DEMONSTRATION OF SPECIFIC TYPES OF PROTEINS AND mRNAs IN NORMAL AND PATHOLOGICAL LIVERS: A LIGHT AND ELECTRON MICROSCOPIC STUDY

THESIS
SUBMITTED FOR FULFILMENT OF
PH.D. DEGREE IN ANATOMY

BY
DR. GAMAL SAID ABD EL-AZIZ
M.B.,B.Ch., M.Sc.

SUPERVISED BY

Prof. Dr. YEHIA YOUSSEF AHMED
Chairman & Head of Anatomy Department
Faculty of Medicine
Ain Shams University

Prof. Dr. ALIA MAHMOUD NASSAR
Professor of Anatomy
Faculty of Medicine
Ain Shams University

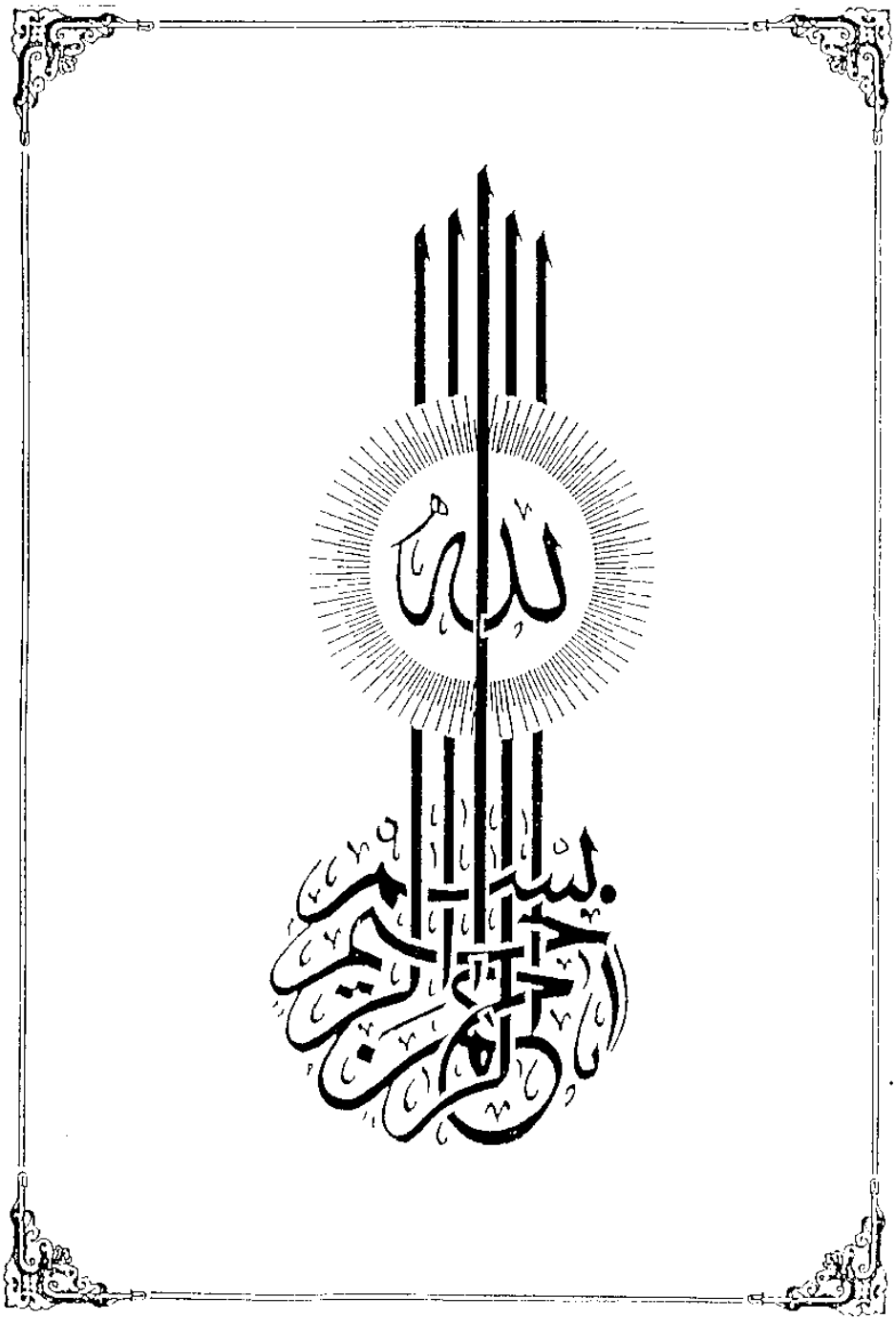
Prof. Dr. FAKHRY AMIN ISKANDER
Professor of Anatomy
Faculty of Medicine
Ain Shams University

Prof. Dr. MOHAMED REDA KHORSHID
Professor of Anatomy
Faculty of Medicine
Ain Shams University

Prof. Dr. KARIMAN MOHAMED EL-GOHARY
Assistant Professor of Anatomy
Faculty of Anatomy
Ain Shams University

Prof. Dr. GUILLOUZO, A.
Professor of Research
Director of Liver Research Unit
Rennes, France.

FACULTY OF MEDICINE
AIN SHAMS UNIVERSITY
1990



This thesis was supported by the **Egyptian Ministry of Education** under the scientific channel program & The Liver Research unit U.49 **INSERM,** Rennes, France.

ACKNOWLEDGEMENT

With my great pleasure, I would like to express my deep gratitude to **Prof. Dr. ALIA MAHMOUD NASSAR**, Professor of Anatomy, Faculty of Medicine, Ain Shams University, and **Dr. GUILLOUZO, A.**, Director of the Liver Research Unit (U-49) of the INSERM, Rennes, France, for their kind attention and direction of my thesis. Really, I am very grateful to their true and fruitful cooperation with me.

My deepest thanks and gratitude to **Prof. Dr. YEHIA YOUSSEF AHMED**, Chairman and Head of the Anatomy Department, Faculty of Medicine, Ain Shams University, for his kind supervision and constant encouragement to me during the growth of this thesis.

I would like to express my deepest thanks to **Prof. Dr. FAKHRY AMIN ISKANDER**, Professor of Anatomy, Faculty of Medicine, Ain Shams University, and **Prof. Dr. MOHAMED REDA KHORSHID**, Professor of Anatomy, Faculty of Medicine, Ain Shams University, for their major help and supervision.

My deepest thanks and gratitude to **Prof. Dr. KARIMAN MOHAMED EL-GOHARY**, Assistant Professor of Anatomy, Faculty of Medicine, Ain Shams University, for her generous and diligent guidance and supervision of my thesis.

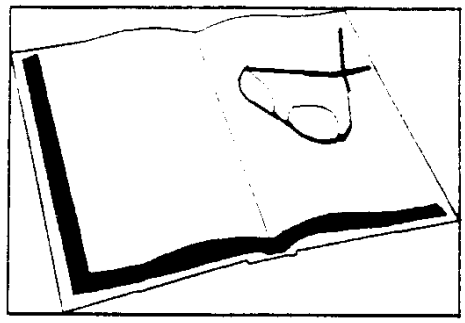
I would like also to pass my best regards to all the members of **The Liver Research Unit (U-49) of INSERM**, in Rennes, France, for their hospitality and warm welcome accorded to me in their nice town. It is noteworthy here to express my appreciation of their generous technical and social aid they extended to me during my stay in France. Also, I have the pleasure to express my hearty thanks to **Dr. CLEMENT, B.; Dr. RATANASAVANH, D.; Dr. RESCAN, P.** and **Mme RISSEL, M.**

My thanks to all the staff and my colleagues in **The Anatomy Department**, Faculty of Medicine, Ain Shams University, for their help and encouragement.

Lastly, grateful acknowledgements are to **My Family** for their invaluable help.

***

CONTENTS



INTRODUCTION AND AIM OF THE WORK

INTRODUCTION

Hepatic fibrosis, which was one feature of liver cirrhosis, occurred in a variety of chronic liver diseases (Popper and Kent, 1975). It consisted primarily of increased deposition of extracellular matrix macromolecules, which were protein in nature, mainly collagens, non-collagenous glycoproteins and proteoglycans (Rojkind, 1982a).

Fibrogenesis, which was the increased deposition of these different types of specific proteins, was a complex process including alterations in both the synthesis and degradation of matrix proteins by different liver cell types. It was generally accepted that once it was established, the fibrosis was irreversible (Popper et al., 1975).

However, previous observation, in man, showing the regression of the periportal fibrosis after the relief of neonatal and acquired extrahepatic biliary obstruction, had led to an important question to arise about the possibility of the reversibility of the hepatic fibrosis after the elimination of the causative agent (Pérez-Tamayo, 1979; Rojkind, 1982b). Besides, other several observations had led to the hypothesis that the experimental hepatic fibrosis was a reversible process following discontinuation of the causative agents. Amongst these reversible fibrotic

reactions were "Schistosomiasis mansoni" in mice (Hutterer, Eisenstadt and Rubin, 1970; Mehlhorn, Frenkel, Andrews and Thomas, 1982) and fibrosis induced by chemicals e.g., CCl_4 and ethionine (Hutterer, Rubin and Popper, 1964; Pérez-Tamayo, 1979). However, the duration of the treatment and the difficulty to identify the causes of fibrosis had made these models in debate.

The most commonly used method of producing experimental cirrhosis involved multiple doses of CCl_4. This toxic agent rapidly induced liver damage and severely altered the metabolism and gene expression of the hepatocytes (Pérez-Tamayo, 1983). Chronic CCl_4 intoxication resulted in extensive necrosis of parenchymal cells and wild inflammation. Such drawbacks and the only few documented observations of human cases had made the reversibility of the hepatic fibrosis in man uncertain.

Extrahepatic cholestasis was another model to investigate the formation of hepatic fibrosis. The choice of the technique of the ligation of the common bile duct for two weeks, followed by the bilio-duodenal anastomosis (Franco, Gigou, Szekely and Bismuth, 1979), had given us the facility to induce hepatic fibrosis in the rats and to assess the reversibility of this fibrosis.

Prolonged obstruction of bile flow or hepatic diseases that lead to anatomical destruction of the biliary tree resulted in morphological and biochemical changes and the development of secondary biliary cirrhosis (Popper and Schaffner, 1985).

The changes induced by the experimental bile duct ligation in the rat had been partly analysed (Carpino, Gaudio, Marinozzi, Melis and Motta, 1981; Kountouras, Billing and Scheuer, 1984). They included an extensive proliferation of bile ducts in enlarged portal spaces with slight inflammation and necrosis together with the formation of periportal fibrosis in less than two weeks after obstruction of the biliary tree (Aronson, De Haan, James, Bosch, Ketel, Houtkooper and Heijmans, 1988).

However, the molecular mechanisms of cholestasis-induced fibrosis were yet unknown. In addition, the possibility that cholestasis-induced fibrosis was a reversible phenomenon had not been explored.

There were many considerable informations in the experimentally induced extrahepatic cholestasis e.g., about the light structural microscopical changes (Moschowitz, 1952; Jacques and Mc Adams, 1957; De Vos, De Wolf-Peters, Desmet, Bianchi and Rohr, 1975) and electron microscopical

changes (Schaffner and Popper, 1961; Robenek, Herwig and Themann, 1980; Carpino, Gaudio, Marinozzi, Melis and Motta, 1981). Also, the biochemical changes in the liver composition had been reported by Toda, Kako, Oka, Oda, and Ikeda (1978); Di Bisceglie, Paterson and Segal (1985).

The quantitative assessment of the cholestatic livers were studied by Aronson et al., (1988). However, the information about the elimination of the common bile duct obstruction on the liver structure and function was not studied before.

During the last few years, attempts to determine the cellular sources of the extracellular matrix proteins in both normal and injured livers had been made, using a variety of techniques, including in vitro studies and in situ localization of matrix proteins or their corresponding mRNAs. It is now admitted that, in addition to the smooth muscle, vascular endothelial and biliary cells in portal spaces, several sinusoidal cell types, including fat-storing cells and the endothelial cells, produced various matrix proteins in vivo (Kent, Gay, Inouye, Bahn, Minick and Popper, 1976; Diegelmann, Guzelian, Gay and Gay, 1983; Clément, Grimaud, Campion, Deugnier and Guillouzo, 1986; Clément, Laurent, Guguen-Guillouzo, Lebeau and Guillouzo, 1988; Clément, Rescan, Baffet, Loréal, Lehry, Campion and

Guillouzo, 1988; Milani, Herbst, Schuppan, Hahn and Stein, 1989).

However, the participation of the hepatocytes in the formation of the extracellular matrix components remained controversial.

Early immunohistochemical studies on tissue sections had shown that the prolyl-hydroxylase, a key enzyme in collagen synthesis, was detectable in the parenchymal cells (Ooshima, 1977; Kuutti-Savolainen, Risteli, Miettinen and Kivirikko, 1979).

More recently, a highly sensitive immunohistochemical procedure, that provides a qualitative assessment of the cellular sources of extracellular matrix proteins in the liver, had been set up by Clément, Rissel, Feyrol, Mazurier, Grimaud and Guillouzo (1985).

In the normal liver, only fibronectin and small amounts of collagen type I were detected in the endoplasmic reticulum of the hepatocytes (Clément, Emonard, Rissel, Druguet, Grimaud, Herbage, Bourel and Guillouzo, 1984), thus suggesting that these cells were not the main producers of extracellular matrix components. These findings were sustained by other immunohistochemical studies

(Martinez-Hernandez, 1984 & 1985; Geerts, Geuze, Slot, Voss, Schuppan, Schellinck and Wisse, 1986; Miyabayashi, Kojima, Inoue, Sasaki, Muragaki and Ooshima, 1987; Takahara, Kojima, Miyabayashi, Inoue, Sasaki, Muragaki and Ooshima, 1989) and by hybridization of matrix protein mRNA. Only in situ hybridization technique (Saber, Zern and Schafritz, 1983), but not Northern-blot analysis (Clément, Laurent, Guguen-Guillouzo, Lebeau and Guillouzo, 1988) allowed the detection of procollagen mRNAs in isolated hepatocytes.

Further studies had shown that, the hepatocytes had the capacity to express laminin and other collagen types in the foetus and during the perinatal period [Rescan, Clément, Yamada, Glaise, Segui-Real, Baffet, Guguen-Guillouzo and Guillouzo, (in press)] and in primary culture (Tseng, Lee, Ells, Bissell, Smuckler and Stern, 1982; Konomi, Hata, Sano, Sunada and Nagai, 1982; Clément, Emonard, Rissel, Druguet, Grimaud, Herbage, Bourel and Guillouzo, 1984; Diegelmann, 1986).

In injured livers, conflicting results had been obtained depending on the technichal approaches that were used to localize the cellular sources of the matrix proteins. By immunohistochemistry, the hepatocytes were found to be slightly stained for various collagens and/or laminin in alcoholic human livers (Bianchi, Biagini,

Ballardini, Genacchi, Faccani, Pisi, Laschi, Liotta and Garbisa, 1984; Clément et al., 1986 & 1988), in CCl_4 treated rat livers (Martinez-Hernandez, 1985) and in bile duct-ligated rat livers (Diegelmann, Guzelian, Gay and Gay, 1983).

In contrast, recent in situ hybridization studies failed to detect procollagen and laminin mRNA in the hepatocyte in either CCl_4-treated (Milani, Herbst, Schuppan, Hahn and Stein, 1989) or bile duct-ligated rat livers (Milani, Herbst, Schuppan, Kim, Riecken and Stein, 1990).

Therefore, the aim of this work was to assess the reversibility of the hepatic fibrosis and to study the distribution and cellular origin of different components of the extracellular matrix in the rat liver after biliary obstruction and after the relief of this obstruction by bilio-duodenal anastomosis. The immunolocalization techniques will be used at both the light and electron microscopic levels.