609–634. New York: Raven Press.

HARTREE, E.F. (1972). Determination of proteins. A modification of the Lowry method that gives a linear photometric response. *Anal. Biochem.*, **48**, 422–427.

KITCHIN, I.T. (1983). Laboratory methods for ten hepatic toxification/detoxification parameters, *Meth. & Find. Exp. Clin. Pharmac.*, **5**, 439–448.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDAL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. biol. Chem.*, **193**, 256–275.

MAINES, M.D. & KAPPAS, A. (1976). The induction of heme oxidation in various tissues by trace metals: Evidence for the catabolism of endogenous heme by hepatic heme oxygenase. *Ann. Clin. Res.*, **8**, (suppl. 17), 39–46.

MAINES, M.D. & KAPPAS, A. (1978). Metals as regulators of heme metabolism. *Science*, **198**, 1215–1221.

MARKS, G.S. (1981). The effects of chemicals on hepatic heme biosynthesis. *Trends pharmac. Sci.*, **2**, 59–61.

MASTERS, B.S.S., WILLIAMS, C.H. & KAMIN, H. (1967). The preparation and properties of microsomal TPNH-cytochrome c reductase from pig liver. In *Methods in Enzymology*. Vol. 10, ed. Estabrook, R.W. & Pullman, M.E. pp. 565–573. New York: Academic Press.

McCOLL, K.E.L., MOORE, M.R., THOMPSON, G.G. & GOLDBERG, A. (1981).Treatment with hemin in acute hepatic porphyria. *Q.J. Med.*, **198**, 161–174.

McKILLOP, D. (1985). Effects of phenobarbitone and β-naphthoflavone on hepatic microsomal drug metabolising enzymes of the male beagle dog. *Biochem. Pharmac.*, **34**, 3137–3142.

MENDENHALL, D.W. (1984). Instability of hematin solutions. *N. Engl. J. Med.*, **311**, 539.

MOORE, M.R., McCOLL, K.E.L. & GOLDBERG, A. (1981). Drugs and the acute porphyrias. *Trends pharmac. Sci.*, **2**, 330–334.

MULLER-EBERHARD, U., EISEMAN, J.L., FOIDART, M. & ALVARES, A.P. (1983). Effect of heme on allylisopropylacetamideinduced changes in heme and drug metabolism in the rhesus monkey *(Macaca Mulatta)*. *Biochem. Pharmac.*, **32**, 3765–3769.

MUSTAJOKI, P. (1976). Red cell uroporphyrinogen I synthetase in acute intermittent porphyria. *Ann. clin. Res.*, **8**, (suppl. 17). 133–138.

NAMKUNG, M.J.,FAUSTMAN-WATTS, E. & JUCHAU, M.R. (1983). Hematin-mediated increases of benzo (a) pyrene monooxygenation in maternal, fetal and placental tissues of inducible and non-inducible mouse strains. *Dev. Pharmac. Ther.*, **6**, 199–206.

OMURA, T. & SATO, R. (1964). The carbon-monoxide-binding pigment of liver microsomes. *J. biol. Chem.*, **239**, 2370–2378.

ORTIZ DE MONTELLANO, P.R. & CORREIA, M.A. (1983). Suicidal destruction of cytochrome P-450 during oxidative drug metabolism. *A. Rev. Pharmac. Tox.*, **23**, 481–503.

PELKONEN, O., KALTIALA, E.H., LARMI, T.K.I. & KÄRKI, N.T. (1973). Comparison of activities of drug-metabolizing enzymes in human fetal and adult livers. *Clin. Pharmac. Ther.*, **14**, 840–846.

PELKONEN, O., PASANEN, M., KUHA, H., CACHALYI, B., KAIRALUOMA, M., SOTANIEMI, E.A., PARK, S.S., FRIEDMAN, F.K. & GELBOIN, H.V. (1986). The effect of cigarette smoking on 7-ethoxyresorufin O-deethylase and other monooxygenase activities in human liver: analysis with monoclonal antibodies. *Br. J. clin. Pharmac.*, **22**, 125–134.

PIERACH, C.A. (1982). Hematin therapy for the porphyric attack. *Semin. Liver. Dis.*, **2**, 125–131.

PIERACH, C.A. (1986). The treatment of porphyric attack with hematin. In *Porphyrins and Porphyrias*. Proceedings of the Second International Congress on Porphyrins and Porphyrias, Paris 19–22 June 1985, ed. Nordmann, Y. pp. 217–224. London: John Libbey.

ROMEO, G. & LEVIN, E.Y. (1971). Uroporphyrinogen decarboxylase from mouse spleen. *Biochem. biophys. Acta*, **230**, 330–341.

SCHNEIDER, W.G. (1948). Intracellular distribution of enzymes. III. The oxidation of octanoic acid by rat liver fractions. *J. biol. Chem.*, **176**, 259–266.

STRAND, L.J., MEYER, U.A., FELSHER, B.F., REDEKER, A.G. & MARVER, H.S. (1972). Decreased red cell uroporphyrinogen I synthetase activity in intermittent acute porphyria. *J. clin. Invest.*, **51**, 2530–2536.

TENHUNEN, R., MARVER, H.S. & SCHMID, R. (1969). Microsomal heme oxygenase. Characterization of the enzyme. *J. biol. Chem.*, **244**, 6388–6394.

TENHUNEN, R., ROSS, M.E., MARVER, H.S. & SCHMID, R. (1970). Reduced nicotinamide adenide dinucleotide phosphate-dependent biliverdin reductase: Partial purification and characterization of the enzyme. *Biochemistry*, **9**, 293–303.

TENHUNEN, R., SAVOLAINEN, H. & JÄPPINEN, P. (1983). Changes in haem synthesis associated with occupational exposure to organic and inorganic sulphides. *Clin. Sci.*, **64**, 187–191.

TENHUNEN, R., TOKOLA, O., LINDEN, I-B. & COTHONI, G.

(1985). Heme arginate: a well tolerated new heme compound. *VIII Meeting of the International Society of Haematolology European and Afrikan Division.* Abstract 377, p. 194, Warsaw: The Polish Society of Haematol and Transfusiology.

TENHUNEN, R., ZITTING, A., NICKELS, J. & SAVOLAINEN, H. (1984). Trinitrotoluene-induced effects on rat heme metabolism. *Exp. Mol. Path.*, **40**, 362–366.

TOKOLA, O. (1987). Barbiturate and ethanol sleeping times and pharmacokinetics of propranolol in mice after intravenous administration of haem arginate. *Pharmac. tox.*, (in press).

WATSON, C.J., DHAR, G.J., BOSSENMAIER, I., CARDINAL, R. & PETRYKA, Z.J. (1973). Effect of hematin in acute porphyric relapse. *Ann. intern. Med.*, **79**, 80–83.

WATSON, C.J., PIERACH, C.A., BOSSENMAIER, I. & CARDINAL, R. (1977). Postulated deficiency of hepatic heme and repair by hematin infusions in the "inducible" hepatic porphyrias. *Proc. natn. Acad. Sci. U.S.A.*, **74**, 2118–2120.

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