Table 2. Nicotinamide concentrations in rats pretreated with nicotinamide

	Nicotinamide (mg/l or mg/kg)
Serum total	1295 ± 57 (9)
CSF	1276 ± 83 (6)
Brain	1240 ± 132 (9)
Serum free	1274 ± 59 (9)
Serum free (unbound) fraction	0.984 ± 0.041 (9)

Values are means ± SD, with the number of animals given in parentheses.

finding agents that may be beneficial against theophylline seizures.

Nicotinamide was chosen as a potential beneficial agent since it offers protection against audiogenic seizures with its effect being enhanced by adenosine [4] and since theophylline's neurotoxicity is presumably related to its effect at adenosine receptors [13], although it is also a GABA and benzodiazepine antagonist [14]. In view of this, the lack of protective effect noted in the current study was surprising. However, this is unlikely to be due to the dose of nicotinamide used since the chosen dose was high and similar to the claimed optimum dose of 400 mg/kg [4]. Similarly, the pretreatment time is probably not inappropriate since the rats received nicotinamide 15 min prior to the start of theophylline infusion giving a pretreatment period of 45 min in most rats which is also the time when nicotinamide showed its maximum protective effect in the audiogenic seizure study [4]. Our negative results may more appropriately reflect the fact that the primary neurochemical action of nicotinamide is on a receptor distinct from the adenosine system, since nicotinamide's anticonvulsant effect has been demonstrated previously in assay systems sensitive to benzodiazepines [7] and GABAergic drugs [6].

In summary, the present study demonstrates that nicotinamide (1000 mg/kg) administered intravenously 45 min before onset of seizures in rats failed to provide protection against theophylline seizures.

Acknowledgement—Ms. DeDonato was supported by a summer scholarship from the School of Pharmacy.

Department of Pharmaceutical Sciences
School of Pharmacy
University of Pittsburgh
Pittsburgh, PA 15261, *U.S.A.*

IQBAL RAMZAN*
VICTORIA DEDONATO

* Author to whom all correspondence should be sent.

REFERENCES

1. B. Scherer and W. Dramer, *Life Sci.* **11**, 189 (1972).
2. J. M. Beaton, G. V. Pegram, J. R. Smythies and R. J. Bradley, *Experientia* **30**, 926 (1974).
3. J. M. Beaton, *Experientia* **32**, 1036 (1976).
4. M. Maitre, L. Ciesielski, A. Lehmann, E. Kempf and P. Mandel, *Biochem. Pharmac.* **23**, 2807 (1974).
5. C. R. Robinson, G. V. Pegram, P. R. Hyde, J. M. Beaton and J. R. Smythies, *Biol. Psychiat.* **12**, 139 (1977).
6. H. Balzer, P. Holtz and D. Palm, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.* **239**, 520 (1960).
7. H. Mohler, P. Polc, R. Cumin, L. Pieri and R. Kettler, *Nature, Lond.* **278**, 563 (1979).
8. D. W. Wooley, *Science* **128**, 1277 (1958).
9. B. Kennedy and B. E. Leonard, *Biochem. Soc. Trans.* **8**, 59 (1980).
10. W. Haefely, *Agents Actions* **7**, 353 (1977).
11. I. M. Ramzan and G. Levy, *J. Pharmac. exp. Ther.* **236**, 708 (1986).
12. C. W. Zwillich, F. D. Sutton, T. A. Neff, W. M. Cohn, R. A. Matthay and M. M. Weinberger, *Ann. intern. Med.* **82**, 784 (1975).
13. T. F. Murray, D. Sylvester, C. S. Schultz and P. Szot, *Neuropharmacology* **24**, 761 (1985).
14. R. F. Squires and E. Saederup, *Brain Res.* **414**, 357 (1987).

Biochemical Pharmacology, Vol. 37, No. 6, pp. 1174–1177, 1988.
Printed in Great Britain.

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

Excretion and metabolism of injected ecdysone in the white mouse

(*Received* 27 *April* 1987; *accepted* 29 *October* 1987)

Ecdysteroids represent a class of steroids which have retained the C-27 skeleton of cholesterol (or in some cases the C-28 or C-29 skeleton of some phytosterols). They are widespread in invertebrates and plants [1–3]. By contrast, they have not yet been found in vertebrates, where steroid biosynthesis and metabolism use different pathways which include an early side-chain cleavage between C-20 and C-22.

In invertebrates, at least in arthropods (there is some, as yet limited, evidence that this could perhaps also apply to annelids and nematodes), ecdysteroids are hormones which control development and reproduction [4–9]. In mammals, although the ecdysteroid structures strongly differ from those of Vertebrate-type steroids, there is evidence that these molecules have several pharmacological effects which include (1) a stimulation of protein synthesis in liver [10], (2) interference with glycaemia controlling factors [11, 12], (3) modifications of enzyme activities, e.g. protein kinase in rat liver [13], glutamic decarboxylase [14] or acetylcholinesterase [15] in rat brain, and (4) alterations in lipid metabolism [16]. These results raise an important question: do ecdysteroids interact with mammalian receptors normally used by other hormones with or without being previously converted into some metabolite(s)?

Moreover, it has been recently claimed that in mammals (including humans) infested with helminths it is possible to detect infestation by the unusual presence of ecdysteroids in blood or urine of infested individuals [17–20]. These compounds would be secreted by parasites in amounts high enough to account for noticeable immunoreactivity in host fluids. This problem is, however, rendered more complex due to the possible presence of ecdysteroids in the food of animals, as these molecules are present in so many plant species [2, 3]. In any case, it may be of interest to learn something about ecdysteroid metabolism in mammals: it is indeed highly probable that mammals are able to transform

these compounds, and if so, it might be more advisable to search for specific metabolites in blood (or urine) instead of the molecules actually present in helminths.

Materials and methods

Animals. Male white mice (*Mus musculus*) supplied by Iffa-Credo (Saint-Germain sur l'Arbresle, France) and weighing *ca.* 30 g were used. These were injected intraperitoneally with [^{3}H]-ecdysone (2 μCi per animal) dissolved in isotonic (0.9%) NaCl solution. When a large amount (0.2 mg per animal) of unlabeled hormone was used, it was first dissolved in 50 μl of propan-2-ol, to which 450 μl of saline were added just before injection. Animals were placed in metabolic cages and their urine and feces were collected together in a beaker containing absolute ethanol (in order to avoid further metabolism by microorganisms) over the 3 days following injections.

A second set of experiments was undertaken in order to determine the short-term kinetics of ecdysone excretion and metabolism, in an attempt to precise the respective roles of kidneys, liver and gut. Animals were injected either intraperitoneally or subcutaneously, and sacrificed at various times after injection. They were dissected as blood, liver, gut, kidneys, bladder and "carcass", and each tissue was extracted for a determination of radioactivity distribution.

Chemicals. Ecdysone (2β,3β,14α,22(*R*),25-pentahydroxy-5β-cholest-7-ene-6-one) was purchased from Simes (Milan, Italy). [23,24-^{3}H] Ecdysone, specific activity *ca.* 100 Ci/mmol, was prepared from labeled 2-deoxyecdysone (a generous gift from Dr Hetru, Strasbourg) by *in vitro* incubation with *Locusta migratoria* Malpighian tubules [21]. Reference 14-deoxyecdysone was from an earlier synthesis [22].

Sample processing. The organs of each animal were extracted twice with 50 ml methanol. The (urine + faeces) samples were extracted first with ethanol (100 ml) then methanol (100 ml), and the extracts were combined. An aliquot was used for radioactivity determination. Other aliquots were evaporated to dryness and directly analyzed by HPLC (see below) without prior purification.

Chromatographic analyses. Both normal phase (NP) or reversed phase (RP) high-performance liquid chromatography (HPLC) were used. RP analyses used a Spherisorb-ODS2 column (250 mm long, 4.6 mm i.d.) eluted with a linear gradient (18–40% in 30 min) of acetonitrile in water containing 0.1% trifluoroacetic acid (TFA) [solvent system 1]. NP analyses used a Zorbax-SIL column (250 mm long; 4.6 mm i.d.) (DuPont) eluted with dichloromethane/propan-2-ol/water (125:20:1.5) [solvent system 2], or with isooctane/propane-2-ol/water (100:30:2) [solvent system 3]. Radioactivity was monitored in-line by using a radioactivity monitor (FLO-ONE model IC, Radiomatic Instruments and Chemicals, Tampa, Florida) (solvent systems 1 and 3). Alternatively, 0.4 min fractions were collected, evaporated and scintillation counted with a Kontron MR300 liquid scintillator (solvent system 2).

Results and discussion

Excretion of injected ecdysone. Injected ecdysone was rapidly eliminated by the animal (Fig. 1). When a larger amount (0.2 mg) of ecdysone was injected, excretion proceeded also at a high rate, and within 3 days no significant radioactivity remained in the injected mice. In fact most of the radioactivity was excreted within the first 24 hours following injection.

Nearly all the injected radioactivity was still present within the animals 2 hr after injection and it was essentially located in the gut and to a lower extent in the liver. By contrast, almost no radioactivity was found in the blood, urine (bladder) and kidney samples, both with intraperitoneally and subcutaneously injected animals.

Short-term kinetic experiments were performed by sampling animals during the first hour following a subcutaneous dorsal injection. Small amounts of radioactivity were detected in the blood during the first 30 min only, together with a very small amount in bladder urine (less than 1% of the injected radioactivity). The analyses of liver and gut contents (Fig. 2) were consistent with the hypothesis that ecdysone was efficiently removed from the blood by liver, then moved (mostly unchanged as evidenced by HPLC analysis—see Fig. 3A) through biliary excretion into the intestine where it accumulated. Within 45 min post injection more than 90% of the injected radioactivity was found in this compartment, and no radioactivity was further detectable in the bladder.

General pattern of metabolites (analytical experiments). RP HPLC analysis of intestine or faeces extracts showed quite simple patterns (Fig. 3): ecdysone was converted to a large extent into a single less polar metabolite (metabolite 1). No polar metabolites (conjugates) were observed. Metabolite 1 could also be separated from ecdysone using

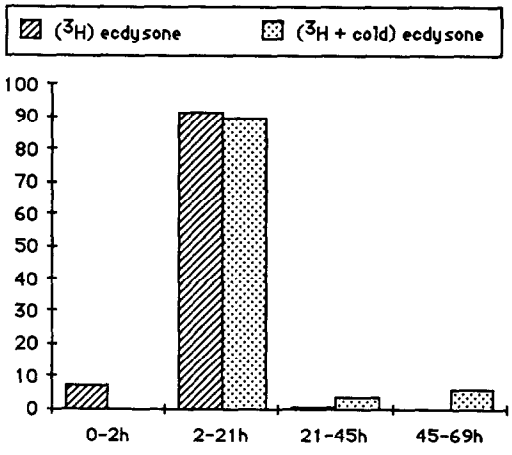

Fig. 1. Kinetics of labelled ecdysone excretion by mice after injection of labelled ecdysone alone or injection of tritiated ecdysone + 0.2 mg cold hormone. Faeces and urine (the latter containing in fact negligible amounts) were collected during the given intervals following injection and processed together. The amounts excreted in a given interval are expressed as a percentage of total recovered tritium.

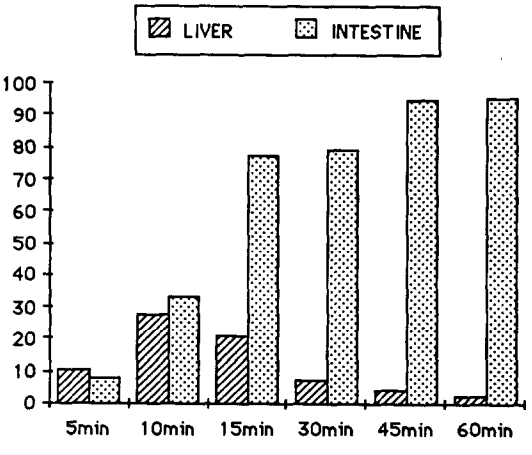

Fig. 2. Kinetics of radioactivity distribution within liver and intestine during the first hour following subcutaneous injection of labelled ecdysone (without cold hormone). Amounts are expressed as a percentage of recovered tritium.

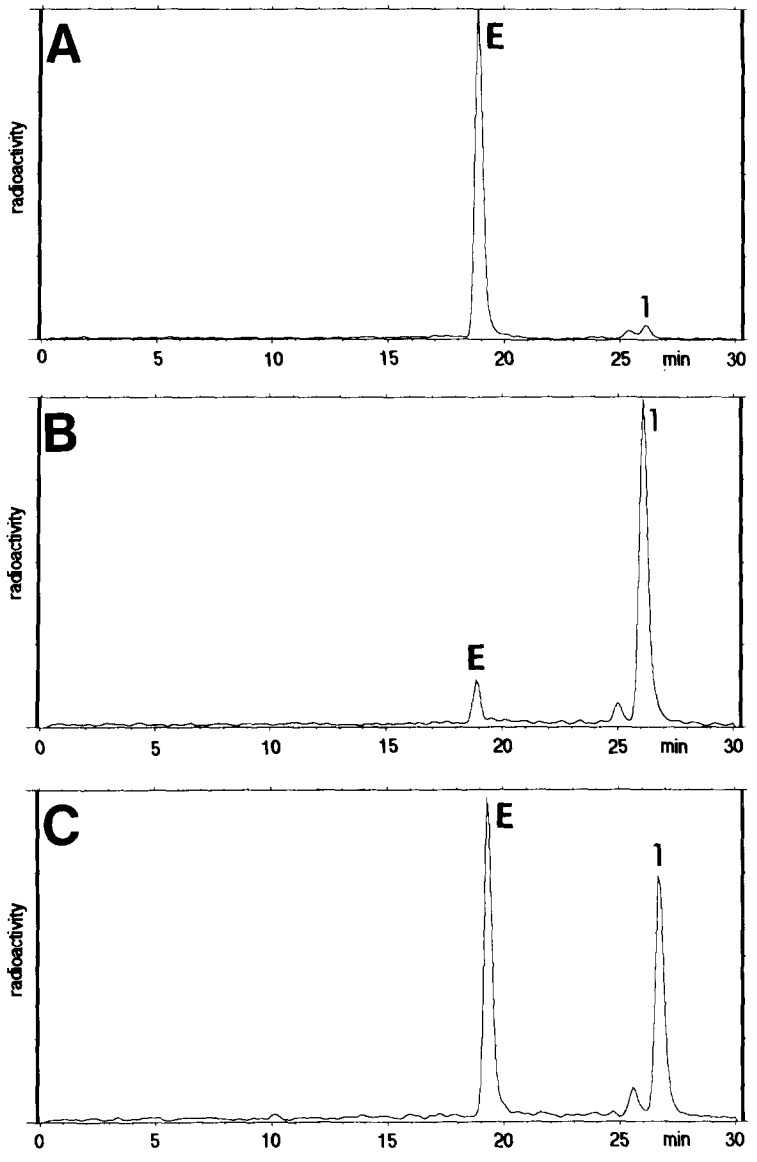

Fig. 3. RP HPLC analysis of intestine or faeces extracts (solvent system 1 at 1 ml/min). A: intestine extract one hour after injection. B: intestine extract 4 hours after injection. C: faeces (+ urine) collected during the 2–21 hr period following injection (E = ecdysone; 1 = metabolite 1).

NP HPLC (Table 1). This compound was identified as 14-deoxyecdysone on the basis of comigration in the three HPLC systems used. Further evidence for its identity has been obtained through preparative experiments [23].

Conclusions. Mice rapidly excrete injected ecdysone. A noticeable part (*ca.* 45%) of the injected compound is eliminated unchanged. As a consequence, it appears possible to find ecdysone in mammals infested with helminths (which already contain this compound besides 20-hydroxyecdysone and 20,26-dihydroxyecdysone [6, 24]). However, we must emphasize that in mice (1) ecdysone was very rapidly removed from the blood, and (2) most of it was excreted within faeces rather than urine. These data are fully consistent with those earlier reported for 20-hydroxyecdysone [25, 26]. The picture might, however, strongly differ between mice and other species, including man, due to a different molecular weight threshold for biliary excretion.

Ecdysone metabolism in mice proceeds essentially through 14-dehydroxylation, which was most probably caused by bacteria from the gut lumen. Similarly, a very efficient 7-dehydroxylation of bile acids takes place in the human gut [27].

Table 1. Chromatographic properties of ecdysone and its major metabolite

	(Retention time in minutes)		
	System 1	System 2	System 3
Ecdysone	19.2	33.8	31.2
Metabolite 1	26.0	11.6	24.3

Flow-rate 1 ml.min^{-1}—see Materials and Methods for solvent systems.

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