SPOTLIGHT ON CARNITINE AND ITS RELATION TO DISEASE

Essay Submitted for
The Partial Fulfillment of the Master Degree in
Clinical and Chemical Pathology
By

Inas Fouad Hanna Awad

M.B.,B.CH

Under supervision of

Prof.Dr.Hany Sobhy Rufail

Professor of Clinical and Chemical Pathology
Faculty of Medicine-Ain Shams University

Dr.Ghada Sadek Sabour

Lecturer of Clinical and Chemical Pathology
Faculty of Medicine-Ain Shams University

Dr.Manal Mohamed Abd Al Aziz

Lecturer of Clinical and Chemical Pathology
Faculty of Medicine-Ain Shams University

Faculty of Medicine
Ain Shams University
1999

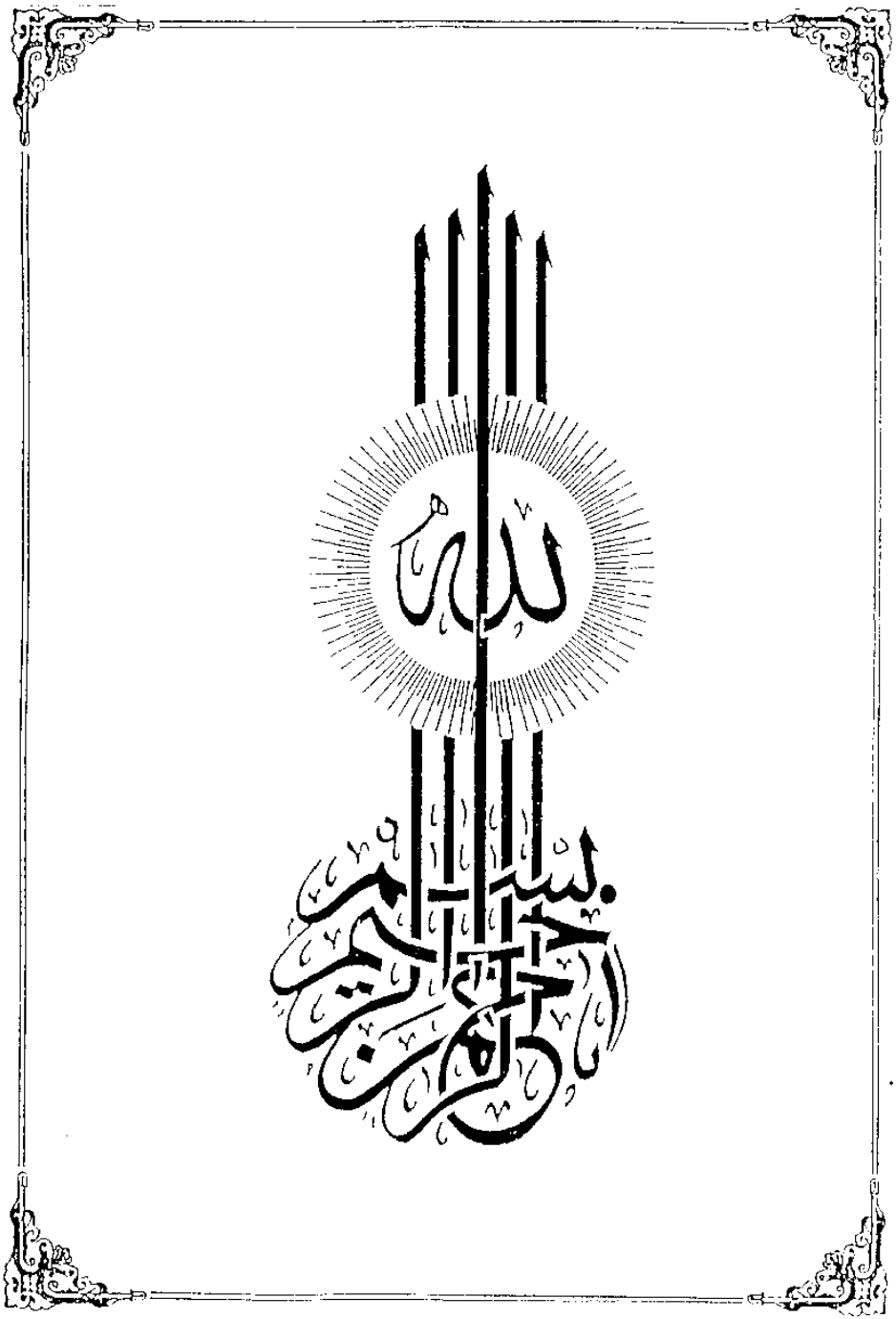
Acknowledgment

First of all, thanks to God. I wish to express my thanks and gratitude to Prof. Dr. Hany Sobhy Rufail, Professor of Clinical Pathology, Ain Shams University, for his constructive criticism, valuable comments and kind advice.

My deepest thanks and special regards are due to Dr. Ghada Sadek Sabour, Lecturer of Clinical Pathology, Ain Shams University, for her generous help and continuous encouragement. She provided me with valuable comments and enthusiastically revised the essay.

I am profoundly grateful to Dr. Manal Mohamed Abd Al-Aziz, Lecturer of Clinical Pathology, Ain Shams University, for her devotion, all the time and effort she scarified and the valuable friendship she offered me in order to start and complete this work.

Last, but not least, no words could even express my deepest gratitude to every member of my family . Their favor is unforgettable.



CONTENTS

	Page
List of Abbreviations	*i*
List of Tables	*ii*
List of Figures	*iii*
Introduction and Aim of the Work	*1*
Review of Literature	
I-BIOCHEMISTRY OF CARNITINE	
A) Historical Background	*3*
B) Occurrence and Distribution	*3*
C) Biosynthesis	*4*
D) Regulation of Carnitine Biosynthesis	*8*
E) Uptake and Transport of Carnitine	*9*
F) Tissue Distribution	*11*
G) Excretion	*13*
H) Role of Carnitine in Fatty Acid Oxidation	*14*
II- CARNIINE IN PATHOLOGICAL CONDITIONS	
1- Primary Carnitine Deficiency	*22*
2- Secondary Carnitine Insufficiency	*28*
3- Cystic Fibrosis	*32*

4- Intermittent Claudication..... 34
5- Cardiovascular Diseases..... 37
6- Chronic Liver Disease..... 39
7- Chronic Renal Disease..... 42

III-Determination Of L.Carnitine

A) Sample Precautions..... 45
B) Methods of Assay:
1- Radio-isotopic Assay..... 45
2- Photometric Assay..... 47
(a) Using Thiol- group colour reagents. 48
(b) Enzymatic ultraviolet test..... 53
3-High Performance Liquid Chromatography..... 55
Summary..... 59
Recommendations..... 64
References..... 65
Arabic Summary..... ١

LIST OF ABBREVIATIONS

ACS	Acetyl COA synthetase
ADP	Adenosine-5-diphosphate
AMP	Adenosine-5-monophosphate
ATP	Adenosine-5-triphosphate
CAT	Carnitine acetyltransferase
COA	Coenzyme A
COASH	Coenzyme A- sulfhydryl group.
CPT	Carnitine palmitoyl transferase
D-isomer	Dextro isomer
DTNB	5,5-dithio-bis-2-nitrobenzoate
HMG	Hydroxy-3-methylglutaryl
HPLC	High-performance liquid chromatography
IC	Intermittent claudication
K_3EDTA	Potassium salt of ethylene diamine tetra acetic acid
LDH	Lactate dehydrogenase
L-isomer	Levo isomer
MK	Myokinase
NADH	Reduced nicotinamide adenine dinucleotide
P.K	Pyruvate kinase
PEP	Phosphophenol pyruvate
PP_1	Inorganic pyrophosphate
TCA	Tricarboxylic acid
TNB	5-thio-2-nitrobenzoate

LIST OF TABLES

Table (1) *Urinary concentrations of carnitine metabolites (μmol/mol creatinine).....* **33**

Table (2) *Plasma venous concentrations of carnitine and its esters in control subjects and patients in group IC1 and IC2.....* **36**

Table (3) *Serum free carnitine values of normal subjects, patients with chronic renal disease, and patients with chronic renal disease undergoing hemodialysis.....* **44**

LIST OF FIGURES

Figure (1)	*Carnitine*.....	**4**
Figure (2)	*Enzyme steps in the biosynthesis of carnitine*.....	**6**
Figure (3)	*Beta oxidation of fatty acids*.....	**16**
Figure (4)	*Role of carnitine in the transport of long-chain fatty acids through the inner mitochondrial membrane*.....	**18**
Figure (5)	*Effects of L-carnitine administration on mitochondrial metabolism in vivo*.....	**29**

Introduction & Aim Of Work

INTRODUCTION

Mitochondrial β-oxidation of long-chain fatty acids provides a major energy source in human both in the resting state and during fasting and exercise, particularly in the heart (myocardium), liver and skeletal muscle (Chalmers et al., 1997).

L-carnitine is the carrier molecule for long-chain fatty acids to cross the mitochondrial membrane. L-carnitine is involved in fatty acids β-oxidation as it plays a key role in facilitating the transport of long-chain fatty acids into the mitochondrial matrix, where they undergo β-oxidation and release energy (Wan and Hubbard, 1998) and (Scaglia and Longo, 1999).

L-carnitine insufficiency is a general phenomenon in disorders of organic acid metabolism. In patients with multiple acyl-coenzyme A dehydrogenase deficiency as an example of organic acid metabolism, demonstration of an abnormal acyl-carnitine profile is diagnostic (Poplawski et al, 1999). Also carnitine deficiency have been reported to cause variety of pathologies ranging from mild forms of muscle weakness to severe forms of hypoglycemia and cardiomyopathy (Marques, 1998).

Since long-chain fatty acids are involved in the synthesis of phospholipids such as lung surfactant. It has been reported that carnitine deficiency contributes to the aggravation of cystic fibrosis of lung and to sudden infant death syndromes

(Lloyd-Still et al., 1993). Furthermore, acylcarnitines levels in amniotic fluid may be a useful tool for prenatal prediction and diagnosis of organic acidemias (Shigematsu et al., 1996).

Carnitine metabolism is altered in peripheral arterial diseases as in patients with intermittent claudication (Brevetti et al., 1996). Also in patients with ischemic cardiovascular disease, there is decrease in the myocardial levels of free carnitine and accumulation of long-chain acylcarnitine (Arsenian, 1997).

Moreover, in patients with various chronic liver diseases, plasma L-carnitine levels are either normal or elevated (Krahenbuhl and Reichen, 1997). Also, in patients with chronic renal disease, whether these patients undergo dialysis or not, there is increase in serum carnitine levels (Marge and Brewste, 1996).

Therefore, carnitine seems to play an important role in various pathological conditions involving various body systems.

AIM OF THE WORK

This work aims at giving a detailed account on the role of carnitine in the normal state, emphasizing on pathological conditions arising from its disorders. Spotlight will be given as well on various methods for its assay.

Review

I. BIOCHEMISTRY OF CARNITINE

A. Historical Background:

Carnitine has a long history in biochemistry .It has been discovered to be a quantitatively important compound in muscle tissue in 1905 (Fraenkel and Friedman, 1957) and its chemical structure was determined in 1927 (Tomita and Sendju, 1927). In 1952, Carter et al. showed new interest in carnitine when they established that it is a growth factor for the meal worm: Tenebrio molitor [hence carnitine's other name, vitamin BT (T for tenebrio)] .

Subsequent studies showed that carnitine-deficient larvae died "fat" when they were starved as they were unable to utilize their fat stores in order to survive (Carter et al, 1952)

B. Occurrence and Distribution:

Carnitine is present in all animal species, in many micro-organisms and in many plants (Mitchell, 1978). The concentration of carnitine varies in different species and in various tissues over a wide range. It has been reported that the highest concentration is in rat epididymal fluid (Brooks et al, 1974). On the other hand, carnitine least concentration is found in plants. In humans, carnitine is present in

many tissues as liver, kidney, heart, skeletal muscle, brain, epididymis and red blood cells (Borum et al, 1985).

C. Biosynthesis:

Carnitine (β-hdroxy-γ-trimethylammonium butyrate) (Fig.1) is a seven carbon organic acid It is water soluble compound of 161 D molecular weight.

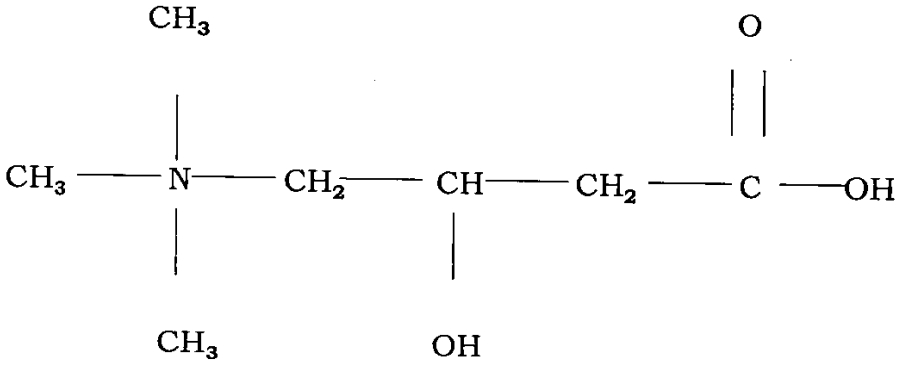


Figure (1) Carnitine

Carnitine is synthesized from lysine and methionine in liver, kidney and brain. In yeast, free lysine is methylated with S-adenosyl methionine as the methyl donor (Horne et al,1971) In animals, however 6-N trimethyllysine is formed by the methylation of lysine residues in proteins such as myosin, actin and histones (Paik and Kim, 1975). Carnitine is formed from this 6-N- trimethyllysine after its liberation in protein breakdown, presumably in the lysosomes (Dunn

and Englard, 1981). In the next step the trimethyllysine is hydroxylated to 3-hydroxy–6-N-trimethyllysine by 6-N trimethyllysine-2-oxoglutarate dioxygenase enzyme (Hoppel et al, 1980). The hydroxy trimethyllysine is cleaved to butyrobetaine aldehyde and glycine by an enzyme called 3-hydroxy-6-N-trimethyllysine aldolase (Novak et al, 1980). The butyrobetaine aldehyde is then oxidized to butyrobetaine by butyrobetaine aldyhyde dehydrogenase enzyme (Hulse and Henderson, 1980). Finally butyrobetaine is hydroxylated to carnitine by γ-butyrobetaine-2-oxoglutarate dioxygenase (Rebouche and Engel, 1981).