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Species Differences in the Aromatization of Quinic Acid *in vivo* and the Role of Gut Bacteria

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1. The fate of (−)-quinic acid has been investigated in 22 species of animals including man. 2. In man and three species of Old World monkeys, i.e. rhesus monkey, baboon and green monkey, oral quinic acid was extensively aromatized (20–60%) and excreted in the urine as hippuric acid, which was determined fluorimetrically. 3. In three species of New World monkeys, i.e. squirrel monkey, spider monkey and capuchin, in three species of lemurs, i.e. bushbaby, slow loris and tree shrew, in the dog, cat, ferret, rabbit, rat, mouse, guinea pig, hamster, lemming, fruit bat, hedgehog and pigeon, oral quinic acid was not extensively aromatized (0–5%). 4. In the rhesus monkey, injected quinic acid was not aromatized, but largely excreted unchanged. 5. In rhesus monkeys pretreated with neomycin to suppress gut flora, the aromatization of oral quinic acid was considerably suppressed. 6. In rats and rhesus monkeys [^{14}C]quinic acid was used and this confirmed its low aromatization in rats and its high aromatization in the monkeys. 7. Shikimic acid given orally was excreted as hippuric acid (26–56%) in rhesus monkeys, but not in rats. 8. The results support the view that quinic acid and shikimic acid are aromatized by the gut flora in man and the Old World monkeys.

Lautemann (1863) showed that in man ingested (−)-quinic acid (1,3,4,5-tetrahydroxycyclohexanecarboxylic acid), a compound occurring in tea, coffee, fruits and vegetables, was excreted to a large extent as hippuric acid. This has been confirmed by several authors (Quick, 1931; Beer, Dickens & Pearson, 1951; Bernhard, Vuilleumier & Brubacher, 1955; Cotran, Kendrick & Kass, 1960). Beer *et al.* (1951) found little or no aromatization of orally administered quinic acid in rabbits and rats or of the subcutaneously injected acid in guinea pigs and cats. Both Bernhard *et al.* (1955) and Cotran *et al.* (1960) have reported a substantial conversion of oral quinic acid into hippuric acid in guinea pigs. A high conversion has been reported in sheep but a low one in man and dog (Vasiliu, Timosencu, Zaimov & Coteleu, 1940; Bernhard, 1937). Cotran *et al.* (1960) have shown that the administration of neomycin in doses sufficient to inhibit bacterial multiplication in the intestine prevents the conversion of quinic acid into hippuric acid in man. They also showed that in the guinea pig aromatization occurs when quinic acid is given by mouth, but not when given intraperitoneally. Asatoor (1965) found that aromatization occurs in rats given quinic acid orally but not in neomycin-treated rats. It thus appears that there may be species differences in the extent of aromatization of quinic acid and that these differences may be related to gut bacteria, which may be responsible for the conversion of quinic acid into benzoic acid. Davis & Weiss (1953) have shown that coliform bacteria can aromatize quinic acid, but Mitoma, Posner & Leonard (1958) found no aromatization of quinic acid by guinea-pig liver homogenates capable of aromatizing cyclohexanecarboxylic acid or by a human liver homogenate, which, however, could not aromatize cyclohexanecarboxylic acid. Some of the results quoted in this paper have been briefly reported (Adamson, Bridges & Williams, 1966; Adamson, Bridges, Evans & Williams, 1969).

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MATERIALS AND METHODS

Chemicals. (−)-Quinic acid, m.p.164°C and $[\alpha]_D^{20}$ −42.2° (*c* 1.9 in water) (Hopkin and Williams Ltd., Chadwell Heath, Essex, U.K.), (−)-[G-^{14}C]quinic acid

(5mCi/mmol) (New England Nuclear Corp., Dreieichenhain, West Germany), [U-*ring*-^{14}C]benzoic acid (48.2mCi/mmol) (The Radiochemical Centre, Amersham, Bucks., U.K.), shikimic acid, m.p.190°C (Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.) and neomycin sulphate (Burroughs Wellcome Ltd., London N.W.1, U.K.) were purchased. [*carbonyl*-^{14}C]Hippuric acid (m.p.189–190°C; 0.04mCi/mmol) was prepared from [*carboxy*-^{14}C]-benzoic acid and glycine with thionyl chloride, and benzoyl glucuronide, m.p.180°C, was a sample prepared by Baldwin, Robinson & Williams (1960) by feeding turkeys with benzoic acid. Other laboratory chemicals were purchased and purified where necessary for chromatography and fluorimetry.

Animals. Twentyone species of animals were used as listed in Tables 2 and 3. They were obtained from animal dealers mainly in the London area and were maintained on an appropriate diet. All the sub-human primates were kept on the same diet, which consisted of fruit (banana and apple) and diet 41B rat food [Herbert C. Styles (Bewdley) Ltd., Bewdley, Worcs., U.K.]. The animals were kept on a constant diet for several days before the administration of compounds and during the experiments. Quinic acid or shikimic acid was administered orally dissolved in water to which $NaHCO_3$ had been added to bring the pH of the solution to 7–8. To suppress gut flora in rats, neomycin sulphate (100mg) in water was administered twice daily by mouth for 6 days, whereas rhesus monkeys received 2g daily for 4 days before the administration of quinic acid. Urine and faeces were collected daily.

Determination of hippuric acid. The method used was essentially that of Ellman, Burkhalter & La Dou (1961). The fresh 24h urine was centrifuged at 2000*g* for 10min. Then 8μl of the supernatant urine, measured with a Hamilton microsyringe, was added to 3ml of 70% H_2SO_4 (7vol. of A.R. H_2SO_4 to 3vol. of water) and mixed. The fluorescence intensity of the solution was then measured in an Aminco–Bowman spectrophotofluorimeter at 366nm with the excitation set at 259nm. The fluorescence intensities of standards containing 2, 5 and 10μg of hippuric acid in 8μl of water and of the same quantities of hippuric acid added to the urine were also measured. In this way any quenching of the hippuric acid–H_2SO_4 fluorescence by urine could be estimated and allowed for. The fluorescence intensity of hippuric acid in H_2SO_4 was proportional to the amount of hippuric acid from 0–10μg in 3ml of 70% H_2SO_4. Other work in this laboratory (J. W. Bridges, M. R. French, R. L. Smith & R. T. Williams, unpublished work) on the fate of [^{14}C]benzoic acid in various species has shown that hippuric acid is the main metabolite of benzoic acid, given orally at a dose of 50mg/kg, in the urine of all the species studied here except for the ferret and fruit bat. In the latter species, benzoyl glucuronide is the main metabolite and when this is added to 70% H_2SO_4 maximum fluorescence occurs at 388nm with maximum excitation at 358nm. These wavelengths were therefore used for the urines of the ferret and fruit bat. Little free benzoic acid occurred in the urine of any of the species examined.

A number of compounds related to benzoic acid together with quinic acid and shikimic acid were examined for their fluorescence in H_2SO_4 with a view to their possible interference with the determination of hippuric acid. The fluorescence of hippuric acid from H_0−9.6 (17.5M-H_2SO_4) to H_0−2.0 (4.5M-H_2SO_4) was also examined.

Chromatography. The R_F values and colour reactions of quinic acid and its possible metabolites are given in Table 1.

Radiochemical experiments. Rats receiving [^{14}C]quinic acid were kept in Metabowl metabolism cages (Jencons Ltd., Hemel Hempstead, Herts., U.K.) so that expired CO_2, urine and faeces could be collected daily. The $^{14}CO_2$ expired was trapped in 4M-NaOH and then recovered as $Ba^{14}CO_3$, the radioactivity of which was counted on a planchet with a Panax end-window counter (model no. D675; Panax Equipment Ltd., Redhill,

Table 1. *R_F values and detection methods for quinic acid and its possible metabolites*

Whatman no. 1 paper with the descending method was used. The solvent systems were: *A*, butan-2-ol–water–acetic acid (14:5:1, by vol.); *B*, butan-1-ol–ethanol–water–acetic acid (30:10:10:1, by vol.). NR, naphtharesorcinol spray (Bridges, Kibby & Williams, 1965). The dried paper was illuminated with u.v. light (254nm) from a Hanovia Chromatolite lamp and the appearance of the spots observed at room temperature immediately after pouring liquid N_2 on to the paper: q, a dark spot due to quenching of the background fluorescence of the paper; —, neither colour nor quenching.

Compound	R_F value		Colour with NR	Appearance of spot in u.v. light of 254nm	
	Solvent *A*	Solvent *B*		At room temp.	In liquid N_2
Quinic acid*	0.25	0.10	—	—	—
Shikimic acid	0.56	0.50	—	q	q
Benzoic acid	0.87	0.85	—	q	Blue†
Hippuric acid	0.82	0.75	—	q	Blue†
Benzoyl glucuronide	0.75	0.60	Blue	q	Blue†
4-Hydroxybenzoic acid	0.85	0.83	—	q	Blue†

* R_F values determined with [^{14}C]quinic acid.

† Phosphorescence. These spots were visible after removing the source of light.

Surrey, U.K.). The radioactivity of the $Ba^{14}CO_3$ was also counted as a suspension in a dioxan gel with a Packard Tri-Carb scintillation spectrometer (model no. 3200). The urine and faeces were freeze-dried. The dried urine was dissolved in the minimum of 90% (v/v) ethanol (2–5ml) and the dried faeces were suspended in the same volume of ethanol. Samples (0.05ml) of these were then spotted on Whatman no. 1 paper, dried and chromatographed (descending) in solvent *A* or *B* (Table 1) against reference spots of the possible metabolites. After drying, the paper was cut into 3.8cm strips, which were run through a Packard radiochromatogram scanner (model 7200). Monkeys receiving [^{14}C]quinic acid were kept in a metabolism cage which allowed the collection of urine. The radioactive urine was examined as described for rats.

Estimation of hippuric acid. Hippuric acid (0.5g) was added to the radioactive urine (5–20ml), which was adjusted to pH10 with 10M-NaOH. The urine was then adjusted to pH2 with 10M-HCl and extracted with ethyl acetate (3×40ml). The extract was evaporated under reduced pressure and the residue of hippuric acid was recrystallized from water to constant specific radioactivity (m.p.186°C); this was checked by conversion into the *p*-nitrobenzyl ester, m.p.136°C.

Incubation of quinic acid with monkey liver. The liver of a rhesus monkey killed rapidly by a lethal intravenous injection of pentobarbital (150mg/kg) was quickly removed and homogenized in a Waring Blendor with 3vol. (v/w) of 0.15M-KCl containing 1mM-EDTA. The incubation mixtures consisted of liver homogenate (2ml), 0.1M-sodium phosphate buffer, pH7.4 (2ml), and (−)-quinic acid (5, 10 or 20mg in 1ml of water) in test tubes. Controls consisted of benzoic acid (5mg in 1ml of water) or water (1ml). The tubes were kept for 1h at 37°C in a shaking water bath, after which time 25% (w/v) trichloroacetic acid (2ml) followed by ethanol (5ml) was added to each tube. The tubes were shaken mechanically for 15min and then centrifuged at 2000*g* for 10min. Benzoic acid in samples of the supernatant was then determined fluorimetrically as described above for hippuric acid except that the fluorescence wavelength used was 388nm with excitation at 258nm. The recovery of benzoic acid (5mg) from incubation mixtures was 99±1%. To ensure that the homogenate was enzymically active, its ability to hydroxylate biphenyl (Creaven, Parke & Williams, 1965) and to acetylate sulphadimethoxine (Bridges, Kibby, Walker & Williams, 1969) was examined.

RESULTS

Fluorescence. The fluorescences of quinic acid, shikimic acid and 17 benzene derivatives at a concentration of 5μg/ml in 70% sulphuric acid were examined in an Aminco–Bowman spectrofluorimeter. Quinic acid, shikimic acid, phenylacetic acid, 3-hydroxy-, 4-hydroxy- and 3,4-dihydroxybenzoic acids, benzyl alcohol, benzene and toluene showed no fluorescence in this solvent even in concentrations up to 500μg/ml. Benzaldehyde, benzamide, benzonitrile, salicylic acid, *p*-aminobenzoic acid, phenol and catechol showed a weak fluorescence in this solvent, but the maximum wavelengths of the fluorescences were different from those of benzoic acid and hippuric acid and the intensities of the fluorescences were less than 1%, usually less than 0.3%, that of benzoic acid. Benzoic acid and benzoyl glucuronide fluoresced strongly at $\lambda_{max.}$ 388nm (activation, $\lambda_{max.}$ 258nm) and hippuric acid at $\lambda_{max.}$ 366nm (activation, $\lambda_{max.}$ 259nm). The relative intensities of these fluorescences were benzoic acid, 100, and hippuric acid, 54. Benzoic acid and hippuric acid were fluorescent with a constant intensity (stable for about 4h) in 4.5–17.5M-sulphuric acid, but in sulphuric acid less concentrated than 4.5M this fluorescence rapidly diminished and at pH0 (0.5M-sulphuric acid) had virtually disappeared. The fluorescence was partly quenched by urine, but this quenching could be allowed for by using internal standards. Benzoic acid and hippuric acid in 70% sulphuric acid fluoresce maximally at slightly different wavelengths. In most of the animals examined, however, the main metabolite of benzoic acid at a dose of 50mg/kg in the urine is hippuric acid, except in the ferret and fruit bat, in which benzoyl glucuronide predominates (J. W. Bridges, M. R. French, R. L. Smith & R. T. Williams, unpublished work).

Aromatization of quinic acid in various species. The excretion of hippuric acid after oral doses of quinic acid in ten species of primates is shown in Table 2. The hippuric acid was determined fluorimetrically and the amount of conversion of quinic acid into hippuric acid was assessed by subtracting the hippuric acid found in the urine excreted for 24h before dosing from that excreted during the 24h after dosing. Since the normal output of hippuric acid was variable, small conversions of quinic acid into hippuric acid cannot be detected in this way and apparent conversions of 5% may or may not be significant. Low conversions can only be detected by using [^{14}C]quinic acid and determining the conversion by isotope dilution or by radiochromatogram scanning as was done for the rat (see Table 4). Gross conversions, however, are readily shown by the fluorimetric method, and it is clear from Table 2 that in man and the Old World monkeys extensive aromatization of quinic acid occurs. The conversion is over 60% of the dose in man and 20–60% in the three species of Old World monkeys examined. In the three species of New World monkeys and three species of Prosimii (lemurs) examined, aromatization is low or does not occur. In Table 3, one species of bird, the pigeon, and 11 species of lower mammals are listed and none of these convert quinic acid into benzoic acid conjugates to any great extent. All the species listed in Tables 2 and 3 excreted ingested benzoic acid in the urine as hippuric acid except the ferret and fruit bat, as mentioned above. It appears from these tables that extensive aromatization of orally

Table 2. *Conversion of quinic acid into benzoic acid in primates*

(−)-Quinic acid was administered orally at a dose of 350 mg/kg, except in man, where the dose was 6 g or about 75–90 mg/kg. Urinary hippuric acid was determined on the days before and after dosing. Average values of body weight and normal hippuric acid output are given, the numbers of animals used being indicated by superscripts. Individual values for aromatization are given.

Animal	Generic name	Approx. body wt. (kg)	Range of normal hippuric acid output (mg/kg per day)	Sex	Aromatization in 24 h (% of dose)
Anthropoidea					
Man	*Homo*	66–80[3]	9–26	M	64, 67
				F	62
Old World monkeys					
Rhesus monkey	*Macaca*	3–6[10]	32–57	M	60, 23
				F	45, 43, 43, 40, 29*, 28*, 21*, 21*
Baboon	*Papio*	13–16[3]	19–53	M	51, 46, 20
Green monkey	*Cercopithecus*	1.4[2]	48, 66	M	47, 42
New World monkeys					
Spider monkey	*Ateles*	5–6[3]	7–27	M	0†, 10†, 11, 2
Squirrel monkey	*Saimiri*	0.5[3]	0.6–2.1	F	0, 0, 0
Capuchin	*Cebus*	1.7[2]	4.4, 10	M	0
				F	0
Prosimii					
Bushbaby	*Galago*	0.6[2]	7, 9	M	3
				F	0
Slow loris	*Nycticebus*	0.7[2]	3, 12	M	9
				F	3
Tree shrew	*Tupaia*	0.4[3]	0.1–4.4	F	0, 0, 0

* These monkeys had been kept by the animal dealers on a low dose of tetracycline for a week; this occurred just before the quinic acid was given.

† Values obtained on separate occasions on the same animal.

Table 3. *Conversion of quinic acid into benzoic acid in some lower animals*

Quinic acid was administered orally at 300 mg/kg in rabbits, hamsters, guinea pigs, lemmings, pigeons, dogs, cats and ferrets, at 350 mg/kg in hedgehogs and fruit bats, and at 600 mg/kg in rats and mice. Urinary hippuric acid was determined on the days before and after dosing. Average values for body weight and normal urinary hippuric acid are given, the number of animals being indicated by superscripts. Individual values for aromatization are given. The animals used were females.

Animal	Family	Body wt. (kg)	Normal hippuric acid (mg/kg per day)	Aromatization (% of dose)
Carnivores				
Dog (mongrel)	Canidae	10[2]	19, 75	0.2, 2
Cat (mongrel)	Felidae	2.4[2]	53, 59	0, 0.4
Ferret (mongrel)	Mustelidae	0.5[2]	165, 173	0, 0
Lagomorpha				
Rabbit (New Zealand White)	Leporidae	2.5[3]	47–129	3, 5, 5
Rodentia				
Rat (Wistar albino)	Muridae	0.25[6]	12–18	4.8 (3.5–7.7)
Mouse (I.C.I.)	Muridae	0.02[12]	37	0*
Guinea pig (English)	Caviidae	0.4[2]	66, 74	0, 0
Hamster (Golden)	Cricetidae	0.1[3]	250, 368	0, 0, 0
Lemming (Steppe)	Cricetidae	0.02[2]	49, 49	0, 0†
Aves				
Pigeon	Columbidae	0.5[2]	82*	2*
Chiroptera				
Fruit bat (Indian)	Pteropidae	0.3[1]	33	0
Insectivores				
Hedgehog (English)	Erinaceidae	0.9[2]	21, 39	0, 0

* Pooled urine or, for the pigeon, pooled excreta.

† One was male.

Table 4. *Fate of* [^{14}C]*quinic acid in rats and monkeys*

Female Wistar albino rats (wt. 250g) were given orally 85mg each of [^{14}C]quinic acid (sodium salt in water). The dose of ^{14}C was 1.4–2.0 μCi/rat. Female rhesus monkeys (wt. 6kg) received orally 2g of [^{14}C]quinic acid/kg and 2 μCi of ^{14}C/monkey. ^{14}C was determined in the urine, faeces and expired air of rats, but only in the urine of the monkeys. Quinic acid was determined by radiochromatogram scanning and hippuric acid by isotope dilution unless otherwise stated. Abbreviations: o., orally; i.v., intravenously; i.p., intraperitoneally.

		% of dose of ^{14}C excreted												
		Rats								Monkeys				
No. of animal ...		1	2	3	4	5	6	7	8	2†	2†	4	5	6
Route of administration ...		o.	o.	o.	o.	o.	o.	i.v.	i.v.	o.	o.	o.	o.	i.p.
Material examined	Days													
Urine	0–1	43	13	25	30	24	14	49	65	21	25	34	46	67
	0–2	43	—	—	39	29	20	—	—	—	27	62	48	69
Faeces	0–2	20			28	7	32							
Expired air as CO_2	0–2	20			19	54	36							
Carcass	0–5	4			1	4	3							
Total ^{14}C found		87			87	94	91							
Metabolites* in 24h urine														
Quinic acid		33	13	10	5	0	0	47	—	0	0	Trace	Trace	64
Hippuric acid		0.05	0.1	0	0.6	12.7	3.6	0‡	0‡	17	21	27	35	0

* Rat urine contained three other radioactive metabolites, one of which behaved chromatographically like shikimic acid; monkey urine contained only one radioactive metabolite, i.e. hippuric acid.

† Two separate experiments on the same monkey.

‡ Determined on chromatograms by strip scanning.

administered quinic acid is peculiar to man and the Old World monkeys.

The fate of ^{14}C-labelled quinic acid was examined in two species, the Wistar albino rat and the rhesus monkey. In the rat, about 90% of the administered ^{14}C radioactivity was accounted for, some 33% being excreted in the urine and 22% in the faeces, while 32% appeared in the expired air as CO_2 (Table 4). Five radioactive peaks were detected by radiochromatogram scanning in the urine of some of the rats. Labelled hippuric acid was found in the urine of five out of six rats in small amounts, except in one rat in which the output reached 12.7% of the dose as determined by isotope dilution. One of the other metabolites was unchanged quinic acid, which was found in the urine of four of the six rats in amounts varying from 5 to 33% of the dose as determined by radiochromatogram scanning. A third metabolite behaved chromatographically like shikimic acid, but its identity was not proved unequivocally. The other two metabolites, which were found in the urine of three of the six rats, were not identified.

In the rhesus monkey, only the urine was examined and only one metabolite was found by radiochromatogram scanning. This metabolite was hippuric acid, which accounted for about 80% of the ^{14}C radioactivity excreted in 24h (32% of the dose, range 21–46% in four monkeys) as determined by isotope dilution. In this particular experiment 25 (17–35)% of the dose of quinic acid was excreted in 24h as hippuric acid. When [^{14}C]quinic acid was injected intraperitoneally into the monkey, 67% of the ^{14}C radioactivity was excreted in the urine in 24h, and by radiochromatogram scanning and isotope dilution no [^{14}C]hippuric acid was formed and the only radioactive compound found in the urine was quinic acid.

Effect of neomycin on the aromatization of quinic acid in the rhesus monkey. The effect of pretreating three rhesus monkeys with neomycin orally for 4 days on the excretion of hippuric acid after the oral administration of quinic acid is shown in Table 5. In this experiment hippuric acid was determined fluorimetrically and covers a period of 51 days. Table 5 shows that neomycin, which suppresses the gut flora, suppresses not only the aromatization of quinic acid but also the normal output of hippuric acid, as shown on days 5–7. If quinic acid is administered to monkeys intraperitoneally, no increase in hippuric acid output above normal occurs (see days 50 and 51 in Table 5). These results strongly suggest that the aromatization of quinic acid is dependent on the gut flora.

Incubation of quinic acid with rhesus-monkey liver. In two experiments with a liver homogenate from one monkey, no benzoic acid formation from quinic acid was detected. The same liver homogenate was found to acetylate sulphadimethoxine at the rate of 0.16 μmol/h per g of liver and to

Table 5. *Excretion of hippuric acid by rhesus monkeys receiving quinic acid*

Three female rhesus monkeys (nos. 3, 4 and 5) were kept on a constant diet and their urine was collected on relevant days and analysed fluorimetrically for hippuric acid. Quinic acid (2g) in water neutralized with $NaHCO_3$ was given orally except on day 51, when it was injected intraperitoneally. Neomycin sulphate (2g) was given orally. The hippuric acid values are the averages for three monkeys.

		Hippuric acid output	
Day of experiment	Compound administered	Normal values (mg)	After quinic acid (mg)
1	None	107	—
2	Quinic acid	—	541
3	Neomycin	141	—
4	Neomycin	85	—
5	Neomycin	98	—
6	Neomycin	76	—
7	None	76	—
8	Quinic acid	—	141
10	None	97	—
11	Quinic acid	—	126
13	None	138	—
14	Quinic acid	—	265
16	None	159	—
17	Quinic acid	—	248
21	None	135	—
22	Quinic acid	—	197
35	None	135	—
36	Quinic acid	—	434
43	None	138	—
44	Quinic acid	—	500
50	None	140	—
51	Quinic acid (intraperitoneally)	—	100
		116 (average value)	

hydroxylate biphenyl at the rate of 1.3 μmol/h per g of liver.

Conversion of shikimic acid into hippuric acid. Shikimic acid (3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid) is a possible intermediate in the conversion of quinic acid (1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid) into benzoic acid. When this compound was given orally to rats and rhesus monkeys, little or no increase in hippuric acid output occurred in rats, but there was a considerable conversion into hippuric acid in the monkeys, some 41% (range 26–56% in two monkeys in four experiments) of the dose being excreted in the urine as hippuric acid (see Table 6). If, however, the shikimic acid is injected intraperitoneally into the monkeys, little if any increase in the hippuric acid output occurs.

DISCUSSION

The results described in this paper show that, in 22 species of vertebrates examined, extensive conversion of orally administered (−)-quinic acid to hippuric acid occurs only in man and the Old World monkeys (Table 2). In New World monkeys, lemurs, 11 species of lower mammals and one species of bird, the pigeon, the extent of this conversion is either low or non-existent (Tables 2 and 3). The pigeon is known to conjugate benzoic acid with glycine like mammals and not with ornithine as is the case with several other classes of birds (Baldwin *et al.* 1960). Taking the rhesus monkey as an example of an Old World monkey, it was found that in this monkey aromatization of quinic acid did not occur to a significant extent if the acid were given

Table 6. *Conversion of shikimic acid into hippuric acid in rats and rhesus monkeys*

Shikimic acid neutralized with $NaHCO_3$ in water was given orally to female Wistar albino rats (dose 0.6g/kg) and female rhesus monkeys (dose 0.3g/kg) unless otherwise stated. The urine collected for 24h before dosing and 24h after dosing was analysed fluorimetrically for hippuric acid.

		Hippuric acid in urine		
Animal	No. of animal	Before dosing (mg)	After dosing (mg)	Conversion into hippuric acid (% of dose)
Rats	1	13.8	10.3	0
	2	6.8	17.9	5
	3	0.0	13.2	6
	4	3.6	0.0	0
	5	4.8	2.7	0
	6	4.8	7.5	2
Monkeys	6	70	764	56
	7	106	735	44
	6	23	329	38
	7	72	383	26
	6 (intraperitoneally)*	41	53	3
	7 (intraperitoneally)*	34	30	0

* Shikimic acid injected intraperitoneally at a dose of 150mg/kg.

by injection, thus avoiding the gastrointestinal canal, or if the acid were given orally to the monkey after it had been treated with oral doses of neomycin to suppress the gut flora (Table 5). These findings strongly support the view that the gut flora are involved in the conversion of quinic acid into benzoic acid, and suggest that the gut organisms and the conditions necessary for the extensive aromatization of quinic acid occur only in the intestines of man and the Old World monkeys. It appears therefore that the observed species variation in the aromatization of quinic acid is dependent on variations in the gut flora rather than on variations in the activity of enzymes in the tissues of the animals (see Williams, 1967).

It is known that certain bacteria can convert quinic acid into aromatic compounds and that shikimic acid is an intermediate in this conversion (Davis, 1955). Our experiments (Table 6) show that orally administered (but not injected) shikimic acid is, like quinic acid, converted into hippuric acid in the rhesus monkey, but not in the rat. This suggests that the steps in the aromatization from quinic acid to shikimic acid and beyond shikimic acid on the way to hippuric acid are carried out by the gut bacteria. The conversion of quinic acid into hippuric acid may be represented as follows:

Quinate → dehydroquinate → dehydroshikimate → shikimate → several intermediates → benzoate → hippurate

The last step in this sequence is carried out by the liver and/or kidney and probably by no other tissue, and there is no evidence to suggest that bacteria can conjugate benzoic acid with glycine. It is possible therefore that, in the conversion of quinic acid into hippuric acid in man and the Old World monkeys, all the steps to benzoic acid can be carried out by the gut flora. However, it has been shown (Mitoma *et al.* 1958; Babior & Bloch, 1966) that guinea-pig liver at least can convert cyclohexanecarboxylic acid via cyclohex-1-ene-1-carboxylic acid into benzoic acid, so that the tissues of certain animals are capable of aromatizing certain cyclohexane derivatives.

In the animals that produce little or no urinary hippuric acid from quinic acid, it is possible that benzoic acid is formed in the gut but is destroyed before being absorbed, for certain bacteria are known to metabolize benzoic acid to non-aromatic products (e.g. see Dutton & Evans, 1969). However, other work in this laboratory has shown that most of the animals used in this work excreted orally administered [^{14}C]benzoic acid almost entirely in the urine, usually as hippuric acid, so that this explanation is unlikely. It is possible that the gut flora of these animals convert quinic acid into aromatic compounds other than benzoic acid. In the rat some 20–50% of the administered quinic acid is oxidized to CO_2 (Table 4), and the urine contained small amounts of hippuric acid, some quinic acid, probably some shikimic acid and two other metabolites that were not identified but did not appear to be aromatic. However, the possibility that the lower animals after dosing with quinic acid are excreting aromatic metabolites other than benzoic acid cannot be ruled out on the evidence available.

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