Metabolism of D- and L-Tryptophan-*7a*-^{14}C in Rats and the Effects of Unlabeled Enantiomers[1] (34661)

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DL-Tryptophan metabolism has been studied extensively in man and animals (1, 2). Nutrition studies in the rat have shown that L- and D-tryptophan were of approximately equal value in the diet (3). Studies of the metabolism of L- vs. D-kynurenine in rats (4) or L- vs. D-hydroxykynurenine in rats and mice have shown that the L-isomer was efficiently metabolized to CO_2 while the D-isomer was not (5). Humans and rabbits given D-tryptophan excreted D-kynurenine in their urine (6, 7). In view of the species variation in their ability to handle D-isomers, it becomes important to know the extent of

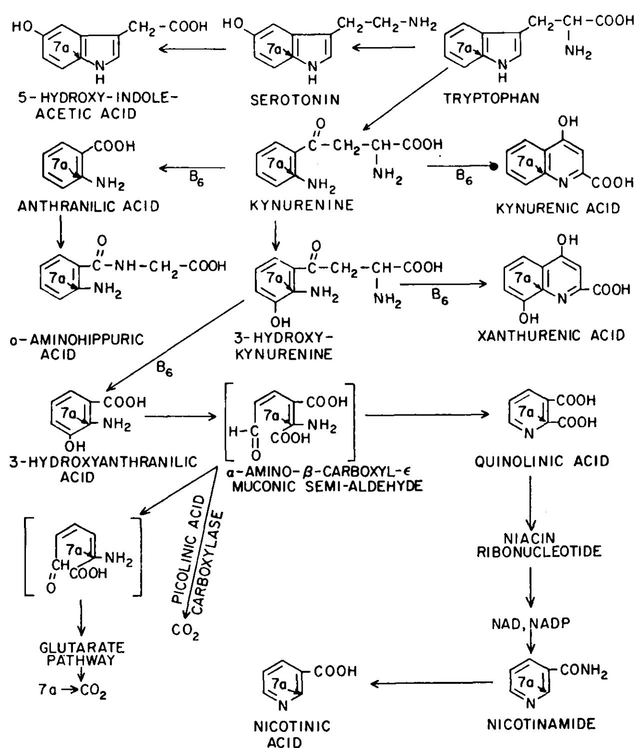

FIG. 1. Pathways of tryptophan metabolism in animals and man.

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utilization of the enantiomers in the metabolism of tryptophan to CO_2. A meaningful interpretation of the metabolism of DL-tryptophan cannot be made without knowledge of the metabolism of the unnatural isomer and the possible effects it might have on the metabolism of the natural isomer.

The work reported deals with the metabolism of L- and D-tryptophan-$7a$-^{14}C along the kynurenine pathway and the influence of doses of the opposite unlabeled isomer on their *in vivo* metabolism in rats. Figure 1 shows the metabolites concerned in this study and their relationships.

Experimental Methods. The D- and L-isomers of the tryptophan-$7a$-^{14}C used in this study were prepared by the resolution of DL-tryptophan-$7a$-^{14}C obtained commercially.[3] The resolution of the D- and L-isomers was done with a paper pulp column (10 × 170 cm) using a solvent system consisting of methanol, benzene, *n*-butanol, and water; 2:1:1:1 (8). With this system D-tryptophan (unnatural isomer) has been shown to have a faster R_f than the L-isomer on ascending paper chromatograms. The purity of the labeled isomers was determined by ascending paper chromatography and by autoradiography of the paper chromatograms. The unlabeled D- and L-isomers of tryptophan were obtained commercially. Sprague-Dawley strain rats of 430 to 530 g weight were used in the study. Freshly prepared aqueous solutions of the labeled and unlabeled isomers were injected intraperitoneally into pairs of rats in three ways: (a) either D- or L-tryptophan-$7a$-^{14}C alone, 0.715 mg/kg body weight; (b) D- or L-tryptophan-$7a$-^{14}C, 0.715 mg/kg in a single solution with an equal amount of the opposite unlabeled isomer; (c) D- or L-tryptophan-$7a$-^{14}C, 0.715 mg/kg of preceded by a loading dose of 28.6 mg/kg of the unlabeled opposite isomer given 30 min earlier. The loading dose is indicated in Table II as "D-load" or "L-load," and would be equivalent to a 2.0-g loading dose of tryptophan in a 70-kg man. The volumes of solution injected were 0.44 to 0.98 ml for a single injection and 1.69 to 1.99 ml, when a loading dose was injected separately.

[3] Tracerlab Inc., Waltham, Mass.

TABLE I. Dosages of D- or L-Tryptophan-$7a$-^{14}C Injected Intraperitoneally into Rats and Percentage of ^{14}C Recovered.

Group no.[a]:	87[d]	88[d]	98[d]	92[f]	93[f]	95[e]	96[e]	74[d]	75[d]	100[d]	80[f]	82[f]	84[e]	85[e]
Doses														
D-Tryptophan-$7a$-^{14}C (μCi)[b]	1.62	1.53	1.50	1.49	1.52	1.48	1.34							
(mg)	0.75	0.71	0.69	0.69	0.71	0.69	0.62				26.39	25.38	0.66	0.74
L-Tryptophan-$7a$-^{14}C (μCi)[c]								1.66	1.83	1.63	1.59	1.57	1.57	1.77
(mg)				28.28	28.28	0.69	0.62	0.69	0.77	0.68	0.66	0.66	0.66	0.74
Recoveries (% administered dose)														
$^{14}CO_2$ (0–12 hr)	16.37	12.21	18.85	31.78	34.08	22.17	22.32	19.22	16.23	18.77	32.00	31.59	17.75	18.45
Urine (0–12 hr)	12.03	13.49	12.56	12.04	11.60	11.00	10.03	12.45	13.00	13.82	12.82	13.91	12.94	11.29
(12–24 hr)	1.75	4.02	2.54	1.96	2.57	1.98	2.19	2.21	1.09	1.97	2.56	2.57	2.22	2.04

[a] Each group contained 2 rats housed together in the metabolism cage. Dosages indicated are the totals given to both rats of the group.

[b] ^{14}C D-isomer injected at 1.54 μCi/kg.

[c] ^{14}C L-isomer injected at 1.71 μCi/kg.

[d] D- or L-^{14}C isomers only.

[e] Equimolar amounts of the opposite unlabeled isomer.

[f] Loading dose of the ^{12}C opposite isomer administered 30 min before the ^{14}C sample.

TABLE II. Percentage of Dose of ^{14}C in Urinary Metabolites Excreted by the Rat After Injection of D- or L-Tryptophan-7a-^{14}C.

Group[a]	Tryptophan[b] Labeled	Tryptophan[b] Unlabeled	Kynurenine	Kynurenic acid	Xanthurenic acid	o-Aminohippuric acid	Quinolinic acid	Picolinic acid	Nicotinic acid
87	D-^{14}C		0.01	0.25	0.11	0.04	0.12	0.12	0.01
88	D-^{14}C		0.07	0.20	0.08	0.05	0.10	0.12	0.03
98	D-^{14}C		0.16	0.31	0.18	0.04	0.17	0.16	0.02
92	D-^{14}C	L-Load[c]	0.20	0.31	0.12	0.05	0.28	0.26	0.02
93	D-^{14}C	L-Load[c]	0.13	0.46	0.16	0.06	0.37	0.26	0.02
95	D-^{14}C	L-Equimolar	0.12	0.28	0.18	0.03	0.15	0.16	0.01
96	D-^{14}C	L-Equimolar	0.10	0.33	0.11	0.05	0.13	0.15	0.01
74	L-^{14}C		0.04	0.14	0.06	0.04	0.15	0.17	0.01
75	L-^{14}C		0.03	0.21	0.10	0.05	0.24	0.14	0.02
100	L-^{14}C		0.04	0.16	0.13	0.05	0.13	0.15	0.02
80	L-^{14}C	D-Load[c]	0.03	0.19	0.10	0.04	0.30	0.22	0.02
82	L-^{14}C	D-Load[c]	0.04	0.19	0.11	0.06	0.30	0.22	0.01
84	L-^{14}C	D-Equimolar	0.02	0.18	0.12	0.04	0.15	0.13	0.01
85	L-^{14}C	D-Equimolar	0.02	0.12	0.10	0.05	0.16	0.13	0.01

[a] Each group contained a pair of rats.
[b] Labeled tryptophan injected at level of 0.715 mg/kg (sp act of 2.1537 μCi/mg for D- and 2.3882 μCi /mg for L-form).
[c] Loads injected at 28.6 mg/kg 30 min prior to isotope injection.

The exhaled CO_2 was collected continuously over a 12-hr period and the urine was collected for two consecutive 12-hr periods utilizing toluene and acetic acid as preservatives. All urine specimens were immediately analyzed for 5-hydroxyindoleacetic acid by the method of Weissbach (9) and then stored frozen. Analysis of the urines for total activity showed that the 12–24-hr urines contained such a small amount of the activity that the samples were not used for isolations of the labeled urinary components. Urinary quinolinic acid, picolinic acid, and nicotinic acid were isolated by carrier additions to the urines and fractionation on columns (10, 11). Kynurenic acid, kynurenine, xanthurenic acid, hydroxykynurenine, and *o*-aminohippuric acid were separated on Dowex 50W (H^+) columns by a modification of the procedure of McCoy and Chung (12). Carrier was added to the appropriate fractions and the isolated metabolites were recrystallized to constant specific activity, which made it possible to accurately determine the percentage of the dose excreted in the urine as these metabolites. The respired CO_2 released from samples of the alkaline absorption column fluid and the CO_2 from wet combustion of the isolated metabolites was counted in Pyrex tubes by the gas proportional method of Van Slyke *et al.* (13). Samples containing less than 100 dpm/mg of carbon were proportionally counted in quartz tubes in a low-background anticoincidence circuited system, which compares in sensitivity and accuracy with radiocarbon dating systems (14).

At the end of 24 hr the animals were anesthetized with ether and blood was drawn in heparinized syringes by heart puncture. Tissues were taken as rapidly as possible and immediately frozen. All plasma and tissue samples were stored frozen for future analysis. The plasma and tissues were analyzed for carbon-14 by a liquid scintillation counting method (15).

Results and Discussion. Table I shows that the amount of labeled isomer given in terms of weight and radioactivity was a constant factor for each group. Rats receiving either the labeled D- or L-tryptophan excreted comparable total amounts of isotope in breath plus urine (29.7–48.2 and 30.3–48.1%, respectively). The rats receiving the D-isomer alone (groups 87, 88, 98) expired 15.81% of

the dose as $^{14}CO_2$ which was comparable to the 18.07% of the dose for those receiving the L-isomer alone (groups 74, 75, 100). In the urine, 12 to 13.5% of the radioactivity of the D-isomer appeared in the first 12 hr and 1.75 to 4% more in the second 12 hr compared to 12.5 to 13.8% in the first 12 hr and 1.1 to 2.2% more in the second 12-hr urine, when the L-isomer was given alone. These data show that the rat can metabolize either isomer with equal ability.

The injection of the loading dose of unlabeled L-tryptophan 30 min prior to the labeled D-isomer (groups 92, 93) more than doubled the $^{14}CO_2$ output but the urinary levels of ^{14}C were not affected (Table I). This response may have been the result of a stimulation of tryptophan oxygenase but if this were so, there should have been an increase in the urinary ^{14}C also. The increase in the $^{14}CO_2$ was more likely the result of two things (i) saturation of tissue demands for tryptophan (the load tended to decrease ^{14}C in tissues) thus making more tryptophan available to enter the kynurenine pathway, and (ii) an adaptation or increased efficiency of rate-limiting enzymes leading to CO_2.

The injection of a solution containing equimolar amounts of both isomers with the D-isomer labeled, increased the level of $^{14}CO_2$ to 22.25% but did not change the urinary level. Under these conditions the production of $^{14}CO_2$ from the D-isomer was about equivalent to that produced by the L-isomer. This suggests that the rat has enzymes which can utilize the D-isomer, or that the rat can convert the D-isomer into a form which can be metabolized through the normal pathway. Inversion of the D-isomer in the intestinal tract is probably not involved in this case since the route of administration was intraperitoneal and the production of $^{14}CO_2$ was so rapid.

The injection of a loading dose of D-tryptophan 30 min prior to injection of labeled L-isomer (groups 80, 82) increased the expired $^{14}CO_2$ twofold to a level of 31.8% but did not increase the urinary level above that seen when the labeled L-isomer was given alone. When the labeled L-isomer was given with an equimolar amount of D-tryptophan (groups 84, 85) the $^{14}CO_2$ excretion was not increased as it was when the labeled D-tryptophan was given with an equimolar amount of L-tryptophan. There was no change in the urinary ^{14}C levels compared to the group given the L-labeled isomer alone.

The kynurenine levels in the urines were in general higher in those groups receiving the labeled D-isomer than in those receiving the labeled L-isomer, but were exceedingly low in all cases. The average kynurenine values for all groups receiving the labeled L-isomer were relatively the same. Those rats given D-tryptophan-^{14}C with a prior load of L-tryptophan (groups 92, 93) excreted slightly more ^{14}C in kynurenine than did the other groups. Kynurenic acid demonstrated a pattern similar to that seen for urinary kynurenine. The groups which received D-tryptophan-^{14}C had higher kynurenic acid values than those which received L-tryptophan-^{14}C, with those getting the load of unlabeled L-tryptophan 30 min prior to the radioactive D-isomer (groups 92, 93) having the highest levels of kynurenic acid.

The hydroxykynurenine carriers isolated from the urines of rats given labeled L-tryptophan contained less than 1 dpm/mg of carrier as measured on a very sensitive low-background counting system (14), and those carriers from rats given labeled D-tryptophan were only slightly higher. Therefore, for all practical purposes, there was no activity in hydroxykynurenine fractions. Apparently any hydroxykynurenine formed in the rat was rapidly metabolized to other components before excretion, although the possibility exists that this relatively unstable aminophenol spontaneously decomposed in the urine before or during the isolation procedure. The latter seems unlikely in that recovery of added hydroxykynurenine to aliquots of urine was uniformly high. Since xanthurenic acid values were quite low, hydroxykynurenine was not converted to appreciable amounts of this metabolite. The levels of xanthurenic acid were about the same for all groups, which signified no appreciable effect of either isomer on the production of xanthurenic acid.

The levels of labeled *o*-aminohippuric acid in the urine were the same for those groups

receiving a labeled isomer singly or in equimolar amounts with the opposite unlabeled isomer, and were not appreciably increased in groups where a load of the opposite isomer was given before the labeled isomer.

The D-isomer of tryptophan was metabolized into urinary quinolinic acid to about the same extent as a similar dose of labeled L-tryptophan given alone. The equimolar doses of an unlabeled isomer had no effect on the percentage of the opposite ^{14}C isomer which appeared as quinolinic acid. Administration of loads of either isomer 30 min prior to the labeled dose caused an approximate doubling of the activity in isolated quinolinic acid. Picolinic acid excretion patterns were similar to those for urinary quinolinic acid. The percentage of the dose excreted as nicotinic acid was quite small and was about the same for all groups with a suggestion of decreased levels in those groups receiving equimolar doses of unlabeled isomers (groups 95, 96, and 84, 85).

The activity found in the urinary kynurenine, kynurenic acid, picolinic acid, quinolinic acid, and nicotinic acid (Table II) agrees with our previous report (4) on D-kynurenine-keto-^{14}C that the rat has systems that can convert D-isomers into these metabolites. In the rat, both isomers of labeled kynurenine were shown to produce labeled kynurenic acid, 3-hydroxykynurenine, xanthurenic acid, quinolinic acid, nicotine acid, nicotinic acid, and N^1-methylnicotinamide although D-kynurenine was not appreciably converted to $^{14}CO_2$ (4). These results also agree with a report that both isomers of hydroxykynurenine-^{14}C can give rise to labeled quinolinic acid, nicotinic acid and N^1-methylnicotinamide (5) and that large doses of D-kynurenine in rats give rise to hydroxykynurenine, kynurenic acid, and xanthurenic acid (4).

In previous studies (4) we observed that D-kynurenine was converted into kynurenic acid 10–15 times more efficiently than L-kynurenine. In the present study the formation of kynurenic acid from D-tryptophan was only about twice as great as from the L-isomer. This suggests that either D-tryptophan does not give rise to much D-kynurenine in the rat or the D-kynurenine formed from D-tryptophan was not utilized the same way as the D-kynurenine given in the previous study (4). Furthermore, there is the possibility that the rat has a D-amino acid oxidase enzyme (16–18) capable of rapidly converting D-tryptophan into indolepyruvic acid, which utilizes an additional pathway in its conversion into the isolated metabolites and CO_2. Berg *et al.* (19) reported that the addition of indolepyruvic acid to a tryptophan-deficient diet gave a growth rate comparable to that of an equivalent amount of tryptophan. Other studies showed that D-tryptophan was almost as good as L-tryptophan in supporting growth of rats (3). These data suggest that D-tryptophan may have been converted to L-tryptophan in the rats as has been observed enzymatically in rat kidney preparations (17, 18). The rapid conversion of D-tryptophan to CO_2 in the present study and the poor conversion of D-kynurenine to CO_2 observed previously (4) suggests that D-tryptophan was not appreciably converted to D-kynurenine in the rat. The minor differences observed in urinary levels of tryptophan metabolites in rats given the D- or L-isomers might be the result of differences in the rates of absorption (20) or in the rate of conversion of the isomers to a utilizable form. The low levels of urinary kynurenine observed in the present study were not unexpected since previous administration of labeled L- and D-kynurenine gave rise to low levels of this metabolite in the urine (4).

As stated previously, the loading dose of unlabeled L-tryptophan given 30 min before the D-^{14}C isomer increased the $^{14}CO_2$ levels by 2-fold but did not change the ^{14}C levels in the urine. However, the rather low levels of activity in these urines show that very little of the administered D-isomer was excreted in the urine unchanged. The levels of kynurenine, kynurenic acid, and xanthurenic acid were rather low in this group but they still were higher than those seen in the urine of rats receiving the labeled L-isomer after a load of unlabeled D-isomer. The quinolinic acid and picolinic acid levels in this group were slightly higher than those seen in the group receiving the labeled D-isomer alone.

The load of unlabeled D-tryptophan almost doubled the $^{14}CO_2$ from the tracer dose of L-tryptophan which was opposite to the effect of a load of L-kynurenine on the production of $^{14}CO_2$ from labeled D-kynurenine observed in a previous study (4). Apparently the D-tryptophan was not converted to D-kynurenine, otherwise one should have seen high levels of kynurenic acid in the urine as was observed in a previous study with labeled D-kynurenine. These observations all suggest that D-kynurenine was not formed in any significant amount from D-tryptophan.

The similarities in the quinolinic acid, picolinic acid, and nicotinic acid levels when either labeled isomer was given, indicates that comparable quantities of D- or L-isomer were converted into these urinary components via a similar if not identical pathway.

Only 4.8 to 12.9% of the urinary activity was accounted for as urinary tryptophan metabolites when either the D- or L-isomer was given.

The L-isomer of tryptophan gave rise to such small quantities of xanthurenic acid and kynurenic acids in comparison to a tracer dose of L-kynurenine (4) that the question arises as to how much tryptophan actually went through the pathway. Assuming the $^{14}CO_2$ and isolated urinary metabolites arose by metabolism through the kynurenine pathway, a total of about 16 to 33% of the dose of either isotope entered this pathway, the higher figures being from those rats given loading doses of tryptophan prior to the tracer dose.

Table III shows the effect of loading with either isomer on the quantitative excretion of 5-hydroxyindoleacetic acid, and this gives an indication of what part of the overall tryptophan load may pass through the serotonin pathway. Since the urinary levels of 5-hydroxyindoleacetic acid were unchanged by the loads of D- or L-tryptophan used, this important pathway is a very minor one quantitatively and does not account for more than a very small portion of the administered dose with loads of such small size (21, 22).

The distribution of the radioactivity was determined in the plasma and tissues (Table IV). Of the tissues studied, liver contained the most total radioactivity, followed by plasma and kidney. This is not surprising since liver and kidney are the major sites for the metabolism of tryptophan. Administration of loads of D- or L-tryptophan 30 min before the labeled compound resulted in a consistent reduction in tissue radioactivity which is reflected in an enhanced conversion to CO_2 in the loaded rats.

TABLE III. Amounts of 5-Hydroxyindoleacetic Acid Excreted in the Urine After D- or L-Tryptophan Administration.

	Tryptophan		5-Hydroxyindoleacetic acid (μg)	
Group	Labeled	Unlabeled	0–12 hr	12–24 hr
87	D-^{14}C		182	117
88	D-^{14}C		127	124
98	D-^{14}C		93	70
92	D-^{14}C	L-Load	65	90
93	D-^{14}C	L-Load	80	129
95	D-^{14}C	L-Equimolar	141	65
96	D-^{14}C	L-Equimolar	144	235
74	L-^{14}C		93	126
75	L-^{14}C		87	87
100	L-^{14}C		141	136
80	L-^{14}C	D-Load	68	103
82	L-^{14}C	D-Load	126	128
84	L-^{14}C	D-Equimolar	132	66
85	L-^{14}C	D-Equimolar	151	113

Summary. DL-Tryptophan was chromatographically resolved into the D- and L-isomers on powdered paper columns. Both isomers of tryptophan were metabolized to $^{14}CO_2$ equally well by the rat. A load of either isomer increased the $^{14}CO_2$ production from the opposite labeled isomer almost twofold, but did not affect the urinary ^{14}C levels. The kynurenic acid levels were slightly elevated when the D-isomer was given, but nothing like the 30% concentrations seen when labeled D-kynurenine was given to rats in previous studies. The loading doses of either isomer slightly increased the urinary kynurenic acid, quinolinic acid, and picolinic acid levels and the similarities in urinary levels of these components showed that com-

TABLE IV. Percentage of Activity Found in the Body Components 24 hr After Intraperitoneal Administration of D- or L-Tryptophan-$7a$-^{14}C.[a]

Component:	Tryptophan		Liver	Plasma[b]	Kidney	Testes	Heart	Spleen	Brain
Group no.	Labeled	Unlabeled							
87, 88, 98	D-^{14}C		7.06 ± 0.73	6.83 ± 1.06	2.69 ± 0.26	0.56 ± 0.05	0.27 ± 0.02	0.25 ± 0.03	0.23 ± 0.03
92, 93	D-^{14}C	L-Load	5.96 ± 0.10	4.68 ± 0.76	1.99 ± 0.12	0.41 ± 0.02	0.23 ± 0.01	0.24 ± 0.03	0.18 ± 0.005
95, 96	D-^{14}C	L-Equimolar	7.52 ± 0.26	6.57 ± 1.30	2.81 ± 0.11	0.61 ± 0.04	0.29 ± 0.01	0.28 ± 0.02	0.22 ± 0.01
74, 75, 100	L-^{14}C		8.30 ± 0.40	5.15 ± 0.57	2.08 ± 0.10	0.53 ± 0.04	0.29 ± 0.01	0.26 ± 0.01	0.18 ± 0.02
80, 82	L-^{14}C	D-Load	4.70 ± 0.17	3.73 ± 0.30	0.92 ± 0.06	0.32 ± 0.03	0.14 ± 0.01	0.15 ± 0.01	0.11 ± 0.01
84, 85	L-^{14}C	D-Equimolar	6.90 ± 0.57	4.68 ± 0.37	1.61 ± 0.11	0.53 ± 0.03	0.25 ± 0.02	0.23 ± 0.03	0.20 ± 0.01

[a] Entries are means ± $[\Sigma d^2/n(n-1)]^{1/2}$.

[b] Plasma volume = body wt (g) × 0.0313 (23).

parable quantities of either isomer were metabolized via similar pathways. The concentration of activity from either isomer in the tissues decreased in the order of liver, plasma, kidney, testes, heart, spleen, and brain. The level of ^{14}C in the tissues was depressed when a load of either unlabeled isomer was given in the presence of the opposite labeled isomer. No detectable activity appeared in urinary 3-hydroxykynurenine when L-tryptophan was given. Urinary levels of 5-hydroxyindoleacetic acid indicated that this pathway was of minor importance quantitatively.

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