Effect of Niacin Precursors on the Excretion of Niacin Metabolites.* (17740)

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The interchangeability of tryptophan and niacin in the nutrition of rats receiving rations low in protein has indicated the utilization of this amino acid in the synthesis of the vitamin(1-3). It has been shown in work with Neurospora(4,5) and later with animals(6,7) that kynurenine, hydroxy-kynurenine and hydroxy-anthranilic acid are intermediates in this conversion. Investigations by Ellinger *et al.*(8) have shown that an increase in the nicotinamide content of suspensions of rat cecal contents occurs when glutamic acid, arginine or ornithine was added to the media. On the basis of these observations, they suggested that nicotinamide was synthesized from glutamic acid and arginine with ornithine as an intermediate. The observation by Teply *et al.*(9), that the type of carbohydrate in the diet markedly influenced the niacin level of rat cecal contents, indicated some synthesis of niacin by the intestinal bacteria. However, work with enterectomized rats has suggested that a larger percentage of the synthesis occurs in the tissues(10). Further work with isolated tissues has indicated the liver and kidneys of the rat are the possible sites of synthesis(11).

Recently Henderson(12) found that quinolinic acid was the compound in rat urine, which on acid treatment (decarboxylation) gave niacin activity for *L. arabinosus*. On the basis of this observation he suggested that quinolinic acid was probably the intermediate compound between hydroxy-anthranilic acid and niacin. Further work by Henderson(13) has shown that the incubation of liver homogenates with added 3-hydroxy-anthranilic acid caused an increase in the quinolinic acid content of the suspension. Additional experiments by Bonner(14) with mutant strains of Neurospora have shown that one of the mutants, grown in the presence of 3-hydroxy-anthranilic acid, can also accumulate quinolinic acid. However, these workers have not been able to find a mutant of Neurospora which can utilize quinolinic acid. Bonner suggests that quinolinic acid is not the intermediate compound, but that after the 3-hydroxy-anthranilic acid ring is split in the 3,4 position a decarboxylation occurs with subsequent ring closure to form niacin.

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In addition to the compounds proposed as intermediates in synthesis of niacin another possible intermediate between 3-hydroxy-anthranilic acid and niacin is isocinchomeronic acid, which would be the dicarboxylic acid obtained if hydroxy-anthranilic acid had been split in the 2,3 position. A study was therefore conducted to determine the actual extent of synthesis of niacin and its metabolites in the intact animals, when these compounds were fed.

Experimental. Rats of the Sprague-Dawley strain weighing 170-290 g were placed in individual metabolism cages for a period of several weeks and fed (*ad libitum*) a 9% casein basal which has been used repeatedly for tryptophan and niacin studies(11). Control samples of urine and feces were collected from each animal over a 3-day period and then the animals were divided into groups of three. After the feeding of the compounds under study, three-day samples of urine and feces were again collected from each animal. Three-day periods were used because previous work in this laboratory(10) showed that after an injection of a massive dose (300 mg) of tryptophan the urine niacin values returned to normal within this time. The compounds fed were anthranilic acid, ornithine, quinolinic acid, isocinchomeronic acid, tryptophan and anthranilic acid plus ornithine.

The isocinchomeronic acid was made by the oxidation of quinaldine according to the method of Soine(15), while the other compounds were obtained commercially. All compounds were fed on an equimolar basis with 50 mg of tryptophan. In the first experiment the compounds (in aqueous solution) were given by stomach tube to the animals in 2½ ml doses, which were spaced three hours apart. In a second experiment the compounds were given in a single, 5 ml dose. The urine samples were collected under toluene. At the end of the collection periods, they were made up to volume, filtered and stored at 5°C under toluene until analyzed.

N'methyl nicotinamide was determined by the acetone-fluorometric method of Huff *et al.*(16). Another portion of each urine was neutralized, diluted, and assayed for niacin by the microbiological assay(17). This value was termed "free" niacin. Work by Henderson(18) has shown that the complete decarboxylation of quinolinic acid in urine is obtained by treatment with glacial acetic acid. Therefore, a third portion of each urine (2 ml) was evaporated to dryness and autoclaved for 3.5 hours at 15 pounds pressure with 0.5 ml of glacial acetic acid. After neutralizing, "total" niacin was determined microbiologically(17). Samples of feces (0.2 g) from the control animals were hydrolyzed by autoclaving for 3.5 hours at 15 lb pressure with 0.5 ml of glacial acetic acid. All feces from the experimental period were ground in a mortar before sampling and then given the same treatment for determination of "total" niacin content. The compounds used in this study gave no activity for niacin when assayed with *L. arabinosus*.

Results and discussion. An increase in the free niacin content of the urine excreted by all animals could be detected after the feeding of the various metabolites (Table I). However, this increase was not significant in the case of ornithine, anthranilic acid or isocinchomeronic acid. It is evident that the increase in free niacin excretion due to the feeding of quinolinic acid was not as great as that obtained with an equimolar quantity of tryptophan. A much greater increase in free niacin should be obtained with this metabolite, if it were a direct precursor of niacin. On the other hand it is possible that the quantity of quinolinic acid administered was too large and it had a depressing effect on the enzyme system converting quinolinic acid to niacin. The much higher excretion of free niacin obtained after tryptophan administration was probably due to the fact that the chain of reactions for niacin synthesis from this amino acid is long enough to prevent any toxic ef-

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TABLE I.
Effect of Precursors on Excretion of Nicotinic Acid and Its Derivatives by Rats.*

Supplement	Animal wt	μg per 24 hr							
		Urinary excretion						Fecal excretion	
		Free nicotinic acid		Total nicotinic acid after acid treatment		N'methyl-nicotinamide†		Total nicotinic acid after acid treatment	
		Control	Metabolite	Control	Metabolite	Control	Metabolite	Control	Metabolite
50 mg tryptophan	239	14	56	46	1427	14.2	272	14.8	49
41 mg quinolinic acid	210	12.8	26.8	41	1344	12.5	456	19.4	1539
32.4 mg ornithine	252	12.6	15.5	62	64	8.2	47	18.2	29
41 mg isocinchomeronic acid	272	13	20	57	208	10.3	164	21.2	17
33.5 mg anthranilic acid	217	13.2	15	59	68	8	207	16.7	20
33.5 mg anthranilic acid + 32.4 mg ornithine	218	10.4	15.8	64	62	8.7	153	18.4	28

* All values in table are the average of 3 animals.
† Grateful acknowledgment is made to Richard L. Lyman for conducting the N'methyl nicotinamide determinations.

fects of a massive dose of the starting material. These results compare very well with excretion data obtained by Henderson(16). In his experiments he compared the effect of daily injection of equimolar quantities of tryptophan, 3-hydroxy-anthranilic acid and quinolinic acid.

It is evident that the material in urine active for *L. arabinosus*, after acid treatment (quinolinic acid), was markedly increased when either tryptophan, quinolinic acid or isocinchomeronic acid was administered. The rather large increase after the feeding of tryptophan or quinolinic acid could be due to a limiting rate of reaction in the formation of niacin from quinolinic acid. The decarboxylation of quinolinic acid to niacin could be a much slower reaction than any of those preceding it, therefore the excess quinolinic acid spills over into the urine. This theory apparently can be applied only to tryptophan and quinolinic acid. Preliminary work with isocinchomeronic acid indicated that the autoclaving with glacial acetic acid did not alter (decarboxylate) the isocinchomeronic acid molecule. This compound gave no niacin activity for *L. arabinosus* either before or after treatment with glacial acetic acid. With this thought in mind it is evident that the increase in the total niacin value after the feeding of isocinchomeronic acid, could not be the result of decarboxylation of isocinchomeronic acid, but must have come from the decarboxylation of quinolinic acid. Apparently the isocinchomeronic acid either stimulated the enzyme system involved in the formation of quinolinic acid or it protected the quinolinic acid from destruction by the tissue enzymes.

The N'methyl nicotinamide content of the urines increased 15 to 40 times over the controls after the administration of the various metabolites. Again the increases obtained with tryptophan, quinolinic acid and isocinchomeronic acid were the greatest. However, in this case, quinolinic acid gave a much higher value than tryptophan. It is possible that the animal metabolized some of the quinolinic acid to nicotinamide and then to N'methyl nicotinamide. On the other hand, it is also possible that the massive dose of quinolinic acid administered to the animals caused

the formation of a methylated quinolinic acid, which was active in the fluorometric method used. It is interesting that several of the compounds caused increases in the excretion of N'methyl nicotinamide without a corresponding increase in the free and total niacin. Apparently these compounds stimulate the formation of a compound in urine which is active in the N'methyl nicotinamide determination.

The analyses of the feces for nicotinic acid revealed that the influence of the various compounds in the intestinal tract was quite variable. Increases in fecal niacin occurred with the feeding of all compounds except isocinchomeronic acid. Evidently the high level of this compound in the diet was toxic to the bacteria in the intestinal tract, and it depressed the synthesis of the vitamin. If the increases in the fecal niacin obtained in the anthranilic acid and ornithine groups are considered significant, these compounds undoubtedly acted by stimulation of the bacteria in the intestinal tract. It may also be suggested that these two compounds are apparently utilized by the bacteria in the tract and not by the tissues for the synthesis of the vitamin. The niacin synthesized in the tract in this case could be in a bound (unavailable) form. The high fecal niacin value for the animals receiving quinolinic acid was obviously due to the quantity of the compound that was not absorbed. However, this quantity amounts to only about one-eighth of the quinolinic acid fed and indicated that the compound is fairly well absorbed. If the increase in the various niacin metabolites in the urine and in the feces of the quinolinic acid group are summated and calculated back to quinolinic acid, the value represents only 30% of the quinolinic acid fed, which indicates that about 70% of it was metabolized by the animal. Whether it was metabolized as niacin or passed out into the urine as some other metabolite such as the pyridone(19) is not known.

The data obtained in the second experiment in which the compounds were fed in a single 5 ml dose compared very well with the data already discussed. The only variation was a slightly lowered excretion of N'methyl nicotinamide for those animals receiving quinolinic acid.

It is evident from the data reported that isocinchomeronic acid is not involved in the synthesis of niacin. Ornithine and anthranilic acid apparently have some stimulatory effect on the bacteria in the tract which synthesize the vitamin. Considering the effect of these two compounds on the free niacin in the urine, evidently they do not influence the synthesis of the vitamin in the tissues. The data presented on quinolinic acid neither prove nor disprove that this compound is a possible intermediate between 3-hydroxy-anthranilic acid and niacin. However, it is evident that quinolinic acid is utilized by some enzyme system, which may be hindered by the presence of isocinchomeronic acid, and thereby cause the accumulation of quinolinic acid, which is eventually excreted. The combination of anthranilic acid plus ornithine was fed, because growth data in other experiments showed that the combination of these two compounds overcame the growth reduction due to the supplementation of 0.2% DL-phenylalanine to a 9% casein ration(20).

Conclusions. 1. The effect of tryptophan, quinolinic acid, ornithine, isocinchomeronic acid, and anthranilic acid on the ability of the rat to form niacin derivatives has been determined.

2. Isocinchomeronic acid does not appear to be an intermediate in the synthesis of niacin.

3. Ornithine and anthranilic acid seem to stimulate the synthesis of niacin in the intestinal tract only.

4. The results neither prove nor refute the theory that quinolinic acid is a possible intermediate in the tryptophan niacin synthesis scheme.

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