3-HYDROXYKYNURENINE-KETO-C^{14}: A PRECURSOR OF URINARY QUINOLINIC ACID*

L. V. Hankes and R. R. Brown

Medical Research Center, Brookhaven National Laboratory, Upton, New York, and Cancer Research Hospital, University of Wisconsin Medical School, Madison, Wisconsin

Received December 13, 1960

Previous work has shown the ability of rats to convert tryptophan-3a, 7, 7a-C^{14} (Henderson and Hankes, 1956), kynurenine-keto-C^{14} (Hankes, 1958), and carboxyl-labeled 3-hydroxyanthranilic acid (Hankes and Henderson, 1957) into quinolinic acid (QA) and N'methylnicotinamide. Species variations inability to convert tryptophan and hydroxyanthranilic acid to pyridine carboxylic acids were noted (Suhadolnik *et al*, 1957). It was observed that hydroxyanthranilic acid was metabolized similarly *in vivo* by cats and rats but differently *in vitro*. Other studies (de Castro *et al*, 1957) showed quantitative enzymic differences *in vitro* between cats and rats which at least partly explain the inability of cats to utilize tryptophan as a niacin precursor (Carvalho da Silva, 1952) and also explain the absence of tryptophan metabolites in the urine of cats (Brown and Price, 1956). In the present study, labeled 3-hydroxykynurenine was synthesized and administered to a cat and rat in further studies on the conversion of tryptophan to niacin and in an attempt to further define the biochemical differences between these species.

*This research was supported by the U.S. Atomic Energy Commission.

Experimental

3-Hydroxy-DL-kynurenine-keto-C^{14} was synthesized by a procedure to be published elsewhere (Brown et al). It was administered intraperitoneally in doses of 1.01 mg. per rat (weight 370 gm.) and 3.952 mg. per cat (weight 1413 gm.). The respiratory $C^{14}O_2$ was collected for 12 hours and the urine for 24 hours. The urine was analyzed for QA by microbiological assay (Henderson, 1949) and the QA was isolated by column chromatography (Henderson and Hirsch, 1949). All CO_2 and isolated QA samples were analyzed for C^{14} by the wet combustion technique of Van Slyke, Steele and Plazin (1951).

Results and Discussion

When 3-hydroxy-DL-kynurenine-keto-C^{14} was administered to a rat and a cat, QA-C^{14} was excreted in the urine. The results of the analysis of the expired CO_2 (Table I) indicated that

Table I

Rate of Expiration of $C^{14}O_2$ from 3-Hydroxy-DL-Kynurenine-Keto-C^{14}

Rat Rec'd 1.01 mg, 1.064 μc, in 1 ml. isotonic NaCl soln. intraperitoneally			Cat Rec'd 3.952 mg, 4.173 μc, in 3.8 ml isotonic NaCl soln. intraperitoneally		
Time from admin. (min.)	% admin. C^{14} expired per min.	% admin. C^{14} expired cumul. total	Time from admin. (min.)	% admin. C^{14} expired per min.	% admin. C^{14} expired cumul. total
0-90	0.212	19.1	0-60	0.100	6.05
90-180	0.093	27.44	60-120	0.173	16.40
180-270	0.033	30.47	120-180	0.111	23.07
270-360	0.019	32.16	180-270	0.040	26.70
360-540	0.011	34.10	270-360	0.018	28.30
540-720	0.003	34.64	360-540	0.008	29.80
			540-720	0.002	30.31

hydroxykynurenine is rapidly metabolized in both the rat and cat. The specific activity of the expired CO_2 reached a maximum value within 90 minutes in the rat and between 60 and 120 minutes in the cat. In both animals approximately 25 per cent of the isotope was expired in the first 3 hours.

The urine samples collected from the rat and cat during the 24 hours after injection of hydroxykynurenine-C^{14} contained much more C^{14} activity than did the CO_2. The rat excreted approximately 1 per cent of administered activity as urinary QA while 49.62 per cent of the dose was present in the urine as unidentified metabolites (Table II). In both

Table II

Urinary Quinolinic Acid from 3-Hydroxykynurenine-Keto-C^{14}

Animal	Rat	Cat
3-hydroxykynurenine inj., mg.	1.01	3.952
C^{14} inj., μc.	1.064	4.173
Quinolinic Acid		
Q-Acid excreted, mMole	0.0006	0.0011
Sp.activity of excreted Q-acid, μc/mMole	16.11	89.37
Carbon-14 excreted in Q-acid, μc	0.0097	0.0984
% of inj. carbon-14 in Q-acid	0.917	2.36
Ratio = $\frac{\text{sp.act. of excreted Q-acid}}{\text{sp.act. of admin. 3-OH KYN}}$ =	0.0682	0.3785
Sp.act. of inj. 3-hydroxykynurenine	236.1 μc/mMole	

species, failure to obtain $C^{14}O_2$ by decarboxylation of isolated QA in the two position showed that the C^{14} activity was either in the 3-carboxyl group or in the pyridine ring.

The cat, which received 3.952 mg. of hydroxykynurenine-C^{14},

disposed of 56.23 per cent of the activity in the urine in 24 hours. Of this, over 95 per cent or 53.87 per cent of the test dose was present as unidentified metabolites.

These preliminary results suggest that the cat can metabolize 3-hydroxykynurenine-keto-C^{14} to CO_2 to about the same extent as the rat. Both species converted this precursor to urinary quinolinic acid; however, the specific activity of urinary quinolinic acid from the cat was 5.5 times that of the rat, suggesting very small body pool sizes of hydroxykynurenine and quinolinic acid in the cat.

Acknowledgments

The authors wish to acknowledge the technical assistance of Mr. M. Schmaeler and Mr. R. Kawashima.

References

Brown, R. R., and Price, J. M. J. Biol. Chem. 219, 985 (1956).
Brown, R. R., Hankes, L. V. and Kawashima, R., unpublished.
Carvalho da Silva, A., Fried, R., and de Angelis, R. C. J. Nutrition 46, 399 (1952).
de Castro, F. T., Brown, R. R., and Price, J. M. J. Biol. Chem. 228, 777 (1957).
Hankes, L. V., and Henderson, L. M. J. Biol. Chem. 225, 349 (1957)
Hankes, L. V. 134th Mtg., Am. Chem. Soc., Chicago, Sept. 1958, abstracts, p.45c.
Henderson, L. M., and Hirsch, H. M. J. Biol. Chem. 181, 667 (1949)
Henderson, L. M. J. Biol. Chem. 181, 677 (1949).
Henderson, L. M., and Hankes, L. V. J. Biol. Chem. 222, 1069 (195
Suhadolnik, R. J., Stevens, C. O., Decker, R. H., Henderson, L. M., and Hankes, L. V. J. Biol. Chem. 228, 973 (1957).
Van Slyke, D. D., Steele, R., and Plazin, J. J. Biol. Chem. 192, 769 (1951).