Lysine and Pipecolic Acid Metabolism in the Domestic Fowl[1] (35083)

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Chicks which consume a diet containing L-lysine in excess of the nutritional requirement have an increased need for arginine to maintain a normal rate of growth (1). Excess lysine also increases chick kidney arginase activity (2). The biochemical basis of these observations has not been explained.

The detoxication of many aromatic acids by the fowl is accomplished by ornithine conjugation (3). The enzymes for diacyl ornithine synthesis are located in the kidney of the fowl (4, 5). The only natural source of ornithine for this conjugation reaction is dietary arginine because the fowl is unable to synthesize ornithine from any other carbon source (6, 7).

Three of the proposed intermediates in the degradative pathway of lysine for the rat (8) and the turkey (9) are L-pipecolate, Δ^1-piperideine-2-carboxylic acid, and Δ^6-piperideine-2-carboxylic acid. It was considered possible that one or more of these compounds could be detoxified by ornithine conjugation and excreted in the urine as the diacyl ornithine. Pyridine-2-carboxylic acid (picolinic acid), a suggested model compound, is detoxified by ornithine conjugation (10). The consumption of benzoic acid, which is also conjugated with ornithine, increases the chick requirement for arginine (6).

If ornithine were required to detoxify a product of lysine degradation it could explain the increased arginine requirement when excess lysine is consumed, and it might also explain the lysine-induced increase in arginase activity. The objectivities of this research were to determine: (i) if the fowl metabolizes lysine and pipecolic acid similarly to the rat, and (ii) if the fowl conjugates pipecolic acid, or a metabolite of pipecolic acid or L-lysine with ornithine.

Methods and Procedures. Adult single comb White Leghorn pullets were prepared with colostomies in order to obtain urine uncontaminated by feces from unrestrained birds (11). Quantitative urine collections were made by means of polyethylene containers held in place with a harness. To preserve the urine, 40 mg of sodium fluoride were added to the collection vessel prior to attachment. Urine was collected 3 to 4 times daily and frozen until analyzed. The pullets were given free access to a nutritionally complete diet. Feed consumption was recorded.

In Exps. 1 and 2, D-lysine and L-pipecolic acid were given intraperitoneally in a single dose. Urine was collected for 36 hr. In Expt. 3, solutions of arginine or lysine plus arginine in 2 to 1 molar ratio were given by crop tube in 7 divided doses over 36 hr. Urine was collected for 48 hr. In Expt. 4, DL-pipecolic acid and alpha picolinic acid were given in 2 divided oral doses 4 hr apart and urine was collected for 24 hr.

For conjugated ornithine analysis, urine was analyzed for ornithine by the Chinard method (12) before and after hydrolysis for 10 hr in 6 N H_2SO_4 at 121°.

Free pipecolic acid in the urine was determined by the method of Schweet *et al.* (13) after first separating pipecolic acid from interfering substance by ion exchange column chromatography (14, 15). Recovery of added pipecolic acid to the column was 97 to 100%. The tabulated urinary excretion values for free pipecolic acid were corrected for an

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TABLE I. Urinary Excretion of Pipecolate.

Hen no.	D-Lysine ip dose (mmole)	Pipecolate excreted (mmole)	Pipecolate excreted (% of dose)
Expt. 1. After intraperitoneal dose of D-lysine			
1	2.28	0.012	0.53
2	2.28	0.009	0.40
Expt. 2. After intraperitoneal dose of L-pipecolate			
	L-Pipecolate ip dose (mmole)		
1	2.22	0.043	1.9
2	2.30	0.129	5.6
Expt. 3. After oral dose of L-lysine			
	L-Lysine oral dose (mmole)		
3	34.0	0.053	0.16
4	27.2	0.057	0.21

amount of pipecolic acid or unknown interfering substance equivalent to 6 to 12 μmoles of pipecolic acid/day. This amount was present in the urine of pullets during the control collection period which preceded and followed the test periods. The actual amount was characteristic of the individual pullet and did not appear to be influenced by the amount of diet eaten.

Results and Discussion. Experiment 1 (Table I) indicated that only 0.40 to 0.53% of a dose of D-lysine was excreted as free pipecolic acid in the urine of fowl. In contrast, Grove and Henderson (15) observed that rats excrete 20–31% of a dose of D-lysine as pipecolate. Boulanger *et al.* (16) reported that germ-free rats excrete 98% of a dose of L-pipecolic acid free and unchanged in the urine. The fowl, however, excreted only 1.9 to 5.6% of the dose as free pipecolate in the urine within a 36-hr period (Expt. 2, Table I). The major portion of this was excreted in the first 24 hr. If D-lysine is degraded by the fowl via the pipecolic acid pathway then pipecolate must be rapidly metabolized or appears in the urine as a conjugate rather than free pipecolate. A conjugate other than that of ornithine was not sought.

TABLE II. Effect of L-Lysine on Conjugated Ornithine Excretion.

Hen no.	Dose (mmoles/36 hr) L-Arg	Dose (mmoles/36 hr) L-Lys	Conjugated ornithine excreted (mmoles/48 hr)
3	0	0	0.90
	17.2	0	0.69
	17.2	34.0	0.65
4	0	0	0.65
	13.8	0	1.17
	13.8	27.2	1.13
5	17.2	34.0	0.83

Experiment 3 indicated that after a 4- to 5-g oral dose of L-lysine, the fowl excretes essentially no pipecolic acid in the urine (Table I). The rat excretes less than 4% of a dose of L-lysine as pipecolate (15). The rat (17) and human (18) are known to possess the saccharopine pathway of L-lysine metabolism. Pipecolic acid is not an intermediate in the pathway.

The question of whether or not pipecolic acid or a metabolite of L-pipecolic acid or L-lysine was excreetd as an ornithine conjugate in the urine was investigated (Tables II, III). L-lysine produced no increase in the

TABLE III. Conjugated Ornithine Excretion of Chickens Given an Oral Dose of Alpha Picolinate or DL-Pipecolate.

Period	Dose (mmoles/4 hr) L-Arg	Dose (mmoles/4 hr) Alpha Picolinate	Dose (mmoles/4 hr) DL-Pipecolate	Conjugated ornithine excreted (mmoles/24 hr) Hen no. 1	Conjugated ornithine excreted (mmoles/24 hr) Hen no. 2
1	6.76	—	—	0.26	0.39
2	6.76	—	6.76	0.22	0.32
3	6.76	—	—	0.26	0.33
4	6.76	6.76	—	2.81	2.62
5	6.76	—	—	0.18	0.47

amount of conjugated ornithine excreted, and DL-pipecolate produced no increase. Alpha picolinic produced a 5- to 12-fold increase in conjugated ornithine excretion (Table III). Picolinic acid is known to be detoxified as the ornithine conjugate by the fowl (10). If the excretion product is assumed to be entirely dipicolinyl ornithine then from 66 to 76% of the alpha picolinic acid was excreted as the ornithine conjugate. These results indicate that the metabolism of L-lysine in the fowl does not give rise to an ornithine conjugate in the urine which would serve to explain the increased dietary requirement for arginine when an excess of lysine is fed.

The results of the study of the fate of intraperitoneally injected L-pipecolic acid suggest that the fowl unlike the rat may be able to metabolize pipecolic acid. Therefore, further studies to determine the major pathway of L-lysine degradation in the fowl are indicated.

Summary. Experiments were conducted with colostomized adult Leghorn pullets to determine the urinary excretion products produced from the metabolism of D- and L-lysine and L-pipecolic acid. The results indicate that the fowl, unlike the rat, excretes very little free pipecolic acid after an intraperitoneal dose of either D-lysine or L-pipecolate. The urine was analyzed for an ornithine conjugate of pipecolic acid or a degradation product of lysine metabolism. None was detected. It was concluded that the increase in the chicken's requirement for arginine when surfeit lysine is consumed is not due to a need for ornithine to detoxify a lysine metabolite.

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