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EFFECTS OF ESTRADIOL-17β ON THE SYNTHESIS OF NAD AND NADP IN THE RAT UTERUS

by

Mary Sue O'Dorisio

A DISSERTATION

Presented to the Faculty of

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For the Degree of Doctor of Philosophy

Department of Biochemistry

Under the Supervision of Professor Kenneth L. Barker

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TITLE

Effects of Estradiol-17β on the Synthesis of NAD and NADP in the Rat Uterus

BY

Mary Sue O'Dorisio

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NOTATION

A_{260} ..... Absorbancy at 260 mμ

ADH ..... Yeast Alcohol Dehydrogenase

ADP ..... Adenosine Diphosphate

ADPR ..... Adenosine Diphosphate Ribose

AMP ..... Adenosine Monophosphate

ATP ..... Adenosine Triphosphate

cpm ..... counts per minute

dNAD ..... deamido Nicotinamide Adenine Dinucleotide

dNMN ..... Nicotinic Acid Mononucleotide

dpm ..... disintegrations per minute

E_2 ..... Estradiol-17β

Estrogen Estradiol-17β

Estradiol Estradiol-17β

fp ..... Flavoprotein

G6PDH ..... Glucose-6-Phosphate Dehydrogenase

NAc ..... Nicotinic Acid

NAD ..... Nicotinamide Adenine Dinucleotide

NADH ..... Reduced Nicotinamide Adenine Dinucleotide

NADP ..... Nicotinamide Adenine Dinucleotide Phosphate

NADPH .....Reduced Nicotinamide Adenine Dinucleotide Phosphate

NAm ..... Nicotinamide

NMN ..... Nicotinamide Mononucleotide

PCA ..... Perchloric Acid

PRPP ..... Phosphoribosyl Pyrophosphate

UV ..... Ultraviolet

INTRODUCTION

The pyridine nucleotides NAD, NADH, NADP and NADPH are best known for their role as coenzymes in dehydrogenase enzyme reactions. They serve as cofactors for a variety of cytoplasmic enzymes, including enzymes catalyzing key steps in glycolysis and pentose metabolism, but they have important functions in other cellular compartments as well.

NAD is a substrate for the nuclear enzyme, NAD glycohydrolase, which releases nicotinamide and polymerizes ADP-ribose moieties, linking ADP ribose covalently to the histones associated with nuclear chromatin. Thus, nuclear pyridine nucleotides may participate in regulation of DNA synthesis. NAD serves as an AMP donor for the DNA ligase reaction in E. coli and as such participates directly in DNA synthesis and repair. NADH, and NADPH via transhydrogenase, are electron donors for oxidative phosphorylation. Hence, pyridine nucleotides, by their abundance or lack, may control the mitochondrial formation of ATP. Reduced nucleotides donate hydrogen for fatty acid synthesis in cytoplasm and in mitochondria and for steroid synthesis in mitochondria and in microsomes. Again, metabolism may be regulated by the availability of pyridine nucleotides. These nucleotides can also activate or inhibit enzymes by either competitive or allosteric mechanisms.

No other nucleotide can assume such varied roles as substrate, coenzyme, electron donor, activator and inhibitor and can function

in nucleus, cytoplasm, mitochondria and microsomes. Because of the ubiquitous and versatile nature of the pyridine nucleotides, control of cellular metabolism may conceivably be mediated through changes in the levels, oxidative states or cellular distribution of NAD and NADP.

The specific aims of the research to be reported in this dissertation were as follows:

1) To determine whether estrogen-induced increases in uterine NAD and NADP are the result of an increased rate of de novo synthesis of these coenzymes.

2) To determine which pathway or pathways for biosynthesis of NAD and NADP are active in the uterus.

3) To determine which metabolic step(s) in the biosynthesis of NAD and NADP in the uterus are increased in response to administration in vivo of estradiol.

LITERATURE REVIEW

Synthesis of NAD

The principal precursors for the biosynthesis of NAD and NADP are tryptophan, glycerol-aspartate, acetate-formate, nicotinamide and nicotinic acid (Fig. 1).

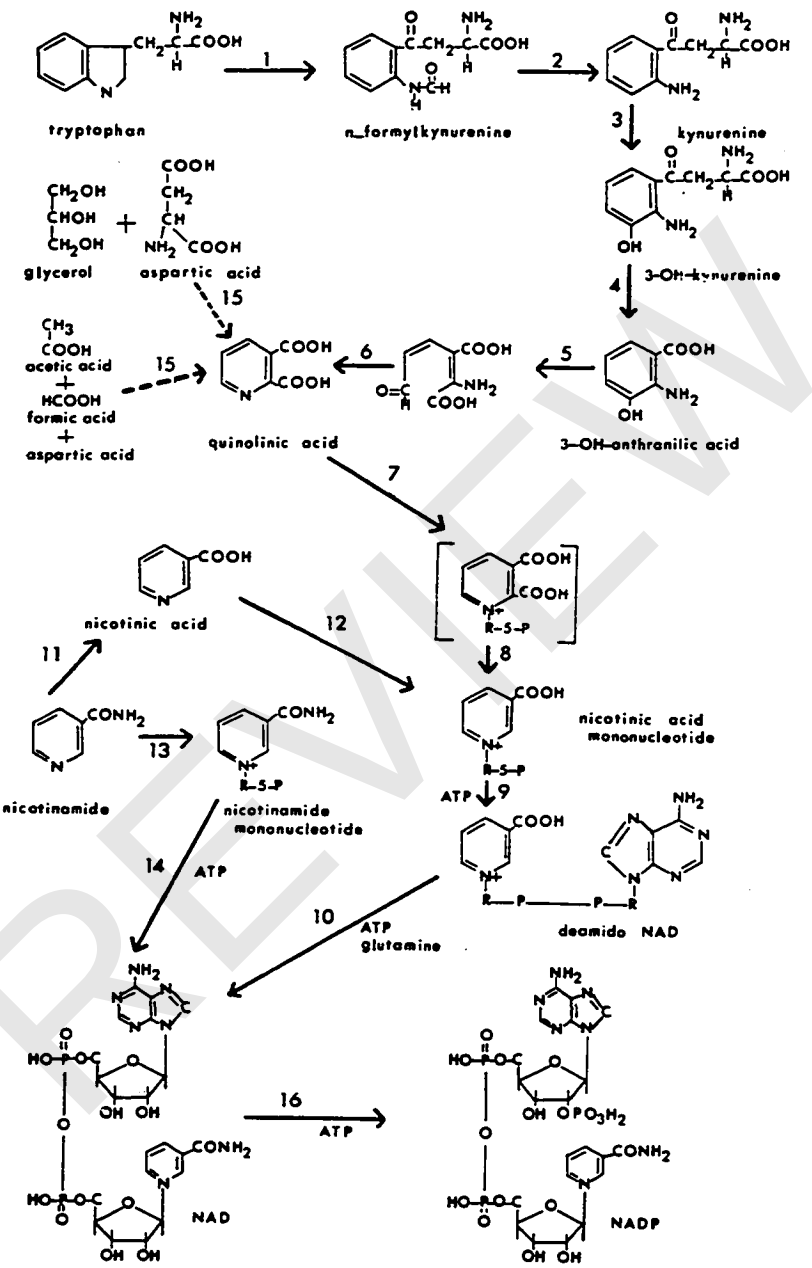
Tryptophan Pathway

It was known as early as 1945 (Krehl et al.) that tryptophan was an adequate dietary substitute for niacin in rats. Nishizuka and Hayaishi (1963) demonstrated the presence of a pathway by which tryptophan can ultimately be converted to the niacin portion of NAD. Tryptophan oxygenase (L-Tryptophan oxygen oxidoreductase EC 1.13.1.12) converts tryptophan to N-formyl kynurenine by splitting the ring and adding H_2O. Enzymatic removal of a CO group results in formation of kynurenine to which a hydroxyl is added to form 3-OH-kynurenine. Alanine is split out resulting in 3-OH-anthranilic acid and the ring is opened to give α-amino-β-carboxymuconic ε-semialdehyde.

This product has two possible fates. It may spontaneously lose H_2O and cyclize to form quinolinic acid or it may be used as a substrate by picolinic carboxylase for formation of α-aminomuconic-ε-semialdehyde, a Coenzyme A precursor. Thus NAD synthesis from tryptophan is inversely related to picolinic carboxylase activity. When carboxylase activity decreases, as in diabetes, NAD synthesis from tryptophan increases (Mehler et al., 1958). Picolinic

FIGURE 1.

Biosynthesis of NAD and NADP



The enzymes which catalyze the reactions depicted above are:

1. Tryptophan Oxygenase
2. Kynurenine Formylase
3. Kynurenine-3-hydroxylase
4. Kynurenine hydrolase
5. 3-OH-anthranilic acid Oxidase
6. Spontaneous
7. Quinolinate Transribosylase
8. Quinolinate Transribosylase
9. dNAD Pyrophosphorylase
10. NAD Synthetase
11. Nicotinamide Deamidase
12. NAc Phosphoribosyl Transferase
13. NAm Phosphoribosyl Transferase
14. NAD Pyrophosphorylase
15. Unknown enzymes
16. NAD Kinase

carboxylase activity is very high in cat liver. In this animal, tryptophan cannot fulfill the requirement for niacin. Since all of the α-amino-β-carboxymuconic-ε-semialdehyde formed from tryptophan is converted to α-aminomuconic-ε-semialdehyde and ultimately to Coenzyme A, no NAD can be synthesized from tryptophan (Nishizuka and Hayaishi, 1971 and Henderson and Swan, 1971).

When picolinic carboxylase activity is low, the quinolinic acid formed is converted to nicotinic acid ribonucleoside and then to nicotinic acid mononucleotide, deamido NAD and NAD. Thus, the tryptophan pathway has two possible regulatory steps, those catalyzed by tryptophan oxygenase and by picolinic acid carboxylase. Partridge et al., (1952) reported the conversion of tryptophan to niacin in the rat, but Nishizuka and Hayaishi (1963) established conclusively that niacin is not a free intermediate in the tryptophan pathway. McDonald et al., (1968) presented the metabolic steps in the conversion of tryptophan to nicotinic acid mononucleotide. This sequence of reactions is also given in Fig. 1.

The tryptophan pathway is operative in mouse liver (McDonald et al., 1968) but tumors from these animals are unable to utilize tryptophan for NAD synthesis. Apparently, the tumors are unable to decarboxylate quinolinic acid. The ability to synthesize NAD from tryptophan is not seen in chick embryos until the sixth day (Shimayama et al., 1969). The tryptophan pathway is operative in mammals and in Neurospora, but only one bacterial species, Xanthomonas pruni is known to possess the necessary enzymes (Saxton et al.,

Glycerol-Aspartate Pathway

Higher plants and many microorganisms synthesize quinolinic acid from glycerol and aspartate. Since quinolinic acid can be converted to dNMN, the condensation of glycerol and aspartic acid is considered a de novo pathway for NAD synthesis (Rowen and Kornberg, 1951). The sequence of reactions by which quinolinic acid is formed from glycerol and aspartate is unknown. Chandler et al., (1970) postulated that a direct condensation occurs but have no evidence yet as to whether the nitrogen-carbon bond or carbon-carbon bond is formed first. Saxton and coworkers (1969) found this pathway to be operative in E. coli, B. subtilis and B. megaterium. They found the pathway to be inhibited when the bacteria were grown in medium containing high levels of nicotinic acid.

Acetate-Formate Pathway

In Clostridium butylicum, acetate and formate are combined with aspartate to form quinolinic acid which then enters the nicotinic acid synthetic pathway at the nicotinic acid mononucleotide step (Isquith and Moat, 1966).

Because quinolinic acid (derived from any of these three sources) is an intermediate of NAD biosynthesis in mammalian tissue and bacteria, many studies of NAD biosynthesis have been done using labeled quinolinic acid. From these studies it is generally concluded that these three pathways (tryptophan, glycerol-aspartate or acetate-formate) are relatively inactive in all organisms unless there is a deficiency

of nicotinic acid and nicotinamide (Chaykin, 1967). While this conclusion is probably valid the interpretation of studies using labeled quinolinic acid as a precursor may be biased due to the relatively poor penetration of this compound into liver cells (Ijichi et al., 1966).

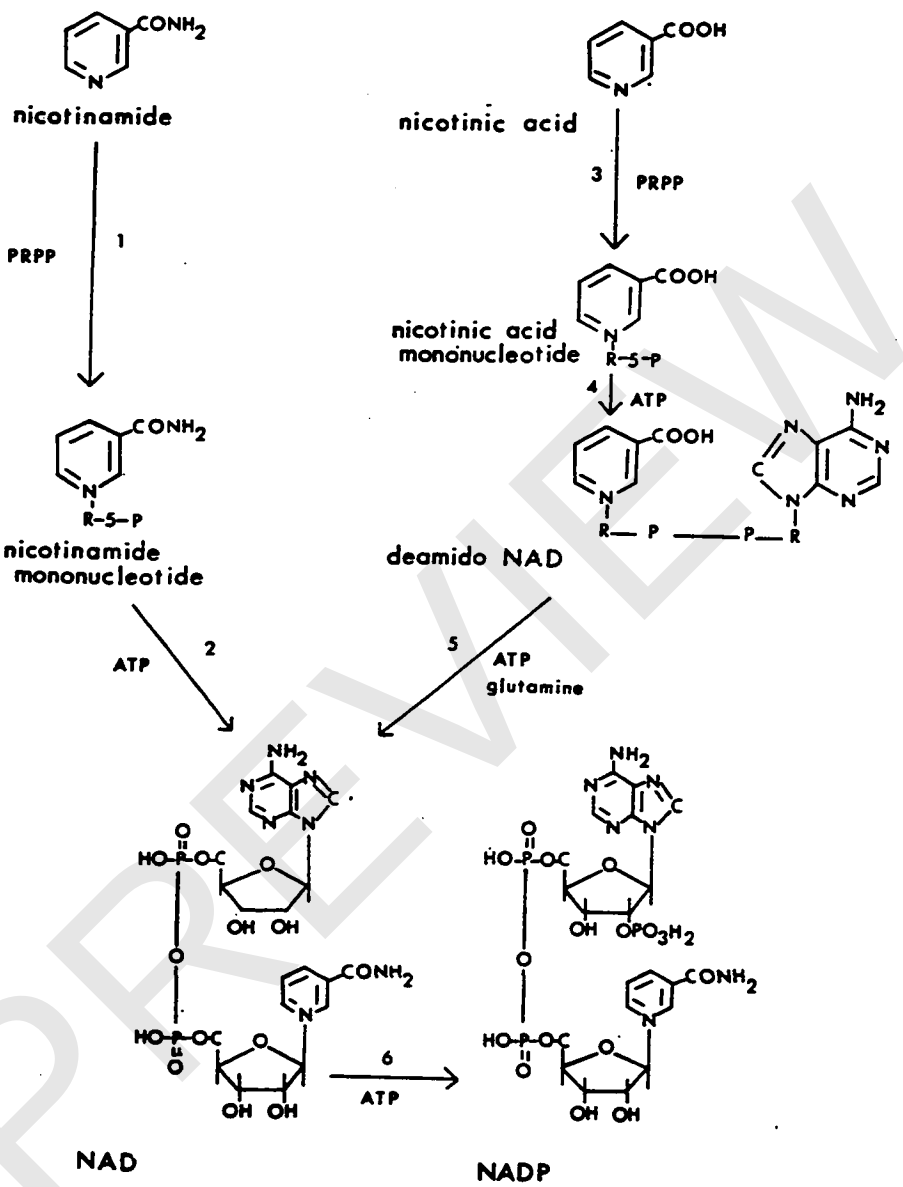
Nicotinic Acid and Nicotinamide Pathways

Nicotinamide and nicotinic acid can serve as NAD precursors in almost all mammalian tissues. Prior to the isolation and determination of the structure of NAD it was shown that administration of nicotinic acid to humans, both normal and pellagrins, caused an increase in blood levels of "V factor" (Kahn, 1938; Hoagland and Ward, 1942; Hoagland et al., 1943; Handler and Kahn, 1943). A smaller increase in "V factor" levels could be effected by administration of nicotinamide but the increase was always less than the increase caused by nicotinic acid (Hoagland et al., 1943 and Handler and Kahn, 1943). "V factor" was later shown to be primarily NAD.

A major discovery was made by Priess and Handler (1958a). They isolated two new compounds thought to be intermediates in NAD biosynthesis and identified them as nicotinic acid mononucleotide (dNMN) and nicotinic acid dinucleotide (dNAD). They were able to demonstrate the presence in human erythrocytes of all the enzymes necessary for the direct synthesis of NAD from nicotinic acid (see Fig. 2). The sequence in the Preiss-Handler pathway for NAD synthesis

FIGURE 2.

Biosynthesis of NAD and NADP



The enzymes for the Nicotinamide Pathway are:

1. Nicotinamide Phosphoribosyl Transferase
2. NAD Pyrophosphorylase

The enzymes for the Nicotinic Acid Pathway are:

3. Nicotinic Acid Phosphoribosyl Transferase
4. dNAD Pyrophosphorylase
5. NAD Synthetase
6. NAD Kinase

was defined as:

$$\text{1) nicotinic acid + PRPP} \xrightarrow[\text{nicotinic acid phosphoribosyl transferase}]{} \text{dNMN + PPi}$$

$$\text{2) dNMN + ATP} \xrightarrow[\text{dNAD pyrophosphorylase}]{} \text{dNAD + PPi}$$

$$\text{3) dNAD + glutamine + ATP} \xrightarrow[\text{NAD synthetase}]{} \text{NAD + glutamate + ADP + Pi}$$

They also found an enzyme capable of converting nicotinamide to NMN and proposed a second sequence for the synthesis of NAD from nicotinamide as follows:

$$\text{4) nicotinamide + PRPP + ATP} \xrightarrow[\text{nicotinamide phosphoribosyl transferase}]{} \text{NMN + PPi + ADP + Pi}$$

$$\text{5) NMN + ATP} \xrightarrow[\text{NAD pyrophosphorylase}]{} \text{NAD + PPi}$$

Nicotinamide: pyrophosphate phosphoribosyltransferase (EC 2.4.2.12 and Reaction 4) has a very high K_m for nicotinamide and the activity of the next enzyme, (ATP: NMN adenyltransferase EC 2.7.7.1), is very low in human erythrocytes (Table I). This observation, coupled with the earlier observations that nicotinic acid was a better NAD precursor than nicotinamide, led Preiss and Handler to postulate that the nicotinic acid pathway was the predominant route for NAD synthesis in mammalian tissues.

The relative efficacy of nicotinamide versus nicotinic acid as NAD precursor and the relative importance of each pathway was a subject of controversy for 15 years.

Table 1. Enzymes Involved in Biosynthesis of NAD and NADP: Characteristics and Kinetics.

Enzyme[a]	Reaction	Organism, Cell Location	K_m	pH opt	Inhibitors and Activators	Isolation Purification
Nicotinamide: pyrophosphate phosphoribosyl transferase - EC 2.4.2.12 (NMN pyrophosphorylase)	NAm + PRPP + ATP → NMN + ADP + PP + Pi	Lacto bacillus fructosus cell supernatant	NAm $2x10^{-6}$M PRPP $4x10^{-5}$M	6-7	Inhibitors NMN NAD Unknown endogenous NAm analogues Activators Mg^{++} ATP	Ohtsu et al., 1967
		Rat liver cytoplasmic (acid soluble)	ATP $6.7x10^{-4}$M	8.0		Dietrich et al., 1966
ATP: NMN Adenyl-transferase - EC 2.7.7.1 (NAD pyrophosphorylase)	NMN + ATP → NAD + PP rat liver nuclei	Lactobacillus fructosus protamine SO_4 precipitate of acid supernatant	NMN $6.7x10^{-4}$M $2.7x10^{-3}$M ATP		Inhibitors hypophysectomy adrenalectomy Activators α-amanatin	Ohtsu et al., 1967 Strip and Corte 1968 Traub et al., 1968

(continued next page)

Table 1. (continued)

Enzyme[a]	Reaction	Organism, Cell Location	K_m	pH opt	Inhibitors and Activators	Isolation Purification
Nicotinate: pyrophosphate phosphoribosyl transferase - EC 2.4.2.11 (Nicotinic acid phosphoribosyl-transferase)	NAc + PRPP + ATP → dNMN + PP + ADP	Baker's yeast acid supernatant	NAc $1.85x10^{-6}$M ATP $1.2x10^{-4}$M PRPP $7.7x10^{-6}$M	8.0	Inhibitors ADP dNMN hypoxathine NaF Activators Mg^{++} ATP	Honjo, 1971
ATP: dNMN adenyl-transferase - EC 2.7.7.18 (dNAD pyrophos-phorylase)	dNMN + ATP → dNAD + PPi	Hog liver acid supernatant E. coli acid soluble	dNMN $3x10^{-5}$M ATP $5x10^{-4}$M	7.5	Inhibitors None tested Activators Mg^{++}	Preiss and Handler, 1958 Imsande, 1961

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