

# Cerebral Anomalies in Congenital Murine Toxoplasmosis: A Preliminary Report

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**Gravid Nya:** NYLAR strain mice were infected with *Toxoplasma gondii* on embryonic day 7. During the remaining 12 days of gestation, considerable fetal wastage occurred. At birth, pups were sickly and growth-stunted, and postnatal mortality was high. After 1 month, surviving weanlings were killed and autopsied. Coronal sections of the cerebrum revealed: numerous cortical and periventricular cystic cavitations, some undergoing calcification; ventricular enlargement and marked periventricular edema; subependymal nodules bulging into the ventricles; loss of subependymal germinal cells; and meningeal and perivascular inflammation. We suggest, as a working hypothesis, that hematogenously transported parasites invaded the vascular endothelium of the fetal cerebrum, inducing endothelial cell activation, recruitment of inflammatory cells, and focal inflammatory lesions in the endothelium. These inflammatory lesions triggered an intravascular coagulopathy, leading to the formation and deposition of microthrombi in cerebral capillaries and the development of numerous infarcts. Hypoxic-ischemic necrosis of the infarcted tissues led to the creation of the cystic cavitations.

**Keywords :** Toxoplasmosis, Congenital, Cerebrum, Anomalies

## INTRODUCTION

Approximately 60 years ago, in a now classical series of papers, Wolf and colleagues [4, 8, 9] published the first detailed observations on the pathology of what was then an obscure parasitic disease afflicting human infants, i.e., congenital toxoplasmosis. Their findings, broadened and extended by other investigators [2, 5, 10], succeeded in establishing congenital toxoplasmosis as a clinically important problem in pediatric medicine. However, despite much progress achieved during the following decades, the pathogenetic mechanisms underlying the ravages of congenital toxoplasmosis still remain poorly defined.

The purpose of the experiments reported herein was to gain insight into the pathogenesis of congenital toxoplasmosis, via the manipulation of a practical and reproducible model of experimental toxoplasmosis in mice [6, 7]. In this brief report, we present some preliminary observations on anomalies developing in the cerebrum of

young weanling mice infected *in utero*, plus speculations concerning the etiology of the cerebral malformations.

## METHODS AND MATERIALS

### **Mice, parasites, and infection procedure**

Nya: NYLAR strain female mice, 18-20 wks of age, were obtained from Griffin Laboratory, New York State Department of Health, Albany, New York. Details concerning the origin, development, and genetic characteristics of the Nya: NYLAR outbred strain have been reported previously [1]. Pairs of NYLAR female mice were cohabited with experienced NYLAR males overnight. The following day, females with vaginal plugs (embryonic day 0) were caged individually. On embryonic day 7, the gravid NYLAR mice were inoculated with brain emulsion containing cysts of the nonlethal Cornell (CS) strain of *T. gondii* [7]. Control mice were inoculated with frozen-thawed aliquots of the same brain emulsion. The procedure for preparing both infective and control brain emulsions have been described

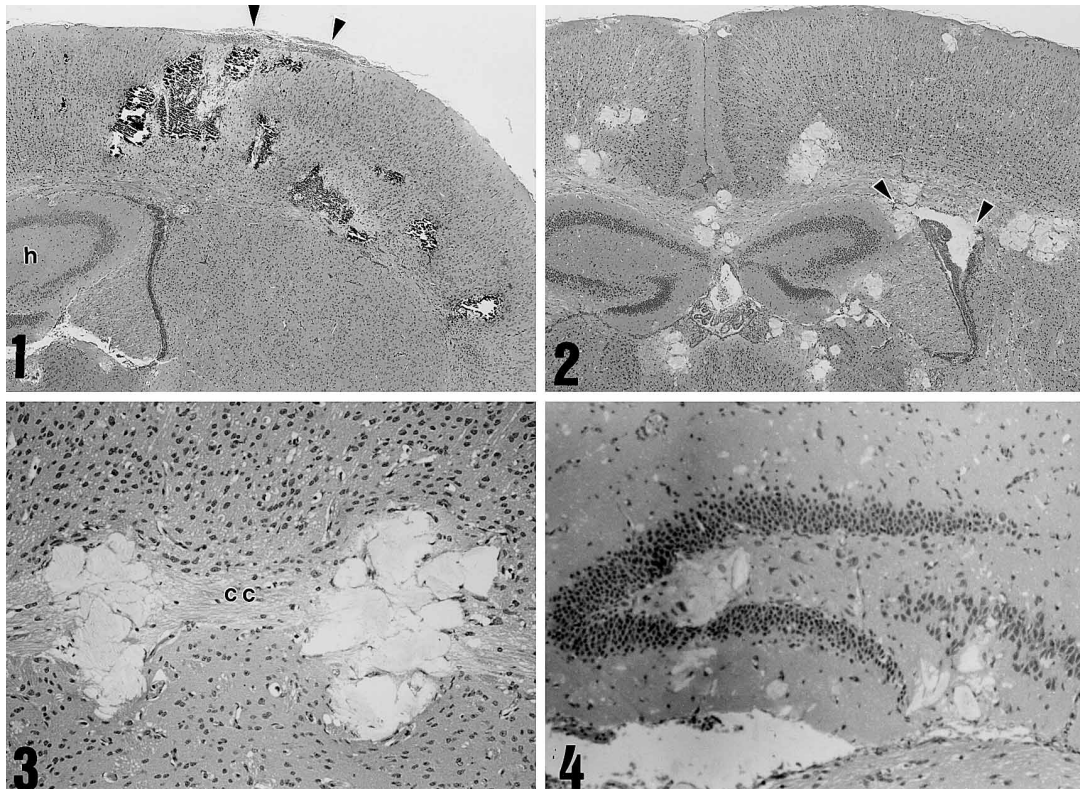
previously [6]. Infected mice received unlimited food and water, and were monitored without further handling to avoid undue stress. Following parturition, it soon became apparent that the infected NYLAR dams were killing the newborn pups. Therefore, to prevent infanticide, the NYLAR litters were quickly exchanged with newborn Balb/c litters. The Balb/c foster mothers proved quite tolerant and accepted the NYLAR litters.

### Necropsy and tissue processing

NYLAR pups surviving the first month of postnatal life were killed and their brains processed by standard histologic procedures. Coronal sections of the cerebrum, cut at 4 microns, were stained with H & E or with PAS. Immunohistochemical procedures for glial fibrillary acidic protein (GFAP) were as described previously [6].

### RESULTS

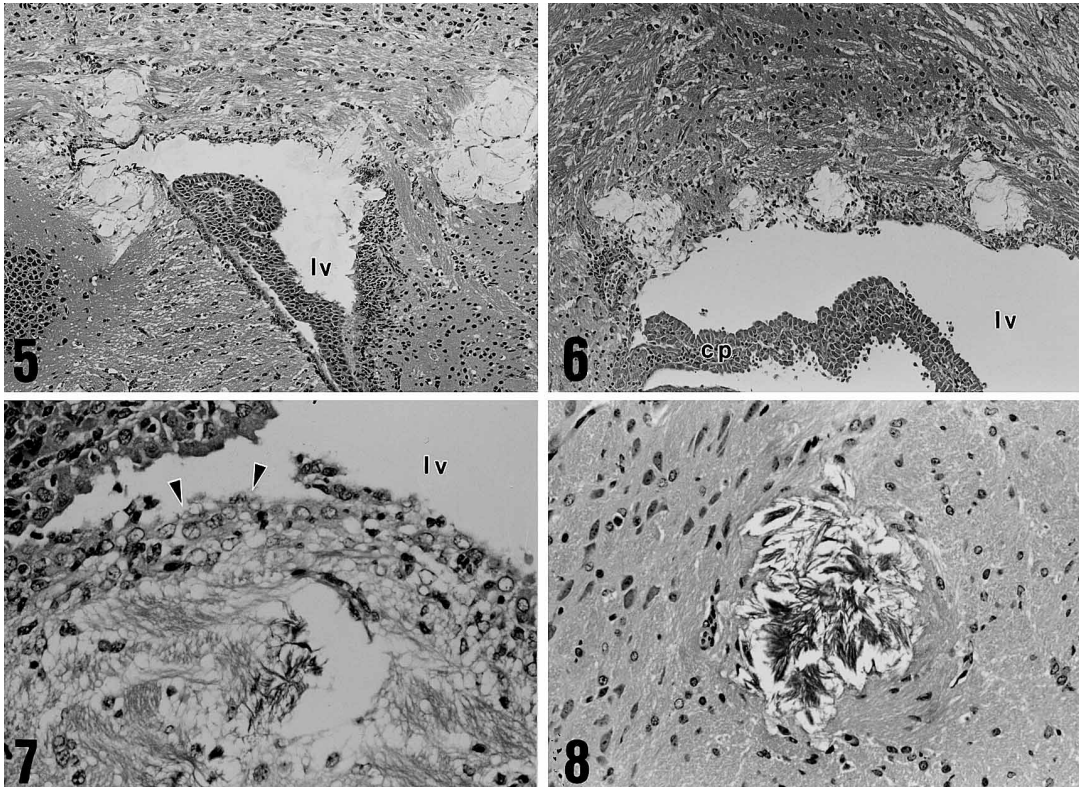
In the pups of control mice, neither parasites nor neuropathologic changes were observed, signifying that the toxoplasmas in the infective brain emulsion did not survive the repeated freeze-thawing. The absence of overt pathology also alleviated concern over possible viral contaminants in the brain emulsion [3]. In contrast, fetal wastage, postnatal morbidity and mortality, neuropathologic changes, and toxoplasma cysts were consistently found in the litters of infected dams, attesting to an infectious process acquired *in utero*. Toxoplasma cysts, either singly or in small clusters, generally were free of inflammation, although some were observed within microabscesses. The most conspicuous anomalies were multiple cystic cavitations and large necrotic calcifying



**Fig. 1** Large calcifying necrotic lesions in cerebral cortex. Note meningeal inflammation (arrowheads). h = hippocampus. H & E;  $\times 40$   
**Fig. 2** Numerous cystic cavitations scattered throughout the cerebrum. Note rupture of dorsolateral angles of the lateral ventricle (arrowheads). H & E;  $\times 40$   
**Fig. 3** Interruption of corpus callosum (cc) by two large cystic cavitations. The cavitations appear lobulated due to the meshwork of trabeculae. H & E;  $\times 100$   
**Fig. 4** Cavitations developing within dentate gyrus of hippocampus. H & E;  $\times 100$

lesions (Fig. 1) developing throughout the gray and white matter of the cerebrum (Fig. 2). Some of the cavities exhibited collagenous trabeculae and a tenuous meshwork of threadlike (glial?) fibrils, but otherwise were largely free of necrotic debris and cells (Fig. 3). Necrosis and depletion of cells in the pyramidal layers of the hippocampus were evident (Fig. 4). The most destructive lesions, however, were located in the periventricular region. The integrity of the lateral ventricles frequently was compromised, with multilocular cavitations destroying extensive segments of the ventricular walls, especially around the angles of the ventricle (Fig. 5). In some instances, the subependymal germinal layer underlying the lateral wall of the ventricle was markedly affected, with gross depletion of germinal cells and formation of subependymal cavitations (Fig. 6, 7).

Periventricular edema was a constant finding, markedly affecting the myelinated fibers of the corpus callosum above the lateral ventricles. The ventricles frequently were enlarged although the enlargement rarely was bilaterally symmetrical. The cystic cavitations varied in size, and were scattered throughout the cerebrum in apparently random fashion. Some cavitations contained sheaves of filariform spicules undergoing mineralization (Fig. 8). Occasionally, large porencephalic-like cystic cavitations were found in the cortex, extending from the roof of a lateral ventricle to the leptomeninges, grossly distorting the corpus callosum and hippocampal architecture. Inflammatory lesions of the vasculature, manifested by perivascular cuffing, hyperemic and swollen venules, endothelial cell swelling, venous stasis, PAS-positive deposits in the walls of the



**Fig. 5** Herniation of angles of lateral ventricle (lv). H & E;  $\times 100$

**Fig. 6** Subependymal cavitations in wall of lateral ventricle (lv). cp = choroid plexus. H & E;  $\times 100$

**Fig. 7** Large calcifying cavitation in wall of lateral ventricle (lv). Note disruption of ependymal layer (arrowheads). H & E;  $\times 200$

**Fig. 8** Calcifying cavitation in thalamus, below lateral ventricle. H & E;  $\times 200$

venules, and meningitis were present in every congenitally infected weanling mouse. Numerous reactive astrocytes, visualized by GFAP immunohistochemistry, were conspicuous around the ventricles, in the hippocampus, and encircling the cystic cavitations. The foot processes of the reactive astrocytes typically were swollen, markedly so in edematous areas and around small blood vessels. However, dense periventricular mats of reactive astrocytes, common in chronically infected adult mice, were not seen in the weanlings.

### DISCUSSION

The nature, severity, and widespread dissemination of the cerebral lesions encountered in the infected mice demonstrate convincingly the pathogenic potential of congenital toxoplasmosis, certainly in mice and, by analogy, in humans as well. Despite the disparity between mouse and man, the brains of both respond in similar fashion to infection with *T. gondii*. Indeed, during a review of the early literature on congenital toxoplasmosis, it became evident that most, if not all, of our present neuropathological findings in mice had been observed years ago, during postmortem examination of congenitally infected human infants [4, 8, 9].

The present study, although still only in a preliminary stage, nevertheless has yielded an impressive array of CNS malformations that permits meaningful speculation. For example, we consider the numerous cystic cavitations as the most significant pathologic finding in the infected mouse cerebrum. With regard to the putative etiology of these cavitations, we suggest a) that widespread microvascular endothelial cell damage led to impairment of normal hemostatic mechanisms, thereby initiating an intravascular coagulopathy and the subsequent formation and deposition of microthrombi in the cerebral vasculature and b) that the cystic cavitations are foci of ischemic coagulation necrosis resulting from infarcts caused by capillary occlusion, rather than from the cytotoxic effects of intracellularly multiplying *T. gondii* tachyzoites. Other observations contributing to our hypothesis are the perivascular cuffings, hyperemic and swollen venules, endothelial cell swelling, venous stasis, PAS-positive deposits in the walls of venules, and

the distinctive microcephalic-hydrocephalic changes present in every congenitally infected weanling mouse managing to survive the first month of postnatal life.

In summation, we postulate that perfusion failure of the developing brain, of both mouse and man, underlies much of the pathologic sequelae of congenital toxoplasmosis. We are well aware that our working hypothesis very likely is an over-simplification of what undoubtedly is a multifaceted and interlocking pathogenic sequence of events. Obviously, validation of our working hypothesis concerning the pathogenesis of congenital toxoplasmosis will require further investigation and substantial experimental evidence.

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### REFERENCES

- 1) Benson LM, Abelseth MK: Investigation of the histocompatibility of the Nya: NYLAR mouse colony by skin grafting. *Lab Anim Sci* 27: 333-335, 1977.
- 2) Frenkel JK, Friedlander S: Toxoplasmosis. Pathology of neonatal disease. Pathogenesis, diagnosis, and treatment. Public Health Service Pub No. 141, U. S. Government Printing Office, Washington, D. C., 1-108, 1951.
- 3) Grimwood BG: Viral contamination of a subline of *Toxoplasma gondii* RH. *Infect Immun* 50: 917-918, 1985.
- 4) Paige BH, Cowen D, Wolf A: Toxoplasmic encephalomyelitis. V. Further observations of infantile toxoplasmosis; intrauterine inception of the disease; visceral manifestations. *Amer J Dis Child* 63: 474-514, 1942.
- 5) Remington JS, McLeod R, Desmonts G: Toxoplasmosis. In *Infectious Diseases of the Fetus and Newborn Infant*, 4th edit., Remington JS, Klein JO (Eds), W B Saunders Co., Philadelphia, 140-267, 1995.
- 6) Stahl W, Kaneda Y: Murine toxoplasmic hydrocephalus. *Parasitol* 114: 219-229, 1997.
- 7) Stahl W, Turek G: Chronic murine toxoplasmosis: clinicopathologic characterization of a progressive wasting syndrome. *Ann Trop Med Parasitol* 82: 35-48, 1988.
- 8) Wolf A, Cowen D: Granulomatous encephalomyelitis due to an encephalitozoon (Encephalitozoic encephalomyelitis). A new protozoan disease of man. *Bull Neurol Inst N. Y.* 6: 306-371, 1937.
- 9) Wolf A, Cowen D, Paige BH: Toxoplasmic encephalomyelitis III. A new case of granulomatous encephalomyelitis due to a protozoan. *Amer J Pathol*

- 15: 657-694, 1939.
- 10) Zuelzer WW: Infantile toxoplasmosis with a report of three new cases, including two in which the patients were identical twins. *Arch Pathol* 38: 1-19, 1944.