

MITOCHONDRIA IN RELATION TO LIPIDS STRUCTURE  
IN ~~SOME~~ MICROORGANISMS

By

Mohamed Ramadan Abou Shady  
B.Sc. (Special Botany)  
Excellent Degree

Thesis

Submitted for a Partial Fulfilment  
of the Degree of  
MASTER OF SCIENCE

Ain Shams University  
Faculty of Science  
Botany Department



3278



1969

## ACKNOWLEDGEMENT

The author wishes to express his thanks and gratitude to Professor Dr. A.H. Montasir, Dr. A. El-Kabarity and Dr. S.S. Radwan, for their kind supervision.

The author offers his gratitude and thanks to Dr. S.S. Radwan lecturer in Microbiology for suggesting the point, directly supervising the work, offering continuous constructive suggestions, and help in presenting the thesis.

Thanks are also due to Dr. A. El Kabarity Assistant Professor for Cytology for his help in the Cytological part of the work.

Thanks are also due to Professor Dr. M.G. A. Hafez, head of Botany Department for his continuous encouragement.

Sincere thanks are due to Dr. S.H. Elwan, Assistant Professor, for Microbiology, for his interest, constructive criticism, his suggestions and for reading the manuscript.

Thanks are also due to the authorities of the Ain Shams University Scientific Computing Centre, especially Mr. Sayed A. El Wahab who prepared the program of calculations.



This thesis has not been submitted for any degree for this or any other University. The literature mentioned hereafter, shows how far I have availed myself to the work of the others.

M. R. Abou Shaḍy

*M. R. Abushady*

## CONTENTS

	<u>Page</u>
<u>ACKNOWLEDGEMENT</u>	
PART I	
General preface	1
Historical Introduction	5
Scope of thesis	21
Material	22
General Methods	
A- Preparation of inoculum:.	22
B- Medium	23
C- Aerobic and anaerobic culture conditions	23
D- Preparation of the cultures for mitochondrial and lipid analysis	24
E- Mitochondrial stain and count	25
F- Lipid extraction and analysis	
a) Extraction and purification of the total yeast lipids.	26
b) Thin layer chromatographic analysis of lipids.	27
Statistics	33

Part II

The mitochondrial numbers and the lipid constitution of anaerobic yeast cells at different growth phases	34
--	----

Part III

Effect of supplemented lipids on the mitochondrial numbers and the lipid constitution of anaerobic yeast cells.

Section (1) Effect of Phospholipids	80
Section (2) Effect of Ergosterol	107
Section (3) Effect of mixture of Ergosterol and phospholipids.	120
Section (5) Effect of Triglycerides	155

Part IV

General Discussion and Conclusions	149
Summary	181
Literature	185
Arabic Summary	

PART I

GENERAL PREFACE, HISTORICAL INTRODUCTION  
and  
GENERAL METHODS

## GENERAL PREFACE

All forms of life are summed up in the initials deoxyribose nucleic acid(DNA) and adenosine triphosphate (ATP). DNA stands for the apparatus responsible for the replication of the most important constituents of the living cell: proteins and nucleic acids themselves. Adenosine triphosphate (ATP) is equally important since it supplies the energy for life processes, including replication controlled by DNA. The energy supplier ATP is in fact an energy store. Therefore ATP formation is in and by itself a goal.

Release of electrons from a substance is the way for transforming and storing this substance in the form of energy. This release of electrons takes place both in presence and in absence of oxygen. Chloroplast in green plants, and mitochondrion in animals ( and also microbes lacking green pigment) are systems for generating energy in the form of ATP, more potent than glycolysis or fermentation enzyme

system in absence of oxygen. In the latter, one molecule and in the mitochondrion, two molecules of ATP are the yield for each pair of electrons released. Therefore mitochondria are important sites in the living cell where chemical events take place to supply energy to the cell.

Mitochondria or sites of mitochondrial nature have been detected in all living cells including yeasts and bacteria. Difference is likely to be structural rather than functional. Structural variation (e.g. shape and size) seems to occur not only in one living entity, but also in the cells of even an individual, being affected by various factors. Therefore in a comparative study the environment should be specified with precision of the shape and the numbers are to be among the experimental criteria.

Recently, however, the role of lipids in the mitochondrial function (e.g. Alexander 1966, and Jurtschuk & Schleich, 1969) has been studied. Evidence is given suggesting that lipids are related to the electron transfer system of the mitochondria, although

the mechanism of this relation is still obscure. Evidence is also given correlating mitochondria with the total lipid content of the cell. Many authors (e.g. Kaufmann et al 1966, and Jollow et al 1968) have shown that the total lipid structure of certain microorganisms depends on the presence or absence of oxygen. On the other hand, numbers of mitochondria in yeast cells were found to be reduced under anaerobic conditions (Hagedorn 1963). It is not only oxygen that affects both mitochondria and cell lipids, but also other factors among which are the age of the cell and its biological activity. Therefore mitochondria and cell lipids show similarity in being affected by certain factors. Yeast cell division has been found to be reduced under anaerobic conditions. It becomes therefore of interest to elucidate the effect of supplied lipids on mitochondrial formation under anaerobic conditions, since informations on this point are rather limited.

The above cited considerations stimulated interest in studying formation of mitochondria in relation to cell lipids in microorganisms growing in various media. The microorganism of choice was Saccharomyces cerevisiae to make comparison with previous findings as convenient as possible. Main points of the present investigation will be summarized in the scope of the thesis given hereafter.

## HISTORICAL INTRODUCTION

The biological and biochemical reactions within a living cell are controlled and directed by means of enzymes. The recent biochemical, cytochemical and cytological studies show that these biological catalysts are bound in form of complex systems to some microscopic or submicroscopic structural units of the cell. Every structural unit carries specific types of enzymes. It follows that each of these units undergoes a specific part of the cell metabolism, and the net result of the activities of all units represents the total metabolic activities within the living cell. The cytological units named mitochondria carry certain enzyme systems which are considered among the most essential ones in the living cell as a whole.

Mitochondria represent the chief sites of the enzymes linking phosphorylation to oxidation (Siekevitz, 1952), of cytochrome C (Schneider and Hogeboom 1950; Beinert, 1951; Avers, and Pfeiffer, 1965), of the Krebs cycle enzymes (Schneider and Hogeboom, 1956; Schneider, 1959), and of the enzymes

catalyzing the oxidation of fatty acids (Schneider, 1948; Kennedy and Lehninger, 1949; Green and Wakil, 1960), amino acids (Paigen, 1954), and choline (Kensler and Langemann, 1951; Williams, 1952). It is generally considered that mitochondria possess the full complement of enzymes, co-factors, and accessory substances required for these reactions (Green and Järnefelt, 1959, Lehninger, 1959b).

Since the mitochondria represent the organelles responsible for such important activities, they are expected to exist in the cells of most of the members belonging to the animal, plant and microbial worlds.

Mitochondria, or at least intraplasmatic granules of mitochondrial nature, were as a matter of fact, detected in all animal and plant cells (Hogeboom 1955).

Mitochondria isolated from plant cells (See Tager and Nautanen 1955; and Martin and Morton 1959), especially those isolated from yeast (Bautz and Marquardt, 1953; Linnane and Still).

1955; Yotsuyanagi, 1955; Bautz 1956; Agar and Douglas, 1957, Hagedorn, 1957; Yotsuyanagi, 1959; and Vitols and Linnane, 1961) resemble morphologically and biochemically the mitochondria of animal origin. Even in the relatively minute bacterial cells mitochondria were detected, for e.g. in Stereptococcus pyogenes, Diplo coccus pneumoniae and Corynebacterium diphtheria (e.g. Mudd 1953; Musta Mallio and Jannes 1954.)

Bacterial mitochondria, primitive mitochondria (Chondrioids), as quoted by Van Iterson, 1965, differ from true mitochondria in being just membranous structures possibly open towards the cell wall and having interior structures that are unit membranes or are bordered by an even thinner layer (Van Iterson, 1965).

The presence of mitochondrial analogous was established by cytochemical methods also in the cells of the facultative aerobe, Lactobacillus delbrueckii (Birnyuzova and Nikitina, 1966).

It was concluded by many authors that the mitochondria of living cells are not fixed entities, but

may change with the physiological state of the organism and the condition of the medium. As early as 1918-1924, Cowdry stressed the great sensitivity of the mitochondria to altered normal and abnormal cell activity. The sensitivity of plant mitochondria to external agents was similarly emphasized by Guilhaumon, 1941, as quoted by Dangeard 1958 who described their progressive vesiculation with cell necrobiosis. Yotsuyanagi, 1955 has reported striking changes in the form of mitochondria in yeast, induced by varying the conditions of growth. Striking mitochondrial changes were also noted when the culture medium was altered (Lettre 1954; Biesele, 1955; Yotsuyanagi, 1962, Wallace and Linnane, 1964, Morpugo et al 1964; Polakis et al 1964, 1965; Polakis and Bartley 1965; Linnane 1965, Wallace et al 1968; and Jolley et al 1968).

In addition it was found that the mitochondrial differs from cell to cell in the same organ, and even within the cell (Hogeboom et al, 1948; Huff and Schneider, 1954; Paigen, 1954; and Avers and Pfeiffer, 1965) from spherical to short, long or spiral rods