Title
Tryptophan Metabolism II.

Authors
Heidelberger, C.
Abraham, A.P.
Lepkovsky, S.

Publication Date
1949-03-15

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

BERKELEY, CALIFORNIA

DISCLAIMER

UCRL-309

Unclassified Distribution

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

Contract No. W-7405-eng-48

TRYPTOPHAN METABOLISM. II.

CONCERNING THE MECHANISM OF THE MAMMALIAN CONVERSION OF TRYPTOPHAN INTO NICOTINIC ACID

Charles Heidelberger, Edward P. Abraham, and Samuel Lepkovsky

March 15, 1949

Berkeley, California

TRYPTOPHAN METABOLISM. II.

CONCERNING THE MECHANISM OF THE MAMMALIAN CONVERSION OF TRYPTOPHAN INTO NICOTINIC ACID*

by

Charles Heidelberger**, Edward P. Abraham+, and Samuel Lepkovsky

Radiation Laboratory and Department of Chemistry, University of California, Berkeley and the Division of Poultry Husbandry, University of California, Berkeley, California.

March 15, 1949

ABSTRACT

1. The conversion of tryptophan into nicotinic acid in the rat has been established with certainty by isotopic experiments.

2. The mechanism of this change is identical to that previously shown for Neurospora and consists of a sequence in which the principle compounds are: tryptophan, kynurenine, 3-hydroxyanthranilic acid and nicotinic acid.

3. Carbon atom-3 in the indole ring of tryptophan, the precursor of the carboxyl carbon of the 3-hydroxyanthranilic acid becomes the carboxyl carbon atom of the nicotinic acid.

For publication in The Journal of Biological Chemistry.

---

* The work described in this paper was sponsored by the Atomic Energy Commission.

** Present address: McArdle Memorial Laboratory, The Medical School, University of Wisconsin, Madison, Wisconsin.

+ While on leave from: Sir William Dunn School of Pathology, Oxford University, Oxford, England.

TRYPTOPHAN METABOLISM. II.

CONCERNING THE MECHANISM OF THE MAMMALIAN CONVERSION OF TRYPTOPHAN INTO NICOTINIC ACID*

by

Charles Heidelberger**, Edward P. Abraham+ and Samuel Lepkovsky

Radiation Laboratory and Department of Chemistry, University of California, Berkeley and the Division of Poultry Husbandry, University of California, Berkeley, California.

Although the conversion of tryptophan into nicotinic acid had been demonstrated by numerous nutrition experiments for the past several years, relatively little was known about the mechanism of this change until the recent studies by Mitchell and Nyc (1) on mutant strains of Neurospora proved that 3-hydroxyanthranilic acid is a key intermediate in the metabolic sequence. That the same compound is involved in the mammalian conversion is indicated by the feeding experiments of Mitchell, Nyc and Owen (2) who reported that it could maintain growth of rats on a nicotinic acid-deficient diet, and by Albert, Scheer and Deuel (3) who found an increased excretion of N-methylnicotinamide by rats fed this substance. In the isotopic experiments (4) the fact that the labeled β-carbon atom of tryptophan

---

* The work described in this paper was sponsored by the Atomic Energy Commission.

** Present address: McArdle Memorial Laboratory, The Medical School, University of Wisconsin, Madison, Wisconsin.

+ While on leave from: Sir William Dunn School of Pathology, University of Oxford, Oxford, England.

(1) H. K. Mitchell and J. F. Nyc, Proc. Nat. Acad. Sci., 34, 1 (1948); J. Am. Chem. Soc., 70, 1847 (1948).
(2) H. K. Mitchell, J.F.Nyc and R.D.Owen, J. Biol. Chem., 175, 433 (1948).
(3) P.W.Albert, B.T.Scheer, and H. J. Deuel, J. Biol. Chem., 175, 479 (1948).
(4) C.Heidelberger, M.E.Gullberg, A.F.Morgan and S. Lepkovsky, J. Biol. Chem.,

did not appear in the nicotinic acid proved that the side-chain is lost in the transformation, and this observation is also consistent with the existence of 3-hydroxyanthranilic acid as an intermediate. However, since we had not demonstrated by tracer experiments the conversion of tryptophan into nicotinic acid, a differently labeled tryptophan-3-C^{14} was synthesized (5) so that this point might be proved. It was also hoped that further light might be shed on the rather obscure mechanism of the transformation of 3-hydroxyanthranilic acid into nicotinic acid.

A preliminary account of this work has already been reported (6) in which the asterisk in the tryptophan formula was incorrectly represented as being over the β-carbon instead of the 3-carbon atom.

EXPERIMENTAL

Three young, male rats were fed for a week with a 12% casein niacin-deficient diet (7) and were then each given by stomach tube a solution of 75 mg. of DL-tryptophan-3-C^{14} in 2 ml. of water. The urines were collected over toluene for 36 hours and were pooled. The N-methylnicotinamide was determined fluorometrically (8) throughout the experiment and the results are indicated in Table I. The radioactivities were also followed and these results are shown in Table II. These measurements were carried out with an

---

(5) C. Heidelberger, J. Biol. Chem.
(6) C. Heidelberger, E. P. Abraham and S. Lepkovsky, J. Biol. Chem., 176, 1461 (1948).
(7) J. M. Hundley, Nutrition, 34, 253 (1947).
(8) J. W. Huff and W. A. Perlzweig, J. Biol. Chem., 167, 157 (1947).

internal proportional counter (Nucleometer) using the direct plating technique (9). The N-methylnicotinamide isolation was carried out according to a known procedure (10).

The urine, (16 ml.) was collected, 14 ml. were diluted to 50 ml. with acetate buffer at pH 4.4, and passed through a Permutit column. Fluorometric analysis indicated that no N-methylnicotinamide was present in the filtrate. The compound was eluted from the column by washing with 15 ml. of hot 25% potassium chloride, followed by 125 ml. of water. Carrier N-methylnicotinamide chloride (76 mg.) was added to the eluate and the mixture was evaporated to dryness in vacuum. The residue was extracted with three 50 ml. portions of 95% ethanol andd the extract again evaporated to dryness. The residue was then re-extracted with 95% ethanol and centrifuged clear. After addition of a solution of picric acid in ethanol to the extract, a precipitate of the picrate of N-methylnicotinamide was obtained which was recrystallized twice from 90% ethanol. The purified picrate, 106 mg., m.p. 187-188°, gave no depression of the melting point on admixture with an authentic sample.

The radioactive picrate, 105 mg., was converted into the corresponding chloride by solution in dilute hydrochloric acid followed by extraction of the free picric acid with ether. The N-methylnicotinamide chloride was heated with 1.5 ml. of concentrated hydrochloric acid in a sealed tube for 27 hours at 250°. The resulting solution was evaporated to dryness, the residue was dissolved in 3 ml. of water and the solution was made slightly alkaline with sodium hydroxide. This solution was then boiled until the

---

(9) C. Heidelberger, M. R. Kirk and M. S. Perkins, Cancer, 1, No. 2, 263 (1948).

(10) M. Hochberg, D. Melnick and B. L. Oser, J. Biol. Chem., 158, 265 (1945).

evolution of ammonia ceased, neutralized and mixed, while hot, with 60 mg. of copper sulfate in 0.5 ml. of water. The mixture was cooled and the copper salt of nicotinic acid was filtered and washed with cold water. The salt was suspended in 5 ml. of water and decomposed with hydrogen sulfide. Evaporation of the solution gave 35 mg. of nicotinic acid, m.p. 218-220° (uncorrected). This melting point was not raised by recrystallization of the compound from moist butanol, nor was it depressed on admixture of an authentic sample of nicotinic acid.

The radioactive nicotinic acid, 13.4 mg., was heated with quinoline and copper chromite catalyst (11) to 285°. The carbon dioxide resulting from the decarboxylation was swept with pure nitrogen into a bubbler containing sodium hydroxide and was then precipitated as barium carbonate. The specific activity of the barium carbonate indicates that the entire radioactivity of the nicotinic acid was present in the carboxyl group.

Table I

Fluorometric Data

	γ of N-methylnicotinamide (Total)
Urine before tryptophan feeding	38
Urine after tryptophan feeding	790
Permutit KCl eluate	175

(11) W. A. Lazier and H. R. Arnold, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York (1943), p. 142.

Table II

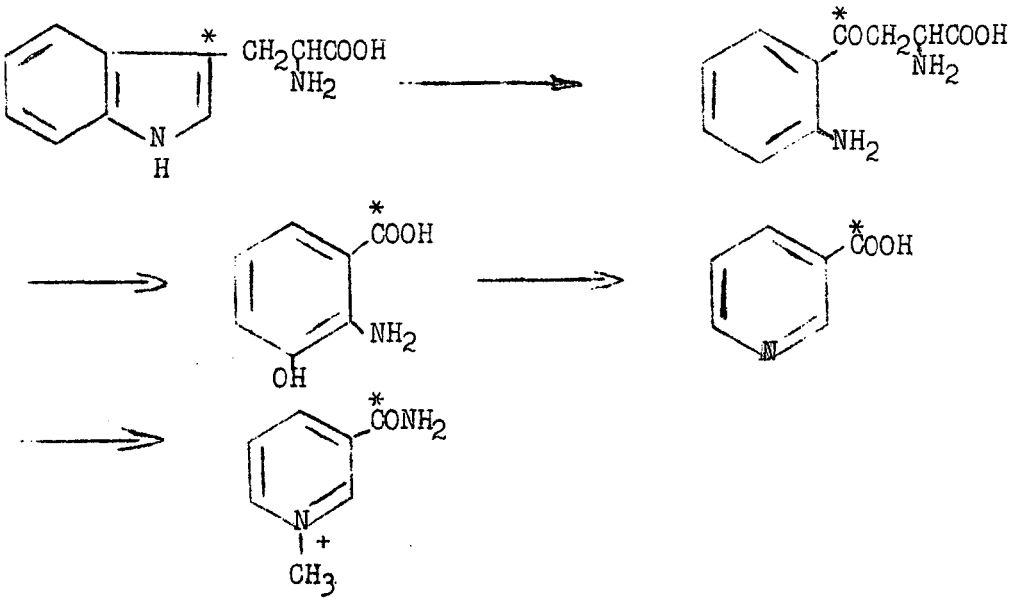
Radioactivities

	Total Cts./min.	Specific Activity cts./min./mg. Found	Calculated
Tryptophan administered	620,000	2740	
Urine	18,800		
N-methylnicotinamide picrate	79	0.75	3.5*
Nicotinic acid	70	2.0	2.2
Barium carbonate from nicotinic acid decarboxylation	21	1.3	1.2

* This figure is calculated as follows, assuming the direct conversion of the administered dose: S.A. tryptophan, 2.74 cts./min/γ. S.A. of N-methylnicotinamide chloride would be 3.25 cts./min/γ. There were 175γ in the eluate, there would be 3.25 x 175 = 570 cts./min. total. Now, 76 mg. of carrier were added, so the S.A. of the N-methylnicotinamide chloride would be 7.5 cts./min./mg. which is equivalent to 3.5 cts./min./mg. of the picrate. Thus, there was a five-fold dilution by the body pools of tryptophan, nicotinic acid (and the various intermediates) in this experiment.

DISCUSSION

These tracer experiments together with the ones using tryptophan-β-C^{14} (4) prove that the conversion of tryptophan into nicotinic acid in the intact mammal takes place by a mechanism identical to that already demonstrated to occur in Neurospora.



The conversion of tryptophan into kynurenine had been demonstrated and evidence is presented in the preceding paper that this occurs directly and by only one mechanism. Now it has been shown that carbon atom-3 in the indole ring of tryptophan becomes the carboxyl carbon of nicotinic acid. (Throughout this work we have referred to nicotinic acid as the end product of this sequence of reactions, although we have in all cases isolated N-methylnicotinamide. Hundley and Bond (12)have shown in experiments with C^{13} that nicotinic acid is excreted almost quantitatively as its N-methyl amide.) Although we have in these experiments no direct evidence for the formation of 3-hydroxyanthranilic acid, our results are consistent with its participation in the metabolic sequence and there can be little doubt from the work of others that it is an intermediate. It is clear that the tagged indole 3-carbon atom is the precursor of the carboxyl carbon of the hydroxyanthranilic acid, so that it has now been demonstrated that the carboxyl group of hydroxyanthranilic acid becomes the carboxyl group of

---

(12) J. M. Hundley and H. W. Bond, J. Biol. Chem., 173, 513 (1948).

nicotinic acid. Although the exact mechanism of this conversion of a benzene into a pyridine derivative still remains to be elucidated, this observation should be of some importance in the final solution of the problem. It seems rather likely that the benzene ring opens, carbon atom-3 which carried the hydroxyl group is lost, and that ring-closure is effected somehow between carbons 2 and the nitrogen atom. However, the details of these reactions remain to be ascertained.

ACKNOWLEDGMENTS

We wish to thank Dr. A. F. Morgan for her generosity in making the facilities of her laboratory available for these investigations. We are grateful to Mrs. Olga Nave, Mrs. Martha Kirk and Mrs. Yvonne Stone for technical assistance and deeply indebted to Mrs. Mary E. Gullberg and Miss Dorothy Mabee who generously volunteered there services for these experiments.

SUMMARY

1. The conversion of tryptophan into nicotinic acid in the rat has been established with certainty by isotopic experiments.

2. The mechanism of this change is identical to that previously shown for Neurospora and consists of a sequence in which the principle compounds are: tryptophan, kynurenine, 3-hydroxyanthranilic acid and nicotinic acid.

3. Carbon atom-3 in the indole ring of tryptophan, the precursor of the carboxyl carbon of the 3-hydroxyanthranilic acid, becomes the carboxyl carbon atom of the nicotinic acid.