

**COMPARATIVE STUDIES BETWEEN CULTURED
FIBROBLASTS/ KERATINOCYTES AND
FOETAL/ ADULT HUMAN SKIN**

Thesis

Submitted for Partial Fulfillment of the M.D. Degree in
Anatomy

Presented By

GAMAL ABDIEN MAHMOUD

M.B., B.CH., M.SC.
Anatomy



52434

Supervisors

611.77
C-1A

Prof. Dr. Alia Mahmoud Nassar
Chairman of Anatomy Department,
Faculty of Medicine, Ain-Shams University

Alia Nassar

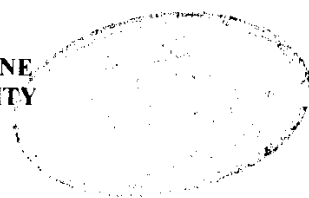
Prof. Dr. Horst Hetzer
Chairman of Theoretical Medicine,
Department. AGW
Osnabruck University, FR Germany

Prof. Dr. Mohamed Reda Khorshid
Professor of Anatomy,
Faculty of Medicine, Ain-Shams University

Prof. Dr. Mostafa Kamel Ibrahim
Professor of Anatomy,
Faculty of Medicine, Ain-Shams University

Prof. Dr. Salah Fadi Taama
Professor of Anatomy,
Faculty of Medicine, Ain-Shams University

**FACULTY OF MEDICINE
AIN-SHAMS UNIVERSITY
1997**







ACKNOWLEDGEMENT

*First and most of all, thank to **God**, the greatest of all, for completing this work.*

*I am very much obliged to the kindness of **Prof. Dr. ALIA MAHMOUD NASSAR**, Chairman of Anatomy Department, Faculty of Medicine, Ain-Shams University, for her helpful discussion, criticism and encouragement. To her I am specially indebted.*

*I would like to express my deepest thanks and gratitude to **Prof. Dr. HORST HETTWER**, Chairman of Theoretical Medicine Department (AGW), Osnabrück University, F.R.Germany, who suggested the topic of this research and assisted me throughout the work. He was very generous in his motivating advice, constructive supervision and insistent encouragement.*

*My deepest thanks also to **Prof. Dr. MOHAMED REDA KHORSHID**, Professor of Anatomy, Faculty of Medicine, Ain-Shams University, for his cooperation, precious advice and repeated revision of every item in this work.*

*Also I would like to express my deepest thanks to **Prof. Dr. MOSTAFA KAMEL IBRAHIM**, Professor of Anatomy, Faculty of Medicine, Ain-Shams University, for his kind supervision of this work sincere help and continuous encouragement.*

*My deepest thanks also to **Prof. Dr. SALAH FADL TAEMA**, Professor of Anatomy, Faculty of Medicine, Ain-Shams University, for his close supervision and valuable suggestions.*

*Finally, I would like to express my thanks and gratitude to **my lab colleagues** in the Theoretical Medicine Department, Osnabrück University, F.R.Germany, for their technical help throughout this work especially **Mr. D. LEHMANN**; Miss, **M. ROBKA** and Miss, **I. KAMPA**. I am very grateful to **Dr. W. HOPPE**, the specialist in the culture unit, who saved no effort in teaching me the most recent technical advances in culture methodology. Also, I can not forget **Miss. G. BARON**, for her help in this work.*

TABLE OF CONTENTS

	<u>Page</u>
INTRODUCTION AND AIM OF THE WORK	1
REVIEW OF LITERATURE	4
• Tissue Culture	4
(A) Culture of keratinocytes	4
(B) Culture of Fibroblasts	15
• Factors Affecting Growth of Epidermal Keratinocytes and Fibroblasts in Tissue Culture	18
• Uses and Applications of Tissue Culture	25
• Dipeptidyl Peptidase IV	31
• Aminopeptidase M or N	55
MATERIAL AND METHODS	67
RESULTS	91
1. Histology of Adult Human Skin	91
2. Histology of the Foetal Human Skin	113
3. The Dermal Fibroblasts in Culture	125
4. Ha Ca T Cells in Culture	137
5. Enzyme Demonstration	146
A) Histochemistry	146
B) Immunohistochemistry	166

C) Immunofluorescence	181
D) Electron Immunogold Histochemistry	199
DISCUSSION	207
SUMMARY	227
REFERENCES	237
APPENDIX	iv
ARABIC SUMMARY.	

***INTRODUCTION
AND
AIM OF WORK***

***REVIEW
OF
LITERATURE***

REVIEW OF LITERATURE

TISSUE CULTURE

CULTURE OF THE EPIDERMAL CELLS

(A) CULTURE OF KERATINOCYTES :

Wheeler, Canby and Cawley (1957) tried to establish a line of human epidermal cells in tissue culture which would be stable enough to grow in culture media for prolonged periods, and could be serially transplanted, and could support the growth and multiplication of a number of viruses. They obtained some full thickness and other split thickness specimens of normal human skin and treated them with trypsin to separate epidermal cells from the dermis. The separated epidermal cells were placed in a growth medium and incubated at 37°C. The cells from most of the obtained specimens either did not grow, grew very little or grew to the stage of keratinization. One specimen of skin, however, yielded a line of cells which grew rapidly, was stable and could be transplanted in series. The cells demonstrated variation in size, shape and staining qualities. Many mitotic figures were present and abnormal mitosis was frequent. The initial cultures had frequently contained large numbers of fibroblasts, and after thirty to sixty days of cultivation "malignant" epithelial-like cells had appeared and these had been passed indefinitely in cell culture. This cell line was able to support the growth of herpes simplex and vaccinia viruses and its morphologic features were indistinguishable from cancer cells.

Later on, **Reaven and Cox (1965)**, succeeded in cultivation of small pieces of postembryonic human skin which explanted under controlled conditions and had resulted in growth and differentiation of epidermal cells leading to the formation of keratohyaline granules and cornification. They found that the production of keratohyaline granules could be influenced by modifying the pH of the growth medium. The authors stated that, although major differences between the *in vivo* and *in vitro* growth of epidermis do exist, there was, nevertheless, in human skin explants, a certain predictable behaviour embodying cell multiplication and epidermal maturation, with the formation of keratohyaline granules and stratum corneum.

Karasek (1966) proved that the cultured human skin epidermal cells were able to form keratin in culture. He found that the primary culture of human skin epithelial cells accumulated an intracellular amorphous material. The isolated material from the culture was studied using histochemical and biochemical methods. The results revealed that the amorphous substance was scleroprotein with properties of native keratin. Electron microscopic studies showed tonofilaments and tonofibrils inside the epithelial cells and they were indistinguishable from those observed in the epithelial cells *in vivo*.

Foetal mouse skin cells were cultivated as a monolayer culture by **Yuspa, Morgan, Walker and Bates (1970)**. The authors described the behaviour of the primary culture. They found that the early cultures