cases reported by French and colleagues were of chronic nature. Antibiotics were required for eradication of the organism in all reported cases except one, in which there was an apparently spontaneous cure.

Protoplasmata gondii infection can occur as a complication of subdural tap. The presence of low-grade fever after the procedure suggests the need for subsequent taps and anaerobic cultures of fluid in order to prevent sequelae of infected subdural hematomas, such as communicating hydrocephalus, cavernous sinus thrombosis, venous infarctions, and septicemia.

Immunohistochemical Demonstration of Toxoplasma gondii

TERRY L. ANDRES, M.D., SANDY A. DORMAN, M.D., WASHINGTON C. WINN, JR., M.D., THOMAS D. TRAINER, M.D., AND DANIEL P. PERL, M.D.

Department of Pathology, University of Vermont, College of Medicine, Burlington, Vermont

paraffin-embedded tissue sections. This immunohistochemical method and electron-microscopic evaluation were used to confirm the presence of Toxoplasma gondii in the infected tissues of an immunosuppressed patient who died of severe toxoplasmosis involving the central nervous system, myocardium, and lungs.

Report of a Case

The patient was a 25-year-old man found to have Stage IV Hodgkin's disease. Several courses of chemotherapy were administered; the last course was given 12 days prior to the patient's final hospital admission. At that time, physical examination showed a febrile, chronically ill man with petechiae over the entire body. The spleen and liver were palpably enlarged; there was no significant lymphadenopathy. Initial laboratory values included a leukocyte count of 1,300/cu mm (50% polymorphonuclear leukocytes), platelet count of 21,000/cu mm, and hematocrit of 25%. The patient was maintained on prednisone and allopurinol, and received platelet and erythrocyte transfusions. After a brief period of clinical improvement, he experienced a sudden attack of left-sided weakness with dysphasia and uncontrolled movements of both arms. Lumbar puncture showed normal pressure. The cerebrospinal fluid was grossly xanthochromic, with a protein concentration of 146 mg/dl and glucose concentration of 38 mg/dl (simultaneous peripheral blood glucose level of 94 mg/dl). Only ten erythrocytes and two lymphocytes were found in the fluid. The patient's condition rapidly deteriorated. He became comatose and died 16 days after admission.

Autopsy revealed enlargement of the liver (3,400 g) and spleen (1,600 g), and the presence of enlarged lymph nodes in the anterior mediastinum and abdominal periortic and inguinal regions. The liver, spleen, lymph nodes, and bone marrow were involved by Hodgkin's disease.
The brain weighed 1,600 g. The cerebral hemispheres, brain stem, and cerebellum demonstrated numerous punctate areas of softening and discoloration. Microscopically, the discolored areas were necrotic. Large numbers of macrophages and plasmacytic-lymphocytic perivascular inflammatory infiltrates were present. In these areas there were small, round to ovoid cysts filled with uniformly sized crescentic trophozoites characteristic of *Toxoplasma gondii*. Ultrastructural study confirmed the presence of toxoplasma organisms both within and outside cysts (Fig. 1). Structures characteristic of *Toxoplasma gondii*, such as conoid, paired organelles, dense bodies, and double-layered pellicle were identified.7-8

Both lungs were edematous and heavy (combined weight, 1,575 g). Microscopically, focal necrotic areas contained toxoplasma cysts. The heart, which was enlarged (400 g), contained focal plasmacytic interstitial infiltrates. Toxoplasma cysts were present in the myocardial fibers.

**Materials and Methods**

The peroxidase-antiperoxidase (PAP) method was performed as described by Sternberger and modified by Taylor.15 Sections were made from paraffin-embedded tissues that demonstrated necrosis or inflammation. The sections were deparaffinized and placed in a bath of methanol-peroxide to block endogenous tissue peroxidase activity. Tris-HCl buffer, pH 7.6, 0.05 M, was used throughout the procedure. Commercially available specific toxoplasma antisera prepared in rabbits and fluorescein-labeled (Wellcome Fluorescent Anti-Toxoplasma gondii Lot #K7505) was used as the primary antibody. This reagent is used for identification of *Toxoplasma gondii* in frozen sections of fresh biopsy material by immunofluorescence technics.9-17 Swine antirabbit IgG and soluble immune complexes of rabbit antiperoxidase and peroxidase (PAP reagent) prepared according to the method of Sternberger18 were used in the procedure. Antisera were diluted with Tris-HCl buffer. Optimum results were obtained with the following antibody dilutions: Wellcome Fluorescent...
Anti-Toxoplasma gondii 1:50; swine antirabbit sera 1:20; and PAP reagent 1:100. In the negative control, phosphate-buffered saline solution was substituted for the primary antibody. The tissue sections were exposed sequentially to each of the antisera. Antibody-bound peroxidase was demonstrated by the addition of diaminobenzidine (0.6 mg/dl) in Tris-HCl buffer and 3% hydrogen peroxide. Sections were then counterstained with Harris hematoxylin, dehydrated through alcohols to xylol, and mounted.

Findings

The PAP method stained both cysts and trophozoites dark brown-black (Fig. 2). Focally necrotic neuropile adjacent to the microorganisms stained less intensely. In histologically normal areas of the brain there was no staining or evidence of nonspecific reactivity. The distinct contrast between the dark brown-black staining toxoplasma and the pale-staining tissue background allowed easier recognition of organisms than in sections stained with hematoxylin and eosin or Giemsa stains. The characteristic histologic features of Toxoplasma gondii trophozoites and cysts were preserved with the PAP method. No staining was observed in the absence of specific Toxoplasma antibody.

Discussion

Although toxoplasmosis is a well-documented cause of fulminant infections in patients with compromised immunologic function, frequently the diagnosis is not made ante mortem. In a large series of patients with acquired toxoplasmosis reported by Ruskin and Remington, only about one quarter received specific
antitoxoplasma chemotherapy. The diagnosis is not entertained with sufficient frequency for immunosuppressed patients, and establishing the diagnosis is very difficult. Serologic confirmation of acute infections is hampered by the widespread prevalence of antitoxoplasma antibodies in the normal population. Histologic confirmation is difficult because of the problems of identifying trophozoites and cysts. Additionally, at the light-microscopic level, toxoplasma in cyst form can be confused with protozoa such as Leishmania donovani or Trypanosoma cruzi. Free forms may closely resemble Histoplasma capsulatum and Pneumocystis carinii. However, these organisms are not usually associated with lesions of the central nervous system. The fine structure of Toxoplasma gondii has been well documented, and in selected cases, electron-microscopic examination of biopsy material has established the diagnosis of toxoplasmosis. Characteristic features include conoid, double-layered pellicle, convoluted structures, paired organelles, dense bodies, and the presence of a nucleus at the rounded posterior end of the organism. Although ultrastructural studies may allow definite morphologic identification of these organisms, the expense and limited availability of electron microscopy preclude its use as a general screening test.

An immunohistochemical technic may be a significant aid is establishing the diagnosis of toxoplasmosis. Dark brown-black staining cysts and trophozoites are easily recognized at low light-microscopic magnifications because of the pale background staining of the tissues. The contrast between microorganisms and background tissue is distinctly improved over that of hematoxylin and eosin- or Giemsa-stained sections. The faint staining of necrotic tissues adjacent to the intact toxoplasma is of interest. The possible presence of Toxoplasma gondii-specific antigen within the infected necrotic tissues may also alert the light microscopist to areas requiring close scrutiny. Commercially available antisera are utilized in the peroxidase-antiperoxidase method, which is an increasingly familiar histologic procedure. In contrast to immunofluorescence, a sensitive immunohistochemical technic as a "special stain" in the diagnostic pathology laboratory. The unlabelled antibody peroxidase-anti­peroxidase method preserves histologic detail and reduces the likelihood of error. Until absorption controls are employed, however, positive results should be referred to a reference laboratory for confirmation.

As acquired toxoplasmosis is more frequently recognized in compromised hosts, clinicians may increase attempts to make the diagnosis by histologic methods. An immunohistochemical technic may be advantageous for specific identification of Toxoplasma gondii in necrotic and inflamed tissues.

Acknowledgment. John E. Craighead, M.D., reviewed the article.

References