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METABOLISM OF C^{14} LABELED ENANTIOMERS OF TRYPTOPHAN, KYNURENINE AND HYDROXYKYNURENINE IN HUMANS WITH SCLERODERMA*

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ABSTRACT

Metabolism of labeled enantiomers of tryptophan, kynurenine and hydroxykynurenine into $C^{14}O_2$ and nine tryptophan metabolites was studied in five patients with scleroderma. The D-isomers of tryptophan, kynurenine and hydroxykyurenine were poorly converted to CO_2 but did give rise to small amounts of activity in several of the urinary metabolites. These observations suggest that man may have D-amino acid oxidase and hydroxylase enzyme systems capable of converting small amounts of the D-isomers into metabolites found in the urine. However, on the basis of previously published animal data, which showed a delayed absorption of the D-isomers from the intestine, it is not possible to exclude metabolites arising from action of the intestinal flora. With both the D- and L-isomers, the C^{14} activity isolated in known urinary metabolites accounted for only a small part of the total urinary C^{14} activity. In several patients given 2 gms of L-tryptophan with C^{14} labeled tryptophan, expiratory $C^{14}O_2$ was depressed and urinary labeled quinolinic acid activity was elevated when compared to patients in previous studies. These factors suggest that some scleroderma patients may have an alteration of a metabolic step in the pathway after hydroxyanthranilic acid. Conclusive evidence of a relationship between amount of $C^{14}O_2$ produced after a 2 gm load of L-tryptophan and metabolic abnormalities was not observed. The experiments showed that the racemic mixture of a labeled tryptophan compound could be used for a $C^{14}O_2$ metabolism study, but that the use of pure, natural L-isomers is necessary for meaningful results in the study of tryptophan metabolism in man.

Racemic mixtures of labeled compounds are commonly used in metabolic studies because they are more readily available than the resolved D- or L-isomers. Previous work with the labeled D- and L-isomers of kynurenine, hydroxykynurenine and tryptophan has shown that the rat and mouse metabolize the enantiomers of these compounds into $C^{14}O_2$ at different rates (1, 2, 3). The rat does not metabolize the D-isomers of kynurenine or hydroxykynurenine efficiently, but it can utilize the D- and L-isomers of tryptophan equally well. Therefore, any study with the DL mixture of these compounds can lead to difficult or erroneous interpretations in some animal species because of the unusual or abnormal metabolism of the unnatural isomer of the mixture. This study was undertaken to compare the metabolic patterns of the D- and L-isomers of tryptophan-7a-C^{14}, kynurenine-keto-C^{14}, and 3-hydroxykynurenine-keto-C^{14} in patients with scleroderma.

Patients with scleroderma were chosen as subjects for investigation for three reasons. First, they provided an opportunity to compare metabolism of tryptophan isomers in humans. Second, they provided an opportunity to evaluate certain aspects of tryptophan metabolism in scleroderma in which aberrations have been reported (4, 5). Third, we wished to check the observation that giving a 2 gm load of carrier tryptophan with a dose of C^{14}-labeled tryptophan produced an appreciable increase in the amount of C^{14} expired as $C^{14}O_2$ (6). If this observation were confirmed, the 2 gm loading dose of L-tryptophan given with the labeled material might provide a useful diagnostic method for clinical detection of aberrations in tryptophan metabolism.

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MATERIALS AND METHODS

Two types of studies were done on patients. The excretion of tryptophan metabolites was measured quantitatively before and after loading with 2.0 gm of L-tryptophan or 1.0 gm of D-tryptophan. At the same time, these subjects were given small tracer doses of labeled tryptophan in order to assess the metabolism of the labeled compound under these loading conditions. Several of the patients were studied again at a later date with tracer doses of D or L-kynurenine or 3-hydroxykynurenine. Thus, they served as their own controls. Studies

are summarized in Table I, which gives pertinent data of patient information, and doses of unlabeled and labeled compounds administered.

All patients gave their informed consent. For a period of five days prior to and during the study period, the patients received a controlled tryptophan diet containing 0.7 to 1.0 gm per day. All forms of drug therapy were avoided during the study period, and all studies were done with patients in the resting state. Labeled compounds were given orally in gelatin capsules one hours after breakfast. The 2.0 gm "loading" doses of L-tryptophan were administered at the same time as the tracer doses, when indicated.

DL-tryptophan-7a-C^{14} and DL-tryptophan-2-C^{14} (Tracerlab Inc., Waltham, Mass.) used here were homogeneous as determined by autoradiography of paper chromatograms. The DL-kynurenine-keto-C^{14} and the 3-hydroxy-DL-kynurenine-keto-C^{14} were synthesized in our laboratories (7, 8). The DL-tryptophan-7a-C^{14}, DL-kynurenine-keto-C^{14} and 3-hydroxy-DL-kynurenine-keto-C^{14} were resolved into the D and L-isomers by a paper powder column technique (Hankes, L. V., Kido, R. and Brown, R. R.: Unpublished procedures.) The specific activities were determined by combustion to CO_2 and analysis in Ballentine gas counting tubes (9).

Urine samples were collected for a 24-hour period prior to the administration of a labeled compound and for three consecutive 24-hour periods thereafter. Urine specimens were collected under toluene in dark glass bottles and stored in the cold until the collections were completed. Total volume was measured and the urine stored frozen in plastic bottles until analyzed.

Twenty-four hour urine samples collected before and after administration of compounds were analyzed for twelve components by chemical (10) or microbiological methods (11, 12). The components determined were kynurenic acid, xanthurenic acid, anthranilic acid glucuronide, o-aminohippuric acid, acetylkynurenine, anthranilic acid, kynurenine, 3-hydroxykynurenine, N^1-methylnicotinamide, N^1-methyl-2-pyridone-5-carboxamide (10), quinolinic acid (11), and nicotinic acid (12). For comparison, the same metabolites were measured, using the same methods, in healthy control women consisting mainly of laboratory personnel (10).

The majority of the metabolites measured were also isolated from the C^{14}-labeled urine collections by carrier techniques applied to appropriate fractions from the analytical columns, recrystallized to constant specific activity and counted by the method of Van Slyke, Steele and Plazin (9). The urinary components isolated were o-aminohippuric acid, kynurenic acid, xanthurenic acid, kynurenine, 3-hydroxykynurenine, N^1-methylnicotinamide (1, 10), quinolinic acid, nicotinic acid (13) and picolinic acid (14). Radioactivity in kynurenine and acetylkynurenine was also determined by liquid scintillation counting of steam volatile o-aminoacetophenone derived from alkaline steam distillation of raw urine (15). Aliquots of each urine were digested with commerical urease and the $C^{14}O_2$ liberated from the urinary urea was trapped in ethanolamine and measured by liquid scintillation counting.

Crystalline D-tryptophan and D-kynurenine carriers were isolated from the 6–12 hour and 12–24 hour urines of patient G-7, (Table II), who received D-tryptophan-7a-C^{14}. This was done by streaking 0.5 ml of the 6–12 hour urine or 1 ml of the 12–24 hour urine on sheets of No. 4 Whatman paper followed by development with a solvent system of methanol, benzene, butanol and water 2:1:1:1 to which was added 1 ml of glacial acetic acid per 100 ml of solvent. Autoradiographs of the paper chromatograms showed an intense black band at Rf 0.55. This labeled band was cut out from two of the chromatograms. The strips were finely divided and suspended in a blender with 50.35 mg of D-tryptophan or 47.57 mg of D-kynurenine and water. The solutions were filtered through sintered glass funnels and concentrated to dryness with a rotary evaporator. The residues were recrystallized to constant specific activities.

Samples of air exhaled by the patients were collected by means of a plastic hood fitting over the patient's head and connected by large diameter plastic tubing to a set of glass absorption towers arranged in parallel, such that the air flow would pass through one column at a time. Fifteen minute collections of CO_2 were made by switching the air flow from one glass absorption tower to the other. Each absorption tower has a capacity to absorb more than 30 minutes of average CO_2 production. The air was pulled through the system at a rate which assured trapping of the CO_2 in the 2N NaOH solution in the absorption tower and, at the same time, kept the patient comfortable. The sodium hydroxide samples were analyzed for C^{14} activity by acidification in a Van Slyke-Neill chamber, followed by transfer of the liberated CO_2 gas into Ballentine gas counting tubes. The CO_2 samples (fifteen minute) were collected continuously for a four-hour period and then at two-hour intervals. The following equations were used to calculate the percent of administered C^{14} expired as $C^{14}O_2$ per minute:

$$\mu c\text{-}C^{14}/\text{mmole } CO_2 \times \text{body surface area } (M^2) \times 4.8$$

$$= \mu c\text{-}C^{14}/\text{min}$$

$$\frac{\mu c\text{-}C^{14} \text{ produced/minute}}{\mu c\text{-}C^{14} \text{ administered}} \times 100$$

$$= \%\ \text{dose expired/minute}$$

The value of 4.8 is the mean normal millimoles of CO_2 expired by resting human subjects per minute per square meter of body surface, according to Consolazio, Johnson and Pecora (16).

RESULTS

Some of the pathways of tryptophan metabolism in animals are shown in Figure 1. The labeled positions of the several compounds used in these studies and the known metabolic routes to the compounds isolated are indicated.

General information about the patients and dose levels are given in Table I. Five female patients were studied, in some cases with more than one labeled compound.

Table II shows the labeled compounds given and the percent of the dose excreted in the urine. Most of the urinary activity from the compounds appeared in the first 0–12 and 0–24 hour periods. Data from urine collections divided into 0–6 and 6–12 hour periods (patients C-6 and G-7) suggest that D-tryptophan is handled differently than the L-isomer.

Table III shows the quantitative excretion of urinary tryptophan metabolites. Any result above or below mean control values by more than two standard deviations was considered abnormal.

Patient C showed a normal urinary metabolite pattern when given the first load test (C-1). How-

TABLE I

Patient data and compounds administered

Patient No.	A	B			C				D		G
Age	49	44	45	46	43	44	45	46	60	60	40
Height (cm)	165.7		152.4			157.48			146.05		154.31
Weight (kg)	54.59	39.04	40.86	40.85	61.06	59.69	62.65	61.00	40.64	40.17	50.17
Body area (sq m)	1.59	1.29	1.32	1.32	1.602	1.59	1.623	1.593	1.28	1.275	1.46
Study No.	A-1	B-1	B-2	B-3	C-1	C-4	C-5	C-6	D-1	D-2	G-7
Labeled compound	DL-TRY-2	DL-TRY-2	L-KYN-K	H-L-KYN-K	DL-TRY-2	D-KYN-K	H-D-KYN-K	L-TRY-7a	DL-TRY-2	L-KYN-K	D-TRY-7a
(mg)	20.48	20.54	28.44	20.73	20.74	31.49	17.84	112.5	19.90	28.38	24.26
(μc)	112.6	113.0	25.46	23.33	114.0	28.18	20.66	10.62	109.5	25.40	49.46
L-Tryptophan (gm)	2.0	2.0			2.0			2.0	2.0		
D-Tryptophan (gm)											1.0

All patients were female Caucasians.

The abbreviations used in the Table are DL-TRY-2, DL-Tryptophan-2-C^{14}; L-KYN-K, L-Kynurenine-Keto-C^{14}; H-L-KYN-K, Hydroxy-L-Kynurenine-Keto-C^{14}; D-KYN-K, D-Kynurenine-Keto-C^{14}; H-D-KYN-K, Hydroxy-D-Kynurenine-Keto-C^{14}; L-TRY-7a, L-Tryptophan-7a-C^{14}; and D-TRY-7a, D-Tryptophan-7a-C^{14}.

TABLE II

*Urinary excretion of carbon-14 from scleroderma patients administered labeled tryptophan metabolites**

Patient	Compounds		Urine collections				
	Labeled	*Tryptophan load*	0–24 hrs.			24–48 hrs.	48–72 hrs.
A-1	DL-Tryptophan-2	2.0 gm-L	35.53			3.29	0.52
B-1	DL-Tryptophan-2	2.0 gm-L	30.67			3.37	0.31
C-1	DL-Tryptophan-2	2.0 gm-L	33.21			1.53	0.20
D-1	DL-Tryptophan-2	2.0 gm-L	37.38			1.82	0.29
D-2	L-Kynurenine-Keto	None	42.56			2.20	0.87
B-2	L-Kynurenine-Keto	None	12.30			1.23	0.68
			0–12 hrs.		12–24 hrs.		
B-3	Hydroxy-L-Kynurenine-Keto	None	24.88		0.51	0.15	0.07
C-4	D-Kynurenine-Keto	None	59.44		9.35	2.25	1.02
C-5	Hydroxy-D-Kynurenine-Keto	None	74.66		5.40	0.90	0.49
			0–6 hrs.	6–12 hrs.			
C-6	L-Tryptophan-7a	2.0 gm-L	3.43	1.09	1.23	1.57	0.60
G-7	D-Tryptophan-7a	1.0 gm-D	4.04	48.07	9.38	4.64	0.44

* All values are percent of dose given.

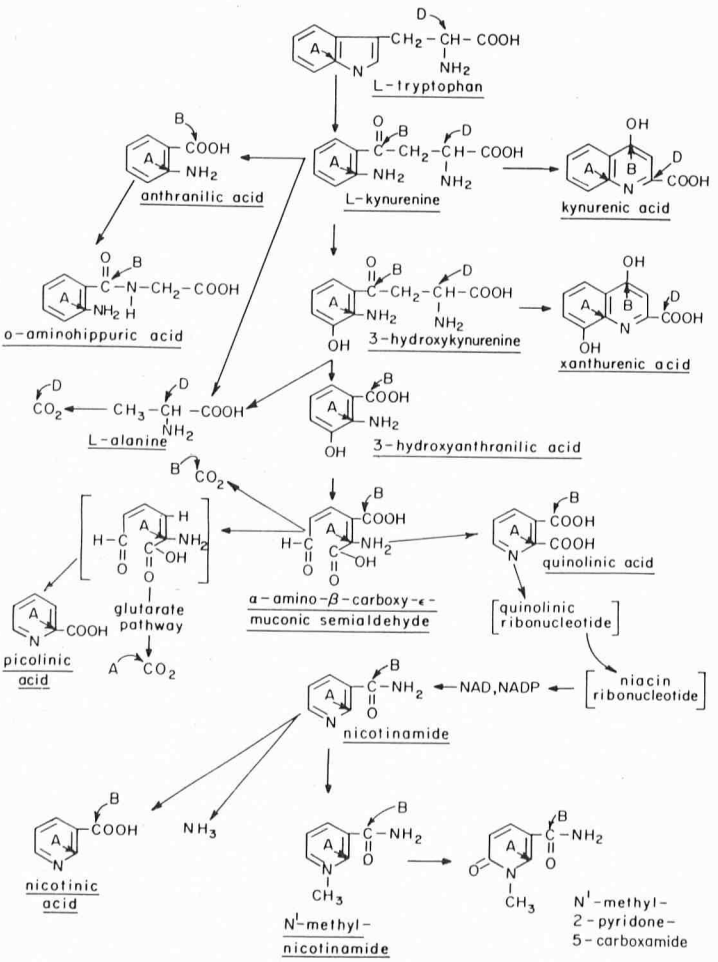

FIG. 1. Letters are given to the labeled carbons of the administered compound and these letters indicate the fate of a particular carbon down through the pathway. The letter A refers to the carbon labeled in the tryptophan-7a-C^{14}. The letter B refers to the carbon labeled in kynurenine-keto-C^{14} and hydroxykynurenine-keto-C^{14}. The letter D refers to the carbon labeled in tryptophan-2-C^{14}.

TABLE III

Urinary excretion of metabolites per 24 hours by patients and control subjects

Patient	Compounds Administered		KA	XA	AAG	OAHA	ACK + AA	KYN	HKYN	MPCA	QA	NA	N^1-MN
	Labeled C^{14}	Tryptophan load[a]											
A-1	None	None	13	7	2	12[c]	9	5	2	41	32.5	7.12	40
	DL-Tryptophan-2	2.0 gm-L	64	31	5.6	23	32[b]	48	36	72	66.5	4.79	41
B-1	None	None	14	10	4.1	11[c]	6	12	11	56	47.7	4.89	12
	DL-Tryptophan-2	2.0 gm-L	52	35	6.6	19	7[c]	33	29	53[c]	86.9	3.53	15
B-2	L-Kynurenine-Keto	None	12	9		13		8	7		31.9	2.94	16
B-3	Hydroxy-L-Kynurenine-Keto	None	7	8		11[c]		8	11		46.2	3.04	16
C-1	None	None	16	9	6.9[b]	21	10	15	13	12[c]	46.8	5.18	21
	DL-Tryptophan-2	2.0 gm-L	77	32	11.4[b]	49	15	43	27	138	116	5.82	57
C-6	L-Tryptophan-7a	2.0 gm-L	109[b]	85[b]		54		57[b]	24		77.3	4.73	128
C-4	D-Kynurenine-Keto	None	18	8		9[c]		49	27		33.2	7.88	5
C-5	Hydroxy-D-Kynurenine-Keto	None	17	8		20		17	30		41.8	3.52	47
G-7	D-Tryptophan-7a	1.0 gm-D	10	9		9[c]		48	55		40.8	4.00	41
D-1	None	None	13	10	4.1	13[c]	15	17	23	79	45.6	4.98	28
	DL-Tryptophan-2	2.0 gm-L	203[b]	261[b]	7.1	37	202[b]	464[b]	397[b]	133	152[b,s]	6.00	47
D-2	L-Kynurenine-Keto	None	11	18[b]		6[c]		9	9		33.1	3.41	3
Female Controls[e]	None	None	13 ± 4	8 ± 2	4.2 ± 1.2	21 ± 4	10 ± 2	10 ± 3	21 ± 14	89 ± 31	46 ± 22[d]	8.5 ± 4.3[d]	32 ± 11
	None	2.0 gm-L	60 ± 19	30 ± 8	6.9 ± 2.1	40 ± 11	18 ± 3	28 ± 13	43 ± 13	131 ± 36	78 ± 22[d]	10.2 ± 6.5[d]	75 ± 66

The abbreviations used are KA, kynurenic acid; XA, xanthurenic acid; AAG, anthranilic acid glucuronide; OAHA, o-aminohippuric acid; ACK + AA, a fraction predominantly N-acetylkynurenine with traces of anthranilic acid; KYN, kynurenine; HKYN, hydroxykynurenine; N^1-MN, N^1-methylnicotinamide; MPCA, N^1-methyl-2-pyridone-5-carboxamide; QA, quinolinic acid; NA, nicotinic acid.

Values are given as micromoles per 24 hours. Control values are the mean values ± standard deviations.

[a] The loading doses of tryptophan were given orally in a single dose at the beginning of the labeled tryptophan collection period.

[b] Indicates values higher than controls by more than 2 S.D. For statistical purposes, the values following tracer doses were compared with basal control values.

[c] Indicates values lower than controls by more than 2 S.D.

[d] QA and NA values are for male controls since this data for female controls are not available.

[e] Control values are from those reported by J. M. Price, R. R. Brown, and N. Yess (10), with the exception of QA, NA, and N^1MN, which are from R. R. Brown and associates (18).

ever, when she was given the load test several years later (C-6) with the labeled tryptophan-7a-C^{14}, she showed abnormal levels of kynurenic acid, xanthurenic acid, and kynurenine.

Patients B-1 and D-1, when given DL-tryptophan-2-C^{14} plus a 2.0 gm load of L-tryptophan, showed similar low $C^{14}O_2$ curves (Fig. 2). However, the tryptophan metabolite levels in their urines were different. The urinary metabolite levels (Table III) of patient B-1, after a tryptophan load, were normal with the exception of low MPCA values, whereas patient D-1 showed abnormally high levels of kynurenic acid, xanthurenic acid, acetylkynurenine, kynurenine, and 3-hydroxykynurenine. Assuming that the normal range of urinary quinolinic acid for females is the same as for males, patient D-1 also had a high quinolinic acid level. It is of interest to note that, at a later date, when patients B and D were given a tracer dose of L-kynurenine-keto-C^{14} with no loading dose of L-tryptophan (B-2 and D-2), no abnormal levels of metabolites were observed by chemical assays. However, when the quantities of C^{14} activity in each component were measured, differences appeared as shown in Table IV. Subject D-2, who was markedly abnormal in response to the 2.0 gm load of L-tryptophan, was also markedly different in response to the tracer dose of L-kynurenine when compared to subject B-2.

Table IV shows the percentage of the labeled dose excreted in the urine as labeled tryptophan metabolites and urea. When L-kynurenine-keto-C^{14} was given to patient B-2 or D-2, the largest percentage of the dose appeared in the labeled quinolinic acid, 3-hydroxykynurenine, and kynurenic acid fractions, with the levels of these components in the urine of patient D-2 being two to four times higher than those of patient B-2. Since the levels of quinolinic acid were so high in the first 24-hour urine of these patients, quinolinic and nicotinic acids were also isolated from their 24–48 hour and 48–72 hour urine specimens. Labeled quinolinic acid levels in the 0–24, 24–48 and 48–72 hour urines of patient B-2 were 2.27%, 0.25% and 0.08% compared to 4.39%, 1.10% and 0.28% found in the urines of patient D-2. These data showed that patient D-2 excreted about twice as much radioactivity in the form of quinolinic acid as patient B-2 for each of the three days. The labeled nicotinic acid levels showed a similar relationship in these urines. These data may reflect a lowered vitamin B_6 activity in some of the later steps of the pathway in patient D-2 since previous work has shown an increased excretion of quinolinic acid in a vitamin B_6 deficiency (17–20). The major labeled metabolites of 3-hydroxy-L-kynurenine-keto-C^{14} in patient B-3 urine were quinolinic acid and xanthurenic acid. Patient C-6, who received L-tryptophan-7a-C^{14}, excreted picolinic acid and kynurenic acid as the major labeled urinary metabolites.

Patient C-4, who was given the D-kynurenine-keto-C^{14}, excreted kynurenine, hydroxykynurenine, kynurenic acid and N^1-methylnicotinamide as major labeled urinary components. Patient C-5, the recipient of 3-hydroxy-D-kynurenine-keto-C^{14}, excreted the most activity in 3-hydroxykynurenine, xanthurenic acid, quinolinic acid and picolinic acid. On the basis of the figures in Table IV, one would assume that kynurenine was the major labeled component in the urine of patient G-7, who received D-tryptophan-7a-C^{14}. However, when the 6–12 and 12–24 hour urines from patient G-7 were chromatographed and the chromatogram autoradiographed, an intense band showed at Rf 0.55. The treatment of this band with either carrier D-tryptophan or D-kynurenine showed that all of the activity from the spot was in D-tryptophan, which accounted for 31.67% of the dose in the 6–12 hour urine and 4.4% of the dose in the 12–24 hour urine.

The values in Table V show that, even though nine components were isolated from the urines, we only accounted for a minimum of 1.32% to a maximum of 71.31% of the urine activity in any particular study. The urine of patient C-4, who received D-kynurenine-keto-C^{14}, contained 4.86% of the activity present in hydroxykynurenine and 2.68% in kynurenic acid, which suggests that the human has a kynurenine hydroxylase capable of hydroxylating D-kynurenine, and a D-amino acid oxidase which can convert D-kynurenine to an α-keto acid that spontaneously cyclizes to kynurenic acid. Quinolinic acid accounted for 5.5 to 18.5% of the urinary activity in all the patients given an L-isomer. Kynurenic acid represented 1.0 to 5.8% of the urine activity in the urine of those patients receiving D or L-kynurenine. Picolinic acid accounted for the largest percentage (7.28) of activity present in the urine of the patient given L-tryptophan-7a-C^{14}.

When labeled kynurenine analyses were done by the distillation of the aminoacetophenone formed from alkaline hydrolysis of urinary kynurenine, the values were four to ten times higher than the values obtained by carrier method in those urines from the patients given D or L-kynurenine-keto-C^{14} (Table IV). Apparently, the carrier method measured the free urinary kynurenine levels, and the distillation methods gave the conjugated plus free kynurenine in the urine.

As shown in Figure 2, when tryptophan-2-C^{14} was given to the scleroderma patients with the 2.0 gm load of L-tryptophan, the production of $C^{14}O_2$ was less than the 22–24% observed in previous studies in all patients except patient A-1 (6). The patients with the lowest output of $C^{14}O_2$ seemed to have the most severe scleroderma from a clinical viewpoint. There was an apparent relationship between rate of $C^{14}O_2$ production after a labeled tryptophan load and the aberrations in tryptophan metabolism observed.

Figure 3 shows a time lag of about one hour before the $C^{14}O_2$ appeared in the breath of those patients receiving L-tryptophan-7a-C^{14} with the 2.0 gm load of L-tryptophan, L-kynurenine-keto-

TABLE IV

*Twenty-four hour excretion of C^{14} labeled metabolites in urine after the administration of labeled enantiomers of tryptophan, kynurenine and hydroxykynurenine**

Patient	G-7	C-6	C-4	C-5	B-2	B-3	D-2
C^{14} Labeled compound given	D-tryptophan-7a-C^{14}	L-tryptophan-7a-C^{14}	D-kynurenine-keto-C^{14}	D-hydroxy-kynurenine-keto-C^{14}	L-kynurenine-keto-C^{14}	L-hydroxy-kynurenine-keto-C^{14}	L-kynurenine-keto-C^{14}
O-Amino hippuric acid	0.008	0.093	0.033	0.007	0.072	0.013	0.065
Kynurenic acid	0.025	0.220	1.840	0.018	0.217	0.003	0.405
Xanthurenic acid	0.003	0.036	0.038	0.490	0.038	0.204	0.280
Kynurenine (distilled)	0.646	0.03	43.61	0.00	1.448	0.00	3.201
Kynurenine (carrier)	0.600	0.148	10.05	0.073	0.105	0.102	0.396
Hydroxykynurenine	0.036	0.038	3.340	0.887	0.156	0.135	0.870
Quinolinic acid	0.018	0.318	0.048	0.145	2.269	3.707	4.392
Nicotinic acid	0.001	0.003	0.003	0.004	0.011	0.012	0.028
N^1-methyl Nicotinamide	0.049	0.051	0.131	0.043	0.02	0.025	0.052
Picolinic acid	0.022	0.419	0.005	0.135		0.008	
Urea	0.006	0.391		0.008	1.180	0.608	0.550
Total recovered†	0.814‡	1.599	49.048	1.737	5.411	4.715	9.843

* Values are percent of dose given.

† Totals are based on the distilled kynurenine values rather than carrier values, since the distillation method also detects acetylkynurenine and other possible conjugates.

‡ An additional 36.07 percent of the total dose was accounted for as unmetabolized D-tryptophan in the urine.

TABLE V

Labeled urinary metabolites as percent of urinary carbon-14

Patient	G-7	C-6	C-4	C-5	B-2	B-3	D-2
Compound given	D-tryptophan-7a-C^{14}	L-tryptophan-7a-C^{14}	D-kynurenine-keto-C^{14}	D-hydroxy-kynurenine-keto-C^{14}	L-kynurenine-keto-C^{14}	L-hydroxy-kynurenine-keto-C^{14}	L-kynurenine-keto-C^{14}
O-Amino hippuric acid	0.012	1.619	0.048	0.008	0.582	0.042	0.152
Kynurenic acid	0.040	3.831	2.677	0.022	1.765	0.011	0.951
Xanthurenic acid	0.005	0.629	0.055	0.613	0.311	0.682	0.658
Kynurenine (distilled)	1.05	0.53	63.4	0.00	9.33	0.00	7.52
Kynurenine (carrier)	0.975	2.581	14.62	0.092	0.852	0.339	0.931
Hydroxykynurenine	0.058	0.656	4.859	1.108	1.267	0.450	2.044
Quinolinic acid	0.029	5.516	0.07	0.181	18.45	12.382	10.32
Nicotinic acid	0.001	0.052	0.004	0.005	0.09	0.04	0.065
N^1-methyl nicotinamide	0.080	0.882	0.191	0.054	0.165	0.083	0.122
Picolinic acid	0.036	7.283	0.007	0.168		0.026	
Urea	0.01	6.80		0.01	9.60	2.40	1.30
Total percent of urinary carbon-14 recovered* (0–24 hrs.)	1.321†	27.798	71.311	2.169	41.560	16.455	23.132

* Totals are based on distilled kynurenine values. This method also detects conjugates of kynurenine.

† An additional 62.79 percent of the total was accounted for as unmetabolized D-tryptophan.

C^{14}, or 3-hydroxy-L-kynurenine-keto-C^{14}. The patient (C-6) given L-tryptophan-7a-C^{14} with the 2.0 gm load of L-tryptophan produced 28% $C^{14}O_2$ which was comparable to the 32% level observed in rats given the same dose on a body weight basis (3).

Figure 4 shows a slightly longer time lag between administration of D-isomers and appearance of $C^{14}O_2$ in expired air of patients, when compared with those receiving L-isomers (Fig. 3). The level of $C^{14}O_2$ produced by the patients receiving D-tryptophan-7a-C^{14} plus 1.0 gm D-tryptophan, D-kynurenine-keto-C^{14} or 3-hydroxy-D-kynurenine-keto-C^{14} was less than 2% in each case.

DISCUSSION

In these studies, there was a time delay of several hours in the production of the $C^{14}O_2$ from D-tryptophan, as compared with $C^{14}O_2$ production from L-tryptophan. Previous studies have shown that some D-isomers were not actively transported by rat intestine (21, 22). With delayed

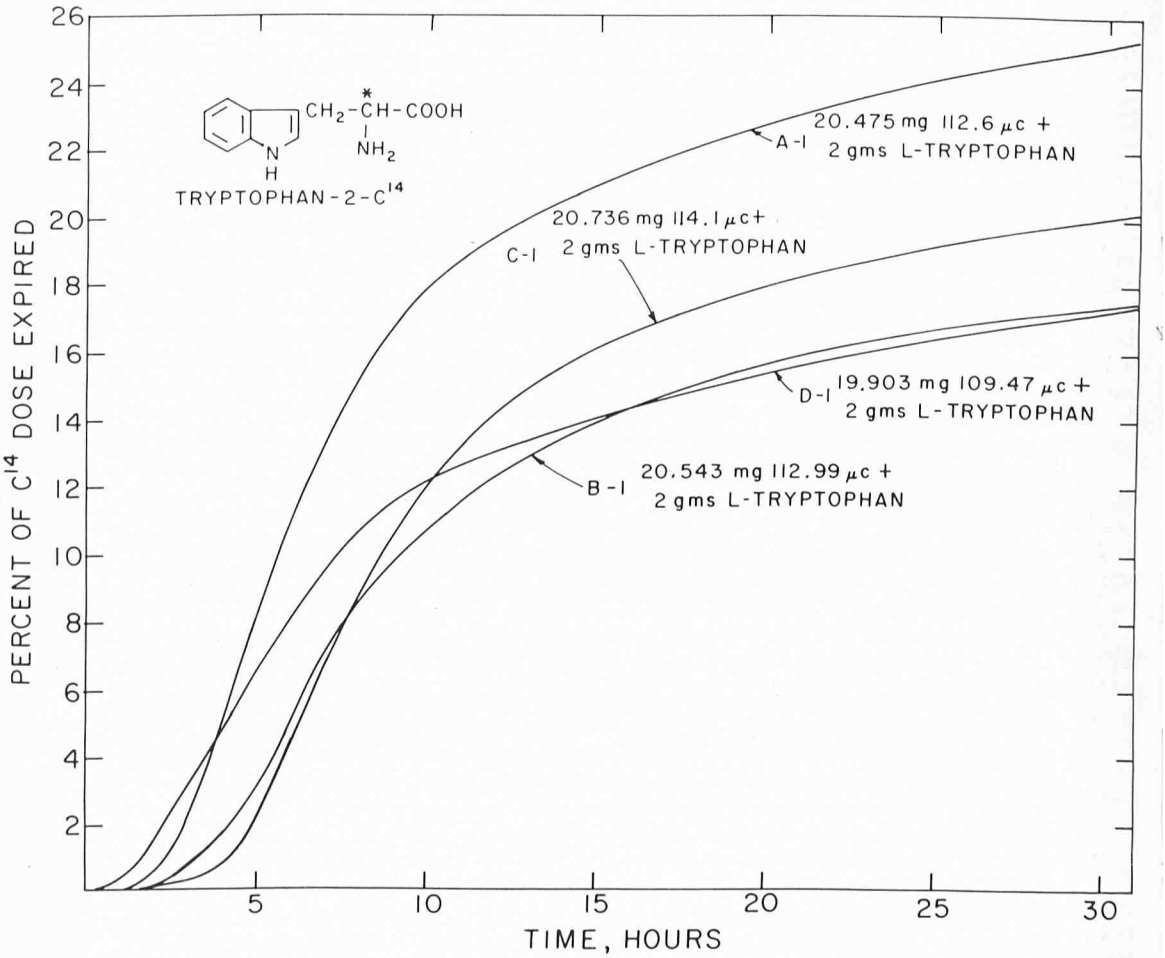

FIG. 2. Expiration of $C^{14}O_2$ by scleroderma patients after oral administration of tracer doses of DL-tryptophan-2-C^{14} with loads of L-tryptophan.

absorption, it is possible that intestinal flora could play a role in the metabolism of these isomers. The delay in the appearance of $C^{14}O_2$ when the D-isomers were given is reflected in the C^{14} content of the urine where the largest quantity of activity appeared in the 6–12 hour period after D-tryptophan ingestion. Some of this could be D-kynurenine formed by the enzymes in the intestinal mucosa. Yamamoto and Hayaishi (23) have shown that the rabbit intestinal mucosa contains an oxygenase capable of converting D-tryptophan into D-kynurenine.

The activity present in the urinary components (other than D-tryptophan) isolated in this study (Table IV) accounted for less than 1% of the dose when D-tryptophan-7a-C^{14} was given. Isolated D-tryptophan-7a-C^{14} accounted for 36% of the dose, but this left 25% of the dose unaccounted for in the urine. This residual C^{14} may represent indole pyruvic acid and related metabolites which would be formed by the action of D-amino acid oxidase or other enzymes.

Some of the scleroderma patients used in this study had normal levels of urinary tryptophan metabolites as evaluated by the 2.0 gm load test. Previous L-tryptophan loading studies in scleroderma patients (4, 5) showed high urinary levels of kynurenine and less elevated levels of hydroxykynurenine in some patients, suggesting that such patients might have an inhibition of the kynurenine hydroxylase system. In the present study, only patient D-1 exhibited markedly abnormal tryptophan metabolism in response to the load test (Table III), and was apparently not lacking in kynurenine hydroxylase, since levels of hydroxykynurenine and xanthurenic acid were also markedly elevated. When this patient was given L-kynurenine-keto-C^{14} seven months later (patient D-2), she showed a depressed $C^{14}O_2$ metabolism (32%) compared to patient B-2 (53%), who also received L-kynurenine-keto-C^{14}, but who had a normal urinary excretion pattern after the 2.0 gm tryptophan load test. The kynurenine and hydroxykynurenine levels in patient D-2, after L-kynurenine-keto-C^{14}, were elevated, but not as much as would be expected if the fault in tryptophan metabolism were in the hydroxylation of kynurenine. Thus there seems to be no demon-

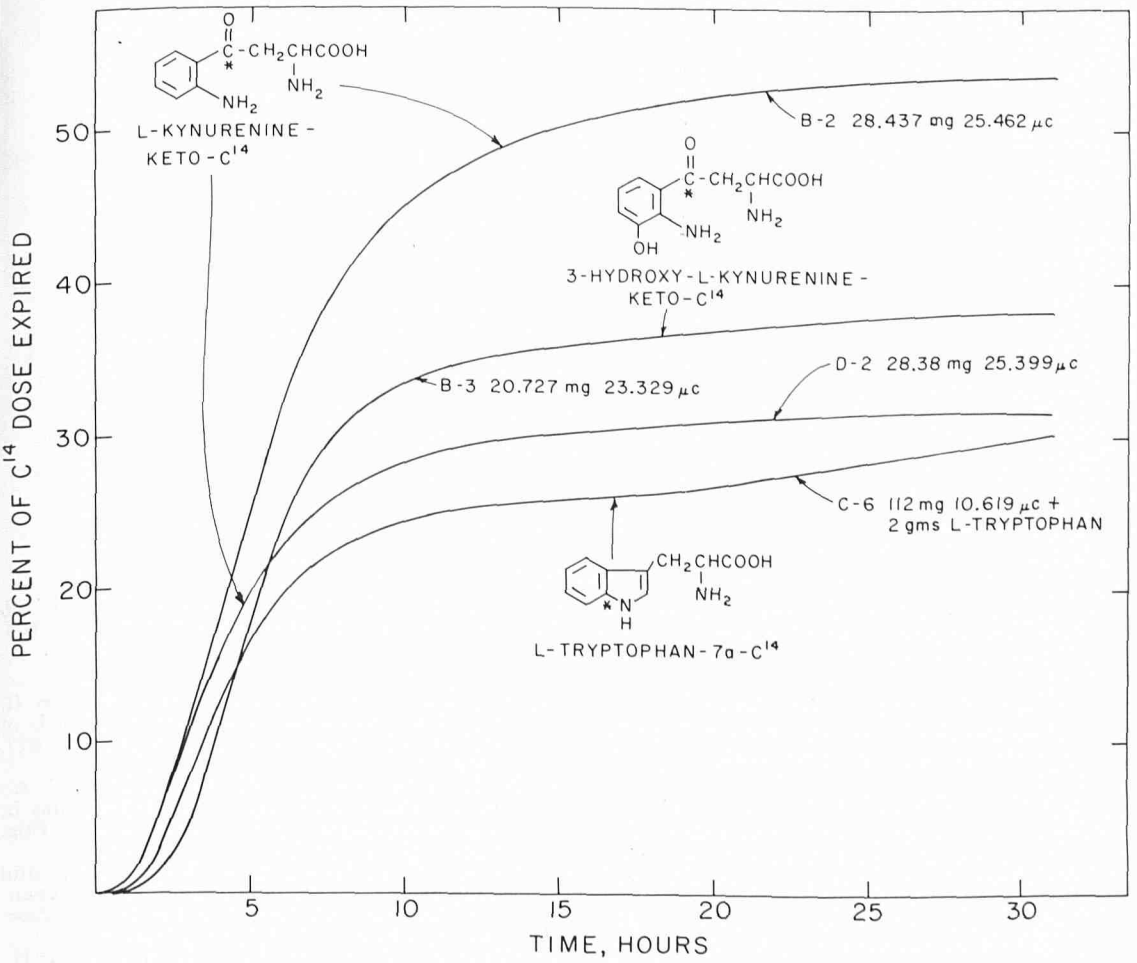

FIG. 3. Expiration of $C^{14}O_2$ by scleroderma patients after oral administration of L-tryptophan-7a-C^{14} with a load of L-tryptophan or tracer dose of L-kynurenine-keto-C^{14} or hydroxy-L-kynurenine-keto-C^{14}. In comparing this figure with Figure 2, note different units of the ordinate axis.

strable defect in kynurenine hydroxylation in this patient.

The fact that the C^{14} activity in all of the components in the pathway (Fig. 1) above quinolinic acid were two to ten times higher in the urine of patient D-2 than in the urine of patient B-2 suggests a difference in her metabolism somewhere after hydroxykynurenine. This response to the L-kynurenine-keto-C^{14} dose cannot be explained by an adapted tryptophan oxygenase or 2.0 gm load stimulation (24, 25) as cause for the increased levels of these components, since the tryptophan oxygenase enzyme step was by-passed when the small tracer doses of L-kynurenine were administered.

The present studies using the D-isomers of labeled tryptophan, kynurenine and hydroxykynurenine in scleroderma patients demonstrated that these isomers contribute very little to the production of $C^{14}O_2$ compared to that contributed by the natural L-isomers. Consequently, in studies of CO_2 production from tryptophan metabolites in man, it would be possible to use readily available DL-compounds with little error. However, these conditions would not be applicable for this type of study in rats, since this species seems to readily convert D-tryptophan to CO_2 (3), but not the intermediates D-kynurenine or D-hydroxykynurenine (1, 2).

The concept of using a 2.0 gm "loading" dose of L-tryptophan with a tracer dose of the labeled DL-tryptophan and measuring $C^{14}O_2$ production may still hold promise as a means of detecting abnormalities in tryptophan metabolism in patients having certain diseases. The depressions in $C^{14}O_2$ excretion observed in the present study, compared to the levels seen in previous studies (6), when DL-tryptophan-2-C^{14} was given with a 2.0 gm load of L-tryptophan, were not consistent enough to indicate a relationship between the presence of scleroderma and tryptophan metabolism. More patients would have to be studied to

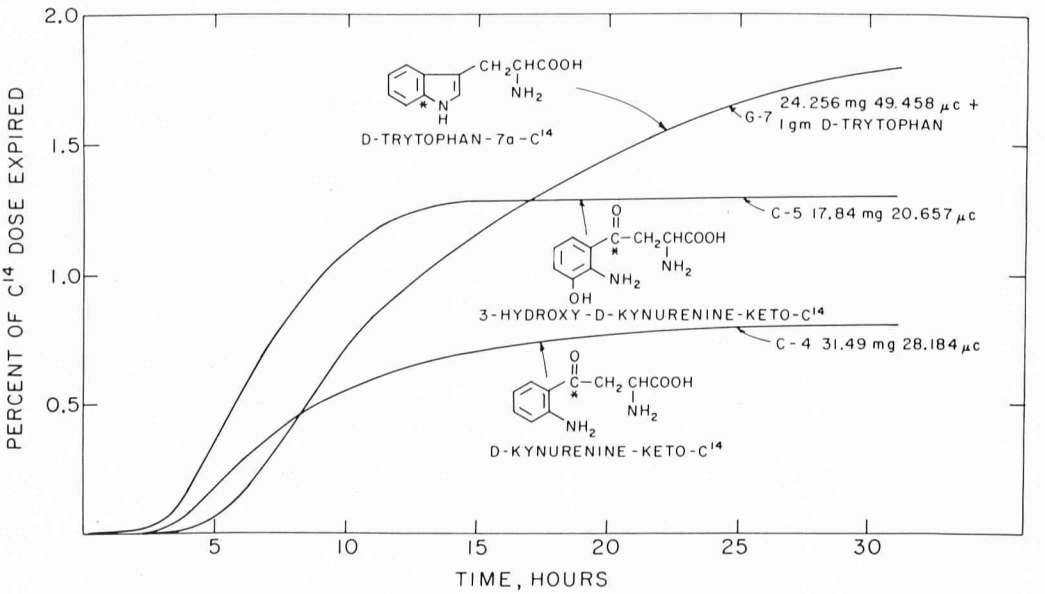

FIG. 4. Expiration of $C^{14}O_2$ by scleroderma patients after oral administration of D-tryptophan-7a-C^{14} with 1.0 gm load of D-tryptophan or tracer dose of D-kynurenine-keto-C^{14} or hydroxy-D-kynurenine-keto-C^{14}. Note the units of the ordinate axis.

determine if a relationship exists between $C^{14}O_2$ production and clinical status, diagnosis, prognosis or urinary metabolites of tryptophan.

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