

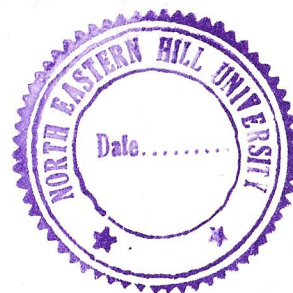
**ROLE OF GLUTAMATE DEHYDROGENASE IN AMMONIA  
MANAGEMENT IN A FRESHWATER AIR-BREATHING  
TELEOST, Heteropneustes fossilis**

**JHARNA RANI DAS**

BIOCHEMICAL ADAPTATION LAB  
DEPARTMENT OF ZOOLOGY  
SCHOOL OF LIFE SCIENCES  
NORTH-EASTERN HILL UNIVERSITY  
SHILLONG-793014 ( INDIA )  
REGISTRATION NO. 431

THESIS SUBMITTED IN FULFILMENT OF THE  
REQUIREMENT OF THE DEGREE OF  
**DOCTOR OF PHILOSOPHY IN ZOOLOGY**

To



**NORTH-EASTERN HILL UNIVERSITY, SHILLONG**

**JANUARY, 1991**



# NORTH-EASTERN HILL UNIVERSITY

Biochemical Adaptation Lab., Department of Zoology, School of Life Sciences, Shillong-793014, India

Professor B. K. Ratha  
Ph. D. (B.H.U.)

January 28, 1991

Certified that the thesis entitled "ROLE OF  
GLUTAMATE DEHYDROGENASE IN AMMONIA MANAGEMENT IN A FRESHWATER  
AIR-BREATHING TELEOST, *HETEROPNEUSTES FOSSILIS*", submitted by  
Miss Jharna rani Das for the degree of DOCTOR OF PHILOSOPHY in  
ZOOLOGY of North Eastern Hill University, Shillong embodies the  
record of original investigations carried out by her under my  
supervision. She has been duly registered and the thesis  
presented is worthy of being considered for the award of the Ph.D.  
Degree. This work has not been submitted for any degree of any  
University.

*B.K. Ratha*

(B.K. Ratha)

Supervisor

*Forwarded*  
*A. Raju Varman*  
*28/1/91*

Head  
Department of Zoology  
School of Life Sciences  
North Eastern Hill University  
Shillong

## CONTENTS

### ACKNOWLEDGEMENTS

I take this delightful opportunity to express my deepest gratitude to Prof. **B.K. Ratha**, Department of Zoology, North-Eastern Hill University, for his constant encouragement, pertinent criticism and infinite patience in seeing me to the completion of my research work.

I am indebted to the Department of Zoology for having provided the necessary facilities and to all the teachers of the Zoology Department for their generous help and encouragement.

The award of fellowship from North-Eastern Hill University, Shillong, is gratefully acknowledged.

I record my appreciation of the special interest which Dr. A. Alam, Department of Biochemistry, took in guiding me in immunological studies.

I gratefully acknowledge the help given by my colleagues Dr. N. Saha, Ms. J. Chakravorty, Ms. J. Dkhar, Ms. B. Choudhury, Ms. R.K. Rana, Ms. A. Saha, Mr. R.M. Lyngdoh and Mr. S. Tarasia. I am also grateful to Mrs. S. Ratha for her encouragement.

I am thankful to my friends M/S Sarfraz, Biplab, Sushil, Alka, Mita and Esther for their timely help. I thank Mr. B.K. Das for the photography Mr. S. Roy Choudhury for the illustrations, Mr. J.P. Rai and Mr. S. Pohiti for maintenance of the animals.

I thank my brother for his neat and meticulous typing of the manuscript.

Finally, I specially thank my father, who has been a constant source of inspiration and encouragement in my academic pursuits. I also thank my mother, brother, sisters and sister-in-law for having urged me with the determination to carry on despite all the impediments faced by any researcher anywhere.

SHILLONG

The 28<sup>th</sup> Jan. 1991

*Jharna Rani Das*

JHARNA RANI DAS

## CONTENTS

	<u>Page</u>
LIST OF ABBREVIATIONS	i-iii
INTRODUCTION	1-18
Ammoniogenesis	
Toxicity of ammonia	
Utilization and detoxification of ammonia	
Classification of GDH	
Purification, molecular characterization and kinetics of GDH	
Plan of Work	
MATERIALS AND METHODS	19-40
Animals	
<u>Experimental set up</u>	
Physiological level, circadian and seasonal variation	
Effect of temperature acclimation	
Effect of water deprivation	
Effect of starvation, refeeding and ambient hyper-ammonia stress	
<u>Tissue processing</u>	
<u>Estimations</u>	
Enzyme assays	
Glutamate dehydrogenase	
Lactate dehydrogenase	
Cytochrome oxidase	
Protein	
Total free amino acid (FAA)	
Purification of hepatic GDH and its physico-chemical properties	
Crude extract	
Ammonium sulphate fractionation	
Ion exchange chromatography on DEAE-Cellulose	
Ion exchange chromatography on DEAE-Sepharose	
Affinity chromatography on Blue Sepharose	
Polyacrylamide gel electrophoresis (PAGE)	
GDH specific staining	
Protein staining	
Absorption spectra	

Temperature optima and thermal stability  
pH optima  
Kinetic studies  
Product inhibition of GDH

Molecular weight determination

Exclusion chromatography  
PAGE  
Preparation of standard protein and GDH sample for SDS-PAGE

Immunological Studies

Preparation of antiserum  
Immuno-diffusion

Chemicals

Statistical analysis and presentation of data

**RESULTS**

**41-52**

Effect of buffer on stability on GDH activity  
ADP and nicotinamide coenzyme requirement  
Physiological level of GDH in various tissues  
Sub-cellular distribution of GDH  
Circadian (24 hr) cycle  
Annual cycle  
Effect of temperature  
Effect of water deprivation  
Effect of Starvation, refeeding and hyper-ammonia stress  
Purification of GDH from liver of H. fossilis  
Kinetics and substrate inhibition  
Absorption spectra  
Temperature optima and thermal stability  
pH optima  
Amino acid (substrate) specificity  
Coenzyme specificity  
Effect of various nucleotides  
Effect of various ions  
Effect of acetylcholine, biogenic amines and some amino acid derivatives  
Effect of varying substrates  
Product inhibition  
Effect of amino acids and keto acids  
Effect of antibody  
Determination of molecular weight  
Immuno diffusion

TABLES 1 - 39

FIGURES 1 - 59

DISCUSSION

53-86

- Effect of buffer on stability of GDH activity
- ADP and nicotinamide coenzyme requirement
- Tissue distribution
- Sub-cellular distribution
- Circadian (24 hr) cycle
- Annual cycle
- Effect of temperature
- Effect of water deprivation
- Effect of starvation, refeeding and hyper-ammonia stress
- Purification of GDH from liver of H. fossilis
- Kinetics
  - Substrate inhibition
  - Absorption spectra
  - Temperature optima and thermal stability
  - pH optima
  - Amino acid (substrate) specificity
  - Coenzyme specificity
  - Effect of metabolites on GDH activity
    - Purine nucleotides
    - Various ions
    - Acetylcholine, biogenic amines and some amino acid derivatives
    - Varying substrates
    - Product inhibition
    - Amino acids and keto acids
- Immunological studies
- Determination of molecular weight

REFERENCES

87-145

## LIST OF ABBREVIATIONS

Ab	- Antibody
ADP	- Adenosine 5'-diphosphate
Ag	- Antigen
Ala	- Alanine
ALT	- Alanine transaminas
AMP	- Adenosine 5'-monophosphate
ARG	- Arginase
ASL	- Arginino succinase
Asp	- Aspartate
ASS	- Arginino succinate synthetase
ATP	- Adenosine 5'-triphosphate
$\alpha$	- Alpha
BLD	- Below limit of detection
BSA	- Bovine serum albumin
$^{\circ}\text{C}$	- Degree centigrade
C	- Competitive inhibitor
Cm	- Centimetre
CPS	- Carbamyl phosphate synthetase
Cys	- Cysteine
Cyt. oxi	- Cytochrome oxidase
DEAE	- Diethylaminoethyl
D	- Dextro rotatory
E.C.	- Enzyme Comission
EDTA	- Ethylenediaminetetra-acetate
ETS	- Electron transport system
FAA	- Free amino acid
FCA	- Complete freund's adjuvant
Fig	- Figure
g	- gram
Xg	- Centrifugal force
GDH	- Glutamate dehydrogenase
GDP	- Guanosine 5'-diphosphate
Glu	- Glutamate
GOT	- Glutamate oxaloacetate transaminase
GOGAT	- Glutamate synthase
GS	- Glutamine synthetase
GTP	- Guanosine 5'-triphosphate
$\gamma$	- Gamma
Hrs	Hours
IMP	- Inosine monophosphate
Ka	- Activation constant
Kav	- Distribution coefficient
KG	- Ketoglutarate
Ki	- Inhibition constant
Km	- Michaelis-Menten's constant of enzyme for particular substrate
l	- Litre

$\lambda$	- Lemda (wave length)
L	- Levorotatory
Lys	- Lysine
LDH	- Lactate dehydrogenase
M	- Molar
mA	- Milliampers
$\mu$ l	- Microlitre
$\mu$ M	- Micromolar
$\mu$ mole	- Micromole
mg	- Miligram
ml	- Millilitre
mM	- Millimolar
min	- Minute
Mw	- Molecular weight
nm	- Nenometer
NAD <sup>+</sup>	- Nicotinamide adenine dinucleotide
NADH	- Nicotinamide adenine dinucleotide, reduced form
NADP <sup>+</sup>	- Nicotinamide adenine dinucleotide phosphate
NADPH	- Nicotimanide adenine dinucleotide phosphate, reduced form
NBT	- Nitroblue tetrazolium
NC	- Non-competitive inhibitor
ND	- Not determined
N.S.	- Non-significant
O.D.	- Optical density
OTC	- Ornithine transcarbamylase
o-u	- Ornithine urea
$\omega$	- Omega
p	- Probability (Level of significance)
PAGE	- Polyacrylamide gel electrophoresis
PCA	- Perchloric acid
PMS	- Phenazine methosulphate
Pi	- Inorganic phosphate
%	- Percentage
Rm	- Relative mobility
S.A.	- Specific activity
S.D.	- Standard deviation
SDS	- Sodium dodecyl sulphate
SDS-PAGE	- Polyacrylamide gel electrophoresis in the presence of SDS
Sec	- Second
T.A.	- Total activity
TCA	- Trichloreacetic acid
TEMED	- N,N,N',N'-tetramethylethylenediamine
Tris	- Tris(hydroxy methyl)aminomethane
Try	- Tryptophan
Tyr	- Tyrosine
UC	- Un-competitive inhibitor
uv	- Ultra violet

- v - volume
- v/v - volume/volume
- Ve - Elution volume
- Vmax - Maximum rate
- Vo - Void volume
- Vt - Total volume of the packed gel
- w/v - weight/volume

## INTRODUCTION

Ammonia plays a central role in nitrogen metabolism in living organisms. It is both a product of catabolism and a precursor for the synthesis of many nitrogen containing biomolecules. It is, however, extremely toxic to animals if allowed to accumulate even at a very low concentration in vivo. Ammonia production, therefore, must be balanced by its excretion and/or conversion to some other less toxic compounds such as glutamate, glutamine, urea, uric acid etc. for temporary storage in vivo (Forster & Goldstein, 1969; Campbell et al., 1972; Campbell, 1973; Watts & Watts, 1974; Hoar, 1984; Nener, 1988; Powers-Lee & Meister, 1988).

### Ammoniogenesis

Ammonia production in animals has been shown by transamination and deamination of amino acids, amides, amines, purines, pyrimidines, nucleosides, nucleotides and hexosamines (Cohen & Brown, 1960; Meister, 1965; Campbell, 1973; Bishop, 1976; Kormanik & Cameron, 1981a,b; Cameron & Heisler, 1985; Evans, 1985; Evans & Cameron, 1986; Randall & Wright, 1987; Powers-Lee & Meister, 1988). The major pathway of ammoniogenesis in animals has been transdeamination of various amino acids (Forster & Goldstein, 1969; Krebs et al., 1978; Walton & Cowey, 1982; Campbell et al., 1983; Lehninger, 1987; Turner & Lushbangh, 1988; Prentø, 1989) besides deamination (Watts & Watts, 1974). Liver has been suggested as the primary site for ammonia production even though the necessary enzymes have also been located in some other tissues such as the kidney, gills and skeletal muscle (Goldstein & Forster,

1961; McBean *et al.*, 1966; Walton & Cowey, 1977, 1982). Substantial amount of hepatic ammonia in fish arises from the oxidative deamination of glutamate catalyzed by glutamate dehydrogenase (GDH) (Pequin, 1962, 1967; Pequin & Serfaty, 1963, 1968; Janssens & Cohen, 1968; Forster & Goldstein, 1969; Wilson, 1973a; Vellas & Serfaty, 1974; van Waarde & Kesbeke, 1981a,b; Campbell *et al.*, 1983; Casey *et al.*, 1983; Chew & Ip, 1987). Glutamate is produced by transamination reaction between  $\alpha$ -ketoglutarate and most amino acids released by hydrolysis of proteins (Hird & Marginson, 1966; Watts & Watts, 1974; Lehninger, 1987; Powers-Lee & Meister, 1988). The number, specificity and sub-cellular localization of various transaminases are still not fully understood. However, it has been clear since Schoenheimer (1942) that most amino acids can undergo reversible transamination. Campbell (1973) suggested that serine and threonine generally are not transaminated in animal tissues. These two hydroxy-amino acids are deaminated by specific dehydratases because of their initial action of removing water. Glutamate is also derived directly from the hydrolysis of protein and in the degradation of glutamine, proline and histidine (Salvatore *et al.*, 1965; Janicki & Lingis, 1970). The overall reaction of liberation of ammonia from amino acids via glutamate, as presented below, is known as transdeamination (Braunstein, 1939).

