UNIVERSITY OF CALIFORNIA

Radiation Laboratory

BERKELEY, CALIFORNIA

DISCLAIMER

UNIVERSITY OF CALIFORNIA
RADIATION LABORATORY

Cover Sheet
Do not remove

INDEX NO. UCRL-112
This document contains 2 pages
and — plates of figures.
This is copy 88 of 108 Series A

[redacted] Issued to: Tech. Div.

[redacted]

RESTRICTED
CLASSIFICATION CANCELLED BY AUTHORITY
OF THE DISTRICT ENGINEER
BY THE DECLASSIFICATION COMMITTEE

Classification

Each person who received this document must sign the cover sheet in the space below.

Route to	Noted by	Date	Route to	Noted by	Date

CONCERNING THE MECHANISM OF THE MAMMALIAN CONVERSION OF TRYPTOPHANE INTO KYNURENINE, KYNURENIC ACID, AND NICOTINIC ACID

by

Charles Heidelberger, Mary E. Gullberg,
Agnes Fay Morgan, and Samuel Lepkovsky

May 26, 1948

UNIVERSITY OF CALIFORNIA
Radiation Laboratory
Berkeley, California

Contract #W-7405-Eng-48

Standard Distribution	Copy Nos.
Argonne National Laboratory	1-10
Armed Forces Special Weapons Project	11
Atomic Energy Commission	12-13
Battelle Memorial Institute	14
Brookhaven National Laboratory	15-22
Carbide & Carbon Chemicals Corp. (K-25 Area)	23-26
Carbide & Carbon Chemicals Corp. (Y-12 Area)	27-30
Columbia University (Failla)	31
General Electric Company	32-35
Hanford Directed Operations	36-42
Iowa State College	43
Kellex Corporation	44-45
Los Alamos	46-48
Massachusetts Institute of Technology	49
Monsanto Chemical Company, Dayton	50-51
National Bureau of Standards	52
Naval Radiological Defense Laboratory	53
NEPA	54
New York Directed Operations	55-57
Oak Ridge National Laboratory	58-69
Patent Advisor, Washington	70
Technical Information Division, ORDO	71-85
UCLA Medical Research Laboratory (Warren)	86
University of California Radiation Laboratory	87-91
University of Rochester	92-93
Western Reserve University (Friedell)	94-97
Declassification Procedure	98-107
Office of Chicago Directed Operations	108
Total	108

Information Division
Radiation Laboratory
University of California
Berkeley, California

CONCERNING THE MECHANISM OF THE MAMMALIAN CONVERSION OF TRYPTOPHANE INTO KYNURENINE, KYNURENIC ACID, AND NICOTINIC ACID

By

Charles Heidelberger, Mary E. Gullberg,

Agnes Fay Morgan, and Samuel Lepkovsky

From the Department of Chemistry and the Radiation Laboratory,
University of California, Berkeley
The Department of Home Economics and the Division of Poultry
Husbandry, Agricultural Experiment Station,
University of California, Berkeley

May 26, 1948

ABSTRACT

dL-tryptophane-β-C^{14} was administered to rabbits, dogs, and rats. The kynurenine and kynurenic acid, isolated from the urines were radioactive, the labeled carbon being the methylene carbon and the 3-position respectively. The N-methylnicotinamide was not radioactive, indicating that it was produced from tryptophane by some mechanism other than ring-closure to a quinoline derivative, followed by oxidation.

This paper is based upon work performed under Contract #W-7405-Eng-48 with the Atomic Energy Commission in connection with the Radiation Laboratory, University of California, Berkeley.

To be published in Journal of Biological Chemistry

CONCERNING THE MECHANISM OF THE MAMMALIAN CONVERSION OF TRYPTOPHANE INTO KYNURENINE, KYNURENIC ACID, AND NICOTINIC ACID

Charles Heidelberger, Mary E. Gullberg,

Agnes Fay Morgan, and Samuel Lepkovsky (1)

From the Department of Chemistry and the Radiation Laboratory, University of California, Berkeley
The Department of Home Economics and the Division of Poultry Husbandry, Agricultural Experiment Station, University of California, Berkeley

May 26, 1948

The metabolic conversion of tryptophane (I) into kynurenine (II) (2) (3), kynurenic acid (III) (4), and nicotinic acid (IV) (5) (6) has been demonstrated by feeding experiments in a variety of mammals. That kynurenine is an intermediate in the conversion of tryptophane into nicotinic acid by neurospora (7) has been established, and recently it has been shown that 3-hydroxyanthranilic acid is also an intermediate in the

---

1. This paper is based on work performed under Contract #W-7405-Eng-48 with the Atomic Energy Commission in connection with the Radiation Laboratory, University of California, Berkeley, California

2. Kotake, Y. and Iwao, J., Zeit. Physiol. 195, 139 (1931).

3. Butenandt,A.,Weidel,W.,Weichert,R., & vonDerjugin,W., Zeit. Physiol., 279, 27 (1943).

4. Ellinger, A., Zeit. Physiol., 43, 325 (1904).

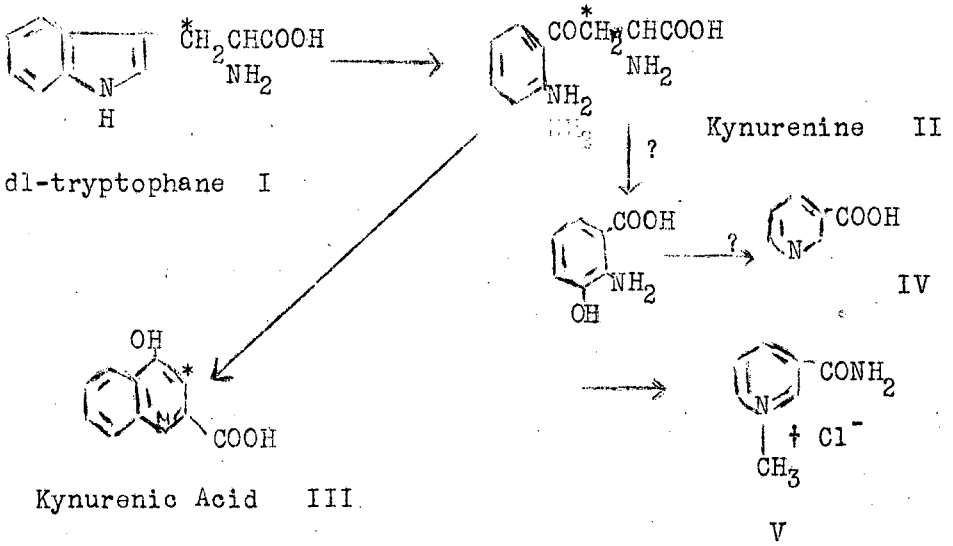
5. Huff, J.W., and Perlzweig, W. H., J. Biol. Chem., 150, 395 (1943).

6. Singal, S.A., Briggs, A.P., Sydenstriker, V.P., & Littlejohn, J.M., J. Biol. Chem., 166, 573 (1946).

7. Beadle, G.W., Mitchell, H.K., Nyc, J.F., Proc. Nat. Acad. Sci., 33, 155 (1947).

neurospora (8) and probably in the rat (9). In animal experiments the nicotinic acid is excreted largely as the N-methyl amide (V), and it has been shown in experiments with C^{13} that when carboxyl-labeled nicotinic acid is fed to rats, the N-methylnicotinamide isolated from the urine retains a very high percentage of the initial label (10).

We have synthesized dl-tryptophane-β-C^{14} (11) and are studying the mechanism of these conversions:



The tryptophane was administered to rabbits, dogs, and rats, and from the urines kynurenine, kynurenic acid, and N-methylnicotinamide, respectively, were isolated by the usual methods (2), (4), (6). The

---

8. Mitchell, H.K., and Nyc, J.F., Proc. Nat. Acad. Sci. 34, 1 (1948).
9. Mitchell, H.K., Nyc, J.F., and Owen, R.D., J. Biol. Chem., in press.
10. Hundley, J.M., and Bond, H.W., J. Biol. Chem., 173, 513 (1948).
11. Heidelberger, C., unpublished material.

specific activities of the kynurenine and kynurenic acid were 37% and 29% of that expected for direct conversion, indicating dilution by the body pool of tryptophane.

The position of the label in the kynurenine (sp. act., 114 counts/min /mg.) was proved by its conversion by means of alkaline hypoiodite to iodoform (sp. act., 81 counts/min/mg; calcd. 78), which could only have originated from the methylene carbon atom. The kynurenic acid (sp. act. 143 counts/min/mg.) was decarboxylated thermally to give inactive carbon dioxide. It was also oxidized to o-carboxyoxanilide (sp. act., 125 counts/min/mg. Calcd. 130)(12) which was hydrolyzed to give active oxalic acid (sp. act. as calcium oxalate, 188 counts/min/mg. Calc'd., 198) and was also decomposed thermally to give radioactive carbon dioxide (sp. act. as $BaCO_3$ 129 counts/min/mg. Calc'd., 128) and inactive carbon monoxide. Formylanthranilic acid, the probable intermediate in this decomposition, liberates carbon monoxide on heating. These reactions strongly indicate, but do not absolutely prove, that the labeled carbon atom is in the 3-position of kynurenic acid.

If the nicotinic acid were produced by oxidation of the benzenoid ring of a molecule such as kynurenic acid, which already contains a pyridine ring, it would be radioactive, following administration of the labeled tryptophane. If, however, hydroxyanthranilic acid is the intermediate (ane we have shown that feeding this compound results in a substantial increase in N-methylnicotinamide excretion) the labeled carbon

---

12. Kretschy, M., Monat 5, 16 (1884).

would be lost, and the nicotinic acid would be inactive. Accordingly, the labeled tryptophane was fed to rats, and after carrier N-methylnicotinamide was added, it was isolated as the picrate (6) and a plate containing 12.3 mg. of picrate was completely devoid of activity. Fluorometric analysis of an aliquot of the Permutit eluate before the carrier was added, indicated the presence of 115 γ (over three times the N-methylnicotinamide found in the urine of rats given no tryptophane). If the compound had been formed through a quinoline intermediate, the plate would have contained 103 counts/min. Less than 0.005% of this could easily be detected with the Nucleometer.

Thus, we have shown as formulated above that kynurenine is formed from tryptophane by direct opening of the pyrrole ring; kynurenic acid by direct ring-closure from kynurenine; and nicotinic acid by a mechanism, probably involving hydroxyanthranilic acid, which does not go through a quinoline intermediate.