British Journal of Nutrition
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British Journal of Nutrition / Volume 48 / Issue 01 / July 1982, pp 119 - 127
DOI: 10.1079/BJN19820094, Published online: 09 March 2007

Link to this article: http://journals.cambridge.org/abstract_S0007114582000178

How to cite this article:
David A. Bender, Bahieldin I. Magboul and David Wynick (1982). Probable mechanisms of regulation of the utilization of dietary tryptophan, nicotinamide and nicotinic acid as precursors of nicotinamide nucleotides in the rat. British Journal of Nutrition,48, pp 119-127 doi:10.1079/BJN19820094

Probable mechanisms of regulation of the utilization of dietary tryptophan, nicotinamide and nicotinic acid as precursors of nicotinamide nucleotides in the rat

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(*Received 13 October 1981 – Accepted 15 February 1982*)

1. The regulation of the utilization of dietary tryptophan, nicotinamide and nicotinic acid as precursors of the nicotinamide nucleotides has been studied in groups of rats fed on diets providing only one of these precursors at a time, in amounts adequate to meet their requirements for nucleotide synthesis.

2. The concentration of nicotinamide nucleotides in the liver of rats receiving a high-tryptophan diet was 56% higher than in animals fed on a diet providing a minimum amount of tryptophan, together with nicotinic acid or nicotinamide. The excretion of N^1-methyl nicotinamide was three times higher in the tryptophan-fed animals than in the other two groups.

3. The concentration of quinolinic acid in the liver was significantly higher in animals receiving the high-tryptophan diet than in the other two groups; that of nicotinic acid was highest in those animals receiving the nicotinic-acid-containing diet. The concentration of nicotinamide was highest in the livers of those animals receiving the high-tryptophan diet, and lowest in those receiving the nicotinic-acid-containing diet.

4. The values of the Michaelis constant K_m of nicotinamide deamidase (nicotinamide amidohydrolase, *EC* 3.5.1.19) and nicotinamide phosphoribosyltransferase (nicotinamide nucleotide: pyrophosphate phosphoribosyltransferase, *EC* 2.4.2.12) were approximately equal, and approximately one-tenth of the concentration of nicotinamide was in the liver. This suggests that both these enzymes normally function at their maximum rate, and a change in the availability of nicotinamide would not affect the rate of its incorporation into nucleotides.

5. The maximum rate of reaction (V_{max}) of nicotinamide deamidase was twice that of nicotinamide phosphoribosyltransferase; this suggests that unless compartmental or other factors are involved, the major route of nicotinamide utilization will be by way of deamidation.

6. The K_m of nicotinate phosphoribosyltransferase (nicotinate nucleotide : pyrophosphate phosphoribosyltransferase, *EC* 2.4.2.11) was less than twice the concentration of nicotinic acid in the liver, so that a change in the availability of nicotinic acid might be expected to lead to a small change in the rate of its utilization.

7. The K_m of quinolinate phosphoribosyltransferase (nicotinate nucleotide : pyrophosphate phosphoribosyltransferase (carboxylating) *EC* 2.4.2.19) was approximately 100 times greater than the concentration of quinolinic acid in the liver, so that any change in the availability of quinolinic acid would be expected to lead to a considerable change in the rate of its utilization. The V_{max} of quinolinate phosphoribosyltransferase was relatively low, so that under conditions of high tryptophan flux, some accumulation of quinolinic acid might be expected. This was observed in animals receiving the high-tryptophan diet.

8. It is concluded that it is unlikely that the utilization of quinolinic acid, arising from tryptophan, for the synthesis of nicotinamide nucleotides is regulated, but that control over tissue concentrations of nucleotides is achieved by hydrolysis of NAD to nicotinamide. Incorporation of nicotinamide into nucleotides seems to be strictly limited by the activity of the enzymes involved.

Both the amino acid tryptophan and the niacin vitamers, nicotinamide and nicotinic acid, can act as precursors of the nicotinamide nucleotides, NAD and NADP. The relevant pathways are shown in Fig. 1.

Gadd & Johnson (1974) suggested that most dietary niacin is present as nicotinamide rather than as the acid, although they did not cite any reference for this statement. The amide is also the immediate product of the hydrolysis of tissue nicotinamide nucleotides, catalysed by NADase (NAD glycohydrolase, *EC* 3.2.2.5). This nicotinamide may either be available for the synthesis of nicotinamide nucleotides, or it may be methylated to N^1-methyl nicotinamide and excreted.

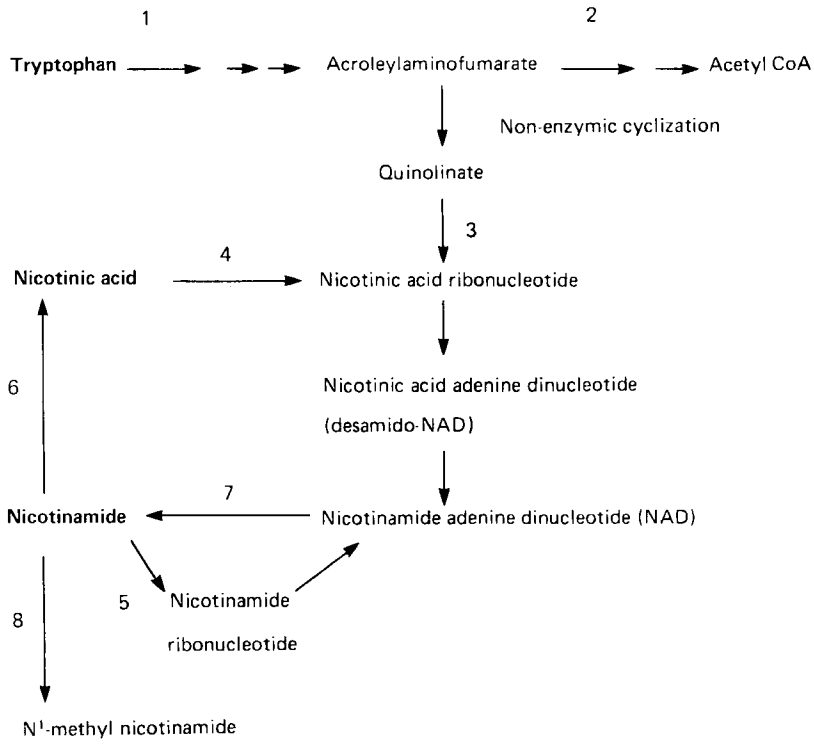

Fig. 1. Pathways of nicotinamide nucleotide synthesis and metabolism. (1) Tryptophan oxygenase (L-tryptophan:oxygen oxido-reductase, *EC* 1.13.1.12); (2) picolinate carboxylase (3'-oxo-prop-2-amino-but-2-ene dioate carboxylase, *EC* 4.1.1.45); (3) quinolinate phosphoribosyltransferase (nicotinate nucleotide:pyrophosphate phosphoribosyltransferase (carboxylating), *EC* 2.4.2.19); (4) nicotinate phosphoribosyltransferase (nicotinate nucleotide:pyrophosphate phosphoribosyltransferase, *EC* 2.4.2.11); (5) nicotinamide phosphoribosyltransferase (nicotinamide nucleotide:pyrophosphate phosphoribosyltransferase, *EC* 2.4.2.12); (6) nicotinamide deamidase (nicotinamide amido-hydrolase, *EC* 3.5.1.19); (7) NADase (NAD glycohydrolase, *EC* 3.2.2.5); (8) nicotinamide *N*-methyltransferase (*S*-adenosyl-methionine:nicotinamide *N*-methyltransferase, *EC* 2.1.1.1).

There is disagreement about the relative importance of the two possible routes of utilization of nicotinamide for nucleotide synthesis: direct incorporation in a reaction catalysed by nicotinamide phosphoribosyltransferase (nicotinamide nucleotide:pyrophosphate phosphoribosyltransferase, *EC* 2.4.2.12) or deamidation to nicotinic acid catalysed by nicotinamide deamidase (nicotinamide amidohydrolase, *EC* 3.5.1.19), followed by incorporation into nicotinic acid nucleotide, catalysed by nicotinate phosphoribosyltransferase (nicotinate nucleotide:pyrophosphate phosphoribosyltransferase, *EC* 2.4.2.11). Petrack *et al.* (1963) showed that there was adequate activity of nicotinamide deamidase in rat liver to account for the utilization of all the normal dietary intake of nicotinamide, without any requirement for a phosphoribosyltransferase. However, Grunicke *et al.* (1974), working with perfused rat liver, showed direct incorporation of [^{14}C]nicotinamide into NAD, with no labelling of nicotinic acid or desamido-NAD. Delabar & Siess (1979) obtained a similar result when isolated guinea-pig atria were incubated with a low (10 μM) concentration of nicotinamide. When the concentration of nicotinamide was increased to 20 mM, they showed that deamidation became an important route of utilization.

It is not known whether the size of the tissue pools of nicotinamide nucleotides is regulated primarily by control of the utilization of the precursors (nicotinic acid, nicotinamide and quinolinic acid), and therefore by regulation of the activities of the phosphoribosyltransferases and nicotinamide deamidase, or whether control is by variation of the activity of NADase, and hence catabolism of nucleotides formed in excess of requirements.

In the present study we have attempted to define regulatory steps in the synthesis of nicotinamide nucleotides from the three possible dietary sources, and also to clarify the relative importance of the two possible routes of nicotinamide utilization (deamidation and direct incorporation into nucleotides). Rats were fed on diets providing their niacin requirements from each of the precursors separately, and tissue concentrations of nucleotides and precursors, urinary excretion of N^1-methyl nicotinamide and the activities of the enzymes involved were assessed. The results suggest regulation of the utilization of nicotinamide and nicotinic acid, but not of quinolinic acid, arising from the oxidative metabolism of tryptophan.

METHODS

Animals and diets

Male Wistar rats, bred in the Courtauld Institute, were used. At 21 d after birth they were weaned onto one of the experimental diets described, and were maintained in groups of five in grid-based cages to minimize coprophagy. After 28 d they were housed individually in stainless-steel metabolism cages (Acme Metal Co, Chicago, Ill, USA), for 24 h to permit collection of urine. They were killed by decapitation; blood was collected into heparinized vessels on ice and was used immediately for the determination of nicotinamide nucleotides. Livers and kidneys were dissected out, frozen in liquid nitrogen within 30 s of decapitation and were stored in liquid nitrogen until they were used for the determination of nicotinamide nucleotides, and nicotinamide, nicotinic acid and quinolinic acid. The remainder of each frozen liver was stored at $-20°$ until it was required for the determination of enzyme activities.

The composition of the diets used is shown in Table 1; they were modified from that described by Carter *et al.* (1977). The vitamin mixture provided the recommended amounts ((US) National Research Council, 1962) of all vitamins except niacin. As described previously (Bender, 1980*b*) the diet was prepared as a jelly, made by dissolving the gelatin in warm water, then adding the mixed dry ingredients. This presentation, which was accepted well by the animals, simplified the addition of small amounts of tryptophan and niacin to the diets, and minimized blockage of the urine-faeces separators of the metabolism cages by particles of food. Three diets were used: diet 1 provided no niacin, but 1·9 g of tryptophan /kg dry matter (DM); diets 2 and 3 provided 0·9 g tryptophan and 15 mg nicotinamide (diet 2) or nicotinic acid (diet 3) /kg DM. These are the (US) National Research Council (1962) recommended amounts of niacin for the rat.

Analytical methods

Tissue and blood concentrations of total nicotinamide nucleotides (NAD^+, $NADP^+$, NADH and NADPH) were measured by the following modification of the methods described by Kaplan *et al.* (1951) and Lowry *et al.* (1961). Approximately 500 mg of tissue were homogenized, while still frozen, in 5 ml ice-cold dilute hydrogen peroxide (5 ml 100 vol. H_2O_2/l) to oxidize the reduced nucleotides. This homogenate was mixed immediately with 5 ml of a solution of 0·1 M-sodium sulphate in 0·02 M-sulphuric acid, and was heated to 100° for 5 min. After cooling and centrifugation to remove denatured protein (2000 *g*, 15 min), the supernatant fraction was neutralized with 0·2 M-sodium hydroxide. Samples of the supernatant fraction (1 ml) were then mixed with 0·5 ml of water, a standard solution

Table 1. *Composition (g/kg) of diets used*

		Diet		
		1	2	3
Maize meal	580			
Gelatin	60			
Vitamin-free casein	28			
Maize oil*	25			
Mineral salt mixture†	41·1			
Amino acid mixture‡	9·2			
Vitamin mixture‖	2			
Tryptophan		1 g		
Nicotinamide			15 mg	
Nicotinic acid				15 mg
Sucrose to 1000 g				

* The following were dissolved in the maize oil to provide (/kg diet): retinol palmitate 10 mg, cholecalciferol 3 μg, menaphthone 1 mg, α-tocopheryl acetate 75 mg.

† The mineral salt mixture provided (/kg diet): $CaHPO_4$ 18 g, $CaCO_3$ 4·5 g, KCl 8·2 g, Na_2HPO_4 7·4 g, $MgSO_4 . H_2O$ 2·8 g, $MnSO_4 . 4H_2O$ 361 mg, ferric citrate . 5 H_2O 349 mg, $ZnCO_3$ 60 mg, $CuSO_4 . 5H_2O$ 47 mg, KIO_3 2 μg.

‡ The amino acid mixture provided (g/kg diet): Met 2·25, Ile 1·70, Thr 1·50, Lys 1·49, Val 1·10, Cys 1·00, Trp 0·13.

‖ The vitamin mixture provided (/kg diet): choline bitartrate 1·8 g, calcium pantothenate 40 mg, thiamin hydrochloride 10 mg, riboflavin 10 mg, folic acid 5 mg, pyridoxine hydrochloride 1·2 mg, biotin 1 mg, cyanocobalamin 50 μg, glucose to 2 g.

of NAD^+ (approximately 40 μM) or a solution of NADase from *N. crassa* (Sigma Chemical Co, Poole, Dorset), containing 30 m-units enzyme activity/ml in 0·2 M-sodium phosphate buffer, pH 7·3. The samples containing NADase were then incubated at 37° for 30 min, while the others were kept on ice. After the incubation, all samples were mixed with 2 ml 6 M-NaOH and heated to 100° for 5 min. The fluorescence due to NAD^+ and $NADP^+$ was measured using an Aminco-Bowman spectrophotofluorimeter (activation 360 nm, emission 460 nm). Preliminary studies (B. I. Magboul, unpublished results) showed that separate internal standards and tissue blanks (in which NAD^+ and $NADP^+$ were hydrolysed by NADase) were necessary for each sample because of the presence of variable amounts of fluorescent material (affecting the tissue blank) and absorbing material (affecting the flurorescence yield of the standard) in different samples of tissue. It was also found that there was a considerable loss of fluorescence due to nicotinamide nucleotides if the tissue was stored for more than approximately 3 h before analysis, even in liquid nitrogen.

The concentration of nicotinamide nucleotides in blood was measured as follows: 200 μl freshly-collected heparinized whole blood was mixed with 200 μl H_2O_2, then 2 ml of the acid solution of sodium sulphate was added, as described previously. After centrifugation and neutralization, 300 μl samples of the supernatant fraction were treated in the same way as the tissue samples.

The following enzyme activities were measured in samples of liver that had been stored at −20° for up to 3 weeks, by methods that have been described previously (Bender, 1980*a*): quinolinate phosphoribosyltransferase (nicotinate nucleotide : pyrophosphate phosphoribosyltransferase [carboxylating], *EC* 2.4.2.19), nicotinate phosphoribosyltransferase, nicotinamide phosphoribosyltransferase and nicotinamide deamidase. Preliminary studies (D. A. Bender, unpublished results) showed that there was no detectable loss of enzyme activity on storage at −20° for up to 4 weeks. For the enzyme kinetic studies, livers from

animals that had been fed on stock animal-house diet (Diet 18; A. Dixon & Sons, Ware, Herts) were used. Enzyme activities were determined as described previously (Bender, 1980*a*), using a range of concentrations of substrate, and the values of Michaelis constant (K_m) and maximum rate of reaction (V_{max}) were determined by means of the Lineweaver–Burk double-reciprocal plot. Best-fitting lines were estimated by unweighted linear regression.

The concentrations of nicotinamide, nicotinic acid and quinolinic acid in liver were determined by the following modification of the method described by Carlson (1966). Tissue was stored in liquid nitrogen for up to 3 h after killing, and was then homogenized while still frozen in 1 ml ice-cold water/g. A 10 ml sample of the homogenate was immediately poured into 60 ml acetone containing 5 ml water or an aqueous solution of nicotinamide, nicotinic acid and quinolinic acid, each at a concentration of 1 mM. After mixing, samples were left to stand for 30 min with occasional shaking, and were then centrifuged at 2000 *g* for 20 min. The supernatant fraction was extracted twice with 10 ml chloroform, and duplicate 3 ml samples of the aqueous phase were evaporated to dryness using a vortex evaporator under reduced pressure at 50°. The dry residue from the samples was redissolved in 100 μl acetone–water (50 : 50, v/v), and 50 μl samples were applied to the origins of paper chromatograms for two-dimensional chromatography. The first solvent was propan-2-ol–ammonium hydroxide–water (100:5:10 v/v); R_f values were: nicotinamide 0·80, nicotinic acid 0·44, quinolinic acid 0. The second solvent (perpendicular to the first solvent) was methanol–pyridine–water (160 : 8 : 40 v/v); R_f values were nicotinamide 0·83, nicotinic acid 0·81 and quinolinic acid 0·74. Spots corresponding to these compounds were located by absorbance under u.v. illumination (254 nm), and were removed.

Nicotinamide and nicotinic acid were eluted from the paper by boiling in water for 3 h, then the volume of the eluate was adjusted to 1·5 ml. Areas of paper containing quinolinic acid were heated at 110° overnight in 3 ml glacial acetic acid in sealed vials, to elute the acid and convert it to nicotinic acid, as described by McDaniel *et al.* (1973). The acid solution was evaporated to dryness in a vortex evaporator under reduced pressure at 50°, and the residue was redissolved in 1·5 ml water. Nicotinic acid and nicotinamide were measured colorimetrically ($A_{410\ nm}$) after reaction with 0·5 ml chloramine T (50 g/l) and 0·5 ml KCN (10 g/l Tris-HCl) as described by Carlson (1966). Since internal standards of nicotinamide, nicotinic acid and quinolinic acid were used, no estimation of the recovery of materials through the procedure was performed. In a series of determinations on duplicate samples from the same tissue, the mean coefficient of variation was found to be 4% for nicotinamide and nicotinic acid and 11% for quinolinic acid.

Urinary N^1-methyl nicotinamide was measured by a small-scale modification of the fluorimetric method of Carpenter & Kodicek (1950), as described previously (Bender, 1980*b*).

RESULTS

The animals maintained on diets 2 and 3 grew less well than those receiving diet 1, which provided no preformed niacin, but contained more tryptophan. All animals weighed between 45–55 g on weaning; after 28 d the mean ($\pm$SE) values were (g): diet 1 175 ± 30, diet 2 109 ± 24, diet 3 108 ± 16. This suggests that tryptophan (and hence protein) may have been a limiting factor in diets 2 and 3.

As can be seen from Table 2, the liver content of nicotinamide nucleotides was significantly higher in the animals receiving the high-tryptophan diet than in either of the other two groups. The concentrations of nicotinamide nucleotides in blood and kidney were not significantly different. The urinary excretion of N^1-methyl nicotinamide was three times higher in the tryptophan-fed animals than in the other two groups.

Table 2. *Tissue concentrations of nicotinamide nucleotides and precursors, and liver enzyme activities in rats fed for 28 d from weaning on diets providing their niacin requirements as tryptophan and nicotinamide or nicotinic acid*

(Mean values with pooled standard error, five animals/group)

Diet no.†	1 Mean	2 Mean	3 Mean	Pooled SEM
Nicotinamide nucleotides:				
Liver (μmol/g)	0·58	0·37*	0·37*	0·06
Kidney (μmol/g)	0·36	0·31	0·31	0·05
Blood (mmol/l)	88·7	82·0	82·8	4·87
Urine N^1-methyl nicotinamide (μmol/24 h)	3·3	1·1***	1·3***	0·22
Enzyme activities (nmol/m per g liver):				
Quinolinate phosphoribosyltransferase (*EC* 2.4.2.19)	4·1	3·8	3·7	0·21
Nicotinate phosphoribosyltransferase (*EC* 2.4.2.11)	0·45	0·33*	0·37*	0·03
Nicotinamide phosphoribosyltransferase (*EC* 2.4.2.12)	0·20	0·16	0·18	0·02
Nicotinamide deamidase (*EC* 3.5.1.19)	4·74	4·39	4·39	0·19
Liver quinolinic acid (nmol/g)	6·2	2·8***	2·7***	0·22
Liver nicotinic acid (nmol/g)	28·7	28·9	32·4*	0·94
Liver nicotinamide (nmol/g)	758	626*	554***	33

Mean values were significantly different from diet 1 (*t* test): *$0·05 > P > 0·001$, ***$P < 0·001$

† For details of diets, see Table 1.

Table 3. *Kinetic measurements of enzymes of nicotinamide nucleotide synthesis in livers from rats fed on animal house stock diet*

(Mean values with their standard errors for the replicate determinations)

	K_m (μM)	SEM	V_{max}(nmol/min per g liver)	SEM
Quinolinate phosphoribosyltransferase	550	36	16	1·3
Nicotinate phosphoribosyltransferase	24	4	2·5	0·04
Nicotinamide phosphoribosyltransferase	42	3·6	7·8	0·8
Nicotinamide deamidase	33	3·6	1·6	2·7

K_m, Michaelis constant; V_{max}, maximum rate of reaction.

The enzymes of nicotinamide nucleotide synthesis showed no significant differences with diet, except that nicotinate phosphoribosyltransferase was lower in the nicotinamide-fed animals than in the other two groups.

The concentration of quinolinic acid in the livers of the tryptophan-fed animals was significantly higher than in the other two groups: the same was true of the liver content of nicotinamide, which was lowest in those animals receiving their niacin as nicotinic acid. The liver content of nicotinic acid was higher in animals fed on diet 3 than on either of the other two diets.

The values for K_m of the enzymes measured are shown in Table 3. Nicotinate phosphoribosyltransferase was inhibited by its substrate; the inhibitor constant (K_i) for nicotinic acid was $1·4 \pm 0·1$ mM, compared with a K_m of 24 ± 9 μM.

DISCUSSION

The higher liver content of quinolinic acid in animals fed on the high-tryptophan diet is to be expected, as is the somewhat higher content of nicotinic acid in the livers of animals receiving this precursor in the diet. The liver content of nicotinamide was higher in animals receiving this vitamer than in those receiving nicotinic acid, as might be expected; however, in the animals fed on the high-tryptophan diet the concentration of nicotinamide in the liver was higher than in either of the other two groups. This suggests that there is a considerable increase in the flux of substrates through nicotinamide nucleotides, with release of nicotinamide from NAD as a result NADase activity. This is supported by the observation of a 3-fold higher excretion of N^1-methyl nicotinamide by the tryptophan-fed animals compared with either of the other two groups, although the liver content of nicotinamide nucleotides was only approximately 56% higher. The enzyme kinetic values shown in Table 3, together with the concentrations of substrates in the livers of animals fed on the three diets, shown in Table 2, suggest some possible sites of regulation. Both nicotinamide deamidase and nicotinamide phosphoribosyltransferase are obviously saturated with substrate under normal conditions: the tissue concentration of nicotinamide is more than 10-fold higher than the K_m of either enzyme. This suggests that the utilization of nicotinamide for nucleotide synthesis will proceed at a more or less constant rate, regardless of changes in the availability of nicotinamide.

Nicotinamide phosphoribosyltransferase appears to act below its V_{max}, as the concentration of nicotinic acid in the liver is less than twice the K_m of the enzyme. It is therefore probable that changes in the availability of nicotinic acid would affect slightly the rate of utilization for nucleotide synthesis. However, the magnitude of the change would be relatively small, since the tissue concentration of substrate is close to the K_m of the enzyme.

Since both nicotinamide deamidase and phosphoribosyltransferase act under conditions of substrate availability so much in excess of K_m, the small difference in K_m between the two enzymes will not affect the choice of route to be followed by nicotinamide. However, the higher V_{max} of the deamidase, and the higher activity of the deamidase observed in the livers of the animals receiving all three diets, compared with the phosphoribosyltransferase, suggest that deamidation may be the more important pathway of nicotinamide utilization, assuming that there are no compartmentation effects in vivo. However, it is possible that transport of nicotinamide between different cell compartments may be an important factor; nicotinamide deamidase is a microsomal enzyme, while the phosphoribosyltransferase was measured in the high-speed supernatant fraction of a liver homogenate, and is therefore probably a soluble enzyme.

Delabar & Siess (1979) showed that in isolated guinea-pig atria, nicotinamide was apparently incorporated directly into nucleotides when it was present in low concentrations, with no exchange of label from [^{14}C]nicotinamide with the tissue-free nicotinic acid. It was only when a relatively high concentration of nicotinamide was used that they observed significant deamidation to nicotinic acid. Petrack *et al.* (1965) reported a considerably higher value of the K_m of nicotinamide deamidase than that reported here, apparently due to the presence of an endogenous inhibitor which is removed in the partially-purified preparation of the enzyme used in this study (Pinder *et al.* 1971). Hence it is still not possible to decide to what extent deamidation or direct incorporation into nucleotides is the major fate of nicotinamide in vivo.

There would appear to be a considerable surplus capacity for the incorporation of quinolinic acid into nucleotides; the K_m of this enzyme is considerably higher than the concentration of quinolinic acid in the liver. This suggests that there is no limitation of the

ability to incorporate this substrate into nuclotides, and such quinolinic acid as is formed from tryptophan will be used for nucleotide synthesis.

The synthesis of quinolinic acid is controlled largely by the activity of tryptophan oxygenase (L-tryptophan : oxygen oxido-reductase, *EC* 1 . 13 . 1 . 12), the first enzyme of the tryptophan oxidative pathway, and by the activity of picolinate carboxylase (3′-oxo-prop-2-amino-but-2-ene dioate carboxylase, *EC* 4 . 1 . 1 . 45), which diverts acroleylamino-fumarate into the pathway leading to total oxidation. It is only when picolinate carboxylase is saturated with its substrate that significant amounts of acroleylaminofumarate accumulate and undergo the non-enzymic cyclization to quinolinic acid (Nishizuka & Hayaishi, 1963).

The activity of tryptophan oxygenase is modified by the availability of tryptophan (Greengard & Feigelson, 1961; Schimke *et al.* 1965*a*) and the hormonal status of the animal (Schimke *et al.* 1965*a*, *b*). In man the ability to utilize tryptophan as a source of nicotinamide nucleotides varies considerably from one individual to another. The widely-quoted equivalence of 60 mg tryptophan : 1 mg nicotinamide was suggested by Horwitt *et al.* (1956) to ensure an adequate excess to cover the wide range observed in their study. Changes in hormonal status that affect tryptophan metabolism, such as pregancy, also affect the apparent equivalence of tryptophan and niacin (Wertz *et al.* 1958). It thus appears that an increase in the rate of tryptophan oxidation will lead to an accumulation of acroleylamino-fumarate at a greater rate than it can be metabolized by picolinate carboxylase, leading to increased formation of quinolinic acid, and increased formation of nicotinamide nucleotides.

The activity of tryptophan oxygenase is also controlled by feedback inhibition. Wagner (1964) showed that both NAD and NADP inhibit the enzyme at a concentration of 5 mM, although at lower concentrations he reported no inhibition. Since the total concentration of nicotinamide nucleotides observed in the livers of animals in the present study was of the order of 0·58 mmol/kg, it is unlikely that feedback inhibition of tryptophan oxygenase will be a significant factor in the regulation of tryptophan and niacin metabolism.

Accumulation of quinolinic acid in the liver has been proposed as one of the mechanisms whereby the administration of tryptophan causes hypoglycaemia; quinolinic acid inhibits phosphoenolpyruvate carboxykinase (*EC* 4 . 1 . 1 . 49) (Lardy, 1971). This suggests that it would be desirable to remove quinolinic acid from the liver as rapidly as possible, so as to avoid accumulation. However, the concentration of quinolinic acid, which is inhibitory, is some 25-fold higher than that which is observed following acute administration of relatively large amounts of tryptophan (McDaniel *et al.* 1973); Smith *et al.* (1979) have demonstrated induction of phosphoenolpyruvate carboxykinase after administration of tryptophan, suggesting that any inhibition will not be physiologically important.

In the animals receiving the high-tryptophan diet, the liver content of nicotinamide nucleotides was approximately 56% higher than in the other two groups of animals, while the excretion of N^1-methyl nicotinamide was approximately 3-fold higher. This suggests that there is control of the tissue content of nicotinamide nucleotides by the activity of NADase; the nicotinamide released would presumably be subject to the same limitation of incorporation into nucleotides as discussed previously, and it is therefore to be expected that much would be methylated and excreted as N^1-methyl nicotinamide. In this context it is interesting to note the observations of Green & Dobrzansky (1967) that administration of streptozotocin leads to a severe depletion of tissue NAD, as a result of increased hydrolysis to nicotinamide, and this is accompanied by an increase in the excretion of N^1-methyl nicotinamide. Shaikh *et al.* (1980) have reported that a number of chemical carcinogens have a similar effect. It thus seems probable that, as suggested by the present kinetic values, the ability to utilize nicotinamide is very limited, and even when tissue levels of nucleotides are severely depleted, the nicotinamide released by hydrolysis cannot be reutilized.

From the present values it would appear that under normal conditions the utilization of nicotinamide for synthesis of nucleotides is very strictly controlled and is limited by the activity of both nicotinamide deamidase and phosphoribosyltransferase, while the utilization of nicotinic acid may be increased slightly in response to an increase in the availability of this vitamer. It seems likely that the utilization of quinolinic acid arising from tryptophan is not limited, and that control over the concentrations of nicotinamide nucleotides in tissues may be achieved by the action of NADase, with limitation of the reutilization of the nicotinamide so released.

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Printed in Great Britain