In summary, ecdysone metabolism was investigated in the white mouse *Mus musculus* in order to determine how vertebrates can modify such molecules, either provided by their food or produced endogenously by parasitic helminths. It appears that mouse concentrate rapidly injected ecdysteroids in their gut, then excrete them mainly within faeces, which contain both the genuine molecule together with a major metabolite, 14-deoxyecdysone.

Ecole Normale Supérieure RENÉ LAFONT
Laboratoire de Biochimie et Physiologie du Développement
C.N.R.S. U.A. 686
46 rue d'Ulm
F-75230 Paris Cedex 05, France

Université René Descartes JEAN-PIERRE GIRAULT
Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques
C.N.R.S. U.A. 400,
45 rue des Saints-Pères
F-75270 Paris Cedex 06, France

Schering Aktiengesellschaft ULRICH KERB
D Arzneimittelchemie B
Postfach 65 03 11
D-1000 Berlin 65
Federal Republic of Germany

REFERENCES

1. P. Karlson, *Hoppe-Seyler's Z. Physiol. Chem.* **364**, 1067 (1983).
2. D. R. Bergamasco and D. H. S. Horn, in *Invertebrate Endocrinology*. Vol. 1, *Endocrinology of Insects* (Eds. R. G. H. Downer and H. Laufer), p. 627. Alan R. Liss, New York (1983).
3. D. H. S. Horn and D. R. Bergamasco, in *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 7, *Endocrinology I* (Eds. G. A. Kerkut and L. I. Gilbert). p. 185. Pergamon Press, Oxford (1985).
4. J. A. Hoffmann and M. Charlet, *Ann. Sci. Nat. Zool.* **7**, 215 (1985).
5. F. Sauber, M. Reuland, J.-P. Berchtold, C. Hetru, G. Tsoupras, B. Luu, M.-E. Moritz and J. A. Hoffmann, *C.R. hebd. Seanc. Acad. Sci. Paris* **296**, 413 (1983).
6. P. Nirdé, G. Torpier, M. L. De Reggi and A. Capron, *FEBS Lett.* **151**, 223 (1983).
7. M. Porchet, N. Gaillet, F. Sauber, M. Charlet and J. A. Hoffmann, in *Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones* (Eds, J. A. Hoffmann and M. Porchet), p. 346. Springer-Verlag, Berlin (1984).
8. J. G. Mercer, *Parasitol. Today* **1**, 96 (1985).
9. M. W. Fleming, *Exp. Parasitol.* **60**, 207 (1985).
10. M. Uchiyama and T. Otaka, in *Invertebrate Endocrinology and Hormonal Heterophylly* (Ed. W. J. Burdette), p. 375. Springer-Verlag, Berlin (1974).
11. T. Yoshida, T. Otaka, M. Uchiyama and S. Ogawa, *Biochem. Pharmac.* **20**, 3263 (1971).
12. S. Ogawa, N. Nishimoto and H. Matsuda, in *Invertebrate Endocrinology and Hormonal Heterophylly* (Ed. W. J. Burdette), p. 341. Springer-Verlag, Berlin (1974).
13. R. E. Catalan, A. M. Martinez and M. D. Aragones, *Comp. Biochem. Physiol.* **71B**, 301 (1982).
14. K. D. Chaudhary, P. J. Lupien and C. Hinse, *Experientia* **25**, 250 (1969).
15. R. E. Catalan, M. D. Aragones, J. E. Godoy and A. M. Martinez, *Comp. Biochem. Physiol.* **78C**, 193 (1984).
16. R. E. Catalan, A. M. Martinez, M. D. Aragones, B. G. Miguel, A. Robles and J. E. Godoy, *Comp. Biochem. Physiol.* **81B**, 771 (1985).
17. P. Nirdé, M. L. De Reggi, G. Tsoupras, G. Torpier, P. Fressancourt and A. Capron, *FEBS Lett.* **168**, 235 (1984).
18. P. Nirdé, G. Torpier, A. Capron, M. Delaage and M. L. De Reggi, in *Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones* (Eds. J. A. Hoffmann and M. Porchet), p. 331. Springer-Verlag, Berlin (1984).
19. J. Koolman, J. Walter and H. Zahner, in *Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones* (Eds. J. A. Hoffmann and M. Porchet), p. 323. Springer-Verlag, Berlin (1984).
20. J. Koolman and H. Moeller, *Insect Biochem.* **16**, 287 (1986).
21. J.-F. Modde, R. Lafont and J. A. Hoffmann, *Int. J. Invert. Reprod. Devl.* **7**, 161 (1984).
22. U. Kerb, G. Schulz, P. Hocks, R. Wiechert, A. Furlenmeyer, A. Fürst, A. Langemann and G. Waldvogel, *Helv. Chim. Acta* **49**, 1601 (1966).
23. R. Lafont, J.-P. Girault and U. Kerb, manuscript in preparation.
24. H. H. Rees and A. H. W. Mendis, in *Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones* (Eds. J. A. Hoffmann and M. Porchet), p. 338. Springer-Verlag, Berlin (1984).
25. H. Hikino, Y. Ohizumi and T. Takemoto, *Yakugaku Zasshi.* **92**, 945 (1970).
26. H. Hikino, Y. Ohizumi and T. Takemoto, *Chem. Pharm. Bull.* **20**, 2454 (1972).
27. I. A. Macdonald, V. D. Bokkenheuser, J. Winter, A. M. McLernon and E. H. Mosbach, *J. Lipid Res.* **24**, 675 (1983).

Biochemical Pharmacology, Vol. 37, No. 6, pp. 1177–1180, 1988.
Printed in Great Britain.

0006–2952/88 $3.00 + 0.00
Pergamon Press plc

Effects of haematoporphyrin derivatives on rat liver endoplasmic reticulum

(*Received* 8 *July* 1987; *accepted* 11 *November* 1987)

Haematoporphyrin derivative (HPD) photodynamic therapy is emerging as a promising treatment for a variety of neoplasms [1]. This is because HPD is selectively retained in tumour tissue, so that subsequent irradiation with high intensity light (wavelength approx 630 nm) will selectively damage the photosensitised tumour tissue.

Recent studies [2] on the cellular and subcellular localization of porphyrins in rat liver, following i.p. administration of HPD, have shown that the porphyrins are initially found in a cytosolic compartment, probably of the hepatocytes. At later time points (24–48 hr), the porphyrins became concentrated in the lysosomes of the Kupffer cells. In addition, HPD has also been shown to inhibit enzyme activities of both the endoplasmic reticulum [3] and the mitochondria [4]. In this study the effect of HPD treatment on the subcellular distribution and morphometry of certain

organelles is examined with particular reference to the endoplasmic reticulum.

Materials and methods

Haematoporphyrin derivative (HPD) was prepared as described in [2]. Analytical subcellular fractionation of rat liver whole homogenate was carried out as described previously [5], using male Sprague–Dawley rats (200–250 g) dosed i.p. with 10 mg of HPD in 0.15 M NaCl (1 ml).

Enzyme assays. The sucrose density gradients were assayed for cytochrome P-450 and NADPH cytochrome *c* reductase as in [6] and RNA levels were measured as described in [7]. Aryl sulphatase C was measured as in [8]. *N*-Acetyl-β-glucosaminidase, neutral α-glucosidase, catalase, succinate dehydrogenase and 5′nucleotidase assays were performed as in [5]. Subcellular fractionation results were computed by the method in [9] and plotted as relative concentration against density.

Electron microscopy and morphometry. For electron microscopy 1 mm cubes of liver were fixed for 2 hr in 3% glutaraldehyde in 100 mM sodium cacodylate buffer pH 7.4 containing 5% sucrose; post-fixed in 1% OsO_4 and 1% potassium ferrocyanide in cacodylate buffer for 2 hr at 4°.

Morphometric methods were employed to estimate the relative proportions of rough and smooth endoplasmic reticulum (ER) in hepatocyte cytoplasm. Ultra-thin sections were obtained from five randomly chosen tissue blocks of experimental and control liver and 30 micrographs at a final magnification of 29,000× were recorded for each according to the method of Weibel *et al.* [10]. A 1 cm point lattice was used to estimate the relative volume fraction (V_v) of rough (rer) and smooth (ser) ER in relation to hepatocyte cytoplasm. All golgi elements were attributed to smooth ER as described in [10]. The ratio V_{vser} to V_{vrer} was calculated for each micrograph and the individual data pooled and logarithmically transformed to enable parametric tests of analysis (*t*-tests) to be performed [11].

Results

Figure 1 compares the distribution profiles obtained for five enzyme activities associated with the endoplasmic reticulum in control animals and in rats treated with HPD. In the control animals (solid line), enzyme activities show a broad bimodal distribution with peaks at modal densities of 1.17 $g.cm^{-3}$ and 1.22 $g.cm^{-3}$ representing the smooth and the rough endoplasmic reticulum, respectively. Animals treated with HPD (dotted line), showed shifts in the distribution of four of the activities, to give a major peak at a density of approximately 1.23 $g.cm^{-3}$ with an apparent decrease in the distribution at 1.17 $g.cm^{-3}$. The distribution of RNA also showed an increase in distribution at the higher density. Quantification of endoplasmic reticulum enzyme activities shown in Fig. 1, by integration of activity over discrete density intervals, indicated that the apparent change in distribution was primarily due to an increase in the amount of enzyme activity associated with the denser membranes rather than selective inhibition of enzyme associated with the smooth endoplasmic reticulum. This is

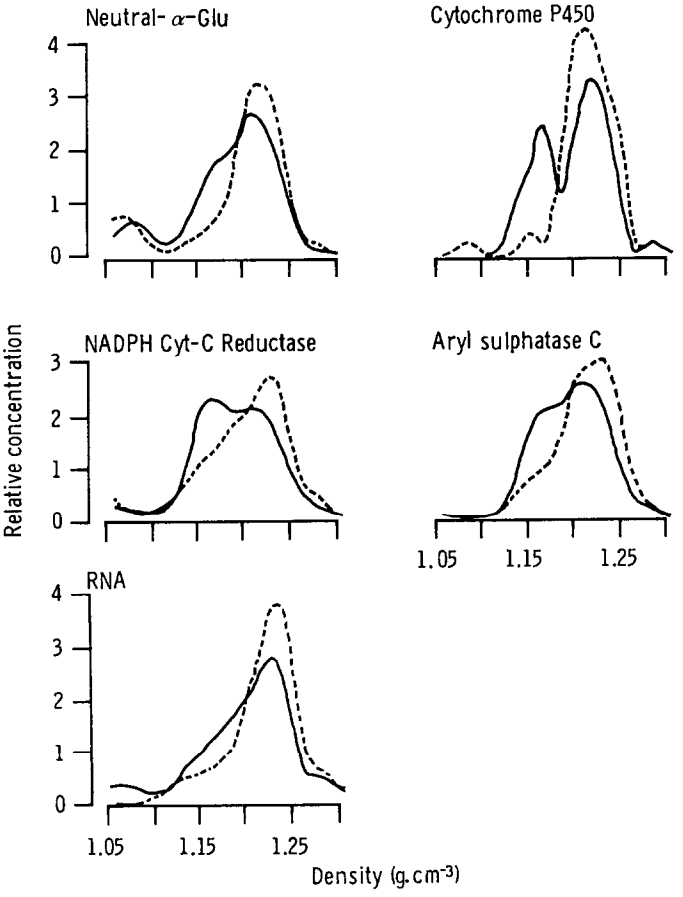

Fig. 1. Comparison of the subcellular distribution of endoplasmic reticulum markers in rat liver homogenates from control animals (——) and animals treated 24 hr previously with HPD (– – –). The distributions represent the mean of three separate experiments.

Table 1. Homogenate values of the measured endoplasmic reticulum activities

Enzyme	Activity	
	Control	HPD-Treated
Neutral-α-glucosidase	206 ± 57	177 ± 17
Aryl-sulphatase-C	288 ± 36	262 ± 51
NADPH cytochrome C reductase	1479 ± 104	1511 ± 333
Cytochrome P-450	7.8 ± 1.8	7.2 ± 1.0
RNA	1.11 ± 0.11	1.37 ± 0.12

The results are the mean of three individual estimates ± SEM and expressed per g of liver. Activities are expressed as milliunits for enzymes, mg for RNA and nmoles for cytochromes.

confirmed by examination of the homogenate activities as shown in Table 1, where no significant differences were observed between the control and HPD treated animals.

Figure 2 shows the distribution of marker enzyme activities for four of the major organelles in control and HPD treated rats: namely plasma membrane (5'nucleotidase), peroxisomes (catalase), lysosomes (*N*-acetyl-β-glucosaminidase), and mitochondria (succinate dehydrogenase). There were no significant differences in distribution profiles between the controls and HPD-treated animals indicating that HPD treatment has not perturbed the equilibrium densities of these organelles.

The livers of control and HPD-treated rats were examined by transmission electron microscopy to investigate morphological changes. The most striking difference was seen in the proportion of rough ER in the hepatocytes. The morphometric analysis indicated that the ratio $V_{vser}:V_{vrer}$ decreased significantly ($P = 1.79 \times 10^{-7}$) from 1.63 (95% confidence limits (CL) = 1.22–2.17) in the control to 0.36 (95% CL = 0.276–1.6) in an HPD-treated liver. The increase in the rough ER seen after HPD treatment was not due to any alteration in the abundance of golgi components. No obvious changes were noted in ER in either endothelial or Kuppfer cells. Representative micrographs are shown in Fig. 3, where a preponderance of rough membrane can be seen in the liver from the HPD-treated rat (B). There were no striking changes in the morphology or abundance of the other major classes of organelles.

Discussion

Previous studies on the effects of HPD on the endoplasmic reticulum have indicated that observed inhibition of cytochrome P-450 was by generation of singlet oxygen and hydroxyl radicals [3]. Enhanced lipid peroxidation was found in hepatic microsomes of HPD-treated rats [12]. However, these effects were only seen after exposure to HPD and subsequent UV irradiation of the membranes. In the present study we show that in the absence of UV radiation there is a significant alteration in the proportion of enzymic activities associated with the rough and smooth endoplasmic reticulum. All four enzyme activities associated with the endoplasmic reticulum showed a similar behaviour, suggesting that this was a general effect on the endoplasmic reticulum, rather than on one particular activity. The shift in the distribution of RNA suggested that this was due to an increased association of ribosomes with the endoplasmic reticulum, thus increasing the proportion of rough membrane. This was confirmed by electron microscopic examination of liver samples, morphometric analysis indicating a fourfold decrease in the ratio of smooth to rough membrane (1.63 to 0.36) following HPD treatment.

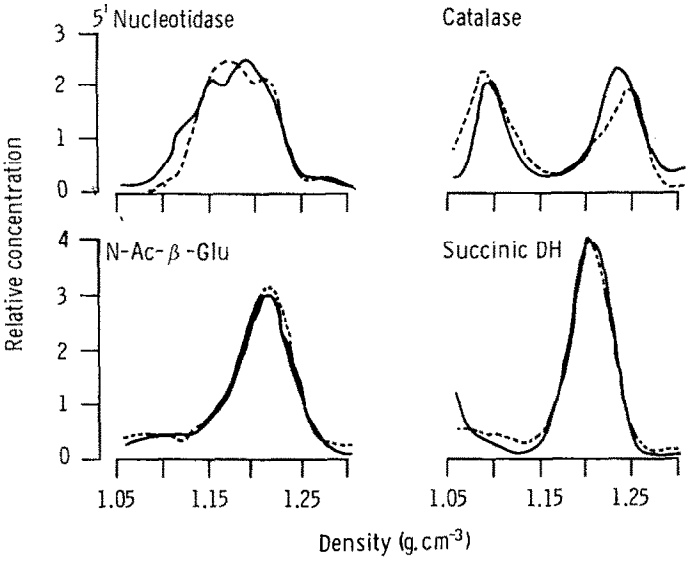

Fig. 2. Comparison of the subcellular distribution of marker enzymes for some of the major organelles in rat liver homogenates from control animals (——) and animals treated 24 hr previously with HPD (– – –). The distributions represent the mean of three separate experiments.

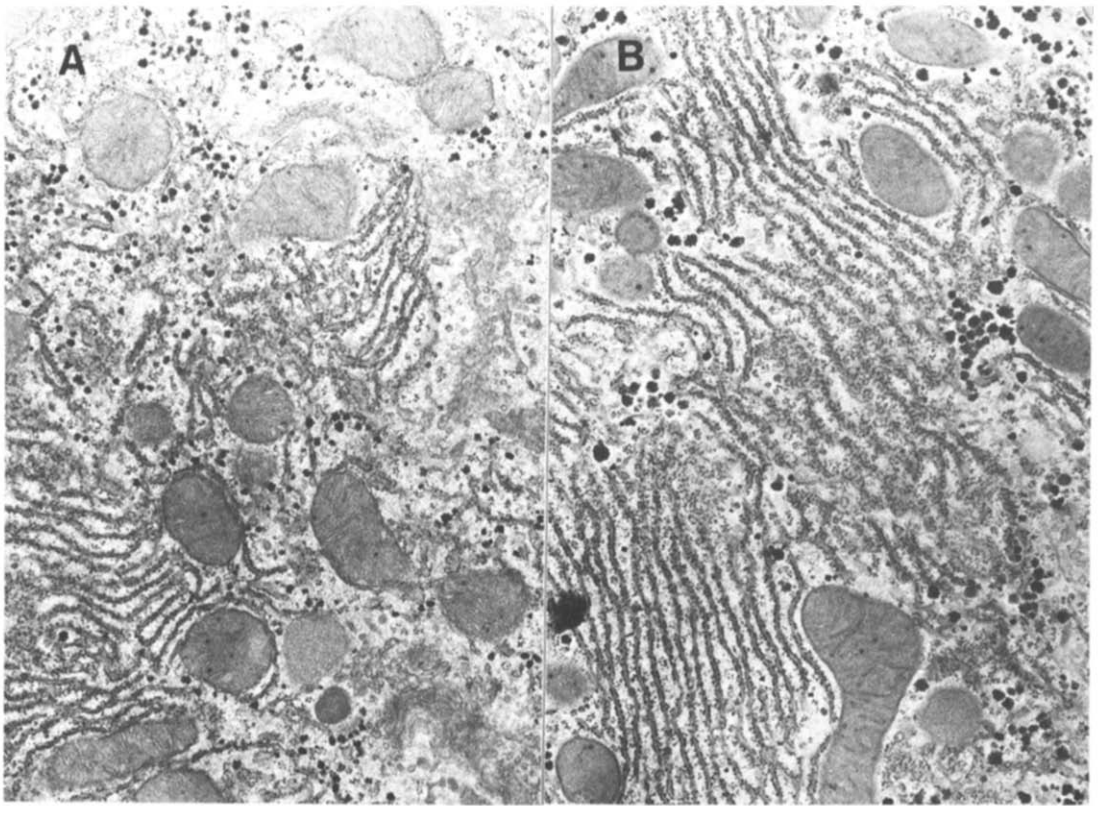

Fig. 3. Electron micrographs (mag. 29,000×) of rat liver from control animals (A) and animals treated 24 hr previously with HPD (B).

Although this study was performed at 24 hr post administration of HPD, a time at which porphyrins are associated with the lysosomes of Kupffer cells [2], these effects seem to be restricted to hepatocytes. However, the previous studies [2] had also suggested a rapid transit of porphyrins to the bile, which would almost certainly involve passage through the hepatocyte cytoplasm. The mechanism by which these changes are mediated is unknown but may involve effects of HPD on protein synthesis. These results also suggest a means by which the ratio of rough to smooth endoplasmic reticulum may be experimentally manipulated.

In summary although we have previously shown that HPD does not accumulate in hepatocytes [2], HPD treatment has a marked effect of the subcellular distribution of endoplasmic reticulum enzymes in the hepatocyte. The alteration in subcellular distribution does not seem to be attributable to inhibition of enzyme activity, but rather to an increase in the proportion of rough membrane. This may be a reflection of increased protein synthetic activity, and certainly indicates that increased use of HPD as an anti-tumour agent should be accompanied by further investigation of its metabolic effects on non-involved tissue.

Acknowledgements—We wish to thank K. Guttridge for expert technical assistance and Gill Smith for typing this manuscript.

* To whom correspondence should be addressed.

Division of Clinical Cell Biology
MRC Clinical Research Centre
Watford Road, Harrow
Middlesex HA1 3UJ, U.K.

BENEDICT A. CARPANINI
GEOFFREY D. SMITH*
PENNY J. COOPER
CHANG K. LIM
TIMOTHY J. PETERS

REFERENCES

1. T. J. Dougherty, *CRC Crit. Rev. Oncol. Haematol.* **2**, 83 (1984).
2. B. A. Carpanini, G. D. Smith, C. K. Limm and T. J. Peters, *Biochem. Pharmac.* **36**, 2759 (1987).
3. D. R. Bickers, R. Dixit and H. Mukhtar, *Biochem. biophys. Res. Commun.* **108**, 1032 (1982).
4. S. L. Gibson and R. Hilf, *Cancer Res.* **43**, 4191 (1983).
5. G. D. Smith and T. J. Peters, *Eur. J. Biochem.* **104**, 305 (1980).
6. H. Beaufay, A. Amar-Costesec, E. Feytmans, D. Thinès-Sempoux, M. Wibo, M. Robbi and J. Berthet, *J. Cell Biol.* **61**, 188 (1974).
7. J. LePecq and C. Paoletti, *Analyt. Biochem.* **17**, 100 (1966).
8. P. G. Canonico, H. Beaufay and M. Nyssens-Jadin, *J. Retic. Soc.* **24**, 155 (1978).
9. G. D. Smith, K. R. S. Osterloh and T. J. Peters, *Analyt. Biochem.* **160**, 17 (1987).
10. E. R. Weibel, W. Staubli, H. R. Gnagi and F. A. Hess, *J. Cell Biol.* **42**, 68 (1969).
11. R. P. Sokal and F. J. Rohlf, *Biometry*, pp. 419–421. Freeman, London (1969).
12. M. Das, H. Mukhtar, E. R. Greenspan and D. R. Bickers, *Cancer Res.* **45**, 6328 (1985).