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Synthesis and Metabolism of Quinolinic Acid Ring Labeled with Tritium.* (22973)

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Since quinolinic acid was identified as a product of tryptophan metabolism in the rat (1), many experiments have been conducted to establish its role in tryptophan metabolism scheme(2). Isotope work has definitely proved that quinolinic acid was a product of tryptophan metabolism(3), and that 3-hydroxyanthranilic acid was converted to quinolinic acid in the rat(4). Various publications have suggested that in the sequence of reactions converting tryptophan to niacin, quinolinic acid functions as a side reaction product(5,6) and that quinolinic acid is not converted to nicotinic acid(7). Synthesis of quinolinic acid labeled in the ring with tritium has made it possible to obtain more dependable information regarding the role of quinolinic acid as an intermediary product in niacin formation. In our experiments labeled quinolinic acid was injected intraperitoneally into rats and labeled N^1-methylnicotinamide was isolated from the urine.

Methods. Preparation of 4,5,6 tritium-labeled quinolinic acid. The tritium ring labeled quinolinic acid was prepared by exposing a mixture of one gram of pure quinolinic acid (recrystallized 12 times) and 110 mg of lithium carbonate to a neutron flux of $1.8 \times 10^{12} n/cm^2/sec$ for 48 hours. This method of tritium-recoil labeling is described by Rowland and Wolfgang(8). After exposure in the nuclear reactor, the quinolinic acid, which had turned from white to tan in color, indicating some decomposition, was dissolved in a small volume of water. This solution was adjusted to pH slightly below 10 with sodium hydroxide to remove the tritium from the carboxyl groups by salt formation. After concentrating the solution to dryness under vacuum and redissolving in water, the quinolinic acid was purified by column chromatography through the following steps: adsorption onto norite at pH of 1.4, elution of norite with 0.1 *N* NH_4OH, concentration of eluate to dryness, extraction of concentrate with acid methanol, and subse-

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TABLE I. Twenty-Four Hour Excretion of N¹methylnicotinamide and Quinolinic Acid after Injection of Ring Labeled Quinolinic Acid.

Rat No.		1	2	3	4
Sp. activity of inj. Q-acid, μc/mMol	(a)	23.29	23.29	23.29	23.29
Quinolinic acid inj., mg		5	5	20	20
Idem mMol		.030	.030	.120	.120
Tritium inj., μc	(b)	.697	.697	2.79	2.79
N¹methylnicotinamide					
N-amide excreted, mg		.235		.770	
Idem mMol	(c)	.0017		.0056	
Carrier added, mg/mg urinary N-amide		973		346.3	
Sp. activity of excreted N-amide, μc/mMol	(d)	5.49		1.78	
Tritium excreted in N-amide					
$\mu c = c \times d$	(e)	.0094		.0100	
% of inj. $= \frac{100\ e}{b}$		1.35		.36	
Ratio, $\frac{\text{Sp. ac. of excreted N-amide}}{\text{Sp. ac. of inj. Q-acid}} = \frac{d}{a}$		.236		.076	
Quinolinic acid					
Q-acid excreted, mg			4.75		13.49
Idem mMol	(f)		.028		.081
Carrier added, mg/mg urinary Q-acid			114		40
Sp. activity of excreted Q-acid, μc/mMol	(g)		23.95		26.11
Tritium excreted in Q-acid					
$\mu c = f \times g$	(h)		.68		2.11
% of inj. $= \frac{100\ h}{b}$			97.6		75.5
Ratio, $\frac{\text{Sp. ac. of excreted Q-acid}}{\text{Sp. ac. of inj. Q-acid}} = \frac{g}{a}$			1.028		1.121

quent adsorption of the methanol extract onto an alumina column. The alumina column was eluted with 0.01 *N* NH_4OH. The eluate fraction of pH 8.8-9.2 was collected and then concentrated to dryness under vacuum. The crude quinolinic acid thus obtained was dissolved in 40% ethanol, treated with activated charcoal, filtered, and precipitated from solution by adjustment to pH 2 with concentrated hydrochloric acid. After several recrystallizations from small amounts of 50% ethanol, the material was paper chromatographed to check for purity. The labeled quinolinic acid gave a single component of the same Rf as commercial quinolinic acid. It had a specific activity of 306,750 counts/minute/mg of compound, when analyzed by gas counting procedure of Christman(9). *Animal experiments.* Male albino rats from Carworth Farms, weighing 280-380 g (raised on stock diet) were transferred to 9% casein-sucrose diet described earlier(10). After 5 weeks, the rats receiving this diet were housed in metabolism cages. Labeled quinolinic acid was administered intraperitoneally in 0.4 to 1.6 ml of 0.9% saline solution. One pair of rats received 5 mg, another pair received 20 mg of labeled quinolinic acid each. Twenty-four hour subsequent urines were collected. All urine samples were assayed for quinolinic acid and N¹methylnicotinamide. Quinolinic acid was determined by microbiological assay(11) and N¹methylnicotinamide was determined by a fluorimetric method using a Lumetron Fluorescence Meter, model 402-EF(12). With the aid of carrier quinolinic acid was isolated from 2 of the urines by previously described method(13). The N¹methylnicotinamide was isolated as the picrate by procedure previously described(14) with the use of carrier N¹methylnicotinamide prepared in this laboratory. (This N¹methylnicotinamide melted at 237° (corrected) and was shown by microbiological assay(11) to be free of nicotinic acid.) The purified quinolinic acid and N¹methylnicotinamide picrate from urines were analyzed for tritium content by the dry combustion and gas counting technic of

Christman(9).

Conversion of quinolinic acid into N¹methylnicotinamide. Table I shows that rats receiving 5 and 20 mg of labeled quinolinic acid excreted, respectively, 1.35 and 0.36% as labeled N¹methylnicotinamide. Activity ratios lead to the calculation that of the excreted amide 23.5 and 7.6%, respectively, in the 2 rats was derived from the injected quinolinic acid. The calculation is based on the assumption that in transformation from quinolinic to N¹methylnicotinamide none of the tritium atoms attached to carbons 4, 5, and 6 of the quinolinic acid is detached. If detachment occurred, the proportion of amide derived from quinolinic acid would be greater than that calculated above.

Quinolinic acid excretion. Quinolinic acid excreted by rats receiving 5 and 20 mg amounted to 95 and 68%, respectively, of the amounts injected. The specific activity was definitely greater in quinolinic acid excreted than in that injected. It appears possible that metabolic destruction of labeled quinolinic acid may have been less than of the unlabeled, permitting a larger proportion of the former to pass into the urine. The excess tritium was greater in excreted quinolinic acid of Rat No. 4, which also apparently metabolized a greater proportion, to judge from the smaller fraction excreted. It does not appear likely that tritium enrichment occurred during recrystallization of the quinolinic acid, since previous recrystallizations of labeled quinolinic acid did not change its activity.

Summary. 1. Quinolinic acid was labeled in the 4, 5, and 6 positions with tritium by the neutron recoil method. 2. Intraperitoneal injection of labeled quinolinic acid into rats was followed by excretion of labeled N¹methylnicotinamide in urine during the next 24 hours. Tritium contents of excreted N¹ methylnicotinamide in 2 experiments indicated that 7.6 and 23.6% originated from the injected labeled quinolinic acid. The evidence is definite that quinolinic acid can serve in the rat as a source of niacin. 3. Most of the injected quinolinic acid was excreted as such in the urine. Quinolinic acid isolated from urines of 2 rats had specific activities of 103 and 112% of injected quinolinic acid. The increase in specific activity suggests that tritium-labeled quinolinic acid may have been less rapidly metabolized than the unlabeled substance.

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