

## THE CONJUGATION OF PHENOL, BENZOIC ACID, 1-NAPHTHYLACETIC ACID AND SULPHADIMETHOXINE IN THE LION, CIVET AND GENET

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### 1. Introduction

In the domestic cat (*Felis catus*) phenol is converted mainly to phenylsulphate with only small amounts of phenylglucuronide [1]. The cat's ability to form the glucuronides of foreign compounds is low compared with other species [2] and it forms little or no glucuronide with phenol [1], benzoic acid [3], sulphadimethoxine [4], or 1-naphthylacetic acid [5]. It was of interest therefore to find out whether cat-like animals were similar to the domestic cat. The above four compounds were administered to weaned lion cubs (*Panthera leo*) and adult African civets (*Viverra civetta*) and forest genets (*Genetta pardina*) and their urine examined for metabolites. These animals showed little or no glucuronide formation with these compounds and they excreted phenol almost entirely as phenylsulphate, benzoic acid mainly as hippuric acid, 1-naphthylacetic acid as the glycine conjugate and sulphadimethoxine as such and as the  $N^4$ -acetyl derivative. As far as conjugation is concerned, these compounds behave in the lion, civet and genet much as they do in the domestic cat.

### 2. Experimental

[U- $^{14}$ C]Phenol, [carbonyl- $^{14}$ C]benzoic acid and 1-naphthyl-[carbonyl- $^{14}$ C]acetic acid were purchased (Radiochemical Centre, Amersham, Bucks., UK).

Sulphadimethoxine (Madribon) was the gift of Roche Products, Welwyn Garden City, Herts., UK.

Seven lion cubs born at the University of Ibadan Zoo were used when they were 4–5 months old and fully weaned and were fed on raw goat and ox meat and weighed 15–25 kg. Two African civets, one adult male (body weight 8 kg) and one adult female (5 kg) and two forest genets both adult males weighing 2 kg each, were also used and kept on a meat diet. For the collection of urine, each animal was placed in a specially constructed metabolism cage (100 × 70 × 70 cm) made of strong wire mesh welded to Dexion angle and with a tray underneath for the collection of excreta.

The labelled phenol, benzoic acid and 1-naphthylacetic acid were dissolved in dilute  $\text{NaHCO}_3$  solution and were administered by injecting the solution into pieces of raw beef which were then eaten by the animals, or they were injected intramuscularly. Sulphadimethoxine was applied to pieces of meat as a paste in water. The 24 hr urine from each animal was collected and preserved by adding 5 ml of a saturated aqueous solution of mercuric chloride. The administration of the compounds and the collection of urine were carried out at the University of Ibadan Zoo. The urines were then transported by air to London and analysed at St. Mary's Hospital Medical School.

Phenol and its metabolites in the urines were determined by quantitative radiochromatography as described by Capel et al. [1], benzoic acid and its

metabolites as described by Bridges et al. [3] and 1-naphthylacetic acid and its metabolites as described by Dixon et al. [5]. Sulphadimethoxine and its metabolites were detected by paper chromatography and colour reactions and estimated by the Bratton and Marshall [6] method as described by Bridges et al. [7].

### 3. Results

The results are shown in table 1. In the case of [ $^{14}\text{C}$ ]phenol some 50% or more of the dose was excreted in 24 hr and the only compound found in the urine under the conditions of these experiments was phenylsulphate which accounted for nearly all the  $^{14}\text{C}$  excreted. Phenylglucuronide was not detected. With [ $^{14}\text{C}$ ]benzoic acid, over 90% of the  $^{14}\text{C}$  was excreted in 24 hr by the lions, but rather less by the

civets (40%) and genets (66%). Most (70–90%) of this  $^{14}\text{C}$  was in the form of hippuric acid, there being no detectable benzoylglucuronide. The output of  $^{14}\text{C}$  in 24 hr after labelled 1-naphthylacetic acid was nearly 60% of the dose in the lions, but much less in the civets (25%) and the one genet used (34%). In this case the main metabolite was the glycine conjugate, naphthylaceturic acid, which accounted for 70–90% of the urinary  $^{14}\text{C}$ . Small amounts of naphthylacetyltaurine were found in the urine of the civets and the genet, but none in that of the lions. Naphthylacetyl glucuronide was not present in lion urine and this was checked by reverse isotope dilution before and after mild alkaline hydrolysis, but trace amounts were found in civet and genet urine. When sulphadimethoxine was given to two of the lions and a civet, over 50% was excreted in 24 hr by the lions and 44% by the civet, but in the genet only 6% of the

Table 1  
The extent of conjugation of phenol and other compounds in the lion, civet and genet

Compound and dose (mg/kg)	Species (No., sex)	Dose of $^{14}\text{C}$ $\mu\text{Ci}/$ animal	$^{14}\text{C}$ excreted in 24 hr, % dose	% of 24 hr excretion found as:			
Phenol [10]				Phenol	Phenylsulphate	Phenylglucuronide	
	Lion (3F)	12	77 (66–85)	–, –, –	99 (99–99)	–, –, –	
	Civet (1F)	3	60	–	97	–	
	Genet (2M)	3	37, 58	–, –	100, 98	–, –	
Benzoic acid (75)				Benzoic acid	Hippuric acid	Benzoyl glucuronide	
	Lion (2M)	10	92, 92	16, 14	82, 85	–, –	
	Civet* (1M, 1F)	5	35, 44	17, 0	77, 95	–, –	
	Genet* (2M)	5	54, 79	28, 23	67, 75	–, –	
1-Naphthylacetic acid (50)				Naphthylacetic acid	Naphthylaceturic acid	Naphthylacetyltaurine	Naphthylacetylglucuronide
	Lion (2F)	15	58, 59	2, 3	95, 93	–, –	0, 0
	Civet* (1M, 1F)	8	25, 25	12, 8	76, 72	4, 8	tr; 4
	Genet (1M)	8	34	9	70	18	tr
Sulphadimethoxine (75)				Sulphadimethoxine	$N^4$ -Acetylsulphadimethoxine	$N^1$ -Glucuronide	
	Lion (2F)	–	53, 82	55, 49	45, 51	–, –	
	Civet (1F)	–	44	34	66	–	
	Genet (1M)	–	6	50	50	–	

The compounds were administered orally as described in the text unless otherwise stated\*. – means not detected; tr = trace; M = male; F = female.

\* Administered intramuscularly.

dose was excreted. Of the drug excreted in 24 hr, half was unchanged and the other half was present as  $N^4$ -acetylsulphadimethoxine as shown by paper chromatography.

#### 4. Discussion

That the domestic cat has a defect in glucuronic acid conjugation has been known for some time (see [8]) but no related species have been examined from this point of view. The lion belongs to the same family, Felidae, as the domestic cat, whereas the civet and genet are cat-like animals belonging to the related family Viverridae [9].

In table 2, the summarized data on the conjugation of phenol, benzoic acid, 1-naphthylacetic acid and sulphadimethoxine in the lion, civet and genet are compared with those obtained previously in the rat and domestic cat [1,3-5].

In the rat, phenol at a dose level of 25 mg/kg is excreted conjugated with sulphate and glucuronic acid in roughly equal amounts, whereas in the cat, it is conjugated almost entirely with sulphate, there being very little conjugation with glucuronic acid [1].

It is clear from table 2, that the lion, civet and genet conjugate phenol almost entirely with sulphate and may have a defect in glucuronic acid conjugation similar to the domestic cat.

At the dose level of 50 mg/kg, benzoic acid is conjugated mainly with glycine in all five species and again the lion, civet and genet are like the cat. In the dog and ferret an appreciable proportion of benzoic acid is known to conjugate with glucuronic acid [3].

1-Naphthylacetic acid is conjugated mainly with glucuronic acid in man, three species of monkeys, the rat and the rabbit, but not in the cat [5]. The values for the rat and cat are quoted in table 2. The cat converts 1-naphthylacetic acid mainly to the glycine conjugate and to a lesser but appreciable extent to the taurine conjugate, but no glucuronic acid conjugation occurs. The lion is similar to the domestic cat in that it forms the glycine conjugate but no glucuronide of naphthylacetic acid, but differs from the cat in not forming a taurine conjugate. The civet and genet are similar to the cat in that they do form small amounts of the taurine conjugate.

Table 2 shows that the lion, civet and genet are able to acetylate the aromatic amino group like most other species except the dog and fox [10]. Some

Table 2  
Conjugation in Felidae compared with the Rat

Compound given	Conjugate found in urine	% of 24 hour excretion in:				
		Rat*	Domestic Cat*	Lion	Civet	Genet
Phenol	Sulphate	54	97	99	97	99
	Glucuronide	42	tr	—	—	—
Benzoic acid	Glycine	99	100	83	86	71
	Glucuronide	tr	tr	—	—	—
1-Naphthylacetic acid	Glycine	23	59	94	74	70
	Glucuronide	51	0	0	tr	tr
	Taurine	0	39	—	6	18
Sulphadimethoxine	$N^4$ -Acetyl-	19	18	48	66	50
	$N^1$ -Glucuronide	8	0	0	0	0

— means not detected; tr = trace

\* Values taken from [1,3,4,5].

species including man and other primates are also able to convert sulphadimethoxine extensively to an  $N^1$ -glucuronide. The domestic cat, the lion, civet and genet, however, do not form any glucuronide with sulphadimethoxine. In some species, e.g. the rat and man, sulphadimethoxine is slowly excreted [4] and it is possible that this is true for the genet and might explain the low output of 6% of the dose in 24 hr, but since only one animal has been examined so far, other explanations are possible such as difficulties in handling and urine collection.

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