Studies in Detoxication

82. THE FATE OF BENZOIC ACID IN SOME DOMESTIC AND OTHER BIRDS*

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In most laboratory mammals benzoic acid is converted into hippuric acid and benzoyl glucuronide (see Williams, 1959), except for the cat, which seems to produce only hippuric acid (Robinson & Williams, 1958, and unpublished data). Jaffe (1877) showed that benzoic acid was converted in hens into ornithuric acid, and benzoyl glucuronide has also been found to be a metabolite of benzoic acid in hens (Baldwin, Robinson & Williams, 1959). It has been assumed that ornithuric acid formation is common to most birds, but, as far as we are aware, there is no evidence to support this. Schachter, Manis & Taggart (1955) have shown that pigeon-kidney slices and extracts form glycine conjugates, whereas kidney preparations from the chicken, duck, goose and turkey do not. They also found that chicken- and duck-kidney powder formed ornithine conjugates. These studies *in vitro* suggest that ducks as well as chickens form ornithuric acids whereas pigeons form hippuric acids. In the present paper we shall show, with the intact bird, that chickens, turkeys, geese and ducks excrete ornithuric acid after being dosed with benzoic acid, whereas the pigeon and the related wood pigeon and dove excrete hippuric acid. Neither hippuric acid nor ornithuric acid was found in the excreta of a parrot and a carrion crow dosed with benzoic acid. All the birds examined formed benzoyl glucuronide.

MATERIALS AND METHODS

Materials. [carboxy- ^{14}C]Benzoic acid was obtained from The Radiochemical Centre, Amersham. L(+)-Ornithuric acid was prepared by benzoylation of L(+)-ornithine; it had m.p. 182° , $[\alpha]_D^{25} + 11.9^\circ$ (c, 1 in 0.1N-NaOH) and light absorption in 0.1N-HCl, λ_{max} 229 (ϵ_{max} 23 250) and 270 m μ (ϵ_{max} 2500). A sample prepared from the excreta of hens dosed with benzoic acid had m.p. 178° and $[\alpha]_D^{25} + 7.9^\circ$ (c, 2 in 0.1N-NaOH). *N*⁵-Benzoyl-L-ornithine, m.p. $243\text{--}245^\circ$ and $[\alpha]_D^{19} + 8.3^\circ$ (c, 0.5 in water) was prepared according to McGilvery & Cohen (1950). *N*⁵-Benzoyloxycarbonyl-L-ornithine (4 g.; Barrass & Elmore, 1957) was benzoylated with benzoyl chloride and dilute NaOH to give *N*⁵-benzoyl-*N*⁵-benzoyloxycarbonyl-L-ornithine (6 g., crude). The latter was dissolved in methanol (100 ml.) and water

(100 ml.), treated with palladium oxide (0.5 g.) and then hydrogenated at a pressure of 38 lb./in.² for 2 hr. The spongy palladium was filtered off and the solution evaporated. The *N*⁵-benzoyl-L-ornithine (1.7 g.) was recrystallized from ethanol-water and had m.p. 239° and $[\alpha]_D^{19} + 4.8^\circ$ (c, 0.9 in water) (Found: N, 11.8. Calc. for $\text{C}_{12}\text{H}_{16}\text{O}_3\text{N}_2$: N, 11.85%). Karrer & Ehrenstein (1926) give m.p. $224\text{--}226^\circ$ and $[\alpha]_D^{20} + 8^\circ$ in water for *N*⁵-benzoyl-L-ornithine prepared by the action of Ba(OH)₂ solution on L-ornithuric acid.

Birds. Brown Leghorn laying pullets (6 months old) were obtained from the A.R.C. Poultry Research Centre, Edinburgh. They were maintained on 100 g. of Superbra Intensive Battery Mash (Poultry Association of Great Britain)/day and allowed ground oyster-shell grit and water without restriction. Pigeons, Muscovy ducks, a goose and a carrion crow were bought on the open market. The two turkeys, Broad-breasted Bronze hens, were gifts. The other birds were obtained on loan from the Zoological Society of London.

Benzoic acid suspended in water containing a little bile salts was administered to the hens, ducks and turkeys by crop tube. With other birds the benzoic acid was enclosed in a small gelatin capsule or compressed into a small tablet and given by mouth.

Collection of excreta. Food, but not water, was withdrawn from the birds on the evening before benzoic acid administration, to reduce the amount of faeces excreted in the first 24 hr. after dosing. With the hens, the urine was collected in a flask containing 2 ml. of saturated aqueous mercuric chloride. The funnel of the metabolism cage was scraped and rinsed with water and, where necessary in the experiments with ^{14}C , the birds' feet were also rinsed with water. With pigeons and the smaller birds, the excreta were collected on a glass plate placed under the wide-mesh bottom of the cage. The excreta were scraped from the plate and cage, which were then rinsed with ethanol and dilute ammonia solution. A thin paste of excreta was thus obtained. For paper chromatography the paste was centrifuged and the supernatant was used. The urine of the turkeys and goose was collected as for hens, but no mercuric chloride was used.

Measurement of radioactivity. All measurements were carried out on solid or liquid samples of 'infinite thickness' in an end-window counter. The specific activities were determined by comparison with a stable reference polymer (The Radiochemical Centre) and sufficient counts to give a standard error of $\pm 2\%$ were taken.

Isotope dilutions

Hippuric acid. This acid (0.5–2 g.) was added to 2–70 g. of homogenized excreta according to the amount of radioactivity expected. The mixture was warmed to dissolve the

* Part 81: Parke & Williams (1960).

acid and then kept at 0° overnight. The mixture was then filtered and the solid residue dissolved in hot ethanol (in the presence of charcoal) and filtered. The hippuric acid that crystallized on cooling was repeatedly recrystallized from water, ethanol and then from mixtures of ethanol and light petroleum (b.p. 60–80°) to constant activity. With hen excreta, repeated recrystallization failed to remove the radioactivity completely, although radioautographs did not reveal any hippuric acid in hen excreta. Paper chromatography in ethylmethylketone–2N-NH₃ soln. (1:1) indicated that a small amount of ornithuric acid remained in the hippuric acid despite repeated crystallizations. The hippuric acid was finally purified by a large-scale paper chromatogram (Whatman no. 3 paper) with the above-mentioned solvent. The hippuric acid was eluted from the paper with ethanol; the eluate was evaporated to dryness on the water bath and the residue was recrystallized from water and ethanol–ethyl acetate mixtures. It was finally converted into methyl hippurate with ethereal diazomethane and then into hippuramide, m.p. 178°. By this procedure it was possible to show that no radioactive hippuric acid was excreted by hens dosed with [¹⁴C]benzoic acid.

Benzoyl glucuronide. Radioautographs of paper chromatograms of hen and pigeon excreta showed that, after feeding with [¹⁴C]benzoic acid, little or no free benzoic acid was excreted. Benzoyl glucuronide in the excreta was therefore estimated as benzoic acid after mild alkaline hydrolysis. The homogenized excreta (1–10 g.) were heated on a boiling-water bath for 1 hr. with 1–10 ml. of N-NaOH. Then carrier benzoic acid (0.5–1 g.) was added and the mixture cooled. The mixture was acidified with 2N-H₂SO₄ and cooled to 0°. The crystals which separated were dissolved in water, treated with charcoal and filtered. The benzoic acid, m.p. 122°, which separated was crystallized to constant activity from water, ethanol and light petroleum (b.p. 60–80°). It was further purified as the

benzylamine salt, m.p. 126°, which was recrystallized from ethyl acetate.

Total benzoic acid. The homogenized excreta (10 g.) were heated for 3 hr. under reflux with 10N-NaOH (10 ml.). After cooling, carrier benzoic acid (1 g.) was added and the mixture was acidified with 30% (v/v) H₂SO₄. After keeping at 0°, the benzoic acid which separated was purified and counted as above.

Ornithuric acid. Carrier L(+)-ornithuric acid (0.5–1 g.) was dissolved in the minimum amount of N-NaOH and the solution was added to the homogenized excreta (1–3 g.). Conc. HCl was added to the mixture until the pH was about 3. The mixture was cooled and the material which separated was collected and washed with boiling water (50 ml.). This material was recrystallized (after treatment with charcoal) from ethanol–ethyl acetate (1:1) to constant activity. The ornithuric acid had m.p. 186–188°. It was converted into methyl ornithurate by treating it in methanol with ethereal diazomethane. The methyl ester, m.p. 142°, was recrystallized from ethanol or methanol to constant activity.

Paper chromatography and colour reactions. The *R_F* values of the relevant compounds are given in Table 1. Benzoic acid, benzoyl glucuronide, hippuric acid and ornithuric acid have a light-absorption band or an inflexion at 270–275 mμ (Baldwin *et al.* 1959). When spots containing these compounds on filter paper are examined under a Hanovia Chromatolite lamp, which emits u.v. light of wavelength about 270 mμ, they appear as dark areas.

N²- and N⁵-Benzoyl-L-ornithine are readily detected (10 μg.) on paper by spraying with a 1% solution of ninhydrin in ethanol and then heating at 100–120° for 1 min., when they show up as purple spots. No other compound in Table 1 gives this test. However, the *R_F* values of these compounds are very similar, but they could be separated in solvent D by allowing the chromatogram to run for 10 hr.

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

Whatman no. 4 paper and the descending technique were used. The *R_F* values were obtained after the front had moved approx. 30 cm. from the origin. The solvents were: A, butanol–acetic acid–water (4:1:5); B, butanol–benzene–water (5:2:2); C, butanol–NH₃ soln. (sp.gr. 0.88)–water (4:1:5); D, ethylmethylketone–2N-NH₃ soln. (1:1); E, propanol–NH₃ soln. (sp.gr. 0.88) (7:3); F, benzene–acetic acid–water (1:1:2); all ratios are by volume. With A, B, C and E the chromatograms were run for 5–6 hr., and with D and F for 2–2.5 hr. All spots showed quenching in ultraviolet light of 270 mμ wavelength.

Solvent system	<i>R_F</i>						Colour reactions		
	A	B	C	D	E	F	Ninhydrin	Dimethyl-aminobenzaldehyde	Dimethylamino-cinnamaldehyde
Benzoic acid	1.0	0.95	0.5	0.1	0.65	0.9	.	.	.
Benzoyl glucuronide*†	0.7	0.15	0.25	0.05–0.1	0.35	0	.	.	.
Ornithuric acid	0.9–1.0	0.7	0.65	0.4	0.8–0.95	0.3	.	.	White spot on yellow background
Hippuric acid	0.85	0.2	0.3–0.4	0.1–0.2	0.05	0.1	.	Orange	Red
N ⁵ -Benzoylornithine	0.5	0.1	0.35	0.01†	0.6	0	Purple	.	White spot on yellow background
N ² -Benzoylornithine	0.46	0.1	0.27	0.01†	0.6	0	Purple	.	White spot on yellow background

* Detected by naphtharesorcinol spray (see Baldwin, Robinson & Williams, 1959).

† See text.

instead of 2 hr., as given in Table 1. During this longer time the solvent front had run off the paper and the *N*⁵ compound had run 7 cm. from the origin and the *N*² compound, 10 cm. Whenever monobenzoylornithines were suspected to be present this technique was used.

Hippuric acid (10 μ g.) is readily detected by spraying the paper with a 1% solution of *p*-dimethylaminobenzaldehyde in acetic anhydride and then heating at 120° for 5 min. Hippuric acid shows up as an orange spot, but none of the other compounds gives a colour.

Ornithuric acid and hippuric acid (10 μ g.) are readily differentiated with a solution of *p*-dimethylaminocinnamaldehyde (0.05%, w/v), in acetic anhydride. After being sprayed with this solution the paper was heated in an oven at 12–140° for 2 min. Hippuric acid shows up as a red spot. Ornithuric acid shows up as a white spot on a yellow background and if the spot is viewed under u.v. light it shows an intense bright-blue fluorescence, and the spot is stable for several weeks. Ornithuric acid is detectable by this method in amounts of 10 μ g.

RESULTS

Hens. Isotope-dilution experiments for two hens are shown in Table 2. Chromatography and radioautography of hen urine showed that only two metabolites were excreted after the administration of benzoic acid, and these were benzoyl glucuronide and ornithuric acid. The examination of the excreta of six hens which had received benzoic acid and one which had received *p*-aminobenzoic acid failed to produce any evidence that hippuric acid or *p*-aminohippuric acid is formed by hens. The isolation of benzoyl glucuronide from hen excreta has been described by Baldwin *et al.* (1959).

Benzamide is also converted into benzoyl glucuronide and ornithuric acid in hens. A hen (1.4 kg.) was given benzamide (0.34 g.) by mouth. The 24 hr. urine (72 ml.) gave a positive naphtharesorcinol reaction and reduced Benedict's reagent. Determination of the glucuronic acid output showed that 42% of the benzamide had been converted into a glucuronide. Chromatography of the urine showed that neither unchanged benzamide nor free benzoic acid was present. The only compounds detected were benzoyl glucuronide and ornithuric acid. Extraction of half of the urine with ether (see section on turkeys) yielded benzoyl glucuronide (8 mg.; m.p. and mixed m.p. 187°, triacetyl methyl ester, m.p. 138°) and ornithuric acid (10 mg.; m.p. and mixed m.p. 187°).

Pigeons. Isotope-dilution experiments with pigeons are recorded in Table 2. The supernatant fluid obtained by centrifuging the excreta as described above was chromatographed on Whatman no. 4 paper with five solvents (A, B, C, D and E in Table 1). Radioautographs were prepared and the papers were also examined by colour reactions. No spot corresponding to ornithuric acid was found. Free benzoic acid and the monobenzoylornithines were not found in the urine.

An adult pigeon was given 135 mg. of benzoic acid by mouth and its excreta were collected for 24 hr. The excreta were mixed with dilute ammonia solution (5 ml.) and ethanol (5 ml.) and then centrifuged. The supernatant, after a 2 ml. portion had been removed for chromatography, was acidified with 2*N*-H₂SO₄ and continuously extracted with ether for 6 hr. The extract was dried with anhydrous Na₂SO₄ and evaporated. The residue was extracted with ether (10 ml.) and the extract was again evaporated to dryness. The second residue was now treated with light petroleum (b.p. 60–80°; 5 ml.) to remove fatty materials and the residue was recrystallized from water. Hippuric acid (2 mg.) was obtained with m.p. and mixed m.p. 187°.

Muscovy ducks. Two ducks, a male (2.5 kg.) and a female (2 kg.), were each given 2 g. of benzoic acid by crop tube, and the urine was collected for 24 hr. The urine reduced Benedict's reagent and gave a strong naphtharesorcinol reaction. Paper chromatography of the urine in solvents A, B, C and D (see Table 1) showed the presence of benzoyl glucuronide and ornithuric acid and the absence of hippuric acid.

Turkeys. Two female turkeys (6 kg. each) were each given 6 g. of benzoic acid on 2 successive days and the excreta were collected for 3 days. As the birds ate little food during the experiment, the excreta contained only small amounts of solid material. Paper chromatography showed that the urine contained no hippuric acid, but large amounts of benzoyl glucuronide and ornithuric acid. The urine was centrifuged to remove solid debris and then brought to pH 2–3 with conc. HCl. It was then continuously extracted with ether for 4 days, a little ethanol being added from time to time to break up emulsions of urine and ether and the ether being changed daily. The ether extracts were evaporated *in vacuo* and the residue was dried overnight in a vacuum desiccator containing

Table 2. Fate of [¹⁴C]benzoic acid in hens and pigeons

Benzoic acid was administered orally.

Expt. no.	Hens (Leghorns)		Pigeons	
	H1	H2	P1	P2
Wt. of bird (kg.)	1.85	1.65	0.3	0.3
Dose of benzoic acid (mg./kg.)	243	242	209	181
Dose of ¹⁴ C (μ C/bird)	8.5	14.4	15.7	4.0
Metabolites excreted (% of dose)				
Time after dosing (hr.)	24	24	18.5	18.5
Benzoyl glucuronide	26.3	32.2	28.8	25.1
Ornithuric acid	47.2	40.3	0.0	0.0
Hippuric acid	0.0	0.0	63.2	60.8
Sum of conjugates	73.5*	72.5	92.0	85.9
Total benzoic acid	86.3*	74.5	94.8	82.6
Total ¹⁴ C in urine	89.0	71.6	93.0	81.0
¹⁴ C in urine after 48 hr.	89.0	78.0	94.0	88.0

* The agreement here is not as good as in the other columns, but no compounds were found other than benzoyl glucuronide and ornithuric acid.

P_2O_5 and anhydrous Na_2CO_3 . The residue was taken up in the minimum quantity of ethanol and 10 vol. of $CHCl_3$ added. On keeping at 0° , crude benzoyl glucuronide (5.4 g.) separated. Two recrystallizations from ethanol (after treatment with charcoal) gave 1.9 g. of pure benzoyl glucuronide, m.p. 187° and $[\alpha]_D^{27} - 25.6^\circ$ (c, 2.5 in water). The glucuronide was further characterized as the methyl ester, m.p. 191.5° and $[\alpha]_D^{27} - 16.1^\circ$ (c, 1 in methanol) and the triacetyl methyl ester, m.p. 145° and $[\alpha]_D^{26} - 15^\circ$ (c, 1 in $CHCl_3$).

The $CHCl_3$ filtrate from the benzoyl glucuronide was evaporated to dryness and the residue extracted with ether (200 ml.). This extract was discarded and the residue was shaken with water (50 ml.) to remove any remaining glucuronide. The residue remaining was now taken up in ethanol (100 ml.) and the solution treated with ether (300 ml.). On keeping for several days L-ornithuric acid (m.p. 182° ; 1.2 g.) separated. It was purified by dissolving in ethanol and then adding ether and had m.p. 187° and $[\alpha]_D^{22.5} + 9.8^\circ$ (c, 1 in N-NaOH). A further crop of 0.5 g., m.p. 184° was obtained by treatment of the mother liquors from the first crop.

Wood pigeon. The wood pigeon (450 g.) was given a pellet of benzoic acid (150 mg.) and its excreta were collected for 24 hr. Benzoyl glucuronide and hippuric acid were shown by paper chromatography to be present in the excreta. No ornithuric acid was found. The excreta were homogenized and brought to pH 3 with conc. HCl and then continuously extracted with ether for 8 hr. Evaporation of the ether left white crystals, which on recrystallization from water (after treatment with charcoal) gave 53 mg. of hippuric acid, m.p. and mixed m.p. 188° .

Barbary dove. The dove (180 g.) was given a pellet of benzoic acid (47 mg.). Paper chromatography of the 24 hr. excreta showed the presence of hippuric acid but not of ornithuric acid. The excreta gave a weak naphtharesorcinol test for glucuronic acid.

Carrión crow. The crow (500 g.) was given a capsule containing 240 mg. of benzoic acid on one day, and a second capsule containing 220 mg. on the succeeding day. The excreta were collected during 3 days. Chromatography of the excreta showed the presence of benzoyl glucuronide, but neither hippuric acid nor ornithuric acid was found. By extraction of the homogenized excreta at pH 3 with ether as described above, benzoyl glucuronide (100 mg.) was isolated, which, after recrystallization, had m.p. 179° , not depressed by the authentic compound, m.p. 184° . It was further identified by its positive naphtharesorcinol reaction and reduction of Benedict's reagent on warming.

Goose. A domestic goose (3.5 kg.) was given 1.5 g. of benzoic acid as small pellets. The 24 hr. urine contained benzoyl glucuronide and ornithuric acid, but no hippuric acid, as shown by paper chromatography. Extraction of the acidified

urine yielded L(+)-ornithuric acid (40 mg. after purification), m.p. 184° , not depressed by an authentic sample.

Parrot. A grey African parrot (350 g.) was given 170 mg. of sodium benzoate in water by subcutaneous injection. It could not be persuaded to take benzoic acid by mouth. The clear urine was collected for 6 hr. The urine gave a strong naphtharesorcinol reaction and readily reduced Benedict's reagent on warming. Paper chromatography of the urine showed the presence of benzoyl glucuronide, but neither ornithuric acid nor hippuric acid was found.

Destruction of ornithuric acid by chicken faeces

The quantitative results for hens quoted in Table 2 were obtained when the urine and a small amount of faeces passed during 24 hr. after dosing were collected in a vessel containing 2 ml. of saturated aqueous mercuric chloride. The use of mercuric chloride became necessary when it was discovered that chicken faeces could destroy ornithuric acid. In one experiment in which no mercuric chloride was used, and in which there was gross faecal contamination of the urine, the ornithuric acid output estimated by isotope dilution amounted to only 0.5 % of the dose (2 m-moles/kg. of $[^{14}C]$ benzoic acid). During this particular experiment samples of urine had been collected by catheter at 4 and 6 hr. after dosing. On chromatography of these urines on paper, only two spots were found by radioautography and colour reactions and these corresponded to ornithuric acid and benzoyl glucuronide. The rest of the urine was allowed to mix with the faeces passed, and the mixture was examined 4 days later. Paper chromatography of this mixture gave spots corresponding to benzoyl glucuronide, benzoic acid and N^5 -benzoylornithine and a weak ornithuric acid spot. The N^5 -benzoylornithine was readily detected by its radioactivity and by ninhydrin. This experiment suggested that the fresh chicken urine contained ornithuric acid, but no monobenzoyl-ornithines, and that chicken faeces were capable of breaking down ornithuric acid. Chicken faeces and urine were therefore examined qualitatively for 'ornithuricase' activity.

Fresh chicken urine was collected by catheterization and 1 ml. was diluted to 10 ml. with 0.067 M-phosphate buffer, pH 7. Fresh chicken faeces (1 g. wet wt.) was made into a suspension of 10 ml. with the same buffer. A solution of L(+)-ornithuric acid containing 2 mg./ml. was made up in the same buffer. The diluted faeces or urine (1 ml.) was mixed with the ornithuric acid solution (1 ml.) and incubated at 35° . The controls consisted of 1 ml. of water plus 1 ml. of ornithuric acid solution, and 1 ml. of boiled faeces or urine (boiled for 30 min.) plus 1 ml. of the ornithuric acid solution. At suitable intervals 0.1 ml. samples of the

solutions were chromatographed on Whatman no. 4 paper with solvent D (see Table 1).

It was found that chicken urine had no apparent action on ornithuric acid at pH 7 even after a week's incubation. The faeces (0.1 g.) destroyed the ornithuric acid (2 mg.) within 21 hr., and although benzoic acid was detectable in the mixture, neither ornithuric acid nor monobenzoylornithines were present. If the faeces were boiled for 30 min., then they had no action on ornithuric acid. However, if the faeces were boiled for only 0.5 min., they still destroyed ornithuric acid, but N^5 -benzoylornithine was readily detected in the incubated mixture after 21 and 28 hr., but after 48 hr. it also had disappeared.

The suspension of faeces (10 ml.) was centrifuged at 4500 rev./min. for 15 min. and the supernatant was used as an enzyme sample. The solid residue was washed with water (2×10 ml.) and again centrifuged, and the residue was finally suspended in 10 ml. of phosphate buffer. The untreated supernatant and samples after boiling for $\frac{1}{2}$ min. and 30 min. were incubated with ornithuric acid solutions as described above. The unboiled supernatant destroyed the ornithuric acid in 21 hr. at which time N^5 -benzoylornithine was readily detected. The supernatant boiled for $\frac{1}{2}$ min. was less active and ornithuric acid was still present after 21 hr., but after 68 hr. N^5 -benzoylornithine became detectable. The supernatant boiled for 30 min. was inactive. The suspension (1 ml.) of the solid residue from the centrifuged faeces was also incubated as described above with the ornithuric acid solution (1 ml.). It readily destroyed ornithuric acid within 21 hr. and although benzoic acid could be detected in the incubated mixture, no monobenzoylornithines were found. If the suspension were boiled for 0.5 min., it still destroyed the ornithuric acid within 21 hr., but now both benzoic acid and N^5 -benzoylornithine were found in the incubated mixture. Boiling the suspension for 30 min. destroyed its 'ornithuricase' activity. This activity is completely inhibited by aqueous 0.035 % mercuric chloride.

These experiments show that chicken faeces, but not the urine, can destroy ornithuric acid and that the activity is mainly associated with the solid particles of the faeces. They also suggest that stepwise removal of benzoyl groups from ornithuric acid may occur, the N^2 -benzoyl group being removed first, as N^2 -benzoylornithine was never detected in any of the experiments.

Further experiments suggested that the faeces destroyed L(+)-ornithuric acid but not the D-isomer, for when DL-ornithuric acid was incubated with fresh faeces at pH 7, benzoic acid and N^5 -benzoylornithine were found within 24 hr., but an ornithuric acid spot was found on the chromato-

grams even after incubation for 3 days and this is due presumably to D-ornithuric acid. In parallel incubations with L(+)-ornithuric acid, the acid had always completely disappeared within 24 hr. The faeces had little action on di-(o-hydroxybenzoyl)- and di-(p-aminobenzoyl)-L(+)-ornithines or hippuric acid.

DISCUSSION

It has been assumed in the past that in birds aromatic acids are excreted conjugated with ornithine, and this assumption has been based largely on observations on the domestic fowl. Tables 2 and 3 show that this assumption is incorrect because, of the nine species of bird examined, only four excreted ornithuric acid in response to ingested benzoic acid. What is common to them all is the excretion of benzoyl glucuronide. Of the orders examined, only the galliformes and anseriformes were found to form ornithuric acid. The columbiformes, which include the pigeons and doves, excreted hippuric acid and not ornithuric acid. The other species examined, which included a carrion crow and a parrot, excreted neither ornithuric acid nor hippuric acid and require further investigation.

Table 2 shows the quantitative aspects of the fate of benzoic acid in the domestic hen and pigeon. In both species benzoyl glucuronide accounts for about a quarter of the dose. In the hen, however, ornithuric acid accounts for 40–50 % of the dose and there is no hippuric acid excreted. In the pigeon, on the other hand, no ornithuric acid is formed, and about 60 % of the dose is excreted as hippuric acid. In both species about 70–90 % of

Table 3. *Metabolites of benzoic acid in some species of birds*

+ Indicates present and -, absent.

	Metabolites isolated or detected		
	Benzoyl glucuronide	Hippuric acid	Ornithuric acid
Galliformes			
Domestic fowl	+	-	+
Turkey	+	-	+
Anseriformes			
Muscovy duck	+	-	+
Goose	+	-	+
Columbiformes			
Pigeon	+	+	-
Wood pigeon	+	+	-
Barbary dove	+	+	-
Passeriformes			
Carrion crow	+	-	-
Psittaciformes			
Grey African parrot	+	-	-

the dose of benzoic acid is eliminated in 24 hr. after dosing. It is known that glycine is necessary for optimum growth in chickens and turkeys, but the glycine requirement of other birds is not known. It has been suggested that this requirement of glycine may be related to the biosynthesis of uric acid, which is a major nitrogenous waste product in birds, including the pigeons (see Arnstein, 1954). It is possible that the formation of hippuric acid is peculiar to the columbiformes, but many more species of birds have to be examined before it could be claimed that hippuric acid formation is characteristic of columbiformes.

SUMMARY

1. Benzoic acid has been administered to nine species of birds, namely the domestic fowl, turkey, goose, Muscovy duck, domestic pigeon, wood pigeon, Barbary dove, carrion crow and grey African parrot.

2. All these species of birds excrete benzoyl glucuronide.

3. The domestic fowl, turkey, goose and Muscovy duck also excreted L(+)-ornithuric acid but no hippuric acid.

4. The domestic pigeon, wood pigeon and Barbary dove also excreted hippuric acid but no ornithuric acid.

5. The carrion crow and grey African parrot did not appear to excrete hippuric acid or ornithuric acid.

6. [*carboxy*-¹⁴C]Benzoic acid was administered to hens and pigeons. The hens excreted about 25–30 % of the dose (240 mg./kg.) as glucuronide and 40–50 % as ornithuric acid in 24 hr. The pigeons excreted 25–30 % of the dose (200 mg./kg.) as glucuronide and 60 % as hippuric acid in 18.5 hr.

7. Chicken faeces contain an enzyme which decomposes L(+)-ornithuric acid via *N*⁵-benzoyl-ornithine. Monobenzoylornithines were not found as metabolites of benzoic acid in hens.

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83. THE AROMATIZATION OF CYCLOHEXANECARBOXYLIC ACID IN HENS

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*cyclo*Hexanecarboxylic acid (hexahydrobenzoic acid) is aromatized to benzoic acid to a considerable extent in man, the rabbit and the dog (Bernhard & Caffisch-Weill, 1945) and to a minor extent in the rat (Beer, Dickens & Pearson, 1951). In man and the rabbit the main excretory product of *cyclo*-hexanecarboxylic acid is hippuric acid. This aromatization reaction has also been studied in tissue slices and homogenates (Beer *et al.* 1951; Mitoma, Posner & Leonard, 1958) and the last-named authors found that aromatization took place in guinea-pig- and in rabbit-liver mito-

chondria in the presence of adenosine triphosphate and glycine. A very weak aromatizing activity was found in rat-liver mitochondria, but no activity was found with liver mitochondria from man, cat, mouse, dog, monkey, toad, pigeon and chicken. This lack of activity was attributed to the lability of the enzyme. With chicken mitochondria replacement of glycine by ornithine did not produce aromatization. Furthermore, there is no evidence in the literature that the intact chicken can aromatize *cyclo*hexanecarboxylic acid. In the present paper it will be shown that chickens can