Metabolism of Hepatic Haem and 'Green Pigments' in Rats Given 2-Allyl-2-isopropylacetamide and Ferric Citrate

A NEW MODEL FOR HEPATIC HAEM TURNOVER

Herbert L. BONKOWSKY,*† John F. HEALEY,* Peter R. SINCLAIR,* Yash P. MAYER† and Raymond ERNY*

**Hepatology and Metabolism Research Laboratory and Departments of Medicine and Biochemistry, VA Medical Centre, White River Junction, VT 05001, U.S.A. and Dartmouth Medical School, Hanover, NH 03755, U.S.A., and †Department of Chemistry, State University of New York, Albany, NY 12222, U.S.A.*

(Received 17 July 1979)

We have studied effects of single doses of 2-allyl-2-isopropylacetamide and ferric citrate on hepatic haem turnover in rats. Haem was pre-labelled by intraperitoneal administration of 5-amino-[4-¹⁴C]laevulinate 4 h before other treatments. Computer-assisted analysis of the haem decay curve showed that at least two exponential components were involved implying two haem pools. In control rats the size of the rapidly-turning-over pool was 38% of the total. Treatment with 2-allyl-2-isopropylacetamide alone resulted in a 2.3-fold increase in the fractional size of this pool. Treatment with ferric citrate alone increased the size of this pool 1.8-fold; treatment with both agents together had no measurable effect beyond that produced by 2-allyl-2-isopropylacetamide. The apparent rate constant for disappearance of labelled haem from the first pool was not affected by treatment with 2-allyl-2-isopropylacetamide or ferric citrate, indicating that these treatments affect hepatic haem turnover primarily by altering the distribution of haem synthesized in the liver. The increased haem degradation after treatment with 2-allyl-2-isopropylacetamide alone was associated with the accumulation of 'green pigments' in the liver. These pigments were detectable 1.5 h after drug treatment; their total amount increased steadily for 28 h and then declined. Despite this increase in amount, radioactivity of the 'green pigment' fraction fell rapidly in a biphasic fashion. Some of the radioactivity that initially was found in the 'green pigment' fraction was later found in an aqueous fraction, not extractable by acidic ethyl acetate. Rats given ferric citrate together with 2-allyl-2-isopropylacetamide accumulated less 'green pigments'.

2-Allyl-2-isopropylacetamide is a well-known porphyrinogenic agent (for reviews see Tschudy & Bonkowsky, 1972; Meyer & Schmid, 1978; De Matteis, 1978*a,b*). Administration of this compound to small rodents, intact embryonated eggs, or chick embryo liver cells in culture, leads to induction of 5-aminolaevulinate synthase, the first and rate-controlling step of haem biosynthesis. According to current concepts, induction of this enzyme by 2-allyl-2-isopropylacetamide results, at least in part, from enhanced degradation of hepatic haem produced by the drug (De Matteis, 1971; Meyer &

Marver, 1971; Tschudy & Bonkowsky, 1972; De Matteis, 1978*a,b*; Ortiz de Montellano *et al.*, 1978). This haem gives rise to 'green pigments', first described by Schwartz & Ikeda (1955), and recently shown to be an adduct of the drug with protoporphyrin (Ortiz de Montellano *et al.*, 1978; De Matteis & Cantoni, 1979).

Iron overload also affects hepatic haem metabolism. In rats, single (De Matteis & Sparks, 1973; Maines & Kappas, 1976; Ibrahim *et al.*, 1979) and repeated (Bonkowsky *et al.*, 1979*a*; Ibrahim *et al.*, 1979) parenteral iron injections decrease hepatic concentrations of cytochrome *P*-450 and increase activity of hepatic haem oxygenase (Maines & Kappas, 1976, 1977; Ibrahim *et al.*, 1979). In contrast, enteral administration of a single dose of

† To whom requests for reprints should be addressed at VA Medical and Regional Office Center, White River Junction, VT 05001, U.S.A.

ferric citrate does not affect hepatic cytochrome *P*-450 (Bonkowsky *et al.*, 1979b), or 5-amino-laevulinate synthase (Stein *et al.*, 1969; Bonkowsky *et al.*, 1979b), although it does exert a synergistic effect upon the induction of the latter enzyme by 2-allyl-2-isopropylacetamide (Stein *et al.*, 1969, 1970; Bonkowsky *et al.*, 1979b) or sulfonal (Stein *et al.*, 1970).

We investigated the turnover of hepatic haem stimulated by 2-allyl-2-isopropylacetamide and studied whether a single enteral dose of ferric citrate affected hepatic haem turnover in the presence or absence of treatment with 2-allyl-2-isopropylacetamide. We also studied the formation and disappearance of the 'green pigments' detectable in rat liver following administration of the drug. Part of this work was presented at the Fourth International Symposium on Microsomes, Drug Oxidations, and Carcinogenesis (Bonkowsky *et al.*, 1980).

Materials and Methods

Treatment of rats

Female Sprague-Dawley rats (Charles River, Cambridge, MA, U.S.A.) were fed Purina Chow and were housed singly in metal cages without absorbent. Rats weighing 100–150 g were starved for 12 h prior to an intraperitoneal injection of 1 μ Ci (18 nmol) of 5-amino[4- ^{14} C]laevulinate (New England Nuclear, Cambridge, MA, U.S.A.), that had been neutralized and diluted just prior to use (Bonkowsky *et al.*, 1978). No food was given thereafter, but water was allowed *ad lib*. Four hours later, when virtually all liver radioactivity is in hepatic haem (Bonkowsky *et al.*, 1978), one group of rats was killed by decapitation. The other groups received one of four types of further treatment: (i) the controls, sodium citrate (2 mmol/kg of body wt.) by gavage and 0.15 M-NaCl (2.7 ml/kg) by subcutaneous injection; (ii) ferric citrate (2 mmol (700 mg)/kg) by gavage and 0.15 M-NaCl by subcutaneous injection; (iii) sodium citrate by gavage and 2-allyl-2-isopropylacetamide [2.88 mmol (400 mg)/kg] by subcutaneous injection; and (iv) ferric citrate by gavage and 2-allyl-2-isopropylacetamide by subcutaneous injection at the above dosages.

Rats from each treatment group were killed by decapitation 7, 10, 13, 17, 32 and 49 h after injection of 5-amino[4- ^{14} C]laevulinate. Some rats from treatment groups (iii) and (iv) were killed 5.5 h after injection of 5-aminolaevulinate. Usually six to eight rats per treatment group were used for each time point. The total number of rats used was 204.

Preparation and extraction of liver homogenates

Livers were thoroughly perfused *in situ* with cold 0.15 M-NaCl/0.1 mM-menadione, rapidly excised,

and weighed. A portion (2 g) was stored frozen at -85°C until extracted, as described below (menadione was used to inhibit lipid peroxidation).

Haem was extracted and purified from liver homogenates as described (Bonkowsky *et al.*, 1978). For 94 separate homogenates, haem recovery by this method averaged $70 \pm 0.9\%$ of the total haem in the homogenate. This value was used throughout to correct results for both total haem content and radioactivity. If correction for haem recovery was not applied, the distribution of hepatic radioactivity was similar to that reported by others (De Matteis, 1971; De Matteis & Unseld, 1976).

Possible contamination of extracted haem by unconjugated bilirubin was ruled out, since unconjugated bilirubin (Pfanstiehl Chemicals, Waukegan, IL, U.S.A.), could be added to the labelled homogenates and then quantitatively removed without loss of radioactivity by pre-extraction with chloroform followed by ethyl acetate. These results confirm and extend the previous conclusion (Bonkowsky *et al.*, 1978) that this method for haem purification is dependable.

'Green pigments' were extracted with 7.5 M-HCl (Schwartz & Ikeda, 1955) from the ethyl acetate fraction, after preliminary removal of porphyrins with 1.5 M-HCl (Bonkowsky *et al.*, 1978). One portion (5 ml) of 7.5 M-HCl removed all extractable 'green pigments' from the ethyl acetate, as judged from the absorbance or fluorescence spectra of further 7.5 M-HCl extracts. No 'green pigments' were detectable in the extracts from control livers, and, in agreement with earlier findings (De Matteis, 1971), only 0.5–1% of total radioactivity appeared in this fraction, associated with a similar proportion of total haem.

Measurement of radioactivity in homogenates and extracts

Portions (0.5–1.0 ml) of crude liver homogenate and subsequent extracts and washes were added to 10 ml of scintillation fluid (ACS, Amersham/Searle) and counted in a Mark III liquid scintillation spectrometer (Searle Analytic, Chicago, IL, U.S.A.) with an efficiency of 80–90%. For counting haem, ethyl acetate was evaporated, the haem was redissolved in alkaline pyridine, counted and quantified (Bonkowsky *et al.*, 1978). The actual d.p.m. of crude homogenates and purified haem were 50–200 times the background rate of about 25 d.p.m.

Quantitative determination of haem and 'green pigments'

Haem in crude homogenates was quantified from measurement of the pyridine haemochromes (Falk, 1964). Relative amounts of 'green pigments' were estimated from the absorption spectrum of the 7.5 M-HCl extract (Maines *et al.*, 1976). 'Green

pigments' were also found to fluoresce when excited at 400 nm, with a peak emission at 630 nm (uncorrected). Fluorescence spectra were obtained with a Perkin-Elmer 512 spectrofluorimeter equipped with an R777 photomultiplier tube (Hamamatsu Co., Hamamatsu, Japan) and a filter in the emission light-path preventing transmission of light at wavelengths less than 595 nm.

Statistics and calculations

Results are given as means $\pm$ S.E.M. unless indicated otherwise. Means were compared by Student's two-tailed *t* test (Dixon & Massey, 1968).

Data for haem disappearance were analysed by computer using the following algorithm. Using a polynomial fitting procedure, a smooth curve was generated from the average of the experimental observations. Using ten computer-generated points falling on this curve, plus the experimental points, an analysis was performed (a) to establish the minimum number of exponentials required; (b) to resolve each of the exponents using a peeling-off procedure (Frost & Pearson, 1961); and (c) to generate final curves minimizing the residual mean square error by iterative adjustment. In this analysis a weighting factor of 1.0 was given to the experimental data points and a weighting of 0.4 to the computer-generated points. The results showed that a minimum of two exponents was involved in haem decay. Apparent rate constants and pool sizes (as a fraction of total haem) were calculated by extrapolating the best-fit semi-logarithmic plots to the time point at 4 h (the time of treatment with 2-allyl-2-isopropylacetamide and ferric citrate).

Disappearance of radioactivity from 'green pigments' with time was plotted semilogarithmically by the method of least squares. The data were fitted to two straight lines of differing slopes, using the peeling-off procedure.

Results and Discussion

Effects of treatment with ferric citrate and 2-allyl-2-isopropylacetamide on liver weight and total liver haem content

As shown in Fig. 1(a), treatment of rats with ferric citrate alone had no effect on mean liver weight, whereas treatment with 2-allyl-2-isopropylacetamide (with or without ferric citrate) produced a significant increase in liver weight. Therefore, turnover data are presented on a 'total liver' basis, rather than 'per g of liver'.

Fig. 1(b) shows the time course of total hepatic haem for all treatment groups. Treatment with 2-allyl-2-isopropylacetamide decreased liver haem up to 32 h, after which time total liver haem was higher than control. Similar changes in cytochrome P-450 concentrations have been observed following

Fig. 1. Time course of the effect of 2-allyl-2-isopropylacetamide and ferric citrate on (a) total liver weights and (b) haem contents

Rats were treated as described in the Materials and Methods section. At various times after treatment, they were killed and liver weights and haem contents were determined. O, Controls; ●, ferric citrate; Δ, 2-allyl-2-isopropylacetamide; ▲, 2-allyl-2-isopropylacetamide plus ferric citrate. Results are plotted as mean $\pm$ S.E.M. for six to eight rats. The vertical arrow (†) indicates the time of administration of ferric citrate and 2-allyl-2-isopropylacetamide. Asterisks indicate times at which values for rats given 2-allyl-2-isopropylacetamide were significantly different (*P* ≤ 0.03) from controls. At 17 and 32 h total haem contents were lower in rats given ferric citrate alone than in controls.

a single injection of 2-allyl-2-isopropylacetamide (Meyer & Marver, 1971; De Matteis, 1973), suggesting that measurements of total haem reflect cytochrome P-450 haem concentrations.

During the first 13 h after treatment, rats given both 2-allyl-2-isopropylacetamide and ferric citrate had hepatic haem contents the same as rats given only 2-allyl-2-isopropylacetamide. Similarly, it was

previously reported that at 17h after treatment ferric citrate did not decrease cytochrome *P*-450 beyond the decrease produced by 2-allyl-2-isopropylacetamide alone (Bonkowsky *et al.*, 1979b). At 32 and 49h, however, total hepatic haem levels were lower in rats given both treatments (Fig. 1b).

In addition, at 17 and 32h, rats given ferric citrate alone had less total liver haem than appropriate controls. These results show a delay before iron affects hepatic haem contents. Other observations of cytochrome *P*-450 concentrations, 24h or more after parenteral iron administration, are compatible with these data (De Matteis & Sparks, 1973; Maines & Kappas, 1976; Ibrahim *et al.*, 1979; Bonkowsky *et al.*, 1979a). In contrast, there is a rapid synergism by enteral ferric citrate on drug-mediated induction of 5-aminolaevulinate synthase within 4h after iron treatment (Stein, *et al.*, 1969, 1970; Bonkowsky *et al.*, 1979b). The temporal disparity of these two iron effects suggests that they may not proceed through the same mechanism. Several possible mechanisms for the effect of iron on hepatic haem metabolism have recently been reviewed (Bonkowsky *et al.*, 1979b).

Hepatic uptake of 5-amino[4-¹⁴C]laevulinate and its incorporation into haem

Five to seven h after injection of 5-amino-[4-¹⁴C]laevulinate, 11% of the administered label was in the liver. This amount was not affected by 2-allyl-2-isopropylacetamide or ferric citrate.

In rats not given 2-allyl-2-isopropylacetamide, 97–104% of total liver radioactivity was in extractable haem during the first 13h after administration of label; thereafter, this proportion decreased (to 74% at 49h, Table 1). Since the half-lives of the haems of catalase, cytochrome *b*₅, and the mitochondrial cytochromes (catalase and *b*₅, 1–2 days; mitochondrial cytochromes, about 6 days) are longer than the haem of cytochrome *P*-450 (<1 day) (Aschenbrenner *et al.*, 1970; Meyer & Schmid, 1978), the extractable radioactivity at 49h is probably accounted for by the mitochondrial cytochromes, catalase, and cytochrome *b*₅. The unextractable 26% probably represents radioactivity in the covalently bound haem of cytochrome *c* that is not extractable without splitting the thioether linkage.

Treatment with 2-allyl-2-isopropylacetamide decreased the proportion of total liver radioactivity in extractable haem; surprisingly, the effect was greatest between 13 and 32h (Table 1).

Kinetic analysis of hepatic haem decay

The time dependence of depletion of labelled haem is shown in Figs. 2(a) and 2(b). For each treatment

Table 1. Time course of total liver radioactivity and distribution of the radioactivity among liver fractions in control rats and in rats treated with 2-allyl-2-isopropylacetamide

The radioactivities in whole homogenates, haem and 'green pigments' were determined and the percentage unextracted was calculated by difference. In some experiments all fractions were counted; the amount of total radioactivity recovered was $99.8 \pm 1.5\%$ for control rats and $98.5 \pm 1.1\%$ for rats given 2-allyl-2-isopropylacetamide. Treatments of rats with sodium citrate or ferric citrate by gavage did not affect results either for control rats or for those given 2-allyl-2-isopropylacetamide. Results are means $\pm$ S.E.M. for groups of 12–16 rats at each time period.																						
Fraction	Control							2-Allyl-2-isopropylacetamide-treated														
	Time after 5-amino[4- ^{14}C]laevulinate injection (h)							Proportion of total radioactivity (%)														
	4	7	10	13	17	32	49	5.5	7	10	13	17	32	49	5.5	7	10	13	17	32	49	
Haem	96.6 ± 3.2 —	97 ± 4.0 —	98.5 ± 3.0 —	103.5 ± 1.7 —	93.9 ± 3.7 —	82.1 ± 4.0 —	74.1 ± 2.8 —	92.5 ± 2.3 5.8	90.8 ± 3.8 11.7	87.6 ± 1.7 7.0	76.3 ± 2.9 6.7	67.3 ± 4.8 6.2	50.4 ± 1.9 5.9	54.1 ± 2.9 4.6	92.5 ± 0.3 ± 0.3	90.8 ± 4.2 ± 4.2	87.6 ± 0.9 ± 0.9	76.3 ± 0.4 ± 0.4	67.3 ± 0.2 ± 0.2	50.4 ± 0.4 ± 0.4	54.1 ± 0.4 ± 0.4	
'Green pigments'	3.4	3.0	1.5	0.0	7.1	17.9	25.9	1.7	0.0	5.4	17.0	26.5	44.1	41.3								
Unextracted	$10^{-3} \times$ Total radioactivity in liver homogenates (d.p.m./liver)																					
	323 ± 6.4	221 ± 12.5	186 ± 9.2	153 ± 8.3	108 ± 7.4	88.0 ± 5.9	61.1 ± 2.6	284 ± 8.2	200 ± 19.0	201 ± 10.0	108 ± 6.5	118 ± 11.7	90.4 ± 7.1	67.7 ± 3.0								

The radioactivities in whole homogenates, haem and 'green pigments' were determined and the percentage unextracted was calculated by difference. In some experiments all fractions were counted; the amount of total radioactivity recovered was 99.8 ± 1.5% for control rats and 98.5 ± 1.1% for rats given 2-allyl-2-isopropylacetamide. Treatments of rats with sodium citrate or ferric citrate by gavage did not affect results either for control rats or for those given 2-allyl-2-isopropylacetamide. Results are means ± s.e.m. for groups of 12–16 rats at each time period.

Fig.2. Decay of radioactivity in liver haem

Groups of rats were treated as described in the Materials and Methods section. At varying times after treatment, they were killed and extractable liver haem radioactivities were determined. (a) O, Controls; ●, ferric citrate. (b) △, 2-Allyl-2-isopropylacetamide; ▲, 2-allyl-2-isopropylacetamide plus ferric citrate. The vertical arrows indicate the time of administration of ferric citrate and 2-allyl-2-isopropylacetamide. Curves were generated by computer as indicated in the Materials and Methods section. The insets show the two components to each curve.

the depletion of labelled haem was adequately accounted for by a rate law of the form:

(Haem radioactivity)_t = P₁ e^{-k₁t} + P₂ e^{-k₂t}

where k₁ and k₂ are the apparent rate constants, and the pre-exponential factors P₁ and P₂ are the fractional depletion through each of the two processes. The kinetic parameters are listed in Table 2.

The insensitivity of the apparent rate constants to treatment with 2-allyl-2-isopropylacetamide or ferric citrate (Table 2) suggests that the rate-determining steps are not influenced by either compound. Instead, the effect of such treatments is to alter the fractional pool sizes of haem. The effect of 2-allyl-2-isopropylacetamide appears dominant when this drug and ferric citrate are given together. These findings indicate that treatment with 2-allyl-2-isopropylacetamide leads to increased degradation of hepatic haem by increasing the size of a rapidly-turning-over pool (P₁).

This conclusion is supported by consideration of the specific radioactivity of haem. In control rats the specific radioactivity of haem fell during the fourth to tenth hour of the experiment, whereas during the equivalent interval following administration of 2-allyl-2-isopropylacetamide, the specific radioactivity did not change (Table 1 and Fig. 2). At the time of drug administration (4h after the label), a good deal of the labelled haem apparently had already 'passed through' P₁ and had been replaced by unlabelled haem that was rapidly degraded; thus, stabilization of the specific radioactivity of total hepatic haem occurred. The conclusion that 2-allyl-2-isopropylacetamide increases the rate of degradation of newly-synthesized haem, which has a rapid turnover, agrees with earlier work (Meyer & Marver, 1971; De Matteis, 1971, 1978a). Our analysis indicates that this increase in degradation is due to an increase in pool size, not to an increase in rate constant.

Products of haem degradation ('green pigments') and their turnover

Treatment with 2-allyl-2-isopropylacetamide led

Table 2. Kinetic parameters of the best-fitting curves for decrease in hepatic haem radioactivities

Rats were treated as described in the Materials and Methods section. Since a minimum of two exponentials describes the decay curves, the data have been fitted to an equation of the form: (haem radioactivity)_t = P₁ e^{-k₁t} + P₂ e^{-k₂t}, as described in the Materials and Methods section. Curves were extrapolated back to the time of drug administration (4 h after 5-amino[4-¹⁴C]laevulinate was given). Mean values ± 2 s.d. (95% confidence limits) are presented.

Group	Treatment	Apparent rate constants (h ⁻¹)		Fractional sizes of pools	
		k ₁	k ₂	P ₁	P ₂
1	Control	0.18 ± 0.03	0.03 ± 0.003	0.38 ± 0.05	0.62 ± 0.05
2	Ferric citrate	0.16 ± 0.03	0.02 ± 0.004	0.67 ± 0.05	0.33 ± 0.03
3	2-Allyl-2-isopropylacetamide	0.21 ± 0.04	0.01 ± 0.003	0.81 ± 0.03	0.19 ± 0.02
4	2-Allyl-2-isopropylacetamide and ferric citrate	0.26 ± 0.02	0.02 ± 0.003	0.81 ± 0.03	0.19 ± 0.02

to hepatic accumulation of 'green pigments' as shown in Fig. 3(a). For unknown reasons, iron given with 2-allyl-2-isopropylacetamide decreased accumulation of hepatic 'green pigments', an effect also seen with cobalt (Maines *et al.*, 1976).

Total radioactivity in the 'green pigments' is plotted in Fig. 3(b). Labelling of this fraction showed

Fig. 3. Time course of the content (a) and radioactivity (b) of hepatic 'green pigments' from rats treated with 2-allyl-2-isopropylacetamide plus ferric citrate

Rats were treated with 2-allyl-2-isopropylacetamide alone (□) or 2-allyl-2-isopropylacetamide plus ferric citrate (■) 4 h (†) after they had received 5-amino[4- ^{14} C]laevulinate. At varying times after treatment they were killed and 'green pigments' were extracted as described in the Materials and Methods section. In (a), total content, as estimated by absorbance of the HCl solution at 423 nm, has been plotted. Asterisks indicate times at which the livers of rats given ferric citrate plus 2-allyl-2-isopropylacetamide contained significantly less ($P \leq 0.03$) 'green pigments' than livers of rats given the drug alone. In (b) total radioactivity in 'green pigments' has been plotted semi-logarithmically.

a biphasic decrease, indicating the existence of at least two pools. The estimated half-life of radioactivity for the first component is 1–2 h, and for the second, 23 h.

As shown in Table 1, second and third rows, some of the radioactivity that earlier was found in the 'green pigments' fraction later appeared to be associated with the aqueous fraction, i.e., was no longer extractable by acidic ethyl acetate. In the first 3 h after 2-allyl-2-isopropylacetamide treatment (7 h after labelled 5-aminolaevulinate) the 'green pigments' fraction contained virtually all the radioactivity not in haem; however, at 28 h after 2-allyl-2-isopropylacetamide treatment, most non-haem radioactivity was in the non-extractable aqueous fraction, suggesting that some label had passed through the 'green pigments' fraction and was now in a more polar form. This increase in the percentage of total radioactivity in the non-extractable aqueous fraction was greater than that in control rats, which was attributed (see above) to the haem of cytochrome c.

Model for hepatic haem metabolism

Fig. 4 presents a model for hepatic haem metabolism compatible with the above results. In the model, treatment with 2-allyl-2-isopropylacetamide and/or ferric citrate is envisioned to increase the amount of labelled 5-aminolaevulinate going into P_1 , the more rapidly turning-over pool of hepatic haem.

Fig. 4. A model for the effects of 2-allyl-2-isopropylacetamide and ferric citrate on hepatic haem turnover

This proposed model is compatible with our kinetic analyses and with other data in the literature. See text for further details. $\oplus$ indicates enhancement of pathway; AIA, 2-allyl-2-isopropylacetamide.

Administration of certain other compounds that enhance hepatic haem turnover (e.g. Co^{2+} and other metallic ions, endotoxin, phenobarbital, and allyl-containing compounds other than 2-allyl-2-isopropylacetamide) may act similarly. The physical basis for this effect is unknown. Perhaps the various treatments alter the membranes of the endoplasmic reticulum or alter the mitochondrial-endoplasmic reticulum complexes (Meier *et al.*, 1978), favoring enrichment of this haem pool. The haem in P_1 may be associated with ligandin, certain species of cytochrome P-450, tryptophan 2,3-dioxygenase, or other haemoproteins.

The P_1 pool may then be envisioned to give rise to an unstable intermediate. The rate constant (k_1) for generation of this intermediate is not altered by treatment with 2-allyl-2-isopropylacetamide or ferric citrate, although the net rate of production of this unstable intermediate is increased by such treatments, owing to the increase in P_1 . This unstable intermediate is rapidly turned over. Previously, such an intermediate has been suggested to be the endogenous substrate for haem oxygenase (Maines, 1977). As shown in Fig. 4, it may also be susceptible to breakdown to 'green pigments' by 2-allyl-2-isopropylacetamide treatment. Therefore, after 2-allyl-2-isopropylacetamide treatment, the haem oxygenase contribution to haem catabolism may be expected to decrease, with decreased bilirubin production, as has been reported (Landaw *et al.*, 1970).

Treatment with 2-allyl-2-isopropylacetamide appears to have two effects that enhance hepatic haem degradation. Firstly, it increases the movement of newly synthesized haem into P_1 , and secondly it leads to production of 'green pigments' from the unstable intermediate. In contrast, ferric citrate treatment appears only to increase P_1 ; this increase, by the law of mass action, leads to an increase in the production of unstable intermediate. An increase in the amount of this intermediate, without stimulation of an alternative catabolic route, may lead to increased haem oxygenase activity as has been described following administration of iron (Maines & Kappas, 1976, 1977; Ibrahim *et al.*, 1979), and other metallic ions, most notably Co^{2+} (Maines *et al.*, 1976; Maines & Kappas, 1977; Maines & Sinclair, 1977).

We wish to thank L. Kaminsky, D. Tschudy and J. Sinclair for helpful comments concerning the manuscript. J. Pomeroy and J. Bement provided able technical assistance. This work was supported by research funds from the U.S. Veterans Administration and by a grant (CA 25012) from the National Cancer Institute, National Institutes of Health.

References

Aschenbrenner, V., Druyan, R., Albin, R. & Rabinowitz, M. (1970) *Biochem. J.* **119**, 157–160

- Bonkowsky, H. L., Bement, W. J. & Erny, R. (1978) *Biochim. Biophys. Acta* **541**, 119–123
- Bonkowsky, H. L., Healey, J. & Carpenter, S. J. (1979a) *Arch. Pathol. Lab. Med.* **103**, 21–29
- Bonkowsky, H. L., Sinclair, P. & Sinclair, J. F. (1979b) *Yale J. Biol. Med.* **52**, 13–37
- Bonkowsky, H., Sinclair, P., Healey, J., Sinclair, J. & Myer, Y. (1980) in *Microsomes, Drug Oxidations and Chemical Carcinogenesis*, vol. 2 (Coon, M. J., Conney, A. H., Estabrook, R. W., Gelboin, H. V., Gillette, J. R. & O'Brien, P. J., eds.), Academic Press, New York, in the press.
- De Matteis, F. (1971) *Biochem. J.* **124**, 767–777
- De Matteis, F. (1973) *Drug Metab. Dispos.* **1**, 267–274
- De Matteis, F. (1978a) *Handb. Exp. Pharmacol.* **44**, 129–155
- De Matteis, F. (1978b) *Pharmacol. Ther.* **2**, 693–726
- De Matteis, F. & Cantoni, L. (1979) *Biochem. J.* **183**, 99–103
- De Matteis, F. & Sparks, R. G. (1973) *FEBS Lett.* **29**, 141–144
- De Matteis, F. & Unseld, A. (1976) *Biochem. Soc. Trans.* **4**, 205–209
- Dixon, W. J. & Massey, F. J., Jr. (1968) *Introduction to Statistical Analysis*, 3rd edn., p. 328, McGraw-Hill, New York
- Falk, J. E. (1964) *Porphyryns and Metalloporphyryns*, Elsevier, Amsterdam
- Frost, A. A. & Pearson, R. A. (1961) *Kinetics and Mechanism*, 2nd edn., John Wiley and Sons, New York and London
- Ibrahim, N. G., Hoffstein, S. T. & Freedman, M. L. (1979) *Biochem. J.* **180**, 257–263
- Landaw, S. A., Callahan, E. W., Jr. & Schmid, R. (1970) *J. Clin. Invest.* **49**, 914–925
- Maines, M. D. (1977) in *Microsomes and Drug Oxidations* (Ullrich, V., Roots, I., Hildebrandt, A., Estabrook, R. W. & Conney, A. H., eds.), pp. 543–550, Pergamon Press, Oxford and New York
- Maines, M. D. & Kappas, A. (1976) *Biochem. J.* **154**, 125–131
- Maines, M. D. & Kappas, A. (1977) *Science* **198**, 1215–1221
- Maines, M. D. & Sinclair, P. (1977) *J. Biol. Chem.* **252**, 219–223
- Maines, M. D., Janousek, V., Tomio, J. M. & Kappas, A. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1499–1503
- Meier, P. J., Spycher, M. A. & Meyer, U. A. (1978) *Exp. Cell Res.* **111**, 479–483
- Meyer, U. A. & Marver, H. S. (1971) *Science* **171**, 64–66
- Meyer, U. A. & Schmid, R. (1978) in *The Metabolic Basis of Inherited Disease*, 4th edn., (Stanbury, J. B., Wyngaarden, J. B., & Fredrickson, D. S., eds.), pp. 1166–1220, McGraw-Hill, New York
- Ortiz de Montellano, P. R., Mico, B. A. & Yost, G. S. (1978) *Biochem. Biophys. Res. Commun.* **83**, 132–137
- Schwartz, S. & Ikeda, K. (1955) *Porphyry Biosynthesis*, *Ciba Found. Symp.*, pp. 209–228
- Stein, J., Berk, P. & Tschudy, D. (1969) *Life Sci.* **8**, 1023–1031
- Stein, J. A., Tschudy, D. P., Corcoran, L. P. & Collins, A. (1970) *J. Biol. Chem.* **245**, 2213–2218
- Tschudy, D. P. & Bonkowsky, H. L. (1972) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **31**, 147–159