PRESERVATION OF TOXOPLASMA GONDII

THESIS

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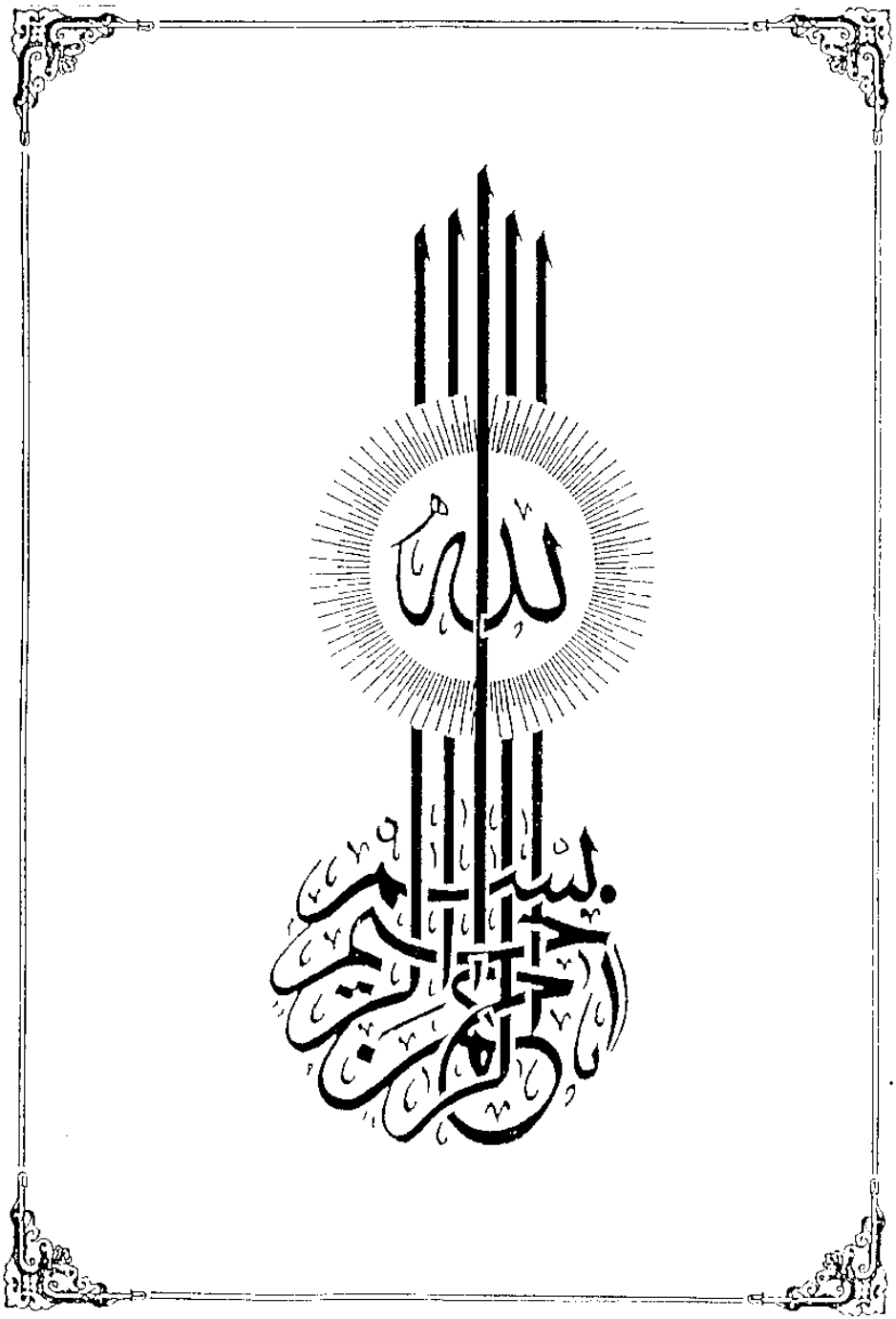

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INTRODUCTION

Toxoplasmosis is a world wide health problem and Toxoplasma gondii is one of the most common infectious pathogenic parasites of man (Russo 1994). More intensive researches about this parasite are taking place nowadays especially with it's increasing rate of infection in imunocompromised patients (AIDS patients). So it is essential to maintain at least one strain of Toxoplasma in parasitological laboratories involved in the performance of experiments related to this parasite and in the preparation of T.gondii antigens required for the diagnosis of toxoplasmosis in population surveis and suspected cases.

Laboratory maintenance of viable T.gondii strains is not an easy task form both man power and economic stand points (Kozojed 1984), therefore cryopreservation of T.gondii is a feasible solution for this problem. Many scientists conducted studies aiming for longer preservation of T.gondii, some of them used simple cheap techniques while others used complicated, expensive and even hazardous techniques. So it was crucial to conduct this study to evaluate different methods used for cryopreservation of T.gondii and to choose the simplest technique to be used in local laboratories, thus

maintaining a source for preparation of T.gondii antigens essential for diagnosis and research.

REVIEW OF LITERATURE

TOXONOMY OF TOXOPLASMA GONDII

Toxonomy of Toxoplasma gondii : according to Beaver et al.(1984):

- **Subphylum** APICOMLEXA (Levine ,1970),
- **Class** SPOROZOA (Leukart,1879),
- **Order** EUCOCOCCIDIORA (Legar,1900),
- **Suborder** EIMERIARINA (Legar,1900),
- **Family** SARCOCYSTIDAE ,(Poche,1913)
- **Genus** TOXOPLASMA (Nicolle and Maceaux,1908),
- **Species** gondii which is the only species of this genus.

HISTORICAL REVIEW

The protozoan parasite Toxoplasma gondii was discovered in 1908 by **Nicolle and Manceaux** in a generalised fatal infection of a captive North African rodent called the gondii. At about the same time **Splendor (1908)** found it in a rabbit. In the ensuing years it was shown to occur in many other animal species. A parasitic cyst was seen in sections of the eye of a blind infant by **Janku in 1923**, that is now regarded as a Toxoplasma cyst. It was not until 1939 that it was properly recognised in man by **Wolf et al.** . Recognition of human infection was an impetus to the development of serological tests. Of the many types of tests which proved to be of greatest use is the "dye test", described by **Sabin and Feldman (1948)**. Many surveys, both serological and parasitological were then done. These indicated that T.gondii had little host specificity, that any warm blooded animal including birds could be infected and that toxoplasmosis was a world wide zoonosis.

BIOLOGY OF THE PARASITE

Toxoplasma gondii is a protozoan parasite that is uniquely adapted for invading and surviving within a wide range of host cells(Sibley 1993).

•Morphology:

According to Anderson and Remington (1975), there are 4 forms of the organism:

1.Tachyzoite (endozoite,trophozoite):

Jacobs (1953) described it as the proliferative form of the parasite seen during acute infection.It is crescentic,pyriform or ovoid in shape, measuring 4-7u long by 2-4u wide and has pointed anterior and rounded posterior ends and a nucleus lying in the posterior half of the organism.

Ferguson and Hutchison (1980) confirmed that the endozoites of T.gondii can multiply by endodyogeny, repeated endodyogeny and fission. However when the types of multiplication were examined quantitatively there were marked differences between the avirulent and virulent strains. In the avirulent strains, only endodyogeny or repeated endodyogeny were observed there was no evidence of multiplication by fission. Fission occured in infection by the virulent RH strains.

Tachyzoites were believed to invade all mammalian cell types except non-nucleated red blood cells (Kaufman et al.,1959). However Schrupp et al.(1978) presented evidence that even non-nucleated mouse red blood cell can become parasitized with T.gondii tachyzoites .

The parasite actively invades the cell, forming a novel vacuole that originates from the host cell plasma membrane. The vacuole membrane is rapidly modified to remove host cell protiens and this compartment subsequently resists fusion with all other host cell endocytic compartments. Shortly after invasion, the parasite secretes a variety of proteins and elaborates an extensive array of membraneous tubules that form a network connecting with the vacuolar membrane. Understanding the formation and modification of this unique vacuole may reveal novel mechanisms for subverting host cell endocytic pathways that lead to intracellular survival (Sibley, 1993). Tachyzoites also remain viable in certain body secretions as peritoneal fluid, milk, urine, saliva or tears for hours to several days (Remington and Desmonts,1976).

2.Tissue cyst :(true cyst , bradyzoite, cystozoite, slowly multiplying trophozoites):

This refers to accumulations of Toxoplasma merozoites characteristically occurring in the brain, retina, myocardium, lung, and muscles of chronically infected animals and man .It's size varies form 10 to 200 u and contains as many as 3000 organisms (Dubey et al.,1970 and Rakel, 1985).

Wanko et al. (1962) reported that the thickness of the cyst wall varies from 0.1-0.25 u and it is the product of interaction between the host cell and the invasive multiplying organism. The organism within the cyst remains viable and reproduces by endodyogeny but at a slower rate than tachyzoites, and hence are called bradyzoites.

Cysts are demonstrable as early as the 8th day of infection in animals and persist viable throughout the life of the host (Lainson,1958).

3.Pseudocyst :

This is formed by a group of multiplying daughter intracellular tachyzoites in parenchymatous cells or macrophages (Goldman et al. 1958). The parasitized cells contain 8-16 or more tachyzoites. They may be spherical, but heavily infected cells are usually irregular. The membranes of the cells show no structural modifications. Pseudocysts have a short life and are destroyed to release tachyzoites (Lainson,1958).