Synthesis and Metabolism of Tritium-Labeled DL-Kynurenine.* (23807)

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Conflicting reports have appeared in the literature concerning the role of kynurenine in the conversion of the amino acid, tryptophan, to the vitamin, niacin. Kynurenine has been reported to stimulate excretion of N^1-methylnicotinamide in rats(1) and to possess growth-promoting activity when it replaces niacin in a diet(2,3). Conversely, it has also been reported that kynurenine does not support growth(4), or stimulate excretion of N^1-methylnicotinamide(5). *In vitro* work has shown that rat liver catalyzes the formation of the niacin precursor, quinolinic acid(6), when 3-hydroxy-kynurenine‡ was in the media, but not when kynurenine was present in the media(3). The failure of the liver to convert kynurenine to 3-hydroxy-kynurenine in these experiments was interpreted as a choice of the wrong tissue or lack of true conditions for the conversion. More recently, De Castro *et al.*(9,10) detected 3-hydroxy-kynurenine paper chromatographically and colorimetrically in the products of a liver mitochondria incubation in the presence of nicotinic acid, L-kynurenine and reduced diphosphopyridine nucleotide.

In the present work kynurenine labeled with tritium has been synthesized and administered to rats to obtain isotopically labeled products from the urine and more definitely establish the role of kynurenine in the tryptophan-niacin metabolic sequence. Labeled kynurenine was injected intraperitoneally into rats and labeled quinolinic acid and N^1-methylnicotinamide were isolated from the urine. The amount of unmetabolized kynurenine in the urine was also determined by carrier technic.

Methods. Preparation of the labeled DL-kynurenine. One gram of DL-kynurenine was exposed to 2.4 curies of tritium gas under 0.39 atmosphere pressure for several weeks. Prior to purification analysis of the DL-kynurenine showed it had an approximate specific activity of 290 mc/g or 60.32 mc/mM. This labeled DL-kynurenine was mixed with cold DL-kynurenine in a ratio of 1:283 and the mixture was recrystallized from 66% ethanol to constant specific activity. Chromatographic and radiographic analysis of this material showed only the D and L isomers of kynurenine. The recrystallized kynurenine was used for the animal experiments; it had an activity of 1.982 mc/g or 0.413 mc/mM, when analyzed by the gas counting method of Christman(11). *Location of tritium in the labeled kynurenine structure.* The benzene ring structure of the DL-kynurenine contained 69.6% of the tritium activity present in the molecule or 0.2875 mc/mM. This was determined by hydrolyzing the kynurenine to ortho-amino-acetophenone with alkali by the method of Spacek(12) and then preparing the ortho-acetamido-acetophenone derivative by the method of Leonard(13). In preparation of the ortho-acetamido-acetophenone derivative dilute acetic acid was the solvent present in the final step of the reaction. It is noteworthy that repeated suspension of the ortho-acetamido-acetophenone in dilute acetic acid (0.4 *N*) for periods of 18 hours showed no exchange of tritium out of the ring positions, such as has been observed on suspension in dilute mineral acids(14). *Animal experiments.* Male albino rats from Carworth Farms, weighing 220-237 g (raised on a stock diet), were transferred to 9% casein-sucrose diet previously described(15). After 5 or more weeks, the rats receiving this diet were housed in metabolism cages. Forty-eight-hour control urines were collected prior to the injection of labeled DL-kynurenine intraperitoneally. The kynurenine was dissolved in 2 *N* H_2SO_4 and the solution adjusted to approximately pH 6.0 with *N* sodium hydroxide. One

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‡ For the system of numbering the carbon atoms of tryptophan and its products, see(7,8).

rat received 1 mg, a second, 6.24 mg, and a third, 12.48 mg of tritium-labeled kynurenine. Urine samples were collected for two succeeding 12-hour intervals under toluene, and these samples were assayed for free nicotinic acid(16), quinolinic acid(17), and N^1-methylnicotinamide(18). Quinolinic acid was isolated from the urine of 2 rats by a carrier method previously described(19). N^1-methylnicotinamide was isolated as the picrate with carrier N^1-methylnicotinamide prepared in this laboratory(6). Unmetabolized kynurenine was isolated from the urine of one rat with the aid of carrier by the method of Kallio and Berg(1). All compounds isolated from the urines were purified and analyzed for tritium by the dry combustion and gas counting technic of Christman(11).

Results. Conversion of DL-kynurenine into nicotinic acid. Table I shows an increase in nicotinic acid excretion by all rats receiving the DL-kynurenine. The increases in nicotinic acid excretion over the control period for each rat were not proportional to the doses given, and they represent 0.76%, 0.96%, and 0.49%, respectively, of the 1, 6.24, and 12.48 mg injected doses. Compared with the results of Henderson, Koski, and D'Angeli(20) these values suggest a greater ability of this strain to convert DL-kynurenine to nicotinic acid or a more efficient utilization of DL-kynurenine, when it is injected at a low level.

Conversion of DL-kynurenine into quinolinic acid. On the basis of the excretion figures in Table I the rats receiving 1, 6.24, and 12.48 mg of DL-kynurenine excreted 2.5, 1.3 and 1.6%, respectively, of the injected compound as quinolinic acid. These quinolinic acid values also represent a greater percentage excretion of this metabolite than the results of Henderson *et al.*(20), but this difference may be a dosage effect. In Table II the activity due to tritium in the ring, *viz.* 287.5 μc/mM, is used for the calculations on rats 2 and 3, since the ring structure was the only part of the molecule appearing in the excreted quinolinic acid and N^1-methylnicotinamide. The data in Table II show that for the 6.24 mg and 12.48 mg rats the percentages of injected DL-kynurenine excreted as quinolinic acid were 1.31 and 0.95%, respectively. The fact that the specific activities of the isolated quinolinic acid samples (179 and 127 μc/mM) shown in Table II were less than the activity of the injected kynurenine (287 μc/mM) may have been the result either of an exchange of ring tritium for hydrogen during metabolic changes, or of dilution with inactive quinolinic acid formed from sources other than the labeled kynurenine. The results indicate, however, that the greater part of the excreted quinolinic acid was formed from kynurenine.

Conversion of DL-kynurenine into N^1-methylnicotinamide. Table I shows that rats receiving 1, 6.24 and 12.48 mg of labeled DL-kynurenine excreted amounts of N^1-methylnicotinamide equivalent per mole to 1.77, 0.77 and 0.43% of the administered kynurenine. These results agree with those of Kallio and Berg(1) in showing that administered kynurenine increases the excretion of N^1-methylnicotinamide. Isotope evidence that the excreted N^1-methylnicotinamide arises from kynurenine is provided in Table II. Here it is shown that rats receiving 8.625 μc and 17.25 μc of DL-kynurenine as tritium excreted 0.359 and 0.562%, respectively, of this tritium as N^1-methylnicotinamide.

TABLE I. Effect of DL-Kynurenine on Excretion of Nicotinic Acid, Quinolinic Acid, and N^1-Methylnicotinamide.

	DL-kynurenine inj.		Excretion per 24-hr period — Quinolinic acid, γ		Nicotinic acid, γ		N^1-methylnicotinamide, γ	
Rat #	mg	mM	Control	Post-inj.	Control	Post-inj.	Control	Post-inj.
1	1.00	.0048	44.61	64.46 (2.5)*	31.6	36.1 (.76)	22.4	34.7 (1.77)
2	6.24	.03	41.89	105.67 (1.3)	9.4	44.9 (.96)	5.8	37.2 (.77)
3	12.48	.06	54.4	213.38 (1.6)	24.4	60.6 (.49)	11.9	59 (.43)

* Figures in parentheses represent % of inj. kynurenine excreted as various metabolites, calculated from the difference between control and post-inj. excretions.

TABLE II. Twenty-Four Hour Excretion of Quinolinic Acid, N^1-methylnicotinamide and Kynurenine after Injection of Labeled DL-Kynurenine.

		Rat No. 1	Rat No. 2	Rat No. 3
Sp. activity of inj. kynurenine, μc/mMole	a	413.0 *	287.5 †	287.5 †
Kynurenine inj., mMole		.0048	.03	.06
Tritium inj., μc	b	1.982	8.625	17.25
Quinolinic acid				
Q-acid excreted, mMole	c		.00063	.00128
Carrier added, mg/mg urinary Q-acid			8492.0	6344.0
Sp. activity of excreted Q-acid, μc/mMole	d		179.1	127.6
Tritium excreted in Q-acid $\mu c = c \times d$	e		.1128	.1634
% of injected tritium $= \frac{100\ e}{b}$			1.308	.947
Ratio $= \frac{\text{Sp. activity of excreted Q-acid } d}{\text{Sp. activity of inj. kynurenine } a}$			.623	.444
N^1-methylnicotinamide				
N^1-amide excreted, mMole	f		.00022	.00034
Carrier added, mg/mg urinary N-amide			15763.0	5676.0
Sp. activity of excreted N-amide, μc/mMole	g		141.0	286.0
Tritium excreted in N-amide $\mu c = f \times g$	h		.031	.097
% of injected tritium $= \frac{100\ h}{b}$			.359	.562
Ratio $= \frac{\text{Sp. activity of excreted N-amide } g}{\text{Sp. activity of inj. kynurenine } a}$			.49	.995
Kynurenine				
Kynurenine excreted, mMole	i	.00033		
Carrier added, mg/mg urinary kynurenine		5376.0		
Sp. activity of isolated kynurenine, μc/mMole	j	412.0		
Tritium excreted in kynurenine $\mu c = i \times j$	k	.136		
% of injected tritium $= \frac{100\ k}{b}$		6.86		

* Total specific activity/mMole.
† Specific activity/mMole based on ring activity alone.

Almost all the activity in the kynurenine ring appears to be located in the 4, 5 and 6 positions, and practically none in the 3 position, as indicated by the following considerations. In tryptophan metabolism the 7 carbon of tryptophan, which becomes the 3 carbon of kynurenine, has been shown to be lost in the formation of N^1-methylnicotinamide (7) If a significant part of the tritium in the kynurenine used were bound to its 3 carbon, it would therefore presumably be lost in the formation of N^1-methylnicotinamide. However, the tritium activity of the N^1-methylnicotinamide (286.0) was the same as the ring activity (287.5) of the injected kynurenine, indicating no such loss. Wilzbach has shown that ring compounds are not uniformly labeled in all positions, when they are labeled by the gas exposure technic(21).

Kynurenine excretion. Rat No. 1 receiving one mg of DL-kynurenine excreted 6.86% of the tritium in unchanged kynurenine (Table II). If a similar percentage of the higher levels (6.24 mg and 12.48 mg) of kynurenine were excreted unchanged, less than 10% of the ring-located, injected tritium was excreted during 24 hours in kynurenine and its products, quinolinic acid and N^1-methylnicotinamide.

Summary. 1. DL-kynurenine was labeled with tritium by the Wilzbach procedure. 2. Intraperitoneal injection of tritium-labeled DL-kynurenine into rats was followed by the excretion of labeled quinolinic acid and N^1-

methylnicotinamide in the urine. The evidence is definite that kynurenine can serve as a precursor of quinolinic acid and N^1-methylnicotinamide. 3. During 24 hours after injection of 1 mg of labeled DL-kynurenine, 6.86% was excreted as unchanged labeled kynurenine.

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A Method for Production of Arrhythmias in the Isolated Rabbit Heart. (23808)

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Rosenblueth(1), Scherf(2), and Harris(3) have described methods for the experimental production of cardiac arrhythmias. These methods, performed on the intact dog *in situ*, are laborious and time consuming. The isolated heart offers a simple and inexpensive procedure for studying cardiac activity; therefore, a series of experiments were undertaken to determine whether it would be feasible to induce arrhythmias in the isolated rabbit's heart.

Materials and methods. Rabbit hearts were perfused by the Langendorff technic(4) with Locke-Ringer's solution prepared with double-distilled pyrogen-free water. The solution is oxygenated, maintained at 37.5° and perfused at 40 mm Hg. It has been found necessary to have both auricles distended by the perfusate, and this can be accomplished by tying off all vessels except the superior vena cava, which is partially ligated. Electrograms are recorded following an adjustment period of 15-20 minutes. Potential difference is measured by placing electrodes at various positions in the myocardium. In order to determine the type of arrhythmia produced, the number and position of electrodes are altered. When a constant pattern is obtained the