Progress in Neuropathology

Volume I

Edited by

H. M. Zimmerman, M.D.

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Foreword

The golden age of neuropathology had its meteoric rise less than twenty years ago. Both in this country and abroad the number of contributions to the scientific literature in this field has multiplied manyfold since the early 1950's. Even more significant, perhaps, has been the large number of young investigators who have sought training in neuropathology during this period. No longer content with purely descriptive morphology, these workers have sought answers to the causes and mechanisms of disease, and in such efforts have employed freely many of the sister sciences such as genetics, virology, immunology, and chemistry, to name a few at random. A tremendous aid in the search for pathogenesis has been the availability of the electron microscope, that has served not only to reveal morphology in its finer details but also to direct attention to altered function of cellular organelles and membranes.

The advances that have been made so rapidly in so few years demand recording not so much for their historical value as for their service as goads and guidelines for continued effort. This is the double purpose of *Progress in Neuropathology*—to present the new and provocative and, on occasion, to provide a forum for a particularly elegant study that brings to a near-close an area of intensive research of the recent past.

There is a serious possibility that the golden age has already run its course. On all sides there are evidences that the pace is slackening. With the abandonment of governmental support for training programs and the drastic curtailment of financial aid to research on the part of government and private foundations, the volume of productivity is bound to diminish. Perhaps, however, its quality can be maintained to some degree with this publication.

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Electron Microscopy in Neuropathology

Asao Hirano, M.D.

When the electron microscope first became available as a biological tool, most neuropathologists were quite skeptical about its usefulness in neuropathology except, perhaps, as a limited research instrument. This feeling was soon reenforced by the poor preservation of fine structure obtained with the techniques available at that time. This defect was especially important when dealing with the central nervous system (CNS). The disappointing results obtained in early work on the CNS, despite tremendous efforts by a number of able investigators, led some to discard the electron microscope as a practical means for the study of the CNS so that there was a long delay in the exploration of its fine structure. Most of the successful earlier work was, in general, confined to the peripheral nervous system (1–9).

Three major advances in technique have finally permitted good preservation and visualization of central nervous tissue. These were the introduction of epoxy resin embeddments, improvement in fixation by perfusion (10) and advances in heavy metal "staining" of sections. However, to utilize all these and other techniques fully, one had to resort to experimental animals. Such studies, of course, have only an indirect application to human neuropathology.

The richest source of human neuropathologic material exists today in formalin-filled specimen jars in the institutional museums. Use of this material resulted in preparations so poor that many electron microscopists have rejected this source out of hand. Better preparations are possible if specimens are obtained at the autopsy table and are immediately fixed in glutaraldehyde or osmic acid rather than formalin, but even here, obvious artifact is apparent, especially when autopsy is delayed. Nevertheless, this is a potentially very rich source of neuropathologic material.

A further improvement of preservation, accompanied by a severe constriction of potential information, is afforded by the use of properly fixed biopsy or surgical specimens. Currently this is probably the most widely used method, and in many cases preservation is quite good, although the information of interest obtainable is severely limited.

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The best preservation of all is afforded by the use of experimental animals when all the optimal conditions for good preservation may be brought to bear. On the other hand, the subject matter is of least interest in terms of human neuropathology.

Thus, a dilemma apparently exists: a choice between either good preservation or significant information directly applicable to human neuropathology. Both aspects are essential and yet seem to be mutually exclusive. A way out of this dilemma is through the thorough study of well-preserved experimental tissue. If we use the results of fine structural studies or experimental animals as a yardstick with which to judge the artifactitious content of poorly preserved material, meaningful information can sometimes be derived even from old, formalin-fixed autopsy material stored for many years in the laboratory storeroom.

In the following, we shall review a number of studies on material derived from the sources cited above. While extensive, this review is not exhaustive and it must be understood that the number of such studies is rapidly increasing as the preparation of this paper goes on.

AUTOPSY MATERIAL STORED IN FORMALIN

This material is the greatest source of information for neuropathologists using the optical microscope. Potentially, it can be used for the study of all documented neuropathologic diseases. Any area of the CNS in many diseases is available at any time, but as pathologists have been fully aware for many years, the longer the duration of storage, the greater the degree of deterioration as evidenced by a decrease in stainability and structural integrity. This is particularly apparent in neurons where the Nissl substance disappears.

Nevertheless, the use of old autopsy material under the electron microscope is sometimes the only way of obtaining the desired information. For example, to study the fine structural changes in the brain stem associated with postencephalitic parkinsonism, we had to resort to old formalin-fixed autopsy material for a number of reasons. The brain stem is obviously inaccessible to biopsy. Postencephalitic parkinsonism is relatively infrequent and nonfatal so that waiting for a case to come to autopsy to use the proper fixation procedure for electron microscopy would be unfeasible from a practical standpoint. Even if such an unlikely combination of events were to occur, the neurons containing the tangles are so few that searching for them in the electron microscope might well be futile. Thus, the examination of these structures was virtually impossible except by the use of accumulated, previously studied, museum specimens.

The material we used had been stored in formalin for about 5 years prior to embedding in plastic, but the structure of the neurofibrillary tangles we observed (Figs. 1 and 2) was essentially the same as that seen in relatively well-preserved cortical biopsies of patients suffering from Alzheimer's disease (11). The melanin granules we found (Fig. 3) were likewise identical to those seen in better preserved, fresh, autopsy material (12, 13) or in animal experiments (14).

The elucidation of the structure of Pick bodies by Schochet and Lampert (15) required the use of old formalin-fixed autopsy material even more than neurofibrillary tangles in postencephalitic parkinsonism. This study, however, is the first of its kind and we must, of course, await further observations of these bodies before a final judgment about their morphology can be made.

Autopsy material was used to demonstrate neuronal intranuclear bundles of 100-A filaments within the anterior horn cells and motor nuclei of the medulla in a patient treated with vincristine for acute lymphocytic leukemia (16). These bundles were remarkably similar to those observed in experimental animals subjected to subarach-

noid injections of various mitotic spindle inhibitors (17) including vincristine (16).

Autopsy material from a case of Alexander's disease was examined under the electron microscope (18). The Rosenthal fibers characteristic of this disease consisted of irregular, granular osmiophilic masses within the cytoplasm of the fibrillary astrocytes. According to the authors, the masses appeared to arise from the conglutination of altered glial filaments. In Alexander's disease (19) Schlote found essentially similar configurations identical to the Rosenthal fibers he observed in surgically removed,

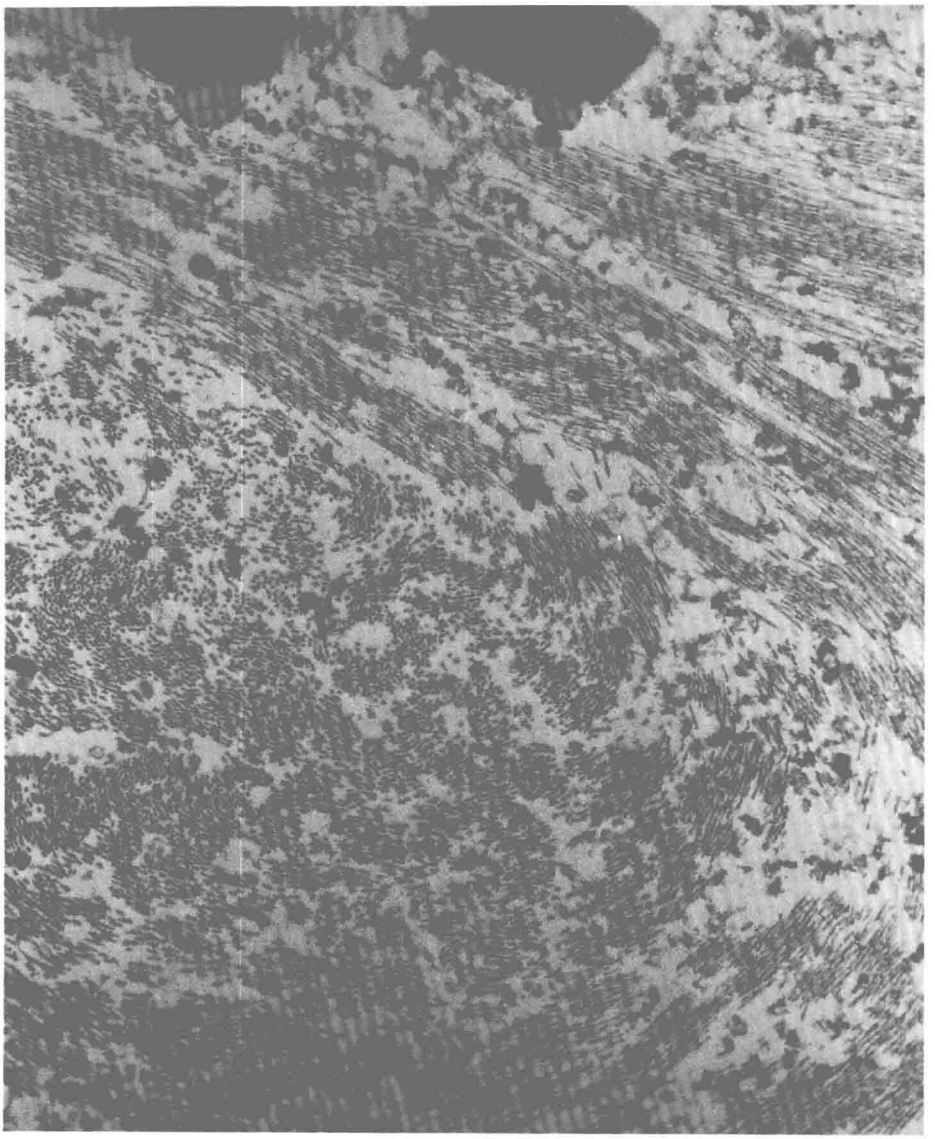

Fig. 1. Neurofibrillary tangle and two dense bodies (melanin) in perikaryon of neuron of locus caeruleus of patient with postencephalitic parkinsonism. Formalin-fixed autopsy material. × 20,000.

osmium-fixed tissue from a spongioblastoma (19, 20).

Fine structural analysis of postmortem material has revealed details of the structure of lipid inclusions in the infantile (21) and late (22) forms of amaurotic idiocy and Peiffer (23) has described other lipid inclusions in gargoylism and metachromatic leukodystrophy. The structure of the lipid inclusions reported by these authors is essentially the same as that found in biopsy specimens of similar material.

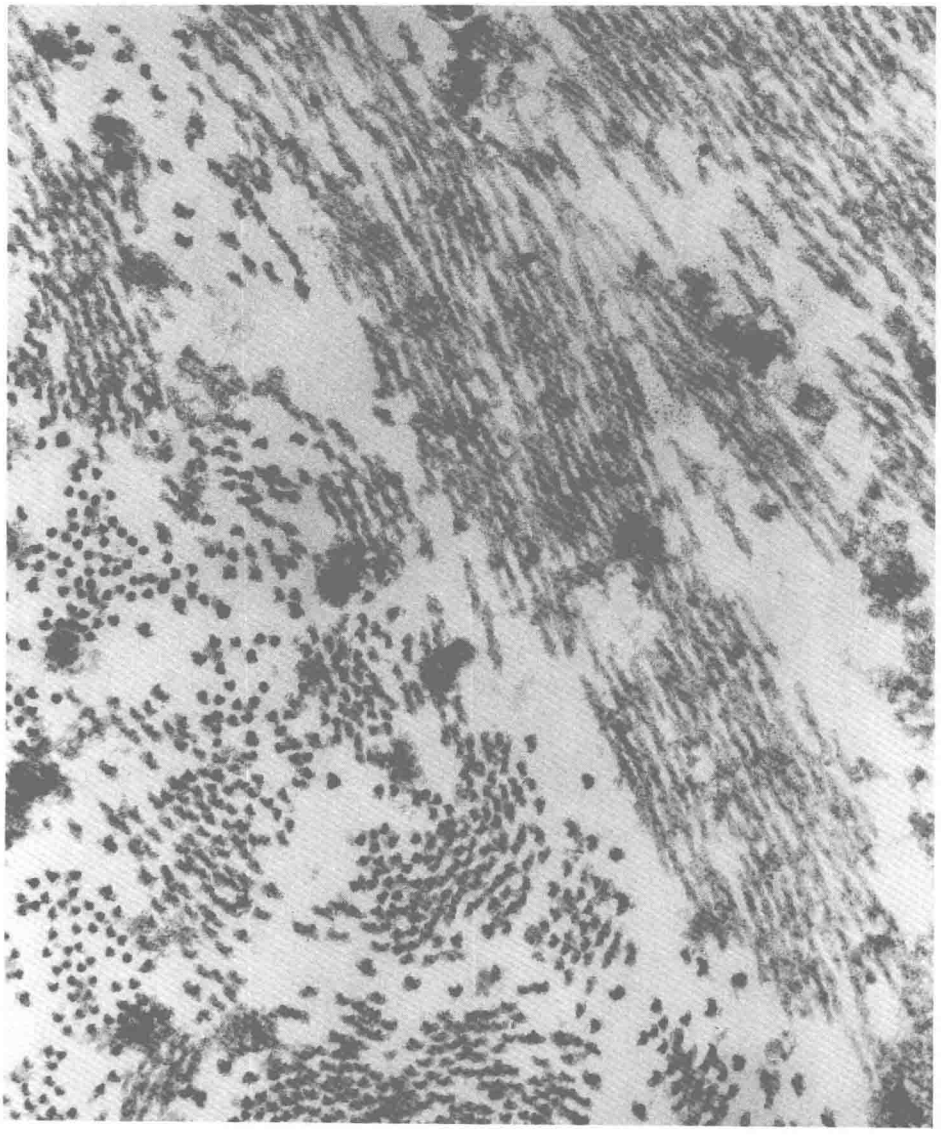

Fig. 2. Higher magnification of neurofibrillary tangles seen in Fig. 1. Both cross and longitudinal sections of fibrils are visible. They are essentially identical to those seen in cortical neurons in Alzheimer's disease (11). × 80,000.

Several infectious diseases of the CNS have also been studied at the fine-structural level in formalin-fixed autopsy material. Toxoplasmosis of the brain, a condition apparently on the increase in adults in recent years (24), has been examined by Poon and Ghatak (25). The structure of the microorganism is essentially the same as that seen in the human heart (26) and in experimental tissue (27). Even more dramatic was the observation of a Popova virus-like particle in glial nuclei in the white matter in a case

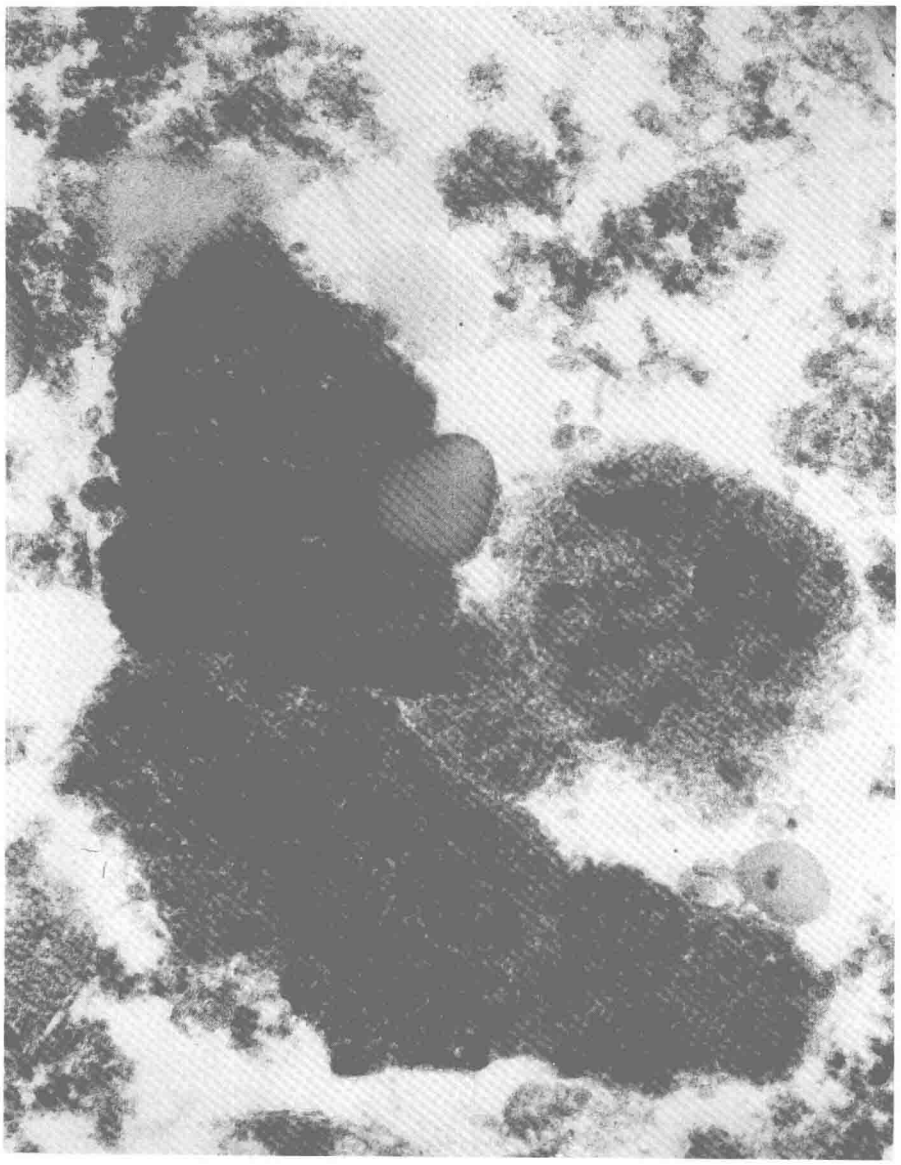

Fig. 3. Higher magnification of melanin pigment is similar to that in Fig. 2. They are essentially the same as those reported in better preserved tissue (12–14). × 74,000.

of progressive multifocal leukoencephalopathy by Zu Rhein and Chou (28) during the examination of a specimen stored in formalin for 2 years. Similar observations were later made by others (29–35). Herpes simplex virus, was observed in formalin-fixed postmortem human brain in cases of acute necrotizing encephalitis (36, 37) and herpes virus-like bodies have been observed widely distributed within nuclei of various cells in a case termed "unusual nuclear inclusion body disease" (38).

Intranuclear inclusion bodies made up of filamentous structures were recently observed by Shaw and his associates (39, 40) in formalin-stored postmortem material from patients who died of subacute sclerosing panencephalitis. According to these authors, these structures were probably myxoviruses. Cortical biopsies from patients with the same disease have been examined (41–46). On the basis of such a biopsy, Herndon and Rubinstein (45), who also observed unusual inclusions, suggested that the etiologic agent for subacute sclerosing panencephalitis may be the measles virus. Another postmortem study of similar material has recently been reported (47).

Under special conditions, even the paraffin blocks of formalin-fixed autopsy material have been used for the fine structural study of viral inclusions. In a case of herpes encephalitis, Morecki and Becker (48) deparaffinized the already prepared paraffin blocks, postfixed them in osmic acid and reembedded them in Epon. Intranuclear viral-like particles were found. This is an extreme example of the use of formalin-fixed tissue and is probably applicable only to the study of those structures extraordinarily resistant to change during fixation, embedding and prolonged storage.

AUTOPSY MATERIAL FIXED IN OSMIC ACID OR GLUTARALDEHYDE

If the autopsy is performed shortly after death and if adequate clinical diagnosis is available, postmortem material fixed in either glutaraldehyde or osmic acid provides distinct advantages. The preservation is considerably better than after formalin fixation, and areas inaccessible to biopsy (including vital areas) are available. One disadvantage is that the preservation is far less satisfactory than after biopsy of living tissue. Unlike stock material for museums, the tissue must be removed as soon after death as possible to avoid excessive autolytic changes. The site of a discrete lesion must be precisely known beforehand through clinical findings.

As an example of the results of such techniques. Duffy and Tennyson (12) have been able to demonstrate the fine structure of Lewy bodies and melanin granules in the substantia nigra and locus caeruleus in parkinsonism. D'Agostino and Luse (13) had earlier studied melanin pigment of normal human substantia nigra. This vital region is approachable only through autopsy, and the use of properly fixed fresh autopsy material has permitted at least the partial elucidation of this important structure. The pigment granules in the substantia nigra and in the locus caeruleus have been examined in monkeys (14).

We have examined the fine structure of Ammon's horn in glutaraldehyde-fixed postmortem material from Chamorro patients who died of parkinsonism dementia and amyotrophic lateral sclerosis on Guam. While not as vital as the brain stem, Ammon's horn is too deep and involves too much risk to the patient to obtain samples by biopsy. The use of this material has enabled us to describe Alzheimer's neurofibrillary tangles, granulovacuolar bodies and eosinophilic rod-like structures (Figs. 4–6) in this area (49, 50) and to examine corpora amylacea in the same material (Fig. 7). Its structure was identical to that reported in biopsy specimens obtained from a similar area in a patient with epilepsy (51).

Multiple sclerosis plaques have been examined in similarly prepared tissue (52). More information concerning the fine struc-

Fig. 4. Electron micrograph of rod-like structure as seen in Sommer's sector of patient with parkinsonism-dementia complex and amyotrophic lateral sclerosis on Guam. Dense, 60–100-A fibrils are found among less dense sheets of unknown material. Glutaraldehyde-fixed autopsy material. × 100,000.