Metabolism of D- and L-Tryptophan in Dogs[1,2]

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ABSTRACT The metabolism of D- and L-[benzene ring-U-^{14}C]tryptophan by dogs was studied. The distribution of label from each isomer in urine, feces, CO_2 and various tissues was determined. Thirteen different urinary tryptophan metabolites were isolated by ion exchange cellulose chromatography. D-[^{14}C]Tryptophan was poorly converted to $^{14}CO_2$ relative to the L-isomer, while giving rise to nearly three times as much urinary ^{14}C as did the L-isomer. The major urinary metabolites of D-tryptophan were unchanged D-tryptophan, D-kynurenine and kynurenic acid. The major urinary metabolite from L-tryptophan was kynurenic acid. Inversion of D-tryptophan to L-tryptophan via indolepyruvic acid appeared to be the major fate of ingested D-tryptophan, with renal excretion of the unchanged D-isomer the next most important fate. The dog apparently utilizes D-tryptophan more efficiently than does the human but much less efficiently than does the rat. The dog appears to be a reasonable animal model for the human in studies of D-tryptophan metabolism. J. Nutr. *106:* 642–652, 1976.

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It is known that the rat is capable of utilizing D-tryptophan as efficiently as L-tryptophan for growth (1). This ability to metabolize the D-isomer has been shown to reside in the rat's ability to convert D-tryptophan to indolepyruvic acid via D-amino acid oxidase and subsequently to transaminate the α-keto acid to L-tryptophan (2). The human (3, 4), the rabbit (5) and the mouse (6) utilize D-tryptophan poorly; nevertheless its ingestion does lead to alterations in the levels of some urinary tryptophan metabolites (5, 7).

In view of the potential human exposure to D-tryptophan through the use of DL-tryptophan in food supplements and in intravenous maintenance supplements as well as the potential for use of D-tryptophan as a sweetening agent (8), it was felt that more information on the metabolism of the D-isomer would be desirable.

Little information about the metabolism of D- or L-tryptophan in the dog is available in the literature, but preliminary experiments indicated that the metabolism of D-tryptophan by this species might be similar to that by the human. Therefore, this work was undertaken to compare D- and L-tryptophan metabolism and to use this information to evaluate the dog as a potential animal model for the human with regard to D-tryptophan metabolism.

The methods utilized in this study were designed to allow complete recovery of all ^{14}C-containing urinary metabolites of both D- and L-[^{14}C]tryptophan. Previous studies on tryptophan metabolism utilizing

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[^{14}C]tryptophan did not fully account for all of the urinary radioactivity.

MATERIALS AND METHODS

Isotopes and chemicals. The D- and L-[benzene-ring U-^{14}C]tryptophan [3] (specific activity 93 μCi/mg and 102 μCi/mg respectively), were purified by paper chromatography in 10 mM mercaptoethanol. Exposure to light during chromatography was minimized. The D-[^{14}C]tryptophan was shown to contain less than 0.5% L-[^{14}C]-tryptophan by a microbiological assay (9).

The following compounds were purchased [4]: D-tryptophan, L-tryptophan, DL-kynurenine, L-kynurenine sulfate, kynurenic acid, xanthurenic acid, quinaldic acid, indole-3-carboxaldehyde, indole-3-acetic acid, 5-hydroxyindole-3-acetic acid, 3-hydroxyanthranilic acid, and indoxyl sulfate-potassium salt (urinary indican). Indoleaceturic acid was a generous gift.[5] 3-hydroxy-DL-kynurenine was from lots previously synthesized. Other chemicals were obtained locally and were of reagent grade. X-ray film was purchased.[6]

Procedure. Eight male beagle dogs, average weight 7.9 kg., were fasted for 12 hours. The dogs had previously been fed a commercial dog food.[7] Four dogs were given D-[^{14}C]tryptophan, diluted with sufficient unlabeled D-tryptophan to provide a dose of 4.4 mg/kg at a specific activity of 0.59 μCi/mg. Four other dogs were given L-[^{14}C]tryptophan similarly diluted with unlabeled L-tryptophan at a dose of 4.4 mg/kg and a specific activity of 0.76 μCi/mg. Doses were aqueous solutions administered by stomach tube. The dogs were then placed in stainless steel metabolism cages, and urine, feces and expired CO_2 were collected. Food and water were permitted ad libitum 4 hours after the tryptophan was administered. Urine was collected in bottles surrounded by dry ice and removed at 12, 24, and 48 hours. Feces were collected and frozen at 24 and 48 hours. Expired CO_2 was collected in 6% NaOH. Collection periods were: 0–4 hours, 6–7 hours, 12–13 hours, and 16–17 hours. The total $^{14}CO_2$ expired was estimated on the basis of these measured periods. Blood samples (approximately 3 ml) were taken at 0, 6, 24 and 48 hours.

At 48 hours the dogs were anesthesized with sodium pentobarbital,[8] bled by heart puncture, and necropsied. The following tissues were taken: liver, kidneys, brain, testes, bone and bone marrow (left femur) and muscle lying along the left femur.

Tissues and feces were combusted in flowing oxygen at 800° in a Vycor tube furnace packed with copper oxide and platinized asbestos. The $^{14}CO_2$ was collected in a solution of monoethanolamine and methyl cellosolve (2/1; v/v). An aliquot of the collecting solution was added to an equal volume of a counting solution and the radioactivity determined in a liquid scintillation counter. Liquid samples were counted directly in an ethanol/toluene (2/3; v/v) scintillation fluid containing 4.0 g 2,5-diphenyloxazole/liter and 0.2 g (1,4-bis[2-(5-phenyloxazolyl)]-benzene/liter.

Analysis of urine for tryptophan metabolites. Determination of the ^{14}C-containing urinary metabolites was accomplished by fractionation of the desalted urine sample on successive ion exchange cellulose columns followed by cellulose thin layer chromatography of the isolated compounds. All column chromatography was done at 4°.

To prepare the urine for chromatography, approximately 25% of the volume of the first urine collection period (usually the 0–12 hour sample) was filtered and 1 to 1.5 mg each of selected carrier compounds were added and dissolved by stirring at 4°. This sample (usually about 50 ml) was then carefully applied to a 4 × 25 cm column of Amberlite XAD-4 [9] and washed with 10 sample volumes of water. The tryptophan metabolites retained on the resin were then eluted with 20 sample volumes of methanol, containing several drops of mercaptoethanol, and the methanol eluate was evaporated at 18 to 20° to a final volume of 25 ml. This constituted the desalted urine samples.

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[3] Amersham-Searle Corp., 2636 N. Clearbrook Drive, Arlington Heights, Illinois 60005.
[4] Sigma Chemical Co., St. Louis, Missouri.
[5] Gift of Dr. R. K. Gholson, Oklahoma State University.
[6] Kodak RP/R54.
[7] Purina Dog Chow, Ralston Purina Co., St. Louis, Missouri.
[8] Nembutal.
[9] Rohm and Haas Co., Independence Mall West, Philadelphia, Pa. 19105.

Ion exchange cellulose chromatography was performed by a modification of the method of Chen and Gholson (10). The first column was a 2.5 × 33 cm column of DEAE-cellulose[10] in the formate form which had been equilibrated with 1 mM triethylamine formate (TEA-F) buffer, pH 4.0. This column was eluted first with 1 mM TEA-F pH 4.0 (100–150 ml), followed by elution with linear gradients of TEA-F, pH 4.0 buffers of increasing concentration. The first gradient was 1 to 250 mM and the second gradient was 250 mM to 1 M. Each gradient had a total volume of 1 liter. The radioactive peak containing carrier tryptophan, kynurenine and 3-hydroxykynurenine which eluted with 1 mM TEA-F, pH 4.0 was lyophilized, reconstituted in 10 ml of water, and applied to the second column. This column was a 2.5 × 50 cm column of CM-cellulose[11] which was washed with 1 liter of 0.5 mM formic acid just prior to application of the sample. Elution was with 0.5 mM formic acid. Tryptophan and kynurenine were eluted simultaneously and this peak was lyophilized, redissolved in 10 ml of water and applied to the third column, a 1.6 × 25 cm column of DEAE-cellulose.[10] This column was then developed with 1 mM TEA-F, pH 8.0, until tryptophan was eluted. This was then followed by a gradient of increasing concentration TEA-F, pH 8.0, to 250 mM. The total volume of this gradient was 300 ml.

The effluents of the columns were assayed for ^{14}C by liquid scintillation counting and the carrier compounds were located by spectrophotometric and fluorometric methods (10). Peaks were pooled; mercaptoethanol was added; and the solution was lyophilized and the residue was redissolved in a small volume. Aliquots were subjected to liquid scintillation counting to determine the ^{14}C content of the peak. This material was also subjected to chromatography on cellulose thin layer sheets[12] in the two-dimensional system of Dalgliesh (11). Mercaptoethanol was present in the TLC solvents at a concentration of 0.1%. Thin layer sheets, after chromatographic development, were examined under ultraviolet light at 254 and 366 nm, as well as by radioautography and by spraying with Ehrlich's reagent (12). Areas located by these methods were cut from the TLC sheets, the cellulose was scraped into scintillation vials, and the ^{14}C content was determined by liquid scintillation counting.

[^{14}C]Urea was determined by incubation of 1 ml aliquots of urine with urease[13] at pH 7.0. The $^{14}CO_2$ released was trapped in ethanolamine/methoxyethanol, (1/2; v/v) which was then subjected to liquid scintillation counting.

Determination of tryptophan specific activity in liver proteins. Lipid-free, trichloroacetic acid precipitates of homogenized liver were subjected to base hydrolysis (13). The neutralized hydrolysate was then passed through a 1.6 × 6 cm column of Amberlite XAD-4.[9] The column was washed with water and the tryptophan was recovered by elution with methanol.[14] This methanol eluate was subjected to descending paper chromatography in 75% ethanol with 0.1% mercaptoethanol present. The band corresponding to tryptophan was located, and the tryptophan was eluted with 0.01 N NH_4OH. Aliquots of this eluate were subjected to liquid scintillation counting and tryptophan determination by the method of Denckla and Dewey as modified by Bloxam and Warren (14).

RESULTS

Figure 1 shows some of the metabolic pathways known to exist in animals for both D- and L-tryptophan. The position of the label in the substrates used in this study is shown.

Table 1 shows the distribution of ^{14}C from D- and L-[^{14}C]tryptophan in urine, CO_2, feces and various tissues. While ingested D-[^{14}C]tryptophan gave rise to 30% as much $^{14}CO_2$ as did the L-isomer, the total amount of urinary ^{14}C excreted from the D-isomer was about three times that excreted from the L-isomer. While 23.6% of the dose from the D-isomer was found

[10] DE-52 Reeve Angel, 9 Bridewell Place, Clifton, New Jersey.
[11] CM-23 Reeve Angel, 9 Bridewell Place, Clifton, New Jersey.
[12] Precoated cellulose TLC sheets, #5537 Brinkmann Instruments Inc., 110 River Road, Des Plaines, Illinois.
[13] Crude Powder from Jack Beans, Sigma Chemical Co., St. Louis, Missouri.
[14] When a mixture of 20 amino acids was passed slowly through a column of Amberlite XAD-4 and the column then washed with H_2O, only tryptophan, plenylalanine, and tyrosine were retained on the column. These were then eluted from the column with methanol.

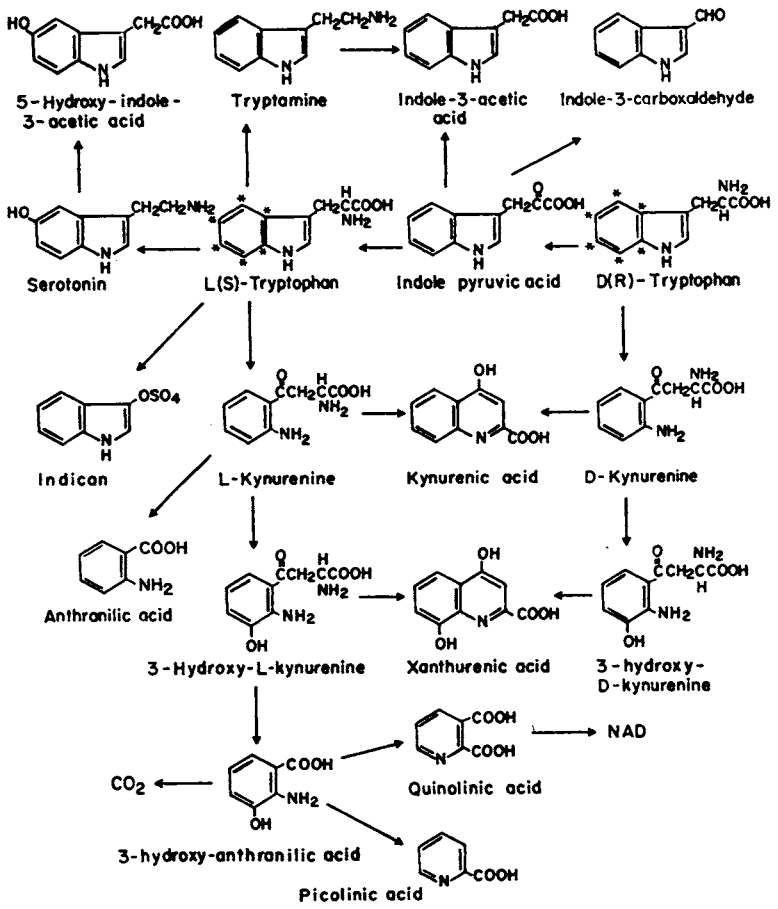

Fig. 1 Pathways of tryptophan metabolism in animals and man.

in the urine in the first 12 hours after ingestion of the dose, only 6.3% of the dose from L-[^{14}C]tryptophan was excreted in this period.

The very low ^{14}C content of the feces indicated that both the D- and L-tryptophan were absorbed in the course of the experiment. The identity of the ^{14}C-containing material in the feces was not determined.

The values for ^{14}C content of the various tissues indicated that the two isomers were nearly equal in their capacity to deposit ^{14}C in these tissues. Samples of the liver were fractionated further, and the distribution of ^{14}C was determined. The results are shown in table 2. Trichloroacetic acid (TCA) soluble and acetone-petroleum ether soluble fractions both contained ^{14}C; however, the majority of the radioactivity in the liver was contained in TCA insoluble material. After base hydrolysis of this fraction, 90% of the radioactivity was found in tryptophan. The tryptophan isolated from the group of dogs given the L-isomer had a specific activity 1.5 times that isolated from the group of dogs given the D-isomer. Because racemization accompanies base hydrolysis, it was not possible to determine whether the tryptophan isolated from the livers of dogs given D-[^{14}C]-tryptophan had retained its configuration. However, it is probable that the tryptophan isolated from the proteins of both groups of dogs was the L-isomer.

Figure 2 depicts the expired $^{14}CO_2$ from D- and L-[^{14}C]tryptophan. The $^{14}CO_2$

TABLE 1

Distribution of ^{14}C in dogs from orally administered D- and L-(benzene ring-U-^{14}C)tryptophan

	[^{14}C] compound administered[a]	
	D-TRP dose recovered	L-TRP dose recovered
	%	%
Total ^{14}C in urine		
0–12 hr	23.6 ±6.5	6.31±1.23
12–24 hr	9.01±5.3	2.80±0.39
24–48 hr	2.10±0.44	2.84±0.47
Distribution of ^{14}C after 48 hr		
Urine	34.7 ±6.0	12.0 ±0.9
CO_2	7.6 ±1.3	23.0 ±2.2
Feces	3.18±0.9	3.9 ±0.6
Liver	5.2 ±0.16	7.9 ±0.5
Kidney	0.95±0.06	1.2 ±0.05
Brain	0.40±0.023	0.55±0.035
Testes	0.05±0.009	0.07±0.009
Total[b]	52.08±5.9	49.0 ±2.9

[a] Mean of four dogs, ± SE of the mean. [b] Total carcass ^{14}C was not determined.

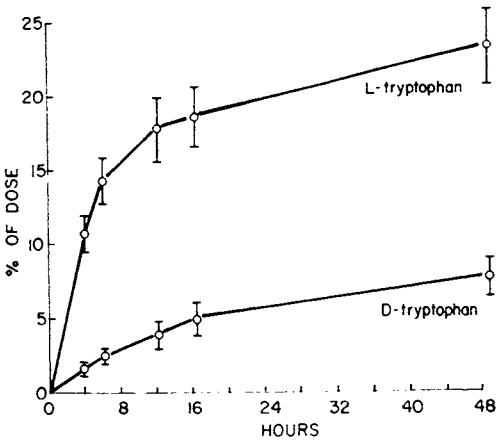

Fig. 2 Expiration of $^{14}CO_2$ by dogs after oral administration of D- or L-(benzene ring-U-^{14}C)tryptophan. Vertical bar = SEM. Each point represents mean values for four animals.

arising from the D-isomer was produced slowly and in a nearly linear manner, while the $^{14}CO_2$ from the L-isomer was initially produced at a rapid rate which then declined and became constant. While the initial rates of $^{14}CO_2$ production from the two isomers differed by a factor of 6.5, the later rates differed by a factor of only 1.5.

Figure 3 depicts the distribution of urinary ^{14}C in a number of metabolites isolated from the urine of dogs which had ingested either D- or L-[^{14}C]tryptophan. The label from the L-isomer was well distributed in a number of compounds, with kynurenic acid accounting for the single largest quantity of urinary ^{14}C (21.45%). The distribution from the D-isomer was dominated by the amounts of urinary ^{14}C in D-tryptophan and D-kynurenine, 71.55 and 5.5% of the urinary ^{14}C, respectively. Other metabolites, with the exception of indole carboxaldehyde and indoleacetic acid, contained lower percentages of urinary ^{14}C when labeled from D-[^{14}C]tryptophan than when they were labeled from the L-isomer.

Tryptophan and kynurenine isolated from the urine of dogs given D-[^{14}C]tryptophan were shown to retain the D-configuration by chromatography in the acidified solvent of Mason and Berg (15).

Table 3 presents the ^{14}C content of all urinary fractions isolated, both identified and unidentified, as percentage of the dose. The ^{14}C represented by these fractions accounted for 90% or more of the urinary ^{14}C from either isomer. Unidentified compounds eluting at the same or similar elution volumes from both groups are generally felt to be the same or similar

TABLE 2

Distribution of ^{14}C in livers of dogs given oral doses of D- or L-(benzene ring-U-^{14}C)tryptophan[1]

	Liver ^{14}C			TCA insoluble ^{14}C in Tryptophan	Specific activity of isolated Tryptophan
	Acetone: petroleum ether soluble	TCA soluble	TCA insoluble		
	%	%	%	%	*dpm/μmole*
D-tryptophan	7.3	4.6	88.1	90	747±104[2]
L-tryptophan	7.8	7.8	84.3	90	1,099± 45

[1] Mean values for four dogs given oral doses of each isomer. [2] Mean ± SEM.

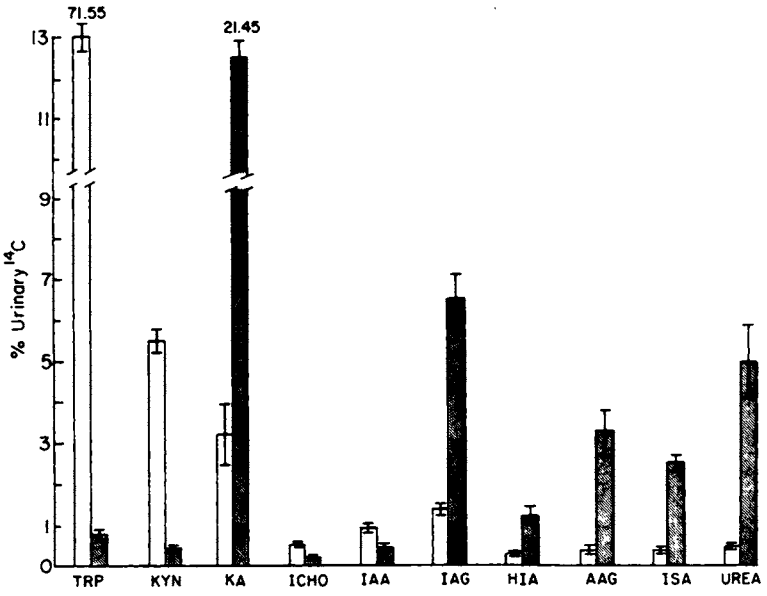

Fig. 3 Distribution of urinary ^{14}C excreted after oral administration of D- or L-(benzene ring-U-^{14}C)tryptophan, as % of urinary ^{14}C. Abbreviations used are: TRP, tryptophan; KYN, kynurenine; KA, kynurenic acid; ICHO, indolecarboxaldehyde; IAA, indoleacetic acid; IAG, indoleaceturic acid; ISA, indican; AAG, anthranilic acid glucuronide; HIA, 5-hydroxyindoleacetic acid. Open bar = D-tryptophan—mean of 4, 0–12 hour samples. Hatched bar = L-tryptophan—mean of 2, 0–12 hour samples and 2, 0–24 hour samples. Inner bar = SEM.

compounds. The first number of the unknown designations used in table 3 indicates which ion exchange cellulose column the material was pooled from, the first (1) or the second (2). The second number indicates the average elution volume of the peak fraction.

Dogs to which D-[^{14}C]tryptophan was administered excreted 17% of the dose as the unchanged D-isomer in the first 12 hours after administration of the compound. No acetyl-D-[^{14}C]tryptophan was observed in the urine of these dogs. D-kynurenine and kynurenic acid, isolated from these urines, accounted for 1.76 and 0.82% of the dose, respectively. Tryptophan and kynurenine isolated from the urine of dogs given L-[^{14}C]tryptophan accounted for only 0.077% of the dose, while kynurenic acid isolated from these urines yielded 1.63% of the dose. Other major urinary metabolites of the L-isomer were: 1–235, 1–830, 1–880, anthranilic acid glucuronide and indoleaceturic acid. Dogs that were administered D-[^{14}C]tryptophan excreted significantly larger quantities of indolecarboxaldehyde and indoleacetic acid than did the dogs given the L-isomer. However, the total amount of indoleacetic acid (free acid plus indoleaceturic acid) produced from the two isomers was very nearly equal. The indoleacetic acid formed from L-tryptophan was excreted primarily as the conjugated form, while that formed from D-tryptophan was excreted both as the free acid and as the conjugated form.

Unidentified compounds 2–300, 2–360 and 2–430 were observed at significantly higher levels from D-tryptophan than from L-tryptophan. Without knowing the identity of these compounds, no real significance can be attached to this observation. Kynurenic acid, 1–235, 1–715, 1–880, anthranilic acid glucuronide, indican and urea isolated from urine of dogs given L-[^{14}C]tryptophan contained a higher percentage of the dose ($P < 0.01$) than did the analogous fractions isolated from urine of dogs given the D-isomer. Component 1–235, which arose from both D- and L-[^{14}C]tryptophan, was shown to be the metabolite of a trace contaminant of the

TABLE 3

Urinary excretion of ^{14}C containing metabolites of orally administered D- and L-(benzene ring-U-^{14}C)tryptophan by dogs, as percentage of dose

	D-Tryptophan[a]	L-Tryptophan[b]
1-100[c]	0.083 ±0.018[d]	0.055 ±0.01[d]
1-235	0.18 ±0.067*	0.826 ±0.048
Indole carboxaldehyde	0.116 ±0.017*	0.015 ±0.003
1-660	0.096 ±0.015	0.046 ±0.006
1-715	n.d.[e]	0.075 ±0.011
3-hydroxyanthranilic acid	0.056 ±0.04[f]	0.032 ±0.011
1-830	0.160 ±0.034	0.220 ±0.074
1-880	n.d.	0.282 ±0.046
Indoleacetic acid	0.247 ±0.009*	0.029 ±0.008
Anthranilic acid glucuronide	0.038 ±0.007‡	0.263 ±0.059
Indoleaceturic acid	0.34 ±0.073	0.503 ±0.096
5-hydroxyindoleacetic acid	0.124 ±0.048	0.119 ±0.018
Kynurenic acid	0.82 ±0.191†	1.626 ±0.355
Indican	0.106 ±0.024	0.159 ±0.037[g]
Xanthurenic acid	0.021 ±0.005	0.036 ±0.005
2-300	0.337 ±0.035*	0.138 ±0.004
2-360	0.325 ±0.052‡	0.120 ±0.007
2-430	0.372 ±0.065‡	0.112 ±0.034
3-hydroxykynurenine	0.019 ±0.004	0.001
Tryptophan	17.0 ±5.05‡	0.050 ±0.010
Kynurenine	1.76 ±0.67*	0.027 ±0.007
Urea	0.12 ±0.02*	0.423 ±0.008

[a] Mean of 4, 0–12 hour samples. [b] Mean of 2, 0–12 hour samples and 2, 0–24 hour samples. [c] Unknown designations—see text [d] Mean, ± SEM. [e] Not detected. [f] Only two values for 3-hydroxyanthranilic acid arising from the D-isomer were obtained. [g] Only 2, 0–12 hour samples were included. The 0–24 hour values were 1.25% of the dose. † Significantly different from the value for L-tryptophan, $P < 0.10$. *t*-test (42). ‡ Significantly different from the value for L-tryptophan, $P < 0.05$. * Significantly different from the value for L-tryptophan, $P < 0.01$.

[^{14}C]tryptophan given to the dogs and was not a metabolite of D- or L-[^{14}C]tryptophan.

A number of fractions contained about the same percentage of the dose from either isomer. Quantitatively, the most important of these were indoleacetic acid, (total acid), 5-hydroxyindoleacetic acid and several of the unidentified fractions. Fractions containing 3-hydroxyanthranilic acid, 3-hydroxykynurenine, and xanthurenic acid contained low levels of ^{14}C from both isomers. No conjugated forms of these three metabolites were detected, but such forms were not searched for rigorously. The label that was found in 3-hydroxykynurenine from D-[^{14}C]tryptophan appeared to be present in both the D- and L-isomers of 3-hydroxykynurenine, but the levels of ^{14}C were too low to determine an accurate ratio of the two forms.

DISCUSSION

In the purification of the D- and L-[benzene ring-U-^{14}C]tryptophan used in these studies it was found that the presence of mercaptoethanol was necessary to attain radiochemical purity in the 98% to 100% range. If the reducing agent were not present, 8 to 10 extraneous compounds comprising 5% to 8% of the [^{14}C]tryptophan were observed on radioautograms of the two-dimensional thin layer chromatograms used to check the purity of the [^{14}C]tryptophan. The isomeric purity of the D-[^{14}C]tryptophan was established by a microbiological method (9) with a sensitivity at least 10 times greater than that which could be obtained with amino acid oxidases or paper chromatography.

The conditions selected for administering the tryptophan were arbitrary. The dogs were fasted long enough that they were in a postabsorptive state, which would reduce the amount of tryptophan incorporated into protein. Feeding again 4 hours after tryptophan administration permitted a return to normal metabolism after the rapid metabolic disposal of tryptophan had occurred. The dosage selected was neither a trace dose nor a massive dose. It represents approximately a 1 day supply for the dogs used. Since it was given in a single intragastric dose it no doubt elevated the tryptophan concentration in plasma and body fluids above those normally existing in the dog.

The methods utilized in this study resulted in greater than 90% recovery of urinary ^{14}C. Recovery of authentic metabolites passed through the procedure ranged between 80% to 100%. The use of the Amberlite XAD-4 [9] resin to prepare the urine samples for DEAE-cellulose chromatography [10] resulted in much better separation of metabolites on smaller columns than was possible with untreated urine. Urinary ^{14}C in the H_2O washes of the XAD-4 columns averaged 1% to 2% from D-[^{14}C]tryptophan and 6% to 20% from the L-isomer. [^{14}C]urea generally comprised about 40% to 50% of this ^{14}C material in the H_2O wash from either isomer, while the remainder was distributed in four or five unidentified compounds.

The data presented here suggest that D-[^{14}C]tryptophan ingested by the dog meets three different fates. It is excreted unchanged, it is metabolized with retention of configuration, and it is inverted to the L-isomer.

The inversion of D-tryptophan to L-tryptophan has been extensively investigated by others and has been shown to involve D-amino acid oxidase conversion of D-tryptophan to indolepyruvic acid followed by transamination to L-tryptophan by tryptophan transaminase (2).

If inversion to the L-isomer is necessary for $^{14}CO_2$ production and for incorporation of [^{14}C]tryptophan into protein, then calculations based on the distribution of ^{14}C found in liver (table 2), when applied to kidneys, muscle, blood, bone and bone marrow indicate that inversion was probably the predominant fate of ingested D-[^{14}C]tryptophan, accounting for at least 30% of the dose. The appearance of label in lipid material and in amino acids other than tryptophan (table 2) is consistent with the known metabolic fate of the benzene ring of L-[^{14}C]tryptophan (16).

The inversion of D-tryptophan is also reflected in the expired $^{14}CO_2$ and in the urinary metabolites that were excreted after the ingestion of D-[^{14}C]tryptophan. Metabolism of D-[^{14}C]tryptophan to $^{14}CO_2$ via D-kynurenine would require hydroxylation to hydroxy-D-kynurenine followed by the action of a "D-hydroxykynureninase" to form 3-hydroxyanthranilic acid. Previous studies with the D- and L-isomers of kynurenine and hydroxykynurenine in the human (4) and in mice and rats (17, 18, 19) have shown that the D-isomers are converted to CO_2 very poorly, relative to the L-isomer, suggesting that very little hydroxylase or hydroxykynureninase activity toward the D-isomers exists. Therefore, since CO_2 arises mainly from the metabolism of tryptophan through 3-hydroxyanthranilic acid, the conversion of D-[^{14}C]tryptophan to $^{14}CO_2$ is probably preceded by inversion to L-[^{14}C]-tryptophan.

The initial rate of the conversion of D-[^{14}C]tryptophan to $^{14}CO_2$ was much slower than the conversion of the L-isomer to $^{14}CO_2$. This could be due to much slower absorption of the D-isomer from the gut, slower tissue uptake of the D-isomer or slow inversion to the L-isomer. Considerable D-[^{14}C]tryptophan was excreted in the urine in the first 12 hours of the experiment, and plasma ^{14}C values at 6 hours after the doses were given (0.011% of the dose/g serum from D-tryptophan as compared to 0.014% of the dose/g serum from L-tryptophan) indicated that absorption of the D-isomer from the gut was keeping pace with renal excretion and tissue uptake. Therefore the rate of absorption of the D-tryptophan from the gut was probably not a limiting factor in the conversion of the D-isomer to CO_2.

Observations with the rat and the rabbit indicate (2, 20) that both the kidney and the liver are involved in the inversion of D-tryptophan to L-tryptophan, with the kidney possessing the greater capacity for inversion. The subsequent metabolism of L-tryptophan so formed, however, takes place predominantly in the liver (2). Studies of the uptake of D- and L-tryptophan by the perfused rat liver (16) indicated that the rate of D-tryptophan uptake was considerably lower than that for the L-isomer. The capacity of the kidney for D-tryptophan uptake is not known. On the basis of this information and the data presented in this study it is not possible to determine if the rate limiting step in the production of $^{14}CO_2$ from D-[^{14}C]tryptophan is at the level of tissue uptake or at the level of inversion to the L-isomer.

The $^{14}CO_2$ expiration from D- and L-[^{14}C]-tryptophan during the last 24 hours of the experiments probably reflects the turnover of tissue proteins and the subsequent release of L-[^{14}C]tryptophan. This is supported by the observation that the final rate of $^{14}CO_2$ expiration from the L-isomer was 1.5 times that from the D-isomer, corresponding directly to the ratio of specific activities of the tryptophan isolated from liver proteins of the two groups of dogs (table 2).

The urinary metabolites excreted by dogs given D-[^{14}C]tryptophan reflect to varying degrees all three of the fates of the D-isomer. The excretion of unchanged D-[^{14}C]tryptophan dominates the urinary ^{14}C pattern and indicates that tubular reabsorption of the D-isomer may be inefficient. This agrees with observations in the chicken (21) which indicated that D-tryptophan had the same renal clearance as inulin. This excretion represents a major fate of D-tryptophan in the first 12 hours after ingestion. The isolation of D-[^{14}C]-kynurenine from the urine of dogs given D-[^{14}C]tryptophan indicates that the in-

doleamine-2,3-dioxygenase described in the rabbit (22) and the rat (23) is present in the dog. D-kynurenine is the major product arising from metabolism of D-tryptophan with retention of configuration.

The [^{14}C]kynurenic acid found in the urine of dogs given D-[^{14}C]tryptophan could have arisen from D-[^{14}C]kynurenine by the action of D-amino acid oxidase (2) or from the action of kynurenine aminotransferase (24) on L-[^{14}C]kynurenine produced by the metabolism of *in situ* generated L-[^{14}C]tryptophan (see fig. 1). It is not possible to differentiate the relative contributions of these pathways, since kynurenic acid no longer contains an asymmetric center.

Indoleacetic acid arising from D-tryptophan is more likely to be excreted without conjugation than is indoleacetic acid arising from the L-somer. More indolecarboxaldehyde is formed from D-tryptophan than from L-tryptophan. These two observations are probably related. Indolecarboxaldehyde and indoleacetic acid were produced from D-tryptophan, but not from L-tryptophan, by normal and vitamin B_6-deficient germfree rats (25). Apparently these compounds arise from indolepyruvic acid (25, 26, 27) produced from D-tryptophan by D-amino acid oxidase in the kidney and the liver (28) (see fig. 1). The indoleacetic acid formed from indolepyruvic acid in the kidney would be likely to be excreted without reaching the liver to become conjugated, while any indoleacetic acid formed in the liver would be conjugated. Indolecarboxaldehyde is presumably excreted without conjugation. Indoleacetic acid, arising from L-tryptophan either generated *in situ* or ingested, could be formed in many tissues (29) and then conjugated in the liver. Therefore, indolecarboxaldehyde and indoleacetic acid (free acid) excreted by dogs given D-[^{14}C]tryptophan probably reflect the flux of material through indolepyruvic acid in the kidney, while indoleaceturic acid excreted probably reflects that amount of indoleacetic acid formed from indolepyruvic acid in the liver as well as the metabolism of *in situ* generated L-[^{14}C]tryptophan by the liver and extrahepatic tissues.

The 5-hydroxyindoleacetic acid excreted by dogs which had ingested D-tryptophan could have arisen by two routes. A tryptophan hydroxylase capable of hydroxylating D-tryptophan at the five position has been reported to be present in rat intestine (30) and the conversion of 5-hydroxy-D-tryptophan to 5-hydroxyindoleacetic acid has been observed in rats (31) and in man (32). Alternatively, *in situ* generated L-tryptophan can give rise to urinary 5-hydroxyindoleacetic acid via the normal metabolic pathways for serotonin (33).

[^{14}C]Indican excreted by dogs given D-[^{14}C]tryptophan probably arises exclusively from *in situ* generated L-[^{14}C]tryptophan (34), however, it is possible that indole may also have been produced directly from the D-isomer (35). It cannot be established whether the unidentified compounds labeled from ingested D-[^{14}C]-tryptophan arise by pathways that require formation of L-[^{14}C]tryptophan *in situ*, whether they represent metabolism of D-[^{14}C]tryptophan, or whether pathways from both isomers contribute to the levels of these compounds observed.

The levels of [^{14}C]kynurenic acid and [^{14}C]anthranilic acid glucuronide excreted by dogs that had ingested L-[^{14}C]tryptophan is in agreement with studies by Brown and Price (36) who found kynurenic acid to be a major metabolite of L-tryptophan in the dog, and who also found the glucuronide to be the major form of anthranilic acid excreted by the dog. Contrary to the findings of Brown and Price (36) however, no ortho-aminohippuric acid was detected in the urine of these dogs. The excretion of indoleaceturic acid from L-tryptophan, may reflect the normal decarboxylation of L-tryptophan by the aromatic L- amino acid decarboxylase (37). The observation that indoleacetic acid is excreted largely as its glycine conjugate by dogs, agrees with the observations of Bridges et al. (38).

The fraction containing 3-hydroxyanthranilic acid isolated from the urine of dogs given L-[^{14}C]tryptophan was also observed to contain the added carrier quinaldic acid. However little or no ^{14}C could be detected in the quinaldic acid isolated from this fraction. It appears that either the dog does not convert kynurenic acid to quinaldic acid as do the human and the rat (39) and the rabbit (40), or it may excrete a conjugated form which has not been identified in this study.

A substantial portion of the urinary ^{14}C from L-[^{14}C]tryptophan is present in compounds that have not yet been identified. These may well represent conjugated forms of tryptophan metabolites, or, as was elucidated for component 1–235, they may have had their origin in trace contaminants of the [^{14}C]tryptophan given to the dogs. Alternatively they may be tryptophan metabolites not normally excreted by other species, hence not previously described. The involvement of the intestinal flora in the production of the unknown metabolites cannot be excluded. While all the known metabolites identified from L- and D-tryptophan can be accounted for by enzymatic activities known to exist in animal tissues (with the exception of indican), the production of these metabolites could also be the result of the action of intestinal flora.

The data indicate that D- and L-tryptophan are metabolized to different extents by the dog and yield urinary metabolite patterns that are qualitatively similar, but differ quantitatively. The dog has considerable capacity to generate a wide variety of tryptophan metabolites from the D-isomer, most of which are also observed when the L-isomer is metabolized.

When these results are compared to those in the literature for the metabolism of D- and L-tryptophan by humans (4, 7) and by rats (41), the dog is seen to be a more efficient utilizer of D-tryptophan than the human, but is considerably less efficient at utilizing the D-isomer than is the rat. The dog and the human both metabolize D-tryptophan in a manner that is different from their metabolism of the L-isomer. Furthermore, the manner in which the two species metabolize D-tryptophan appears to be similar, although they apparently differ in their capacity to invert the D-isomer and to metabolize D-tryptophan to D-kynurenine.

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