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The Fate of Benzoic Acid in Various Species

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1. The urinary excretion of orally administered [^{14}C]benzoic acid in man and 20 other species of animal was examined. 2. At a dose of 50mg/kg, benzoic acid was excreted by the rodents (rat, mouse, guinea pig, golden hamster, steppe lemming and gerbil), the rabbit, the cat and the capuchin monkey almost entirely as hippuric acid (95–100% of 24h excretion). 3. In man at a dose of 1mg/kg and the rhesus monkey at 20mg/kg benzoic acid was excreted entirely as hippuric acid. 4. At 50mg/kg benzoic acid was excreted as hippuric acid to the extent of about 80% of the 24h excretion in the squirrel monkey, pig, dog, ferret, hedgehog and pigeon, the other 20% being found as benzoyl glucuronide and benzoic acid, the latter possibly arising by decomposition of the former. 5. On increasing the dose of benzoic acid to 200mg/kg in the ferret, the proportion of benzoyl glucuronide excreted increased and that of hippuric acid decreased. This did not occur in the rabbit, which excreted 200mg/kg almost entirely as hippuric acid. It appears that the hedgehog and ferret are like the dog in respect to their metabolism of benzoic acid. 6. The Indian fruit bat produced only traces of hippuric acid and possibly has a defect in the glycine conjugation of benzoic acid. The main metabolite in this animal (dose 50mg/kg) was benzoyl glucuronide. 7. The chicken, side-necked turtle and gecko converted benzoic acid mainly into ornithuric acid, but all three species also excreted smaller amounts of hippuric acid.

In a previous paper (Adamson, Bridges, Evans & Williams, 1970) it was shown that in man and certain Old World monkeys orally administered quinic acid was extensively aromatized apparently by gut bacteria to benzoic acid and excreted as hippuric acid. In certain other primates and lower animals this did not occur to any large extent. However, it was possible that in the lower animals the conversion of quinic acid into benzoic acid did occur, but that the latter was destroyed before it had the opportunity of being conjugated and excreted. The fate of [^{14}C]benzoic acid was therefore examined in man and 20 other species of animals, including three species of subhuman primates. In the present paper it is shown that all the species examined excrete orally-administered benzoic acid mainly as its conjugates and this suggests that benzoic acid is not destroyed in these animals. Some new information on the fate of benzoic acid in certain species has been found.

MATERIALS AND METHODS

Materials. [*ring*-^{14}C]Benzoic acid (395μCi/mg) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Benzoic acid, m.p. 122°C, and hippuric acid, m.p. 187°C, were purchased and purified. [*carbonyl*-^{14}C]Hippuric acid (0.022μCi/mg), L(+)-ornithuric acid, m.p. 182°C and $[\alpha]_D^{23}+11.9°$ (*c* 1 in 0.1M-NaOH), and benzoyl glucuronide, m.p. 180°C and $[\alpha]_D^{20}-25.6°$ (*c* 0.7 in water), were samples previously prepared in this laboratory. The purity of these compounds was checked by paper chromatography and, where possible, by radiochromatogram scanning.

Animals and dosing. The animals used are listed in Table 2. They were obtained from animal dealers mainly in the London area and were maintained on an appropriate diet. Sodium [^{14}C]benzoate was administered to the subhuman primates, the dogs and pigs mixed in food and to cats and ferrets dissolved in milk. The rodents, reptiles, fruit bats and hedgehogs received the compound dissolved in water by syringe into the stomach and the birds and rabbits by stomach tube. The animals were kept in metabolism cages appropriate to their size and their urine was collected daily in receptacles containing a few millilitres of saturated aq. $HgCl_2$ solution to prevent bacterial breakdown of conjugates. Birds were starved for 24h before the administration of benzoate to decrease the amount of faeces, and their excreta were collected on galvanized metal trays placed below their cages. All urines were kept frozen after collection until needed, but were analysed for ^{14}C as rapidly as possible after collection.

Chromatography. The R_F values and methods of detection of benzoic acid and its metabolites are given in Table 1.

Radiochemical techniques. The radioactivities of all

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Table 1. *R_F values and colour reactions of benzoic acid and its metabolites*

Whatman no. 1 paper with the descending method was used. The solvent systems were: *A*, butan-1-ol–ethanol–water–acetic acid (3:1:1:0.1, by vol.); *B*, butan-1-ol saturated with a solution of equal volumes of 1.5M-NH_3 and 1.5M-$(NH_4)_2CO_3$; *C*, butan-1-ol–acetic acid–water (4:1:2, by vol.). NR, naphtharesorcinol spray (Bridges, Kibby & Williams, 1965); DMAB, spray of *p*-dimethylaminobenzaldehyde in acetic anhydride (4%, w/v) containing a little sodium acetate, followed by gentle heating of the paper with a hot-air blower; DMAC, spray of *p*-dimethylaminocinnamaldehyde in acetic anhydride (0.05%, w/v), then heating the paper for 2min at 120°C. The dried paper was illuminated with u.v. light (254nm) from a Hanovia Chromatolite lamp: q, quenching of background fluorescence of the paper.

	R_F value			Colour reactions			Appearance
	Solvent *A*	Solvent *B*	Solvent *C*	NR	DMAB	DMAC	in u.v. light
Benzoic acid	0.92	0.45	0.90	—	—	—	q
Hippuric acid	0.83	0.35	0.83	—	Yellow	Red	q
Benzoyl glucuronide	0.62	0.23	0.67	Blue	—	—	q
Ornithuric acid	0.91	0.70	0.89	—	—	White on yellow background*	q

* Blue fluorescence in u.v. light.

urine samples (0.5–1.0ml) were counted in quadruplicate in a dioxan scintillator fluid (20ml) (Bridges, Davies & Williams, 1967). Faeces and tissues (1g) were homogenized in 10 parts (v/w) of water and then made up to 100ml in water. Radioactivities of 0.5ml samples of this solution were counted in triplicate in a gel dioxan scintillator system (Bridges *et al.* 1967) in a Packard Tri-Carb model 3214 scintillation counter. All samples were counted at 0°C and then recounted about a week later to allow for possible errors due to chemiluminescence. Radiochromatogram scans of the urine of every animal given [^{14}C]benzoic acid were prepared with a Packard model 7200 radiochromatogram scanner. The urine (0.1ml) was applied as a band to a strip (3.8cm wide) of Whatman no. 1 paper and dried. The chromatograms were developed during 15h in solvents *A*, *B* or *C* (see Table 1), and then scanned for ^{14}C.

When necessary benzoic acid and hippuric acid were determined by reverse isotope dilution, the acids being isolated from acidified urine and their radioactivities counted before and after conversion into their *p*-nitrobenzyl esters (Adamson *et al.* 1970).

RESULTS AND DISCUSSION

The results of our experiments on the fate of orally-administered [^{14}C]benzoic acid in man and 20 other species are summarized in Table 2. Most of the animals were given the compound at a dose of 50mg/kg, and in most cases 50–100% of the ^{14}C administered was excreted in the urine within 24h of dosing. All the ^{14}C excreted was present as compounds containing benzoic acid. The only animals in which the excretion of ^{14}C was relatively slow were the turtle, which excreted 12% and 39% in 2 and 3 days respectively, and the geckos, which excreted 32% and 39% in 2 and 3 days respectively.

The metabolites of benzoic acid in most species are hippuric acid and benzoyl glucuronide, although some species may produce ornithuric acid or benzoylarginine (see Williams, 1967*a*; Smith, 1968). Table 2 shows that in most of the herbivorous and omnivorous animals benzoic acid when given at 50mg/kg is excreted almost entirely (90–100%) as hippuric acid. These animals include man, rhesus monkey, squirrel monkey, capuchin monkey, pig, rabbit and the six species of rodents (rat, mouse, guinea pig, hamster, lemming and gerbil). The two pigs and two squirrel monkeys examined excreted some 10–20% of the ^{14}C eliminated in 24h as free benzoic acid, and it is possible that this could have arisen by decomposition of the relatively labile benzoyl glucuronide. The result for man is not strictly comparable with the other animals in this group, since the dose given was only 1mg/kg. However, the dose (70mg) was excreted by both subjects entirely as hippuric acid, and 97% of the ^{14}C administered was excreted within 4h after dosing and virtually 100% in 12h.

Three carnivorous species were examined, namely the cat, dog and ferret. In the cat the whole of the ^{14}C excreted was present as hippuric acid; this was not unexpected, since the cat has a defective glucuronic acid conjugation (see Williams, 1967*a*,*b*). Both the dog and ferret, however, excreted appreciable amounts of benzoyl glucuronide. It is known that in the dog the rate of mobilization of glycine for hippuric acid synthesis is relatively low compared with other species, and further, glycine conjugation occurs only in the kidney, whereas in the rat and rabbit for example it occurs in both the liver and the kidney. Therefore in the dog the tendency for benzoic acid to conjugate with glucuronic acid occurs at lower doses than in other

Table 2. *Metabolites of* [^{14}C]*benzoic acid in urine in various species*

Sodium [^{14}C]benzoate was administered orally as described in the text. The first 24 h urine (unless otherwise stated) of each animal was chromatographed on Whatman no. 1 paper (see Table 1 for solvents) and the paper scanned in a Packard radiochromatogram scanner. Where three or more animals were used the results are expressed as averages with ranges in parentheses. Where only one or two animals of a species were used the individual results are given. The amounts of the metabolites were estimated from the chromatogram scans as described in the text. M, male; F, female.

Species (no. and sex)	Family or other description	Dose: Benzoic acid (mg/kg)	Dose: ^{14}C (μCi/animal)	^{14}C excreted in 24 h (% of dose)	% of 24 h excretion found: Benzoic acid	Hippuric acid	Benzoyl glucuronide	Ornithuric acid
Primates								
Man (2M)	*Homo*	1	5.8	99.4, 99.7	0	100	0	0
Rhesus monkey (3F)	*Macaca*	20	6.8	47 (33–59)	0	100	0	0
Squirrel monkey (2F)	*Saimiri*	50	8.8	46, 49	14, 18	81, 83	5, Trace	0
Capuchin (1F)	*Cebus*	50	8.8	57	0	100	Trace	0
Artiodactyla								
Pig (large white) (2F)	*Suidae*	50	10.1	48, 51	15, 7	85, 93	Trace	0
Lagomorpha								
Rabbit (New Zealand White)	*Leporidae*	49	8.4	60 (36–77)	0	100	0	0
(3F)		200	9.7	86 (79–95)	Trace	98 (97–100)	2 (0–5)	0
Rodents								
Rat (Wistar albino) (3F)	*Muridae*	50	1.0	100 (98–102)	1 (Trace–2)	99 (97–100)	Trace	0
Mouse (I.C.I.) (3×10F)*	*Muridae*	56	0.6	55 (43–67)	Trace	95 (94–96)	5 (4–6)	0
Guinea pig (English) (3F)	*Caviidae*	49	5.0	79 (63–94)	Trace	98 (97–99)	3 (2–3)	0
Hamster (golden) (3F)	*Cricetidae*	52	2.1	99 (98–101)	1	97 (97–98)	1 (1–2)	0
Lemming (steppe) (3F)	*Cricetidae*	56	2.5	98 (94–102)	Trace	100 (99–100)	0	0
Gerbil (3F)	*Cricetidae*	29	3.3	75 (66–82)	2 (0–4)	98 (96–100)	0	0
Carnivora								
Cat (mongrel) (2F)	*Felidae*	51	9.7	29, 86	Trace	100	0	0
Dog (mongrel) (2F, 1M)	*Canidae*	51	8.2	94 (87–100)	0	82 (71–100)	18 (Trace–25)	0
Ferret (mongrel) (3F)	*Mustelidae*	50	7.1	69 (57–90)	9 (6–12)	70 (67–72)	22 (21–22)	0
		198	7.5	78 (62–88)	9 (5–12)	47 (40–54)	44 (41–48)	0
		400	7.5	67 (58–74)	22 (21–23)	30 (24–37)	49 (42–53)	0
Insectivora								
Hedgehog (European) (2M)	*Erinaceidae*	50	6.4	67, 78	5, 7	76, 86	19, 7	0
Chiroptera								
Fruit bat (Indian) (2F)	*Pteropidae*	50	7.8	49, 54	12, 30	Trace	88, 70	0
Birds								
Chicken (Light Sussex) (5F)	*Phasianidae*	50	9.3	56 (38–69)	22 (16–28)	21 (10–43)	3 (0–8)	54 (40–69)
Pigeon (3F)	*Columbidae*	50	6.3	87 (84–102)	15 (13–20)	84 (80–87)	1 (Trace–3)	0
Reptiles								
Turtle (side-necked) (1)†	See footnote	50	6.2	39§	10	16	2	72
Gecko (1×3F)‡	*Gekkonidae*	19	2.5	32‖	3	6	6	85

* The urines of ten animals were pooled for each determination.

† Also called hidden-necked turtle; sex not known; sub-order Pleurodira.

‡ The urines of three animals were pooled.

§ 96 h excretion.

‖ 48 h excretion.

species such as the rabbit (for discussion see Williams, 1959). Table 2 shows that the ferret may be like the dog in this respect, for in the ferret about 22% of the 24h excretion after a dose of 50mg of benzoic acid/kg occurs as benzoyl glucuronide. When the dose is increased to 198mg/kg glycine and glucuronic acid conjugation are about equal, and at 400mg/kg glucuronic acid conjugation is the main mechanism. The ferret also excreted appreciable amounts of free benzoic acid, which may well have arisen by breakdown of benzoyl glucuronide. This effect of dose size in the ferret should be compared with that in the rabbit (see Table 2), an animal that has a relatively high rate of mobilization of glycine for conjugation. At 50mg/kg benzoic acid is excreted in this animal entirely as hippuric acid, and on raising the dose to 200mg/kg glycine conjugation of the acid is still nearly complete (97–100%), only small amounts of benzoyl glucuronide being detectable in the urine.

The hedgehog, an insectivore, has been little used in metabolic studies of this type. Adamson *et al.* (1970) have shown that like many other subprimate species it does not aromatize quinic acid. Other work in this laboratory has shown that it can conjugate phenol with sulphate and glucuronic acid and oxidize phenol to quinol (M. R. French, R. L. Smith & R. T. Williams, unpublished work). Table 2 shows that it converts an oral dose (50mg/kg) of benzoic acid into hippuric acid to the extent of about 80% and also forms benzoyl glucuronide. In this respect it is similar to the dog and ferret.

Table 2 suggests that the Indian fruit bat (*Pteroptus giganteus*) may have a defective glycine-conjugation system. Benzoic acid is excreted by this animal entirely as benzoyl glucuronide (70–80% of the 24h excretion) and free benzoic acid. Radiochromatogram scans in solvents *A*, *B* and *C* (Table 1) of fruit-bat urine after dosage with [^{14}C]-benzoic acid showed two peaks corresponding to benzoic acid (minor) and benzoyl glucuronide (major). Reverse isotope dilution for benzoic acid and hippuric acid confirmed that labelled hippuric acid was not present in fruit-bat urine in more than traces. Conjugation with glycine or some other amino acid (e.g. ornithine, arginine etc.; see Smith, 1968) is almost universal among animals, ranging from insects to man. The absence of such a conjugation, however, has been reported in the carrion crow and grey African parrot (Baldwin, Robinson & Williams, 1960) and certain crustacea (Smith, 1968). These observations, however, need confirmation, and the possible absence of hippuric acid synthesis in the fruit bat has now to be investigated at the enzyme level. It is noteworthy that the fruit bat is, like man, unable to synthesize ascorbic acid (Chatterjee, Kar, Ghosh & Guha, 1961; Schmidt-Nielsen, 1967). It does not aromatize quinic acid (Adamson *et al.* 1970), but it conjugates small doses (25mg/kg) of phenol, mainly with glucuronic acid and only to a minor extent with sulphate (M. R. French, R. L. Smith & R. T. Williams, unpublished work).

The findings for the pigeon confirm previous work that this bird converts benzoic acid into hippuric acid and not ornithuric acid (Baldwin *et al.* 1960). With the chicken (Light Sussex) both ornithuric acid and hippuric acid were found. Radiochromatogram scans of chicken excreta after the administration of [^{14}C]benzoic acid showed four radioactive peaks, the largest one being due to ornithuric acid. Although ornithuric acid was the major metabolite, a peak corresponding to hippuric acid was found on the radiochromatogram scans of the excreta of all five hens used. The amount varied from about 5 to 20% of the dose of benzoic acid. In previous work in this laboratory with Brown Leghorn hens (Baldwin *et al.* 1960) it was found that these hens did not form hippuric acid from [^{14}C]benzoic acid, but reverse isotope dilution for hippuric acid in the presence of ornithuric acid is not entirely satisfactory. That glycine conjugation occurs in hens to a minor extent has been reported by Suga (1919) and Chang & Johnson (1956). Both the hen and pigeon excrete appreciable amounts of free benzoic acid (15–20% of 24h excretion), but this could well have arisen from benzoyl glucuronide, which is also present in small amounts in their excreta.

The last two species in Table 2, the turtle and the gecko, excrete the administered [^{14}C]benzoic acid slowly. In both cases, however, the main radioactive metabolite is ornithuric acid, but on the radiochromatogram scans a small peak corresponding to hippuric acid was found. Komori, Sendju, Sagara & Takamatsu (1926) have reported that the turtle excretes hippuric acid after being given benzoic acid, and Khalil (1947) has found hippuric acid in the normal urine of the sea-turtle (*Chelone mydas*), but neither of these papers reported ornithuric acid. Table 2 shows that ornithuric acid (28% of the dose in 96h) is the main metabolite of benzoic acid in the side-necked turtle (species unidentified), although a small amount (6% of the dose) of hippuric acid appears to be present also.

As far as we are aware, the gecko (*Gekko gecko*) has not been previously investigated. It converts benzoic acid mainly into ornithuric acid (27% of the dose in 48h) together with a small amount of what may be hippuric acid (about 2% of the dose) (Table 2). Smith (1958) has found that the green lizard (*Lacerta viridis*) converted benzoic acid into ornithuric acid, but hippuric acid was not detected. Both the gecko and green lizard belong to the reptile sub-order Lacertilia (or Sauria), but to

different infra-orders, namely Gekkota and Scincomorpha respectively.

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