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Haem arginate: a new stable haem compound

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Intravenous administration of haem in acute hepatic porphyrias inhibits the induction of δ-aminolaevulinic acid synthase, reduces the formation of potentially harmful metabolites of porphyrin synthesis and corrects the haem deficiency. Typically, haem therapy has been given in the form of haematin—haem dissolved in alkali. Such haematin solutions are, however, extremely unstable. Thus, the rapid decomposition of this therapeutic agent may have been responsible for the ineffectiveness of treatment in some clinical states and adverse reactions may have been caused by haematin degradation products. There is, therefore, a need for a stable, effective and well-tolerated haem preparation. We have prepared certain highly soluble haem compounds of which haem arginate has proved to be the most promising. Pure haemin was isolated from HIV and hepatitis B negative human blood. The haem derivatives prepared were screened as substrates for haem oxygenase. Haem arginate and haem lysinate were found to be as good substrates as methaemalbumin. Stock solutions of haem arginate were stable for 2 years at +6 °C. After dilution with sterile isotonic saline the haem arginate infusion was clearly more stable than haematin solutions made in the laboratory or prepared by dissolving commercial lyophilized haematin. The antiporphyrogenic effect of haem arginate (even after storage for two years) in 2-allyl-2-isopropylacetamide-induced experimental porphyria of rats was equal to that of freshly prepared haematin. The acute oral toxicity of haem arginate was low compared with the parenterally administered drug, indicating poor oral bioavailability. The acute toxic effects after high intravenous or intraperitoneal doses were directed to the liver. Conventional haematin caused thrombophlebitic reactions at the site of infusion in all rabbits, but haem arginate did not cause any thrombophlebitis even after repeated infusions. The dose of haem arginate used in clinical trials, 0·2 mg of haem kg^{-1} min^{-1} during 15 min (= total dose 3 mg kg^{-1} diluted in 100 mL isotonic saline), did not cause any clinically significant changes in the numerous haemostatic parameters measured in patients, and ten times higher infusion rates or total doses were needed to cause a decrease in the blood pressure of rats. Haem arginate has proven to have many advantages over conventional haematin solutions.

Haem (the term haem is used here to indicate an iron-protoporphyrin IX compound irrespective of the oxidation state of the iron) is an integral part of haemoglobin, myoglobin, cytochromes and many enzymes, and as such is one of the most essential compounds in the living organism. The biosynthesis of haem is regulated enzymatically; functional disorders of the enzymes involved may be either hereditary or induced by external factors (Kappas et al 1983). Biosynthesis of haem in the liver is controlled largely by the rate of production of δ-aminolaevulinic acid (ALA) synthase. The activity of this rate limiting enzyme is feedback regulated by the intracellular concentration of haem (Granick 1966). In red blood cells, on the other hand, the regulation of haem synthesis may not be controlled by ALA synthase alone (Hoffman et al 1980).

The hepatic and erythropoietic porphyrias comprise inherited or acquired disorders characterized by defects in specific enzymes of haem biosynthesis, increased accumulation and excretion of metabolites of haem synthesis, and a variety of clinical manifestations (Kappas et al 1983). Therefore, it seems logical to treat patients with acute porphyrias with haem, which inhibits the induction of ALA synthase, reduces the formation of potentially harmful intermediates of porphyrin synthesis and at least partly corrects the haem deficiency (Bonkowsky et al 1971; Watson et al 1973; Dhar et al 1975).

Preparation of conventional haem solution implies the dissolving of haemin in alkali with the formation of haematin (Bonkowsky et al 1971; Watson et al 1973; Dhar et al 1975). This procedure, which has been carried out in various modifications in many laboratories and hospital pharmacies, is connected with certain problems in the quality of haematin solution and with complications in its clinical use. For instance, haematin solutions are extremely unstable and decomposition of the drug may have been responsible for ineffectiveness in some clinical situations (Mendenhall 1984; Goetsch & Bissell 1986). Haematin solutions have been frequently

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reported to cause thrombophlebitis and disturbances in blood coagulation (Morris et al 1981; Glueck et al 1983). Also these side effects have been suggested to be due to degradation products of haematin (Pierach 1986; Goetsch & Bissell 1986).

Under the Orphan Drug Program of the FDA a lyophilized form of haemin hydroxide (Panhematin) has recently been approved for marketing in the USA. The shelf-life of this product is, however, very short. Contrasting with the various laboratory haematin solutions, which are typically prepared in a hurry, this drug is subjected to numerous quality control procedures. Unfortunately, after reconstitution, Panhematin solutions are rapidly degraded and therefore cannot be kept in store. There are no published reports on the effectiveness or safety of the lyophilized preparation. Clearly, there is need for a well-tolerated, stable haem compound.

We have prepared certain highly soluble haem compounds, of which haem arginate has proved to be the most promising and, as we report in this study, it is stable for several years and is effective in experimental porphyria.

MATERIALS AND METHODS

Drugs

Haem was isolated and recrystallized twice from haemolysed, HIV and hepatitis B negative human blood. On spectroscopic analysis, the alkaline-pyridine complex prepared with this material exhibited an E557/541 ratio of 3·45 (Paul et al 1953).

L-Arginine (E. Merck, Darmstadt), L-lysine (dihydrochloride, Fluga AG, Buchs), piperidine, isopropylamine, morpholine, cyclohexylamine (free base), triethylamine, ethylamine, and diethanolamine (all Sigma, St Louis MO) were of analytical grade. Haem derivatives of these compounds were prepared as follows: 11·5 mmol of each compound was mixed with 10 g of ethanol and 40 g of 1,2-propanediol then water added to a total volume of 100 mL. Whilst this was stirred vigorously with a magnetic stirrer, 3·85 mmol of haemin was added. After stirring for 15 min, the solution was passed through a 0·22 μm Millipore filter then the volume of filtrate adjusted to 100 mL with sterile water. The sterile solution was sealed in glass ampoules. The Millipore filtration resulted in a loss of less than 2% of the haem content.

For the preparation of the haematin solution, haem was dissolved in 0·25 M Na_2CO_3 and, after adjusting the pH to 8 with 0·1 M HCl, the solution was diluted with 0·15 M NaCl to a haem concentration of 7 mg mL^{-1}. Subsequent filtration through a 0·22 μm Millipore filter resulted in a loss of less than 10% of the haem content.

The haem arginate preparation (Normosang, Huhtamäki Oy Pharmaceuticals, Medica, Helsinki, Finland) contained haem arginate corresponding to haemin 25 mg mL^{-1} in aqueous solution with 1,2-propanediol (40%) and ethanol (10%) in ampoules of 10 mL. The ampoules were stored in a refrigerator (at +6 °C).

The lyophilized haematin preparation (Panhematin, Abbott Laboratories, North Chicago, Illinois) was kept frozen (at −80 °C) until the time of reconstitution and the solution was prepared and infused as described in the manufacturer's data sheet.

Determinations of haem

Haem with its derivatives as well as porphyrins were measured by high performance liquid chromatography using a fluorescence detector (Salmi & Tenhunen 1980).

In stability studies, haem concentrations were measured by high performance liquid chromatography using μBondapak C_{18} (30 cm, 10 μM) as a solid phase and methanol–water–acetic acid (75:25:1) as a solvent system. The flow rate was 1·5 mL min^{-1} and the pH was adjusted to 4·2 using 1 M sodium hydroxide; UV-detection was at 360 nm (Häkli et al unpublished).

Ability to serve as substrates for physiological enzyme systems in-vitro

Microsomal haem oxygenase activity was measured by the method of Tenhunen et al (1969). When various haem compounds were used as substrates the haem concentration in each experiment was adjusted to 17 μM in the final reaction mixture. A 20 000*g* supernatant of rat spleen homogenate 1:2 (v/v) in 0·1 M potassium phosphate buffer pH 7·4 was used as the enzyme source for haem oxygenase measurements. Protein was measured by the method of Lowry et al (1951).

Effects in experimental porphyria

For induction of experimental porphyria in rats 2-allyl-2-isopropylacetamide (AIA), generously donated by Hoffman-La Roche Co. AG (Basel, Switzerland), was used. Male Sprague-Dawley rats, 190–230 g, housed in metabolic cages, were fasted 18 h before AIA administration and then throughout the experiment. Water was freely available. AIA dissolved in 0·15 M NaCl to a concentration of 20 mg mL^{-1} was administered by subcutaneous

injection in a dose of 300 mg kg^{-1} day^{-1} on two consecutive days. Haem arginate and haematin were given intraperitoneally 30 min before AIA administration in a dose of 10 mg haem kg^{-1}. Urine was collected as 24 h fractions. The rats were killed 24 h after the second AIA, AIA and haem arginate or AIA and haematin injections. For measurement of the 24 h excretion of porphobilinogen and δ-aminolaevulinic acid the methods of Mauzerall & Granick (1956) were used.

Safety

Acute toxicity (LD50) in NMRI mice was determined after intravenous, intraperitoneal and oral administration of haem arginate. Ten or 20 male mice (18–22 g) per dose, were randomly placed in plastic cages. The room temperature was 21 ± 2 °C, the lighting was artificial (12 h light and 12 h dark), and food and water were unlimited. The follow-up period was 14 days. During the first day the animals were observed every hour, the second day every second hour and thereafter twice a day. Cageside observations during the first day included changes in the skin and fur, eyes and mucous membranes, and the behavioural pattern. Macroscopic evaluation of the organs was performed during necropsy. The LD50 values (mg haem kg^{-1}) with 95% confidence limits were calculated according to Litchfield & Wilcoxon (1949).

Venous tolerability of various haem compounds was tested in Californian white rabbits weighing 3·0–3·5 kg. The amount of haem administered was 5 mg kg^{-1}. The infusion time was in early experiments 2 h and later on 15 min; after the infusion, the vein was rinsed with isotonic saline injection. The injection sites were observed and photographed 2, 5, 24 and 48 h after the termination of the infusion. The evaluation of venous reactions was always done by the same person, who did not know the codes of the infusions given.

Effects of haem arginate infusions on the blood pressure of normotensive male Sprague-Dawley rats (260–305 g) were measured for up to 1 h. The rats were urethane-anaesthetized and the mean arterial blood pressure in the carotid artery measured by a Harvard transducer. The infusion rates were 0·25, 1·25, 2·5, 5 and 10 mg haem kg^{-1} min^{-1}.

The blood coagulation parameters of volunteers (i.e. activated partial thromboplastin time [APTT], Quick time, thrombin time, ethanol gelation, prothrombin, factor V, factor VII, factor VII : C, factor VIII : Ag, factor VIII : Rcof, antithrombin III functional, factor IX, factor X, protein C and plasminogen function) were measured by the laboratory of the Finnish Red Cross transfusion service using routine methods. Thromboxane B_2 concentrations in human plasma and serum were measured by radioimmunoassay (Viinikka & Ylikorkala 1980) before and 15, 30 min, 1 and 2 h after haem arginate infusion. The dose was 3 mg haem kg^{-1}. Complete blood screens were carried out by the routine methods of the hospital laboratory. The study protocol was accepted by the local ethical committee and informed consent was obtained from each subject before the study.

RESULTS

Preparation

Haem forms a soluble compound with L-arginine with no precipitate when centrifuged for 10 min at 20 000*g*. The loss of haem when passed through a Millipore 0·22 μm filter is less than 2%. Three moles of arginine are necessary to solubilize one mole of haem.

The stability of haem arginate in stock solution is at least 96% in 2 years (Table 1). When diluted with sterile physiological saline this decreases and is dependent on the concentration of haem arginate, but it is always better than that of haematin solutions (Table 2). When diluted to the same concentration as

Table 1. Stability of haem arginate solutions (25 mg haem mL^{-1}) during storage at +6 °C. The concentration of unchanged haem as well as pH values at different time points are shown. Haem is measured by high performance liquid chromatography using UV-detection.

Batch no.	Starting values		Storage time: 6 months		1 year		2 years		2½ years	
	pH	Haem	pH	Haem	pH	Haem	pH	Haem	pH	Haem
8201	8·7	25·4	8·7	25·4	8·7	26·1	8·8	25·5	8·7	25·5
8308	8·5	26·4	8·9	25·6	8·8	25·2	8·8	24·1	[a]	[a]
8401	8·7	25·2	8·9	24·5	—	24·5	8·7	24·2	[a]	[a]

[a] Not yet done.

Table 2. Diluted haem arginate solutions and reconstituted lyophilized haematin: comparison of stabilities. The storage temperature for haem arginate was +6 °C and for lyophilized haematin −80 °C before dilution. After dilution both haem solutions were kept at room temperature (21 °C). Isotonic saline was used for dilution and reconstitution. Haem concentrations were measured by HPLC using UV-detection.

Time after dilution (min)	Haem (% of stated content)		
	Haem arginate[a]	Haem arginate[b]	Lyophilized haematin[b]
5	97	100	54
60	94	100	50
120	90	99	46
240	79	99	39

[a] Diluted for administration to 2·5 mg haem mL^{-1}.
[b] Diluted for administration to 7·3 mg haem mL^{-1}.

recommended for Panhematin it is clearly more stable.

Screening as substrates for haem oxygenase

The ability of different haem compounds to serve as substrates for haem oxygenase varies greatly (Table 3). Haem arginate and haem lysinate are at least as good substrates as methaemalbumin.

Table 3. The ability of haem compounds to serve as substrates for microsomal haem oxygenase. Methaemalbumin was used as a reference (=100%). Haem oxygenase activity was measured according to Tenhunen et al (1969) using rat spleen 20 000*g* supernatant as an enzyme source.

Compound	Relative haem oxygenase activity (%)
Methaemalbumin	100
Haem arginate	106
Haem lysinate	106
Haem piperidine	78
Haem isopropylamine	71
Haem morpholine	64
Haem cyclohexylamine	31
Haem triethylamine	23
Haem ethylamine	21
Haem diethanolamine	13

Effects

In AIA-treated rats the daily urinary excretion of porphobilinogen (PBG) and δ-aminolaevulinic acid (ALA) increased from mean values of 66 and 120 to 4850 and 4440 nmol day^{-1}, respectively. Haematin and haem arginate showed equal antiporphyrogenic action (Table 4) by inhibiting the induction of ALA synthase and inhibiting the increase of PBG and ALA excretion.

Table 4. Antiporphyrogenic effects of haematin and haem arginate in experimental porphyria of the rats. Urinary excretion of porphobilinogen, PBG (nmol day^{-1}) and δ-aminolaevulinic acid, ALA (nmol day^{-1}), after treatment of rats on two consecutive days with allylisopropylacetamide, AIA (300 mg kg^{-1} s.c. day^{-1}) alone and combined either with haematin (10 mg of haem kg^{-1} i.p. day^{-1}) or with haem arginate (10 mg of haem kg^{-1} i.p. day^{-1}). Mean values and standard deviations of excretion in each group of five rats are shown.

Treatment	Day	PBG	ALA
AIA	0	66 ± 53	120 ± 65
	1	430 ± 160	380 ± 150
	2	4850 ± 880	4440 ± 2450
AIA + haematin	0	18 ± 8	110 ± 79
	1	38 ± 13	96 ± 100
	2	130 ± 60	180 ± 80
AIA + haem arginate	0	34 ± 38	120 ± 68
	1	28 ± 20	110 ± 26
	2	180 ± 110	92 ± 36

Safety

Acute toxicology in mice. LD50 (mg haem kg^{-1} with 95% confidence limits) was 56·3 (46·8–67·7), 112·5 (103·3–122·6) and >5000 after intravenous, intraperitoneal and oral administration, respectively. In the highest intravenous dose group (87·5 mg haem kg^{-1}) piloerection and restlessness occurred 2 h after injection. A yellow skin colour was noted in hairless areas. Death was preceded by reduced motility, piloerection and respiratory disorders. The animals that died during the first day of observation had an infarcted liver; those that died after the first day had a necrotic liver with varying localization and extension of necrotic areas, and a necrotic pancreas. A few animals in every dose group also had black mucous in the duodenum. The muscles and fat tissue were yellow coloured during the first two days.

Venous tolerability in rabbits. Haem arginate infused either once or repeatedly did not cause thrombophlebitis up to 24 h after infusion. In contrast conventional haematin caused thrombophlebitic reactions in all rabbits.

Effects on the blood pressure of rats. The constant infusion rate of 0·25 mg kg^{-1} min^{-1} caused no effect on the blood pressure of the rats during 60 min (total dose 15 mg kg^{-1}). When the total dose of haem was more than 30 mg kg^{-1} the decrease in blood pressure was marked (Fig. 1).

Blood coagulation parameters in volunteers. Minor changes were observed in coagulation parameters. Factors IX and X decreased ($P < 0·05$–$0·01$) after

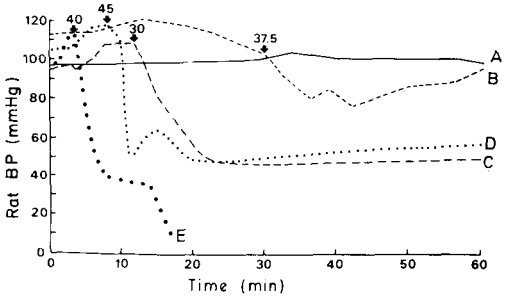

FIG. 1. Effects of haem arginate on the mean arterial blood pressure of normotensive, urethane-anaesthetized male rats.

Key: Graph	Infusion rate (mg kg^{-1} min^{-1})	Total dose (mg kg^{-1})
A	0·25	15
B	1·25	75
C	2·5	150
D	5	300
E	10	130

Numbers on the figure are the total dose of haem (mg kg^{-1}) received to the time marked.

haem infusion. Other changes were statistically insignificant (Table 5). Plasma and serum thromboxane B_2 values did not change after the haem arginate infusions; the individual mean values, 5 measurements per subject (with ranges from time 0 to 2 h) were: No. 1: serum 376·3 ng mL^{-1} (327·6–408·1), plasma 223·0 pg mL^{-1} (190·5–243·6), No. 2: 115·7 ng mL^{-1} (103·3–142·5) and 205·9 pg mL^{-1} (158·5–352·1), respectively.

DISCUSSION

Haem has been shown to be a controlling factor in porphyria synthesis in various systems, e.g. *R. spheroides* (Lascelles 1960), chick embryo liver cells (Granick 1966), and rat liver (Marver et al 1966). In porphyrias, which are characterized by defects in specific enzymes of haem biosynthesis, a decreased formation of haem leads to a compensatory induction of ALA synthase with increased accumulation and excretion of intermediates of porphyrin synthesis. The crucial experiments that led to the initial trial of haem therapy in acute porphyria (Bonkowsky et al 1971) were the studies of Waxman et al (1966), which demonstrated that intravenously administered haematin effectively repressed hepatic ALA synthase in porphyric rats.

In the haem therapy of acute porphyrias, commercial animal haemin has been used most frequently. Due to the poor solubility of haemin in neutral aqueous solutions it has been dissolved in an alkaline, usually sodium carbonate, solution with the formation of haematin. It is known, however, that haems and porphyrins tend to aggregate in both aqueous and non-aqueous solutions (Brown et al 1980) and the 2,4-vinyl groups of protohaem appear to increase the aggregation substantially, compared with haems with less electron-withdrawing substituents. Physiologically, these aggregates seem to be less active than the monomeric form. Addition of alcohols is known to disperse dye aggregates (Rabinowitch & Ebstein 1941). Moreoever, haematin solutions have proved to be prone to rapid spontaneous degradation, which can only be inhibited to a small extent by the addition of alcohols (sorbitol, mannitol).

Table 5. Effects of haem arginate on haemostasis. Changes of coagulation parameters in six volunteers 15 min and 4 h after intravenous infusion of haem arginate (haem 3 mg kg^{-1}).

	Starting values (mean ± s.d.)	After haem infusion: 15 min mean change	After haem infusion: 4 h mean change
APTT(s)	34·5 ± 1·97	−0·17	+0·17
Quick time (s)	17·0 ± 1·10	+0·33	+0·17
Thrombin time (s)	18·7 ± 0·82	+0·17	+0·17
Prothrombin (%)	91·5 ± 5·68	−0·83	−4·33
Factor V (%)	94·3 ± 27·68	+0·50	+1·50
Factor VII (%)	87·5 ± 8·76	+0·83	−4·00
Factor VIII : C (%)	111·5 ± 38·76	−4·50	−16·67
Factor VIII : Ag (%)	127·5 ± 43·61	−7·00	−11·00
Factor VIII : Rcof (%)	107·8 ± 33·54	−2·33	−11·83
Antithrombin III funct.	104·5 ± 20·20	−6·67	+2·83
Factor IX (%)	110·7 ± 16·54	−17·00*	−11·00*
Factor X (%)	82·4 ± 7·20	−8·40**	−9·20
Prot. C (%)	89·2 ± 14·22	−4·17	−1·83
Platelets (×10^9 L^{-1})	211·3 ± 9·30	+5·67	−7·67
Plasminogen funct. (%)	92·5 ± 8·27	−1·50	−1·25
Ethanol gelation	neg	neg	neg

* $P < 0·05$, ** $P < 0·01$ (paired t).

As shown in this study, in aqueous solutions, haem forms a highly soluble compound with arginine, haem arginate. This solution is stable after addition of 1,2-propanediol and alcohol and on the basis of electron paramagnetic resonance studies haem arginate is in a loosely polymeric form (Sievers et al 1987). When infused undiluted or diluted with physiological saline into the blood, haem dissociates easily from arginine (Lindén et al 1987) forming haem-haemopexin complexes capable of binding to specific receptors e.g. on the plasma membrane of hepatocytes (Smith & Morgan 1979).

Haem arginate is a good substrate for the physiological haem-degrading enzyme system, microsomal haem oxygenase. Accordingly, the infusion of large

amounts of haem arginate in the organism could lead to an expansion of the haem pool with an induction of haem oxygenase activity. This induction, as well as the incorporation of haem into haemoproteins, would prevent the possibility of excess haem causing membrane peroxidation and would be an explanation for the good tolerability of haem arginate.

The incorporation of the haem of haem arginate into cytochrome P450 is suggested by the therapeutic efficacy of haem arginate in the experimental animal porphyria induced by AIA. This chemical is known to reduce hepatic cytochrome P450 content by stripping the *N*-alkylated porphyrin from its apoprotein (Ortiz de Montellano et al 1984). With haem arginate the hepatic cytochrome P450 could be reconstituted in AIA-induced porphyria as effectively as with haematin. The AIA-induced disorder of porphyrin metabolism in rats resembles acute intermittent porphyria in patients. The AIA model is useful for comparing therapeutic efficacy (i.e. decrease of urinary excretion of porphyrin precursors and inhibition of hepatic δ-aminolaevulinic acid synthase) of a new treatment with older ones (Tokola et al 1987). Theoretically, being easily soluble and stable, haem arginate should be at least as effective as haematin in the treatment of porphyric attacks in man.

The acute toxic effects after high doses were directed to the liver. The binding capacity of haemopexin and albumin is exceeded after toxic doses. Smaller doses, but still 3–10 times the therapeutic doses for one month in rats and dogs, significantly increased the activity of hepatic haem oxygenase but decreased concentrations or activities of drug metabolizing enzymes, e.g. cytochrome P450 and ethoxyresorufin *O*-deethylase (Tokola 1987). The iron released from haem may cause infarction and necrosis of the liver in acute toxicity tests after lethal doses, through lipid peroxidation. The therapeutic index seems to be satisfactory for this kind of drug. The LD50 is 20 to 35 times higher than the dose used in the treatment of porphyric patients. The toxicity of the compound when given orally is low and the great difference between the oral and parenteral LD50s indicates poor oral bioavailability.

Haematin has been reported to cause a chemical phlebitis at the site of infusion in up to 30% of the patients treated (McColl et al 1981). In this study too, phlebitis of the ear vein was seen in all rabbits treated with haematin, whereas Panhematin caused only mild irritation of the ear vein in one rabbit. Haem arginate, even after repeated infusions, did not cause any phlebitic reactions.

Overdoses of oral iron salts and even therapeutic doses of parenteral iron may cause cardiovascular collapse and cardiac dysrhythmias (Reinicke 1984). Therefore, effects on the blood pressure of rats were studied as part of the pharmacological profile of the compound. In clinical trials and in the treatment of patients we have used a haem dose of 3 mg kg^{-1} administered by intravenous infusion, which means 0·2 mg of haem kg^{-1} min^{-1} during 15 min. This dosage has a wide enough safety margin, because ten times higher total doses or infusion rates were needed to cause a clear decrease in the blood pressure of rats.

Disturbances of haemostasis have also been reported during haematin treatment of patients (Morris et al 1981; Glueck et al 1983). Characteristically there is a prolonged prothrombin time and partial thromboplastin time, reptilase time and thrombin time. In addition to this anticoagulant effect haematin has been shown to aggregate platelets, probably by inducing ADP release. Both these abnormalities closely parallel the serum haematin levels being most marked 10 min after infusion and substantially resolved at 5 h. Haem arginate did not have any significant effects on the following parameters of blood coagulation when tested in six persons: activated partial thromboplastin time, thrombin time, the Quick prothrombin time, prothrombin concentration, factor V concentration, factor VII concentration, factor VIII : C concentration, factor VIII : Ag concentration, factor VIII : Rcof concentration, antithrombin III (functional) activity, protein C concentration or plasminogen functional activity. The small changes observed in concentrations of factors IX and X in man after haem arginate infusion do not have any clinicial importance. Furthermore, haem arginate neither reduces the platelet count nor activates thrombocytes.

On the basis of several studies it is evident that both the anticoagulant properties as well as the thrombophlebitic effect of haematin after intravenous administration are due to decay products, which appear to result from reaction of the vinyl substituents of protoporphyrin with molecular oxygen (Goetsch & Bissell 1986). Furthermore, the antiporphyrogenic effect of stored decayed haematin is sometimes diminished or even absent (McColl et al 1981; Goetsch & Bissell 1986).

As shown in this study, haem arginate is remarkably stable. Probably due to this its antiporphyrogenic effects remain relatively constant during storage. It also seems to be free from the side effects associated with haematin therapy.

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