

CENTRAL NERVOUS SYSTEM VASCULITIS IN CYTOMEGALOVIRUS INFECTION

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SUMMARY

A 51-year-old man received cyclophosphamide, vincristine, procarbazine and prednisone in the treatment of a small-cell undifferentiated lymphoma. Two years later, he developed a rapidly progressive neurological syndrome characterized by a decline in alertness, deafness, blindness and paraplegia. Examination of his eyes revealed severe hemorrhagic chorioretinitis. Leg weakness was thought to be due to transverse myelopathy at a thoracic level. He had a grand mal convulsion and died from terminal bronchopneumonia.

Autopsy examination of the eyes revealed sweeping destruction of the retina due to inclusion body chorioretinitis. The brain and spinal cord showed multiple small infarcts accounting for the deafness and paraplegia. The lesions were due to occlusive arteritis in gray and white matter. Veins were also involved.

Tissue surrounding the foci of necrosis contained cells with intranuclear and intracytoplasmic inclusion bodies. Some of the Cowdry type A inclusion bodies were large, measuring 30 μm in diameter and were located in enlarged cells.

Electron microscopy of retina and brain tissue disclosed virus particles compatible with cytomegalovirus.

The subject of cerebral and ocular angiitis due to herpes virus infections is reviewed.

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INTRODUCTION

Chemotherapy of malignant neoplasms and anti-rejection therapy after organ transplantation have a now well recognized side effect, "opportunistic infection". Immunosuppression is deliberately sought in organ recipients and is an undesirable but often inescapable effect of cancer therapy with cytotoxic drugs. "Opportunistic" describes infections that are relatively uncommon in previously healthy persons. It implies that decreased cellular and humoral immunity pave the way for infection. The central nervous system (CNS) is not exempt and is especially vulnerable to infection by fungi such as *Cryptococcus neoformans*, *Aspergillus sp.* and *Candida sp.*, and by *papovavirus*, *Toxoplasma gondii* and *Listeria monocytogenes* in immunosuppressed patients. Among the herpes viruses, the cytomegalovirus (CMV) is a relatively common organism found in systemic opportunistic infections but CMV encephalitis is unusual (Schneck 1965; Dorfman 1973). Immunosuppression may also invite infection of the CNS by varicella-zoster (McCormick et al. 1969; Hotson and Pedley 1976) and measles viruses (Coulter et al. 1979). Atypical herpes simplex encephalitis is known from 4 cases where chemotherapy or anti-rejection drugs were used (Price et al. 1973; Hotson and Pedley 1976). Herpes simplex type 1 encephalitis with most unusual clinical and neuropathological features occurred in a patient on chemotherapy for Hodgkin's disease (Price et al. 1973). Encephalitis also affected 3 cardiac transplant recipients who had disseminated (cutaneous and/or pulmonary) herpes simplex infections (Hotson and Pedley 1976). Instead of the usual necrosis and inflammatory response, their brain tissue revealed mostly microglial nodules. An intranuclear inclusion was present in one case and herpes simplex virus was isolated from the brain of another. Presumably all viruses of the herpes group should be counted among opportunistic viruses with potential for CNS infection.

Infections by the described viruses may occur by new entry or by reactivation of latent infection. Whatever the mechanism, most opportunistic infections of the CNS carry a grave prognosis.

Though postnatal CMV infection is extremely common, as determined by a steady age-related increase of persons with CMV-antibodies (Stern and Elek 1965; Weller 1971), spontaneous clinically apparent CMV infection of the CNS is uncommon in healthy adults. In the CNS, infection presents as an encephalitis or a meningoencephalitis (Signer 1962; Sterner et al. 1970; Perham et al. 1971; Chin et al. 1973; Bäck et al. 1977; Phillips et al. 1977; Duchowny et al. 1979), in the peripheral nervous system occasionally as a Guillain-Barré syndrome (Klemola et al. 1967). The prognosis in cases without prior immunosuppression would appear quite favorable, if one judges from pertinent publications. In contrast, immunologically suppressed and/or debilitated adults may have overwhelming CNS infections that are rapidly fatal.

We wish to report a patient who received chemotherapy for 2 years prior to CMV infection of the brain. The infection was restricted to the CNS and the eyes and was associated with vasculitis. Cerebral symptoms were due primarily to

infarction rather than to necrotizing encephalitis. To our knowledge, this tissue reaction has not been recognized heretofore in CMV infection of the CNS though it has been reported in association with herpes zoster (Rosenblum and Hadfield 1972; Rosenblum et al. 1978; Linnemann and Alvira 1980).

CASE REPORT

In January 1977, at age 51, the patient experienced shortness of breath that worsened over a 2-week-period. On admission to hospital, physical examination and chest X-ray confirmed a large left pleural effusion. Thoracentesis produced a milky fluid that was acellular and contained a total protein of 5.5 g/100 ml, and a glucose of 100 mg/100 ml. It was sterile. After transfer to another hospital he was found to have a palpable abdominal mass. An upper gastrointestinal barium X-ray series, an intravenous pyelogram, ultrasound examination and gallium scan pointed to a retroperitoneal tumor. It was biopsied at the time of laparotomy and found to be a nodular small-cell undifferentiated lymphoma. A bone marrow biopsy disclosed focal infiltration by abnormal lymphocytes.

Several courses of chemotherapy were given and medications included cyclophosphamide, vincristine, procarbazine and prednisone. He suffered from recurrent pleural effusions that required thoracentesis. Pneumothorax occurred on one occasion and necessitated underwater seal drainage.

He did reasonably well until October 1979, when he was dizzy and unsteady on his feet. Acute right-sided otitis media was diagnosed and he received oral ampicillin. Discomfort in the ear subsided but he complained of progressive hearing loss and failing vision in the right eye. Shortly after admission to hospital the right leg became weak and there was also urinary retention.

Neurological examination showed that the hearing defect could not be attributed to conduction loss but rather to a bilateral eighth nerve lesion of unknown etiology. Visual acuity in the right eye was greatly reduced and the optic disc appeared pale. There was weakness of the legs that was more pronounced on the right side. The muscle stretch reflexes of the lower extremities were abolished and there were bilateral Babinski signs. He had no sensory loss.

Laboratory examinations

A complete blood count was as follows: white blood cells, 8,600/mm³; red blood cells, 3.61 × 10⁶/mm³; hemoglobin 11.2 g/100 ml; Hct, 33.1%; mean corpuscular volume 91 μm³; mean corpuscular hemoglobin concentration 34 g/100 ml; platelets 220,000/mm³; white blood cell differential was 78% polymorphnuclears, 5% bands, 11% lymphocytes, 6% monocytes. Several determinations of blood electrolytes, blood urea nitrogen, uric acid, serum glutamate oxalacetate transaminase, serum glutamate pyruvate transaminase, creatine phosphokinase, total protein, albumin, globulin, calcium, phosphorus, bilirubin, and creatinine were normal. Activities of alkaline phosphatase and lactate dehydrogenase were mildly elevated with respective levels of 120 IU and 245 IU (normal values for alkaline phosphatase, 30–85 IU; for lactate dehydrogenase, 100–200 IU).

The CSF was examined on 3 separate occasions. It was always clear and colorless and under normal pressure. The first specimen contained 60 atypical lymphocytes/mm³, a total protein of 135 mg/100 ml, glucose 55 mg/100 ml. Cultures were negative. Subsequent lumbar punctures revealed similar values for protein and glucose but a decline of the white cell count to 16 and 10/mm³.

Radiographs of the skull revealed only a small osteoma of the left frontal sinus. A CAT scan of the head and a radionuclide brain scan were normal. An audiogram confirmed profound bilateral neurosensory hearing loss.

Skin tests for coccidioidomycosis, candida and protein purified derivative were negative but reactive to a streptokinase-streptodornase preparation.

Hospital course

The abnormal lymphocytes in the CSF were interpreted as evidence of metastatic lymphoma of the central nervous system. The patient received a course of cranial radiation (total dose 3400 rads; duration, 21 days) and 3 intrathecal injections of methotrexate (15 mg) and cortisol (30 mg). There was no improvement in his condition. He became totally blind and deaf but was able to speak. He read letters and numbers written on his palm and recognized large preformed and shaped letters of the alphabet. His bladder was entirely paralyzed and an indwelling catheter was necessary.

He was transferred to the Veterans Administration Medical Center, Albany, NY, for terminal

care. The possibility of a treatable opportunistic infection was considered and additional examinations and laboratory tests followed.

Examining neurologists thought that his mental status had deteriorated though blindness and deafness made evaluation of this difficult. He no longer spoke or took an interest in offered letters. There was no vision and no pupillary light reaction. The fundi revealed widespread hemorrhagic chorioretinitis with patches of pale atrophic and swollen regions. The optic discs were pale. He had weak arms and flaccid paraplegia, absent leg reflexes and prominent Babinski signs. A sensory level at D₄ was suspected.

A complete blood count was unchanged but the technician remarked on the presence of Döhle inclusion bodies. The following levels of immunoglobulins were obtained (in mg/100 ml): IgA, 22.1 (normal: 60–350); IgM, 63.9 (normal: 40–260); IgG, 230 (normal: 620–1400). *Toxoplasma gondii* serology was non-reactive, complement fixation antibodies to cytomegalovirus was present in a titer of 1 : 16 (low). A second CAT scan was again normal but a further EEG record revealed severe diffuse high amplitude slow waves.

The patient suffered a grand mal seizure and remained comatose afterwards. He died with pneumonia on January 14, 1980, after approximately 3 years of illness.

General pathological findings

The immediate cause of death was bilateral severe bronchopneumonia. There was mild atherosclerosis of the coronary arteries. Scattered collections of abnormal lymphocytes in the bone marrow were the only indication of residual lymphoma. Lymphnodes and spleen showed no neoplastic tissue.

Eyes, brain and spinal cord were removed at autopsy and fixed in formaldehyde solution.

Pathological observations on the eyes

Both eyes were equally affected. After sectioning the globe in the horizontal plane, massive pigment dispersion in vitreous and retina was apparent. The macula revealed white patches.

Microscopic examination of the retina showed sweeping destruction so that only remnants were recognizable (Fig. 1). Bruch's membrane was nearly denuded of normal pigment epithelial cells. Retinal pigment was located either in detached pigment epithelial cells or within macrophages. Eosinophilic intranuclear inclusion bodies were present in occasional retinal cells and occupied about 50% of the nuclear volume. A clear halo surrounded the inclusions and separated them from the internal surface of the nuclear membrane. The choroid was slightly edematous and contained patchy inflammatory cells, mostly lymphocytes and plasma cells. The sclera was unremarkable.

The optic nerves were totally necrotic. Their entire cross section was occupied by large macrophages that contained myelin debris retaining the staining properties of normal myelin (Luxol Fast Blue–periodic acid, Schiff). Bodian stains revealed total axonal loss. The ophthalmic artery on one side revealed arteritic changes (Fig. 2). The arterial lesions were similar to those found in the brain and a detailed description follows below.

Samples of formaldehyde-fixed tissue from retina and choroid were prepared for electron microscopy by standard methods. Virus particles were abundant. Though preservation was poor due to delayed fixation and autolysis, nuclear membranes were retained and most virus particles were located within nuclei. They measured

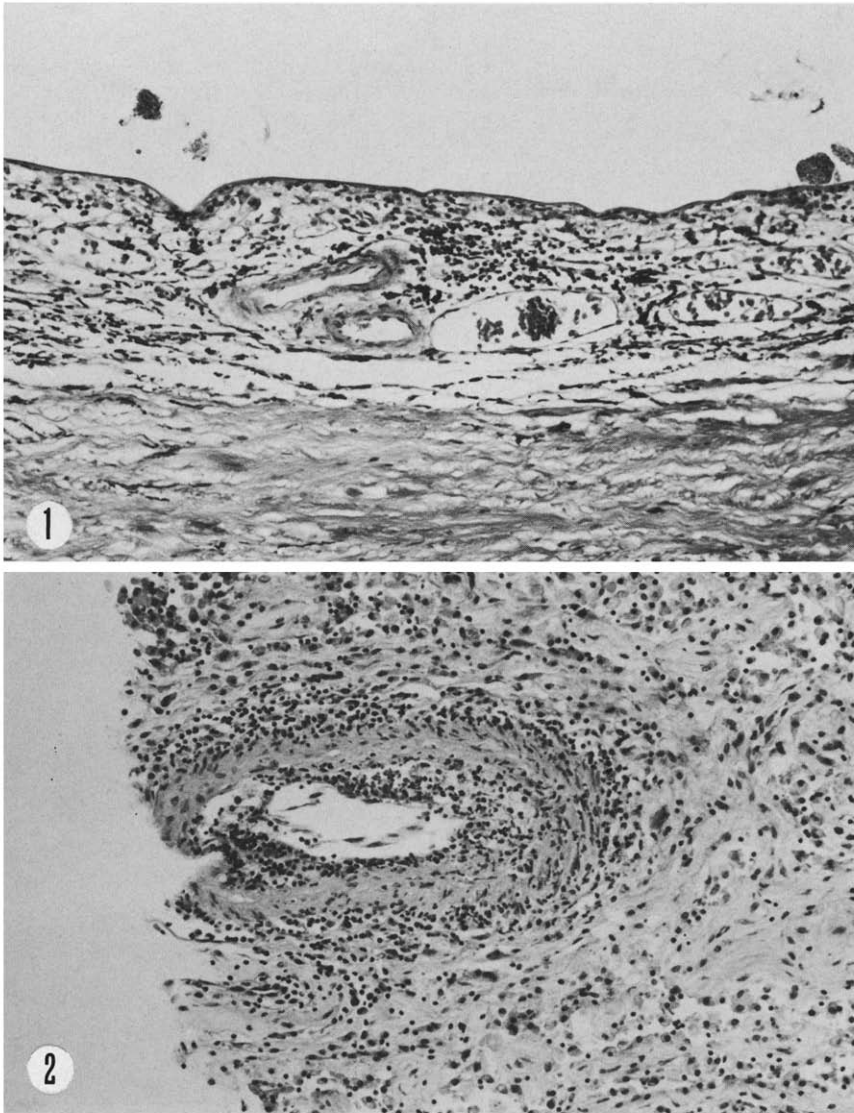
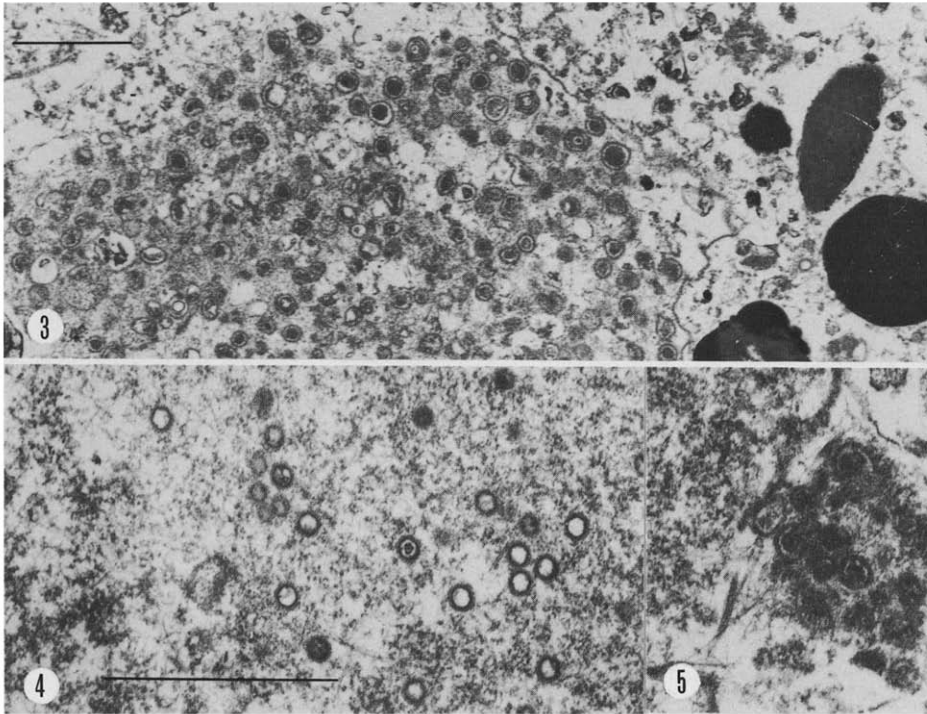


Fig. 1. Retina and choroid. The normal structure of the retina is no longer recognizable. The relatively hypocellular tissue on the bottom is the sclera. Residual retinal and choroidal tissue is infiltrated by inflammatory cells and pigmented phagocytes. H&E, original magnification, $\times 40$.

Fig. 2. Ophthalmic artery. The entire arterial wall is infiltrated by inflammatory cells. The parenchyma of the nerve surrounding the inflamed vessel is necrotic and macrophages are the predominant cell type. H&E, original magnification, $\times 40$.

100–120 nm in diameter and many had a distinct hexagonal shape. Many nucleocapsids had cores of variable size, configuration and density (Figs. 3–5). Transformation of the nuclear membrane was observed in several locations and complete virions of high electron density with an average diameter of 150 nm were present



Figs. 3-5. Retina and choroid.

Fig. 3 illustrates a disrupted pigment-containing cell of retina or choroid with innumerable nucleocapsids. Most virus particles contain an electron-dense core of variable size. Bar represents 1000 nm. Electron microscopy, $\times 21,200$.

Fig. 4 shows an intranuclear collection of virus particles, predominantly empty nucleocapsids.

Fig. 5 shows an intracytoplasmic collection of complete virions with a dense outer coat, derived from the nuclear membrane (to left).

Magnifications of Fig. 4 and 5 are identical. Bar represents 1000 nm. Electron microscopy, $\times 41,350$.

in the cytoplasm (Figs. 3-5). Collections of cytoplasmic virions with a surrounding membrane were not seen (Donnellan et al. 1966).

Neuropathological observations

The weight of the unfixed brain was 1310 g. Examination after fixation revealed total destruction of the optic nerves, the chiasm and the optic tracts. Serial coronal sections of the cerebrum revealed focal damage to the lateral geniculate bodies and their neighboring tissues. The lateral geniculate bodies were no longer recognizable. The brainstem at the level of the pontomedullary junction showed cystic necrosis. The lesions lay near the entry zone of the eighth nerves and occupied the region of the dorsal cochlear nuclei and extended into the ventral cochlear and lateral vestibular nuclei.

The cervical spinal cord was not affected. At midthoracic levels, the left lateral fasciculus revealed a cystic myelomalacia that involved the dorsal horn and extended

through almost all thoracic segments. The lumbosacral spinal cord showed a similar gross lesion in the right dorsal column.

Microscopic examination

The optic nerve, chiasm and tracts were devoid of normal myelin sheaths and axons. The tissue was replaced by large macrophages containing cellular constituents that still gave the staining of myelin with appropriate stains. There were no inflammatory cells in the substance of these tracts. Anterior and posterior pituitary tissue were sampled with the optic chiasm. The posterior pituitary contained a focus of plasma cells, lymphocytes and histiocytes.

Right and left lateral geniculate bodies were replaced by foci of encephalomalacia. The main necrotic focus was surrounded by smaller satellites of softening (Fig. 6). Normal tissue was replaced by macrophages but astrocytes were quite sparse in the relatively preserved neighboring brain tissue. Numerous corpora amylacea had been phagocytized by macrophages. Several small infarcts occupied the region of the dorsal cochlear, ventral cochlear and lateral vestibular nuclei on both sides. Additional smaller foci of ischemic necrosis were present in the pontine tegmentum and the brachium pontis on the left.

At the midthoracic level of the spinal cord, the left lateral funiculus was frankly necrotic (Fig. 7). The lesion contained numerous swollen axons but there were neither inflammatory cells nor macrophages.

In the right dorsal column of the lumbar cord, a similar lesion of slightly older age was present. Numerous macrophages and polymorphonuclear leukocytes were present.

Inclusion bodies

Cowdry type A intranuclear and intracytoplasmic inclusion bodies were very abundant at the edges of the lesions (Figs. 8–13). Some were as large as 30 μm in diameter and brightly eosinophilic. They occupied mostly glial nuclei though some cells had the triangular outlines of neurons. In some, the host cell was no longer recognizable. Margination of nuclear chromatin was commonly seen even if no inclusion bodies were present. Intracytoplasmic inclusion bodies, measuring about 5 μm in diameter, were more basophilic than intranuclear inclusions (Fig. 13). Most intranuclear inclusions were surrounded by a clear halo unless they occupied the nucleus totally (Fig. 12).

Vasculitis

Extensive sampling revealed lesions of vasculitis in the posterior pituitary, in the depth of hemispheric sulci, in the interpeduncular fossa, in the ambient cistern, and on the dorsal aspects of the thoracic spinal cord (Figs. 14–17). Most foci of encephalomalacia revealed at least one neighboring arterial profile with arteritis. In the interpeduncular cistern, parenchymatous lesions were absent though several affected vessels were present in the same section.

Arteries and veins of medium and small caliber were involved, veins with

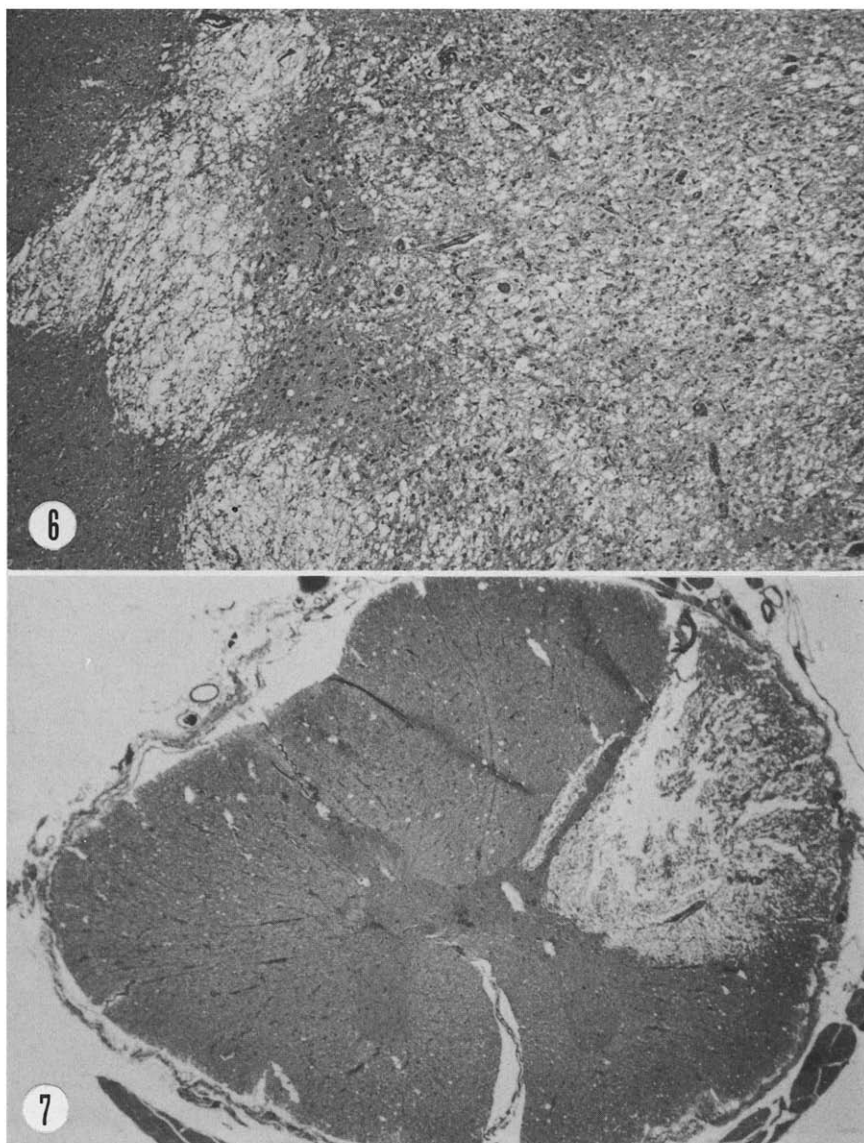
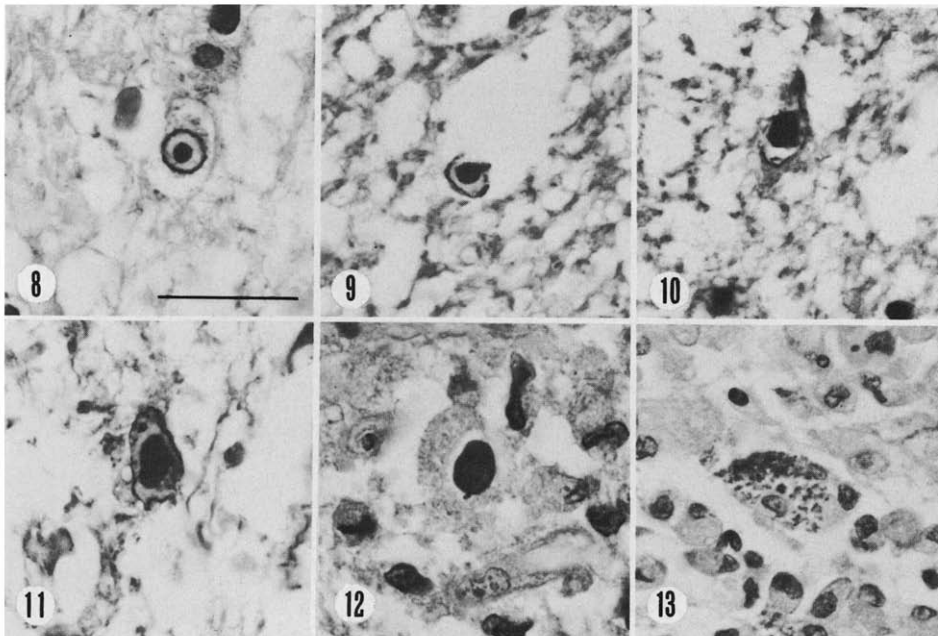


Fig. 6. Lateral geniculate body. The lesion consists of confluent foci of necrotic tissue. Intranuclear inclusion bodies are most abundant near the edges. H&E, original magnification, $\times 10$.

Fig. 7. Thoracic spinal cord. A focus of necrosis occupies the left anterolateral funiculus and dorsal horn. An occluded artery of the pial arterial plexus was found nearby. H&E, original magnification, $\times 4$.

lesser severity. The entire circumference of the vessel wall was generally involved but longitudinal involvement was quite variable. On serial sections, made for special stains (phosphotungstic acid-hematoxylin for fibrinoid necrosis and smooth muscle cells; elastica), arterial and venous lesions rapidly disappeared implying nodular involvement.



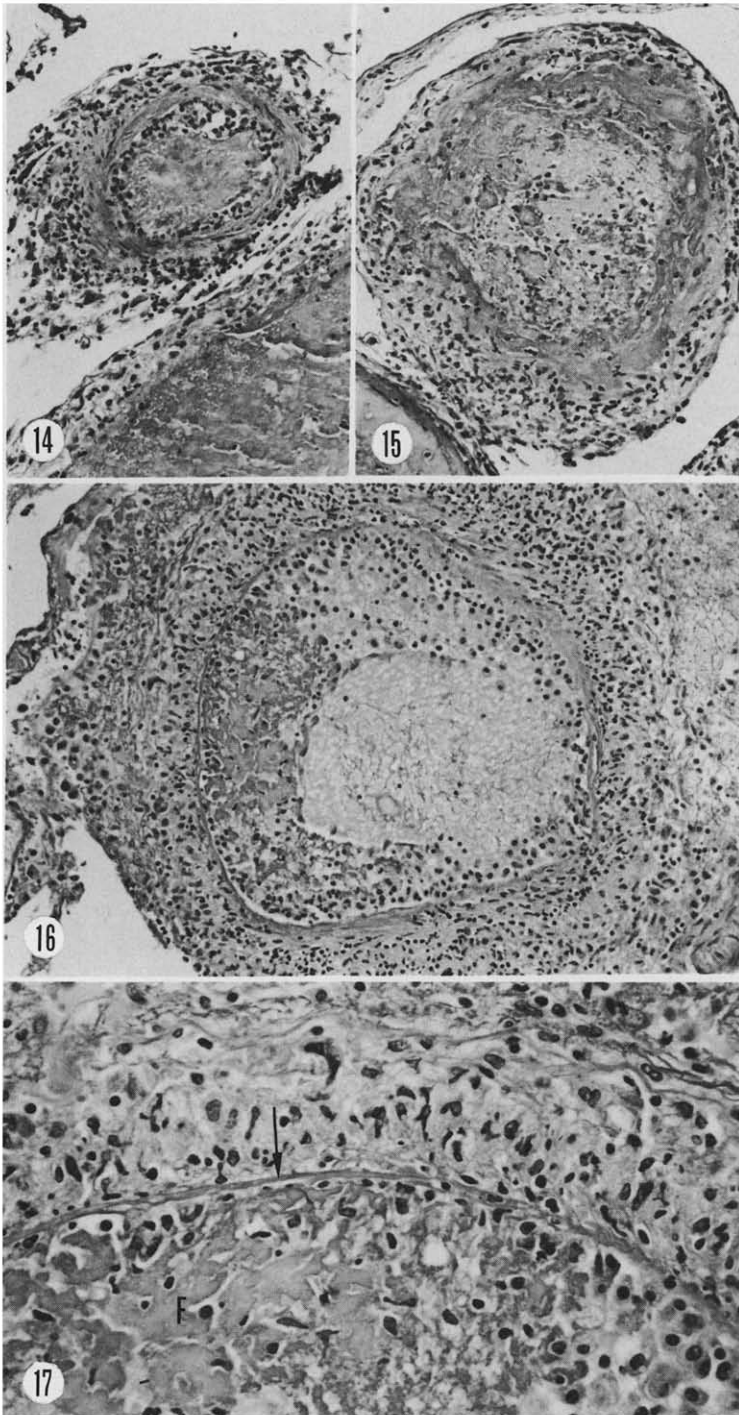
Figs. 8–13. Inclusion bodies.

Figs. 8–12 show intranuclear Cowdry type A inclusion bodies. The cell types are not identifiable with certainty. A glial origin is likely for the inclusions in Figs. 8, 9, and 11. Other cell types (neurons?) may be involved in Figs. 11 and 12.

Fig. 13. Intracytoplasmic basophilic inclusion bodies of much smaller size are present. The cell resembles a pseudocyst of *Toxoplasma gondii*. Magnification is identical for all figures, bar represents 100 μm . H&E, original magnification, $\times 225$ (oil immersion).

The endothelial lining of the arteries was disrupted and remaining endothelial cells were swollen. The intima showed focal collections of phagocytes that lifted the endothelial covering off the elastica interna and produced a bulge into the lumen of the vessel. Occasionally the entire endothelium was denuded and replaced by a layer of fibrin.

The elastica interna was fragmented in many arteries and fibrin deposition was common in its place. In the media, smooth muscle cells were disrupted by inflammatory cells. Inflammatory infiltration was most intense in the adventitia but occasionally all layers were infiltrated. In the infiltrate, young polymorphnuclear leukocytes predominated, and lymphocytes and histiocytes were rare. Only one giant cell was found. Fibroblastic proliferation was present in some arterial walls but fully healed arteritis was absent. Most affected arteries appeared to have the same stage of involvement implying comparable duration of the process. Fibrinoid necrosis and fibrin deposition were difficult to distinguish in major lesions. In some vessels, fibrin was found as a lining of the luminal surface where endothelium had disappeared. Some vessels revealed large globular deposits of fibrin in their walls (Figs. 16 and 17). Very large collections penetrated the endothelium and projected



Figs. 14–17. Arteritic lesions.

Fig. 14. A pituitary artery reveals inflammatory infiltration of its wall but relative preservation of wall structures. The lumen contains red blood cells and appears patent. H&E, original magnification, $\times 40$.

Fig. 15. The artery shows serious disruption of its wall, inflammatory infiltration, fibrin deposition and occlusion of its lumen by fibrin and cellular debris.

Fig. 16. The artery shows dense inflammatory infiltration of its wall, retention of the elastica interna and focal fibrin deposition in the intima. H&E, original magnification, $\times 40$.

Fig. 17 is a higher power photograph of the same artery to illustrate the elastica (arrow) and the fibrin deposit (F). The predominant inflammatory cell in the wall structures is the polymorphnuclear leukocyte. H&E, original magnification, $\times 100$.

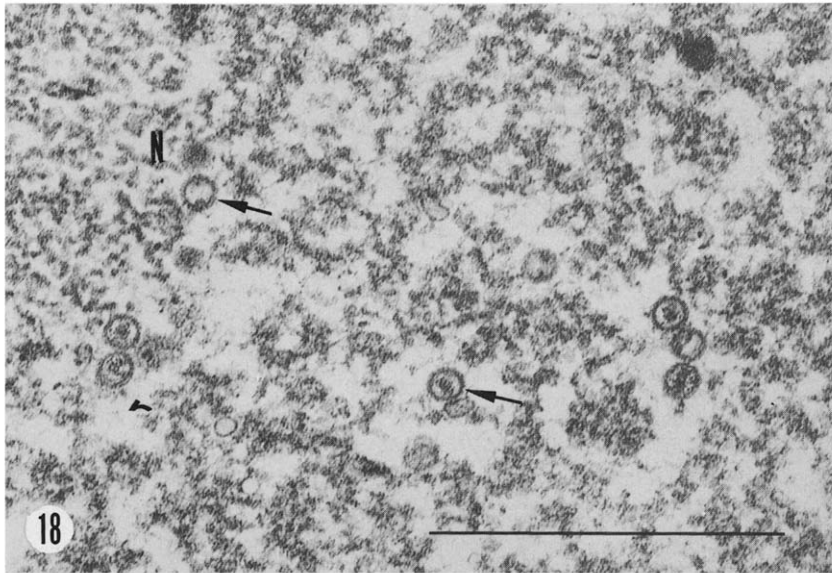


Fig. 18. Brain tissue near necrotic focus in left lateral geniculate body. The arrows indicate examples of nucleocapsids with and without established core. The nuclear membrane (N) is to the left and the bar represents 1000 nm. Electron microscopy, $\times 46,800$.

into the lumen. In the most dramatic examples, the entire wall was occupied by fibrin and occlusion of the lumen by a fibrin thrombus had taken place.

Veins also showed some fibrin deposits in their walls. Inflammatory infiltration was less severe than in arteries and restricted to the adventitia.

Electron microscopy

Virus particles were much less abundant than in the retina and choroid. Intranuclear particles were of similar size (100–120 nm) and contained occasional cores (Fig. 18). There were no convincing examples of cytoplasmic virions and no specific transformations of the nuclear envelope.

DISCUSSION

Clinical features

Progressive CNS symptoms and signs in our patient and abnormal lymphocytes in the CSF suggested lymphoma, and intrathecal chemotherapy followed. The overwhelming retinopathy could have supported the diagnosis of CMV infection (Smith 1964; De Venecia et al. 1971; Wyhinny et al. 1973; Hotson and Pedley 1976), but the low level of antibody against CMV in the patient's blood after several weeks of retinopathy militated against such an infection. In retrospect, the lack of antibody rise can perhaps be explained by the restriction of the infection to eyes, pituitary and brain and by his immunosuppressed state. Since no lesions of cytomegalovirus

disease were found in extracerebral organs, reactivation of latent cerebral infection may be considered a distinct possibility. A recommendation can be derived from experience with this patient. Spinal fluid cells should perhaps be examined with the electron microscope where lymphomatous involvement of the CNS is considered. The success of antiviral therapy in some cases with CMV encephalitis, albeit in immunologically intact patients, makes similar treatment in immunosuppressed patients at least worthwhile.

Ocular pathology

CMV chorioretinitis in adults is uncommon. In a review of 54 cases up to 1962 of adult CMV disease, Wong and Warner (1968) did not report CMV infection of eyes or brain. The lesions in the eyes of our patient are more serious but entirely comparable to the earlier 17 cases summarized by Murray et al. (1977). An additional case following cardiac transplantation was reported by Hotson and Pedley (1976). Otherwise the underlying conditions were renal transplantation in 14 patients, Hodgkin's disease in one (Smith 1964) and carcinoma of the breast in another (Murray et al. 1977). In one patient an underlying disease was not stated but all others received immunosuppressive drugs. Electron-microscopic examination of the retina was performed in 2 earlier cases (De Venecia et al. 1964; Wyhinny et al. 1973).

Retina and choroid in our patient did not reveal vasculitis but the optic nerve was infarcted due to arteritis of the ophthalmic artery (Fig. 2). The appearance of the retina and choroid together with the abundance of virus on electron microscopy is perhaps the strongest argument that the lesion of eye *and* brain was due to CMV infection. Choroiditis as a complication of herpes zoster ophthalmicus may occasionally show granulomatous giant cell arteritis of the posterior ciliary arteries (Naumann et al. 1968). This observation is of greater interest if one considers the vasculitic lesions of the brain in our case.

Herpes simplex virus may produce a fulminating retinitis with or without simultaneous encephalitis (Minckler et al. 1976). In the described case the clinical course was quite dramatic and chorioretinitis was more acute than in our case. Electron microscopy revealed abundant herpes simplex virus.

Neuropathology

In previous cases of CMV infection of the CNS in adults, where post mortem examination was possible, microglial nodules were a cardinal feature while CMV inclusion bodies were either sparse or could not be found at all. In the absence of typical inclusions the diagnosis has to be based on either serological evidence or the recovery of virus by infecting tissue cultures.

The case of simultaneous infection by herpes simplex virus and CMV reported by Yanagisawa et al. (1975) must be viewed as an exception, and it is not certain which of the morphological features in this case of encephalitis can be attributed to one or the other virus.

Our case differed greatly from the described patients with CMV encephalitis in several points: (a) arteritis; (b) abundance of inclusion bodies; (c) infarctions due to arterial occlusion. Inflammatory cells were not abundant in the brain parenchyma but were present in the posterior pituitary. Intranuclear inclusion bodies were found in large numbers about the necrotic regions but only unconvincing examples were seen in endothelial cells. Intracytoplasmic inclusions were frequent (Fig. 13).

Investigation of this case poses two questions: Is the identity of CMV proved? Did CMV produce arteritis?

Since electron microscopy cannot reliably distinguish between the viruses of the herpes group there is only circumstantial evidence that CMV was indeed responsible for the lesion. The inclusion bodies were frequently quite large, intracytoplasmic inclusions were present and sweeping viral chorioretinitis make CMV the likely if not established agent.

Simultaneous infection with another organism, such as aspergillus had to be considered because arteritis is a feature of such infection, and multiple cerebral infections in immunosuppressed persons are well known (ZuRhein and Varakis 1974; Mathews et al. 1976). Special stains for fungi were negative and most arteritic lesions did not reveal the severe necrosis that is characteristic of, for example, aspergillosis. In contrast to herpes simplex virus, immunohistochemical demonstration of CMV in fixed tissues proved unsatisfactory and antibodies against human CMV for immunocytochemistry are difficult to raise in experimental animals. An effort was made to demonstrate virus with the method recently described by Swoveland and Johnson (1979). The technique involves deparaffinization and rehydration of tissue sections, incubation with trypsin and indirect immunofluorescent staining of viral antigens. After inspection of the sections with the fluorescence microscope, the cover slips can be removed and ordinary cell stains can be applied. In this fashion, the fluorescent tissue constituents can be identified with precision and the viral antigen can be localized to nuclei and/or cytoplasm. Human serum with a high titer against CMV was obtained from the Virology Laboratory of the New York State Department of Health. After removal of paraffin and rehydration of the sections, the tissue was incubated with trypsin as described by Swoveland and Johnson (1979). After washing, the sections were treated with the serum containing antibodies against CMV. Under the assumption that IgA, IgM and IgG of the serum would be fixed to viral antigen in the section, the further procedure involved their visualization by the described method. An alternate method is the demonstration of IgA, IgM and IgG by immunoperoxidase methods. Neither procedure revealed CMV antigen. Subsequent staining of the sections processed for fluorescence microscopy showed abundant intranuclear inclusion bodies so that virus should have been revealed if the antigen had remained active.

Cytomegalovirus in infected tissue cultures can be demonstrated by related methods. Ultimately, these established procedures may be of value for fixed or unfixed frozen sections of brain and other organs.

It is possible that CMV antigenicity is lost by fixation in formaldehyde

solutions, prolonged storage, paraffin embedding and the various other steps involved in tissue processing.

We do not believe that the arteritis was part of this patient's lymphoma as was described by Rewcastle and Tom (1962) and Sams et al. (1968). In their cases, granulomatous arteritis was present throughout the entire course of the illness. Our patient had a short progressive course.

Arteritis in association with recent and remote herpes zoster has been known for many years (Feyrter 1954). Granulomatous angiitis of the CNS also has occurred after herpes zoster infection of the skin (Rosenblum and Hadfield 1972; Rosenblum et al. 1978; Linnemann and Alvira 1980). Particles compatible with herpes virus have been seen by electron microscopy in granulomatous angiitis of the CNS, with and without previous varicella-zoster infection (Reyes et al. 1976; Linnemann and Alvira 1980). The observations by Linnemann and Alvira (1980) (generalized varicella-zoster) lend weight to the interpretation that virus is directly responsible for the arteritis because these workers demonstrated virus particles in the affected arterial wall. We located arteritic lesions by light microscopy and then sampled the matching paraffin block for electron microscopy but could not demonstrate virus in cells of the affected arterial wall. Preservation was inadequate.

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Addenda

Since completion of the manuscript, two articles on cytomegalovirus retinitis in immunosuppressed hosts were published. They are:

- Pollard, R. B., P. R. Egbert, J. G. Gallagher and T. C. Merigan (1980) Cytomegalovirus retinitis in immunosuppressed hosts, Part I (Natural history and effects of treatment with adenine arabinoside), *Ann. int. Med.*, 93: 655-664.
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Recently, a specific guinea pig serum against human CMV (AD-169 strain) was made available by Dr. Larry E. Davis, Albuquerque, NM and the Communicable Disease Center, Atlanta, GA (U.S.A.). Antibodies were raised according to the instructions of Huang et al. (1974). Both antisera were used for indirect immunofluorescence and immunoperoxidase techniques on paraffin sections from eye, brain and spinal cord of the described patient. An autopsy specimen of CMV-infected lung and surgical biopsy material from a case of CMV-pericarditis (both in paraffin) served as positive controls. All sections were treated with trypsin. Fluorescence and immunoperoxidase reaction product were inadequate for a conclusive diagnosis in any of the sections. It would appear that the antigenicity of CMV is lost quite rapidly and that prompt fixation in cold ethanol is necessary for preservation (Davis et al. 1981).

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