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IMMUNOFLOURESCNCE AND ULTRASTRUCTURE COMPARATIVE STUDIES ON BRAIN OF MICE EXPERIMENTALLY INFECTED WITH R.H. STRAIN OF *TOXOPLASMA GONDII* AND THOSE CHALLENGED POST-VACCINATION

(With 11 Figures)

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دراسات مناعية فلوروسينية وميكروسكوبية دقيقة مقارنة على مخ الجرذان المحقونة تجريبيا بالعترة R.H للتوكسوبلازما جوندى والأخرى المحقونة بعد التحصين

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تـم إجـراء دراسـات مناعية فلوروسينية وميكروسكوبية دقيقة على انسجة المخ للجرذان المحقونـة تجريبـيا بالعترة R.H للتوكسوبلازما جوندى والأخرى المحقونة بعد التحصين. أثبتـت الدراسات المناعية الفلوروسينية أن هناك اختلافا فى التعبير عن وجود المستضد فى انسـجة المخ فى كلا المجموعتين حيث اظهرت أنسجة المخ للجرذان المعدية بالطفيل وجود تجمعـات عديـدة للطـور النشط للطفيل (Tachyzoites) إما داخل الخلايا فى سيتوبلازم الخلايـا العصـبية أو خلايا الغراء العصبى فى حين أظهرت خلايا المخ للجرذان المحصنة تعبـيرا اقل عن وجود مادة المستضد وكانت أساسا داخل الخلايا مع قلة تواجدها فى خارج الخلايا كما كانت شدة صبغة التفاعل أقل نسبيا مقارنة بالجرذان المعدية. أظهرت الدراسات الميكروسـكوبية الدقـيقة لأنسجة المخ للجرذان المعدية وجود طور نشط أو أكثر إما بداخل الخلايـا العصـبية أو خلايـا الغراء العصبى خاصة الخلايا النجمية أو خارجها. وظهرت الأطـوار النشـطة للطفيل داخل الخلايا محاطة بفراغ أو فجوة طفيلية شفافة إلكترونيا. كما أظهـرت خلايا العائل وجود تجمعات عديدة للحبيبات الخيطية (الميتوكوندريا) حول الجدار المحـدد للفـراغ أو الفجوة الطفيلية فى حين أظهرت بعضا من هذه الحبيبات الخيطية وجود استحالة وتنكرز مع فقد لحوافها (Cristea). وظهرت أنوية الخلايا المصابة بالطفيل محددة الكروماتيـن المتبايـن مع عدم انتظام للغلاف النووى وتحلل محتويات كلا من السيتوبلازم والـنواة، بيـنما أظهرت أنسجة المخ للجرذان المحصنة تشوهات شكلية للطور النشط داخل الخلايا تمثلت فى عدم انتظام محيط الغشاء الخارجى للطور النشط وانقباض النهاية الأمامية وأيضـا انكمـاش الأنويـة وتجزء محتويات خلايا الطفيل الى أجسام معتمة إلكترونيا. كما

أظهـــرت بعض الخلايا المصابة بالطفيل احتفاظها نسبيا بالتركيب الدقيق لعضيات الخلية مع تعـــرج ظاهر لغشاء النواة الى مجموعة من التجاويف الغير منتظمة كما أظهر عدد قليل من الخلايـــا تفتـــتا لمحتوياته الخلوية كما ظهرت أيضا دلائل طفيفة لانقسام الطور النشط داخل خلايا المخ فى الجرذان المحصنة.

SUMMARY

Immunoflourescnce and ultrastructure comparative studies were performed on brain tissues of mice experimentally infected with R.H. strain of *T. gondii* and those challenged post-vaccination. Immunoflourescence studies revealed that there were variable expressions of antigenic materials between the two groups. The brain tissue of infected mice showed positivily reacted numerous clusters of extra or intra-cellular tachyzoites within the cytoplasm of neurons or neuroglia cells meanwhile the brain tissue of the vaccinated group, exhibited little expression of antigenic materials mainly of intracellular location with less evidence of extracellular ones and the intensity of the staining reaction was relatively less than that of infected ones. Ultrastructure studies of brain tissue of infected mice showed one or two intracellular tachyzoites within the cytoplasm of neurons or neurologia cells particularly in astrocytes. The intracellular tachyzoites were surrounded by electron-lucent parasitophorous vacuole. The host cells revealed collection of numerous mitochondrial elements around the limiting membrane of the parasitophorous vacuole meanwhile, other mitochondria appeared to be degenerated and/or necrotic with loss of its cristea. The nuclei of parasitized cells depicted marginated nuclear heterochromatin, wrinkled nuclear envelope or complete lysis of the cytoplasmic and nuclear components. The brain tissue of vaccinated group showed different morphological deformities of the intracellular tachyzoites represented by irregular contours of the parasite pellicle, constriction of its anterior conoidal end, pyknosis of its nuclei and fragmentation of its cellular constituents into more electron dense bodies. Moreover, some parasitized cells relatively maintained the fine structure of its cytoplasmic organelles, with significant irregularities of the nuclear envelope into series of concavities, meanwhile few cells appeared disintegrated with fragmentation of its cellular constituents. There were little evidence of multiplication of intracellular tachyzoites in the vaccinated group.

Key words: *Toxoplasma, Electron microscopy, Immunoflourscence, Vaccine, Mice, Brain*

INTRODUCTION

T. gondii is a ccocidian obligate intracellular protozoan that causes serious illness in all worm blooded animals and humans (Dubey and Beattie, 1988; Guiterriez, 1990 and Dubey, 1998).

T. gondii is responsible for great losses to the livestock industry represented by embryonic death, resorption, foetal death, mummification, abortion, still birth and neonatal death (Dubey, 1998). In recent years many trials have been performed in order to establish a new vaccine which has the ability to reduce the incidence of abortion resulted from transplacental infection and to minimize the risk of human exposure resulted from ingestion of infected meat with the subsequent risk of foetal infection (Sayles *et al.*, 2000 and Hiramoto *et al.*, 2002). Suzuki and Remington (1990) and Milon and Louis (1993) pointed out that the major mechanism of resistance against *T. gondii* is considered to be cell mediated. However, Chao *et al.* (1993) stated that macrophages and monocytes represent the key cell types that prevent intracellular multiplication of *T. gondii*.

Many trials have been done to determine the cellular biology of *T. gondii* through ultrastructure studies which provide a critical foundation for improved antimicrobial agent as well as development of protective preparations (Ferguson and Hutchinson, 1987; Ferguson *et al.*, 1991; Mcleod *et al.*, 1991; Speer *et al.*, 1997 and Halonen *et al.*, 1998).

Bjerkas and Landsverk (1986) stated that electron microscopic studies seem to be useful for identification of protozoan parasite and are alternative to the immunoperoxidase methods. However, Tang *et al.* (1986) pointed out that electron microscopic diagnosis of *T. gondii* is faster, more direct and safer than any other methods. Recently electron microscopy is used for identification of *T. gondii* infected patients using biopsy or necropsy.

The objectives of the present study were to assess and compare the immunoflourscence and ultrastructural pathology of brain tissue of mice experimentally infected with R.H. strain of *T. gondii* and those challenged post-vaccination.

MATERIALS and METHODS

1. Experimental animals:

A total of 70 male white Swiss mice of 8 weeks old were used in the present study, 50 mice were vaccinated then challenged post-

vaccination and 20 mice were kept as controls (infected with R.H. strain of *T. gondii*).

2. Vaccination protocol:

A. Antigen of *Toxoplasma gondii* (R.H. strain) was prepared from the peritoneal exudates of the infected mice according to Beverley *et al.*, (1971).

B. Adjuvant: both Bordetella pertussis (Vacsera) and complete Freund's adjuvant (Difco) were used in the vaccinated group.

C. Each mouse in the vaccinated group received 20 µg of antigen with CFA at 1:1, V:V ratio and 2.5 X 10^8 m.o. of BP, S/C (sensitizing dose), which was followed, 14 days later with a vaccinating dose of 100 µg of antigen, CFA and 10^{10} m.o. of P.B., S/C (Micheal and Frenkel, 1984).

3. Challenge:- One month after the vaccinating dose each mouse in the 2 groups was challenged with 3 X 10^4 organisms I/P (Beverley *et al.*, 1971).

4. Fluorescent antibody technique:

Biological reagents:

A. Antisera against *T. gondii*:

Antisera against *T. gondii* were prepared by intradermal infection of Boscat white rabbit with 2 mg of antigen emulsified with 0.5-1 of CFA (Complete Freund's adjuvant). Two weeks later, the rabbits received a second intradermal dose consisted of the same amount of antigen emulsified with incomplete Freund's adjuvant. After two weeks from the 1st injection blood was collected from ear vein for sera separation. Sera were kept at - 20°C till used.

B. Anti-rabbit conjugated flourscein: as a secondary antinody against rabbit IgG supplied by Sigma Comp., Germany.

Paraffin sections (3 micron) of the brain tissue of both infected and vaccinated groups were screened for detection and comparison of antigenic materials by indirect immunoflourescnce technique according to Kawamura (1977).

5. Transmission electron microscopy (TEM):

Small portions (1 mm cubes) from different parts of the brain tissue of mice (cerebrum and cerebellum) were immediately fixed in 4% cold glutaraldhyde in phosphate buffer then in osmic acid solution. They were dehydrated in different grades of alcohol and embedded in epoxy resin. Semithin sections were made and stained with toulidin blue. Ultrathin sections were stained with uranyl acetate and lead citrate prior to examination with a joel 100 CX electron microscope according to Hayat (1989).

6. Measurement of vaccine efficiency:

A. Interferon-gamma level in serum:

It was assayed by estimating the level of interferon-gamma in the sera of both vaccinated and control groups of mice. It is the principal marker of the measurement of cell mediated immunity (Khan and Kasper, 1996).

Sera were collected from 5 mice in each group, pooled in one sample and stored at -20°C till used. It was assayed by ELISA technique using ELISA reader model Dynateck 700 R and kit (Pethyl Research Diagnostic, Inc., USA), as previously described by Pestka (1986).

B. Survival rate: Retardation of death of the vaccinated mice in comparison to the infected ones was considered by many authors as indicator of vaccine success (Mcleod *et al.*, 1988 and Araujo, 1994).

RESULTS

A. Immunofluorescence studies:

The brain tissue involved both the cerebrum and cerebellum from both groups of mice infected with R.H. strain of *T. gondii* and those challenged post-vaccination were screened for detection of antigenic materials.

In the group of infected mice, the *T. gondii* antigens were demonstrable either free in the brain tissue particularly in the malacic zones or in the form of numerous intracellular clusters of tachyzoites within the cytoplasm of neuron or neuroglia cells (Fig., 1 A).

The brain tissue of mice challenged post-vaccination exhibited intracellular expressions of antigenic materials with little evidence of free organisms (Fig., 1B). Moreover, some positively reacted tachyzoites were recognized within the lumen of some cerebral blood vessels.

The immunoflourescnce staining reactions in the brain tissue of infected group was more intense and deep in comparison to those challenged post-vaccination which had moderate to mild expressions of antigenic materials.

B. Ultrastructure studies:

Ultrastructure pathology of brains of mice experimentally infected with R.H. strain of *T. gondii*.

The proliferative stages of *T. gondii* (tachyzoites) were detected in the cerebral cortex either free extracellulary in the neuropil or intracellularly in neurons or neuroglia cells particularly astrocytes. The tachyzoites were pear in shape in longitudinally sectioned parts (Fig., 2)

meanwhile it exhibited oval shape with centrally located rounded nucleus in transversely sectioned parts as in Fig. (3 A & B).

The tachyzoites were surrounded by electron lucent parasitophorous vacuole (Fig., 3) which sometimes appreared only as a double membrane (Fig., 2). In many instances, the intracellular tachyzoites were recognized at the stage of endodyogeny (a special form of asexual reproduction) where two daughter cells were produced (Fig., 3).

The intracellular tachyzoites occupied a large area of the cytoplasm of parasitized cells or they might compress the cytoplasmic organelles and the nucleus to one side.

Sometimes the parasitized cells appeared to be surrounded by more or less electron lucent zone suggested accumulation of oedema fluid at the pericellular region (Fig., 2).

The parasitized cells in either neuroglia or nerve cells exhibited a variety of ultrastructural alterations represented by close associations of numerous host cell mitochondria toward the limiting membrane of the parasitophorous vacuoles. The mitochondria of some of the parasitized cells appeared swollen, degenerated with loss of most of its cristea (Fig., 4 A & B).

In one parasitized cell, autophagosome of one degenerated mitochondria was detected where it is surrounded by a membrane bound vesicle (Fig., 4 A). The rough endoplasmic reticulum in the cytoplasm of some parasitized cells revealed absence of ribosomes from its sides. In many instances some neurons and neuroglia cells exhibited dissociation or lysis of most of its cytoplasmic organelles depicted washed out cytoplasmic matrix associated with pyknotic nucleus (Fig., 5 A) which manifested by the picture of encephalomalacia by light microscopy. Sometimes, the parasitized cells showed partial disintegration of the host cell plasmalemma associated with focal loss of the nuclear envelop (Fig., 5 B). The nuclei of the parasitized cells exhibited various alterations emphasized by marginated nuclear heterochromatin pattern in some cells (Fig., 1 & 2) or the nuclear heterochromatin appeared in the form of discrete blocks associated with wrinkled nuclear envelop and mild dilatation of the perinuclear cisterne (Fig., 6 A). Moreover, other cells revealed marked dissociation or lysis of the nucleoplasm content with few randomly distributed heterochromatin which assumed the appearance of washed out nuclear interior (Fig., 6 B). Some cells revealed few cytofilaments in their cytoplasm.

Sometimes, the intracellular tachyzoites were detected at the stage of multiplication by a sexual form called endodyogeny with the formation of two daughter cells from each parent parasite with intact parasitophorous vacuole. The parasitized cells in which the division process occurred were disintegrated with lysis of most of its cytoplasmic organelles (Fig., 7 A & B).

The parasitophorous vacuole depicted the presence of intravacuolar microtubules which connected the vacuole to the parasite pellicle (outer membrane) meanwhile conoid was observed in one of the divided tachyzoites (Fig., 7 B).

II. Ultrastructure pathology of brains of mice challenged with R.H. strain of *T. gondii* post-vaccination:

The most striking features were recognized in both cerebral and cerebellar cortex which comprised numerous discrete aggregates of microglia cells at different parts of the neuropil suggested microglial nodules by light microscopy (Fig., 8 A). Some parasitized cells either neurons or neuroglia cells revealed intracellular tachyzoite with intact shape and cellular constituents. Meanwhile other cells showed marked morphological deformities in the shape of the profile of the intracellular tachyzoites represented by irregular contours of its pellicle (outer membrane) where it thrown into numerous variable size and shape processes and edentations (Fig., 8 B) and (Fig. 9, A & B). Some intracellular tachyzoites showed marked constriction of its anterior conoidal end (Fig., 8 B). While other intra-cellular tachyzoites revealed fragmentation of most of its cellular constituents into variable size and shape electron dense discrete bodies (Fig., 10 A & B) with inconspicuousness of definite cellular constituents. The nuclei of the tachyzoites depicted pyknosis manifested by shrunken and condensation of chromatin materials (a heterochromatic nucleus, Fig. 11). The nuclei of some intracellular tachyzoites illustrated multifocal loss of the nuclear envelope.

Most of the parasitized cells were surrounded by a clear halo represented pericellular oedema fluid. The cytoplasmic organelles of the parasitized cells relatively maintained its fine structure with the appearance of few mitochondria around the limiting membrane of the vacuole. Meanwhile few cells appeared disintegrated with lysis of its cellular constituents (Fig., 11).

The nuclei of the parasitized cells displayed variable alterations emphasized by irregularities of the nuclear envelope into series of

concavities, marked depletion of nuclear euchromatin and aggregated heterochromatin pattern (Fig., 8 B & Fig., 9 A).

The brain tissue in a vaccinated group depicted little evidence of tachyzoite replications than that seen in the infected group.

C. Interferon-gamma level in the serum of the vaccinated mice was significantly elevated (498.3 ± 6.1 I.U./ml) above its level in the controls (130.1 ± 3.2 I.U./ml).

D. Survival rate: vaccinated mice showed prolonged survival post-challenge in comparison to the non-vaccinated controls, i.e. While the controls died between the 3rd and 5th day post-challenge (d.p.c.), the vaccinated ones died between the 18th an 20th d.p.c.

DISCUSSION

Immunoflourscence and electron microscopic studies were used to compare the neuropathological lesions associated with infection of mice with R.H. strain of *T. gondii* and those challenged post-vaccination. Our study revealed that there were variable expressions of antigenic materials in both groups. However the intensity of the staining reactions of antigenic materials was less in the vaccinated group than that of infected ones. These findings reflect either low concentrations of antigenic materials in the brain tissue of a vaccinated group or a possible degradation of the antigenic materials within the host cells as those hypothesized by Tropier *et al.* (1993).

The recognition of some positively reacted tachyzoites within the lumen of some cerebral blood vessels were in agreement to that mentioned by Sims and Hay (1995) who explored that *T. gondii* antigens leave the CNS through the vascular route.

The ultrastructural investigations of brain tissue of mice experimentally infected with R.H strain of *T. gondii* revealed presence of one or two intracellular tachyzoites (the proliferative stage of *T. gondii*) within the cytoplasm of neurons or neuroglia cells, meanwhile extracellular forms could be detected within neuropil. The intracellular tochyzoites were surrounded by electron lucent parasitophorous vacuole. These pictures were previously described by (Ferguson *et al.*, 1989), (Speer *et al.*, 1997) and (Dubey, 1998). In some instances the parasitophorous vacuole may be appreciated only as a double cell membrane as those mentioned by Guiterriez (1990). Moreover, Dubey (1998) suggested that parasitophorous vacuoles were derived from both the parasite and the host cells.

Endo and Yagita (1990) pointed out that the invasion processes of the tachyzoites into the mammalian cells occurred through alterations of extracellular ion concentration and maintained in the cells by prevention of acidifications of parasitophorous vacuole. The majority of the tachyzoites were recognized mainly in neurons and astrocytes, these observations coincided with the notion of Hulinska *et al.* (1990) who pointed out that the predilection seats for tachyzoite development were mainly in neurons and astrocytes. Moreover, Peterson *et al.* (1993) speculated that astrocytes may provide a safe harbor for tachyzoite survival and multiplication.

The most important ultrastructural alterations detected in the brains of mice experimentally infected with *T. gondii* were close association of host cell mitochondria toward the limiting membrane of the parasitophorous vacuole. A finding which previously detected by Ferguson and Hutchison (1987), McLeod *et al.* (1991), Lindsay *et al.* (1993) and Kurz *et al.* (1998).

Tanabe and Murakami (1984), accounted for the abundance of mitochondrial elements around the limiting membrane of the vacuoles to the fact that after invasion *T. gondii's* mitochondrial membrane potential was reduced therefore the host cell mitochondria collect around the vacuole membrane, meanwhile Speer *et al.* (1997) considered this phenomena a feature denotes that the parasitophorous vacuole membrane might be metabolically highly active. The recognition of some mitochondrial elements at the stage of degeneration with loss of most its cristea beside absence of ribosomes from the sides of rough endoplasmic reticulum accentuate the hypothesis of Ferguson *et al.* (1989) who postulated that nutritional requirements of the parasite for maintaince, multiplication and growth were obtained from the host cells. The nuclear changes encountered in the parasitized cells emphasized by marginated nuclear heterochromatin pattern, wrinkled nuclear envelope, washed out appearance of the nuclear interior in many cells and focal loss of the nuclear envelope which were incriminated as morphologic signs of degenerative and necrotic entities by Dickersin (1988), Bibbo (1991), Eyden (1996) and Kumar *et al.* (1997). Moreover, the cytoplasmic organelles of many cells were completely lysed gave the appearance of washed out cytoplasmic matrix. These features were previously recorded by (Ferguson *et al.*, 1989 and Bibbo, 1991) who attributed the cell necrosis to mitochondrial dysfunction with lack and depletion of ATP which it self constituted a lethal event. Milon and Louis (1993) and Kumar *et al.* (1997) attributed lysis of cytoplasmic

organelles to sensitized $CD8^+$ T. cells which has an ability to lyse target cells before replication of the infected organism through secretion of a perforin molecule that drill holes in the target cells and cause osmotic lysis. Jones *et al.* (1997) referred to the mechanism by which the infected cells were killed by cell mediated cytotoxicity as apoptosis which characterized by markedly electron dense chromatin materials and gave rise to apoptotic bodies which represented in the present study by a membrane bound vesicle contained degenerated mitochondria.

Sims *et al.* (1988) attributed the necrotic changes in the parasitized cells to the disintegration of the microtubular microfilamenteus components of the host cells as a response to highly antigenic stimulation.

The necrotic changes were also encountered in other neurons or neuroglia cells within the brain tissue rather than parasitized cells, these results supports the hypothesis of Saavedra *et al.* (1990) who mentioned that *T. gondii* produce soluble antigens which diffuse into the neuropil and come in contact with other cells.

Concerning the ultrastructural pathology of brain of mice challenged post vaccination. It was found that partial protective effect was obtained against *T. gondii* infection demonstrated by both immunoflourscence and ultrastructural studies.

Eid *et al.* (2004) reported that the protective effect evidenced by long survival rate of avaccinated mice, increased interferon gamma level and less evident tissue changes.

Since the vaccination tools used in the present study depended primarily on activation of cell mediated immunity represented by phagocytes and CD^8T and CD^4T lymphocytes which release cytokines such as interferon γ.

Our study explored that brain tissue of avaccinated group of mice displayed relatively less necrotic changes of the neural tissue in comparison to infected ones as well as morphological deformities of the intracellular tachyzoites. These findings were in correlation to that described by (Sayles *et al.*, 2000) and (Johnson and Sayles, 2002) who showed that T. lymphocytes and interferen γ are knowen mediators of resistance against *T. gondii*. On the other side, Milon and Louis (1993) suggested that $CD^{8+}T$ cells able to lyse *T. gondii* infected cells.

Moreover, Pfefferkorn *et al.* (1986) mentioned that interferon γ suppress growth of intracellular tachyzoites by depletion of host cell tryptophane.

The brain tissue of avaccinated group of mice revealed multifocal aggregations of microglia cells which appreciated as microglial nodules by light microscopy. This entity supports the hypothosis of (MacGavin *et al.,* 2001) who mentioned that microglia cells are the first cell to react with any infectious insults of the CNS. Moreover, Chao *et al.* (1993) suggested that activation of microglia by IFY γ inhibited intracellular *T. gondii* multiplication. The majority of intracellular tachyzoites exhibited variable ultrastructural changes emphasized by irregular contours of the parasite pellicle with constriction of its anterior conoidal end. These changes proposed that the intracellular tachyzoites underwent degenerative changes with little tendency to invade other cells since the conoidal end is probably associated with penetration of the tachyzoite through the host cell membranes (Mcleod *et al.,* 1991) and (Dubey, 1998). Also, *T. gondii* outer membrane (pellicle) appeared to form moving, tight junction through utilization of surface membrane components of the host cell during invasion process as described by (Kasper and Mineo, 1994).

The fragmentation of the cellular constituents of some tachyzoites into more electron dense bodies associated with pyknosis of the nuclei together with irregularities of its pellicle all of these changes seem to be an evidence denotes degeneration of the intra cellular tachyzoites.

The changes encountered in some parasitized cells in the vaccinated group represented by irregular nuclear envelope and depletion of the nuclear euchromatin (the highly metabolic part of the nuclear chromatin) provide an evidence for the early signs of cell degeneration as those mentioned by Bibbo (1991) and Eyden (1996). Meanwhile, few cells appeared disintegrated with fragmentation of its cellular constituents seem to be an element in the favour of partial protection achieved in the present study.

We can concluded that, the criteria for partial protective immunity against R.H. strain of *T. gondii* recognized immunoflourscencally and ultrastructurally may provide important targets for further investigations of development of immunological strategies against this infection.

REFERENCE

Araujo, F.G. (1994): "Immunization against *T. gondii.*" Parasitology Today, 10 (9): 358-360.

Beverley, A.K.J.; Archer, F.J.; Watson, A.W. and Fawcett, R.A. (1971): "Trial of a killed vaccine in the prevention of ovine abortion due to toxoplasmosis." Br. Vet. J. 127: 529- 534.

Bibbo, M.M.D. (1991): "Comperhensive cytopathology." W.B. Sounders company. Harcourt Brace Jovanovich. Inv U.S.A.

Bjerkas, I. and Landsverk, T. (1986): "Identification of *Toxoplasma gondii* and *Encephalitezoon cuniculi* by immunoperoxidase techniques and electron microscopy." Acta Veterinaria Scandinavca. 27: 1, 11- 22.

Chao. Ghun, Shuxian Hu, Genya Gekker, William, J. Novick, Jack, S., Remigton, and Phillip, K. Peterson (1993): "Effect of cytokines on multiplication of *Toxoplasma gondii* in microglia cells." The Journal of Immunology, 150, 8, 3404- 3410.

Dickersin, G.K. (1988): "Diagnostic electron microscopy: A text/ Atlas." IGAKU- Shoin medical publishers, Inc.

Dubey, J.P. (1998): "Toxoplasmosis cited in Topely and Wilson's microbiology and microbial infections 9th Edition Vol. 5 parasitology Chep. 16." Arlond, London Sydney, Aukland.

Dubey, J.P. and Beattie, C.P. (1988): "Toxoplasmosis of animals and man." CRC Press, Boca Raton.

Eid, R.A.A.; Abd El- Aal, A.A.; Barakat, A.M.A. and Fatma, M.M. Darwish (2004): "A trial for vaccination of mice against toxoplasmosis." Egypt. J. Comp. Path. Clinc. Path. 17, 2, 1- 25.

Endo, T. and Yagita, K. (1990): "Effect of extracellular ions on motility and cell entry in *Toxoplasma gondii*." J. of Protozology. 37, 133- 138.

Eyden, B. (1996): "Organelles in tumour diagnosis." IGAKU- Shoin Medical Publisher.

Ferguson, D.J.P. and Hutchison, W.M. (1987): "An ultrastructural study of the early development and tissue cyst formation of *Toxoplasma gondii* in the brains of mice." Parasitology Research, 73: 383- 491.

Ferguson, D.J.P.; Hutchison, W.M. and Pettersen, C. (1989): "Tissue cyst rupture in mice chronically infected with *Toxoplasma gondii*." An immunocytochemical and ultrastructure study. Parasit. Res. (75): 599- 603.

Ferguson, D.J.P.; Graham, D.I. and Hutchison, W.M. (1991): "Pathological changes in the brains of mice infected with *Toxoplasma gondii* a histological, immunocytochemical and

ultrastructural study." International J. of Experimental Pathology 72: 4, 463- 474.

Guiterriez, Y. (1990): "Diagnostic pathology of parasitic infections with clinical correlations." Lea and Febiger Philadelphia USA.

Halonen, S.R.; Weiss, L.M. and Chiu, F.C. (1998): "Association of host cells intermediate filaments with *Toxoplasma gondii* cysts in murine astrocytes in vitro." Int. J. Parasitology 58 (5), 815- 23.

Hayat, M.A. (1989): "Principles and techniques of electron microscopy biological applications third edition." Macmillan Press Ltd. USA.

Hiramoto, R.; Galisteo, J.; Nascimento, N. and Androde, H. (2002): "200 Gy sterilized *Toxoplasma gondii* tachyzoites maintain metabolic functions and mammalian cell invasion, eliciting cellular immunity and cytokine response similar to natural infection in mice." Vaccine (20), 2072- 2091.

Hulinska, D.; Sykora, J. and Zastera, M. (1990): "Effect of cortisone on *Toxoplasma gondii* infection studied by electron microscopy." Folia- Para. 37 (3): 207- 12.

Johnson, L.L. and Sayles, P.C. (2002): "Deficient humoral responses underlie susceptibility to *Toxoplasma gondii* in CD4-deficient mice." Infection and Immunity. 70 (1): 185- 91.

Jones, T.C.; Hunt, R.D. and King, N.W. (1997): "Vet Pathology 6th Ed., William and Wilkins, Awavely company, Baltimore, Philadelphia London."

Kasper, L.H. and Mineo, J.R. (1994): "Attachment and invation of host cells by *Toxoplasma gondii.*" Parasitology Today 10 (5), 184- 188.

Kawamura, J.R. (1977): "Fluorescent antibody techniques and their applications, second edition." University of Tokyo press.

Khan, A.I. and Kasper, H.L. (1996): "Il15 augmenta $CD9^+$ T cell-mediated immunity against T. gondii infection in mice." J. Immunology. 157: 2103- 2108.

Kumar, V..; Cotran, R. and Robbins, L. (1997): "Basic pathology." 6th Ed., W.B. Saunders comp., Harcourt Brace & comp. London, Tokyo."

Kurz, B.; Bockeder, W. and Buse, E. (1998): "In Vitro- model for *Toxoplasma gondii* invasion into neuroepithelial cells." Anat. Anz. 180 (4): 299- 305.

Lindsay, D.S.; Mitschler, R.R.; Toivio- Kinnucau, M.A.; Upton, S.J.; Dubey, J.P. and Blagburn, B.L. (1993): "Association of host

cell mitochondria with developing *Toxoplasma gondii* tissue cysts." American J. of Vet Research. 54: 10, 1663- 1667.

MacGavin, M.D.; Carleton, W.W. and Zachary, J.F. (2001): "Thamson's special Vet. Path. Mosby." Harcourt health Science Comp., London.

McLeod, R.; Frenkel, J.K.; Estes, R.C.; Mack, D.G.; Eisenhauer, P.B. and Gibori, G. (1988): "Subcutaneous and intestinal vaccination with tachyzoites of *T. gondii* and acquisition of immunity to per oral and congenital toxoplasma challenge." J. of Immunology, 140 (5): 1632-1637.

McLeod, R.; Mack, D. and Brown, C. (1991): "*Toxoplasma gondii*- new advances in cellular and molecuolar biology." Experimental Pathology, 72: 109- 121.

Micheal, R.E. and Frenkel, J.K. (1984): "Immunity to toxoplasmosis in hamsters." Am. J. of Vet Res, 45 (12), 2668- 2674.

Milon, G. and Louis, J. (1993): "$CD8^{+}$T cells and immunity to intracellular pathogens." Parasitology today, 9, 6, 196- 197.

Pestka, S. (1986): "Interferon standards methods in enzymology." Academic Press, New York 119: 12- 23.

Peterson, P.K.; Gekker, G.; Shuxian, H. and Chao, C. (1993): "Intracellular survival and multiplications of *Toxoplasma gondii* in astrocytes." J. Infectious Diseases, 168: 1472-1478.

Pfefferkorn, E.R.; Eckel, M. and Rebhun, S. (1986): "Interferon- gamma suppresses the growth of *Toxoplasma gondii* in human fibroblasts through starvation for tryptophane." Molocular and Biochemical Parasitology 20, 215- 222.

Saavedra, R.; Meutcr, F. and Hcrion, P. (1990): "Monocolonal antibodies identify new *Toxoplasma gondii* soluble antigens." Hypridoma 9, 753- 63.

Sayles, C.P.; Gibson, G.W. and Johson, L.L. (2000): "B. cells are essential for vaccination- induced resistance to virulent *Toxoplasma gondii*." Infection and Immunity 68. 3. 1026- 1033.

Sims, T.A. and Hay, J. (1995): "Host- parasite relationship between congenital toxoplasma infection and mouse brain : role of small vessles." Parasitology, 110, 123- 127.

Sims, T.A.; Hay, J. and Talbot, I. (1988): "Host- Parasite relation- ship in the brains of mice with congenital toxoplasmosis." J. of Path., 156, 255- 61.

Speer, C.A., Dubey, J.P.; Blixt, J.A. and Prokop. (1997): "Time lapse video microscopy and ultrastructure of penetrating sporozoites, types 1 and 2 parasitophorous vacuoles and the transmission of sporozoites to tachyzoites of VEG strain of *Toxoplasma gondii*." J. Porositology, 83 (4), 565- 574.

Suzuki, Y. and Remington, J. (1990): "The effect of anti- IFN- Y antibody on the protective effect of Lyt- 2^+ immune T. cells against toxoplasmosis in mice." The J. of Immunology. 144. 1954- 1956.

Tanabe, K. and Murakami, K. (1984): "Reduction in the mitochondrial membrane potential of *Toxoplasma gondii* after invasion of host cells." J. of Cell Science. 70, 73- 81.

Tang, T.T.; Harb, J.M.; Dunne, W.M.; Wells, R.G.; Meyer, G,A.; Chmsid, M.J.; Casper, J.T. and Camilla, B.M. (1986): "Cerebral toxoplasmosis in an immunocompromised host a precise and rapid diagnosis by electron microscopy." American J. of Clinical Pathology, 85- 1, 104- 110.

Tropier, G.; Charif, H.; Darcy, F.; Liu, J.; Darde, M. and Capron, A. (1993): "*Toxoplasma gondii* differential locations of antigens secreted from encysted tachyzoite." Experimental Parasitology, 77: 13- 22.

LEGENDS FOR FIGURRES

Fig. 1: A. Cerebrellar cortex of a mouse infected with R.H. strain of *T. gondii* revealing intense immunoflourscence reactions of intracellular tachyzoites within the neuroglia cells (X 400).

B. Cerebral cortex of a mouse challenged post-vaccination against *T. gondii* illustrating mild to moderate immunoflourscence reactions of intracellular tachyzoites (X 400).

Fig. 2: TEM of brain of a mouse experimentally infected with *T. gondii* showing parasitized nerve cell depicting longitudinal section of intracellaulr tachyzoite surrounded by scant cytoplasmic organelles. The nucleus of a parasitized cell revealed marginated nuclear heterochromatin pattern. The parasitized cell is surrounded by electron lucent zone of odema fluid (X 8000).

Fig. 3: TEM of brain of a mouse experimentally infected with *T. gondii* revealing:

A. A parasitized cell contained transverse section of two intracellular tachyzoites at the stage of division surrounded by electron lucent parasitophorous vacuole and small part of cytoplasmic organelles. The extracellular neural tissue revealed numerous mylinated nerves and mitochondria of synaptic end processes of adjacent neurons (X 7200).

B. Higher magnification illustrating two oval shaped tachyzoites with centrally located nuclei. (X 18000).

Fig. 4: TEM of a brain of a mouse experimentally infected with *T. gondii* revealing:

A. Close association of host cell mitochondria toward the limiting membrane of the parasitophorous vacuole (right arrow) and autophagosome of one degenerated mitochondria which surrounded by a membrane bound vesicle (left arrow) (X 6000).

B. Depicting higher magnification illustrating various changes in the host cell mitochondria represented by degeneration and loss of its cristea (X 15.000).

Fig. 5: TEM of a brain of a mouse experimentally infected with *T. gondii* illustrating:

A. Lysis of most of the cytoplasmic organelles with pyknotic nucleus (washed out cytoplasmic matrix). (X 3600)

B. Marginated electron dense heterochromatin with multifocal loss of the nuclear envelope associated with partial loss of host cell plasmalemma. (X 8000).

Fig. 6: TEM of a brain of a mouse experimentally infected with *T. gondii* depicting:

A. nuclear heterochromatin appreciated as discrete blocks, wrinkled nuclear envelope and mild dilatations of prenuclear cisterne (X 6000).

B. dissociation or lysis of the nucleoplasm content with washed out appearance of the nuclear interior and few randomly distributed heterochromatin. Few cytofilaments appeared in the cell cytoplasm. (X 6000).

Fig. 7: TEM of a brain of a mouse experimentally infected with *T. gondii* illustrating:

A. asexual reproduction of the tachyzoites with the formation of two doughter cells in intact parasitophorous vacuole. The host cell appeared to be disintegrated with lysis of most of the cytoplasmic organelles (X 4000).

B. Higher magnification of divided tachyzoites showing intravacuolar microtubules within the parasitophorous vacuole (left arrows) and conoid (right arrow). X 10.000

Fig. 8: TEM of a brain of a mouse challenged post vaccination against *T. gondii* revealing:

A. Aggregations of numerous microglia cells (X 4000).

B. Preicellular odema around the parasitized cell. The intracellular tachyzoite exhibited irregularities of the parasite pellicle (outer membrane) with constriction of the anterior conoidal end (arrow), marginated heterochromatin pattern and few mitochondria around the limiting membrane of the vacuole. (X 8000).

Fig. 9: TEM of a brain of a mouse challenged post vaccination against *T. gondii* illustrating:

A. Irregular contorus of the parasite pellicle (outer membrane) where it thrown into variable shape and size numerous processes and edentations (X 6000).

B. Higher magnification of a parasitized cell depicating numerous processes and edentations of the tachyzoite pellicle associated with pericellular odema of the adjacent neural tissue. Two neuroendocrine granules appeared at the vicnity of the tachyzoite (X 10000).

Fig. 10: A & B TEM of a brain of a mouse challenged post vaccination illustrating irregular contours of the parasite pellicle associated with fragmentation of the cellular constituents into more electron dense discrete bodies (A. X 8000; B X 15000).

Fig. 11: TEM of a brain of a mouse challenged post vaccination demonstrating irregular contours of the parasite pellicle, shrunken and karyo-pyknosis of its nucleus and fragmentation of the cellular constituents into more electron dense discrete bodies with inconspicuosness of definite organelles. The parasitized cell showed disintegration of its cellular constituents (X 6000).

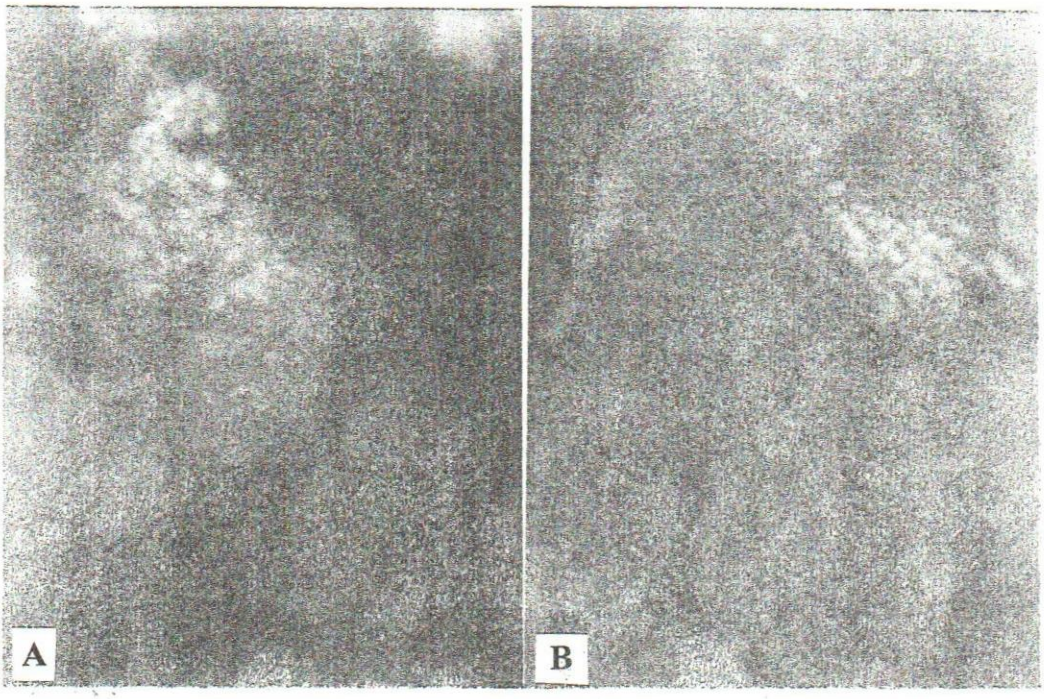

Fig. 1

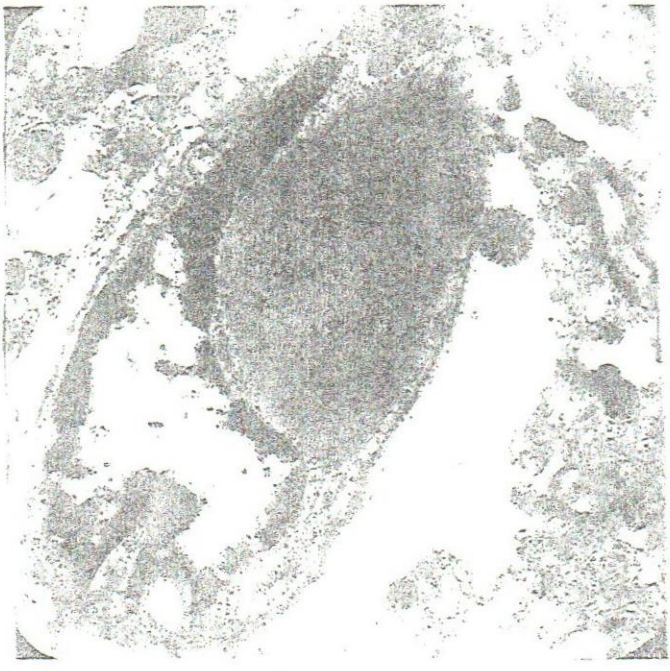

Fig. 2

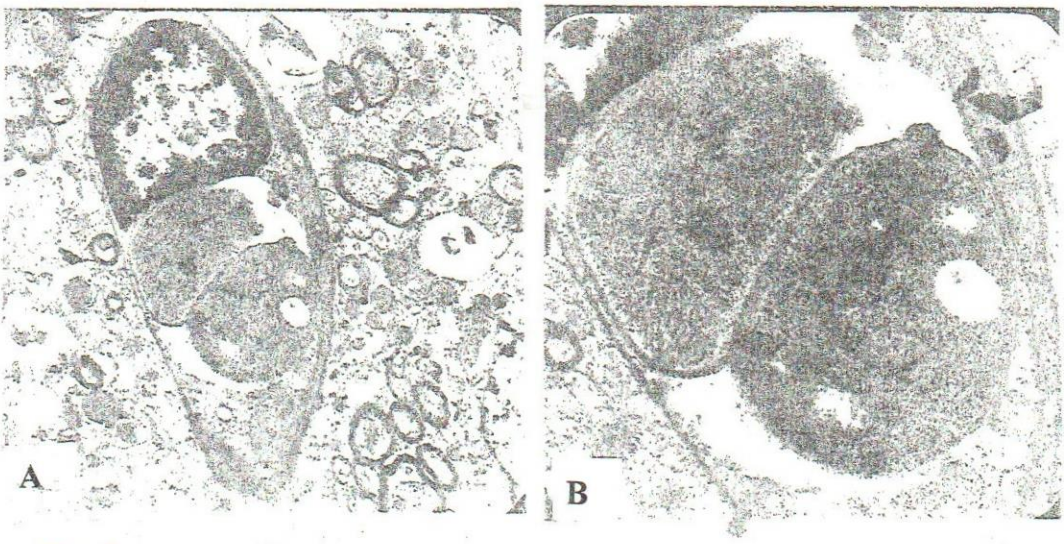

Fig. 3

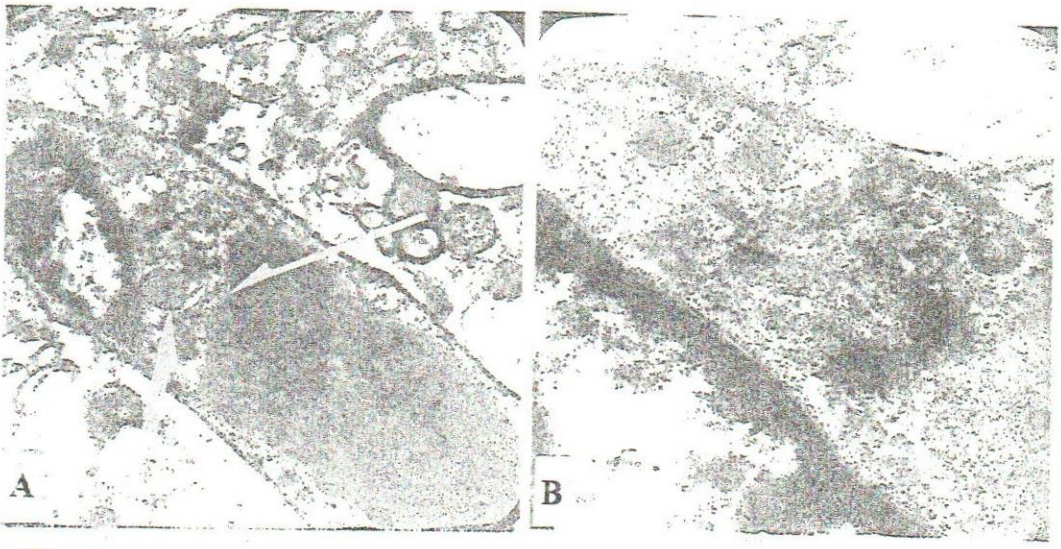

Fig. 4

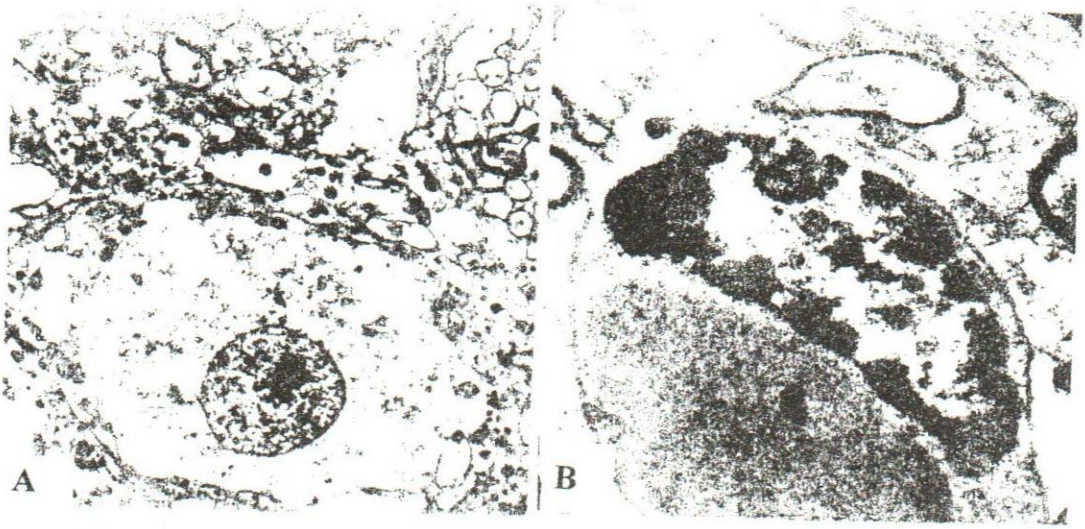

Fig. 5

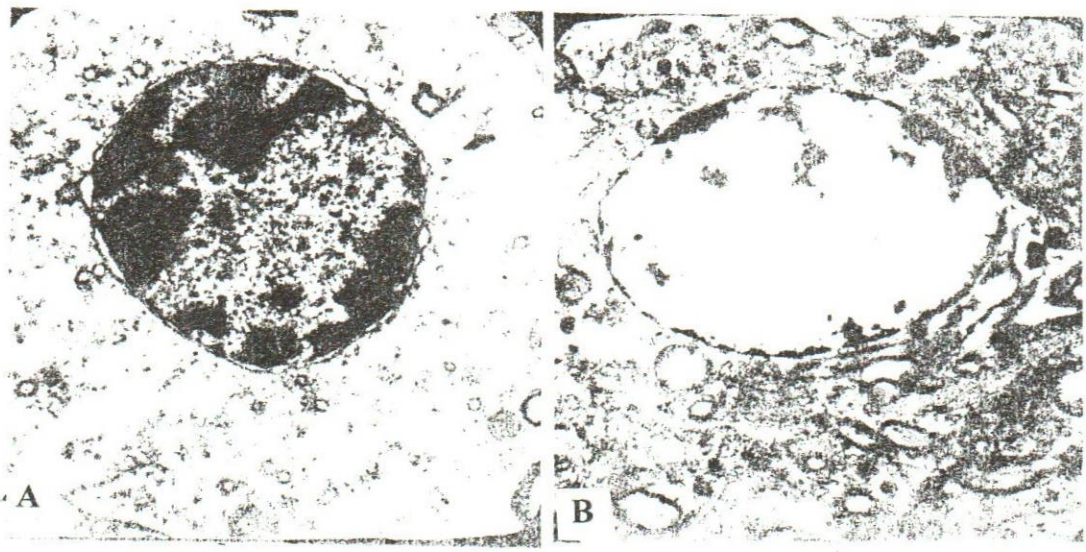

Fig. 6

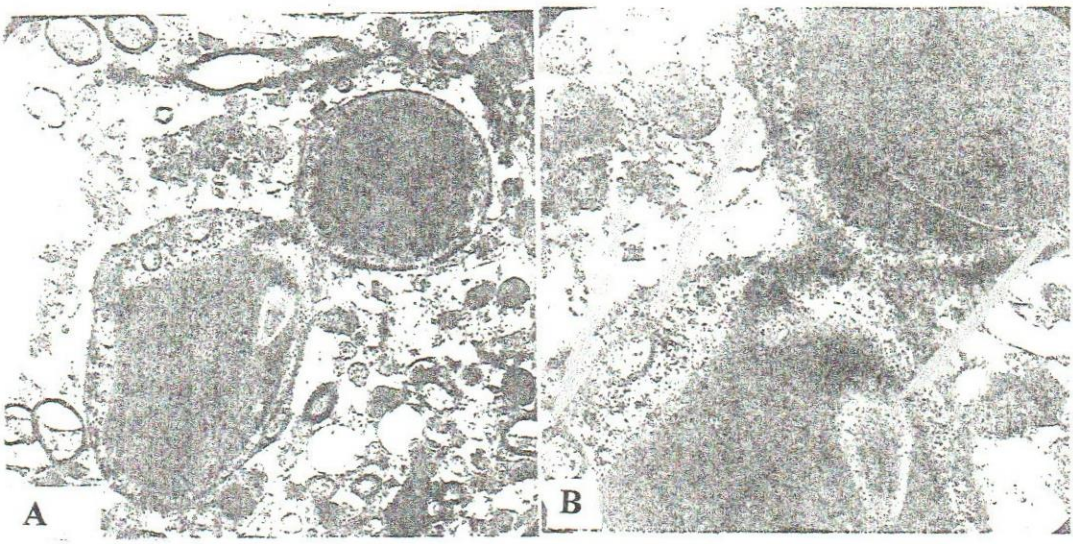

Fig. 7

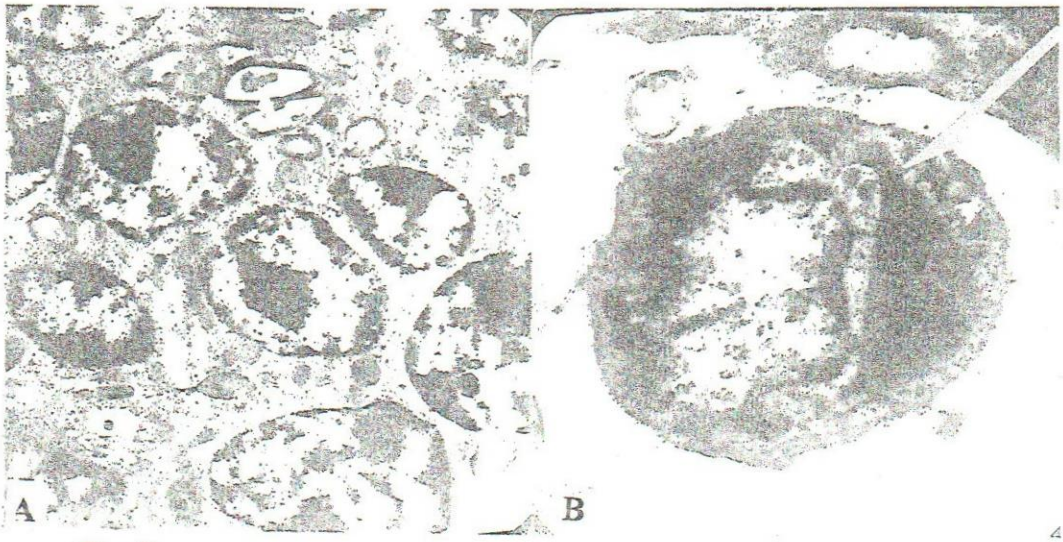

Fig. 8

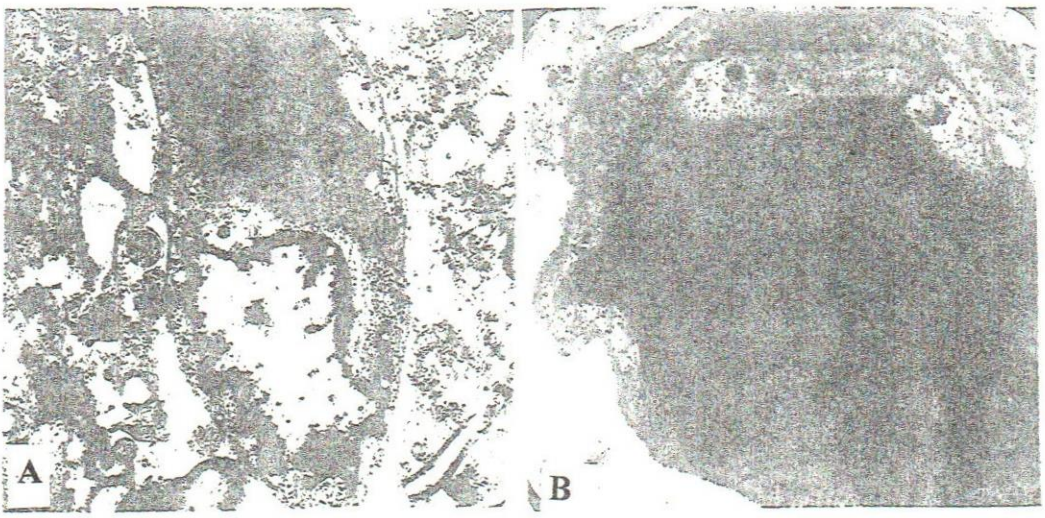

Fig. 9

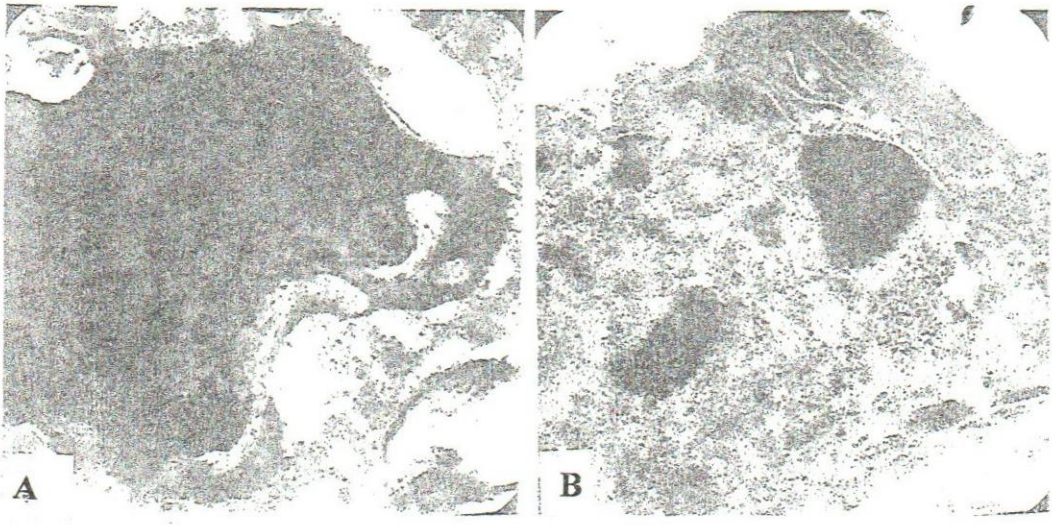

Fig. 10

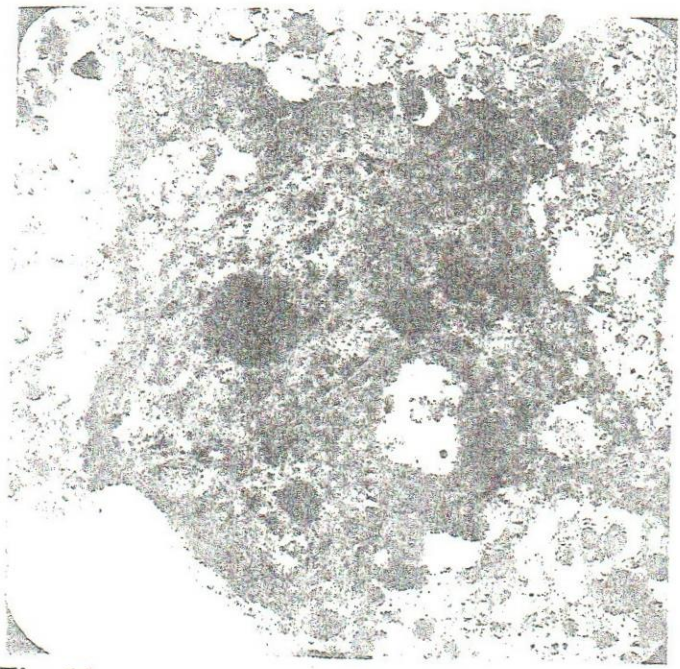

Fig. 11