Ornithine Utilization by the Chick (35946)

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Ornithine is an important metabolite in the rat. It is a key intermediate in the conversion of proline or glutamic acid to arginine (1, 2) and has a significant role in the urea cycle (3). However, ornithine is thought to have only minimal significance in the chicken which lacks a functional urea cycle (4). Synthesis of ornithine from either proline or glutamic acid is considered of minimal significance in the fowl (5, 6). Hence, neither proline nor glutamic acid can spare the chick arginine need (7).

Ornithine is produced in the chicken, as in the rat, as a by-product of creatine synthesis in the liver and as a result of arginine degradation by kidney arginase (8). However, to our knowledge the catabolic pathway of ornithine has never been examined in the chicken. In light of our previous findings that proline is unequivocably a dietary essential for the young chick (9–10), it seemed desirable to establish whether ornithine could be converted to proline, and if so, to determine the significance of this conversion. Also of interest was an evaluation of ornithine's ability to furnish nonspecific amino nitrogen for growth of the chick.

Materials and Methods. Chicks resulting from the mating of New Hampshire males to Columbian females were used in all assays. Their preexperimental care, selection and allotment to experimental treatments has been described previously (12). The composition of the reference standard crystalline amino acid diet (RS) is shown in Table I. Unless otherwise indicated, the experimental diets were offered on an *ad libitum* basis. All dietary modifications were made at the expense of cornstarch.

Because no clear-cut evidence had been advanced to show that dietary L-ornithine HCl could be absorbed from the gastrointestinal tract, the first assay was designed to assess the chick capacity to absorb this amino acid. Beginning on day 8 posthatching, 35 female chicks were fed the complete RS diet (Table I) for 2.5 days. On the morning of day 11 following an overnight fast, 5 chicks were bled by heart puncture for fasting plasma amino acid values. Equal portions (2 ml) of blood from individual chicks were pooled. The remaining 30 chicks were equally distributed at random to one of three dietary treatments (RS, RS + 0.5% L-ornithine HCl, and RS + 1.0% L-ornithine HCl) in two replicate lots and allowed to eat for 30 min after which they too were bled and blood from individual chicks was pooled by replicate lot. After preparing the plasma, it was deproteinized with crystalline sulfosalicylic acid (50 mg/ml). Analysis for free plasma amino acids was accomplished by automated ion-exchange chromatography.

The second assay was designed to ascertain whether the chick can metabolize ornithine. Six chicks were fed the complete RS diet from days 8 to 12 posthatching. After an overnight fast, they were injected intraperitoneally with 10 μCi of ornithine-U-^{14}C which was suspended in 1 ml of saline. Four of the birds were immediately placed in individual all-glass metabolism cages and the CO_2 expired in a 4-hr period was trapped in a 1:2 mixture of ethanolamine and ethylene glycol monomethyl ether. An aliquot was added to a scintillation vial, mixed with a scintillation solution (13) and counted using a liquid-scintillation spectrometer.

At 4 hr postinjection, all 6 chicks were

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TABLE I. Composition of Reference Standard Diet.

Ingredient	% of diet
L-Arginine HCl	1.21
L-Histidine HCl·H_2O	0.41
L-Lysine HCl	1.19
L-Tyrosine	0.45
L-Tryptophan	0.15
L-Phenylalanine	0.50
DL-Methionine	0.35
L-Cystine	0.35
L-Threonine	0.65
L-Leucine	1.20
L-Isoleucine	0.60
L-Valine	0.82
Glycine	1.20
L-Proline	0.20
L-Glutamic acid	10.00
Corn oil	15.00
Salt mixture[a]	5.37
Cellulose	3.00
$NaHCO_3$	1.00
Choline chloride	0.20
Vitamins[b]	+
Ethoxyquin (125 mg/kg)	+
Cornstarch	to 100.00

[a] Dean and Scott (18).
[b] Klain *et al.* (19).

sacrificed by cervical dislocation and the livers were excised rapidly and frozen for analysis. Protein was isolated from the livers as described by Herrara and Renold (14) with the exception that no protein carrier was employed. After drying the isolated protein, approximately 25 mg were added to a scintillation vial, wetted with 0.1 ml of distilled water and left at room temperature for 30 min. Two milliliters of a solubilizer (quaternary ammonium base in toluene) were added to each vial to digest the samples. After standing for 2 days at room temperature, 15 ml of the toluene scintillation cocktail were added to each vial and the samples were counted.

Another 25 mg sample of the isolated liver protein was acid hydrolyzed under vacuum at 110° for 22 hr. After removing the acid by flash evaporation and adjusting the sample pH, automated ion-exchange chromatography was used to quantitate the amino acids. A determined amount of glutamic acid, proline, and valine was collected as they eluted from the column (prior to reacting with Ninhydrin) and the radioactivity of each amino acid fraction was determined by liquid scintillation.

The remaining two studies were growth assays in which weight gain and gain/feed ratio were the sole criteria. Assay 3 was conducted to examine the capacity of ornithine or its principle precursor, arginine, to spare the need for dietary proline. Assay 4 was designed to ascertain if dietary ornithine could be used as an effective source of nonspecific amino nitrogen. Hence, the basal diet for Assay 3 was devoid of L-glutamic acid.

The data were analyzed when appropriate by analysis of variance, and both orthogonal and nonorthogonal single degree-of-freedom comparisons were used to test for treatment differences.

Results. The results of Assay 1 (Table II) clearly indicate that the young chick can absorb ornithine. In fact, over the range of ornithine intakes studied, a straight-line relationship ($r = 0.99$) existed between ornithine intake (mg) and plasma ornithine concentration (μg/ml of plasma). Kelly and Scott (15) reported similar findings for lysine, an amino acid known to be absorbed efficiently from the gastrointestinal tract. Relative to the fasting values, diet consumption increased the plasma concentration of all amino acids studied, but only plasma ornithine was influenced to any marked degree by the level of ornithine fed. The fasting plasma ornithine value was extremely low, probably a reflection of reduced glycine transamidinase and arginase activities upon feed withdrawal. Since feeding the ornithine-free diet resulted in a 100-fold increase in plasma ornithine compared with the fasting value, a significant amount of ornithine synthesis must have occurred during the 30 min feeding period.

The tracer study (Assay 2) revealed that nearly 50% of the administered dose of ^{14}C was recovered as CO_2 in a 4 hr period (Table III). Radioactivity was also found in the liver protein fraction, and since ornithine itself is not a constituent of avain protein, conversion of ornithine to other amino acids

TABLE II. Effect of Feeding Graded Levels of Ornithine on Plasma Concentration of Ornithine and Related Amino Acids (Assay 1).

		(μg/ml of plasma)		
		Dietary L-ornithine HCl (%);		
Amino acid	Fasting[a]	0[b]	0.5[b]	1.0[b]
Ornithine	0.3	29.8 ± 3.9	90.4 ± 1.4	158.0 ± 8.9
Arginine	12.2	71.5 ± 9.0	69.3 ± 6.1	71.6 ± 6.4
Proline	21.4	33.2 ± 2.5	29.0 ± 1.5	27.4 ± 0.8
Glutamic acid	32.8	134.4 ± 10.8	112.6 ± 11.1	107.8 ± 2.2

[a] Mean of 1 lot of 5 chicks.

[b] Mean ± SEM of 2 lots of 5 chicks each.

must have occurred. Two metabolically related amino acids, proline and glutamic acid, accounted for 60% of the total label in the liver protein fraction. Valine, which is not synthesized *in vivo*, accounted for but a trace of the total ^{14}C found in liver protein. These results support the view that ornithine (i) is metabolized *in vivo* and (ii) can be converted to both proline and glutamic acid.

Despite the findings that dietary ornithine is absorbed and is capable of being converted to proline, neither supplemental ornithine HCl nor arginine was able to eliminate the dietary need for proline (Table IV). The small but remarkably consistent response to supplemental proline is in agreement with earlier reports from our laboratory (9–11). Supplemental glutamic acid produced a small but nonsignificant gain and gain/feed ratio response and this may suggest, as shown previously (10), a slight sparing effect of glutamic acid on the dietary requirement for proline.

Ornithine proved to be an effective source of nonspecific nitrogen as indicated by data in Table V. Chicks fed 3.45% supplemental L-ornithine HCl gained as fast as those fed an equimolar quantity of L-glutamic acid HCl. Both sources of nitrogen resulted in close to a 40% growth response over that observed with chicks fed the nitrogen-limiting basal diet. Gain/feed ratio was also improved

TABLE III. *In Vivo* Metabolism of Ornithine by the Young Chick (Assay 2).[a]

Criterion	Radioactivity[b]
Recovered as CO_2 (% of dose)	47.5 ± 0.1
Liver protein (% of dose)	1.3 ± 0.3
Liver amino acids (% of liver protein)	
Proline	45.5 ± 2.4
Glutamic acid	14.2 ± 1.8
Valine	Trace

[a] Chicks were injected ip with 10 μCi of ornithine-U-^{14}C and CO_2 was collected over a 4 hr period after which chicks were sacrificed for liver analysis and counting.

[b] Mean ± SEM for 4 chicks for CO_2 recovery and 6 chicks for liver protein and amino acid analysis.

TABLE IV. Capacity of Ornithine and Arginine to Spare the Need for Dietary Proline (Assay 3).

Dietary treatment[a]	Daily gain (g)[b,c]	Gain/feed
1. Basal (B)	13.1 ± 0.2	0.66
2. B + 0.4% L-proline	14.0 ± 0.3	0.69
3. B + 1.17% L-ornithine HCl[d]	12.8 ± 0.2	0.67
4. B + 1.21% L-arginine	12.8 ± 0.1	0.67
5. B + 1.02% L-glutamic acid	13.4 ± 0.1	0.68

[a] Basal diet (Table I) was modified to be devoid of L-proline; proline precursors were provided at a level to furnish 2 moles of precursor/mole of proline.

[b] Mean ± SEM for triplicate groups of 8 male chicks for the experimental period 8–14 days posthatching; average initial weight was 83 g.

[c] Treatment 2 greater ($p < .05$) than treatment 1, 3, 4, or 5.

[d] 0.59% supplemental $NaHCO_3$ was added to diet 3 so that it would be equimolar to the HCl furnished by 1.17% L-ornithine HCl.

TABLE V. Capacity of Ornithine to Furnish Nonspecific Nitrogen for Growth of the Chick.

Dietary treatment[a]	Daily gain (g)[b c]	Gain/feed[c]
1. Basal (B)	4.8 ± 0.2	0.42
2. B + 3.45% L-ornithine HCl	6.8 ± 0.1	0.59
3. B + 3.74% L-glutamic acid HCl	6.6 ± 0.1	0.52

[a] Basal diet (Table I) was modified to be devoid of L-glutamic acid and to contain 2.0% additional $NaHCO_3$; 3.74% L-glutamic acid HCl is equimolar to 3.45% L-ornithine HCl.

[b] Mean ± SEM for duplicate groups of 9 male chicks for the experimental period 8–14 days posthatching; average initial weight was 61 g.

[c] Treatment 2 or 3 greater ($p < .01$) than treatment 1.

markedly by providing supplemental nitrogen. In fact, chicks fed the diet containing supplemental ornithine HCl exhibited greater gain/feed ratios than those fed the diet containing supplemental glutamic acid HCl. However, it is pertinent to note that the ornithine HCl addition, while equimolar to the glutamic acid HCl addition, provided twice as much nitrogen as that provided by glutamic acid HCl.

Discussion. Our results indicate that the chick can readily absorb and metabolize orally administered ornithine. That liver proline and glutamic acid were highly labeled suggests a sequence of ornithine catabolism similar to that in the rat. That is, ornithine is converted to glutamic semialdehyde which can give rise to either glutamic acid or proline. This is supported by our finding that ornithine nitrogen can be utilized as a source of nonspecific nitrogen and the finding of Klain and Johnson (16) that the administration of uniformly labeled arginine-^{14}C to chicks results in labeling of liver glutamic acid and proline. In the rat the pathway from ornithine to either proline or glutamic acid is reversible (17). In contrast, the chick appears incapable of converting proline or glutamic acid to ornithine (5, 6).

Even though proline was shown to be synthesized from ornithine, dietary L-ornithine HCl could not spare the dietary proline requirement. This suggests that the flux of the pathway from ornithine to proline may be insufficient to sustain maximal growth rate.

Summary. Assays employing a chemically defined diet were conducted to determine if the young chick could utilize dietary ornithine. A plasma assay and a tracer study clearly demonstrated that the chick can both absorb and metabolize ornithine. Moreover, a significant quantity of ^{14}C from injected ornithine-U-^{14}C was recovered in liver protein as proline and glutamic acid. Growth assays demonstrated that orally administered L-ornithine HCl was an effective source of nonspecific amino nitrogen but it was ineffective in eliminating the dietary requirement of the chick for proline.

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