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*KYNURENINE AS AN INTERMEDIATE IN THE FORMATION OF
NICOTINIC ACID FROM TRYPTOPHANE BY NEUROSPORA**

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For a number of years it has been clear that nicotinic acid is synthesized by at least some mammals. The amount of this vitamin retained in the body of a rat plus that excreted may far exceed the dietary intake.¹⁻³ On a diet containing adequate protein the dog appears not to need exogenous nicotinic acid.⁴ Recently it has been shown that nicotinic acid synthesis in the rat,^{3, 5-7} man,^{8, 9} the horse¹⁰ and the cotton rat¹⁰ is increased when the diet is high in tryptophane.

It has likewise been shown by Woolley¹¹ and by Kodicek, Carpenter and Harris¹² that nicotinic acid and tryptophane are interchangeable in counteracting the pellagragenic effects of 3-acetyl pyridine, indole-3-acetic acid, and a factor present in corn that may be related to one of these.

As pointed out by Rosen, Huff and Perlzweig⁵ these facts suggest that tryptophane may serve as natural precursor of nicotinic acid. Other interpretations are possible, however.¹³

Evidence in support of the hypothesis that tryptophane is a precursor of nicotinic acid and that kynurenine is an intermediate in the conversion comes from a study of a mutant strain of *Neurospora crassa*. This mutant strain, designated 65001, descended from a conidium irradiated with 9400 ergs of ultraviolet per square millimeter. The manner of treatment and of isolating and detecting mutant strains has been described elsewhere.¹⁴

Mutant strains of *Neurospora* requiring exogenous tryptophane for growth are known.¹⁵ Other strains have been studied that will not grow unless nicotinic acid or a related compound is supplied in the medium.¹⁶ In certain of these strains the requirements are specific. Tryptophaneless strain 10575 will not respond to nicotinic acid, and nicotinic acid-requiring strains 3416 and 4540 will not grow on tryptophane. Strain 65001, on the other hand, grows if supplied with *either* tryptophane or nicotinic acid.

In outcrosses to wild-type strains, mutant 65001 behaves as though it differed from normal by a single gene. Asci from the cross carry four ascospores that give rise to cultures that grow in the absence of both tryptophane and nicotinic acid and four spores that give cultures which, like the mutant parent, require either but not both of these growth factors. The gene in which 65001 and wild type differ is not sex linked. Its genetic relation to genes carried in mutant form by previously reported tryptophaneless and nicotinicless strains has not yet been completely established.

The behavior of strain 65001 suggested to us that it, like the rat, might be able to make nicotinic acid from tryptophane. Kynurenine, known as a product of tryptophane metabolism in mammals,^{17,18} insects^{19, 20} and bacteria,²⁰ suggested itself as a possible intermediate in this process. Tests of *l*-kynurenine, isolated from the urine of rabbits and kindly made available to us by Professor S. Lepkovsky of the University of California, showed it to be active in promoting growth of mutant 65001. As determined by three-day growth in 125-ml. flask cultures,¹⁶ approximately 1.6 micromoles of *l*-kynurenine gives half-maximum growth. The corresponding values for nicotinamide and *l*-tryptophane are 1.4 and 9.8 micromoles, respectively.

Following the method of Butenandt, *et al.*^{21, 22} *dl*-kynurenine was synthesized. It proved to have half the activity of the natural material showing that the *d*-isomer is not utilized as a growth factor by 65001.

Like the mammal, strain 65001 produces an excess of nicotinic acid (or amide) when grown in the presence of an excess of tryptophane but not when grown on an amount just sufficient for growth. Thus four 500-ml. flask cultures of 65001, made up with 100 ml. of culture medium each, two containing 2 mg. *dl*-tryptophane per 100 ml. medium and two containing four times as much, were grown for seven days and the culture medium tested for nicotinic acid. The lesser amount of tryptophane was adequate for growth of strain 65001. Following replenishment of sugar and biotin, the culture media were tested for ability to support growth of mutant 3416, a strain which appears to be specific for nicotinic acid or nicotinamide.¹⁶ The media initially high in tryptophane gave good growth while those initially low in tryptophane gave very little. While it is conceivable that the compound accumulated is not nicotinic acid or nicotinamide but a compound of unknown nature with similar biological activity, this seems unlikely.

An experiment made in a similar manner shows that when 65001 is supplied *dl*-kynurenine in an amount just sufficient for maximum growth (500 micrograms per 20-ml. medium), no nicotinic acid is given off in the culture medium in 12 days. But when the kynurenine is increased fourfold, nicotinic acid accumulates in the culture medium in an amount sufficient for maximum growth of strain 3416—at least 0.5 microgram per milliliter of culture medium.

These experiments lend strong support to the view that *Neurospora* converts tryptophane to nicotinic acid by way of kynurenine.

Since kynurenine is active as a growth factor for strain 39401 which was used as an assay strain by Bonner and Beadle¹⁶ in the isolation of two nicotinic acid precursors accumulated by strain 4540, the question arises as to the relation of kynurenine to these precursors. Elementary analyses of the strain 4540 precursors reported by Bonner and Beadle show that they cannot be kynurenine, a conclusion consistent with independent evidence indicating that they both contain a pyridine ring.¹⁶ A reëxamination of the properties of mutant 39401 shows that it, like mutant 65001, grows when supplied tryptophane. These two strains may be alike genetically; attempts to cross them have so far been unsuccessful.

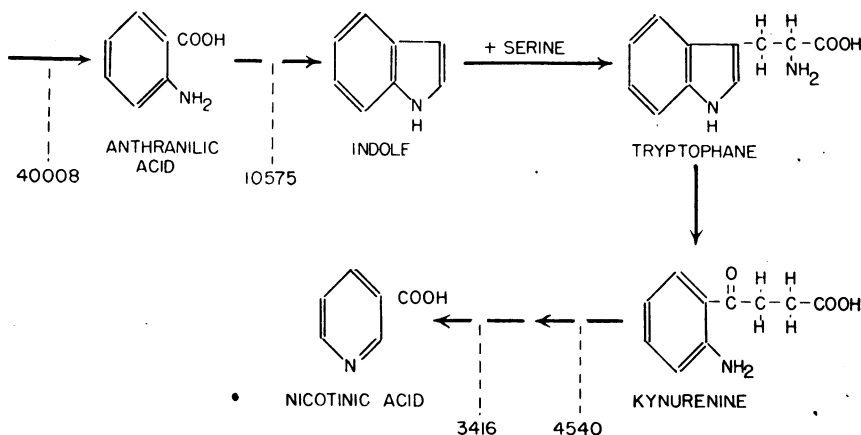


FIGURE 1

Proposed scheme of synthesis of tryptophane and nicotinic acid in *Neurospora*.

On the basis of the evidence so far available, we suggest that the synthesis of tryptophane and nicotinic acid in *Neurospora* takes place according to the scheme shown in figure 1. It is clear that this scheme is incomplete. The part played in it by the gene in which strain 65001 differs from wild type is not yet entirely clear but we suspect that it is concerned with one of the steps between anthranilic acid and indole. In addition the evidence strongly suggests that one of the functions of nicotinic acid is to act catalytically in the synthesis of tryptophane.

Summary.—On the basis of the growth responses of *Neurospora* mutant strains to tryptophane, nicotinic acid and kynurenine, it is postulated that nicotinic acid is normally formed from tryptophane with kynurenine as an intermediate. If this conclusion is correct, kynurenine has a biological significance not heretofore suspected. Whether kynurenine serves as an in-

intermediate between tryptophane and nicotinic acid in the mammal remains to be determined.

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ON THE EVOLUTION OF THE GENUS *NICOTIANA**

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Taxonomic Status.—The relatively advanced family *Solanaceae* is allied to such families as the *Labiatae*, *Hydrophyllaceae*, *Boraginaceae* and *Polemoniaceae* but most closely related to the *Nolanaceae* and *Scrophulariaceae*. The approximately seventy-five genera have been organized in five to nine