Biochem. J. (1986) 238, 263–268 (Printed in Great Britain)

N-Alkylation of exogenous haem analogues caused by drugs in isolated hepatocytes

Structural isomerism and chirality of the resulting porphyrins

Francesco DE MATTEIS,* Carolyn HARVEY* and Stephen R. MARTIN†

*M.R.C. Toxicology Unit, M.R.C. Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF, U.K., and †Division of Physical Biochemistry, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.

1. Isolated rat hepatocytes incubated with two suicide substrates of cytochrome *P*-450, 2-allyl-2-isopropylacetamide and 3,5-diethoxycarbonyl-4-ethyl-1,4-dihydro-2,6-dimethylpyridine (4-ethyl-DDC), convert exogenous mesohaem and deuterohaem into *N*-alkylated mesoporphyrins and deuteroporphyrins respectively. 2. The *N*-alkylated mesoporphyrins can be separated by h.p.l.c. from the corresponding *N*-alkylated protoporphyrins originating from endogenous haem; in this way the contribution of both endogenous and exogenous pools of haem can be studied in the same experiment. 3. *N*-Alkylated mesoporphyrin exhibits chiral properties, and its isomeric composition and/or amount are dependent on the particular cytochrome *P*-450 enzyme predominating in the cell. 4. These findings provide additional and more direct evidence that exchangeable haem is taken up by cytochrome *P*-450 before being *N*-alkylated.

INTRODUCTION

Two classes of suicide substrates of cytochrome *P*-450, drugs with unsaturated side chains and 4-alkyl-substituted dihydropyridines, both convert the haem prosthetic group of the cytochrome into *N*-alkylated porphyrins, i.e. porphyrins substituted at one of their pyrrole nitrogen atoms. The detailed mechanism involved differs in the two cases, as the substituent found on the pyrrole nitrogen is the whole drug plus an oxygen atom in the case of the unsaturated compounds, but only a small fragment of the drug, the 4-alkyl group, in the case of the dihydropyridines (De Matteis *et al.*, 1982*a*; Ortiz de Montellano & Correia, 1983; White, 1984). Exogenous protohaem can be utilized in both types of *N*-alkylation reactions, as shown by (*a*) its ability to undergo drug-dependent destruction *in vitro* (Unseld & De Matteis, 1978), (*b*) recovery of radioactivity from labelled haematin in purified *N*-alkylated porphyrins *in vivo* and *in vitro* (Correia *et al.*, 1979; De Matteis *et al.*, 1982*c*), and (*c*) the increased yield of alkylated porphyrins observed when isolated hepatocytes are incubated with drugs in the presence of excess haematin (De Matteis *et al.*, 1982*c*, 1983).

It has been suggested that exogenously administered haematin and other exchangeable pools of haem, including the endogenous 'regulatory' haem pool, must first be taken up by the apoprotein of cytochrome *P*-450 in order to be subjected to *N*-alkylation (De Matteis, 1978; Correia *et al.*, 1979, 1981). This concept, which has important implications for the utilization of haem, and for the regulation of liver haem biosynthesis, is supported by the finding that cytochrome *P*-450 made haem-deficient by treatment with drugs can be partially reconstituted by supplying excess exogenous haem (Sardana *et al.*, 1976; Farrell *et al.*, 1981). However, there is no direct evidence that *N*-alkylation of exchangeable pools of haem takes place within the apoprotein of cytochrome *P*-450. The findings by Correia *et al.* (1981), that the di-isopropyl ester derivative of haem does not reconstitute cytochrome *P*-450 *in vitro* and is not converted into *N*-alkylated porphyrins, cannot be interpreted unequivocally. The ester differs markedly from the physiological (free carboxylate) form of haem in solubility properties and may therefore be inaccessible to alkylation, whether inside or outside cytochrome *P*-450.

The main purpose of the present paper is to show that the protohaem analogue mesohaem can be converted by isolated hepatocytes into *N*-alkylated porphyrins of the two classes defined above as effectively as protohaem. Since the *N*-alkylated mesoporphyrins thus produced can be separated by h.p.l.c. from the corresponding alkylated protoporphyrins originating from the cellular protohaem, the *N*-alkylation of endogenous and exogenous pools of haem can be studied simultaneously and the contributions of both pools determined in the same sample. The *N*-alkylmesoporphyrin produced by the hepatocytes exhibits chiral properties and its isomeric composition is influenced by the particular cytochrome *P*-450 species predominating in the cell. This provides additional and more direct evidence that exogenous (exchangeable) haem is *N*-alkylated at the cytochrome *P*-450 active site. Some of these findings have been presented elsewhere in a preliminary form (De Matteis *et al.*, 1985*a*).

Abbreviations used: 4-ethyl-DDC, 3,5-diethoxycarbonyl-4-ethyl-1,4-dihydro-2,6-dimethylpyridine; AIA, 2-allyl-2-isopropylacetamide.

MATERIALS AND METHODS

Treatment of animals and experiments with hepatocytes

Male fed rats of the Porton (Wistar-derived) strain, ranging in body weight from 200 to 230 g, were used throughout. Some were pretreated with inducers of cytochrome *P*-450 isoenzymes as previously described (De Matteis *et al.*, 1983). Hepatocytes were prepared by a collagenase perfusion technique (Paine & Legg, 1978), and viability (Trypan Blue exclusion) was 70–94% at the beginning of the incubation. The method used for incubation of hepatocytes has already been described (De Matteis *et al.*, 1982*c*). Protohaem was added as methaemalbumin, which had been prepared, with the use of crystalline human albumin, by the method of Tenhunen *et al.* (1968). Mesohaem and deuterohaem were prepared from mesoporphyrin IX and deuteroporphyrin IX by the ferrous sulphate method described by Falk (1964), and were added to hepatocyte suspensions complexed to human albumin as for protohaem.

Isolation and characterization of *N*-alkylated porphyrins

At the end of the incubation the hepatocytes were obtained by centrifugation, and the *N*-alkylated porphyrins were extracted from the hepatocyte pellets, isolated by column chromatography on Sephadex LH-20 and determined in the column eluate by using the same $\epsilon = 228\ mM^{-1} \cdot cm^{-1}$ for the Soret absorption of the dication derivative, regardless of the type of alkylated pigment under study. The porphyrins were further purified by t.l.c., and their methyl ester derivatives were chromatographed on an h.p.l.c. column (4.6 mm × 250 mm) of Nucleosil 5, with isocratic elution with dichloromethane/methanol/conc. NH_3 (sp.gr. 0.88) (250:250:1, by vol.) and a flow rate of 2 ml/min. This system separates the *N*-ethylprotoporphyrin IX into two chromatographic fractions (F_1 and F_2) each consisting of a pair of structural isomers, and both *N*-ethylmesoporphyrin IX and *N*-ethyldeuteroporphyrin IX are completely resolved into all four structural isomers (De Matteis *et al.*, 1985*b*). The amounts of the various isomers were calculated from the area under the h.p.l.c. elution profile, with correction for overall recovery by comparison with amount of pigment isolated from the Sephadex LH-20 column, as described previously (De Matteis *et al.*, 1983). The various alkylated porphyrins were identified by recording their electronic spectra, and in the case of the *N*-ethylated porphyrins also by comparing the retention times obtained in the above h.p.l.c. system for the four structural isomers with those of the authentic synthetic *N*-ethylated porphyrins (De Matteis *et al.*, 1985*b*). However, the isomeric type of each structural isomer of *N*-ethylmesoporphyrin and of *N*-ethyldeuteroporphyrin (i.e. which of the four pyrrole nitrogens was ethylated in each isomeric fraction) was not established.

Samples for c.d. spectroscopy were prepared at a concentration of approx. 14 μM in dichloromethane. Exact concentrations were determined by using $\epsilon = 127800\ M^{-1} \cdot cm^{-1}$ for the Soret absorption of neutral porphyrin in spectra in both cases. C.d. spectra were recorded on a Jasco J41C spectropolarimeter equipped with a model J-DPY data processor. Digitized spectra collected by the processor were transferred to a PDP 11/23 computer and processed for baseline subtraction, normalization and smoothing. Spectra were routinely recorded from 500 to 380 nm at a sensitivity of 0.5 mdegrees/cm with an instrumental time constant of 4 s. The spectra reported here were recorded at 22 °C in 10 mm cuvettes and are the averages for four scans. The spectra are presented as molar circular dichroism, $\Delta\epsilon$. Molar ellipticity may be obtained from (Bayley, 1980) $[\theta] = 3300\Delta\epsilon$.

Source of special chemicals

Authentic *N*-ethylprotoporphyrin IX, *N*-ethylmesoporphyrin IX and *N*-ethyldeuteroporphyrin IX (all dimethyl esters) were prepared from their respective parent porphyrins as described previously (De Matteis *et al.*, 1985*b*). 4-Ethyl-DCC was prepared by the method given in De Matteis & Prior (1962), starting with propionaldehyde, and AIA was a gift from Roche Products. Methanol and dichloromethane (h.p.l.c. grade) were obtained from Rathburn Chemicals.

RESULTS AND DISCUSSION

Production of *N*-ethylated porphyrins by 4-ethyl-DDC

When exogenous mesohaem was added to hepatocytes suspension, the production of *N*-alkylated porphyrins due to either 4-ethyl-DDC or AIA was significantly stimulated. Compared with the previously reported effect of protohaem addition, mesohaem was at least as effective at increasing the yield of *N*-alkyl porphyrins with both AIA and 4-ethyl-DDC (Table 1). Evidence that the protohaem analogue was itself accepted for *N*-alkylation, giving rise to *N*-alkylated mesoporphyrins, is described below.

The alkylated pigments produced by isolated hepatocytes on incubation with 4-ethyl-DDC alone could be resolved by h.p.l.c. into two different components with retention times characteristic of the two *N*-ethylprotoporphyrin isomeric pairs (the two isomers with ring A and B ethylated being eluted first, followed by the ring C and D isomers). In contrast, when mesohaem was added to the incubation, in addition to the first two peaks corresponding to *N*-ethylprotoporphyrin, four more peaks could be detected in the h.p.l.c. elution profile, each corresponding in retention time to a different structural isomer of *N*-ethylmesoporphyrin (Fig. 1). In one experiment, in which deuterohaem was added, in addition to the two peaks attributable to *N*-ethylprotoporphyrin isomers, four more peaks could be demonstrated, with retention times very similar to those of the four isomers of authentic *N*-ethyldeuteroporphyrin (results not shown). These findings indicate that 4-ethyl-DDC can transfer its 4-ethyl group, not only to endogenous cellular protohaem (thus producing *N*-ethylprotoporphyrin), but also to exogenous haem analogues (mesohaem and deuterohaem), giving rise to the corresponding *N*-ethylated porphyrins.

The yield of *N*-ethylmesoporphyrins produced from exogenous mesohaem was increased by pretreatment of the rat *in vivo* with either phenobarbitone or β-naphthoflavone, the classical inducers of cytochrome *P*-450 and cytochrome *P*-448 respectively (Table 2). In addition, the isomeric composition of the *N*-ethylmesoporphyrin was found to be influenced by such pretreatments, particularly by phenobarbitone, so that the ratios between the various isomers differed significantly according to the type of cytochrome *P*-450 predominating in the hepatocytes (see Table 2, where the IV/I isomeric

Table 1. Comparison between exogenous protohaem and mesohaem as precursors of *N*-alkylated porphyrins in hepatocyte suspensions incubated with either AIA or 4-ethyl-DDC

Hepatocytes isolated from rats pretreated with either phenobarbital or β-naphthoflavone *in vivo* were incubated for 4 h in a suspension (40 ml) to which AIA (16 mg) or 4-ethyl-DDC (0.6 mg) was added both at the beginning and after 2 h incubation, each time in 0.1 ml of ethanol. Exogenous haems were added at the start of the incubation to a final concentration of 6.25 μM. Results are given as means ± S.E.M. for at least three observations (each obtained with the pooled hepatocytes of two separate incubations) or averages with individual observations in parentheses. The results obtained with protohaem [marked with an asterisk (*)] are from previous work (De Matteis *et al.*, 1982*c*; 1983) and are given for comparison.

Pretreatment of the rat *in vivo*	Drug	Exogenous haem	*N*-Alkylated porphyrins: Amount (nmol/100 × 10^6 cells)	*N*-Alkylated porphyrins: Increase due to exogenous haem (%)
Phenobarbital	AIA	None	13.1 ± 0.8 (3)	108*
		Protohaem	27.4 ± 2.9 (3)	
Phenobarbital	AIA	None	11.6 ± 2.5 (3)	92
		Mesohaem	22.3 ± 4.4 (3)	
Phenobarbital	4-Ethyl-DDC	None	4.9 ± 0.3 (8)	94*
		Protohaem	9.5 ± 0.4 (6)	
Phenobarbital	4-Ethyl-DDC	None	4.2 (3.5, 5.0)	293
		Mesohaem	16.5 (13.2, 19.8)	
β-Naphthoflavone	4-Ethyl-DDC	None	7.1 (7.0, 7.2)	89*
		Protohaem	13.4 (14.1, 12.8)	
β-Naphthoflavone	4-Ethyl-DDC	None	4.0 (3.3, 4.8)	212
		Mesohaem	12.6 (10.7, 14.5)	

ratios are given as an example). Similar findings have been reported for the *N*-ethylprotoporphyrin produced from the endogenous protohaem of the hepatocytes, although in that case changes in isomeric composition seen after induction of the two main cytochrome *P*-450 isoenzymes showed a different pattern and were more pronounced (De Matteis *et al.*, 1983). These findings are compatible with a role of the apo-cytochrome in directing alkylation on to different pyrrole nitrogen atoms and therefore suggest that the exogenous haem is taken up by the apoprotein before undergoing *N*-alkylation.

The identity of each structural isomer of *N*-ethylmesoporphyrin has not been established, so a direct comparison of the isomeric profile presented in this work (Table 2) and that reported for the *N*-ethylprotoporphyrin, obtained under similar conditions from protohaem (De Matteis *et al.*, 1983), is not possible. However, the differences in isomeric pattern referred to above suggest that the four pyrrole nitrogen atoms of the tetrapyrrolic macrocycles are ethylated in different proportions in the two cases. More work is necessary to establish whether this reflects differences between protohaem and mesohaem in the way in which they are bound at the cytochrome *P*-450 active site or in the relative reactivity of the four pyrrole nitrogen atoms.

Effect of AIA

Evidence for *N*-alkylation of exogenous mesohaem and deuterohaem was also obtained after treatment of hepatocytes with AIA. In addition to the peak of alkylated protoporphyrin obtained from endogenous protohaem, a second peak was also seen on addition of either mesohaem or deuterohaem, and this was identified as *N*-alkylmesoporphyrin (or deuteroporphyrin) from the electronic absorption spectrum (Table 3 and Fig. 2). Previous work (Smith & Farmer, 1982) has shown that the absorption spectrum of a *N*-alkylated porphyrin is diagnostic for the type of porphyrin that is *N*-substituted and is usually unaffected by the nature of the alkyl group

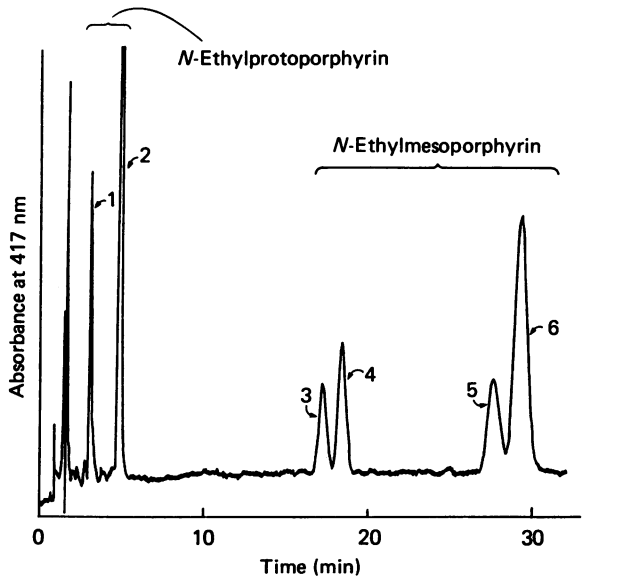

Fig. 1. H.p.l.c. elution profile of the *N*-ethylated porphyrins obtained from hepatocyte suspensions incubated with 4-ethyl-DDC in the presence of mesohaem

Hepatocytes from a rat pretreated with β-naphthoflavone were incubated for 4 h as indicated in the legend to Table 1. Peaks 1 and 2 were identified as structural isomers of *N*-ethylprotoporphyrin (each peak containing two isomers) and peaks 3–6 as the individual isomers of *N*-ethylmesoporphyrin (see the text).

Table 2. Effect of pretreatment of rats with phenobarbital or β-naphthoflavone *in vivo* on amount and isomeric composition of *N*-ethylmesoporphyrin formed from exogenous mesohaem by isolated hepatocytes treated with 4-ethyl-DDC

Hepatocytes were incubated with 4-ethyl-DDC and mesohaem for 4 h as indicated in the legend to Table 1. Results are those of individual observations, each obtained with the pooled hepatocytes of two separate incubations. The four isomers are given in the order in which they were eluted from the h.p.l.c. column.

Pretreatment of the rat *in vivo*	*N*-Ethylmesoporphyrin produced					
	Amount (nmol/100 × 10^6 cells)	Isomer composition (%)				IV/I isomeric ratio
		I	II	III	IV	
None	4.21	12.1	21.1	20.4	46.4	3.8
	4.60	11.6	22.6	18.3	47.5	4.1
Phenobarbitone	9.69	5.3	4.0	30.0	60.6	11.4
	14.82	6.0	7.6	31.4	55.0	9.2
β-Napthoflavone	9.74	9.9	15.9	18.4	55.8	5.6
	7.41	9.2	17.2	18.1	55.4	6.0

Table 3. Retention times on h.p.l.c. and neutral spectra of *N*-alkylated porphyrins produced by hepatocytes incubated with AIA

Hepatocytes obtained from rats pretreated with phenobarbital *in vivo* were incubated for 4 h with AIA, in the presence or in the absence of an exogenous haem, as indicated in the legend to Table 1. The alkylated porphyrins were analysed by h.p.l.c. as described in the Materials and methods section, and the porphyrins eluted as individual peaks were isolated and their electronic spectra recorded. The following absorption maxima were obtained for authentic *N*-methylated protoporphyrin, mesoporphyrin and deuteroporphyrin respectively: values given are those of the Soret band, band IV and band I in this order: 419, 513 and 652 nm; 411.5, 506 and 641.5 nm; 410.5, 504 and 641 nm.

Exogenous haem added	Alkylated porphyrin detected	Retention time on h.p.l.c. (min)	Absorption maxima of bands (nm)		
			Soret	IV	I
None	Protoporphyrin	1.53	417	512	651
Protohaem	Protoporphyrin	1.52	417	512	651
Mesohaem	(*a*) Protoporphyrin	1.53	417	512	650
	(*b*) Mesoporphyrin	1.92	411	505	641
Deuterohaem	(*a*) Protoporphyrin	1.56	417	512	651
	(*b*) Deuteroporphyrin	1.65	412	506	642

present on the pyrrole nitrogen atom. The close similarity of the two spectra shown in Fig. 2 to those of authentic *N*-methylated protoporphyrin and mesoporphyrin indicates that the two pigments obtained from hepatocytes incubated with AIA and mesohaem contain protoporphyrin and mesoporphyrin respectively as their tetrapyrrolic nucleus.

Hepatocyte suspensions were incubated with AIA and mesohaem for different periods of time, so as to compare endogenous and exogenous haems as precursors of *N*-alkylated porphyrins. At short times of incubation the major proportion of *N*-alkylated porphyrin was of the protoporphyrin type, but the alkylated mesoporphyrin gradually increased in time, representing the major proportion of the accumulating pigment after 4 h incubation (Fig. 3). A likely interpretation for these findings is that the endogenous haem of cytochrome *P*-450 is first alkylated, then leaves the apoprotein free to take up exchangeable pools of haem (represented in this case mostly by exogenously administered mesohaem). A functional cytochrome *P*-450 is again obtained (Bornheim *et al.*, 1984), and this can then catalyse the suicidal alkylation of its new prosthetic group, producing alkylated mesoporphyrin.

The c.d. spectra of the two purified pigments were also recorded (Fig. 4): both pigments showed a prominent band of negative ellipticity at about 410 nm; in addition, the protoporphyrin-containing pigment, but not the mesoporphyrin, showed a small positive band at about 430 nm. Thus both pigments exhibited chiral properties, as would be expected if the *N*-alkylation reaction (responsible for their production) was taking place on an enzymic template (Ortiz de Montellano & Kunze, 1981; De Matteis *et al.*, 1982*b*; Ortiz de Montellano *et al.*, 1983) in both cases. This finding is therefore entirely compatible with the exogenous haem being taken up by apoprotein of cytochrome *P*-450 before being *N*-alkylated.

GENERAL CONCLUSION

The evidence produced in the present paper provides support for the view that exchangeable pools of haem can be utilized for conversion into *N*-alkylated porphyrins

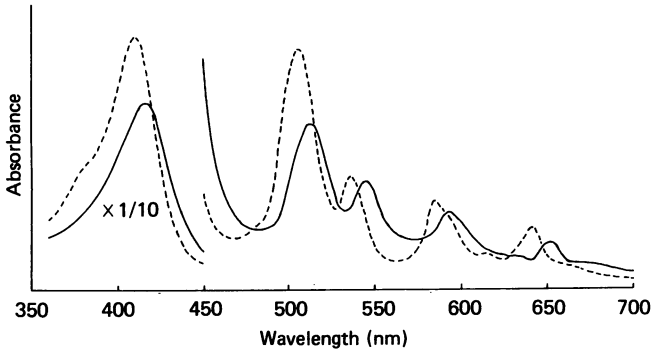

Fig. 2. Electronic spectra of the two alkylated products obtained on incubation of hepatocytes with AIA in presence of mesohaem

Hepatocytes from rats pretreated with phenobarbital were incubated with AIA and mesohaem for 4 h as indicated in the legend to Table 1. The two alkylated porphyrins were separated by h.p.l.c., and the neutral spectra were recorded in chloroform: ——, *N*-alkylprotoporphyrin; ------, *N*-alkylmesoporphyrin. Note that the scale of the ordinates has been decreased 10-fold for the Soret region of the spectra.

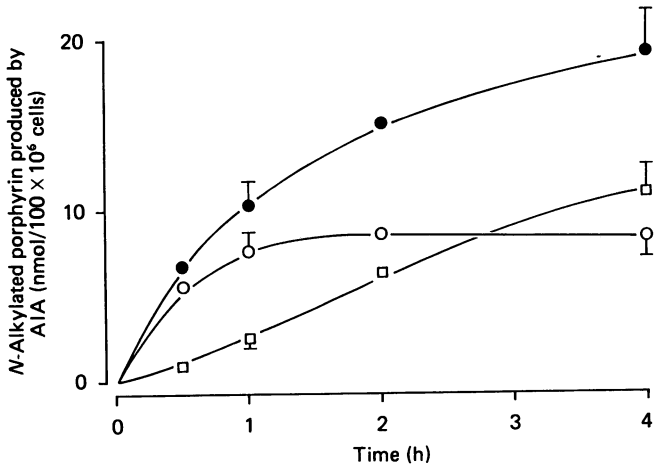

Fig. 3. Time course of the AIA-dependent accumulation of *N*-alkylprotoporphyrin and *N*-alkylmesoporphyrin in hepatocyte suspensions

Hepatocytes from rats pretreated with phenobarbital were incubated with AIA and mesohaem for different periods of time as indicated in the legend to Table 1. The methods for isolation of the alkylated products and h.p.l.c. analysis are given in the Materials and methods section. Results are averages for two observations, each obtained with the pooled hepatocytes of two separate incubations, or means ± S.E.M. for seven observations: ●, total *N*-alkylated porphyrins; ○, *N*-alkylprotoporphyrin; □, *N*-alkylmesoporphyrin.

after treatment with two classes of suicide substrates of cytochrome *P*-450, examples of which are AIA and 4-ethyl-DDC. It is now found that the protohaem analogue mesohaem can be utilized in both *N*-alkylation pathways at least as effectively as protohaem. As in this case the products of *N*-alkylation of endogenous and exogenous haem (*N*-alkylprotoporphyrin and *N*-alkylmesoporphyrin respectively) can be separated by h.p.l.c., this allows a study of the contribution of both haem pools in the same experiment. The *N*-alkylmesoporphyrin exhibits chiral properties, and its isomeric composition and/or amount are dependent on the particular cytochrome *P*-450 enzyme predominating in the cell. This provides additional and more direct evidence that exchangeable haem is taken up by cytochrome *P*-450 before being *N*-alkylated. These experiments offer a new approach to the study of the mechanisms by which exogenous haem is taken up by the hepatocytes, enters the exchangeable pool of intracellular free haem and is utilized for cytochrome *P*-450 by the intact cell *in vivo*.

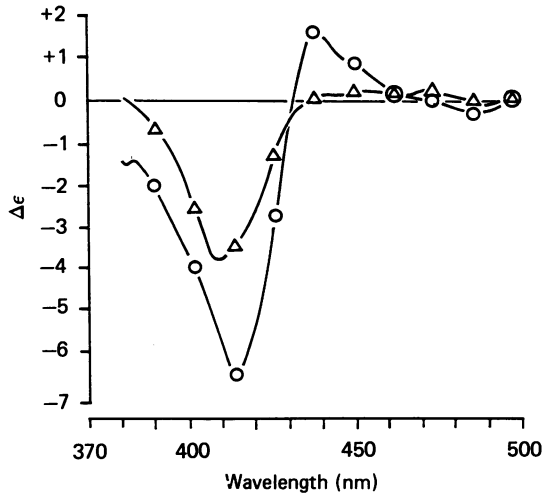

Fig. 4. C.d. spectra of the two alkylated products obtained from hepatocytes incubated with AIA in the presence of mesohaem

The two pigments were obtained as indicated in the legend to Fig. 2. Spectra were recorded at a concentration of the porphyrin dimethyl ester of approx. 14 μM: ○, *N*-alkylprotoporphyrin; △, *N*-alkylmesoporphyrin.

We thank Dr. P. M. Bayley for helpful discussion and Mr. A. H. Gibbs for preparing the standard *N*-alkylated porphyrins.

REFERENCES

Bayley, P. (1980) in An Introduction to Spectroscopy for Biochemists (Brown, S. B., ed.), p. 151, Academic Press, London

Bornheim, L. M., Correia, M. A. & Smith, K. M. (1984) Biochem. Biophys. Res. Commun. **121**, 95–101

Correia, M. A., Farrell, G. C., Schmid, R., Ortiz de Montellano, P. R., Yost, G. S. & Mico, B. A. (1979) J. Biol. Chem. **254**, 15–17

Correia, M. A., Farrell, G. C., Olson, S., Wong, J. S., Schmid, R., Ortiz de Montellano, P. R., Beilan, H. S., Kunze, K. L. & Mico, B. A. (1981) J. Biol. Chem. **256**, 5466–5470

De Matteis, F. (1978) Handb. Exp. Pharmacol. **44**, 129–155

De Matteis, F. & Prior, B. (1962) Biochem. J. **83**, 1–8

De Matteis, F., Gibbs, A. H., Farmer, P. B., Lamb, J. H. & Hollands, C. (1982*a*) Adv. Pharmacol. Ther. Proc. Int. Congr. 8th **5**, 131–138

De Matteis, F., Jackson, A. H., Gibbs, A. H., Rao, K. R. N., Atton, J., Weerasinghe, S. & Hollands, C. (1982*b*) FEBS Lett. **142**, 44–48

De Matteis, F., Hollands, C., Gibbs, A. H., de Sa, N. & Rizzardini, M. (1982*c*) FEBS Lett. **145**, 87–92
De Matteis, F., Gibbs, A. H. & Hollands, C. (1983) Biochem. J. **211**, 455–461
De Matteis, F., Gibbs, A. H. & Hollands, C. (1985*a*) in Microsomes and Drug Oxidations (Boobis, A. R., Caldwell, J., De Matteis, F. & Elcombe, C. R., eds.), pp. 265–273, Taylor and Francis, London
De Matteis, F., Gibbs, A. H. & Harvey, C. (1985*b*) Biochem. J. **226**, 537–544
Falk, J. E. (1964) BBA Libr. **2**, 133–135
Farrell, G. C., Gollan, J. L., Correia, M. A. & Schmid, R. (1981) J. Pharmacol. Exp. Ther. **218**, 363–367
Ortiz de Montellano, P. R. & Correia, M. A. (1983) Annu. Rev. Pharmacol. Toxicol. **23**, 481–503
Ortiz de Montellano, P. R. & Kunze, K. L. (1981) Biochemistry **20**, 7266–7271
Ortiz de Montellano, P. R., Kunze, K. L. & Beilan, H. S. (1983) J. Biol. Chem. **258**, 45–47
Paine, A. J. & Legg, R. F. (1978) Biochem. Biophys. Res. Commun. **81**, 672–679
Sardana, M. K., Rajamanickam, C. & Padmanaban, G. (1976) in Porphyrins in Human Disease (Doss, M., ed.), pp. 62–70, S. Karger, Basel
Smith, A. G. & Farmer, P. B. (1982) Biomed. Mass Spectrom. **9**, 111–114
Tenhunen, R., Marver, H. S. & Schmid, R. (1968) Proc. Natl. Acad. Sci. U.S.A. **61**, 748–755
Unseld, A. & De Matteis, F. (1978) Int. J. Biochem. **9**, 865–869
White, I. (1984) Pharm. Res. 141–188

Received 3 February 1986/9 April 1986; accepted 28 April 1986