The origin of urinary aromatic compounds excreted by ruminants 3. The metabolism of phenolic compounds to simple phenols

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1. Dietary phenolic cinnamic acids are hydrogenated in the side-chain, demethylated and dehydroxylated in the rumen and are responsible for the large urinary output of benzoic acid by ruminants.

2. Decarboxylation of phenolic acids to simple phenols is another reaction of the intestinal microflora and experiments were made to determine the extent of this reaction in the rumen of sheep.

3. In five experiments phenolic compounds, quinic acid or casein were infused into the rumen or abomasum of sheep and increments in urinary outputs of phenolic acids and phenols determined by thin-layer and gas-liquid chromatography.

4. Production of phenols was almost exclusively confined to reactions in the rumen.

5. Rumen administration of phenolic benzoic or phenylacetic acids which contained a 4-hydroxy substituent yielded large increments in urinary phenol outputs. Other phenolic benzoic and phenylacetic acids were not decarboxylated. Rumen decarboxylation of 4-hydroxy-3-phenylpropionic acid did not occur and decarboxylation of 4-hydroxycinnamic acids was slight.

6. Nearly half the tyrosine content of rumen-administered casein was excreted as *p*-cresol, a decarboxylation product of 4-hydroxyphenylacetic acid. *p*-Cresol was the principal phenol found in sheep urine.

7. Catechol and phenol were consistently found in sheep urine samples and *p*-ethylphenol, resorcinol, quinol, 4-methylcatechol, orcinol and pyrogallol were also found when suitable precursors were infused to the rumen.

8. It is concluded that *p*-cresol is a rumen metabolite of tyrosine. The other phenols found are microbial metabolites of phenolic precursors which are either widely distributed in plants such as 4-hydroxybenzoic, protocatechuic and vanillic acids or of more limited distribution such as the orcinol glycosides of some Ericaceous plants.

Urinary phenols have for a long period been regarded as intestinal microbial metabolites of tyrosine (Baumann, 1879). This role of microbial metabolism has been supported by two observations; first, oral treatment of man and laboratory animals with antibiotics has almost completely inhibited urinary phenol output (Bernhardt & Zilliken, 1959; Smith, 1961; Curzon & Pratt, 1964; Bakke, 1969*d*; Tamm & Villako, 1971) and second, the greatly increased urinary excretion of phenol and *p*-cresol found in man with gastrointestinal disorders which cause increased exposure of the digesta to the gut microflora (Duran *et al.* 1973).

Rumen fermentation of food would therefore be expected to cause the production of large amounts of phenols. There is little quantitative evidence available (Drasar & Hill, 1974). The considerable urinary benzoic acid (BA) output of ruminants has been shown to be a consequence of microbial reduction of the unsaturated side-chain, demethylation and dehydroxylation of phenolic cinnamic acids to 3-phenylpropionic acid (3-PPA) in the rumen (Martin, 1982*b*); these studies also showed that phenolic BA and phenylacetic acid (PA) derivatives did not undergo similar reactions. The intestinal microfloras of rats have been shown to decarboxylate some phenolic BA and PA derivatives to phenols (Scheline, 1966*a*, *b*, 1967, 1968*a*, *b*). Ruminant urine has been shown to contain phenol (Braden *et al.* 1967; Suemitsu *et al.* 1970), *m*-cresol (Suemitsu *et al.* 1965), *p*-cresol (Bassett *et al.* 1955; Braden *et al.* 1967; Suemitsu *et al.* 1970), *p*-ethylphenol (Grant, 1948; Braden *et al.* 1967; Suemitsu *et al.* 1970), 2-methoxyphenol (Suemitsu *et al.* 1970), catechol (Von Euler & Lishajko, 1959; Suemitsu *et al.* 1970) and *m*-*n*-propylphenol (Suemitsu *et al.* 1970).

Most ruminant foods do not contain any simple phenols. These compounds are, however, produced during ensilage (Clarke & Humphreys, 1971; Kibe & Kagura, 1976; Sakata &

Ishiyama, 1978) and quinol (Thieme & Winkler, 1971) and orcinol (Harborne & Williams, 1969) glycosides occur in some Ericaceous plants.

The present studies were made to determine the contribution of rumen metabolism of protein and of dietary phenolic compounds to urinary phenol excretion by sheep. Rumen metabolism of tyrosine produced *p*-cresol which was the principal phenol found in sheep urine; other phenols were products of rumen metabolism of dietary 4-hydroxy-substituted BA or PA.

EXPERIMENTAL

Experimental design. These studies were made as part of Expts 2–6 inclusive described by Martin (1982*a*) where descriptions of the sheep used, experiments and rations, collection of faeces and urine and experimental plans were given. The eighteen phenolic and one hydroxyalicyclic compound (quinic acid, QA) infused as continuous drips in a saline (Martin, 1982*a*) solution to the rumen (in twenty-nine experimental periods) or abomasum (in eighteen experimental periods) of sheep have been described previously (Martin, 1982*b*).

Creatinine. The daily output was determined in all experiments to assess the efficiency of urine collection and if necessary correct for losses (Martin, 1982*a*).

Free phenolic acids in urine. In Expts 3 and 4 the assay of urinary aromatic acid–glycine conjugates in diethyl ether extracts of unhydrolysed urine enabled additionally the determination of the urinary output of some free phenolic acids (Martin, 1982*b*). The titratable acids in diethyl ether extracts of alkali hydrolysed urine were also determined (Martin, 1973).

Urine phenol analyses

Experiment 2. Triplicate acid hydrolyses in 0·75 M-sulphuric acid were made with all control and infusion period samples (A.K. Martin, unpublished results). Additionally, when quinic, 4-hydroxybenzoic (4OH-BA) and 3,4,5-trihydroxybenzoic (gallic, 3,4,5OH-BA) acids were infused, urinary phenol conjugates were hydrolysed by incubating with an enzyme preparation (ex *Helix pomatia*, Type H-2; Sigma London Ltd, Poole, Dorset). To a diluted sample in 0·03 M-acetate buffer (pH 5·0) containing 5 ml of urine/l of daily urine output an amount of enzyme preparation containing 1·0 i.u. β-glucuronidase (*EC* 3.2.1.31) and 32 i.u. arylsulphatase (*EC* 3.1.6.1) was added. Incubations were for 18 h at 39°.

Phenols were extracted from the hydrolysates at pH 1. In two infusion periods (casein and 3-methoxy, 4-hydroxy-cinnamic acid (3MeO, 4OH-CA)) the combined solvent extracts were washed with sodium bicarbonate to remove acids (Bakke & Scheline, 1969).

The amounts of phenols in the extracts were determined both by gas-liquid chromatography (GLC) of free phenols on 5% w/w neopentylglycol succinate (NGS) columns and of phenyl acetates on 3% w/w OV 17 columns. Eugenol was used as internal standard. The presence of *m*-cresol in the unresolved *m*- plus *p*-cresol peaks was shown by GLC of free phenols on a 2·5% w/w tricresylphosphate (TCP) column and quantitative assay of the *m*-cresol present by a modification of the thin-layer chromatographic (TLC) separation of phenol isomers described by Smith & Sullivan (1964). The identity of phenols and phenolic acids found on GLC was confirmed by TLC (Scheline, 1968*a*) of extracts of hydrolysed urine.

Expts 3 and 4. Triplicate diluted urine samples were hydrolysed and extracted. Two samples were hydrolysed in 1·5 M-H_2SO_4 at 100° for 1 h and the remaining one incubated with a β-glucuronidase plus sulphatase enzyme preparation (ex *Helix pomatia*, Type H-1; Sigma London Ltd, Poole, Dorset). To a diluted urine sample containing 5 ml of urine/l daily urine output an amount of enzyme preparation containing 1·25 i.u. β-glucuronidase and 15–54 i.u. arylsulphatase activity was added and the incubations kept at 39° for 16 h.

Phenols were extracted from the hydrolysates at pH 1. Acids were removed from the combined solvent extracts of the enzyme and one of the acid-hydrolysed extracts by shaking with an aqueous solution of 50 g sodium bicarbonate/kg.

Portions of each extract were acetylated and the phenols present assayed by GLC using columns packed with a support containing 3% w/w OV17. The apparent identities of the phenols found on GLC were confirmed by TLC of underivatized extracts (A. K. Martin, unpublished work).

Expts 5 and 6. Three diluted urine samples were hydrolysed at 100° for 1 h in 0·75 M-H_2SO_4 and up to six samples were hydrolysed for 48–72 h at 39° with a β-glucuronidase–arylsulphatase Type H-1 preparation containing 1·0 i.u. β-glucuronidase.

One acid hydrolysate and one enzyme hydrolysate were extracted at pH 1 and the remaining samples at pH 7. None of the solvent extracts were washed with bicarbonate solution.

Portions of the extracts were used for the preparation of acetate and of trimethylsilyl (TMS) derivatives of phenols. They were assayed by GLC of their acetates on 10% w/w OV 1 and 10% w/w OV 17 columns and of the TMS derivatives on a 5% w/w trixylenyl-phosphate (TXP) column. The homogeneity of peaks found on GLC or urine phenol extracts was determined by trapping the peaks eluting from OV 1 and OV 17 columns by manual preparative chromatography. Trapped derivatives were hydrolysed and identified by uv spectrophotometry and TLC.

RESULTS

Control urine phenol outputs

In Expt 2 the means of assays of acid-hydrolysed urines in which phenols were separated on NGS and OV 17 columns were used. In Expts 3 and 4 monohydroxyphenol assays were means of all acid- and enzyme-hydrolysed urine assays. Significant amounts of dihydroxyphenols were lost on bicarbonate washing of solvent extracts (A. K. Martin, unpublished results) so catechol and 4-methylcatechol assays in non-bicarbonate-washed acid-hydrolyses only were used. In Expts 5 and 6 the mean phenols found in pH 7 extracts of enzyme-hydrolysed urines assayed on OV 1 and OV 17 were used.

The mean control urine phenol outputs of sheep when control saline infusions were made are shown in Table 1. In some experiments the *m*-cresol component of the combined *m*- plus *p*-cresol GLC peak was determined; it was always small. In Expt 2 *m*-cresol accounted for (mean and standard deviation) $4·0 \pm 1·8\%$ (n 5) of the combined peak area and in Expts 5 and 6 only $0·3 \pm 0·4\%$ (n 8) was due to *m*-cresol. *p*-Cresol was the principal phenol found in sheep urine with smaller amounts of catechol and phenol always present. *p*-Ethyl phenol and 4-methylcatechol were found in some samples. When phenol precursors were infused to sheep the basal output of phenols was calculated as the product of the control output of urine phenols/g creatinine and the urinary creatinine output during the infusion period. Increments in urine phenol output were the differences between the observed urine phenol output and the calculated basal value.

Infusion of aromatic and alicyclic acids

BA (4·9 g/d), PA (5·6–5·9 g/d), 3PPA (6·0–6·5 g/d), cinnamic (CA, 5·7–6·3 g/d) and cyclohexanecarboxylic (4·7–4·8 g/d) acids when infused into the rumen or abomasum of sheep (Martin, 1982*a*) did not yield urinary increments of phenols. When 6·1–8·0 g QA/d was infused into the rumens of sheep no increment in urinary phenols was found, but when 37·0 g/d was infused into the rumen of a sheep (Expt 2, sheep no. 2, Martin, 1982*a*) a small urinary increment in catechol excretion equivalent to 0·5% of the acid infused was detected.

Table 1. *The mean urinary output of creatinine (g/d) and phenols (mg/d) by sheep in control periods*
(Mean control period outputs and standard deviations; no. of control periods given in parentheses)

Expt no.	Sheep no.	Creatinine		Phenol		*m*- plus *p*-Cresol		*p*-Ethyl phenol		Catechol		4-Methylcatechol	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2	2(5)	1·57	0·05	28	8	947	57	16	17	144	30	30	17
3	3(4)	1·16	0·05	20	2	347	29	0	—	132	11	6	1
	4(3)	1·15	0·06	23	2	377	31	1	0	141	11	4	1
	9(4)	1·29	0·07	26	1	493	27	1	0	167	9	5	1
	10(3)	1·19	0·08	26	2	477	30	0	—	154	9	0	—
4	3(5)	1·16	0·08	20	1	441	23	0	—	125	7	0	—
	4(4)	1·22	0·06	22	1	537	21	0	—	147	6	1	0
	9(4)	1·23	0·06	25	2	528	30	0	—	112	6	1	0
	10(4)	1·20	0·06	34	1	652	27	0	—	125	5	1	0
5	5(1)	1·51	0·05	64	—	575	—	4	—	140	—	31	—
	6(2)	1·52	0·07	64	—	515	—	4	—	145	—	25	—
	7(2)	1·49	0·04	58	—	360	—	2	—	128	—	25	—
	11(2)	1·81	0·05	63	—	633	—	4	—	147	—	29	—
6	5(2)	1·53	0·05	22	—	854	—	0	—	58	—	8	—
	6(2)	1·58	0·02	11	—	711	—	0	—	52	—	8	—
	7(2)	1·50	0·05	18	—	834	—	2	—	63	—	9	—
	11(2)	1·55	0·09	22	—	1062	—	0	—	60	—	8	—

Infusion of phenolic compounds

Infusion of 5·9 g salicylic acid (2OH-BA)/d to the rumen of a sheep (no. 6, Expt 6) yielded no increment of urinary phenols. 2OH-BA is soluble in toluene and therefore extracted on assay of aromatic acids in alkali-hydrolysed urine (Martin, 1982*a*). At least 33% of the infused acid was excreted unchanged in the urine. The following acids were infused to the rumen and abomasum of sheep without increments in urinary phenols being found: 3-hydroxybenzoic (3OH-BA, 5·6–5·8 g/d), gentisic (2,5OH-BA, 5·1–6·1 g/d), phloretic (4OH-3-PPA, 6·9–7·1 g/d), *o*-coumaric (2OH-CA, 6·6–6·8 g/d) and *m*-coumaric (3OH-CA, 6·5–7·4 g/d) acids.

Increments in diethyl ether-soluble titratable acids on infusion of 3OH-BA showed that after infusion by either route all the infused acid was excreted as acidic metabolites. Between 20 and 34% (Expt 3, abomasal and rumen infusions respectively) were excreted as free 3OH-BA in urine but the nature of conjugated metabolites was not studied. In Expt 4 urinary excretion of free 4OH-BA was found after abomasal infusion of 4OH-3-PPA (equivalent to 9% of the infused acid) and *p*-coumaric acid (4OH-CA, equivalent to 18% of the infused acid). After rumen infusion of 4OH-3-PPA and 4OH-CA no such increments were found. These assays were not made when 2OH-CA and 3OH-CA were infused. After rumen or abomasal infusion of 2,5OH-BA increments in diethyl ether-soluble titratable acids showed that the infused acid was completely recovered as acidic metabolites in urine. The methyl ester of 2,5OH-BA did not elute from the column used in the assay of some free phenolic acids (p. 498) but 2,5OH-BA was identified by TLC in the phenolic acid fraction (bicarbonate soluble) of acid-hydrolysed urine extracts after both rumen and abomasal infusions.

When phenolic benzoic acids containing a 4-hydroxy group were infused to the rumen of sheep decarboxylation and demethylation (vanillic acid, 3MeO, 4OH-BA) took place but these reactions did not occur significantly after abomasal infusion (Table 2). The identity of phenol as a metabolite of 4OH-BA was confirmed by spectrophotometry of extracts of hydrolysed urine coupled with diazotized *p*-nitroaniline (λ_{max}, 499 nm; A. K. Martin, unpublished results); quinol was also identified as a metabolite by TLC of extracts of acid-hydrolysed urine. Catechol, which was a component of control urines, was excreted in enhanced amounts on rumen infusion of protocatechuic acid (3,4OH-BA) and 3MeO, 4OH-BA; its identity was confirmed by TLC of free phenols in extracts of hydrolysed urine. The increments in diethyl ether-soluble acids observed on rumen infusion of 3MeO, 4OH-BA did not contain any free 3MeO, 4OH-BA but after abomasal infusion 14% of the infused acid was excreted in the urine in this form (GLC of methyl esters of diethyl ether extracts of unhydrolysed urine and TLC). The identities of resorcinol and pyrogallol as metabolites of rumen-infused 3,4,5OH-BA was confirmed by TLC. Unchanged 3,4,5OH-BA was only found on TLC of extracts of hydrolysed urine after abomasal infusion.

4-Hydroxyphenylacetic acid (4OH-PA) was also decarboxylated on infusion to the rumen of sheep but not on abomasal administration (Table 3); increased urinary *p*-cresol excretion was found on rumen infusion. After abomasal infusion of 4OH-PA, 88% of the infused acid was found to be excreted in the urine as the free acid (GLC of methyl esters of diethyl ether extracts of unhydrolysed urine); none was found following rumen infusion. Rumen infusion of casein also yielded enhanced urinary *p*-cresol output. In addition to identification of *p*-cresol by GLC of extracts of hydrolysed urine on NGS, TCP and OV 17 columns, identification was confirmed by coupling these extracts with diazotized *p*-nitroaniline which gave solutions with a λ_{max} at 545 nm, close to that of *p*-cresol (A. K. Martin, unpublished work). Both rumen and abomasal infusions of the quinol glycoside, *p*-arbutin, yielded similar substantial urinary increments of quinol (Table 3) on assay of extracts of acid-hydrolysed

Table 2. *The amounts (%) of some 4-hydroxy substituted benzoic acids recovered as phenols in the urine of sheep following administration to the rumen or abomasum*

Acid infused	Infusion route	Expt no.	Sheep no.	Wt infused (g/d)	Amounts (%) of infused acids recovered in the urine as: Diethyl ether-soluble acids	Phenol	2-Methoxy phenol	Catechol	Resorcinol	Quinol	Pyrogallol
4-Hydroxy benzoic	Rumen	2	2	10·9	35	53	0	0	0	10	0
3,4- Dihydroxy benzoic	Rumen	6	5	5·9	nd	0	0	63	0	0	0
3-Methoxy-4-hydroxy benzoic	Rumen	4	3	7·5	21	0	4	60	0	0	0
	Abomasum	4	9	7·1	39	0	0	4	0	0	0
3,4,5-Trihydroxy benzoic	Rumen	2	2	23·2	34	0	0	0	41	0	15
		4	4	7·0	10	0	0	0	0	0	7
	Abomasum	4	10	7·4	35	0	0	0	0	0	0

nd, Not determined.

Table 3. *The amounts (%) of some phenolic substances recovered as simple phenols in the urine of sheep following administration to the rumen or abomasum*

Substance infused	Infusion route	Expt no.	Sheep no.	Wt infused (g/d)	*p*-Cresol	Quinol	4-Methylcatechol	Orcinol
4-Hydroxy phenylacetic acid	Rumen	3	3	4·1	44	0	0	0
	Abomasum	3	9	3·7	0	0	0	0
Casein	Rumen	2	2	26·8*	43†	0	0	0
p-Arbutin	Rumen	3	3	4·8	0	71	0	0
	Abomasum	3	9	4·9	0	69	0	0
Orcinol	Rumen	3	3	2·0	0	0	0	73
	Abomasum	3	9	2·0	0	0	0	69
Rutin	Rumen	4	3	7·3	0	0	18	0

* g Nitrogen.
† Calculated from the tyrosine content (392 mg/g N) of the casein infused.

urines. Lesser amounts were recovered from enzyme-hydrolysed urines. Infused orcinol was also recovered in urine in similar amounts after rumen or abomasal administration (Table 3). These urine phenol assays were of bicarbonate-washed, pH 1 diethyl ether extracts, of enzyme-hydrolysed urines. Bicarbonate washing reduced the recovery of orcinol (A. K. Martin, unpublished work) so these recoveries were incomplete. Acid-hydrolysis of the orcinol conjugates in these urines gave only approximately 40% of the amounts of orcinol found on enzyme hydrolysis (A. K. Martin, unpublished work). When a flavonol glycoside, rutin, was administered to the rumen at least 18% was excreted in the urine as 4-methylcatechol. Recovery of 4-methylcatechol has probably been underestimated as the pH 1 diethyl ether extracts of acid-hydrolysed urine were washed with bicarbonate to remove acids interfering in the GLC of 4-methylcatechol acetate on OV 17. The identity of 4-methylcatechol was confirmed by TLC of a diethyl ether extract of acid-hydrolysed urine.

Phenolic cinnamic acids containing 4-hydroxy groups were only decarboxylated to a limited extent to yield simple phenols. In four infusions in which 4OH-CA (6·0–8·5 g/d) was administered to the rumen, 2·1 ± 0·7% (mean ± SE) of the infused acid was excreted in the urine as *p*-ethylphenol; none was found after abomasal infusion. The presence of *p*-ethylphenol was confirmed by GLC of TMS phenols on a TXP column. 4OH-CA was not found on TLC of diethyl ether extracts of hydrolysed urine after infusion by either route. After abomasal infusion only of 4OH-CA, GLC of phenyl acetates on a 10% w/w OV 17 column produced an unknown peak (RR71; nonadecane standard) in slightly smaller quantity than the *p*-ethylphenol peak produced on rumen infusion. The peak was trapped by manual preparative GLC, and on TLC (toluene:methanol:glacial acetic acid, 46:5:4, by volume) gave, on spraying with fast blue B and alkali, an orange-brown spot at R_f 0·46. Its identity was not studied further. Rumen infusions of caffeic (3,4OH-CA; 5·5–6·8 g/d), ferulic (3MeO,4OH-CA, 7·6–12·1 g/d) or chlorogenic acid (3-*O*-caffeoylquinic acid (CHL), 4·4–5·2 g/d) gave no increments in urinary phenol outputs. However, on abomasal infusion of 3MeO,4OH-CA (4·9–7·7 g/d) approximately 5% of the infused acid was excreted as an unknown substance which when acetylated yielded a peak at *RR* 0·77 on 10% w/w OV 1 and at *RR* 104 on 10% w/w OV 17 columns (nonadecane standard). Lesser amounts were seen on abomasal infusion of 3,4OH-CA (6·8 g/d) and CHL (5·6 g/d). The peak was isolated by manual preparative GLC and shown to contain two phenolic components on TLC of acid-hydrolysed eluates from the traps. The spots had R_f values of 0·62 and 0·68 in toluene:methanol:glacial acetic acid (46:5:4, by volume) and gave purple and brown spots respectively on spraying with fast blue B and alkali.

DISCUSSION

Phenol and *p*-cresol have long been thought of as microbial metabolites of tyrosine and Baumann (1879) proposed the following scheme for their production:

$$\text{Tyrosine} \xrightarrow{1} \text{4OH-3-PPA} \xrightarrow{2} p\text{-ethylphenol} \xrightarrow{3} \text{4OH-PA} \xrightarrow{4} p\text{-cresol} \xrightarrow{5} \text{4OH-BA} \xrightarrow{6} \text{phenol}$$

Suemitsu *et al.* (1968) have suggested that these and related reactions are responsible for the presence of phenols in the urine of cows. Bakke (1969*a*) was unable to demonstrate that this pathway was operative in the rat. The present experiments show that it does not occur in the sheep either; *p*-cresol was the principal phenol excreted with only small amounts of phenol being produced. Reaction 1 is known to occur in the sheep rumen (Scott *et al.* 1964) but reaction 2 has not been observed. 4OH-3-PPA was quantitatively dehydroxylated in the rumen to yield 3-PPA as the end-product of microbial metabolism (Martin, 1982*b*). In contrast, 4OH-PA on rumen metabolism gave considerable urinary increments of the

decarboxylation product, *p*-cresol (reaction 4) and the soluble readily-fermented protein, casein, yielded a similar portion of its tyrosine as *p*-cresol (Table 3). Also, 4OH-BA infusion to the rumen gave large urinary increments in its decarboxylation product, phenol (Table 2). Rumen micro-organisms metabolized these phenolic compounds in a similar manner to the microflora of the rat caecum which decarboxylated 4-hydroxy-substituted benzoic acids (Scheline, 1966*a*) and 4-hydroxy-substituted phenylacetic acids but did not decarboxylate 4OH-3-PPA (Scheline, 1968*a*).

Rumen micro-organisms differed from those of the rat caecum in that only small amounts (mean $\pm$ SE, $2{\cdot}1 \pm 0{\cdot}7\%$) of 4OH-CA were decarboxylated and reduced to yield *p*-ethylphenol; *p*-ethylphenol was also only a minor component of the urinary phenols excreted by sheep (Table 1). This variation in microbial metabolism is probably due to differences in composition of the microflora of the rat caecum and sheep rumen. Peppercorn & Goldman (1971) isolated several species of human faecal micro-organisms and did not find any capable of more than one of the sequences of reactions involved in dehydroxylation, decarboxylation or side-chain reduction of phenolic cinnamic acids. In the rumen, the reduction of 4OH-CA to 4OH-3-PPA, which cannot be decarboxylated, appears to take precedence over decarboxylation and subsequent reduction to *p*-ethylphenol found in other species (Scheline, 1968*a*; Bakke, 1969*a*). *p*-Ethylphenol has been isolated from the urine of goats (Grant, 1948) and is the principal rumen metabolite (Braden *et al.* 1967) of the oestrogenically-active isoflavones, biochanin A (5,7OH,4MeO-isoflavone) and genistein (5,7,4′OH-isoflavone). Isoflavones are of restricted distribution in ruminant feedstuffs and would not be found in those offered in the present experiments (dried grass or hay–sugar-beet pulp, Martin, 1982*a*). The observation that 4OH-PA is the preferred Stickland reaction product of tyrosine in the rumen (Scott *et al.* 1964) and the failure to detect increases in *p*-ethylphenol excretion when abnormally large amounts of tyrosine were fed to rats (Bakke, 1969*b*) or when dietary proteins were exposed to unusually intensive microbial metabolism (Duran *et al.* 1973) suggest that *p*-ethylphenol is not an intermediate in tyrosine catabolism in the intestine. In the present studies the rumen metabolism of casein, 4OH-3-PPA and 4OH-PA did not yield any increment in urine output of phenol. An oral dosage of *p*-cresol (Bray *et al.* 1950; Bakke, 1970) was partially oxidized by rats to 4OH-BA, and *p*-ethylphenol (Bakke, 1970) was oxidized to 4OH-PA, but these are reactions of the body tissues and require biliary excretion to allow decarboxylation reactions 4 and 6 to occur. This did not happen in the sheep used in the present experiments.

Phenol can be produced directly from tyrosine by the microbial enzyme tyrosine ammonia lyase (*EC* 4.1.99.2.; Brot *et al.* 1965). Spoelstra (1978) showed that in pig faeces, tyrosine was metabolized to phenol at pH 7–8·5 but not at acid pH values; this observation may explain the apparent absence of this reaction in the rumen.

In the present experiments catechol was the second most abundant phenol in urine of fed sheep (Table 1) but, in common with all phenols other than *p*-cresol, was not excreted when sheep were fasted (A. K. Martin, unpublished work). There are several possible dietary precursors. Quinic acid can yield catechol as a minor product of the metabolism of intestinal micro-organisms (Indahl & Scheline, 1973). In the present experiments rumen infusion of a large amount of quinic acid (p. 499) was required to produce an increment in urinary catechol excretion. Catechol is also a minor oxidation product of phenol in the body tissues (Parke & Williams, 1953) but in the present experiments large increments in urine catechol output were only found on rumen administration of either 3,4OH-BA or 3MeO,4OH-BA (Table 2). Scheline (1966*a*) found that 3,4OH-BA was extensively decarboxylated by rat caecal micro-organisms and that 3MeO,4OH-BA was demethylated and decarboxylated in similar circumstances. Dacre & Williams (1968) deduced that 30% of an oral dose of 3,4OH-BA given to rats was decarboxylated; the greater exposure of precursors

to microbial metabolism in the rumen of sheep increased the extent of decarboxylation (Table 2). In anaerobic methanogenic cultures of organisms utilizing 3,4OH-BA, Balba & Evans (1980) found catechol to be an intermediate leading to complete degradation of the aromatic ring by the reductive pathway (Balba & Evans, 1977). In the intestine, catechol appears to be an end-product of metabolism. Simpson *et al.* (1969) reported that catechol was not utilized by cultures of rumen micro-organisms, and rabbits given oral doses of catechol excreted most of the dose as glucuronic acid or sulphate conjugates in the urine (Garton & Williams, 1948).

o-Dihydroxyphenols can be O-methylated in the body tissues and it may be expected that some catechol would be excreted as guaiacol (2-methoxyphenol). Guaiacol has been reported as a minor component of both the urine phenols of the rat (Bakke, 1969*c*) and of cows (Suemitsu *et al.* 1970); 7% of an oral dose of catechol was excreted as guaiacol by rats (Bakke, 1970). In the absence of demethylation, guaiacol would be the expected decarboxylation product of 3MeO,4OH-BA in the rumen. However, only 4% of rumen-infused 3MeO,4OH-BA was excreted in the urine as guaiacol (Table 2) and 60% as catechol. Demethylation in the rumen was extensive and little methoxylation took place in the body tissues of the sheep.

Pyrogallol (1,2,3OH-benzene) and resorcinol (1,3OH-benzene) were only found in urine after rumen infusion of gallic acid (3,4,5OH-BA). Decarboxylation of 3,4,5OH-BA by intestinal micro-organisms yielded pyrogallol (Scheline, 1966*b*, 1968*b*) which can be dehydroxylated to give resorcinol. The latter reaction varies with different microbial cultures: rat faecal but not caecal cultures of 3,4,5OH-BA or pyrogallol produced resorcinol (Scheline, 1966*b*) whereas rabbit faecal cultures only produced traces of resorcinol from pyrogallol (Scheline, 1968*b*). The sporadic incidence of resorcinol excretion has also been noted by Curzon (1957) who found it in only 2–3% of human urine samples examined; it was subsequently shown to be an intestinal microbial metabolite of tea polyphenols (Curzon & Pratt, 1964). In ruminant urine also, the amounts of resorcinol and pyrogallol excreted varied. In different sheep given different foods (dried grass in Expt 2; hay–sugar-beet pulp in Expt 4) and different amounts of rumen-infused 3,4,5OH-BA (23·2 or 7·0 g/d; Table 2) the extent of phenol excretion differed widely with resorcinol only found when the larger amount of precursor was infused. Whether these observations were due to differing rumen microbial populations in the two experiments and consequent differences in either the production or degradation of the two phenols in the rumen is not known. Some phenols are degraded in anaerobic conditions by micro-organisms isolated from the rumen; e.g. the trihydroxy phenol phloroglucinol can be completely utilized (Tsai *et al.* 1976) but this particular organism did not utilize resorcinol. However, in non-ruminants too the urinary recovery of resorcinol and pyrogallol may be incomplete; Fuji & Ito (1972) found in rat urine only 4% of an oral dose of 50 mg pyrogallol/kg and Garton & Williams (1949) found in rabbit urine 77% of oral doses of 100 mg resorcinol/kg.

Quinol is known to be an oxidation product of phenol in body tissues (Parke & Williams, 1953; Capel *et al.* 1972) and when large amounts of phenol were produced on rumen metabolism of 4OH-BA in the present experiment 10% of the administered acid was excreted in the urine as quinol (Table 2). Similar but incomplete urinary recoveries (69–71%. Table 3) of the quinol moiety of *p*-arbutin were found after rumen or abomasal infusion. Quinol is readily oxidized in alkaline urine to yield dark-coloured polymerization products of *p*-benzoquinone (Miller *et al.* 1973). No such colours were observed during the present experiments. Urine was collected in dilute acid (maximum pH 4) and no free quinol was found in extracts of unhydrolysed urine so it is unlikely that the incomplete urine quinol recoveries were due to oxidation of quinol in the sample.

Small amounts of 4-methylcatechol were found in sheep urine in most of the present experiments. Bakke (1969*c*) found it to be a minor constituent of the phenolic compounds

in the urines of rats given foods containing plant material. It is known to be an intestinal microbial metabolite of 3,4OH-PA (Scheline, 1967) in the rat. 3,4OH-PA itself is a microbial metabolite of the flavonol glycoside, rutin (3-*O*-rutinosyl-5,7,3′4′OH-flavonol) in the rabbit intestine (Booth *et al.* 1956) and bovine rumen (Krishnamurty *et al.* 1970). In the present experiments rumen administration of rutin yielded 18% of the dose as urinary 4-methylcatechol (Table 3).

Orcinol has not been reported as a metabolite of flavonols which could conceivably give rise to orcinol on intestinal microbial metabolism (Griffiths & Smith, 1972). Its presence in urine appears to depend on the consumption of plants containing orcinol glycosides.

What then are the most likely dietary precursors of the phenols found in sheep urine? Phenol is produced readily in the rumen from 4OH-BA but it is likely that conditions are unfavourable for its production from tyrosine through the action of tyrosine ammonia lyase. 4OH-BA is of almost ubiquitous occurrence in plants (Harborne & Simmonds, 1964) although the concentrations found appear, with a few exceptions (Bate-Smith, 1968), to be small as is normal urinary phenol output. 4OH-BA has been found in oat (*Avena sativa*) seed (Durkee & Thivierge, 1977), lucerne (*Medicogo sativa*) leaf (Newby *et al.* 1980), and various straws (Guenzi & McCalla, 1966; Kuwatsuka & Shindo, 1973; Salomonson *et al.* 1978) and ryegrass (*Lolium multiflorum*) plant cell walls (Hartley & Jones, 1977).

p-Ethylphenol excreted in small amounts is probably a minor ruminal metabolite of 4OH-CA which is widely distributed in ruminant feeds (Martin, 1982*b*). When excreted in larger amounts, it could be a rumen metabolite of 4′hydroxy- or 4′-methoxy-substituted 5,7OH-isoflavones.

Both 3,4OH-BA and 3MeO,4OH-BA yielded catechol as their principal rumen metabolite. The consistent presence of this phenol in ruminant urine is no doubt due to the ubiquitous presence of these acids in plants which contain lignin (Harborne & Simmonds, 1964).

Quercetin is one of the most abundant flavonols in plants (Harborne, 1979) and its rumen metabolism to 3,4OH-PA and 4-methylcatechol can explain the presence of the latter phenol in ruminant urines.

Hydrolysable tannins which are esters of 3,4,5OH-BA or its derivatives are of more restricted distribution than flavonols (Swain, 1979) and hence resorcinol and pyrogallol are less common urinary metabolites of ruminants.

When quinol is found in urine in the absence of large amounts of phenol it is likely that the animal's food contains conjugates of simple phenols. These are uncommon. Thieme & Winkler (1971) have reported that some Ericaceous plants may contain as much as 6% of quinol as its glycoside *p*-arbutin. Heather (*Calluna vulgaris* L.) may contain (g/kg) 0·2–0·8 quinol and 1·3–3·6 orcinol in young shoot dry matter (A. K. Martin, unpublished results). Orcinol occurs as a monoglycoside in several species of the *Ericaceae* (Harborne & Williams, 1969). It is possible that urinary output of a phenol such as orcinol could be used to determine the intake of an orcinol-containing component of a mixed sward by grazing animals.

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REFERENCES

Bakke, O. M. (1969*a*). *Scand. J. Gastroenterol.* **4**, 603.
Bakke, O. M. (1969*b*). *J. Nutr.* **98**, 217.
Bakke, O. M. (1969*c*). *J. Nutr.* **98**, 209.
Bakke, O. M. (1969*d*). *Scand. J. Gastroenterol.* **4**, 419.
Bakke, O. M. (1970). *Acta pharmac. tox.* **28**, 28.
Bakke, O. M. & Scheline, R. R. (1969). *Analyt. Biochem.* **27**, 451.

Balba, M. T. & Evans, W. C. (1977). *Biochem. Soc. Trans.* **5**, 302.
Balba, M. T. & Evans, W. C. (1980). *Biochem. Soc. Trans.* **8**, 452.
Bassett, E. G., Sewell, O. K. & White, E. P. (1955). *N.Z. J. Sci. Technol.* **36**, 437.
Bate-Smith, E.-C. (1968). *J. Linn. Soc. (Bot.)*, **60**, 325.
Baumann, E. (1879). *Ber. dt. chem. Ges.* **12**, 1450.
Bernhardt, F. W. & Zilliken, A. (1959). *Archs Biochem. Biophys.* **82**, 461.
Booth, A. N., Murray, C. W., Jones, F. T. & De Eds, F. (1956). *J. biol. Chem.* **223**, 251.
Braden, A. W. H., Hart, N. K. & Lamberton, J. A. (1967). *Austr. J. agric. Res.* **18**, 335.
Bray, H. G., Thorpe, W. V. & White, K. (1950). *Biochem. J.* **46**, 275.
Brot, N., Smit, S. & Weissbach, H. (1965). *Archs Biochem. Biophys.* **112**, 1.
Capel, I. D., French, M. R., Millburn, P., Smith, R. L. & Williams, R. T. (1972). *Xenobiotica* **2**, 25.
Clarke, E. G. C. & Humphreys, D. J. (1971). *J. Sci. Fd Agric.* **22**, 208.
Curzon, G. (1957). *Biochem. J.* **66**, 27*P*.
Curzon, G. & Pratt, R. T. C. (1964). *Nature, Lond.* **204**, 383.
Dacre, J. C. & Williams, R. T. (1968). *J. Pharm. Pharmac.* **20**, 610.
Drasar, B. S. & Hill, M. J. (1974). *Human Intestinal Flora*, 1st ed., p. 98. London: Academic Press.
Duran, M., Ketting, D., De Bree, P. K., Van der Heiden, C. & Wadman, S. K. (1973). *Clinica chim. Acta* **45**, 341.
Durkee, A. B. & Thivierge, P. A. (1977). *J. Fd Sci.* **42**, 551.
Fuji, K. & Ito, H. (1972). *Arnzneimittel-Forsch.* **22**, 777.
Garton, G. A. & Williams, R. T. (1948). *Biochem. J.* **43**, 206.
Garton, G. A. & Williams, R. T. (1949). *Biochem. J.* **44**, 234.
Grant, J. K. (1948). *Biochem. J.* **43**, 523.
Griffiths, L. A. & Smith, G. E. (1972). *Biochem. J.* **130**, 141.
Guenzi, W. D. & McCalla, T. M. (1966). *Agronomy J.* **58**, 303.
Harborne, J. B. (1979). In *Herbivores: Their Interaction with Secondary Plant Metabolites*, pp. 622, 637 [G. A. Rosenthal and D. H. Janzen, editors]. New York: Academic Press.
Harborne, J. B. & Simmonds, N. W. (1964). In *Biochemistry of Phenolic Compounds*, p. 85 [J. B. Harborne, editor]. London: Academic Press.
Harborne, J. B. & Williams, C. A. (1969). *Phytochemistry* **8**, 2223.
Hartley, R. D. & Jones, E. C. (1977). *Phytochemistry* **16**, 1531.
Indahl, S. R. & Scheline, R. R. (1973). *Xenobiotica* **3**, 549.
Kibe, K. & Kagura, S. (1976). *J. Sci. Fd. Agric.* **27**, 726.
Krishnamurty, H. G., Cheng, K.-J., Jones, G. A., Simpson, F. J. & Watkin, J. E. (1970). *Can. J. Microbiol.* **16**, 759.
Kuwatsuka, S. & Shindo, H. (1973). *Soil Sci. Pl. Nutr.* **19**, 219.
Martin, A. K. (1973). *Br. J. Nutr.* **30**, 251.
Martin, A. K. (1982*a*). *Br. J. Nutr.* **47**, 139.
Martin, A. K. (1982*b*). *Br. J. Nutr.* **47**, 155.
Miller, J. J., Powell, G. M., Olavesen, A. H. & Curtis, C. G. (1973). *Biochem. Soc. Trans.* **1**, 1163.
Newby, V. K., Sablon, R.-M., Synge, R. L. M., Castelle, K. V. & Van Sumere, C. F. (1980). *Phytochemistry* **19**, 651.
Parke, D. V. & Williams, R. T. (1953). *Biochem. J.* **55**, 337.
Peppercorn, M. A. & Goldman, P. (1971). *J. Bact.* **108**, 996.
Sakata, K. & Ishiyama, S. (1978). *J. agric. chem. Soc. Japan* **52**, 471.
Salomonson, A.-C., Theander, O. & Åman, P. (1978). *J. agric. Fd Chem.* **26**, 830.
Scheline, R. R. (1966*a*). *J. Pharm. Pharmac.* **18**, 664.
Scheline, R. R. (1966*b*). *Acta pharmac. tox.* **24**, 275.
Scheline, R. R. (1967). *Experientia* **23**, 493.
Scheline, R. R. (1968*a*). *Acta pharmac. tox.* **26**, 189.
Scheline, R. R. (1968*b*). *Acta pharmac. tox.* **26**, 332.
Scott, F. N., Ward., P. F. V. & Dawson, R. M. C. (1964). *Biochem. J.* **90**, 12.
Simpson, F. J., Jones, G. A. & Wolin, E. A. (1969). *Can. J. Microbiol.* **15**, 972.
Smith, A. A. (1961). *Nature, Lond.* **190**, 167.
Smith, G. A. L. & Sullivan, P. J. (1964). *Analyst, Lond.* **89**, 312.
Spoelstra, S. F. (1978). *Appl. environ. Microbiol.* **36**, 631.
Suemitsu, R., Fujita, S.-I., Joshimura, M., Gen, H., Yuasa, A. & Ushijima, J.-I. (1970). *Agric. biol. Chem.* **34**, 957.
Suemitsu, R., Fujita, S.-I. & Matsubara, H. (1965). *Agric. biol. Chem.* **29**, 908.
Suemitsu, R., Fujita, S.-I., Mishima, Y. & Yoshimura, M. (1968). *Bull. Chem. Soc. Japan* **41**, 1381.
Swain, T. (1979). In *Herbivores: Their Interaction with Secondary Plant Metabolites*, p. 664 [G. A. Rosenthal and D. H. Janzen, editors]. New York: Academic Press.
Tamm, A. & Villako, K. (1971). *Scand. J. Gastroenterol.* **6**, 5.
Thieme, A. & Winkler, H.-J. (1971). *Pharmazie* **26**, 419.
Tsai, G.-C., Gates, D. M., Ingledow, W. M. & Jones, G. A. (1976). *Can. J. Microbiol.* **22**, 159.
Von Euler, U. S. & Lishajko, F. (1959). *Nature, Lond.* **183**, 1123.

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