Ann. Rev. Nutr. 1983. 3:289–307

NIACIN

LaVell M. Henderson

Department of Biochemistry, College of Biological Sciences, University of Minnesota, St. Paul, Minnesota 55108

CONTENTS

INTRODUCTION

The history of niacin as a nutrient for man largely preceded the chemical identification of the vitamin. This history was reviewed in a recent symposium on the history of pellagra (60). The coenzyme functions of the pyridine nucleotides and general metabolism of niacin have been under investigation for about 50 years, and have been reviewed periodically (18,33,45,61,120). The regulation of NAD synthesis and degradation and the recycling of the niacin amide moiety have also been reviewed (10,120). During the past 15 years the discovery of non-redox functions of NAD has led to exciting developments which bear on the regulation of cellular processes. This area has also been the subject of frequent reviews (42,49,81,91,96,107).

0199-9885/83/0715-0289$02.00

USE OF DIETARY SOURCES

It seems safe to assume that in uncooked foods of animal origin the major forms of niacin are the cellular pyridine nucleotides, NAD(H) and NADP(H). Documentation on this matter is not easily found, and I found no evidence for the extensive occurrence in foods of free acid or amide. Little attention has apparently been given to how heat and pH during cooking affect the degradation of the pyridine nucleotides. Enzymatic hydrolysis of the coenzymes might result from food processing, especially through the action of widely distributed glycohydrolases and pyrophosphatases.

Digestion

The digestion of pyridine nucleotides by mammalian digestive enzymes appears to have received little attention. In 1961, Turner & Hughes (116) extended their study of the absorption of B-vitamins by surviving rat intestine preparations by doing similar experiments with bound forms, including NAD. Their results indicated that this coenzyme is split chiefly by mucosal enzymes. They presented some evidence for NMN (nicotinamide ribonucleotide) formation, but the major product was nicotinamide (NAm), whose formation was attributed to NADase (NAD glycohydrolase). Baum et al (5) recently reported that the particulate matter from rat intestine homogenates hydrolyzes NAD largely to NAm at pH 5–6, but at pH 7 the product was principally NMN. In a more detailed study (39) using a variety of preparations ranging from whole animals to mucosal homogenates, the conclusion was reached that in rat, NAD is quickly digested by the action of a pyrophosphatase present in the intestinal contents. The resulting NMN is then rapidly hydrolyzed to nicotinamide riboside (NR) which accumulates in the lumen of the upper small intestine. Therefore, the rate limiting digestion step is the release of NAm from NR by hydrolysis or phosphorolysis or both, under the influence of mucosal enzyme(s). That the absorbed form of niacin is the amide seems to be widely accepted, though the possibility of its hydrolysis by nicotinamidase is an idea that has persisted for many years.

Ijichi et al (57), on the basis of in vivo studies with mice, concluded that NAm given in rather large doses (82 μmol) intraperitoneally or intraportally accumulated in the gastrointestinal tract one hour after injection. The accumulated amide was hydrolyzed to nicotinic acid (NA) within three hrs, presumably by the action of bacterial nicotinamidase (113). The NA was then transported to the liver, where it was converted to NAD via the Preiss-Handler (95) pathway. Collins & Chaykin (21) failed to observe the accumulation of NAm in the digestive tract of mice, and the products in the liver and urine from intraperitoneal ^{14}C-NAm precluded significant deamidation to NA. They concluded, on the basis of experiments in mice with [^{14}C]-NAm and [^{14}C]-NA given by

stomach tube and intraintestinal injection, that little or no NAm was hydrolyzed in the digestive tract. Streffer & Benes reached similar conclusions (109).

Experiments with rats (46,47) in which [^{14}C]-NAm and [^{14}C]-NA were administered intralumenally to perfused intestine and to live animals reinforced this view. These observations and the very rapid absorption of NAm from the perfused rat intestine (46) indicate that NAD is hydrolyzed to NAm, which is the dominant absorbed form of this vitamin when the dietary sources are pyridine nucleotides at the usual level of intake.

Absorption

Using everted sacs of rat and hamster intestine, Turner & Hughes (115) studied the absorption of a number of B vitamins, including nicotinic acid, and concluded that the latter is absorbed by passive diffusion. Because of the unphysiological nature of the everted sac—and the insensitivity of the nonisotopic analytical methods used—the conclusions may be questioned. Since the accumulation of NAm in the intestinal tract had been reported (57) and since erythrocytes are very active in the synthesis of NAD, care must be exercised in choosing an uncomplicated physiological system for investigating absorption of niacin. The one-pass intestinal perfusion system without erythrocytes, adapted from the procedure described by Windmueller & Spaeth (121), has been used to investigate absorption of both NAm and NA (46). The rate of absorption of [^{14}C]-NAm into the perfusate exceeded that for [^{14}C]-NA and was proportional to dose from 0.16–1600 μmol. On the other hand, the uptake of NA into the perfusate and intestinal tissue was saturable above a dose of 160 μmol (concentration 160 mM). Uptake by the intestinal tissue from the perfusate was more rapid for NA than for NAm. Little transport from the perfusate to the lumen was observed with NA, but the counter transport of the amide was pronounced, especially when the luminal NAm was removed by continuous irrigation.

Sadoogh-Abasian & Evered (102) reached somewhat different conclusions based on their observations with everted sacs of rat small intestine. Neither NA nor NAm was absorbed against a concentration gradient, but they interpreted inhibition by ouabain or a low sodium medium as supporting carrier-mediated, facilitated diffusion of both compounds at low concentrations. At higher concentrations passive diffusion masked the mediated process. Concentrations used ranged from 1–12 mM. In the vascular perfusion experiments (46) (0.16–1600 mM), the transport measured included that in the perfusate and in the intestinal cells. The dose did not influence the distribution of transported NAm between perfusate and tissue, but NA was preferentially transported to the intestinal cells at high dosages.

Limited observations in humans indicate that nicotinic acid (3 g by mouth) was nearly completely absorbed (79). A more recent report showed that this

form of the vitamin is absorbed rapidly from the stomach, but more rapidly from the small intestine (6).

BIOSYNTHESIS OF NIACIN

Quinolinate appears to be the immediate precursor for de novo synthesis of niacin in all living forms that have been studied. Plants (24) and microorganisms synthesize quinolinate from aspartate and a 3-carbon compound—identified as dihydroxyacetone phosphate in bacteria (86). Many mammals and *Neurospora* obtain some of their niacin from the degradation of tryptophan via the kynunenine-3-hydroxyanthranilate pathway (44). The observation that animals (87) and bacteria (3) convert quinolinate to nicotinic acid ribonucleotide by phosphoribosyl transfer from 5-phosphoribosyl-1-pyrophosphate with loss of CO_2 completed the discovery of the steps involved in converting the indole nucleus of tryptophan to the pyridine nucleus of NAD. In recent years examination of the factors that influence the efficiency of this conversion has continued, but the details of regulation remain obscure. Some suggest that the quinolinate-phosphoribosyl transferase catalyzed reaction is the rate limiting step in rats and that its rate is inversely related to the protein content of the diet (103).

The efficiency with which tryptophan replaces dietary niacin has been investigated extensively. In the growing chick, Baker et al (4) observed that when tryptophan or niacin was added to a diet containing enough tryptophan (0.13%) to give optimum growth with an excess of niacin (10 mg/100 g), additional tryptophan at 0.09% or niacin at 2 mg/100 g gave equivalent responses, indicating that conversion of tryptophan to niacin is about 2% in this species, not unlike the rat (68).

The extent of the conversion of tryptophan to niacin in humans under various dietary conditions continues to be important because of the major role played by the tryptophan content of the proteins in certain types of diets in meeting the requirements for niacin. The elimination of "Niacin Equivalents" for tryptophan from the 1974 (8th) edition of the *Recommended Dietary Allowances* (RDA) (99) prevented food manufacturers from estimating the niacin obtained from tryptophan in their products, as they had done using the earlier edition of the RDA. Therefore, nutritional labeling in accordance with the Food and Drug Administration regulations could not include the niacin from this source. Since the niacin content of foods that contain milk and eggs is often less than 2% of the RDA, these foods must be labeled inadequate in niacin even though they may be excellent sources of niacin because of their tryptophan content. The 1980 edition of the RDA restored the "niacin equivalent" approach. A recent report (53) discussed the data upon which the conversion factor of 1 mg of niacin for 60 mg of tryptophan was based (85, 90).

TRANSPORT AMONG ORGANS

The current literature indicates that most tissues take up both forms of this vitamin by simple diffusion and metabolic trapping as the nucleotides and dinucleotides. One well-documented exception is the facilitated diffusion of niacin into erythrocytes (69). The anion transport protein (band 3 from SDS gel electrophoresis) representing 30% of the protein in erythrocyte membranes is the carrier for part of the transport of nicotinate into erythrocytes (D. Wunderlich & L. M. Henderson, unpublished observations). The conclusion that band 3 protein is responsible for the facilitated diffusion of NA is largely based upon the irreversible inhibition of NA transport into erythrocytes by 4,4'-diisothiocyano-2,2'-stilbene-disulfonic acid (DIDS), one of a group of stilbene sulfonic acid derivatives that binds at the substrate binding site and completely inhibits chloride and sulfate transport. This system, necessary for the chloride-bicarbonate exchange, is not the major carrier for some organic anions (25). However, it provides an explanation for the facilitated diffusion of niacin in erythrocytes.

Brush-border membranes from rabbit kidneys (13) exhibit a Na^+ gradient-dependent, saturable, electro-neutral transport system for nicotinic acid. Studies of the renal excretion of niacin indicated that as plasma concentration was increased the clearance ratio declined, suggesting the saturation of a secretory system. Clearance was not affected by pH between 6.2 and 8. In brain slices and choroid plexes concentrative uptake by energy-dependent processes appears to be mediated by metabolism (108).

When niacin reaches the plasma, it is rapidly removed by the liver and erythrocytes and converted to NAD by the Preiss-Handler pathway (95). NAD glycohydrolases then release the pyridine nucleus as NAm, which circulates and serves as the precursor of NAD in other tissues, all of which are obliged to synthesize their own NAD. Most mammalian cell lines can utilize either NA or NAm for synthesis of NAD, though some can utilize only NAm (97).

METABOLISM

Methods

Mention should be made of the methods developed for analyzing and isolating NA, NAm, and intermediates formed during the synthesis and degradation of pyridine nucleotides. Analysis of the acid or amide by microbiological and the acid by chemical methods–based upon cyanogen bromide fission of the pyridine ring followed by formation of a chromogen with aromatic amines–has been the standard procedure for analyzing suitable extracts of biological samples (34). In biological systems, the only interfering substances are NAm and pyridine nucleotides. They can be hydrolyzed to NA, and the CNBr method can

be used for total vitamin content. The inconsistent results obtained using chemical hydrolysis of the amide have been overcome (35) by hydrolysis with yeast nicotinamide deamidase. When one needs determination of the separate forms, they must first apply separation methods. Liquid chromatographic methods have been described (9) that separate as many as 20 metabolites of niacin and niacinamide on the basis of the ionic strength of the eluant used to develop the Dowex-1-formate column. The enzymology and assay methods for intermediates in the metabolism of NAm nucleotides have been reviewed (93).

The predominant products arising from NA, NAm, and NAD have also been separated by high-voltage electrophoresis supplemented with paper chromatography for purposes of identification and quantitation (39,47). This provides a much more rapid separation than the Dowex-1-X8 column used in most past metabolic studies.

The Huff & Perlzweig flurometric method (54) for N^1-methylnicotinamide has been adapted to measure NAm in serum after quantitative conversion to N^1-methylnicotinamide with Iodomethane (19).

Pyridine Nucleotide Formation

The controversy regarding the alternate routes for pyridine nucleotide synthesis was resolved nearly ten years ago, when it was shown that both the Preiss-Handler (95) and the Dietrich (28, 30) pathways were functional in the mouse (21,109) and in various rat tissues (66, 72), including erythrocytes (73). The difficulty in demonstrating the involvement of the Dietrich pathway came from complications in interpreting isotope data. The scheme shown in Figure 1 will

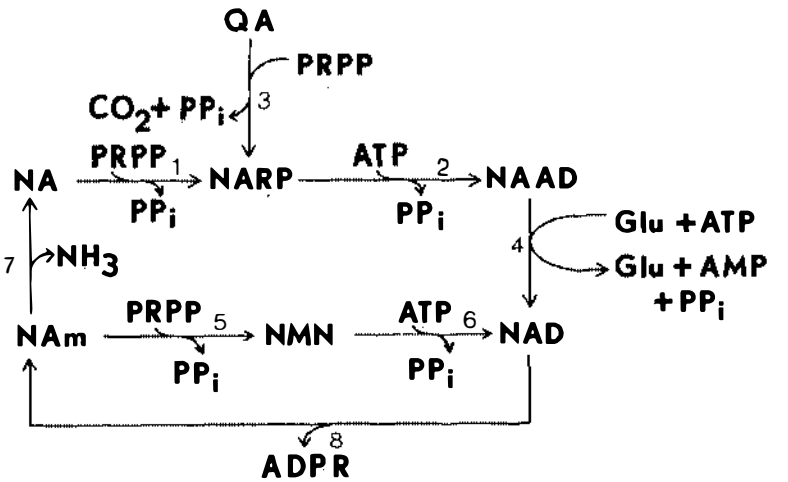

Figure 1 Reactions involved in the synthesis of NAD and the recycling of the pyridine nucleus.

serve to illustrate these complications and to focus on the pyridine nucleotide cycles that function in mammalian species.

The widespread occurrence of nicotinamidase (reaction 7, Figure 1) in homogenates of various mammalian tissues led to the assumption that they would hydrolyze NAm to NA in vivo . This proved to be a false assumption (21,26,66,72,73,109) for physiological concentrations of NAm. The second factor that complicated interpretations of isotope data was the possible exchange of [^{14}C]NAm with the pyridine moiety of NAD, catalyzed by NAD glycohydrolase, thus incorporating isotope without net synthesis of NAD. The extensive exchange of NAm seems unlikely in rat erythrocytes (73) in view of the failure of the cells, in contrast to hemolysates, to release [^{14}C]NAm from NAD synthesized from [^{14}C]NA. The direct incorporation of NAm during synthesis of NAD is supported by the extensive labeling of NMN by [^{14}C]NAm (reaction 5), but not by [^{14}C]NA. A third complication to full acceptance of the Dietrich pathway was the reported high Km value for NAm (0.1 M) of the NMN pyrophosphorylase (Reaction 5) of human erythrocytes. In rat erythrocytes the Km is 0.067 μM, well below the physiological concentration of NAm (1–50 μM) (18). The high Km value for this enzyme in several human tissues has not yet been adequately explained.

The pyridine nucleotide cycle (37) is accepted for bacteria where the cycle includes NA (reactions, 8, 7, 1, 2, 4, and possibly others; Figure 1) (120), and for mammals where NAm is recycled through reactions 8, 5, and 6. This serves as a salvage pathway for recovering the NAm released when one or more of the glycohydrolases in liver forms ADP-ribose or polyADP-ribose.

Bernofsky (10) postulated that the concentration of NAm in extracellular fluid controls tissue levels of NAD and that NAm concentrations in the plasma are regulated by hormonally controlled release of NAm from "storage NAD" in the liver. The storage NAD does not appear to equilibrate with the "functional NAD" involved in redox processes. The long-recognized large increase in NAD concentration in the liver in response to NAm intake is accounted for chiefly by the increase in the storage NAD. The remainder of this control system functions by disposing of excess NAm. This function resides in the liver enzymes, which form irreversibly the N^1-oxide (12) and N^1-methyl derivatives of NAm excreted by the kidney. The free NAD behaves differently from enzyme-bound (functional) NAD as indicated by the fact that glyceraldehyde 3-phosphate dehydrogenase-bound NAD will not serve as a substrate for glycohydrolase (11). The free NAD is a substrate and therefore a source of ADP-ribose for ADP-ribosylation, a process dealt with in more detail below.

The temporary increase in free NAD levels (10 times normal) in the liver in response to exogenous NAm suggests that the enzymes of the Dietrich pathway are poorly regulated in this organ. The nicotinamide phosphoribosyl transferase, which catalyzes the first reaction of this sequence, is influenced posi-

tively by ATP and negatively by NAD (26) in a structurally specific manner and also by hormones (29). None of these controls is very effective in the liver and this may account in part for the observed toxicity of NAm. The toxicity could be the result of depletion of cellular ATP and/or PRPP used in NAD synthesis (101). Suppression of DNA synthesis and inhibition of t-RNA methylation in liver have been suggested as explanations for the toxicity of NAm at levels of 5–10 m mol per kg of body weight, the dosage required to stimulate NAD synthesis in rats and mice (10). This seeming lack of regulation of NAD in the liver, in fact, regulates NAm levels in the plasma and thereby the NAD levels in other tissues. Prolonged treatment with these high levels of NAm (4 m mol/kg/day) to rats for several weeks resulted in a permanent increase of several hundred precent in the NAD in liver, but little increase in NADP and NADPH (110). The excretion of nicotinuric acid under these circumstances indicates that NAm deamidase functions at these high dosages. Additional dietary glycine and methionine partially relieved the observed liver damage.

Pyridine Nucleotide Fission

NAD GLYCOHYDROLASES This group of enzymes has been discussed in connection with NAD digestion, with the salvage pathway, and with the release of NAm from the liver and possibly other tissues to provide a source of this vitamin for tissues generally. The glycohydrolases often associated with membranes catalyze the hydrolytic cleavage of the β-N-glycosidic linkage of NAD, yielding ADP ribose and NAm (equation 1).

$$ADPR-NAm^+ + H_2O \rightarrow ADPR + NAm + H^+ \quad 1.$$

The β-N-riboside linkage is a high-energy bond with a free energy of hydrolysis of –8.2 kcal/mole at pH 7 and 25°C. The full significance of this bond energy was not realized until the transfer of the ADPR to macromolecules was discovered about 1967. Some NAD glycohydrolases also catalyze the exchange of NAm with other bases to provide a method of preparing NAD analogs in which the pyridine base is replaced (122), (Equation 2).

$$ADPR-NAm^+ + Base \rightarrow ADPR-Base^+ + NAm \quad 2.$$

In Reaction 1, the receptor of the ADPR moiety is water; in Reaction 2, another nitrogenous base.

The discovery that some glycohydrolases have transglycosidase activity, manifested in the transfer of ADPR, to macromolecules sparked renewed interest in glycohydrolases. The simple type found in bacteria, fungi, seminal plasma, and snake venom possesses no transglycosidase activity, whereas the typical mammalian glycohydrolase also catalyzes reaction 2. These enzymes are membrane bound and are inhibited by NAm (123). The glycohydrolase from *Neurospora* is not inhibited by NAm nor does it catalyze the exchange reaction (64). Meda et al (76) reported a rat liver chromatin glycohydrolase insensitive to NAm, unlike most mammalian glycohydrolases.

ADP-RIBOSYLATION ADP-ribosyl transferases catalyze the transfer of ADPR to a macromolecule (M), (Equation 3).

$$ADPR-NAm^+ + M \rightarrow ADPR-M + NAm + H^+ \quad 3.$$

Many well-studied examples of this type of enzyme are known. In procaryotes, diphtheria toxin is perhaps the best known ADPR-ribose transferase. It inhibits protein synthesis in HeLa cells without affecting other cellular functions, but only in the presence of NAD. This NAD requirement was explained in 1968 when Honjo et al (51) demonstrated that elongation factor-2 (EF-2) was ADP-ribosylated, blocking the translocation event on ribosomes and hence protein synthesis. Further work showed that the enzymatic activity resides in a fragment of mol wt = 24,000, prepared by proteolytic cleavage of the intact toxin (42). The site of ADP-ribosylation of EF-2 has been identified as position N_1 on a modified histidyl residue. This residue, diphthamide, is 2-[3-carboxyamido-3-(trimethyl-ammonio)propyl] histidine (117). The enzyme ADP ribosylates all of the EF-2s, but no other proteins. This specificity is accounted for, at least in part, by the unique modified histidyl residues on EF-2. *Pseudomonas* toxin appears to function in the same manner (55, 56).

The action of cholera toxin provides (81) another example of mono-ADP ribosylation in bacteria and adds the monomer to membrane-bound protein that regulates adenylate cyclase (16, 80). The amino acid residue that is ADP-ribosylated is not known, but free arginine and other guanido groups will serve as acceptors. The ADP-ribosylation catalyzed by both diphtheria and cholera toxins occurs with inversion of the N-glycosidic bond from β to α (89).

POLY ADP-RIBOSYLATION All types of eukaryotes have enzymes in their nuclei that catalyze the transfer of many ADPR residues to the protein acceptor in a manner expressed by Equation 4 to form an attached polymer of ADPR.

$$n\ ADPR-NAm^+ + M \rightarrow (ADPR)_n-M + n\ NAm + nH^+ \quad 4.$$

The poly-(ADPR) synthetase (polymerase) is found also in mitochondria and in the cytoplasm, bound to ribosomes. The nuclear polymerase is closely associated with chromatin and requires DNA for activity. The major acceptor of poly-(ADPR) in rat liver nuclei is the polymerase itself (88). The precise function of poly(ADP-ribosylated) protein in the nucleus is not known, though evidence suggests its participation in DNA replication (22), DNA repair (32), or cell differentiation (58, 92). This nonredox function of NAD probably accounts for the rapid turnover of NAD in human cells. The half-life of NAD is 60 min in these cultured cells whose generation time is 24 hr. In contrast, in *E. coli* whose generation time is 30 min, the half life is 2 hr (98).

NAm inhibits the polymerase (94). The nature of the attachment of poly (ADPR) to the protein (80), the identity of proteins that are so modified, the evidence for the structure of the polymer, the enzymology of formation, and the

degradation and the function of poly (ADPribosylated) proteins are all dealt with in excellent reviews of this subject (42,91,96).

That niacin status may affect the extent of ADP-ribosylation of proteins was suggested by the observations (14) that when the NAD level of the rat liver was increased 2.5-fold by NAm treatment, the protein bound mono(ADPR) residues increased 1.5-fold in spite of the inhibitory effect of NAm on ADP transribosylase. Lower NAD levels than normal in neonatal rat liver or in ascites hepatoma cells accompanied much reduced mono(ADPR) residues (14). The variation in NAD levels influenced the number of acceptor sites reacting, not the average length of the $(ADPR)_n$ chains. The glycohydrolases, which have transferase activity, could be involved in integrating cellular processes by modulating enzyme activity by ADP-ribosylation. The "storage form" or free form of NAD is the substrate for these glycohydrolases. While this system of regulation may be adequate in some tissues, it would seem to be of limited value in the liver, where NAD concentrations can increase 8–10-fold in 8–12 hr in response to high intake of NAm.

Further experimentation will be required to clarify the role of these interlocking control mechanisms for NAD synthesis, NAD hydrolysis, and ADP-ribosylation. Hormonal modulation has been reported for NAD synthesis and for tryptophan conversion to niacin (10). Insulin and epidermal growth factor stimulate after long-term treatment (105). Others (100) observed isopreterenol stimulation of NAD-dependent ADP-ribosyl transferase.

EXCRETORY PRODUCTS

The studies of excretory products of niacin and niacinamide conducted before 1970 using isotopically labeled vitamin in rodents established a spectrum of urinary products including nicotinic acid, nicotinamide, N^1-methylnicotinamide, nicotinamide-N-oxide, N^1-methyl-4-pyridone-5-carboxamide, N^1-methyl-2-pyridone-5-carboxamide, nicotinuric acid, 6-hydroxynicotinamide, and 6-hydroxynicotinic acid. The intestinal microflora does not appear to be involved in the formation of these excretory products (71), though 6-hydroxynicotinic acid is formed by a nicotinic acid-fermenting *Clostridium* and several other bacterial species (50).

Investigations of the urinary excretion products of niacin in humans have been more limited. The species differences in this regard are largely quantitative, not qualitative. With single doses ranging from 100-3000 mg, the response in urinary excretion by humans using HPLC methods was examined (82). For nicotinic acid at doses of 1000 mg or less, the major product was the N^1-methyl-2-pyridone-5-carboxamide. At increasing doses, nicotinuric acid and N^1-methylnicotinamide increased disproportionately to become the dominant excretory products at a dose of 3 g. The spectrum of products and the

quantities excreted in response to NAm showed a similar result to that for NA, except that nicotinuric acid and NA were absent, and considerable free amide was excreted by this route. Nicotinamide N^1-oxide was a minor product from NA and NAm. The ratio between the 2-pyridone and the 4-pyridone was nearly constant at 5.3–6.3. The 6-hydroxy derivatives of NA and NAm were not detected.

PHYSIOLOGICAL AND PHARMACOLOGICAL EFFECTS

Glucose Tolerance Factor

The observation (78) that nicotinic acid is a component of a biologically active form of chromium present in yeast made it appear that there may be another function of this vitamin. This complex, which exhibits an insulin potentiating effect by increasing glucose tolerance in rats and other species, has been called the glucose tolerance factor (GTF). Synthetic complexes containing Cr^{III} and nicotinic acid, and stabilized by the incorporation of glycine and glutamic acid or glutathione (114), compare in biological activity to the natural GTF, but they are not absorbed as readily (77). The niacin in these synthetic complexes is available to rats for growth. Free niacin does not affect glucose tolerance at the concentration at which the complex is active.

Atherosclerosis

The first report that large doses of NA reduce serum cholesterol levels in man appeared in 1955 (1). Several subsequent studies indicated that NA lowers plasma triglycerides as well. In spite of substantial lowering of blood lipids by NA, its use has been limited by the well-known flushing of the face that results from large doses. The conclusions reached by a collaborative research group (23) indicated that it was a safe drug, that it caused a 9% lowering of plasma cholesterol, and that it reduced the recurrence rate of myocardial infarction by 29%. It seems unlikely that NA acts by influencing the redox function of the pyridine nucleotides since a much higher dose of NAm, which is more effective than NA in stimulating NAD synthesis, did not influence the redox-state of the cytosolic NAD system (111). The metabolic changes observed appeared to be the result of reduced lipolysis. In the fasted animal the same metabolic changes were the result of increased gluconeogenesis (112). The same effect has been reported for NA, but the antilipolytic effect of NAm is of longer duration. The early developments in this field were exhaustively reviewed in 1971 (36). The recently observed (40) lowering of plasma cholesterol by 22%, and plasma triglycerides by 52%, confirms earlier experience with NA as a plasma lipid lowering drug. These authors wrote, "To our knowledge, no other single agent has such potential for lowering both cholesterol and triglycerides."

The mechanism by which NA lowers plasma cholesterol and triglycerides is not known. Some suggest that the synthesis of lipoprotein is reduced in the liver as a result of reduced lipolysis in the adipose tissue (118). Niceritrol (pentaerythritol tetranicotinate) has been used at a dosage of 3 g daily. Others have observed that flushing, itching, and rash prevents the use of such high doses in a substantial percentage of the patients. NA esters have frequently been used in combination with dietary modification and medication with clofibrate (118, 119).

Pellagra

The association between pellagra and corn continues to attract attention. One explanation for corns being more pellagragenic than its niacin content would suggest is the imbalance of amino acids created by the low tryptophan content of corn. In addition, the nonavailability of much of the niacin in corn continues as a consideration in the etiology of pellagra in populations in the past (15). The amino acid imbalance that precipitates the niacin deficiency in rats fed 9% casein diets supplemented with corn products is caused by the threonine and methionine-cysteine provided by the corn (41). The reports that an imbalance created by adding leucine to marginal diets for humans (38), monkeys (8), and pigs (7) created controversy for many years. Most attempts to confirm these observations in dogs (75), rats (74,84), chicks (74), and humans (83, 90) proved unsuccessful. These human experiments provided more data regarding the extent of tryptophan conversion to niacin under various dietary conditions, discussed above (85,90).

The prevalence of pellagra in the United States and Southern Europe in the early 1900s when corn was a staple item in the diet continues to be a challenging puzzle. The fact that Central American diets do not cause pellagra, in spite of a very high corn content, has been attributed to the cooking in lime the corn receives in tortilla preparation. Some authors estimate that about 33% of the niacin is discarded in the washings, yet this alkaline treatment reduces the pellagragenic effect. Carpenter (15) has reminded us that pellagra became a health problem in this country about the same time that degermination became commonplace in the large corn processing centers. He suggests this could account for the rather sudden outbreak of pellagra in urban institutions between 1906 and 1910. Persons in rural areas were protected because they continued to use the ground whole grain. The milling removed both tryptophan and niacin to give 67% as much in niacin equivalents in the endosperm as in the whole corn meal. It is difficult to exclude an unknown factor in corn that might cause a reduction in the already ineffecient conversion of typtophan to niacin (<3%) and account for the pellagragenic effect of this grain.

Considerable evidence indicates that cereal components, including wheat bran, contain niacin in a form not released by digestion. It is esterified with

glucose, imbedded in a glycopeptide. Alkali, or even NH_3 vapor, will release the vitamin in a form useful to man and other animals. With available niacin coming to an unknown degree from bound niacin—and from tryptophan to a degree that is dependent upon the balance of essential amino acids—it seems unlikely that niacin status of populations will be very predictable on the basis of analysis of diets for tryptophan and niacin. Therefore, the assessment of human requirements will probably remain rather tentative. Currently, the RDA for adults is 6.6 mg niacin equivalents/1000 kcal (99), or not less than 13 niacin equivalents for caloric intakes of less than 2000 kcal for adults. This increases for infants, children, and lactating or pregnant females. Average diets in the United States provide 16–34 mg of niacin equivalents (99).

Antagonists

As discussed in the above section on the transglycosidase activity of glycohydrolase, this class of enzymes provides a convenient laboratory method to convert NAm analogs to NAD and NADP analogs. Experience with pig brain and calf spleen preparations show that nonpyridine nitrogenous bases do not participate in the base exchange catalyzed by these glycohydrolases. A large number of pyridine derivatives have been incorporated by exchange with NAm, and many of these are coenzymatically active NAD analogs. Anderson (2) tabulated and discussed their NAD analogs. The following substituents on the 3-position of the pyridine nucleus will substitute for $-CONH_2$ and give NAD analogs that have some activity: -CHO, $-COCH_3$, $-COCH(CH_3)_2$-$CH_2CH_2CH_3$, $-COC_6H_5$, -CONHOH, $-CONHNH_2$, $-CH{=}NOH$, $-CSNH_2$, $-COCH_2Cl$, -CN, -Cl, -Br, and -I. That the reaction is base exchange, and not reversal of the hydrolytic cleavage of the N-riboside bond, was proved by the failure of the enzyme to form [^{14}C]NAD from [^{14}C]NAm and ADP-ribose.

Until recently, the primary concern in making NAD analogs was to produce compounds that would be useful in studying the redox function. Consequently, the emphasis has been on modification of the pyridine ring, where the hydride transfer occurs. More recently, interest increased in modifying the adenine portion, and many such analogs serve as coenzymes for dehydrogenases (2). Such analogs should be very useful in the ADP-ribosylation reactions. For this function, the pyridine moiety must be an effective leaving group such as NAm.

The NAD-ase catalyzed exchange was first demonstrated with isonicotinic acid hydrazide (124) where the product was inactive as a coenzyme. When 3-acetylpyridine, (3-AP), a nicotinamide antagonist in animals (43) was incorporated, the resulting NAD analog functioned with yeast alcohol dehydrogenase (63). The 3-AP forms analogs of NAD in the brain, spleen, and tumor tissue (62), but it was oxidized to NA in the liver where it stimulated NAD synthesis.

This in vivo incorporation of NAm analogs into NAD created considerable interest in their use in various biological systems. One of the most studied analogs is 6-aminonicotinamide (6-AN). The NAD analog containing this compound is neither a coenzyme for most dehydrogenases, nor a substrate for most glycohydrolases (2).

In 1955, 6-AN was shown to cause paralysis in laboratory animals (59). Continued administration causes degeneration of the neurons in adults and retarded differentiation of the ependyma and choroid plexus in developing animals (17). Herken et al (48) demonstrated that the levels of 6-phosphogluconate in neural tissue dramatically increased (170-fold) 6 hr after intraperitoneal administration of 7 mg of 6-AN to mice. This compound was presumably inhibiting the pentose phosphate pathway. The 6-AN analog of NADP forms (67) via base exchange, and is a potent competitive inhibitor of 6-phosphogluconate dehydrogenase. The Ki for 6-AN adenine dinucleotide phosphate for this enzyme is at least ten times lower than for other NADP-dependent dehydrogenases in the brain. The accumulation of 6-phosphogluconate inhibits glycolysis by its effect on phosphoglucoisomerase (70). In spite of the apparent disruption of the pentose phosphate cycle, the cerebral energy use was maintained (65).

A report (52) on the effect of 6-AN on the central nervous system of the rat suggests that the lesions produced are similar to those seen in presenile Creutzfeldt-Jackob disease in humans. Others have recognized the similarity between the lesions of this disease and those seen in pellagra encephalopathy, suggesting that there may be dysfunction of pyridine nucleotide–requiring enzymes in Creutzfeldt-Jackob disease.

The teratogenic effect of 6-AN has been investigated in a series of experiments with chick embryos during the past ten years. As in mammals, it forms 6-amino NAD, a competitive analog of NAD. It inhibits dehydrogenases needed for ATP synthesis and interferes with development. Studies of chondrogenesis have suggested that sulfate incorporation was 30% of control values. The molecular weights of glycosaminoglycans were reduced and less chondriotin-6-sulfate and more chondroitin-4-sulfate was found (104). These changes can explain the shortened limbs observed in the embryos as well as the cleft palate and other teratogenic effects in rats (31). The fact that 6-AN is 15 times more toxic than 6-aminonicotinic acid suggests the incorporation of 6-AN into the NAD analog in vivo through the glycohydrolase catalyzed exchange with NAm. In *E coli,* which has no NAD glycohydrolase activity, the bacteriostatic action of 6-amino nicotinic acid results from the synthesis of 6-AN through the pyridine nucleotide cycle (20). Such incorporation probably does not occur in mammals (27). The strong carcinostatic effect of 6-AN is reversed by nicotinamide (106).

Literature Cited

1. Altschul, Z., Hoffer, A, Stephen, J. D. 1955. Influence of nicotinic acid on serum cholesterol in man. *Arch. Biochem. Biophys.* 54:558–59
2. Anderson, B. M. 1982. Analogs of pyridine nucleotide coenzymes. In *The Pyridine Nucleotide Coenzymes,* ed. J. Everse, B. Anderson, K. S. You, pp. 91–133. NY: Academic
3. Andreoli, A. J., Ikeda, M., Nishizuka, Y., Hayaishi, O. 1963. Quinolinic acid: A precursor to nicotinamide adenine dinucleotide in *Escherichia coli. Biochem. Biophys. Res. Commun.* 12:92–97
4. Baker, D. H., Allen, N. K., Kleis, A. J. 1973. Efficiency of tryptophan as a niacin precursor in the young chick. *J. Anim. Sci.* 6:299–302
5. Baum, C. L., Selhub, J., Rosenberg, I. H. 1981. The hydrolysis of nicotinamide adenine dinucleotide (NAD) by intestinal brush border membranes (BBM). *Am. J. Clin. Nutr.* 32:615 (Abstr.)
6. Bechgaard, H., Jespersen, S. 1977. Gastrointestinal absorption of niacin in humans. *J. Pharm. Sci.* 66:871–72
7. Belavady, B., Madhavn, T. V., Gopalan, C. 1967. Production of nicotinic acid deficiency (black tongue) in pigs fed diets supplemented with leucine. *Gastroenterology* 53:749–53
8. Belavady, B., Rao, P. V. 1973. Production of nicotinic acid deficiency in monkeys fed leucine supplemented diets. *Int. J. Vitam. Res.* 43:454–60
9. Bernofsky, C. 1980. Isolation and analysis of pyridine nucleotides and related compounds by liquid chromatography. *Methods Enzymol.* 66:23–39
10. Bernofsky, C. 1980. Physiologic aspects of pyridine nucleotide regulation in mammals. *Mol. Cell. Biochem.* 33:135–43
11. Bernofsky, C., Pankow, M. 1971. Role of dehydrogenase binding in protection of nicotinamide adenine dinucleotide from enzymatic hydrolysis. *Biochim. Biophys. Acta* 242:437–40
12. Bonavita, V., Narrod, S. A., Kaplan, N. O. 1961. Metabolites of nicotinamide in mouse urine: effects of azaserine. *J. Biol. Chem.* 236:936–39
13. Bowmendil-Podevin, E. F., Podevin, R. A. 1981. Nicotinic acid transport by brush border membrane vesicles fom rabbit kidney. *Am. J. Physiol.* 240:F185–91
14. Bredehorst, R., Lengyel, H., Hilz, H., Stark, D., Siebert, G. 1980. Increase of mono (ADP-Ribose) protein conjugate level in rat liver induced by nicotinamide administration. *Hoppe-Seylers Z. Physiol. Chem.* 361:559–62
15. Carpenter, K. J. 1981. Effects of different methods of processing maize on its pellagragenic activity. *Fed. Proc.* 40:1531–35
16. Cassel, D., Pfeuffer, T. 1978. Mechanism of cholera toxin action: covalent modification of the guanyl nucleotide-binding protein of the adenyl cyclase system. *Proc. Natl. Acad. Sci. USA* 75:2669–73
17. Chamberlain, J. G. 1972. 6–aminonicotinamide (6–AN)-induced ependyma and choroid plexus as seen with the scanning electron microscope. *Teratology* 6:281–88
18. Chaykin, S. 1967. Nicotinamide coenzymes. *Ann. Rev. Biochem.* 36:149–70
19. Clark, B. R. 1980. Fluorometric quantitation of picamole amounts of 1-methylnicotinamide and nicotinamide in serum. *Methods Enzymol.* 66:5–8
20. Cobb, J. R., Pearcy, S. C., Gholson, R. K. 1977. Metabolism of 6-aminonicotinic acid in *Escherichia coli. J. Bacteriol.* 131:789–94
21. Collins, P. B., Chaykin, S. 1972. The management of nicotinamide and nicotinic acid in the mouse. *J. Biol. Chem.* 247:778–83
22. Colyer, R. A., Burdette, K. E., Kidwell, W. R. 1973. Poly ADP-ribose synthesis and DNA replication in synchronized mouse L-cells. *Biochem. Biophys. Res. Commun.* 53:960–66
23. The Coronary Drug Project Research Group. 1975. Clofibrate and niacin in coronary heart disease. *J. Am. Med. Assoc.* 231:360–81
24. Dawson, R. F., Christman, D. R., Byerrum, R. U. 1971. Biosynthesis of nicotinic acid in plants and microbes. *Methods Enzymol.* 18B:90–113
25. Deuticke, B., Beyer, E., Forst, B. 1982. Discrimination of three parallel pathways of lactate transport in human erythrocyte membrane by inhibitors and kinetic properties. *Biochim. Biophys. Acta.* 648:96–110
26. Dietrich, L. S. 1971. Regulation of nicotinamide metabolism. *Am. J. Clin. Nutr.* 24:800–4
27. Dietrich, L. S., Friedland, I. M. 1960. 6-aminonicotinamide and 6-aminonicotinic acid metabolism in nucleated and non-nucleated erythrocytes. *Arch. Biochem. Biophys.* 88:313–17

28. Dietrich, L. S., Fuller, L., Yero, I. L., Martinez, L. 1966. Nicotinamide mononucleotide pyrophosyphorylase activity in animal tissue. *J. Biol. Chem.* 241:188–91
29. Dietrich, L. S., Martinez, L., Muniz, O., Farinas, B. 1967. Effect of hypophysectomy and adrenalectomy on enzymes concerned with nicotinamide adenine dinucleotide synthesis. *Endocrinology*. 81:779–82
30. Dietrich, L. S., Muniz, O., Powanda, M. 1968. NAD synthesis in animal tissues. *J. Vitaminol.* 14:123
31. Diewert, V. M. 1979. Correlation between mandibular retrognathia and induction of cleft palate with 6-aminonicotinamide in the rat. *Teratology* 19:213–27
32. Durkacz, B. W., Omidiji, O., Gray, D. A., Shall, S. 1980. (ADP-ribose)n participates in DNA excision repair. *Nature* 283:593–96
33. Foster, J. W., Moat, A. G. 1980. Nicotinamide adenine dinucleotide biosynthesis and pyridine nucleotide cycle metabolism in microbial systems. *Microbiol. Rev.* 44:83–105
34. Freed, M. 1966. *Methods of Vitamin Assay*, pp. 169–95. The Assoc. of Vitamin Chemists Inc. NY: Interscience. 3rd ed.
35. Fuller, L. 1980. Determination of nicotinamide. *Methods Enzymol.* 66:3–4
36. Gey, K. F., Carlson, L. A., eds. 1971. *Metabolic Effects of Nicotinic Acid and Some of its Derivatives*. Bern, Switzerland: Huber. 1251 pp.
37. Gholson, R. K. 1966. The pyridine nucleotide cycle. *Nature* 212:933–34
38. Gopalan, C., Rao, K. S. J. 1975. Pellagra and amino acid imbalance. *Vitam. Horm. (NY)* 33:505–28
39. Gross, C. J., Henderson, L. M. 1983. Digestion and absorption of NAD by the small intestine of the rat. *J. Nutr.* In press
40. Grundy, S. M., Mok, H. Y. I., Zechs, L., Berman, M. 1981. Influence of nicotinic acid on metabolism of cholesterol and triglycerides in man. *J. Lipid Res.* 22: 24–36
41. Hankes, L. V., Henderson, L. M., Elvehjem, C. A. 1949. Effect of cystine and threonine on the growth of rats receiving tryptophan-deficient rations. *J. Biol. Chem.* 180:1027–35
42. Hayaishi, O., Ueda, K. 1977. Poly (ADP-ribose) and ADP-ribosylation of proteins. *Ann. Rev. Biochem.* 46:95–116
43. Hayman, S., Shahinian, S. S., Williams, J. N., Elvehjem, C. A. 1955. Effect of 3-acetylpyridine on pyridine nucleotide formation from tryptophan and niacin. *J. Biol. Chem.* 217:225–28
44. Henderson, L. M. 1949. Quinolinic acid metabolism: II. Replacement of nicotinic acid for the growth of the rat and *Neurospora*. *J. Biol. Chem.* 181:677–85
45. Henderson, L. M. 1956. The metabolism of niacin. In *Symposium on Vitamin Metabolism*, pp. 28–48. NY: Natl. Vitamin Found.
46. Henderson, L. M., Gross, C. J. 1979. Transport of niacin and niacinamide in perfused rat intestine. *J. Nutr.* 109:646–53
47. Henderson, L. M., Gross, C. J. 1979. Metabolism of niacin and niacinamide in perfused rat intestine. *J. Nutr.* 109:654–62
48. Herken, H., Lange, K., Kolbe, H. 1969. Brain disorders induced by pharmacological blockade of the pentose phosphate pathway. *Biochem. Biophys. Res. Commun.* 36:93–100
49. Hilz, H., Stone, P. 1976. Poly (ADP-ribose) and ADP-ribosylation of proteins. *Rev. Physiol. Biochem. Pharmacol.* 76:1–58
50. Holcenberg, J. S., Stadtman, E. R. 1969. Nicotinic acid metabolism. III. Purification and properties of a nicotinic acid hydroxylase. *J. Biol. Chem.* 244:1194–1203
51. Honjo, T., Nishizuka, Y., Hayaishi, O. 1968. Diphtheria toxin–dependent adenosine diphosphate ribosylation of aminoacyl transferase II and inhibition of protein synthesis. *J. Biol. Chem.* 243:3553–55
52. Horita, N., Isii, T., Izuniyama, Y. 1980. Ultrastructure of 6-aminonicotinamide (6-AN) induced lesions of the central nervous system of rats. II. Alterations in the nervous susceptibility with aging. *Acta Neuropathol.* 49:19–27
53. Horwitt, M. K., Harper, A. E., Henderson, L. M. 1981. Niacin-tryptophan relationships for evaluating niacin equivalents. *Am. J. Clin. Nutr.* 34:423–27
54. Huff, H. W., Perlzweig, W. A. 1947. The fluorescent condensation product of N^1-methylnicotinamide and acetone: II. A sensitive method for the determination of N^1-methylnicotinamide in urine. *J. Biol. Chem.* 167:157–67
55. Iglewski, B. H., Kabat, D. 1975. NAD-dependent inhibition of protein synthesis by *Pseudomonas aeruginosa* toxin. *Proc. Natl. Acad. Sci. USA* 72:2284–88
56. Iglewski, B. H., Sadoff, J., Bjorn, M. J., Maxwell, E. S. 1978. *Pseudomonas aeruginosa* exoenzyme S: an adenosine diphosphate ribosyltransferase distinct

from toxin A. *Proc. Natl. Acad. Sci. USA* 75:3211–15
57. Ijichi, H., Ichiyama, A., Hayaishi, O. 1966. Studies on the biosynthesis of nicotinamide adenine dinucleotide: comparative in vivo studies on nicotinic acid, nicotinamide and quinolinic acid as precursors of NAD. *J. Biol. Chem.* 241:3701–7
58. Ikai, K., Ueda, K., Fukushima, M., Nakamura, T., Hayaishi, O. 1980. Poly (ADP-ribose) synthesis. A marker of granulocyte differentiation. *Proc. Natl. Acad. Sci. USA* 77:3682–85
59. Johnson, W. J., McColl, J. D. 1955. 6-aminonicotinamide, a potent nicotinamide antagonist. *Science* 122:834
60. Jukes, T. H., Sebrell, W. H., Roe, D. A., Krehl, W. A., Carpenter K. J. 1980. The conquest of pellagra. *Fed. Proc.* 40:1519–37
61. Kaplan, N. O. 1961. Metabolic pathways involving niacin and its derivatives. In *Metabolic Pathways*, ed. D. Greenberg, 2:627–72. NY: Academic
62. Kaplan, N. O. 1976. Target directed cancer chemotherapeutical agents. In *Cancer Enzymology*, ed. J. Schulz, F. Ahmad, 12:201–24. NY: Academic
63. Kaplan, N. O., Ciotti, M. M., Stolzenbach, F. E. 1956. Reaction of pyridine nucleotide analogues with dehydrogenases. *J. Biol. Chem.* 221:833–44
64. Kaplan, N. O., Colowick, S. P., Nason, A. 1952. *Neurospora* diphosphopyridine nucleotidase. *J. Biol. Chem.* 191:473–83
65. Kauffman, F. C., Johnson, E. C. 1974. Cerebral energy reserves and glycolysis in neural tissue of 6-aminonicotinamide-treated mice. *J. Neurobiol.* 5:379–92
66. Keller, J., Liersch, M., Grunicke, H. 1971. Studies on the biosynthesis of NAD from nicotinamide and on the intracellular pyridine nucleotide cycle in isolated perfused rat liver. *Eur. Biol. Chem.* 22:263–70
67. Kohler, E., Barrach, H-J., Neubert, D. 1970. Inhibition of NADP dependent oxidoreductases by the 6-aminonicotinamide analogue of NADP. *FEBS Lett.* 6:225–28
68. Krehl, W. A., Sarma, P. S., Teply, L. J., Elvehjem, C. A. 1946. Factors affecting the dietary niacin and tryptophan requirement of the growing rat. *J. Nutr.* 31:85–106
69. Lan, S. J., Henderson, L. M. 1968. Uptake of nicotinic and nicotinamide by rat erythrocytes. *J. Biol. Chem.* 243:3388–94
70. Lang, K., Kolbe, H., Keller, K., Herken, K. 1970. Der Kohlenhydratstoffwechsel des Gehirns nach Blockade des pentose-phosphate Weges durch 6-Aminonicotinasaueramid. *Hoppe-Seylers Z. Physiol. Chem.* 351:1241–52
71. Lee, Y. C., Gholson, R. K., Raica, N. 1969. Isolation and identification of two new nicotinamide metabolites. *J. Biol. Chem.* 244:3277–82
72. Lin, L-F., H., Henderson, L. M. 1972. Pyridinium precursors of pyridine nucleotides in perfused rat kidney and the testis. *J. Biol. Chem.* 247:8023–30
73. Lin, L-F. H., Lan, S-J., Richardson, A. G., Henderson, L. M. 1972. Pyridine nucleotide synthesis. Purification of nicotinamide mononucleotide pyrophosphorylase from rat erythocytes. *J. Biol. Chem.* 247:8016–22
74. Manson, J. A., Carpenter, K. J. 1978. The effects of a high level of dietary leucine on the niacin status of chicks and rats. *J. Nutr.* 108:1883–88
75. Manson, J. A., Carpenter, K. J. 1978. The effect of a high level of dietary leucine on the niacin status of dogs. *J. Nutr.* 108:1889–98
76. Meda, V., Fukushima, M., Okayama, M., Hayaishi, O. 1975. Nicotinamide adenine dinucleotide glycohydrolase from rat liver nuclei; isolation and characterization of a new enzyme. *J. Biol. Chem.* 250:7541–46
77. Mertz, W. 1975. Effects and metabolism of glucose tolerance factor. *Nurt. Rev.* 33:129–35
78. Mertz, W., Toepfer, E. W., Roginski, E. E., Polansky, M. M. 1974. Present knowledge of the role of chromium. *Fed. Proc.* 33:2275–80
79. Miller, O. N., Hamilton, J. G., Goldsmith, G. A. 1960. Investigations of the mechanism of action of nicotinic acid on serum lipid levels in man. *Am J. Clin Nutr.* 8:480–90
80. Moss, J., Stanley, S. J. 1981. Amino acid-specific ADP-ribosylation: Identification of an arginine-dependent ADP-ribosyltransferase in rat liver. *J. Biol. Chem.* 256:7830–33
81. Moss, J., Vaughan, M. 1979. Activation of adenylate cyclase by choleragen. *Ann. Rev. Biochem.* 48:581–600
82. Mrochek, J. E., Jolley, R. L., Young, D. S., Turner, W. J. 1976. Metabolic response of humans to ingestion of nicotinic acid and nicotinamide. *Clin. Chem.* 22:1821–27
83. Nakagawa, I., Ohguri, S., Sasaki, A., Kajimoto, M., Sasaki, M., Takahashi, T. 1975. Effects of excess intake of leucine and valine deficiency on tryptophan and niacin metabolites in humans. *J. Nutr.* 105:1241–52

84. Nakagawa, I., Sasaki, A. 1977. Effect of an excess intake of leucine, with and without additions of vitamins B_6 and/or niacin on tryptophan and niacin metabolism in rats. *J. Nutr. Sci. Vitaminol.* 23:535–40
85. Nakagawa, I., Takahashi, T., Suzuki, T., Masana, Y. 1969. Effect in man of addition of tryptophan or niacin on the excretion of their metabolites. *J. Nutr.* 99:325–30
86. Nasu, S., Wicks, F. D., Gholson, R. K. 1982. L-Aspartate oxidase, a newly discovered enzyme of *Escherichia coli,* is the B protein of quinolinate synthetase. *J. Biol. Chem.* 257:626–32
87. Nishizuka, Y., Hayaishi, O. 1963. Enzymic synthesis of niacin nucleotides from 3-hydroxyanthranilic acid in mammalian liver. *J. Biol. Chem.* 238:483–85
88. Ogata, N., Ueda, K., Kawaichi, M., Hayaishi, O. 1981. Poly (ADP-ribose) synthetase, a main acceptor of Poly (ADP-ribose) in isolated nuclei. *J. Biol. Chem.* 256:4135–37
89. Oppenheimer, N. J., Bodley, J. W. 1981. Diphtheria toxin, site and configuration of ADP-ribosylation of diphthamide in elongation factor 2. *J. Biol. Chem.* 256:8579–81
90. Patterson, J. I., Brown, R. R., Linkswiler, H., Harper, A. E. 1980. Excretion of tryptophan-niacin metabolites by young men: effects of tryptophan, leucine, and vitamin B_6 intakes. *Am. J. Clin, Nutr.* 33:2157–67
91. Pekala, P. H., Anderson, B. M. 1982. Non-oxidation-reduction reactions of pyridine nucleotides. In *The Pyridine Nucleotide Coenzymes,* ed. J. Everse, B. Anderson, K. S. You, pp. 326–69. NY: Academic
92. Pekala, P. H., Lane, M. D., Watkins, P. A., Moss, J. 1981. On the mechanism of preadipocyte differentiation: masking of poly (ADP-ribose) synthetase activity during differentiation of 3T3-L1 preadipocytes. *J. Biol. Chem.* 256:4871-76
93. Pinder, S., Clark, J. B, Greenbaum, A. L. 1971. The assay of intermediates and enzymes involved in the synthesis of the nicotinamide nucleotides in mammalian tissue. *Methods Enzymol.* 18B:20–46
94. Poirier, G. G., Savard, P., Rajotte, D., Morisset, J., Lord, A. 1978. Properties of poly (ADP-ribose) synthetase from rat pancreas and poly (ADP-ribosylation) of basic nuclear proteins. *Can. J. Biochem.* 56:784–90
95. Preiss, J., Handler, P. 1958. Biosynthesis of diphosphopyridine nucleotide I. Identification of intermediates. *J. Biol. Chem.* 233:488–500
96. Purnell, M. R., Stone, P. R., Whish, W. J. D. 1980. ADP-ribosylation of nuclear proteins. *Biochem. Soc. Trans.* 8:215–27
97. Rechsteiner, M., Hill, K. R. 1975. Autoradiographic studies of nicotinic acid utilization in human-mouse heterokaryons and inhibition of utilization in newly-formed hybrid cells. *J. Cell. Physiol.* 86:439–52
98. Rechsteiner, M., Hillyard, D., Olivera, B. M. 1976. Turnover of nicotinamide adenine dinucleotide in cultures of human cells. *J. Cell. Physiol.* 88:207–18
99. Recommended Dietary Allowances. 1980. Washington DC: Food & Nutri. Board, Natl. Acad. Sci. 9th ed.
100. Reilly, T. M., Beckner, S., McHugh, E. M., Blecher, M. 1981. Isopreterinol-induced ADP-ribosylation of cultured differentiated RL-PR-C hepatocytes. *Biochem. Biophys. Res. Commun.* 98:1115–20
101. Revel, M, Mandel, P. 1962. Effect on an induced synthesis of pyridine nucleotides in vivo on the metabolism of ribonucleic acid. *Cancer Res.* 22:456–62
102. Sadoogh-Abasian, F., Evered, D. F. 1980. Absorption of nicotinic acid and nicotinamide from rat small intestine in vitro. *Biochim. Biophys. Acta* 598:385–91
103. Satyanarayana, W., Rao, B. S. 1977. Effect of dietary protein level on some key enzymes of the tryptophan-NAD pathway. *Br. J. Nutr.* 38:39–45
104. Seegmiller, R. E., Horowitz, A. L., Dorfman, A. 1980. Alterations in the chondroitin sulfate synthesized in chick embryo cartilage cultured in the presence of 6-aminonicotinamide. *J. Embryol. Exp. Morphol.* 59:207–16
105. Shimizu, Y., Shimizu, N. 1981. Insulin and epidermal growth factor stimulate poly ADP-ribosylation. *Biochem. Biophys. Res. Commun.* 99:536–43
106. Shapiro, D. M., Dietrich, L. S., and Shils, M. E. 1957. Quantitative biochemical differences between tumor and host as a basis for cancer chemotherapy. V. Niacin and 6-aminonicotinamide. *Cancer Res.* 17:600–4
107. Smulson, M., Shall, S. 1976. Poly (ADP-ribose). *Nature* 263:14
108. Spector, R., Kelley, P. 1979. Niacin and niacinamide accumulation by rabbit brain slices and choroid plexus *in vitro*. *J. Neurochem.* 33:291–98

109. Streffer, C., Benes, J. 1971. Nicotinamide monoculeotide. Determination of its enzymatic formation in vitro and its physiological role for the biosynthesis of nicotinamide-adenine dinucleotide in mice. *Eur. J. Biochem.* 21:357–62
110. Sturm, G., Stark, D., Spengler, U., Nittinger, J., Jaus, H. H., Grassle, B., Siebert, G. 1980. Long-term perturbation of pyridine nucleotides in rat liver. *Hoppe-Seylers Z. Physiol. Chem.* 361:551–58
111. Talke, H., Keller, J., Schmahl, F. S. 1970. Effect of nicotinamide on the regulation of carbohydrate metabolism in rat liver. *Horm. Metab. Res.* 2:147–52
112. Talke, H., Maier, K. P., Kersten, M., Gerok, W. 1973. Effect of nicotinamide on carbohydrate metabolism in rat liver during starvation. *Eur. J. Clin. Invest.* 3:467–74
113. Tanigawa, Y., Shimoyama, M., Murashima, R. 1970. The role of microorganisms as a function of nicotinamide deamidation in rat stomach. *Biochim. Biophys. Acta* 201:394–97
114. Toepfer, E. W., Mertz, W., Polansky, M. M., Roginski, E. E., Wolf, W. R. 1977. Preparation of chromium-containing material of glucose tolerance factor activity for brewer's yeast extracts and by synthesis *J. Agric. Food Chem.* 25:162–66
115. Turner, J. B., Hughes, D. E. 1962. The absorption of some B-group vitamins by surviving rat intestine preparations. *Q. J. Exp. Physiol.* 47:107–23
116. Turner, J. B., Hughes, D. E. 1962. The absorption of bound forms of B-group vitamins by rat intestine. *Q. J. Exp. Physiol.* 47:124–33
117. VanNess, B. G., Howard, J. B., Bodley, J. W. 1980. ADP-ribosylation of elongation factor 2 by diphtheria toxin, NMR spectra and proposed structures of ribosyl-diphthamide and its hydrolysis. *J. Biol Chem.* 225:10710–16
118. Vessley, B., Lithell, H., Gustafsson, I-B., Boberg, J. 1979. Pronounced lipoprotein lipid reduction obtained by combined lipid lowering treatment of patients with atherosclerotic disease. *Atherosclerosis* 33:457–77
119. Vessley, B., Lithell, H., Gustafsson, I–B., Boberg, J. 1980. Changes in the fatty acid composition of the plasma lipid ester during lipid lowering treatment with diet, clofibrate and niceritol. *Atherosclerosis* 35:51–65
120. White, H. B. 1982. Biosynthetic and salvage pathways of pyridine nucleotide coenzymes. See Ref. 91, pp. 225–348
121. Windmueller, H. G., Spaeth, A. E. 1972. Fat transport and lymph and plasma lipoprotein biosynthesis by isolated intestine. *J. Lipid Res.* 13:92–105
122. Yost, D. A., Anderson, B. M. 1981. Purification and properties of the soluble NAD glycohydrolase from *Bungarus fasciatus* venom. *J. Biol. Chem.* 256:3647–53
123. Zatman, L. J., Kaplan, N. O., Colowick, S. P. 1953. Inhibition of spleen diphosphopyridine nucleotidase by nicotinamide, an exchange reaction. *J. Biol. Chem.* 200:197–212
124. Zatman, L. H., Kaplan, N. O., Colowick, S. P., Ciotti, M. M. 1954. Effect of isonicotinic hydrazide on diphosphopyridine nucleotidase. *J. Biol. Chem.* 209:453–66