GLYCINE CONJUGATION IN THE INDIAN FRUIT BAT

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1. Introduction

In an earlier study from this laboratory [1], it was found that the Indian fruit bat did not excrete hippuric acid when injected with benzoic acid, the major metabolite excreted being benzoylglucuronide. This suggested that the fruit bat had a defect in glycine conjugation. These studies have now been extended to phenylacetic acid and it has been found that the major metabolite of this acid in the fruit bat is the glycine conjugate, phenaceturic acid. The metabolism of benzoic acid has been re-examined in the fruit bat and the absence of the glycine conjugation of this acid has been confirmed. There is thus a sharp difference between the metabolism of benzoic acid and its homologue, phenylacetic acid, in the fruit bat. It is well known that these acids differ in their metabolism in man, benzoic acid forming hippuric acid and phenylacetic acid, phenacetylglutamine [2]. In the rat, however, both acids are conjugated with glycine. The Indian fruit bat, therefore, has a glycine conjugation, but its occurrence depends upon substrate.

2. Materials and methods

[Carbonyl-^{14}C]phenylacetic acid and [carbonyl-^{14}C]benzoic acid were purchased (Radiochemical Centre, Amersham, Bucks, U.K.). Phenaceturic acid and benzoylglucuronide were available in this laboratory. Indian fruit bats (*Pteropus giganteus*) were purchased from local dealers and were maintained as described by Bababunmi et al. [1]. The labelled acids in 1 ml dilute $NaHCO_3$ solution were injected intraperitoneally and the urine was collected for 24 hr. The urine which had pH 7–8 was brought to pH 6 with 2N acetic acid stored at 0°C after adding a little mercuric chloride solution as a preservative.

The output of ^{14}C was determined by scintillation counting (Packard Tri-Carb scintillation spectrometer, model 7200). Urines (5 μl) were chromatographed on thin-layer using aluminium based fluorescent silica gel $60F_{254}$ (E. Merck, Darmstadt, W. Germany) and the chromatograms were scanned in a Packard radiochromatogram scanner Model 3320. An approximate estimate of the relative amounts of metabolites was obtained by scraping out the silica gel from the areas of peak radioactivity on the chromatograms and determining the ^{14}C in the silica by scintillation counting. Phenacetylglycine and phenylacetic acid were estimated by reverse isotope dilution as described by James et al. [3] and hippuric acid by Bridges et al. [4]. The presence of glucuronic acid conjugates on chromatograms was detected with naphtharesorcinol and glycine conjugates with *p*-dimethylaminobenzaldehyde as previously described [1].

3. Results

3.1. *Phenylacetic acid*

Each of the urines of five fruit bats which had been injected with [^{14}C]phenylacetic acid showed on radiochromatograms three clear peaks (labelled 1, 2 and 3 in table 1). The R_F values of phenylacetic acid and phenaceturic acid are shown in table 1. These R_F values varied to some extent and this depended upon the age of solvents A and B and upon the concentration of urine used to spot them on thin layer. Standards of phenylacetic and phenaceturic acid

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Table 1
T.l.c. of phenylacetic and phenaceturic acid

	R_F value in solvent				Colour tests	
	A		B		NR	DMBA
	w	u	w	u		
Standards						
Phenaceturic acid	0.17,	0.22	0.45,	0.77	–	orange
Phenylacetic acid	0.60,	0.70	0.90,	0.93	–	–
Urine peaks						
1	0.02–0.10		0.08–0.16		blue	–
2 (main peak)	0.22–0.39		0.40–0.57		–	orange
3	0.53–0.69		0.79–0.90		–	–

Solvents: A, benzene–acetone–acetic acid (6:3:1); B, chloroform–methanol–acetic acid (12:4:1) (proportions by vol). Chromatograms were run on aluminium based fluorescent silica gel $60F_{254}$. Standard R_F values were measured after the compounds had been applied to the chromatogram dissolved in water (w) and in fruit bat urine (u). The range of R_F values of ^{14}C peaks in urine are those found in the urines of 5 fruit bats, the two extremes being quoted. The naphtharesorcinol (NR) and 4-dimethyl-aminobenzaldehyde (DMBA) sprays were used as described by Bababunmi et al. [1].

Table 2
Metabolites of [^{14}C]phenylacetic acid in fruit bats

	^{14}C excreted in 24 hr	Peak No. 1		Peak No. 2			Peak No. 3		
		A	B	A	B		A	B	
Bat No. and Sex	% Dose		p.s.		p.s.,	i.d.*		p.s.,	i.d.**
1 F	38.5	7.3	11	30	87,	78	1.4	2.3,	3.7
2 M	81.2	12	18	65	78,	80	4.6	3.8,	5.7
3 F	45.2	4.5	10	36	85,	79	2.7	5.3,	6.1
4 F	54.4	6.0	11	38	75,	70	4.3	14,	7.8
5 F	26.6	5.4	8	19	87,	73	1.8	4.4,	6.6
	Mean values		12		82,	76		6,	6

* Reverse isotope dilution with phenaceturic acid
** Reverse isotope dilution with phenylacetic acid

Each bat was injected intraperitoneally with [^{14}C]phenylacetic acid (14.8 mg and 10 μCi) in 1 ml in N $NaHCO_3$ solution. Urines were collected for 24 hr and chromatographed on thin-layer and ^{14}C counted as described in the text. The results (given to the nearest two numbers) are expressed in two ways, A as % of dose and B as % of the ^{14}C excreted in 24 hr. p.s. indicates that the value was obtained by scraping the plate and scintillation counting; i.d. means determined by reverse isotope dilution. Peak No. refers to peaks on radiochromatograms (see table 1).

were therefore run at the same time as each unknown to allow for these variations. The nature of the three peaks was elucidated as follows. Peak No. 1 (table 1) appeared to consist mainly of an ester glucuronide, probably phenacetyl glucuronide, since it gave a blue colour with the naphtharesorcinol spray for glucuronic acid and largely disappeared on alkaline hydrolysis. Peak No. 2 was the major one and corresponded in R_F value to phenaceturic acid. It also gave an orange colour with the 4-dimethylaminobenzaldehyde spray for glycine conjugates. Peak No. 3 corresponded to phenylacetic acid and on hydrolysis of the urine and rechromatographing, it increased considerably at the expense of the other two peaks.

Table 2 shows the recovery of administered ^{14}C in the 24-hr urine and washings. The recoveries of ^{14}C in 24 hr after dosing varied from 27% for bat No. 5 to 81% for bat No. 2 and simply illustrate some of the difficulties in collecting urine from fruit bats which hang upside down in their cages [1]. This table also shows the % of dose of ^{14}C in each peak (A) and the proportion (B) of the 24 hour output which each peak represents. Both phenaceturic acid and phenylacetic acid were determined by isotope dilution and it is clear that 76% (range 70-80%) of the ^{14}C excreted is present as phenacetylglycine and 6% (3.7–7.8) as the unchanged acid. Peak No. 1 accounted for 12% (8–18) of the 24-hour output of ^{14}C, and appeared to contain some phenacetyl-glucuronide.

3.2. *Benzoic acid*

Three fruit bats (Nos. 1, 2 and 4) were each injected with 44.7 mg (8.3 μCi) of [^{14}C]benzoic acid in 1 ml of $NaHCO_3$. The recoveries of ^{14}C in the urine in 24 hr were No. 1, 43.8%, No. 2, 9.7% and No. 4, 44.0% of the dose. Thin-layer chromatograms were prepared using solvents A and B of table 1, and R_F values were compared with authentic samples of benzoylglucuronide, hippuric acid and benzoic acid. Two significant peaks were found, a large one corresponding to benzoylglucuronide and a small one to benzoic acid as shown by Bababunmi et al. [1]. Isotope dilutions were carried out for hippuric acid and the results obtained were, bat No. 1, $< 0.12\%$, No. 2, $< 0.75\%$ and No. 4, $< 0.16\%$ of the dose, these figures being on the limit of the method with ^{14}C available in the sample. These results confirm our previous finding [1] that hippuric acid is not a significant metabolite of benzoic acid in the Indian fruit bat.

4. Discussion

Some species have been found to be defective in certain conjugation mechanisms which occur in man. These include glucuronide formation in cats (and related species, see [5]), sulphate conjugation in pigs and acetylation of aromatic amines in the dog and fox [6]. Further investigation, however, has shown that these apparent defects are substrate dependent [7] since cats can form glucuronides and pigs ethereal sulphates with certain compounds [8]. It is now clear that in the Indian fruit bat glycine conjugation does not occur with benzoic acid but does with phenylacetic acid. When defining a species defect in conjugation, the substrate used must also be defined.

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