

THE UNIVERSITY OF MANITOBA
" THE EFFECT OF NIACIN DEFICIENCY ON THE
REGULATION OF PYRIDINE NUCLEOTIDES AND
ENZYMES IN VARIOUS TISSUES OF JAPANESE
QUAIL "

BY
IN KOOK PARK

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the University of Manitoba in partial fulfillment of the requirements
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ABSTRACT

The addition of 0.5% sulfaguanidine or high leucine to niacin free diets did not induce niacin deficiency symptoms in mature Japanese quail. Niacin deficiencies in growing quail resulted in the typical growth depression, poor feather development, blindness, and lowering of NAD and NADPH levels in the breast muscle. However, there were no significant differences in pyridine coenzyme levels in liver, brain, and heart as compared to niacin fed quail. Similarly, there were no significant differences in activities of liver aldolase, lactic dehydrogenase, glutamic dehydrogenase, tryptophan pyrrolase, fructose diphosphatase, and malic enzyme. Niacin deficiency or food-restriction led to a reduction in breast muscle lactic dehydrogenase or glyceraldehyde-3-phosphate dehydrogenase activity.

6-Aminonicotinamide as an antagonist of niacin did not affect the levels of pyridine coenzyme, ATP, lactic dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase activity in liver or breast muscle whereas specific activities of liver malic enzyme and fructose diphosphatase of the control group were significantly higher than those of the 6-aminonicotinamide treated group.

The apparent turnover rates of radioactive products in breast muscle following the injection of 7-¹⁴C-nicotinic acid were extremely low while the turnover rates in liver were much higher than those in breast muscle. Turnover rates in

heart and brain were intermediates to those of liver and breast muscle. The apparent turnover rates of niacin products in all tissues in the niacin deficient quail were the slowest whereas those of quail fed a high level of niacin were the fastest. The apparent half-lives of nicotinamide, NAD, and NADP in liver of the niacin deficient quail were estimated to be 80.3, 100.2, and 626.0 hours, respectively, whereas the corresponding values for the food-restricted quail were 92.1, 87.7, and 469.2 hours, respectively. In breast muscle the apparent half-life of nicotinamide in the food-restricted quail was about 10 times shorter than that of the niacin deficient quail. The apparent half-life of NAD in the food-restricted quail was 490.1 hour whereas the corresponding value was infinite in the niacin deficient quail.

Thermal stability studies on liver and breast muscle enzymes from control, niacin deficient and food-restricted quail showed that there were no significant differences in the thermal stability of liver malic enzyme, glyceraldehyde-3-phosphate dehydrogenase, lactic dehydrogenase, and aldolase among treatments. Similarly, no significant differences in thermal stability of breast muscle lactic dehydrogenase and aldolase were observed. However, breast muscle glyceraldehyde-3-phosphate dehydrogenase activity in niacin deficient quail was significantly reduced as compared to that from the control and food-restricted quail. Among all of the liver and breast muscle enzymes tested, breast muscle glyceraldehyde-3-phosphate dehydrogenase appeared to be the most

sensitive of all enzymes to heat inactivation in the absence of NAD or NADP in the incubation mixture.

The effects of niacin deficiency on relative turnover rates of proteins showed that liver had the fastest turnover rates, that heart and brain had the intermediate turnover rates and breast muscle had the slowest turnover rates of proteins irrespective of dietary treatments. The $^3\text{H}/^{14}\text{C}$ ratios of both control and niacin deficient quail were significantly higher than those of the food-restricted quail but there were no significant differences between the former groups. This suggests that the relative turnover rates of proteins in the niacin deficient quail were much greater than those of the food-restricted quail. The resolution of breast muscle proteins by isoelectric focusing showed greater degradation of certain specific acidic proteins in niacin deficient quail relative to control quail.

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ABBREVIATIONS

- A₂₆₀: Absorption at 260nm of a solution in a cuvette with a one centimeter lightpath.
- ADH: Alcohol dehydrogenase.
- ADP: Adenosine-5'-diphosphate.
- ADRP: Adenosine diphosphoribose.
- AMP: Adenosine-5'-monophosphate.
- 6-AN: 6-Aminonicotinamide.
- ATP: Adenosine-5'-triphosphate.
- CoA: Coenzyme A.
- DNA: Deoxyribonucleic acid.
- DEAE: Diethylaminoethyl.
- EDTA: Ethylenediaminetetraacetic acid.
- G.I.Tract: Gastrointestinal tract.
- GSH: Reduced glutathione.
- IDH: Isocitrate dehydrogenase.
- IU: International unit.
- LDH: Lactic dehydrogenase.
- NAD: Nicotinamide adenine dinucleotide.
- NADP: Nicotinamide adenine dinucleotide phosphate.
- NFD: Niacin free diet.
- O.D.: Optical density.
- Pi: Inorganic phosphate.
- PMSF: Phenylmethylsulfonyl fluoride.
- Poly U: Polyuridylic acid.

POPOP: 1,4 bis-{2-(5-phenyloxazolyl)} benzene.
PPi: Pyrophosphate.
PRPP: 5-phosphoribosyl-1-pyrophosphate.
RNA: Ribonucleic acid.
mRNA: Messenger RNA.
tRNA: Transfer RNA.
SDS: Sodium dodecyl sulphate.
TCA: Trichloroacetic acid.
Tris: Tris(hydroxymethyl) aminomethane.

INTRODUCTION

Niacin is a precursor of pyridine coenzymes. They have been implicated in most of the major metabolic pathways. As the prosthetic group of several dehydrogenases, they are involved in glycolysis, fat synthesis and tissue respiration. Recently NAD has been shown to have functions beyond its well known role as an electron carrier in various biological oxidation-reduction systems.

One such area is that NAD is consumed in certain metabolic reactions in which it serves as a substrate. In eukaryotic cells, the most intriguing of these reactions is the cleavage of NAD to form nicotinamide and a unique polymer, poly adenosine diphosphoribose (poly ADPR); the reaction is catalyzed by the enzyme poly ADPR synthetase (Chambon et al., 1963). Other well studied reactions involving the destruction of NAD include the cleavage of NAD to form AMP and NMN (nicotinamide mononucleotide) by bacterial DNA ligases (Olivera and Lehman, 1967) and the breakdown of NAD (with the concomitant inactivation of protein synthesis) by the diphtheria toxin (Collier and Pappenheimer, 1964). Thus, one could envision a rather high turnover of NAD in various cells and this emphasizes the importance of reutilizing nicotinamide in an efficient manner.

The objective of this thesis was to investigate in greater detail the development of niacin deficiency and its effect on turnover of pyridine coenzymes and to establish

the role which niacin plays in the turnover of tissue proteins, particularly those proteins that have a coenzyme requirement for pyridine nucleotides.

More specific objectives were:

1. To establish the reliability of methods for the extraction and determination of pyridine nucleotide contents. This included:
 - a) the recovery of pyridine nucleotides in the extraction medium and assay mixture.
 - b) the stability of pyridine nucleotides as affected by post-mortem changes, duration of homogenization time and storage in the presence of reducing reagents.
2. To induce niacin deficiency in mature female quail (127 - 128 g). This included a study on the effect of high leucine or sulfaguanidine in a niacin free diet on growth, liver weight, liver pyridine nucleotide content and liver enzyme activity levels.
3. To induce niacin deficiency in immature quail (55 - 62 g) in a manner similar to that outlined above.
4. To determine the effect of niacin deficiency in growing quail of varying ages. The response in growth, pyridine nucleotide contents and enzyme activity levels to graded levels of nicotinic acid and to a high level of tryptophan in a niacin-free diet was followed.
5. To determine if 6-aminonicotinamide (6-AN), as an anti-niacin, produced a pattern of symptoms or metabolic dis-