CONJUGATION OF FOREIGN COMPOUNDS IN THE ELEPHANT AND HYAENA

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1. Introduction

Little is known about the metabolism of foreign compounds in exotic animals. French et al. [1] have examined lions, genets and civets and Ette et al. [2] and Idle et al. [3] have studied the Indian fruit bat. In this paper we report some observations made on the African elephant (Loxodonta) and the spotted hyaena (*Crocuta crocuta*). There is a report in the literature that the elephant (Indian?) forms hippuric acid [4], but no data seems to have been obtained on the hyaena.

In this work it will be shown that like most species of mammals, the African elephant converts benzoic and phenylacetic acid into their glycine conjugates and phenol into its sulphate and glucuronic acid conjugates. In the case of the hyaena phenol, at a level of 10 mg/kg, forms only sulphate conjugates and is thus similar to the cat, lion, civet and genet and also the caracal [5]. The hyaena also does not acetylate the aromatic amino group of sulphadimethoxine and is in this respect like the domestic dog [6] rather than the cats which acetylate the drug [1]. The hyaena, however, converts phenylacetic acid to phenacetylglycine like many other species. 1-Naphthylacetic acid is conjugated in the hyaena mainly with glycine and to a lesser extent with taurine as it is in both the cat [7] and the dog [8], but there is also a substantial glucuronide conjugation of this acid in the hyaena which does not occur in the cat [7] and only slightly in the dog [8].

2. Experimental

The ^{14}C-labelled compounds used and their sources are described by French et al. [1] and Ette et al. [2].

The elephant used was a young female African elephant (Loxodonta) about 6 months old and weighing about 200 kg. Compounds were administered to it dissolved in a slurry of rice and milk which the elephant sucked from a bottle. Urine was collected either by persuading the animal to urinate into a bucket or by keeping the animal in a suitable pen whereby urine and washings could be channelled into a large bottle. The urine was preserved with mercuric chloride.

The hyaenas were the adult spotted variety (*Crocuta crocuta*), weighing about 50 kg. The compounds were administered concealed in meat which the animals bolted. The animals were kept in a pen whereby urine and washings could be channelled into a large bottle. The technique is a rough one but necessary under the circumstances, since hyaenas are dangerous animals. The administration of the compounds and the collection of urine were carried out at the University of Ibadan Zoo. Preserved samples of urine were then transported by air and analysed at St. Mary's Hospital Medical School.

Mercuric ions present in urine preserved with mercuric chloride were removed by precipitation with 10 M-NaOH added dropwise, centrifugation and immediate neutralisation of the supernatant or by passing the urine through a suitably prepared column

of Amberlite XAD-2 resin (BDH Chemicals Ltd., Poole, Dorset, UK) as described by Mulé et al. [9].

The compounds and their metabolites were determined by quantitative radiochromatography as already described for phenol [10], benzoic acid [11], phenylacetic acid [2] and 1-naphthylacetic acid [7] and by paper chromatography and colorimetry for sulphadimethoxine [12].

3. Results

3.1. *[U-^{14}C] Phenol*

In the elephant nearly 50% of the ^{14}C was recovered in 18 h after dosing (table 1). The mercury free urine fraction showed on paper chromatography, two ^{14}C peaks, one corresponding to phenylsulphate (35.1% of dose) and the other to phenylglucuronide (12.6%). On cutting up the paper and counting the various areas by liquid scintillation counting, traces of quinol sulphate (0.4%) and quinol glucuronide (0.2% of dose) were also found. The identities of these areas were confirmed by chromatography in two different solvent systems.

With two hyaenas, one male and one female, the recovery of ^{14}C was poor in one case (male), being only 15.2% of the dose in 24 h, being only 15.2% of the dose in 24 h, but satisfactory (47%) in the other (female), thus illustrating the technical difficulties. However, chromatography and scintillation counting indicated that only phenylsulphate and quinol sulphate were present in both urines, the ratio of phenylsulphate to quinol sulphate being about 15/1 in one case and 7/1 in the other (see table 1). The oxidation of phenol to quinol was much greater in the hyaena than in the elephant.

3.2. *[carboxy-^{14}C]Benzoic acid*

This was only examined in the elephant. The recovery of ^{14}C (63.8% of dose in 12 h) was satisfactory and this ^{14}C was present mainly as hippuric acid. By t. l. c., 89.7%, and by reverse isotope dilution, 87.5% of the urinary ^{14}C was present as hippuric acid. Radiochromatograms showed two ^{14}C peaks, the main one being hippuric acid and a more one, benzoic acid (8.8% of the urinary ^{14}C by chromatography). At the dose level of 100 mg benzoic acid/kg benzoylglucuronide was not found (< 0.6% of the dose).

3.3. *[carboxy-^{14}C]Phenylacetic acid*

This was administered to both the elephant (100 mg/kg) and the hyaena (25 mg/kg). In 6 h nearly 31% of the dose of ^{14}C was recovered from the elephant and this was present entirely as phenaceturic acid. No conjugate with glucuronic acid, taurine or glutamine or unchanged phenylacetic acid were found. In the male hyaena, the recovery was poor being only 12.6% of the dose in 24 h. The urine showed two ^{14}C peaks corresponding to phenaceturic acid (11% of dose) and unchanged phenylacetic acid (1.6%). Glucuronic acid, glutamine or taurine conjugates were not found.

3.4. *[carboxy-^{14}C]1-Naphtylacetic acid*

This acid was fed to a female hyaena. Nearly 50% of the ^{14}C was recovered in 24 h in the urine which showed four peaks of radioactivity on thin-layer chromatograms. The main peaks were due to 1-naphthylacetylglycine (23% of dose) and 1-naphtylacetylglucuronide (19.9%), which were determined by reverse isotope dilution. 1-Naphthylacetyltaurine (5.3%) and unchanged 1-naphthylacetic acid (1.8%) were also found.

3.5. *Sulphadimethoxine*

5 g of sulphadimethoxine were fed to a male hyaena. When the urine was analysed for free and total sulphadimethoxine by the Bratton and Marshall method [13] the results (free 13.9% of dose, total 13.5%) suggested that the drug was excreted mainly unchanged, or possibly as labile N^4-conjugates or N^1-conjugates. Paper chromatography of the urine and analysis of the appropriate areas of the chromatogram as described by Bridges et al. [12], revealed that the main metabolite was the unchanged drug (10% of the dose). Two other areas indicated the presence of the N^4-glucuronide (3.8%) and a small amount of N^1-glucuronide (0.5%). No N^4-acetylsulphadimethoxine or sulphadimethoxine N^4-sulphamate were found (< 0.1%).

4. Discussion

The order Carnivora is divided into two superfamilies, Canoidea and Feloidea [14], the hyaenas (family Hyaenidae) belonging to the latter to which

Table 1
Conjugation in the hyaena and elephant

Compound	Species (dose mg/kg)	Dose of ^{14}C μCi/animal	^{14}C excreted % dose (hours)	% of 24 h excretion found as			
Phenol				Phenylsulphate	Phenylglucuronide		Quinol sulphate
	Hyaena (10)	20	15.2, 47.0 (24)	93, 86	0, 0		6, 13
	Elephant (10)	30	49.2 (18)	73	25		1
Benzoic acid				Benzoic acid	Hippuric acid		
	Elephant (100)	30	63.8 (12)	9	90[a]		
Phenylacetic acid				Phenylacetic acid	Phenaceturic acid		
	Hyaena (25)	25	12.6 (24)	13	87		
	Elephant (100)	43	30.7 (6)	0	100		
1-Naphthylacetic acid				Naphthylacetic acid (NA)	NA-glycine	NA-glucuronide	NA-taurine
	Hyaena (1)	25	49.7 (24)	4[a]	46[a]	40[a]	11
Sulphadimethoxine				Sulphadimethoxine (SDM)	SDM-N^4-glucuronide	SDM-N^1-glucuronide	N^4-acetyl-SDM
	Hyaena (100)	–	13.7 (24)	73	23	4	0

[a] Determined by isotope dilution.

The compounds were administered orally and the urine collected as described in the text. The metabolites were separated by thin layer and/or paper chromatography. ^{14}C in the urine and on chromatograms was determined by scintillation counting. The values for the metabolites are given to the nearest whole number.

Table 2
Conjugation in the hyaena compared with other carnivores

Compound given	Conjugate found in urine	% of 24 h excretion in					
		Canoidea		*Feloidea*			
		Dog[a]	Ferret[a]	Hyaena	Cat[a]	Civet[a]	Genet[a]
Phenol							
	Sulphate	50	58	90	90	97	99
	Glucuronide	18	40	tr	tr	0	0
1-Naphthylacetic acid							
	Glycine	56	6	46	59	74	70
	Glucuronic acid	7	26	40	0	tr	tr
	Taurine	25	63	11	37	6	18
Sulphadimethoxine							
	N^4-Acetyl	0	27	0	18	66	50
	N^1-Glucuronide	19	0	4	0	0	0

0 means not detected; tr = trace
[a] Values taken from [1,5,6,7,15].

the true cats (Felidae) and the genet and civet (Viverridae) also belong. Of the Canoidea, the domestic dog (Canidae) and the ferret (Mustelidae) have been examined in this laboratory. The metabolites of phenol, 1-naphtylacetic acid and sulphadimethoxine found in the hyaena have been compared with those found in certain members of the two superfamilies for which we have data, as was done by French et al. [1] for the cats. This comparison is shown in table 2, the figures in the table indicating approximately the extent of conjugation. On the data available, the table suggests that in the conjugation of phenol the hyaena is like the other Feloidea but not like those Canoidea shown in the table. In the metabolism of 1-naphtylacetic acid, the hyaena is like the other species in the table in forming both glycine and taurine conjugates, but unlike them in producing considerable amounts of 1-naphthylacetylglucuronide. The ferret appears unique in that it excretes large amounts of 1-naphthylacetyltaurine [15]. In the metabolism of sulphadimethoxine the hyaena is like the dog in not acetylating the aromatic amino group, a reaction which is carried out by the ferret, cat, genet, civet and lion. Further studies are needed to support these suggestions, and similar studies on the metabolism of foreign compounds should be extended to wild animals in general in view of world interest in pollution of the environment and its impact on wild life.

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