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The Oxidation and Kidney Clearance of D- and L-Tryptophan by Chickens[1] (38454)

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(Introduced by D. B. McCormick)

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The nutritional usefulness of D-tryptophan by chickens is less than that of the L-isomer. Grau and Almquist (1) were the first to report that the D-enantiomer in a DL-mixture was not appreciably utilized by the chick. Anderson *et al.* (2) found little or no utilization of D-tryptophan by chicks fed diets containing glucose (cerelose) as the source of carbohydrate. The unnatural isomer was of some value in diets containing corn starch in place of cerelose. They hypothesized a change in composition of intestinal microflora to account for the altered metabolism of D-tryptophan (2). Wilkening and Schweigert (3) indicated that the D-isomer was from 17 to 40% as active as the L-form for growing chicks. Contrary to other reports, West *et al.* (4) reported no difference in the utilization of DL- versus L-tryptophan for growing chickens.

Morrison *et al.* (5) refined previous experiments by feeding strictly the D-isomer in basal diets and compared growth rate of those chicks to others fed diets containing the L-isomer. Fourteen times more dietary D-tryptophan was required to support optimal growth of chicks compared to L-tryptophan. They suggested that D-tryptophan was poorly absorbed by chicks although no direct measurements were made. Some utilization of D-tryptophan by growing chicks has been shown employing crystalline amino-acid diets (6).

In preliminary studies in our laboratory (DiLorenzo, unpublished data, 1972), a large proportion of a dose of ^{14}C-DL-tryptophan was recovered as tryptophan in excreta of chicks following an intraperitoneal injection. The studies reported in this paper were intended to investigate the relative proportions of D- and L-tryptophan metabolized to $^{14}CO_2$ or excreted in urine by chickens and to determine the reabsorption of the two compounds by the kidney.

Materials and Methods. Separation of labeled tryptophan isomers. Racemic tryptophan (benzene ring-^{14}C; specific activity, 224 μCi/mg) was purchased from Amersham/Searle Corporation and was separated into D- and L-tryptophan by a modification of the procedure of Contractor and Wragg (7). The radioactive tryptophan was dissolved in 0.1 *N* NaOH at an approximate concentration of 1 mg/ml and the solution was neutralized using 1 *N* HCl. Glass plates 20 × 50 cm were used as supports for the stationary phase (Cellulose Powder, CC 41; Whatman, approximately 0.25 mm thick). Radioactive tryptophan samples were applied in a strip at the origin at a maximum concentration of 12 μg/cm. In a separate lane, 10 μg/cm of unlabeled DL-tryptophan were applied to serve as a standard. The plate was developed for 16 hr with a solvent system consisting of *n*-butanol:pyridine:water (1:1:1). After development was complete, the plates were allowed to air-dry under a hood. The unlabeled DL-tryptophan lane was sprayed with Fluram (Roche Diagnostics) dissolved in acetone (20–30 mg/100 ml acetone), and the two isomers were detected using ultraviolet light. D-Tryptophan has a higher R_f than L-tryptophan in this system (0.6 for D-, 0.5 for L-tryptophan). Cellulose in the areas of the thin-layer plate corresponding to the expected position of the D- or L-isomer was removed from the glass plate and transferred to a test tube to which was added

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5 ml of water. The test tube was shaken several times over a 4–6 hr period. The tube was centrifuged and the supernatant saved. The cellulose pellet was washed once, recentrifuged and the washing combined with the original supernatant. The purity of the separated isomers was checked by rechromatography of 20 μl of the supernatant solutions. An equal quantity of cold tryptophan standard was spotted with the radioactive material to aid in identification of the two isomers after plate development. The plate was developed and sprayed with Fluram. The two isomers were located, removed and counted in Bray's solution. The entire supernatant solutions were freeze-dried separately and rechromatographed a second time to eliminate contamination of one isomer with the other. The final preparations used were a D-isomer estimated to contain about 3.7% L-tryptophan and an L-isomer contaminated with 1.8% D-tryptophan.

Preparation of animals. The 12 male chicks used in metabolism experiments ranged in age from 13 to 17 days. After hatching, birds were fed a commercial chick starter. Four days prior to the experiment all chicks were fed a purified diet, mixture "a" described by Scott, Nesheim and Young (8). The diet contained about 0.16% tryptophan. On day prior to the experiment, chicks were fed the same purified diet to which tryptophan was added to a level of 1.2%. Birds were injected with 1 ml of D- or L-^{14}C ring-labeled tryptophan having a concentration of 955,364 and 1,253,950 dpm/cm^3, respectively.

Metabolism cages and peripheral apparatus. Chicks were housed individually in air tight Plexiglas metabolism cages (diameter, OD, 15; height, 20 cm). A 0.167-hp vacuum pump was used to move air through a column of dessicant into a metabolism cage and through a Delmar trapping tower filled with 100 ml methylcellosolve:monoethanolamine (2:1) for trapping metabolic CO_2. Four cage units with trapping towers were connected in parallel to the vacuum pump, allowing the concurrent monitoring of four birds.

Sampling procedure. All chicks received intraperitoneal injections of ^{14}C-tryptophan in the morning. Labeled CO_2 was collected each hour for 6 hr by removing the trapping solution. Blood and excreta samples were obtained at the end of each 6-hr experiment.

Renal clearance studies. White Leghorn hens (about 12 mo of age) served as experimental animals. All were fed a commercial laying ration prior to the renal clearance experiments. Twelve hens were infused through a wing vein with specific isomers of tryptophan (four with D-tryptophan at 4 μmoles/min; four with L-tryptophan at 4 μmoles/min; four with L-tryptophan at 8 μmoles/min). The surgical procedure and methodology for urine collection, blood sampling, and infusion were described by Wang and Nesheim (9). Urine samples were collected at 20-min intervals over a 3-hr period. Infusion of a specific isomer began immediately following the collection of sample No. 1. Blood was withdrawn from the right brachial vein at the midpoint of each 20-min urine collection period. Inulin was determined by the resorcinol method (10) and a fluorescence assay was used to determine tryptophan concentrations (11). Renal clearances were calculated by standard methods (12).

Results. Metabolism of labeled tryptophan isomers. Results of the experiment employing ring-labeled D- or L-tryptophan are shown in Table I and Fig. 1. Only 3.7% of the original radioactive D-tryptophan dose was recovered as CO_2, compared to 29.4% recovery of $^{14}CO_2$ from injected L-^{14}C-tryptophan over the 6-hr collection period. The differences between recovery of $^{14}CO_2$ from the two isomers were statistically significant ($P < 0.01$). L-Tryptophan was catabolized to CO_2 rapidly during the first 2 hr following intraperitoneal injection of the isotope (Fig. 1) while very low levels of $^{14}CO_2$ were detected from chicks receiving the D-isomer. Over the 6-hr collection period, 62.7% of the ^{14}C injected was recovered in excreta from chicks given D-tryptophan compared to 20.8% from chicks given the L-isomer. These differences were highly significant ($P < 0.01$).

Renal clearance studies. Results of the renal clearance experiments are shown in Fig. 2. Plasma concentration of tryptophan (μg/ml) was greater when D-tryptophan was infused at 4 μmoles/min compared to chicks given L-tryptophan at the same rate (Fig. 2). Over the 3-hr collection period, the cumulative concentration of plasma tryptophan (μg/ml) from D-tryptophan infusions at 4 μmoles/min was twice the comparable values for L-tryptophan given at the same infusion rate. The differences were significant ($P < 0.01$). When the infusion rate of L-tryptophan was doubled, the cumulative plasma tryptophan concentration increased

TABLE I. Metabolism of Labeled Tryptophan Isomers.

Isomer	Body wt (g)[a]	N	Plasma tryptophan (μg/ml)	% Recovery of isotope[b]	
				CO_2	Excreta
L	100.2 ± 1.1	5	25.2 ± 1.9	29.4 ± 0.7	20.8 ± 2.1
D	101.6 ± 3.2	8	24.9 ± 1.1	3.7 ± 0.3	62.7 ± 3.8

[a] $\bar{X}$ ± SE.

[b] Cumulative total over a 6-hr collection period.

2.4 times. There was no significant difference in plasma tryptophan concentration when L-tryptophan was infused at 8 μmoles/min compared to D-tryptophan infused at 4 μmoles/min.

The data in Fig. 2 clearly show that tryptophan excretion (μg/20 min collection period) was markedly increased when D-tryptophan was infused. During the collection periods, substantially more tryptophan was excreted when D-tryptophan was given compared to L-tryptophan when both were infused at 4 μmoles/min. A doubling of the L-tryptophan infusion rate did not result in urinary excretion of tryptophan comparable to that observed when the D-isomer was administered.

The ratio of tryptophan clearance relative to inulin clearance is also shown in Fig. 2. As D-tryptophan infusions were started, tryptophan clearance quickly approached inulin clearance. By 40 min after D-tryptophan infusion began, tryptophan clearance was equal to or somewhat above inulin clearance for the remainder of the experiment. This indicated that D-tryptophan was cleared from plasma by the kidney essentially at the same rate as inulin. There was no evidence of any reabsorption of D-tryptophan by the kidney tubule after 40 min of infusion of the isomer.

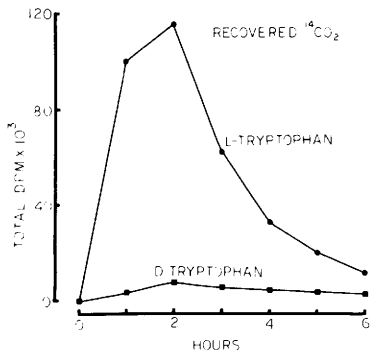

FIG. 1. The hourly rate of $^{14}CO_2$ produced following a single intraperitoneal injection of labeled D- and L-tryptophan.

In contrast, the reabsorption of L-tryptophan was extremely efficient. The average ratio of L-tryptophan clearance to inulin clearance was 0.04 and 0.07 when the L-isomer was infused at 4 and 8 μmoles/min, respectively. This indicates efficient reabsorption of the L-isomer by the kidney, in marked contrast to the reabsorption of D-tryptophan.

Discussion. These data suggest that D-tryptophan is excreted rapidly with little opportunity for it to be metabolized. There seems to be little evidence of D-tryptophan reabsorption by the kidney. Tissue uptake from the blood also appears to be very slow. Consequently, blood levels rose much more rapidly when D-tryptophan was infused than when L-tryptophan was administered. Absorption from the gastrointestinal tract was not measured, but D-tryptophan may also be poorly transported across the intestinal mucosa. Thus the poor utilization of D-tryptophan by chickens may be a combination of poor absorption from the gastrointestinal tract, slow tissue uptake and rapid excretion via the kidneys.

The $^{14}CO_2$ produced from ^{14}C D-tryptophan and nutritional studies (5) suggest some D-tryptophan utilization in chickens. To the best of our knowledge, the mechanism of D-tryptophan utilization in chickens is unknown. Insight into possible mechanisms of D-isomer utilization perhaps can be obtained from the extensive studies conducted on the laboratory rat. In contrast to the chicken, early nutritional studies in the rat suggested that DL- and L-tryptophan were of equal value in the diet (13). More recent studies have tested the requirements of L- and D-tryptophan for optimum growth in diets lacking nicotinic acid (14). Their findings showed that in diets containing 0.15 or 0.20%

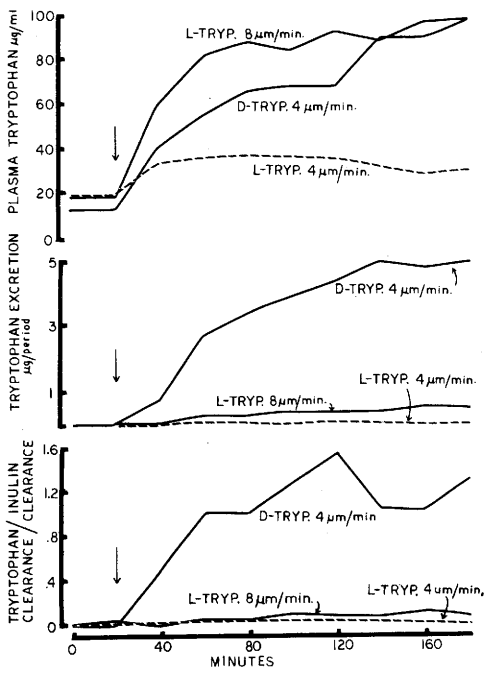

FIG. 2. Plasma and urinary concentration tryptophan and the ratio of tryptophan clearance to inulin clearance during a 3-hr infusion of D- or L-tryptophan. Arrows mark a beginning of infusion of each isomer. TRYP = tryptophan.

tryptophan, the D-isomer was about 75% as effective as the L-form. Metabolic studies with ring-labeled D- or L-tryptophan have shown that rats receiving either labeled D- or L-tryptophan excreted comparable total amounts of isotope in urine as well as respired CO_2 (15). These data are additional evidence supporting comparable utilization of either isomer. A relevant finding from excretion studies showed that in rats pulse-fed D-tryptophan, considerable quantities of urinary indolepyruvic acid were detected (16). Since indolepyruvic acid is an excellent dietary substitute for L-tryptophan, the data of Mason and Berg suggested that rats have the enzymatic capability to invert D-tryptophan to the L-form (17). Inversion of D-tryptophan to L-tryptophan has recently been verified to account for the ability of rats to utilize the unnatural isomer (18). In addition, extracts of rat and rabbit liver and intestine have been shown to convert D-tryptophan to D-kynurenine (19, 20). It is not known whether two separate pyrrolase enzymes exist to catabolize each isomer or if one enzyme degrades both enantiomers; however, evidence favors the latter interpretation (21).

The enzymatic ability of chickens to convert D- to L-tryptophan or D-kynurenine has not been investigated. The lack of kidney retention of D-tryptophan by chickens may limit the amount of metabolism that can occur even if appropriate enzymes are present. Man (22) and the mouse, like the chicken, seem to have difficulty utilizing D-tryptophan to support nitrogen equilibrium or promote growth (17).

Summary. Ring-labeled D- or L-tryptophan was injected intraperitoneally into chicks. The major portion of L-tryptophan was recovered as CO_2 while the D-isomer was primarily detected in excreta. Renal clearance studies showed that infused L-tryptophan was efficiently reabsorbed by the kidney while D-tryptophan behaved similarly to inulin. Compared to L-tryptophan, the D-form was poorly utilized in chickens.

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