

بسم الله الرحمن الرحيم





شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم



جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار





بعض الوثائق الأصلية تالفة





بالرسالة صفحات لم ترد بالأصل



**Studies on Nuclear Proteins of the Fresh Water
Snail *Biomphalaria alexandrina***

**Thesis
Submitted in Partial Fulfillment For
the Master Degree of Science (M.Sc.)
in Zoology (Physiology).**

**By
Yasser Ezzat Abdel Aziz Shahein
B.Sc. Zoology
Faculty of Science
Tanta University
1991**

Supervised by

M. F. Bayomy
Prof. Dr.
Mohamed Fathy Farag Bayomy
Professor of Physiology
Zoology Dept.
Faculty of Science
Menoufia University

Ragaa R. Hamed
Prof. Dr.
Ragaa Reda Hamed
Professor of Biochemistry
Molecular Biology Dept.
Genetic Engineering Division
National Research Centre

**Dr.
Imam Abdel Salam Hassouna**
Lecturer of Physiology
Zoology Dept.
Faculty of Science
Menoufia University

**Menoufia University
Faculty of Science
Department of Zoology
(1997)**

B
11529

Acknowledgment

I would like to express my sincere thanks to *Prof. Dr. Mohamed Fathy Bayoumy*, Prof. of Physiology, Department of Zoology, Faculty of Science, Menoufia University for his close supervision, continuous guidance, and valuable criticism.

I owe special thanks, deepest feeling and sincere gratitude to *Prof. Dr. Ragaa Reda Hamed*, Prof. of Biochemistry, Department of Molecular Biology, National Research Centre for suggesting this point, valuable supervision, sympathetic help, and encouragement. It was a great honor to work under her supervision.

It is a pleasure to offer my thanks and personal indebtedness to *Dr. Imam Abdel Salam Hassouna*, Lecturer of Physiology, Department of Zoology, Faculty of Science, Menoufia University for kind help and cooperation done to make this work.

Deepest gratitude is indebted to *Dr. Mahmoud Abdel-Aziz Ibrahim*, Researcher of Physiology, Department of Molecular Biology, National Research Centre for his valuable guidance during the practical work of this thesis.

Finally last I would like to thank the members of Molecular Biology Department, National Research Centre.

*To my parents,
my wife, Amira &
my son, Ahmed.*

Table of Contents

Item	Page
List Figures	I
List of Tables	IV
Introduction	1
Aim of Work	3
Review of Literature	4
Materials and Methods	31
Materials	31
I- Snail material	31
II- Chemicals	31
Buffers	31
Methods	32
I- Preparation of nuclear components	
1- Isolation and purification of snail nuclei	32
2- Extraction and fractionation of nuclear components	
a- Histones	32
b- Nucleic acids	33
c- Nonhistone proteins	33
3- Preparation of calf thymus histones	33
4- Separation of histone H1 and core histones from <i>B. alexandrina</i>	34
II- Analytical procedures	
1- Determination of protein	34
2- Determination of DNA	35
III - Enzyme assays	
1- Acid and nutral phasphatase activities	38
2- Catalase activity	41
IV- Electrophoresis	
1- SDS-PAGE	42
2- Tricine SDS-PAGE	45
3- Staining	
a- Coomassie brilliant blue R-250	47
b- Silver nitrate staining	47
4- Molecular weight determination	49

V- Gel exclusion chromatography	49
VI- Immunological procedures	
1- Preparation of anti H1 antibodies	53
2- Enzyme Linked Immuno Sorbent Assay (ELISA)	53
VII- Subcellular fractionation.....	55
VIII- Statistical analysis	57

Results

I- Effect of the relative centrifugal force on the nuclear yield	58
II- Isolation and purification of nuclei from	
<i>Biomphalaria alexandrina</i> snails	58
III- Criteria of nuclei purity	
1- Morphologic criterion	60
2- Biochemical criterion	60
IV- Determination of the different nuclear content in both	
clean and <i>S.mansoni</i> infected snails	
1- Histone content and its related DNA content	60
2-Nonhistone nuclear proteins and their related DNA content	63
V-Electrophoretic pattern of <i>B. alexandrina</i> histones	
1- 18% SDS-PAGE	67
2- Tricine-SDS-PAGE	69
3- Electrophoretic comparison between clean and infected snail histones	69
4- Molecular weight determination of snail histones	69
5- Electrophoretic comparison between clean and infected snail	
nonhistones extracted by both NaOH & Triethanolamine	73
VI- Chromatographic separation of histones on Bio-gel P-60	
1- Calf thymus histones	76
2- Clean snail histones	80
VII- Extraction of histone H1 and core histones	83
VIII- Purification of histone H1	83

IX- Production of antihistone H1	86
X- Optimal antigen (H1) and Antibodies concentration	86
XI- Optimal alkaline phosphatase conjugate concentration	88
XII- Specificity of antihistone H1 of	
<i>Biomphalaria alexandrina</i>	88
XIII- Determination of H1 in <i>B. alexandrina</i> snails during	
weeks of infection exposure	92
XIV- Acid phosphatase activity in clean and <i>S. mansoni</i>	
infected <i>B. alexandrina</i> cell fractions	94
XV- Effect of polycations on acid phosphatase activity of cell	
fractions of clean & infected <i>B. alexandrina</i>	97
XVI- Effect of tartarate on acid phosphatase of clean and	
infected <i>B. alexandrina</i> cell fractions	97
XVII-Neutral phosphatase activity in clean and <i>S. mansoni</i>	
infected <i>B. alexandrina</i> cell fractions	104
Discussion	110
Summary	126
References	131
Arabic summary	

Fig. (20): 18% SDS-PAGE of clean and infected <i>B. alexandrina</i> non-histones extracted by NaOH and Triethanolamine	74
Fig. (21): Chromatographic elution profile of calf thymus histones on Bio gel P-60	77
Fig. (22): 18% SDS-PAGE of calf thymus histone fractions separated by column chromatography on Bio gel P-60	79
Fig. (23): Chromatographic elution profile of clean <i>B. alexandrina</i> histones on Bio gel P-60	81
Fig. (24): 18% SDS-PAGE of clean <i>B. alexandrina</i> histone fractions separated by column chromatography on Bio gel P-60	82
Fig. (25): 18% & Tricine-SDS-PAGE of perchloric acid soluble fraction and perchloric acid insoluble fraction	84
Fig. (26): 18% SDS-PAGE of perchloric acid extract of clean snail fractions eluted from Bio gel P-60 column and stained with silver nitrate	85
Fig. (27): Developmental profile of antibodies to clean snail H1 raised in the rabbit	87
Fig. (28): A checker board to determine the optimum concentration of the antigen and antibody used in performing the ELISA test	89
Fig. (29): Titration curve of ALPase labelled anti-rabbit IgG	90
Fig. (30): ELISA titration curve for the specificity of anti-H1	91
Fig. (31): ELISA absorbance values of infected snail H1 level during the first 4 weeks of exposure to infection and 3 weeks post shedding	93
Fig. (32): Distribution of APase in cell fractions of clean & infected <i>B. alexandrina</i>	96

Fig. (33): APase activity in presence of histones in cell fractions of clean & infected <i>B. alexandrina</i>	99
Fig. (34): APase activity in presence of protamines in cell fractions of clean & infected <i>B. alexandrina</i>	100
Fig. (35): APase activity in presence of 1 μ mole tartrate in cell fractions of clean & infected <i>B. alexandrina</i>	102
Fig. (36): APase activity in presence of 5 μ mole tartrate in cell fractions of clean & infected <i>B. alexandrina</i>	103
Fig. (37): Distribution of NPase in cell fractions of clean & infected <i>B. alexandrina</i>	106
Fig. (38): NPase activity in presence of histones in cell fractions of clean & infected <i>B. alexandrina</i>	108
Fig. (39): NPase activity in presence of MgCl ₂ in cell fractions of clean & infected <i>B. alexandrina</i>	109

List of Tables

Table number	Page
Table (1): Classification of histones	6
Table (2): Monitoring the purity of nuclei by detection of catalase activity	62
Table (3): The acid soluble proteins of the clean and <i>S. mansoni</i> infected snail tissues	64
Table (4): The nuclear DNA of the clean and <i>S. mansoni</i> infected snail tissues	65
Table (5): Nonhistone contents of clean and infected snail tissues	66
Table (6): Molecular weights of snail histones determined by Tricine -SDS-PAGE using calf thymus histones as molecular weight markers	72
Table (7): The molecular weights of the major non-histone proteins from clean and infected snails	75
Table (8): Summary of the purification of calf thymus and clean snail histons on Biogel P-6	78
Table (9): Acid phosphatase activity in <i>B. alexandrina</i> cell fractions	95
Table (10): Effect of polycations on APase activity in clean and infected <i>B. alexandrina</i>	98
Table (11): Effect of tartrate on APase activity of the cellular fractions of clean and infected <i>B. alexandrina</i>	101
Table (12): NPase activity in <i>B. alexandrina</i> cell fractions	105
Table (13): Effect of histones and $MgCl_2$ on NPase activity of the cellular fractions of clean and infected <i>B. alexandrina</i>	107

List of Abbreviations

AC	Acetylated
ADP	Adenosine diphosphate
Ala	Alanine
ALP	Alkaline phosphatase
APase	Acid phosphatase
APS	Ammonium persulphate
Arg	Arginine
<i>B. alexandrina</i>	<i>Biomphalaria alexandrina</i>
BSA	Bovine serum albumin
bP	Base pair
C	Cytosol
C-terminal	Carboxy-terminal
DNA	Deoxy ribonucleic acid
ELISA	Enzyme Linked Immuno Sorbent Assay
Gly	Glycine
H1	Histone 1
H2A	Histone 2A
H2B	Histone 2B
H3	Histone 3
H4	Histone 4
HMG	High mobility group
KDa.	Kilo-Dalton
Lys	Lysine
Mc	Microsomal
Mt	Mitochondrial