EXPERIMENTAL OTITIS MEDIA IN THE GUINEA PIG

THESIS

Submitted in Partial Fulfilment for the
Doctor Degree in Otolaryngology

By

MAGED MAHMOUD BAHER NAGUIB , M.S.

Supervised by :

Prof. Dr. Mamdouh El-Gohary, M.D.
Professor of Otolaryngology
Faculty of Medicine
Ain Shams University

Dr. Newton J. Coker, M.D.,F.A.C.S.
Associate Professor of Otolaryngology
Baylor College of Medicine
Houston, Texas, U.S.A.

Dr. M. Mahgoub Kairy, M.D.
Assistant Professor of Otolaryngology
Military Medical Academy

Dr. Charles M. Henley, Ph.D.
Assistant Professor of Otolaryngology
Baylor College of Medicine
Houston, Texas, U.S.A.

Faculty of Medicine
Ain Shams University
1991

February 23 , 1991

BAYLOR
COLLEGE OF
MEDICINE
Texas Medical Center
Houston, Texas 77030
Department of
Otorhinolaryngology and
Communicative Sciences
Otological Research Laboratory
(713) 798-5927

Professor Mamdouh El Gohary, M.D.
Department of Otolaryngology
Faculty of Medicine
Ain Shams University
Cairo , Egypt.

Dear Professor El Gohary:

This is to inform you that the thesis of Dr. Maged Mahmoud Baher Naguib entitled " Experimental Otitis Media in the Guinea Pig " has been accomplished as per protocol presented. We feel that the thesis is ready for defence.

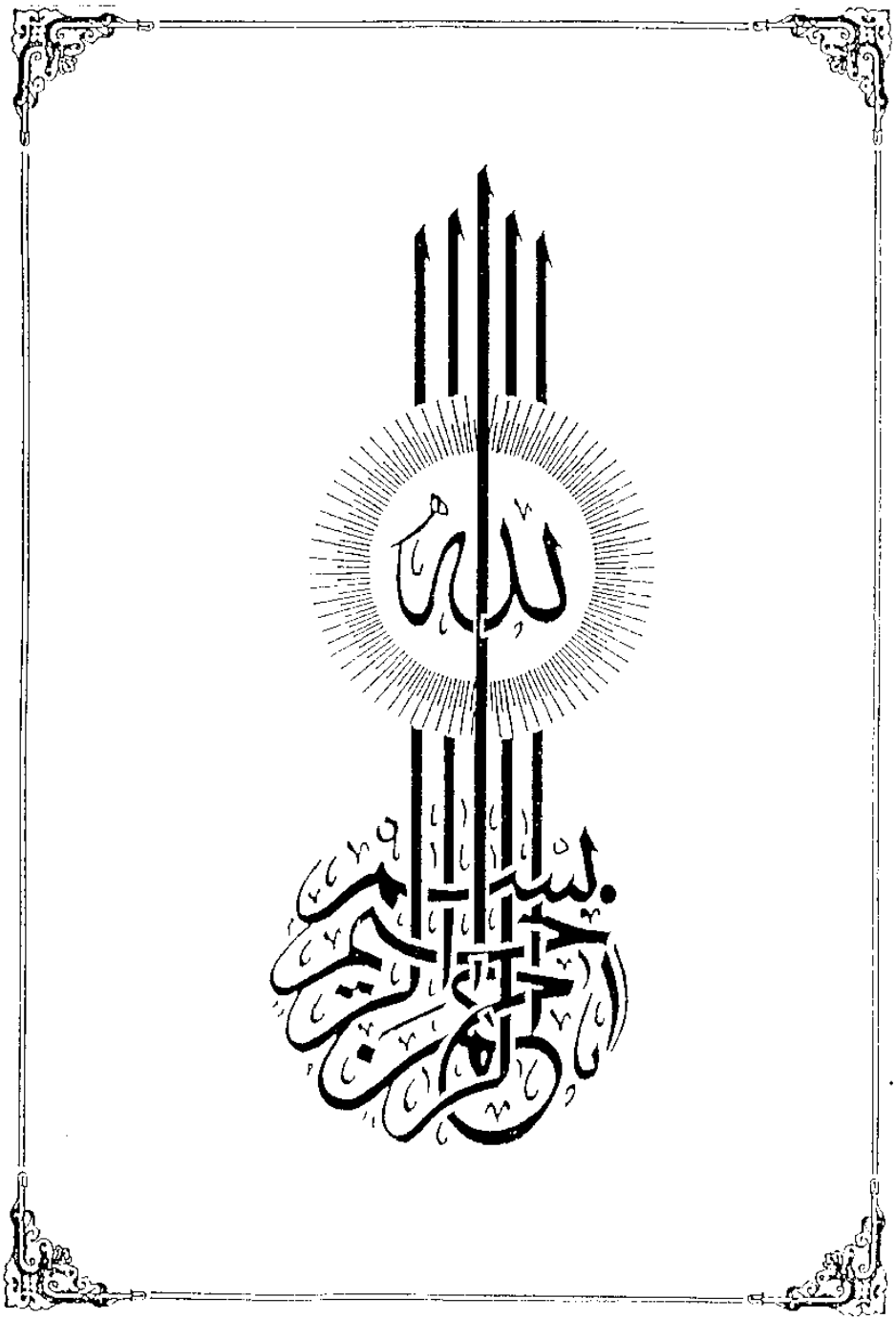
As we express our pleasure to cooperate with you, please accept our personal regards.

Sincerely,

Newton J. Coker, M.D., F.A.C.S.
Associate Professor

Charles M. Henley III, Ph.D.
Assistant Professor

NJC: lgp



بسم الله الرحمن الرحيم

إِنَّ السَّمْعَ وَالْبَصَرَ وَالْفُؤَادَ كُلُّ أُولَئِكَ كَانَ عَنْهُ مَسْئُولاً

الإسراء ٣٦

Dedicated
To My
Wife

ACKNOWLEDGEMENT

"We are all like dwarfs seated on giant's shoulders. If we can see far, it is not because we are tall, but it is because we are seated on giant's shoulders" (Bernard De Charters, 12 th. Century).

I would like to express my sincere gratitude to **Prof. Dr. Mamdouh El-Gohary, M.D.** Professor of E.N.T. Ain Shams University for his enthusiastic supervision, support and sincere guidance; to whom I feel deeply indebted.

Dr. Newton J. Coker, M.D. and **Dr. Charles M. Henley, Ph.D.,** Assistant Professors, E.N.T. Department, Baylor College of Medicine, Texas, U.S.A., have during my whole work been of the greatest importance as teachers, collaborators and advisors. They have in every possible way, supported my studies and through stimulating criticism contributed most directly to the present results.

I am extremely grateful to **Dr. Mohamed Mahgoub Kairy, M.D,** Assistant Professor, of E.N.T., Military Medical Academy, for all what he taught me and for his kind assistance and guidance that are extremely appreciated and remembered.

During my work at Baylor College of Medicine I have had the privelage of working with many cooperative scientists: It is a pleasure to thank **Prof. Dr. Makoto Igarashi, M.D.,** Professor of E.N.T. for interest and revision of the inner ear results. I am also indebted to **Prof. Dr. Donald Greenberg, M.D.,** Professor of Pathology, who provided valuable advice regarding histopathology of the middle ear.

A special thanks goes to Suzanne Moore, Daniel Leibel and Donna Heims for important technical assistance with specimen preparations, electron microscopy , graphs and photographic work.

I am deeply indebted to my parents; my mother who supported me in times when I strongly needed support. And my father, the finest man I will probably ever meet, who laid the foundation.

CONTENTS

INTRODUCTION

Acute otitis media is one of the most common infectious diseases of childhood. It is also one of the most common complications of upper respiratory tract infections in preschool children, and so far there is no sign that the quantitative importance of middle ear disease is diminishing (Ingvarsson et al., 1987).

It is likely that humans have always suffered from acute infection of the middle ear and its suppurative complications. Temporal bone studies of a 2600 -year- old Egyptian male mummy done by Lynn and Benitez (1974) reveal perforation of the tympanic membranes in otherwise normal middle ears. They considered this as an evidence of acute otitis media and probable defective hearing among ancient Egyptians.

The economic, psychological and developmental ramifications of this disease are considerable and range from minor conductive hearing loss to cholesteatoma, brain abscess, and death. Inspite of its tremendous impact on health care, the pathophysiology of otitis media is incompletely understood and the appropriate treatment is a subject of much debate. Retardation of progress in these areas is, in part, due to the difficulties surrounding the accurate diagnosis of otitis media and recognition of its more subtle complications and sequelae (Meyerhoff et al., 1984).

Although large number of clinical, epidemiological, and bacteriological studies dedicated to the study of acute otitis media were published, yet, experimental animal studies on the disease however are relatively sparse. This is a surprising phenomenon since experimental animal studies seem to offer an ideal instrument for a systematic approach to the knowledge of the course of otitis media and short and long-term sequelae.

REVIEW OF THE LITERATURE

The Ultrastructure of the Middle Ear Mucosa.

The lining of the middle ear was for many years considered to consist normally of nonciliated flat or cuboidal cells (Wolf, 1943, Schwarzbard, 1958, Friedmann, 1963). It was also termed mucosa despite the supposed inability to produce mucus and the absence of cilia (Zollner, 1942, Friedmann, 1955 a & b, Suehs, 1952).

However, several investigators noted the presence of a few ciliated cells or goblet cells in humans and in dogs near the opening of the tube or in the hypotympanum (Polvgot and Babb, 1940, Terracol et al., 1949, Holmgren, 1934).

In the past, no specific histochemical stains were applied to study the middle ear mucosa. At first, because they were not available, and later because the dictum that the middle ear mucosa is devoid of cilia or mucus-secreting elements had become a dogma.

The first systematic histological study in which the normal middle ear lining was observed to possess mucociliary properties was reported by Sadé (1966). He studied the lining of the tympanic cavity at autopsy in 80 cases not involving otologic diseases. On examination of serial sections microscopically, it became apparent that the middle ear mucosa is, to an important degree, a respiratory mucosa which bears cilia and synthesizes and secretes mucus. The extent of the mucociliary epithelium varied from ear to ear, being more extensive in the young. The mucociliary covering ended at the line of the facial nerve, beyond which no columnar or cuboidal cells were found. Instead, a flat epithelium was seen to cover the posterior attic, antrum, and mastoid. Inferior and anterior to the facial nerve line, he divided the specimens into three groups and found that : In one third,

respiratory mucosa covered the whole middle ear up to the facial nerve line. In another third, only two-thirds of the tympanic cavity was covered with ciliated and goblet cells. The remaining third had only the anterior tympanic cavity covered with mucociliary cells. The mucus producing region usually extended posteriorly beyond the area bearing ciliary cells. Cells which bore cilia were either columnar or cuboidal. The anterior part of the tympanic cavity usually contained taller ciliated cells than the posterior. And the inferior ciliary tract in the hypotympanum was seen as more predominating than the superior tract and the roof.

The really important breakthrough was the realization that the middle ear is lined with a respiratory epithelium with all the physiological (i.e. mucociliary transport), pathological (metaplastic instabilities), and immunological implications. This also provides insight into the origin of mucus and other epithelial secretions in the various otitis entities.

Hussl and Lim (1969) reported their electron microsocopic study of the middle ear mucosa of the guinea pig confirming its "respiratory" nature.

Furthermore, they described three types of secretory cells:

a) The goblet cells which are filled with large light granules. These cells have the overall appearance resembling those of goblet cells described elsewhere. They possess the classical, chalice shape. These cells are numerous close to the tubal orifice and become increasingly rare towards the bulla.

b) Intermediatry cells containing both light and dark granules in proportions that change from one cell to another. The shapes of these cells varry, some resemble goblet cells, others are columnar or cuboidal. The free surface of some of the cells can be seen bulging toward the lumen. The nucleus is located in the basal part of the cell. A well developed Golgi apparatus is present.

c) Dark- granulated cells containing dark granules. Their shape depends upon their location. They are slender columnar, cuboidal, or even squamous. Almost consistently the free cell surface bulges toward the lumen. In some cells the top portion protrudes and displaces cilia of the neighboring cell. The nucleus is located at the base of the cell. Smooth surfaced endoplasmic reticulum is present beneath the nucleus. The Golgi apparatus is well developed.

On the basis of the relative amount of neutral and acidic polysaccharides contained in the secretory units, Hussl and Lim (1969) classified the secretory units into:

1- Mucus units containing large amounts of acidic carbohydrates.

2- Serous acini containing varying amounts of neutral polysaccharides but almost no acidic carbohydrates.

3- Seromucus acini, possessing an appreciable quantity of both neutral and acidic carbohydrates.

Accordingly goblet cells correspond best to the seromucus category. Intermediatry and dark granulated cells fall into the serous category. However, Hentzer (1970) pointed out that the dark granules have not been proven to be secretory, as he considered them to be precursors of the light granules.

Histopathologic Changes in the Mucoperiosteal Lining of the Middle Ear

Changes in the mucoperiosteal lining of the middle ear were reported after attempts to produce inflammation and / or effusions in the middle ear in order to study the resulting pathological changes.

Friedmann (1955 a) injected virulent strains of various organisms into the middle ear of guinea pigs. He observed metaplasia from the flattened endothelial like membrane to a columnar ciliated epithelium with numerous goblet cells and gland - like structures. He also added that he had rarely observed squamous metaplasia in his experimental material. Senturia et al. (1962) attempted induction of middle ear effusion in dogs by electrocauterizing the nasopharyngeal orifice of the eustachian tubes and examined the middle ear mucosa at planned intervals. They reported absence of squamous metaplasia in the epithelium of the tympanum and bulla, but reported the metaplastic changes to be confined to the production of simple glands lined by columnar often ciliated, epithelium in the bulla.

The term "metaplasia" of the middle ear mucosa to the secretory pattern was rejected by some authors as Sadé (1966), Lim and Klainer (1971) and Lim and Birck (1971). They reported that since light microsocopic investigations of Zechner et al. (1968), Friedmann (1963) and Senturia et al. (1962), Showed proliferation of the mucus secreting epithelium in both humans and experimental animals, and since it was proved that the normal middle ear lining is a true mucosa. They believed this change to be due to the nature of the middle ear epithelium which could proliferate into the secreting epithelium as a normal response to infection as seen in other mucosa covered organisms, and not metaplasia. In other words, a hyperplastic transformation of the mucosa which represents an exaggerated state of the normal. According to Lim and Shimada

(1971) basal cells are hyperplastic up to three months and they differentiate into ciliated or secretory cells as soon as the injured epithelium sloughs and degenerates.

On the other hand, Senturia et al. (1962) emphasized that the major morphologic changes are not in the epithelium but in the lamina propria of the lining membrane of the tympanum and bulla. This was verified by Paparella et al. (1985) who defined the role of the subepithelial space in various forms of otitis media. They reported that their findings proved that the subepithelial space was more actively involved in all forms of otitis media than had been thought.

The normal subepithelial space was defined by Paparella et al. (1985) as being a connective tissue consisting of ground substance, an amorphous substance rich in proteins and mucopolysaccharides, collagen fibres and a few elastic fibres, cells including fibrocytes and inflammatory cells such as lymphocytes, plasma cells, macrophages, and occasionally mast cells, blood vessels and lymphatic vessels, and nerve fibres.

The most typical findings in the subepithelial space in purulent otitis media are large numbers of neutrophils and increased thickness of the subepithelial space caused by oedema, severe capillary dilation, and vascular congestion. Some fibroblasts could also be seen. According to Lim and Klainer (1971) 72 hours after infection of the middle ear, the leucocytes become less predominant, and lymphocyte, plasma cells, and round cells predominate. This was attributed by Paparella et al. (1985) to represent a later phase of purulent otitis media involving a slower specific immune response after rapid neutrophilic response, as documented also by Lim and Klainer (1971).

Grote and van Blitterswijk (1984) reported the middle ear bone response in acute otitis media. In their experimental model, they reported that three days after infection, osteoblastic activity was evident resulting in impressive osteogenesis after one week of infection. Similar results were witnessed by Lim and Klainer (1971), and Senturia et al. (1962). The function of the new bone formation was postulated by Grote and van Blitterswijk (1984) to be reconstructive and / or isolatory.

Inner Ear Pathology And Otitis Media

Sensorineural hearing loss was associated with otitis media as early as 1884 by Politzer. In recent years, cochlear dysfunction in the course of otitis media has been documented and confirmed by a number of authors (Paparella et al., 1972 , Nakai and Igarashi, 1976, Arnold et al., 1977, Goycoolea et al., 1980 a &b Munker, 1981, Paparella, 1983, Morizano et al., 1985, Cusimano et al., 1989).

According to Paparella et al. (1984) otitis media, can, by passage of inflammatory agents through the round windows, cause temporary threshold shift due to toxic labyrinthitis, or permanent threshold shifts, due to permanent dysfunction of the organ of Corti.

The round window membrane is a semi-permeable membrane, and is the only portion of the inner ear that lacks a bony wall between the middle ear and the inner ear. Studies done on its permeability showed that low molecular weight substances, such as electrolytes and antibiotics, as well as macromolecular substances, such as horseradish peroxidase and isolated bacterial toxins, can pass through an intact round window membrane (Schucknecht, 1957, Wersall et al., 1969, Goycoolea et al., 1980 a & b, Okuno and Nomura, 1984, Nomura, 1984, Saijo and Kimura, 1984, Juhn et al., 1988). Moreover, albumin and ferritin which do not pass a normal round window membrane, have been traced in the perilymph after their application in the middle ear cavity during inlfammation (Goldberg et al., 1981, Nomura, 1984). This appears to be due to a change in the passage dynamics secondary to inflammation (Hellstrom et al., 1984).

A number of animal studes on experimentally induced otitis media by injection of bacterial toxins into the middle ear cavity (Lim et al., 1987, Kawauchi et al., 1988, Spandow et al., 1989), or by inoculation of bacterial organisms including *Streptococcus pneumoniae* (Hodges et al., 1984), were done