Distribution of some urea cycle enzymes in the insect fat body

Species	Stage in development	OTCase	ASase	Arginase* μmoles urea formed per h per g tissue	Arginase* μmoles urea formed per h per mg protein
B. cranifera	2nd instar nymphs	B. L. D.	–	–	–
	6th instar nymphs	B. L. D.	–	63	1.8
				47	1.2
	9th instar nymphs	B. L. D.	B. L. D.	66	0.9
				75	1.5
	11th instar nymphs	B. L. D.	–	88 ± 22 (3)	2.0 ± 0.4 (3)
	Adults	B. L. D.	B. L. D.	126 ± 27 (10)	2.3 ± 0.6 (10)
H. gloveri	Diapause pupae	B. L. D.	B. L. D.	112 ± 34 (9)	1.0 ± 0.1 (8)
	Adult moths	B. L. D.	B. L. D.	2453 ± 907 (9)	24.5 ± 5.1 (9)

* Wherever 3 or more assays were done, the mean values and the standard deviations are given with the number of assays in the parentheses. Otherwise individual observations are given. B. L. D.: Below the level of detection.

quite sensitive and have been successfully employed to demonstrate these activities in the tissues of vertrebrates, as well as invertebrates like snails, flatworms and annelids[11,12].

These results are consistent with those of Porembska and Mochnacka[13], who failed to detect the synthesis of citrulline and arginine in the fat body and muscle extracts of Celerio euphorbiae, and Kameyama and Miura[14], who could not detect the NH_4-dependent carbamoylphosphate synthetase and OTCase in Aldrichina grahami. Thus it would appear that the insects studied by us, as well as Celerio euphorbiae[13], are not only incapable of synthesizing citrulline from NH_3, CO_2 and ornithine, but also cannot affect the conversion of citrulline to arginine. However, the nutritional studies of Hinton[6] on Drosophila, and Davis[7] on Oryzaephilus surinamensis, as well as the isotopic studies of Inokuchi, Horie and Ito[15] on Bombyx mori, suggest that some insects at least are capable of converting citrulline to arginine. The possibility remains that some insects have lost the entire arginine biosynthetic pathway making arginine irreplaceable in their diet, whereas others have retained a part of the pathway enabling them to substitute dietary citrulline for arginine.

Arginase is the only enzyme of the urea cycle present in the fat body of the cockroach and silkmoth (table). The arginase activity increases 20- to 30fold during the metamorphosis of silkmoth pupae into adults (table), and this increased arginase activity has been shown to play a role in the conversion of exogenous arginine to proline, which is necessary in the flight muscle metabolism of the adult moth[16]. There is no such dramatic increase in the fat body arginase activity during development of the cockroach (table) consistent with the limited capacity of this insect to fly.

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Enterohepatic cycling of O-(β-hydroxyethyl) rutosides and their biliary metabolites in the rat

A. M. Hackett and L. A. Griffiths[1,2]

Department of Biochemistry, University of Birmingham, P. O. Box 363, Birmingham B15 2TT (England), 5 August 1976

Summary. O-(β-Hydroxyethyl)rutosides are shown to undergo reabsorption from the intestine following their secretion in bile.

Although biliary excretion has been established as a major route of excretion of O-(β-hydroxyethyl) rutosides following absorption from the gut or parenteral administration to the rat[3,4] the possibility of re-absorption of biliary metabolites of these compounds from the lumen of the intestine and subsequent enterohepatic cycling does not appear previously to have been examined, although evidence has been presented that these glycosides are ultimately excreted largely as their aglycones in faeces[3,4]. Use of an intercannulated rat preparation has now permitted some assessment to be made of the extent of reabsorption of each of the hydroxyethylrutosides and/or their conjugates following their secretion in bile.

Materials and methods. 3′,4′,5,7-Tetra-O-(β-hydroxyethyl)rutoside, (tetra-HR) 3′,4′,7-tri-O(β-hydroxyethyl)-rutoside (tri-HR) (constituents of the therapeutic agent Paroven, Zyma S.A., Nyon, Switzerland) and 7-mono-O-(β-hydroxyethyl)rutoside (7-mono-HR) (the therapeutic value of which is currently under investigation) were

1 To whom reprint requests should be addressed.
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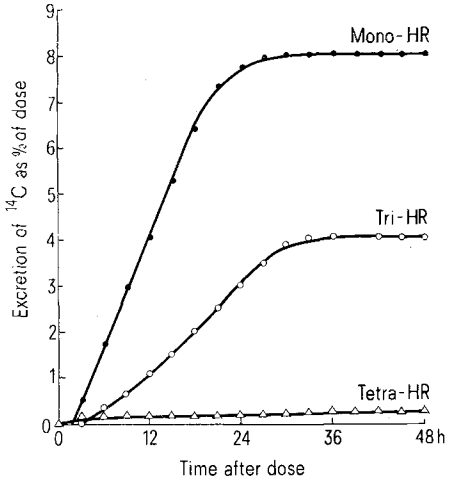
Excretion of ^{14}C (expressed as a percentage of the administered dose) over 48 h from rats of series 2 following administration of specific hydroxyethylrutoside to rats of series 1

	Mono-HR	Tri-HR	Tetra-HR
Bile	8.05 (± 1.02)	4.08 (± 0.47)	0.31, 0.29
Urine	1.56 (± 0.41)	1.02 (± 0.01)	1.10, 2.04
Faeces	45.27 (± 4.86)	37.33 (± 3.60)	24.53, 20.07
Recovered from intestine	5.28 (± 0.9)	11.09 (± 3.09)	2.53, 3.27
Total	64.03 (± 6.42)	54.18 (± 4.88)	28.47, 25.67

The values in columns 1 and 2 are the means of 3 experiments; those in column 3 are the values of duplicate experiments.

supplied by Zyma S.A., Nyon, Switzerland, labelled in C2 of the hydroxyethyl side chains with ^{14}C. The purity of these preparations was checked by methods previously described[3,5]. The specific activities of the hydroxyethyl rutosides (HRs) were adjusted to optimal levels by the addition of cold carrier such that when administered at a dosage level of 7.5 mg/kg body weight, the total radioactivity given to each pair of rats in respect of 3′,4′,5,7-tetra-O-(β-hydroxyethyl-[^{14}C]) rutoside and 3′,4′,7-tri-O-(β-hydroxyethyl-[^{14}C]) rutoside was 0.5 μCi and in the case of 7-mono-O-(β-hydroxyethyl-[^{14}C]) rutoside 0.3 μCi.

In separate experiments, the 3 hydroxyethyl rutosides were administered by injection of the appropriate rutoside dissolved in physiological saline into the caudal vein of rats of series 1. $^{14}Carbon$ in bile and urine samples of rats of series 2 was determined by addition of aliquots of these fluids to a toluene based PPO/POPOP scintillation fluid mixed 2:1 v/v with Triton X100[6]. $^{14}Carbon$ in faeces and intestinal contents was measured by combustion of a portion of freeze dried material in a Harvey Biological Material Oxidizer (ICN-Tracerlab, Hersham, Surrey). The percentage of ^{14}C associated with each metabolite on paper chromatograms was determined by direct liquid scintillation counting of chromatogram segments[6] or by combustion of relevant areas of chromatograms using a Harvey Biological Material Oxidizer (ICN, Hersham, Surrey), the combustion products being trapped in Harvey Scintillation Cocktail (I.C.N., Hersham, Surrey). Recovery of $^{14}CO_2$ by these means was checked by combustion of known amounts of ^{14}C-mannitol (Radiochemical Centre, Amersham, Bucks.) applied to Whatman 3MM chromatography paper and was found to be $>95\%$ with negligible memory effect.



The cumulative excretion of ^{14}C in the bile of rats of series 2 following i. v. administration of rutosides to rats of series 1.

Metabolites in urine and bile were separated on Whatman 3MM paper employing butan-2-ol-acetic acid-water 5-1-2 (by vol.). Metabolites thus separated were compared with previously characterized metabolites derived from the bile of single rat preparations[3] dosed with the same rutoside, in respect of R_F values and reactions following spraying with 1% methanolic $AlCl_3$, diazotised p-nitroaniline and 2% naphthoresorcinol in 33% trichloroacetic acid. R_F values and reactions with spray reagents for rutosides and conjugates were as previously published[3,4,6]

Cannulation procedure. Plastic cannulae were implanted in the common bile ducts of 2 male Wistar rats under nembutal anaesthesia as follows: A cannula was introduced into the common bile duct of the first animal (rat 1). The unattached end of this cannula was then implanted in the distal portion of the common bile duct of the second animal (rat 2) such that bile from rat 1 was delivered into the duodenum of rat 2. A second cannula was then established in the proximal end of the bile duct of rat 2, such that bile from this animal could be collected, the bile duct being ligated between the two cannulae[7]. Under these conditions biliary metabolites arising in the liver of rats of series 1 from the administered hydroxyethulrutoside are transferred to the intestine of rats of series 2. Determination of these metabolites in the bile and urine of rats of series 2 provides a measure of their re-absorption in a first enterohepatic cycle.

Results and discussion. The total values of ^{14}C recovered from bile, urine and faeces of rats of series 2 (table) indicate that biliary metabolites representing minimal mean values of 64%, 54% and 27% of the mono-HR, tri-HR and tetra-HR administered to rats of series 1 were eliminated in the bile of those animals and subsequently transferred by the impanted cannulae to the intestinal lumen of the linked animals (series 2). Summation of the ^{14}C bile and urine levels in respect of each rat of the second series indicates that 9.61%, 5.10% and 1.87% of the administered mono-HR, tri-HR and tetra-HR were recovered, which represents a reabsorption from the intestine of 14.8%, 9.2% and 7.4% of the total biliary excreted metabolites of mono-HR, tri-HR and tetra-HR respectively.

Examination of pooled bile and urine samples over the 48-h-period showed the major metabolite of mono-HR in both urine and bile was chromatographically identical with a compound already shown to be the 3′ or 4′-glucuronide of mono-HR[3]. This represented 82–92% of the ^{14}C activity in bile and 72–77% of that in urine. A second metabolite possibly a 5-glucuronide of mono-HR represented 3–9% activity in bile and 1–10% in urine. The remaining activity in bile and urine was associated with unchanged mono-HR. Following dosage of tri-HR, the unchanged glycoside was found to represent 45–64% of ^{14}C activity in the bile and 3–5% of that in the urine. The residual activity in both cases consisted largely of a 5-glucuronide of tri-HR. Radioactivity recovered from

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faeces and intestinal contents of rats of series 2 consisted mostly of the corresponding aglycones, but small amounts (< 8%) of the glycosides were also detected.
These results indicate that a significant level of reabsorption of [^{14}C] labelled hydroxyethylrutosides occurs after biliary excretion. It is not possible, on the evidence obtained, to state whether metabolic hydrolysis of the hydroxyethulrutoside glucuronides by the intestinal microflora precedes reabsorption although this appears probable. Evidence that hydroxyethylrutoside glucuronides are susceptible to bacterial glucuronidase has been presented earlier[3,8] and the ^{14}C activity of rat faeces following HR administration has previously been shown to be mainly attributable to unabsorbed hydroxyethylquercetins[3,4].

The demonstration that free and conjugated hydroxyethylrutosides can be recovered from the bile and urine of each second animal following reabsorption of the biliary metabolites indicates that a significant level of enterohepatic cycling is operating in animals receiving hydroxyethylrutosides. These findings may be of significance in relation to the maintenance of therapeutic levels of the drugs in man but the possibility of species differences should not be excluded.

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Affinity chromatography of monoamine oxidase

Ch. M. Buess, Judy K. Price, B. D. Roberts and W. R. Carper[1]

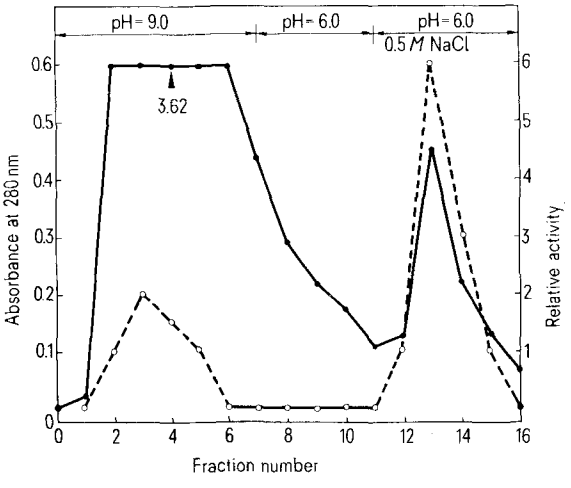
Department of Chemistry, Wichita State University, Wichita (Kansas 67208, USA), 2 August 1976

Summary. A method is described for the purification of pig liver monoamine oxidase by affinity chromatography, using a column with covalently bound pargyline.

Liver mitochondrial monoamine oxidase (MAO) has been isolated from a variety of mammalian sources including the rat[2–5], human[6], beef[7–9] and pig[10–13]. This high molecular weight protein catalyzes the oxidative deamination of primary, secondary, and tertiary amines with a methylene group adjacent to the nitrogen as follows:

$$RCH_2NHR' + O_2 + H_2O \rightarrow RCHO + R'NH_2 + H_2O_2.$$

We recently reported the successful substitution of various divalent metals for copper(II) in pig liver MAO[14]. The preparation of such pseudo-MAOs requires essentially pure enzyme, which prompted the development of the affinity chromatography system described herein.
Monoamine oxidase is inhibited by a variety of basic substances, many of which were included in a recent structure-activity study[15]. Unfortunately, the mode of action of the inhibitors are not completely understood, even with regard to reversibility. Pargyline (N-methyl-N-propargylbenzylamine) is known to react stoichiometrically and irreversibly with bovine kidney monoamine oxidase[16] and the structures of photoaddition products of pargyline and flavoquinone model compounds have been determined[17]. Nevertheless, the inhibition of pig liver monoamine oxidase by pargyline appears to act initially in a reversible manner[18]. Therefore, we attempted to use the pargyline inhibitor for purification by



Elution of MAO from pargyline affinity column in the dark at 4 °C. Absorbance at 280 nm (●) and relative activity (○) using benzylamine as substrate at 30 °C.

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