J Forensic Sci, Nov. 2005, Vol. 50, No. 4
Paper ID JFS2005160
Available online at: www.astm.org

CASE REPORT

Bennet I. Omalu,[1] *M.D., M.P.H.; Joseph A. Mancuso,*[2] *F.D.; Patrick Cho,*[3] *M.D.; and Cyril H. Wecht,*[1] *M.D., J.D.*

Diagnosis of Alzheimer's Disease in an Exhumed Decomposed Brain After Twenty Months of Burial in a Deep Grave*

ABSTRACT: After 20 months of interment in a deep grave, the decomposed body of the 81-year old testator of a will was exhumed to sustain the burden of proof that he lacked testamentary capacity when the will was rewritten two days prior to his death. The brain was mushy and pulverized with complete disappearance of the brainstem, cerebellum and subcortical ganglia. Small foci of relatively intact dorsal frontal neocortex were identified. Sections from these foci were stained with hematoxylin and eosin, bielchowsky silver stain and immunostains for beta amyloid peptide (βA4), tau and alpha-synuclein. Despite severe autolysis and decomposition, the bielchowsky stain and the βA4 immunostains showed preserved frequent neuritic amyloid plaques with very few residual neurofibrillary tangles. Cerebral Amyloid Angiopathy was present. At the present time this case represents the first documented and reported case of direct tissue diagnosis of Alzheimer's Disease pathology in a decomposed brain following long term burial in a deep grave.

KEYWORDS: forensic science, will, Alzheimer's disease, decomposition, long term burial

In the State of Pennsylvania, any person 18 or more years of age, who is of sound mind may make a will (1). However, a will may be contested based on lack of testamentary capacity and lack of mental competence due to a dementia at the time of execution of the will. The executrix of an estate petitioned a Pennsylvania court, contesting the Codicil-ratified will of her 81-year-old uncle that was rewritten two days prior to his death. In the last will, the executrix was disinherited of all earthly possessions, which were transferred to a nephew who took care of the uncle during his terminal illness.

After 20 months of interment in a deep grave, the body of the testator and decedent was exhumed. The objective of the exhumation was to sustain the burden of proof that he lacked testamentary capacity based on significant terminal impairment of intellectual functions and judgment. There was a pre-mortem clinical differential diagnosis of a possible dementia and a post-exhumation autopsy was aimed at establishing a pathologic diagnosis of a dementia if present. Other pertinent pre-mortem clinical history included arteriosclerotic and hypertensive cardiovascular disease, chronic obstructive pulmonary disease, coronary artery by-pass surgery, cardiac defibrillator placement, metastatic pulmonary adenocarcinoma and congestive cardiac failure.

We report a case of autopsy proven Alzheimer's Disease in an exhumed decomposed human brain after long term [twenty months] burial in a deep grave. Following a comprehensive review of the English medical literature, this is the first reported case of direct histochemical tissue diagnosis of Alzheimer's Disease in a decomposed brain using the bielchowsky silver stain and immunostains for beta amyloid peptide.

Materials and Methods

Exhumation and Autopsy

The decedent was an 81-year-old, white male, who had been buried for twenty months in a metal casket, in a standard six-feet grave and in a local cemetery. Exhumation was authorized by the County Court of Common Pleas and autopsy was requested by his daughter, the executrix of his estate. The body laid in a normal state of repose within the casket and was in a fair state of preservation without insect or animal activity. The body was clad in a funeral suit and revealed evidence of embalming procedures. There was patchy mold growth on the skin of the face, neck, right shoulder, chest, arms and legs. The eyeballs and intraorbital soft tissues were markedly dehydrated and recessed. There was minimal skin slippage on the thighs. There was no scalp hair. The gums were partially edentulous. The head, face, neck, trunk and extremities were symmetrical and revealed no evidence of trauma. A complete autopsy was performed by the combined technique of Virchow and Rokitansky (2). The autopsy confirmed the presence of severe atherosclerosis of the coronary arteries and the aorta

[1] Divisions of Forensic Pathology and Forensic Neuropathology, Allegheny County Coroner's Office, Pittsburgh, PA.
[2] Department of Natural Science and Mathematics, Carlow University, Pittsburgh, PA.
[3] Department of Pathology, Allegheny General Hospital, Pittsburgh, PA.
* Presented at the annual meeting of the National Association of Medical Examiners at Nashville, Tennessee in September 2004, and at the annual meeting of the American Association of Neuropathologists (AANP) at Cleveland, Ohio in June 2004.
Received 26 March 2005; and in revised form 14 June 2005; accepted 25 June 2005; published 14 Sept. 2005.

FIG. 1—*Gross photograph of mushy brain showing advanced decomposition and pulpification with obliterated and absent gyro-sulcal convolutions except for focally intact gyri (arrow) in the dorsal frontal lobes, which exhibit overlying arachnoid mater and subarachnoidal vessels.*

with myocardial infarction of the posterior wall of the left ventricle, myocardial fibrosis and a fusiform atherosclerotic aneurysm of the distal abdominal aorta. There was evidence of a remote triple coronary artery by-pass graft. Hypertensive cardiovascular disease was present with cardiomegaly, eccentric ventricular hypertrophy, benign arterial and arteriolar nephrosclerosis, and severe systemic hypertensive arteriolosclerosis. The lungs revealed a diffusely infiltrating, poorly differentiated large cell carcinoma with distant metastasis to lymph nodes and liver. There was bilateral irregular pulmonary emphysema with usual interstitial fibrosis. All the thoracic and abdominal viscerae revealed mild to severe parenchymal autolysis and decomposition.

Neuropathologic Examination

The scalp, calvarium and base of the skull were intact without evidence of hemorrhages or fractures. There were no intracranial hemorrhages and the dura mater appeared intact. The brain was removed and placed in 10% formaldehyde for fixation. The weight of the formalin fixed brain was 775 grams and measured $18 \times 12 \times 11$ cm. The brain was friable and pulpified with complete obliteration and disappearance of the cerebellum and brain stem. The cerebral hemispheres were pulverized into a mushy mass of white-tan tissue with near-complete obliteration and disappearance of the anatomic topography. The gyro-sulcal convolutions were completely obliterated and absent except for identification of focally intact gyri in the dorsal frontal lobes, which exhibited overlying arachnoid mater that contained subarachnoidal vessels (Fig. 1). The basal cerebral vessels of the Circle of Willis were completely obliterated and absent. The cranial nerves were not identified.

Multiple serial coronal sections of the brain revealed a pasty white-tan homogeneous tissue with complete obliteration and disappearance of the subcortical ganglia and structures. Focally intact neocortical gray matter was identified in the dorsal frontal lobes with relatively preserved distinction of the gray-white matter junction (Fig. 2). The region of the centrum semiovale was white tan, pasty and homogeneous with complete obliteration and disappearance of subcortical white matter tracts and structures. No tumors, hemorrhages or other lesions were discernible. The ventricles were absent. Multiple representative and topographically targeted sections of the intact frontal neocortex and accompanying subcortical tissue were taken and examined with hematoxylin and eosin [H/E] stains, Bielchowsky silver stain and immunohistochemical stains for tau [SMI 51, Sternberger and Sternberger], amyloid beta protein [Aβ, 4G8, Senetek, Maryland Heights, MO, USA] and alpha-synuclein (Zymed, San Francisco, CA).

Results

Examination of hematoxylin and eosin stained sections of the neocortex revealed diffusely decomposed, pulpified and eosinophilic cerebral parenchyma and neuropil with identification of few scattered residua of neurons and glial cells, and randomly scattered remnants of lipofuscin pigment (Fig. 3). Histomorphologic remnants of the laminar tissue architecture of the neocortex were focally identified in the frontal cortex. Some leptomeningeal and penetrating parenchymal vessels were relatively preserved and identifiable in the frontal lobes. They showed mild to moderate hyperplastic and hyaline arteriosclerosis and arteriolosIcerosis. Many residual corpora amylacea were identified. The caudate, putamen, globus pallidus, thalamus and other subcortical nuclei were not identified.

Bielchowsky silver stain and immunohistochemical stains for beta amyloid peptide revealed moderate to frequent primitive, classic, mature and burnt-out neuritic amyloid plaques in the frontal neocortex (Fig. 4*a–d*) accompanied by dense intramural immunopositivity of leptomeningeal and penetrating parenchymal vessels for beta amyloid peptide (Fig. 5). Very few scattered residual neurofibrillary tangles were identified with the bielchowsky silver stain (Fig. 6). Immunohistochemical stains for tau protein and alpha-synuclein did not stain any neurofibrillary tangles, dystrophic neuritics, neuropil threads, Lewy bodies or other inclusions.

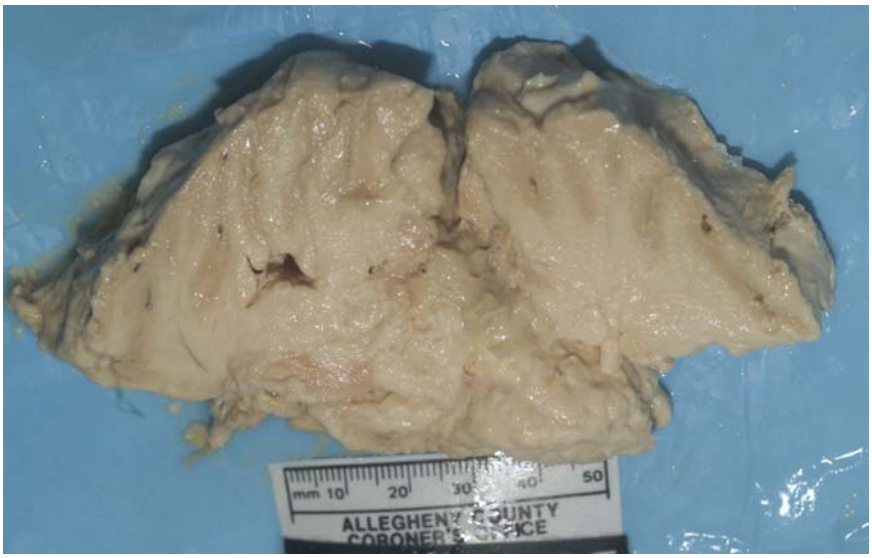

FIG. 2—*Gross photograph of a coronal section of the brain residua showing decomposed, pasty white-tan tissue with focally intact neocortical gray matter and obliteration of subcortical ganglia.*

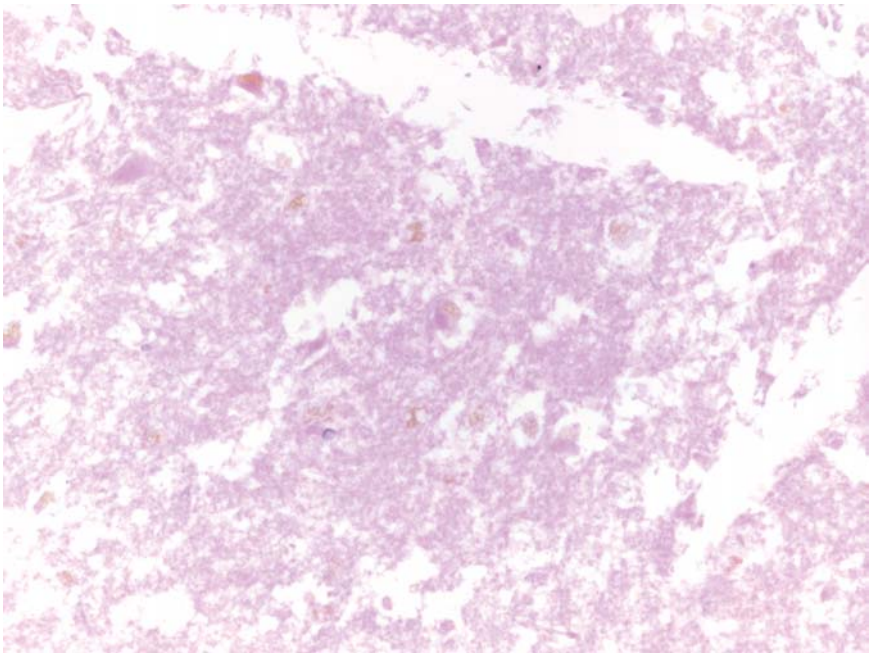

FIG. 3—*Photomicrograph [×200, hematoxylin and eosin] showing the neocortex with diffusely autolysed eosinophilic cerebral parenchyma and neuropil with identification of few scattered residua of neurons and glial cells, and randomly scattered residua of lipofuscin pigment.*

Discussion

This case illustrates an instance of postmortem, histologic identification of Alzheimer's Disease pathology in a markedly decomposed and autolysed brain following long term, twenty-month burial in a deep grave. It shows that bielchowsky silver stain and immunostains for beta-amyloid peptide may be used in identifying neuritic amyloid plaques and neurofibrillary tangles despite advanced autolysis and decomposition. The combination of moderate to frequent neocortical amyloid plaques and neuritic plaques in the frontal neocortex, accompanied by few identifiable neurofibrillary tangles may be applied to the diagnosis of Alzheimer's Disease, in this case, using three diagnostic criteria (3): CERAD [Consortium to Establish a Registry for Alzheimer's Disease] criteria (4), Braak and Braak staging criteria (5), and the National Institute on Aging [NIA]-Ronald and Nancy Reagan Institute working group criteria (6).

The CERAD criteria involve an estimation of an age related plaque score and determination of a diagnostic group for Alzheimer's Disease. For this case, there were moderate to frequent neocortical neuritic plaques. Given the age of 81-years-old, this frequency of neuritic plaques corresponds to an age related plaque score of "C" according to the CERAD criteria (3,6). An age related plaque score of "C" corresponds to a CERAD diagnostic group of "Definite Alzheimer's Disease" with a "clinical history of dementia, and presence or absence of other neuropathologic lesions likely to cause dementia" (3,6). An age related plaque score of "C"

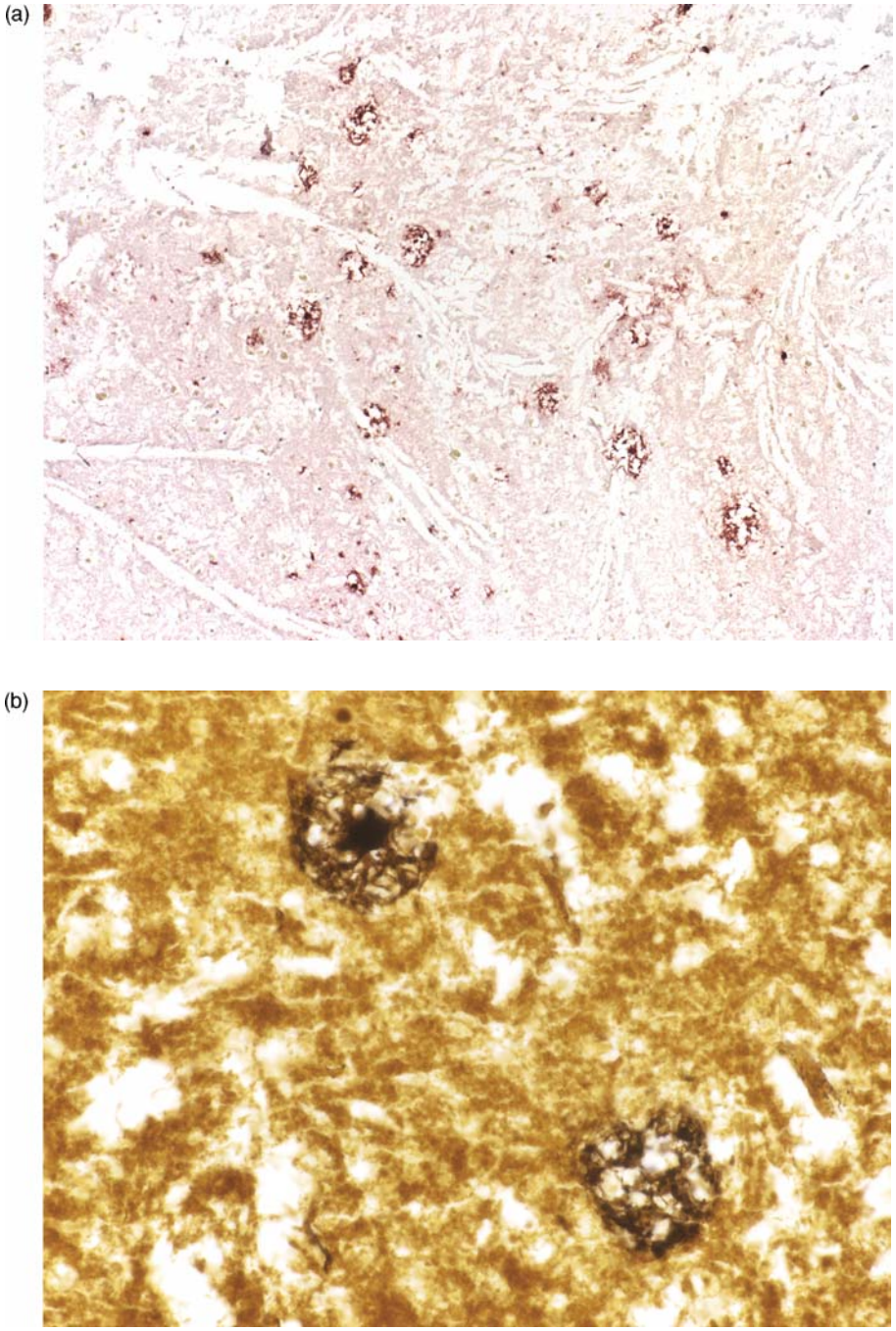

FIG. 4—*Photomicrographs showing [a] frequent neocortical neuritic plaques [×100, beta amyloid peptide immunostain]; [b] a classic {above} and a primitive {below} neuritic plaque [×400, bielchowsky silver stain]; [c] three classic neuritic plaques [×400, bielchowsky silver stain]; [d] another classic neuritic plaque [×400, bielchowsky silver stain].*

may also correspond to a CERAD diagnostic group of "Possible Alzheimer's Disease" if there was absence of clinical manifestations of dementia (3,6).

In this case immunostains for tau protein were negative despite the presence of neuritic amyloid plaques identified with bielchowsky silver stain and with immunostains for beta-amyloid peptide. A possible explanation for this observed finding may be that tau protein, a microtubule associated protein, may decompose and disappear at a faster rate than other components of neocortical plaques and neurofibrillary tangles, and may have disappeared from this markedly decomposed and autolysed tissue after twenty months of burial in a deep grave. This suggested possible explanation, however requires further empirical research for confirmation. Bielchowsky silver stain, in this case, also revealed very few scattered residual neurofibrillary tangles in the neocortex. The Braak and Braak criteria (3,5) for the diagnosis of Alzheimer's Disease is based on the degree of involvement of the defined topographical regions of the brain by neurofibrillary tangles. For this case, the presence of at least few scattered and identifiable neurofibrillary tangles in the frontal neocortex may correspond to Braak and Braak Stage IV (3,5) assuming that this pathology was present in the mesial temporal lobe and other regions of the brain, which have disappeared.

A combination of frequent neuritic plaques [CERAD criteria] and Braak and Braak Stage IV corresponds to an intermediate to high

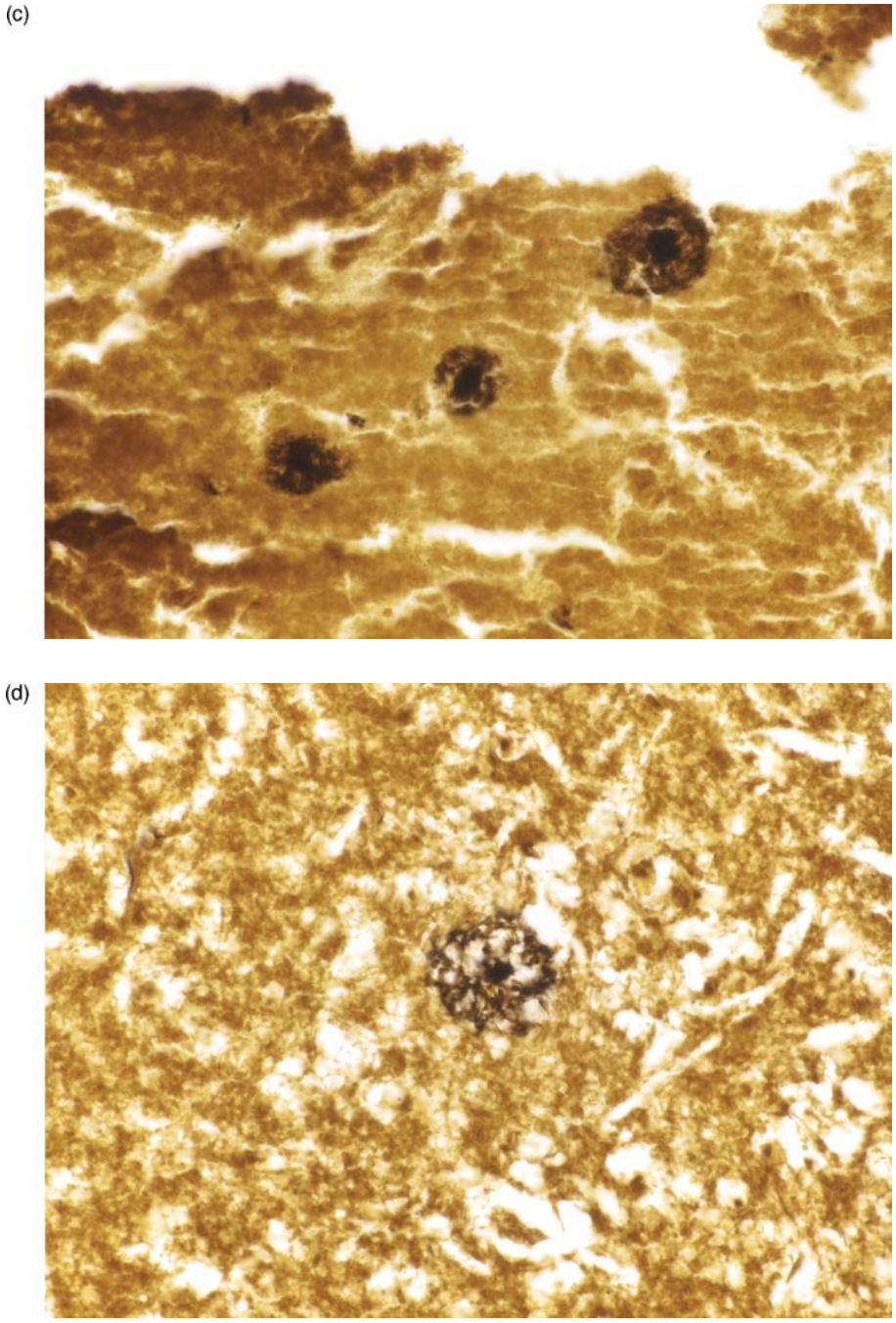

FIG. 4—*Continued.*

likelihood that Alzheimer's Disease pathology was the underlying cause of dementia in this case. This is according to the criteria of the working group of the National Institute for Aging [NIA] and the Ronald and Nancy Reagan Institute of the Alzheimer's Association (3,6).

The presence of beta-amyloid peptide deposits in walls of parenchymal and meningeal blood vessels, in this case, is consistent with Cerebral Amyloid Angiopathy [CAA]. Sporadic or common variants of CAA are the most prevalent forms and are strongly associated with Alzheimer's Disease. In CAA, the media or adventitia of cerebral parenchymal or meningeal vessels (mainly arterioles) are replaced by skeins of microfilaments, which are 7–10 nm thick and show properties of amyloid (3). CAA is demonstrable in over 90% of cases of Alzheimer's Disease (3).

In summary, we present the first documented and reported case of forensic neuropathologic diagnosis of Alzheimer's Disease in a decomposed and autolysed, exhumed brain following long-term, twenty-month burial in a deep grave. Bielchowsky silver stain and immunostains for beta-amyloid peptide [Aβ, 4G8, Senetek, Maryland Heights, MO, USA] were most instrumental for this post-mortem diagnosis. Decomposed tissue with identifiable residual tissue architecture was used for histologic analysis. In this instance only the frontal cortex was identifiable and showed minimally intact arachnoid mater and focal remnants of a neocortical gray ribbon. All the other regions of the brain were reduced to a pasty and pulpified tissue without discernible tissue architecture. Neuropathologic diagnosis of Alzheimer's Disease was based on the presence of frequent neuritic plaques in the frontal neocortex, presence of few,

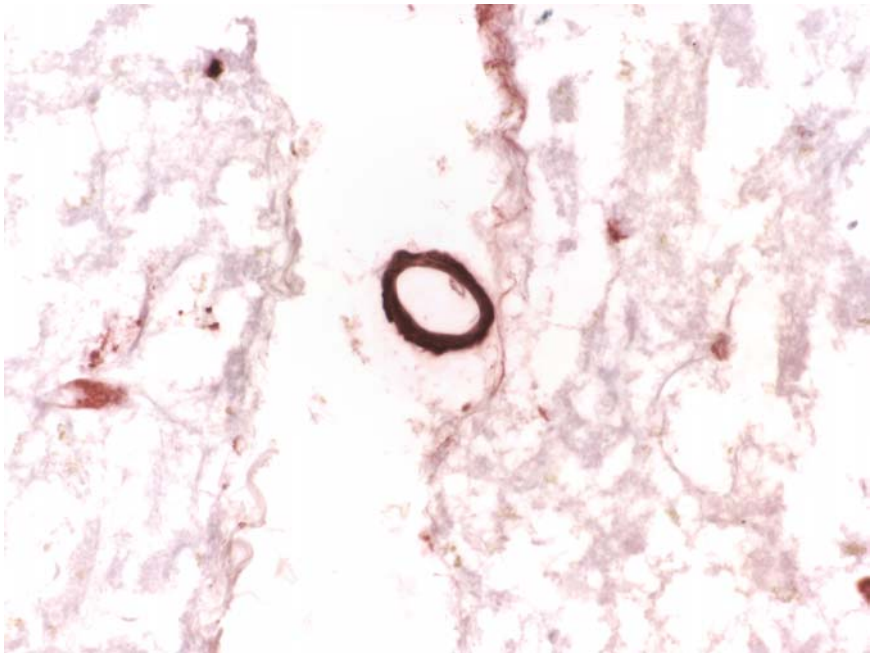

FIG. 5—*Photomicrograph [×400, beta amyloid peptide immunostain] showing dense intramural deposits of beta amyloid peptide in a leptomeningeal arteriole.*

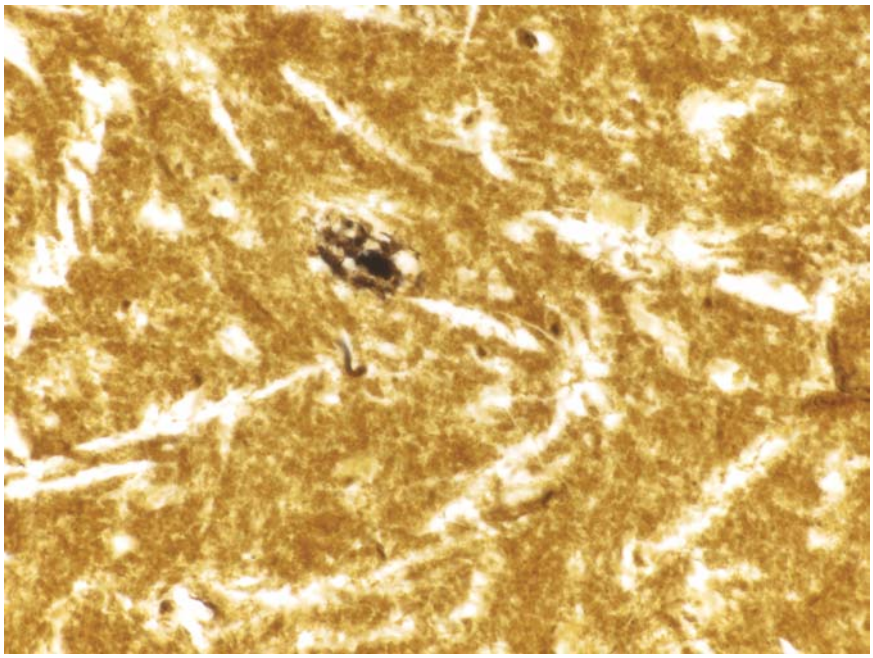

FIG. 6—*Photomicrograph [×400, bielchowsky silver stain] showing a single residual neurofibrillary tangle adjacent to a neuritic plaque. There were very few scattered residual neurofibrillary tangles in the sections of the neocortex stained with bielchowsky silver stain.*

residual neurofibrillary tangles and presence of Cerebral Amyloid Angiopathy.

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Additional information and reprint requests:
Bennet I. Omalu, MD, MPH
7520 Penn Bridge Court
Pittsburgh, Pennsylvania 15221
E-mail: bennet.omalu@verizon.net