Biochemical Pharmacology, Vol. 17, pp. 338–342. Pergamon Press. 1968. Printed in Great Britain

Bacterial degradation of the aromatic amino acid side chain

(*Received* 5 *May* 1967; *accepted* 30 *August* 1967)

DURING studies of the urinary indole-excretion in East Africans living on various local diets it was observed that indolylacrylyl-glycine was frequently present in urine from apparently healthy individuals using plantain (musa sp.) and sweet potato as staple foods.[1] This compound has previously only been observed in quantity in urine of patients suffering from Hartnup disease and from conditions in which there is amino acid malabsorption[2]. It has been suggested that its presence in the urine of the East African plantain eaters could be ascribed to either an unusual gut flora or increased exposure of tryptophan to bacterial degradation in the large gut. As early as 1903 Hopkins *et al.*[3] suggested that tryptophan was converted to indolyl-propionic acid, in fact they had proved the conversion of skatole amido acetic acid to skatole acetic acid as was shown later by Ellinger and Flamand.[4] Milne *et al.*[5] found that i.p. injection of indolyl-propionic acid in rats led to excretion of indolylacrylyl-glycine whereas injection of indolyl-acrylic acid only resulted in excretion of indolylacrylyl-glycine; there was no evidence of the indolyl-propionic acid. On the basis of these results it was assumed that tryptophan was converted to the indolyl-acrylic acid via the propionic acid.

The following experiment was carried out in order to see if the first step in this conversion was a direct conversion of tryptophan to indolyl-propionic acid or to indolyl-acrylic acid or if it was possible to isolate intermediary products. The bacterial degration of tryptophan and two other essential amino acids, phenylalanine and histidine, was studied under similar conditions to try to establish a common mechanism for the bacterial degradation of the aromatic acid side chain.

METHODS

Faeces were collected from monkeys (Cercopithecus Aethiops) fed on diets consisting of plantain or sweet potatoes, who, prior to the experiment, were shown to excrete indolyl-acrylic acid or the glycine conjugate in the urine; 1 g of faeces was mixed with 5 ml physiological saline and 10 mg of one of the following compounds added:

L-tryptophan	Histidine	Phenylalanine
Indolyl-acrylic acid	Urocanic acid	Cinnamic acid
Indolyl-propionic acid	Dihydrourocanic acid	Phenyl propionic acid

Incubations were carried out under both aerobic or anaerobic conditions for two days at 37° after which time no further conversions could be observed. The faecal suspensions were extracted with an equal amount of (a) acetone for tryptophan-metabolites, (b) 0·1 N, HCl for histidine-metabolites, (c) ethylacetate for phenylalanine metabolites.

50 μl of the extracts were examined by TLC. The solvent systems and adsorbents devised to effect separation of the relevant side chain derivatives are given in Table 1.

RESULTS AND DISCUSSION

The results (Table 2) suggest that tryptophan is metabolized via indolyl-acrylic acid to indolyl-propionic acid by the gut bacteria. Tryptophan on incubation with faecal material yielded indolyl-propionic acid and traces of indolyl-acrylic acid; incubation of indolyl-acrylic acid resulted in formation of indolyl-propionic acid but the reverse reaction did not occur in detectable quantity.

A similar pattern was observed in phenylalanine with aerobic incubation producing both the saturated and unsaturated side chains. Under anaerobic conditions cinnamic acid only was found and the saturated derivative was not detectable.

TABLE 1. THIN LAYER SOLVENT SYSTEMS USED FOR SEPARATION OF SIDE CHAIN VARIANTS OF TRYPTOPHAN, PHENYLALANINE AND HISTIDINE

	Adsorbent	Solvent system	Spray	R_f-values
Tryptophan metabolites	Kieselgel G nach stahl (Merck)	*p*-ether 25 (60–80°) (6) ether 75 HCOOH 2	Ehrlich reagent (7)	Indolyl-propionic acid 68 Indolyl-acetic acid 61 Indolyl-acrylic acid 50 Indolyl-carboxylic acid 78 Tryptophan 0
Histidine metabolites	Kieselgel G	acetone 6 methanol 1 methylethylketone 1 acetic acid 2 water 0·5	Sulphanilic acid (7)	Histidine 6 Imidazoyl acetic acid 20 Imidazoyl propionic acid 31 Urocanic acid 73
Phenylalanine*	(1) Kieselgel G	*p*-ether (100–120°) 50 ether 25 HCOOH 1	Bromocresol purple (7)	(1) Benzoic acid 74 Phenyl acetic acid 57·5 Phenyl propionic acid 66 Cinnamic acid 89† Phenyl alanine 0
	(2) Polyamid MN (Macherey-Nagel)	benzene 30 *p*-ether (60–80°) 70 HCOOH 1	2·6 dichlorophenolindophenol (7)	(2) 1 80 2 70 3 86·5 4 64 5 0

* System II was used to separate cinnamic and phenylpropionic acid, whereas system I was used to separate benzoic and phenylacetic acid.

† Brominated by addition of 10 μl of a 5% solution of Br_2 in absolute ethanol directly to the spots on the chromatoplates before exposure to the appropriate solvent system.

TABLE 2. SIDE CHAIN METABOLISM BY FAECAL BACTERIA OF TRYPTOPHAN, PHENYLALANINE AND HISTIDINE

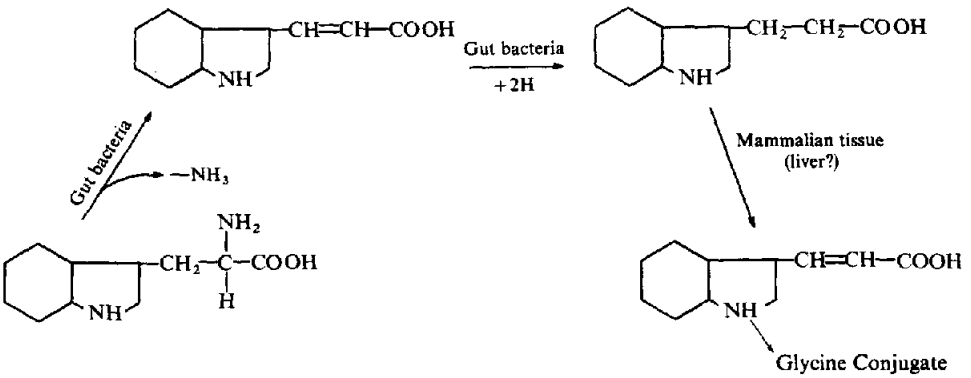
Substrate		Incubation	End products —CH=CH—COOH	End products —CH_2—CH_2COOH
Tryptophan	(Indolyl —CH_2—$CHNH_2$—COOH)	Aerobic (a)	Traces	+
		Anaerobic (an)	−	+
	Indolyl —CH_2—CH_2—COOH	(a)	−	+
		(an)	−	+
	Indolyl —CH=CH—COOH	(a)	+	+
		(an)	+	+
Histidine	(Imidazoyl —CH_2—$CHNH_2$—COOH)	(a)	+	−
		(an)	+	−
Dihydrourocanic acid	(Imidazoyl —CH_2—CH_2—COOH)	(a)	+	−
		(an)	+	−
Urocanic acid	(Imidazoyl —CH=CH—COOH)	(a)	+	−
		(an)	+	−
Phenylalanine	(phenyl—CH—$CHNH_2$—COOH)	(a)	±	±
		(an)	+	−
Phenylpropionic acid	(phenyl—CH_2—CH_2—COOH)	(a)	−	+
		(an)	−	+
Cinnamic acid	(phenyl —CH=CH—COOH)	(a)	+	+
		(an)	+	+

None of the acrylic and propionic acids could be detected in an extract of 19 faeces incubated at 37° for two days.
No ketoacid standards were available but none of the plates showed spots corresponding to α-ketoacids when sprayed with 2·4 dinitrophenylhydrazine.
N.B. Limit of detectability by the method was 10–100 μg/g faeces ~ <1% conversion.
The + indicates concentration ⩾ 1 mg/g faeces ~10% conversion.

On the other hand, histidine incubation led only to the formation of urocanic acid, the unsaturated derivative.

In the case of tryptophan and phenylalanine, the unsaturated side chain could be hydrogenated but the reverse reaction was not detected. It seems likely that the amino acid is directly deaminated to leave the unsaturated side chain which is subsequently hydrogenated. Incubation of the unsaturated side chain derivative of histidine, unlike the previous reactions, did not result in hydrogenation. However, the saturated dihydro-urocanic acid was reduced to the urocanic acid which is again the opposite of the tryptophan and phenylalanine reactions.

The scheme in Figs. 1 and 2 illustrates complete removal of ammonia from tryptophan by the bacteria leaving a molecule which may act as a hydrogen acceptor in the anaerobic state. A small



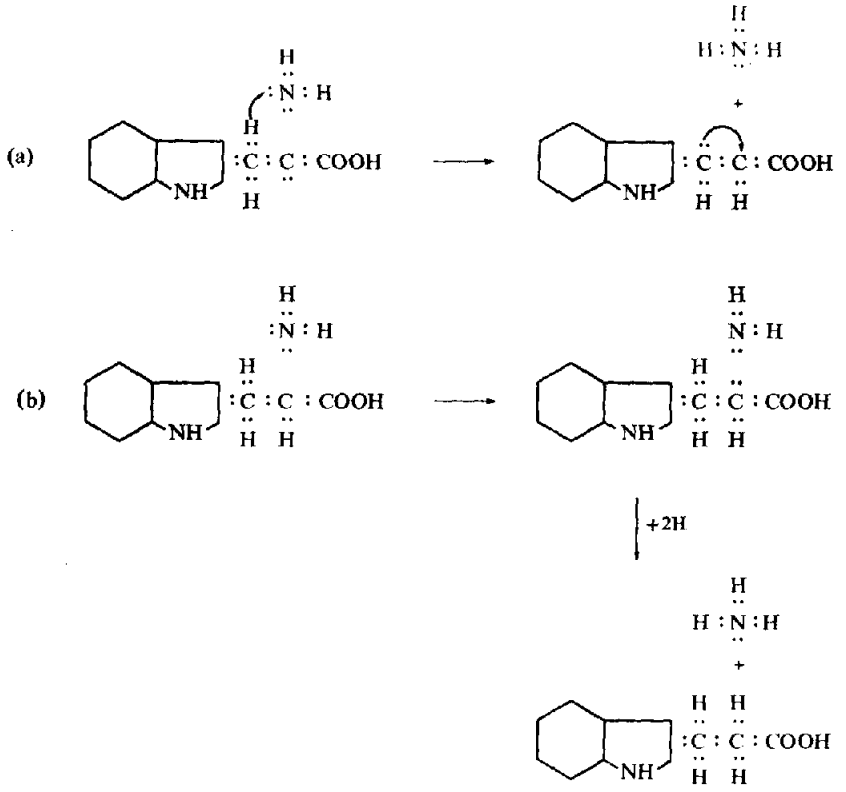
Proposed scheme of tryptophan metabolism via indolyl-acrylic acid in bacteria and tissues.

FIG. 1. Under anaerobic conditions oxidative deamination is unlikely. It would seem likely that cleavage of NH_3 would occur and the bacteria would then make use of the unsaturated side chain as a hydrogen acceptor. The findings of traces of indolyl-acrylic acid on incubation of tryptophan aerobically but not anaerobically (Table 2) and also the failure to observe the acrylic acid under anaerobic or aerobic incubation of the propionic acid supports the production of the acrylic acid as an intermediate.

amount of indolylacrylic acid remained during aerobic incubation when the need for a hydrogen acceptor would be reduced. This suggests that the reaction was via the acrylic acid as incubation of the propionic did not yield the acrylic whereas the reverse reaction of acrylic to propionic occurred readily.

The histidine results confirm the observation by Raistrick [8] that bacterial degradation of histidine can result in urocanic acid; in a recent study by Sen *et al.*[9] it has further been shown that intraperitoneal injection of histidine ^{14}C to rats led to excretion of urocanic acid and dihydrourocanic acid and that intravenous injection of urocanic acid is followed by excretion of dihydrourocanic acid. These findings in combination with those presented here seem to indicate that histidine can be deaminated by the bacteria but the urocanic acid does not act as a hydrogen acceptor under conditions of the bacterial flora.

A possible explanation for the different behaviour of tryptophan and phenylalanine molecules on one hand and histidine on the other might be that the imidazol-nucleus is less electrophilic than the indole and phenol-nuclei. Their different electrophilic natures may place the molecules on one or other side of the redox range of the bacterial enzyme systems, allowing the indole and phenyl derivatives to act as hydrogen acceptors but the imidazoyl as a hydrogen donor.



Proposed rearrangements for bacterial release of NH_3 from tryptophan leaving (a) the unsaturated and (b) the saturated side chain.

FIG. 2. Two possible rearrangements for direct cleavage of the NH_3 are illustrated above. The first is thought to be most likely in view of the production of traces of indolyl-acrylic acid after anaerobic incubation of tryptophan. The proton on the β carbon atom might either migrate to the free electron pair or the nitrogen or alternatively an exchange could take place on the enzyme surfaces with a proton from the medium.

Acknowledgements—We wish to express our appreciation to the Ministry of Overseas Development for a grant in aid of this work.

Department of Biochemistry,
Makerere University College, Kampala, Uganda.

INGE L. HANSEN*
M. A. CRAWFORD†

* Present address: The Neurochemical Institute, Raadmandsgade 58, Copenhagen N., Denmark.
† Department of Biochemistry, Nuffield Institute for Comparative Medicine, Regents Park, London N.W.1.

REFERENCES

1. J. BANWELL and M. A. CRAWFORD, *Biochem. J.* **89**, 69P (1963).
2. M. D. MILNE, M. A. CRAWFORD, C. B. GIRAO and L. LOUGHBRIDGE, *Q. Jl Med.* **29**, 407 (1960).
3. F. G. HOPKINS and S. W. COLE, *J. Physiol., Lond.* **29**, 451 (1903).
4. A. ELLINGER and C. FLAMAND, *Chem. Ber.* **40**, 3029 (1907).
5. M. D. MILNE, M. A. CRAWFORD, C. B. GIRAO and L. LOUGHBRIDGE, *Clin. Sci.* **19**, 165 (1960).
6. I. L. HANSEN and M. A. CRAWFORD, *J. chron. Dis.* **22**, 330 (1966).
7. I. SMITH, *Chromatographic and Electrophoretic Techniques*. Heinemann, London (1960).
8. H. RAISTRICK, *Biochem. J.* **11**, 71 (1917).
9. N. P. SEN, P. L. MCGEER and R. M. PAUL, *Biochem. biophys. Res. Commun.* **9**, 257, (1962).