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*Original Investigations*

# Meningoencephalitis Due to Acanthamoeba SP

## Pathogenesis and Clinico-Pathological Study

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**Summary.** Amebic Meningoencephalitis (AM) and Primary Amebic Meningoencephalitis (PAM) are infectious diseases essentially confined to the Central Nervous System (CNS) and caused by free-living amebas of the genus *Acanthamoeba* (*A.*) and *Naegleria* (*N.*) respectively.

AM due to *A.* sp. (*Acanthamoeba castellanii* and *Acanthamoeba culbertsoni*) have been reported in chronically ill debilitated individuals, some of them under immunosuppressive therapy, or in immunologically impaired patients without a history of recent swimming in contrast to cases due to *N.*sp. which usually occurs in healthy, young individuals with a recent history of swimming in man-made lakes or heated swimming pools.

AM due to *A.*sp. is characterized by a subacute or chronic granulomatous meningoencephalitis involving mainly the midbrain, basal areas of the temporal and occipital lobes and posterior fossa structures. CNS lesions in AM are perhaps secondary and the portal of entry in humans is probably from the lower respiratory tract, genitourinary system or skin reaching the CNS by hematogenous spread. The predominant host reaction is usually composed of lymphocytes, plasma cells, monocytes and multinucleated foreign body giant cells. Necrosis is moderate and hemorrhage scant or absent. Cysts as well as trophozoites may be seen within the CNS lesions.

PAM is due to *Naegleria fowleri* and is characterized by an hemorrhagic necrotizing meningoencephalities with an acute inflammatory response. Only trophozoites are found in lesions. The portal of entry is through the olfactory neuroepithelium.

CNS tissues fixed in formalin may be used for further identification and taxonomical classification of the causative protoza using immunofluorescent antibody techniques (IFAT) and electron microscopic methods.

**Key words:** *Acanthamoeba* – *Naegleria* – Meningoencephalitis – Granulomatous – Free-living amebas – Cysts – Trophozoites.

## Introduction

In human hosts Amebic Meningoencephalitis (AM) and Primary Amebic Meningoencephalitis (PAM) have been reported due to free-living amebic species of the genera *Acanthamoeba* (or *Hartmannella*) (*A.*) and *Naegleria* (*N.*).

In recent years there have been significant advances in the understanding of the epidemiology, pathogenesis and clinicopathological characteristics of these disorders, as well as more accurate taxonomic criteria for the classification of the responsible protozoa. AM, due to *A.*sp. (*Acanthamoeba castellanii* and *Acanthamoeba culbertsoni*) appears to be an opportunistic infection of the central nervous system (CNS) by other ubiquitous free-living amebas not previously known to be pathogenic for human beings. AM occurs in patients who are chronically ill, debilitated or those whose cell-mediated immune responses have been impaired as a result either of the underlying systemic disease or its treatment by immunosuppressive methods [5, 18, 20, 26, 27].

In contrast, *Naegleria fowleri* produces an acute, fulminant, necrotizing hemorrhagic meningoencephalitis affecting mainly the olfactory bulbs, the base of the frontal and temporal lobes and cerebellum in young, healthy individuals with a recent history of practicing water-related sports or swimming in man-made lakes [6–9, 12–15, 17].

Despite individual variations, the clinicopathological features of both entities have been remarkably uniform from case to case. In this paper, particular emphasis will be paid to the nature and distribution of CNS lesions in AM due to *A.*sp., the mode of the CNS involvement, the host-parasite interrelationship, the clinical course and epidemiological considerations.

## Materials and Methods

The findings reported here are based on the review of the clinical histories, autopsy protocols and histopathology of 3 cases of Amebic Meningoencephalitis due to *Acanthamoeba* sp. (Table 1) and comparison with other cases reported in the literature [5, 18, 20, 26].

Case 1 though previously reported [27] as an example of PAM due to *Naegleria* has been reclassified as an example of AM based on IFAT and EM studies.

Light microscopic studies were done from tissues routinely fixed in 10% buffered formalin. After routine processing and paraffin embedding, sections of CNS from different areas and samples of lungs, kidneys, liver, prostate, spleen, heart, gastro-intestinal tract, pancreas, uterus and lymph nodes were cut at 6–8 µm and stained with hematoxylin-eosin. Selected sections from brain, kidneys, and prostate were also stained with Gomori methenamine-silver, PAS-H (Schiff) and acid fast stain (Ziehl-Neelsen). Ultrastructural studies were done using blocks of brain tissue fixed in formalin and embedded in paraffin.

Following examination of the microscopic slides, stained with H.-E., the blocks containing most trophozoites and cysts were selected, and the most promising areas, isolated, deparaffinized and hydrated. Then 1 mm cubes were placed in 4% glutaraldehyde in cacodylate buffer for 4 h, osmicated and dehydrated in graded series of ethanols and propylene oxide and then embedded in Epon 812. One µm sections were cut with glass knives on a Porter-Blum MT 2 ultramicrotome. These sections were stained with paragon PS-1301 or toluidine blue. Ultra-thin sections were made with a diamond knife, mounted on 240 hole copper grids, double stained with uranyl acetate and lead citrate and observed in an Hitachi HS-8F-2 electron microscope.

Immunofluorescent antibody techniques (IFAT) were performed in all three cases using deparaffinized brain sections which were incubated with dilutions of several antisera raised against the different known type species of amebas, e.g. *Naegleria fowleri, Acanthamoeba castellanii, A. rhysodes, A. polyphaga. A. astronyxis, A. palestinensis* and *A. culbertsoni*. These antisera were prepared either by multiple injections [30] of an insoluble extract or of purified plasma membranes of amebas [29, 33] or by intravenous inoculations of whole living cells. The results were read with a Leitz Ortholux Microscope equipped for fluorescence with a ploemopak 2 and a super pressure mercury lamp HBO 50 W. The filter system used was FITC combined with interference filters KP 490, K 510 and K 445.

## Results

### *Clinical Features*

Clinical symptoms of AM due to *A.* sp. are those associated with severe meningeal irritation: headache, nausea, vomiting and stiff neck progressing to coma and death.

Typically, AM due to *Acanthamoeba* sp. may occur in debilitated individuals with chronic alcoholism, diabetes mellitus, liver cirrhosis, or under immunosuppressive therapy and without a history of recent swimming. The incubation period is unknown but perhaps more than 10 days (Table 1).

In cases of infection with *Naegleria fowleri* the premortem diagnosis can be established by finding trophozoites in the cerebrospinal fluid (CSF); this is established by examining a wet preparation by ordinary light microscopy in which actively motile trophozoites can be easily seen. The CSF pattern is that found in bacterial meningitis but sterile for bacteria, namely: neutrophilic pleocytosis, high protein and usually low sugar.

Staining the sediment with the Wright or preferably Giemsa technique the characteristic amebic trophozoites with a dense round nucleolus surrounded by a nuclear rim and abundant cytoplasm can be identified, amidst other cellular elements of the CSF. Gram's stained smears and bacterial cultures are negative. In cases of infection with *Acanthamoeba* sp. no premortem or clinical diagnosis have been made except in the case reported by Callicott et al. [8].

### *Neuropathological Features*

*Gross Pathology.* The leptomeninges, mainly over the most affected cortical areas, contain a moderate amount of cloudy exudate; in some less affected regions the leptomeninges are normal.

The cerebral hemispheres show severe edema and foci of softening, associated with focal hemorrhagic necrosis (Figs. 1–3). Bilateral uncal notching and cerebellar tonsillar herniation may be a prominent feature. Microscopic abscesses were present in all 3 cases. The olfactory bulbs and spinal cord are usually spared (Table 1).

### *Histopathology*

There is inconspicuous, scant exudate in the leptomeninges covering the overlying affected cortex. The exudate is composed of lymphocytes, macrophages, a few polymorphonuclear leukocytes, plasma cells, and histiocytic elements.

Typically parenchymal lesions are a chronic, granulomatous reaction, with multinucleated "foreign-body giant cells" in some areas (Fig. 4). Focal necrosis and recent hemorrhages can be seen. Fossilized or "ferruginated" neurons may be present (Fig. 5) in the most affected areas.

The amebic forms observed are both trophozoites and cysts. Both forms of the organisms are scattered throughout the lesions, but preferentially located around and near perivascular spaces. The trophozoites have a central dense karyosome, surrounded by a clear nuclear halo and abundant spongy cytoplasm. (Fig. 6 inset). The cysts usually reveal a wrinkled double wall with a star-like appearance (Figs. 4 and 5 insets).

Case no. 1 revealed a chronic granulomatous lesion in the renal cortex (Fig. 7) and Case no. 3 revealed granulomatous lesions with "multinucleated giant cells" within the prostate (Table 1).

Case no. 2 revealed many "granulomata" within liver, spleen and uterus.

**Tabelle 1.** Clinical and pathological features of acanthamoeba encephalitis

| Case number | Age/sex (years) Country | Clinical features | Gross pathology | Histopathology | IFAT[a] |
|---|---|---|---|---|---|
| 1 | 7 y/o male Texas (U.S.A.) | Pharyngitis for 3 weeks. Iritis, headache. Nausea. Uveitis. Meningism. Left hemiparesis. Ataxia. Coma. Steroids + Penicillin. CSF: 106 lymphs cu/mm. Death 21 days A.A.[b] | Purulent leptomeningitis, slight. Brain edema with herniations. Foci of cortical necrosis. Necrotizing-hemorrhagic lesion in thalamus and cerebellum | Necrotizing chronic, granulomatous encephalitis with foci of recent hemorrhages and multinucleated giant-cells. Amebic trophozoites and cysts. Chronic granulomatous lesion in one kidney | + for Acanthamoeba sp. (A. castellanii, polyphaga, rhysodes) |
| 2 | 26 y/o female (Venezuela) | Fever for 2 weeks. Headache. Left abdominal pain. Meningism. Left hemiparesis. High fever. Coma. Seizures. Steroids. Death 12 days A.A. | Purulent leptomeningitis, slight. Brain edema. Necrosis on right fronto-temporal occipital lobes, cingulate gyrus, putamen, caudate and right thalamus. Focal necrosis on cerebellar folia and pons | Necrotizing chronic, granulomatous encephalitis with giant-cells and hemorrhages. Amebic trophozoites and cysts. Chronic granulomata in liver, spleen and uterus (Myometrium) | Positive for A. culbertsoni |
| 3 | 20 y/o male (Peru) | Headache. Somnolence. Generalized seizures. Fever. Stiff neck. Right hemiparesis. Steroids + broad-spectrum antibiotics. CSF: 750 lymphs cu/mm. Death 53 days A.A. | Purulent leptomeningitis. Brain edema. Necrosis on left fronto-parietal lobe, right cerebral peduncle and medulla oblongata | Necrotizing chronic, granulomatous encephalitis with focal hemorrhages and multinucleated giant-cells. Amebic trophozoites and cysts. Chronic granulomata in prostate | Positive for A. castellanii |

[a] IFAT = Immunofluorescent antibody techniques
[b] A.A. = After admission

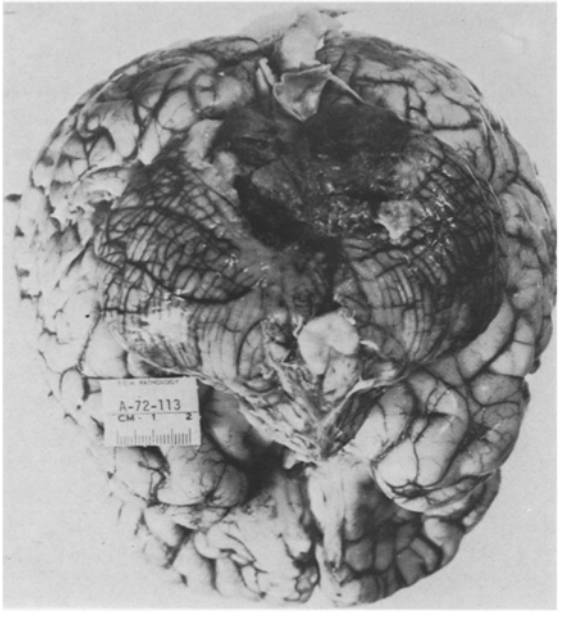



**Fig. 1.** Base of the brain of Case 1. Necrosis of the inferior surface of the cerebellum is prominent

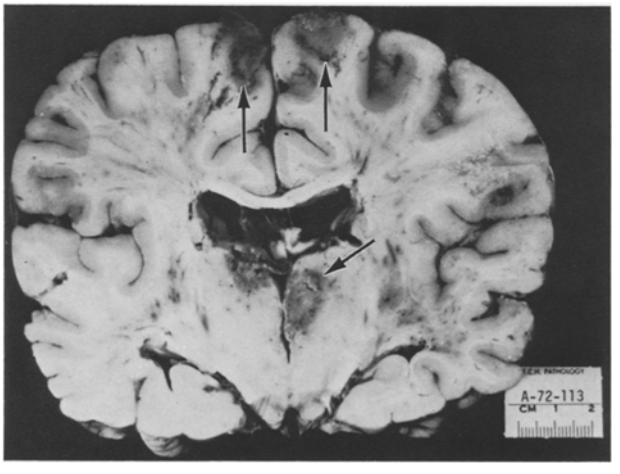
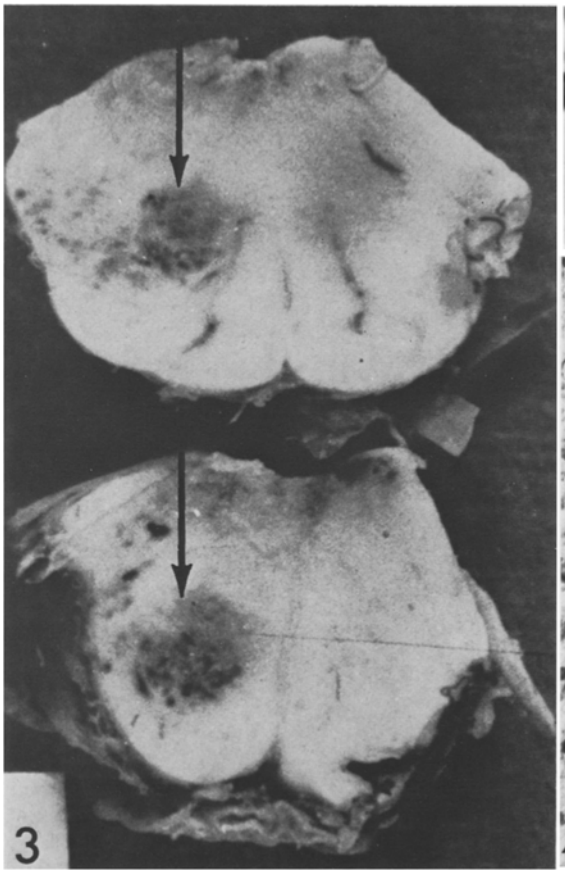



**Fig. 2.** Coronal section of cerebral hemispheres with necrotizing cortical and thalamic lesions (arrows). Case 1



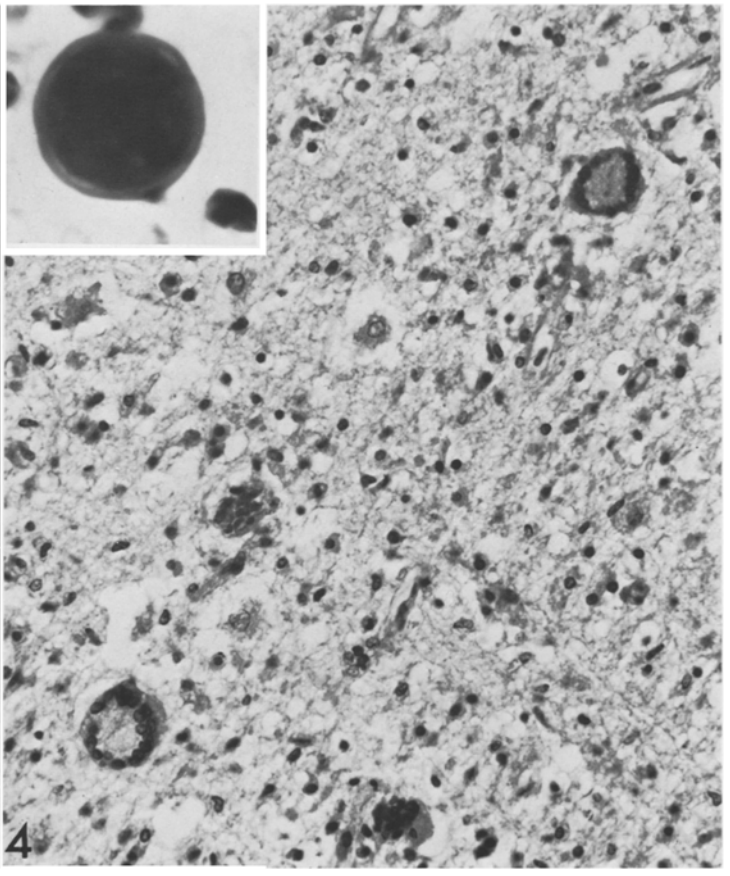




**Fig. 3.** Horizontal sections of lower pons and medulla oblongata with foci of hemorrhagic necrosis (arrows). Case 3

**Fig. 4.** Multinucleated "foreign-body giant cells" within granulomatous-gliotic encephalitic process. H.-E. × 280. Inset: Cyst with prominent double-wall. Case 1. H.-E. × 1690

*Indirect Immunofluorescent Antibody Techniques*

Indirect immunofluorescent identification of the etiological agent. Case no. 1 and Case no. 3 only stained well with the antisera raised against the *Acanthamoeba* species belonging to the group *A. castellanii* – *A. polyphaga* – *A. rhysodes*. The staining titers obtained varied from 1 : 32 to 1 : 8. This important cross reaction obtained with these three antisera do not allow a species specific identification. Case no. 2 only stained with anti-*A. culbertsoni* antisera. The antiserum raised against whole cell, stained at a titter of 1 : 32 and with the purified membrane antiserum better results were obtained (Fig. 8). No other antiserum provided a positive result.

*Electron Microscopic Findings*

Despite severe postmortem autolysis and other postmortem alterations of the CNS tissue, the amebic trophozoites and cysts were well preserved.

Trophozoites were bound by a thin, well defined cytoplasmic membrane (Figs. 6 and 9). They measure 20 μm in average diameter. Cytoplasmic processes or acanthopodia were noted in some trophozoites. The cytoplasm contained abundant round and/or spherical mitochondria with dense cristae, free ribosomes, vesicles of different sizes and large vacuoles. Some trophozoites revealed a prominent cytoplasmic membrane system, such as the Golgi apparatus, smooth and rough endoplasmic reticulum, digestive vacuoles, lysosomes and glycogen granules. In general the nucleus was eccentrically located, measuring 5 μm in average diameter and containing a dense irregularly round nucleolus measuring about 2.5 μm. A clearly defined nuclear membrane was demonstrated. In some instances the nucleus contained a dividing nucleolus (Fig. 6). Other times well defined spherical "nuclear-body-like" structures were present within the evenly distributed nuclear chromatin. Some trophozoites disclosed a "pre-encystment" stage characterized by condensation of the cytoplasm all around the cytoplasmic membrane.

Cysts were characterized by a prominent, thick, wrinkled wall (Figs. 4 and 5 insets and 10). The mean

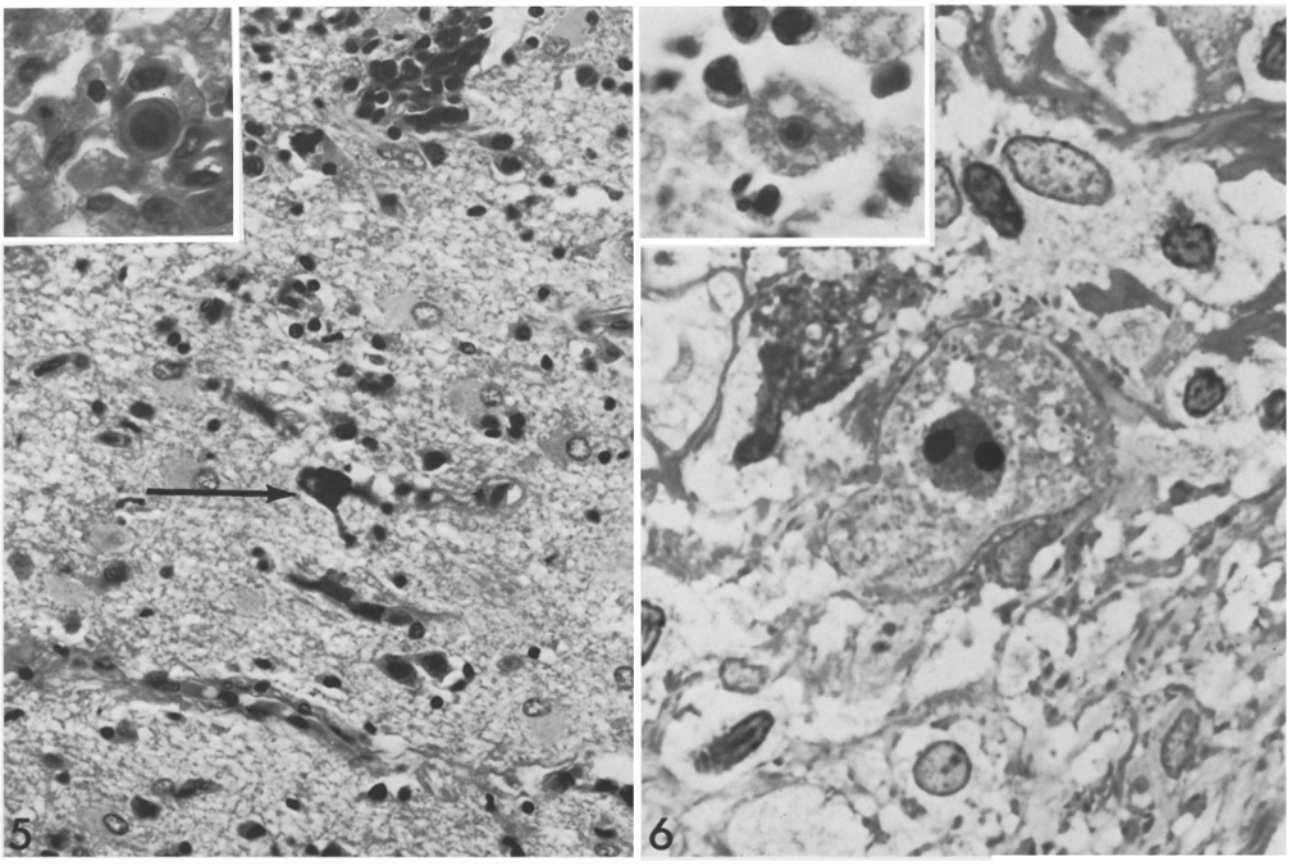


**Fig. 5.** Prominent gliosis and moderate chronic inflammatory exudate within cerebral cortex. Note "mineralized" or "ferruginated" neuron (arrow). H.-E. ×375. Inset: Cyst of *A. castellanii* within necrotizing granulomatous cerebral tissue. Case 3. H.-E. ×600

**Fig. 6.** Trophozoite of *Acanthamoeba castellanii* from cerebellar lesion demonstrating two dense nucleoli. One μm section. Toluidine blue. ×1500. Inset: Typical trophozoite of *A. castellanii* from paraffin embedded CNS tissue

diameter of a mature cyst was 10.5 μm. Empty cysts were also identified. The mature cyst was wrinkled, spherical, sometimes "star-shape". The cyst wall was sometimes composed of concentric parallel layers with partial splitting. Irregular spaces were present between the layers.

## Discussion

The increasing frequency with which protozoal amebic infections are being recognized in all parts of the world reflects many factors: wider awareness of their occurrence; better diagnostic facilities; more accurate diagnosis; the increasing prevalence of "opportunistic" infections.

Free-living amebas belonging to the genera *Acanthamoeba* and *Naegleria* are established animal and human pathogens [2,16,22,24]. Most of the reported human cases have been acute fulminant fatal amebic meningoencephalitis due to *Naegleria fowleri* (PAM) [1,6–8,12,13–15,17]. A smaller number infected with species of *Acanthamoeba* (AM), most of them subacute or chronic, have been observed in man [5, 18,20,26,27] and also in domestic animals [2,16,24]. *A* has not been isolated yet from fatal human cases. The acute hemorrhagic necrotizing meningoencephalitis is evident and persists in all cases of *Naegleria* infection as a distinctive component of the lesions, while the lesion is a subacute or chronic granulomatous encephalitis in cases of *Acanthamoeba* infection.

It is perhaps very significant that lower respiratory tract involvement with numerous lung abscesses is a prominent feature in experimental *A* infection. Mice may develop severe, extensive, bilateral "amebic pneumonitis" containing trophozoites and cysts [22]. Culbertson demonstrated that "amebic rhinitis" and respiratory amebiasis or "amebic pneumonia" were followed by fatal encephalitis in experimental infections in mice, rabbits and monkeys [11]. It is of interest to point out that *A.*sp. has been isolated from throats of normal individuals without any disease [10,32], from a purulent ear discharge [21], and from cases of keratitis [19,25,31], keratoconjunctivitis, and uveitis [19].

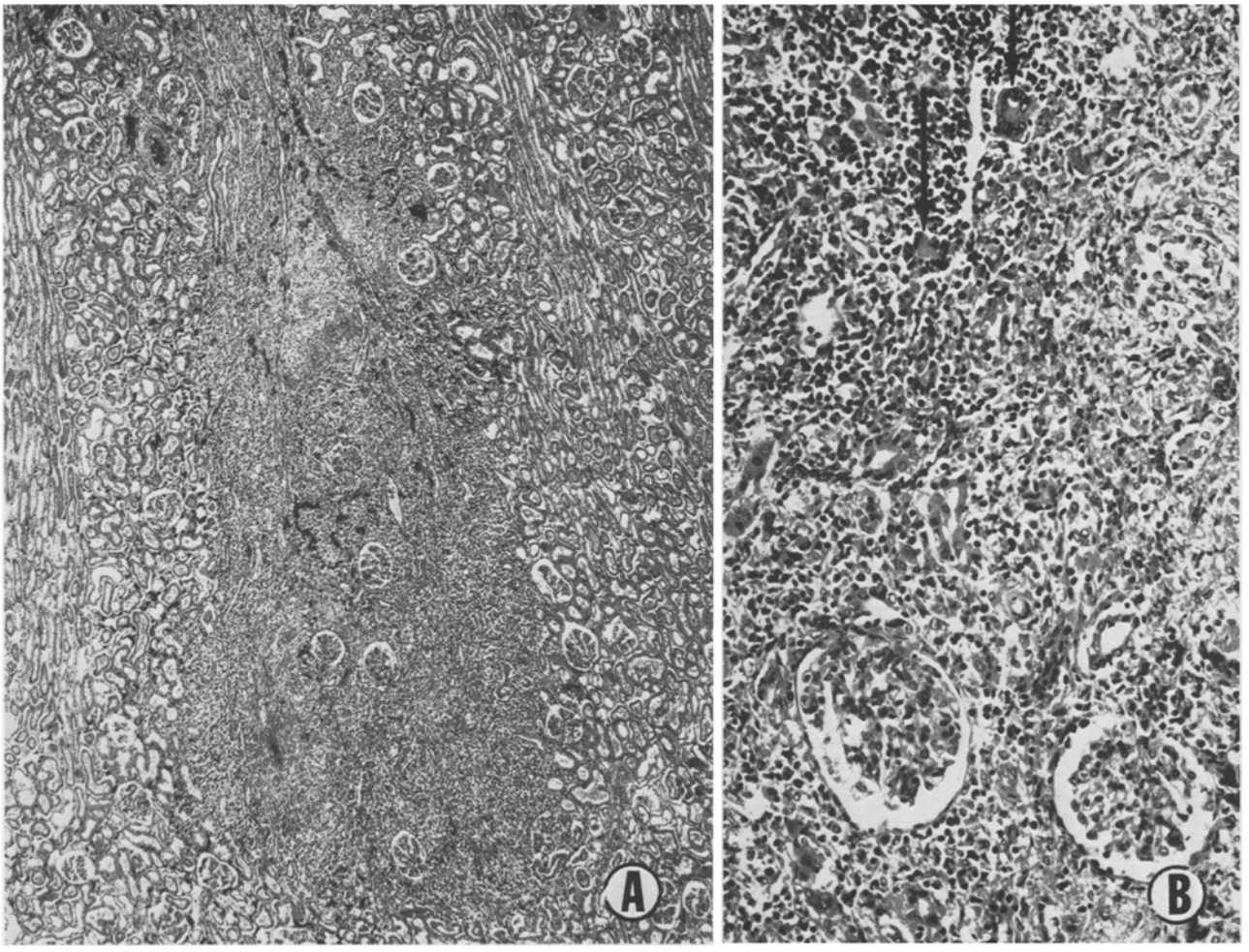



**Fig. 7. A** Granulomatous lesion within renal cortex. H.-E. ×40. **B** Multinucleated giant cells (arrows). H.-E. Case 1. ×360

It has been established experimentally that a portal of entry into the CNS in *N. fowleri* infection is via disruption of the olfactory-mucosa, penetration of the organisms into the submucosal nervous plexus, probably by phagocytosis of the amebas by the sustentacular cells of the olfactory neuroepithelium and passage through the cribriform plate to the subarachnoid space [23]. However, in cases of *Acanthamoeba* sp. the involvement of the CNS appears to be a secondary phenomenon representing metastatic spread from a primary focus in the skin, genitourinary, or respiratory tract. Cutaneous ulceration as a possible point of entrance with hematogenous spread to the CNS [5] and lower respiratory tract infection in experimental animals have been reported [22].

It is of interest to point out that a "granulomatous lesion" with multinucleated foreign body giant cells within the renal cortex was found in Case no. 1 and a chronic granulomatous lesion was found in the prostate of Case no. 3. This suggests that the genitourinary tract might also be a possible portal of entry. However, hematogenous dissemitation of amebas cannot be ruled out from a primary focus elsewhere. No amebas were identified within the kidney in Case no. 1, prostate in Case no. 3, or within the liver, spleen or uterus in Case no. 2. In this connection it might be recalled that even though speculative, according to Batson [3, 4], metastatic abscesses and metastatic tumors may appear in locations not in the line of direct spread from their primary focus, He postulated on the basis of postmortem injection studies that vertebral veins and spino-vertebral venous plexuses with their rich anastomoses connect the thoracic, abdominal and pelvic cavities with the intracranial venous system.

Susceptibility of the CNS might be related to a failure of true abscess formation with walling off of organisms. Trophozoites may be present within CNS tissues, unaccompanied by inflammatory reaction. It is also possible that within the neural tissues there is a deficiency of a specific antibody to amebas. Humoral antibodies have been produced in animals to both amebic organisms, but to date no such anti-

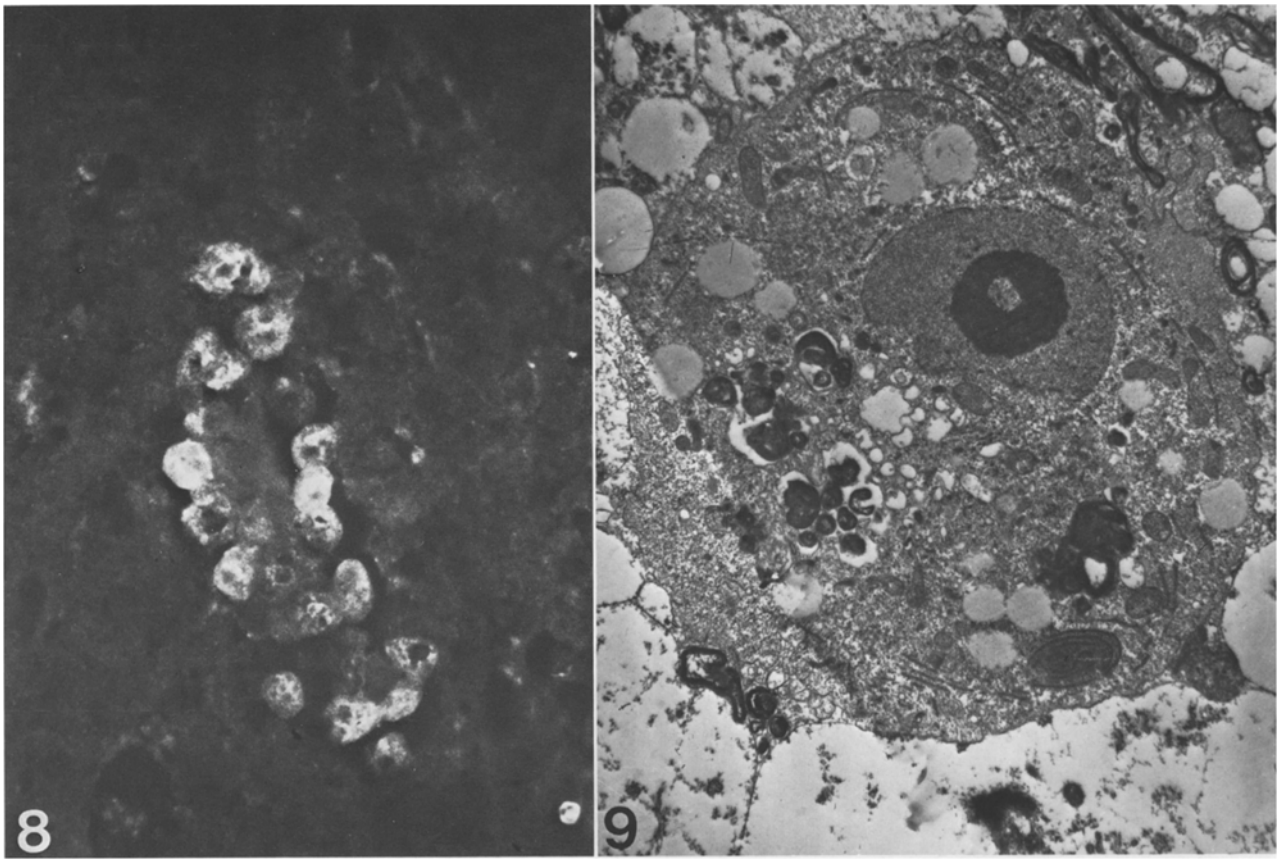



**Fig. 8.** IFAT demonstrating positive fluorescence with *A. culbertsoni* anti-serum. Case 2. ×850

**Fig. 9.** Electron micrograph of a trophozoite of *A. culbertsoni*. The cytoplasm contains numerous "myelin figures", mitochondria, empty vacuoles and stalk of endoplasmic reticulum. Case 2. ×12000

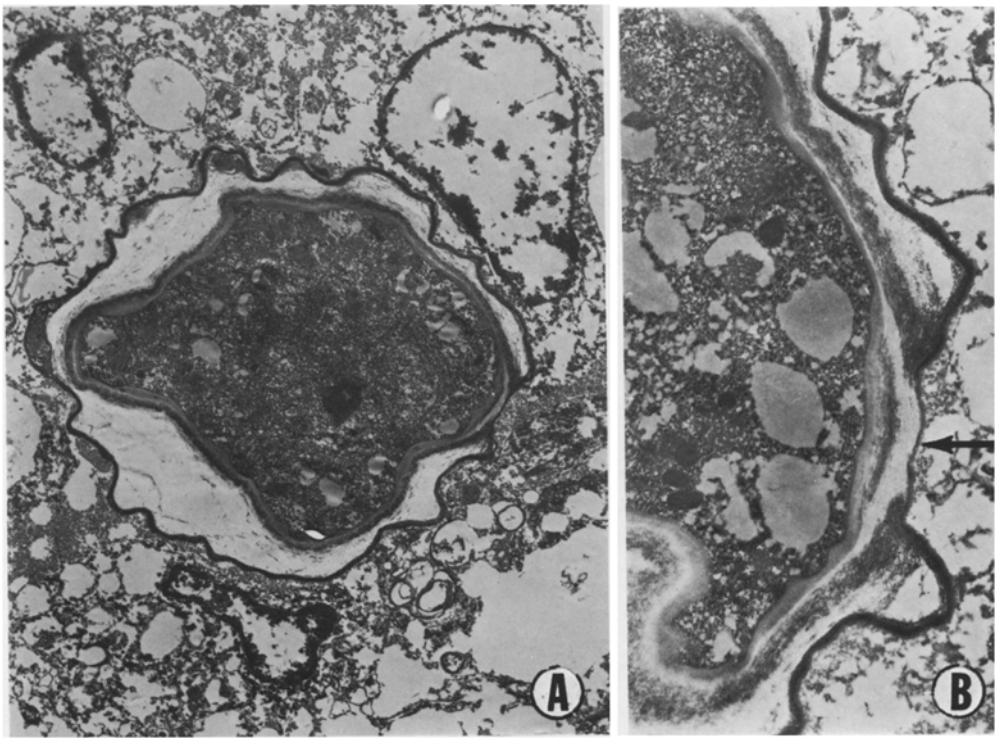



**Fig. 10. A** Mature cyst of *A. culbertsoni* showing a prominent, wrinkled double wall. ×5000. **B** Note the ostiole (arrow) with the distinct circular plug or operculum. Case 2. ×15000

bodies have been demonstrated in human cases. Engulfment of some amebas by phagocytes, the formation of multinucleated giant cells and the close proximity of eosinophils, lymphocytes, plasma cells and phagocytes to the protozoa suggest that some antigen-antibody reaction, a prominent host-immune response or some chemotactic phenomena assist the host cells in their attack upon the protozoa.

Extracellular edema may be prominent near the trophozoites in both types of amebic infection with astrocytic swelling and activation of pericytes and microglia. While this may represent some specific response of brain tissue to the amebas it may well be a nonspecific response to CNS damage.

The positive staining with the *Acanthamoeba* antisera and negative staining with *Naegleria* and *Entamoeba* fit in well with the morphological appearances observed after H&E staining and the presence of wrinkled cysts. However, the morphological characteristics alone do not allow the identification of the species. Our IFAT results allow us to establish that *Acanthamoeba* is the etiological agent. In 2 cases the amebas were identified as belonging to the group *A. castellanii – A. polyphaga – A. rhysodes* and in 1 case *A. culbertsoni*. It is the first identification in human of *A. culbertsoni*, since this species has been established as an animal pathogen by the experimental work of Culbertson et al. [11].

The indirect immunofluorescent staining of CNS sections, fixed in formalin, appears to be a valuable tool in the localization and identification of amebas in the brains of patient who have died from meningoencephalitis.

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**Note Added in Proof**

Since this paper was submitted for publication, another case of "Probably Acanthamoeba Meningoencephalitis in a Korean Child", was reported by J. Ringsted, B. Val Jager, D. Suk and G. S. Visvesvara, in Amer. J. Clin. Path. **66**, 723–730 (1976). The patient sustained a laceration of his left eye with subsequent development of several amebic subcutaneous granulomas and died one year later due to Amebic Meningoencephalitis.