The Autopsy Committee of The College of American Pathologists has prepared this guideline in conjunction with representatives of other organizations to assist pathologists in autopsy procedures for brain, spinal cord, and neuromuscular system. The guideline is to be regarded as being primarily an educational tool. Application of these recommendations on autopsy reporting is to be made on the basis of the judgment of the pathologist engaged in a specific case.

(Arch Pathol Lab Med. 1995;119:777–783)

The American Medical Association is working cooperatively with national medical specialty societies, including the College of American Pathologists (CAP), to guide the development and implementation of practice guidelines. In an attempt to improve the quality and consistency of practice guidelines, attributes have been established to guide their development.1

I. Practice guidelines should be developed by or in conjunction with physician organizations.
II. Reliable methodologies that integrate relevant research findings and appropriate clinical expertise should be used to develop practice guidelines.
III. Practice guidelines should be as comprehensive and specific as possible.
IV. Practice guidelines should be based on current information.
V. Practice guidelines should be widely disseminated.

Several definitions are important to the development, understanding, and use of practice guidelines.

Parameters: Practice parameters are strategies for patient management developed to assist physicians in clinical decision making. Practice parameters include standards, guidelines, and other patient management strategies.

Standards: Standards are accepted principles for patient management. Practice variation due to patient- or physician-specific factors is not expected.

Guidelines: Guidelines are recommendations for patient management that identify a particular management strategy or a range of management strategies. Practice variation is reasonable to the extent that definitions of management strategies or applicable clinical categories allow incorporation of patient- or physician-specific information.

Options: Practice variation is expected since implementation of options requires incorporation of substantial patient- or physician-specific information.

It should be understood that adherence to these guidelines does not guarantee a successful outcome. Rather, these guidelines are provided as an educational tool to assist physicians in providing quality care. The ultimate judgment regarding the propriety of any specific procedure must be made by the pathologist in light of the individual circumstances presented by a specific patient or specimen. Adherence to a guideline is voluntary.

The College recognizes that this document may be used by hospitals and other institutions, managed care organizations, and insurance carriers and other payers. However, this document was not developed for use regarding reimbursement or credentialing. The College cautions that these uses involve considerations that are beyond the scope of this document.

The Autopsy Committee was charged by the CAP with the responsibility to develop and assess practice guidelines for autopsy pathology. Thus far, two practice guidelines for autopsy pathology have been adopted by the CAP Board of Governors: “Practice guidelines for autopsy pathology: autopsy performance” was approved by the CAP House of Delegates on October 14, 1992, and the CAP Board of Governors adopted it as official policy on November 14, 1992. The guideline was published in the Archives of Pathology and Laboratory Medicine.2 “Practice guidelines for autopsy pathology: autopsy reporting” was approved by the CAP House of Delegates on April 13, 1994, and the CAP Board of Governors adopted it as official policy on May 20, 1994. The guideline was published in the Archives of Pathology and Laboratory Medicine.3

This practice guideline, the third developed by the Autopsy Committee, concerns examination of the central nervous and neuromuscular systems. This committee includes representatives of the American Academy of Forensic Sciences, the American Association of Neuropathologists, the American Society of Clinical Pathologists, the

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Armed Forces Institute of Pathology, the Association of Anatomic and Surgical Pathology Directors, the Association of Clinical Scientists, the Association of Pathology Chairmen, the College of American Pathologists, the Society of Pediatric Pathology, and the United States and Canadian Academy of Pathology. This guideline was developed initially by the Professional Affairs Committee of the American Association of Neuropathologists, 1992, with additional input from the CAP Neuropathology Committee.

This practice guideline was subjected to reality testing and then revised by the Autopsy Committee. The CAP House of Delegates approved this guideline on October 26, 1994, and the CAP Board of Governors adopted it as official policy on November 18, 1994. It becomes effective upon publication.

**GENERAL CONSIDERATIONS FOR BRAIN AND SPINAL CORD**

Examinations of the brain and spinal cord are performed at autopsy to identify and diagnose diseases affecting the central nervous system and to provide detailed descriptions of the sites of lesions in relation to the structural and functional systems of the brain. The latter often provides important correlation with premortem clinical and neuroradiologic findings. To detect all macroscopic lesions, closely spaced sectioning of well-fixed brain and spinal cord (“brain cutting”) usually is performed after fixation (eg, in a solution of formaldehyde for at least several days). This policy is intended for routine cases, but in some circumstances it may be necessary to cut the brain in the fresh state (eg, forensic cases in which information is needed during the prosecution or for investigative protocols that necessitate the collection of fresh samples or slices and their placement in nontraditional fixatives). The laterality of lesions and specimens is documented.

If possible, the history and other clinical data are reviewed carefully and the patient’s physician is consulted to determine the specific goals and needs of the autopsy. For example, fresh cerebrospinal fluid and tissue samples may be required for microbiologic culture; tissues may need to be frozen for later biochemical or molecular studies; or tissues may require special fixation for electron microscopy, immunocytochemistry, or in situ hybridization. These and other sampling procedures usually are performed before immersing the brain in the routine fixative.

It also may be important to sample other specific sites or tissues based on the patient’s history (eg, sampling at appropriate vertebral levels in cases of spondylitis, spinal fracture, or bone involved by a metastatic process; sampling of dorsal root ganglia, peripheral nerve, and skeletal muscle in hereditary, degenerative, or neuromuscular disorders; or sampling of dorsal root and trigeminal [Gasserian] ganglia in herpes zoster infection).

Most of the details included in this guideline are most appropriate for the postmortem examination of hospitalized adults and children; comparable methods for perinatal and infant autopsies are included in the upcoming CAP “Practice guidelines for autopsy pathology: 4. The perinatal and pediatric autopsy” (Autopsy Committee of the CAP, unpublished) and for forensic cases in the CAP Handbook of Forensic Pathology.4

**Intracranial Cavity**

The brain and spinal cord are usually removed separately at autopsy, as described in standard texts.5–9 Care is taken to avoid skin incisions on the face. After removal of the calvarium or opening of infant skulls through the sutures, the skull cap may be transilluminated to reveal bone lesions. The interior of the cranial cavity is inspected following removal of the brain. The basilar dura may be separated from the skull to facilitate inspection for fractures. The pituitary may be removed. The internal and middle ear is encased in temporal bone and can be removed en bloc and decalcified, if indicated. If not removed, the cavity of the middle ear may be opened with a bone chisel or one may sample bone from the petrous ridge. The region of the cavernous sinus, the ophthalmic and carotid arteries, and other anatomic structures below the floor of the intracranial cavity can also be visualized and sampled.

Examination of the extracranial sources of the cerebral circulation is important in many cases; if necessary and possible, the neck vessels can be visualized or removed after provisions for preservation of the external carotids are made.7,8 If removal is not appropriate, low-pressure perfusion of the common carotids with water and observation of the presence and rate of flow from the carotid siphon usually provide an assessment of patency.

**Eyes**

In cases in which examination of the eyes is indicated and authorized, their removal can be accomplished by unroofing the orbit or through an anterior orbital approach and sectioning of the extraocular muscles. The anterior orbital or external approach is accomplished by cutting the conjunctival attachments to the bulb, the extraocular muscles, and the optic nerve with the aid of ophthalmic scissors or a thin scalpel. This procedure can be greatly facilitated by using ophthalmic lid retractors to increase exposure and protect the eyelids. The intracranial or internal approach necessitates the breaking and partial removal of the orbital plate with a chisel and hammer, bone forceps, or an oscillating saw. This permits visualization of the orbital contents and removal of the globe. The posterior attachments of the globe are cut and its conjunctival margin is then incised carefully to avoid damaging the eyelids. As an alternative strategy, the posterior portion of the globe may be removed through the intracranial approach. Standard prostheses or cotton balls can then be placed in the orbit. Vitreous fluid for biochemical analyses can be aspirated with a needle-syringe while the globe is in situ.

**Dura**

The dural cap often is dissected free from the skull in the course of removing the brain. The dura is inspected for evidence of subdural or epidural hematoma and, if present, the volume of the hematoma is estimated. The dura also is examined for lesions, such as meningiomas. It may be appropriate to sample the dura for histologic examination. The superior sagittal sinus is palpated and inspected after opening longitudinally or by multiple cross sections. A sample cross section of the superior sagittal sinus, including ample tissue on each side (to include arachnoid granulations) may be taken in cases where blockage of the absorption of cerebrospinal fluid is suspected (eg, postsubarachnoid hemorrhage, meningeal carcinomatosis, or other causes of communicating hydrocephalus).
Brain

After examination of the dura, the brain is removed, weighed, and its weight recorded. Normal weights for age and sex are given by Dekaban and Sadowsky and Ho et al. The surface of the brain is inspected, lightly palpated for abnormalities, and the vasculature is examined. Abnormal findings are described, measured, and, when appropriate, photographed.

In cases with massive subarachnoid hemorrhage, the search for a suspected aneurysm is facilitated by washing away the clotted and unclotted blood prior to fixation. If an aneurysm is identified, it may then be perfused with water to validate leakage. When an aneurysm or another vascular malformation is suspected, but is not visible by external inspection even after the subarachnoid space is cleared of blood, it may be visualized by gently perfusing the suspected vessel with water or by injecting the cerebral arteries with contrast material (eg, barium-gelatin suspension) followed by roentgenography-assisted visualization. In this and other instances in which there is substantial hemorrhage, the formaldehyde solution may be changed after 1 to 2 days to facilitate adequate fixation.

Occasionally, the brain needs to be cut prior to fixation. In such a situation, placing a dry paper towel onto each tissue slice prior to fixation is advised to prevent shrinkage artifact. Most often, however, the entire brain is placed in an appropriate fixative (usually a formaldehyde solution) for a suitable period of time (usually at least several days). To prevent distortion, the brain is promptly suspended (usually by a string tied loosely around the basilar artery) in a bucket of fixative without contact to the sides or bottom.

The infant brain contains more water and less lipid than adult brain and consequently does not fix as well. After 1 to 2 weeks in formaldehyde solution, infant brains may be “hardened” by immersion in changes of graded alcohols, starting with a 70% solution.

Before the fixed brain is cut, the patient’s history and other clinical data are reviewed with attention to potential clinicopathologic correlation. Computerized tomographic scans, magnetic resonance images, or other radiologic studies may be reviewed and, if possible, are available at the brain-cutting session. Useful instruments for brain cutting may include a brain knife, scissors, scalpel handle with blade, forceps without teeth, and a rule. The brain surface is again inspected and palpated for abnormalities, such as dysmorphism, atrophy, edema, asymmetry, or focal softening. The cranial nerves and the major vessels at the base of the brain are inspected. The distribution and extent of atheroma are described. The cingulate, medial temporal, and cerebellar tonsillar regions are examined for evidence of herniation. Surface abnormalities may be photographed before sectioning the brain.

The brainstem and cerebellum usually are separated from the cerebrum by a straight transverse slice with a scalpel, often midway between the pons and the mammillary bodies (at the level of the cerebral peduncles). In doing so, care is taken to make a single smooth incision so as to leave a flat surface of brainstem from which a sample of substantia nigra-midbrain can be taken. The cerebrum is usually cut coronally at 1.0-cm intervals; alternatively, it may be cut in the plane of available computed tomography or magnetic resonance imaging films, horizontally, or sagittally, with or without the cerebellum and brainstem attached, to provide optimal clinicopathologic correlation.

The cut surfaces of the slices are examined methodically and carefully; gross lesions can be sampled for histologic examination only if they are detected at this step. Lesions are measured, described, and, when appropriate, photographed and sampled. If appropriate, thinner slices are cut (0.5 to 0.75 cm) to search for elusive metastases, infarcts, etc, as might be indicated by radiologic or clinical information or by initial findings at brain cutting. In certain cases, thicker slices (2.0 to 5.0 cm) may be required because of the friability of the brain, notably in the setting of massive intraparenchymal hemorrhage, tumor, or cases of postmortem autolysis.

The cerebellum and brainstem may be cut in many different ways. Some pathologists prefer to leave the cerebellum and brainstem attached to each other and to cut in a plane transverse to the axis of the brainstem, usually at 0.5 to 1.0-cm intervals. The cut surfaces of these slices are then inspected carefully, and lesions are measured, described, photographed, and sampled as appropriate. Other pathologists separate the cerebellum from the brainstem by cutting through the cerebellar peduncles. The cerebellum can then be cut sagittally to demonstrate atrophy of the anterior vermis (as in alcoholism or certain cerebellar system degenerations) prior to axial sectioning. Alternatively, the cerebellum can be cut in still another plane, such as one parallel to the axis of the brainstem.

To ensure an accurate sagittal section of the brainstem, the brain can be placed upside down (circle of Willis uppermost), allowing a smooth ventral to dorsal sagittal cut to be made. While the cut is being made, the brainstem, which is often twisted slightly to one side during fixation, is clearly visualized and positioned so that it is cut precisely in the midline.

Spinal Cord

Removal of the spinal cord, when indicated, is usually accomplished through an anterior approach after the contents of the thoracic and abdominal cavities have been removed. The paravertebral muscles are freed from the vertebra, permitting visualization of the vertebral pedicles and the emerging peripheral nerves and plexuses (cervical and lumbar). Cutting the pedicles is accomplished with an oscillating saw; many pathologists prefer a fan-tailed blade. The angle of cut to the vertebral body varies from horizontal in the lumbar spine, oblique at thoracic levels, and nearly vertical to the cervical spine. Care is taken not to cut too deeply into the spinal canal. Horizontal cuts through intervertebral disc spaces permit a partial or extended removal of the vertebral column and spinal cord. Whether the approach is anterior or posterior, the dura at the foramen magnum needs to be transected completely. This is accomplished more easily from an intracranial approach, but can be done with considerable difficulty from below by inserting the scalpel blade between the dura and surrounding bone for the entire circumference of the cord.

After the column of vertebral bodies is removed, the cord in its dural enclosure is examined in situ. The cord is gently lifted from the spinal canal by cutting through the nerves just distal to the dorsal root ganglia as they reside in the intervertebral foramina. Removal of the cervical cord, in cases of neck trauma or malformations (eg, Klippel-Feil or Arnold-Chiari syndrome) is more difficult and may necessitate posterior removal. If a posterior ap-
proach is necessary, the midline skin and the paraspinal muscles are incised, permitting visualization of the vertebral lamina. The laminae are cut with an oscillating saw about 2.0 cm from the midline and at an angle to the lamina of about 45°. The posterior arches are then removed, exposing the cord, which can be removed after in situ examination. One avoids compression or tension on the cord, because handling artifacts can disfigure normal anatomy as well as gross and microscopic lesions. After removal of the spinal cord and nerve roots, the anterior and posterior faces of the spinal canal are examined for masses that could impinge on the spinal cord or for other lesions, which can be matched to the corresponding level of the spinal cord. The anatomic levels of spinal cord can be identified following criteria given by Hughes, Hirano, and Weller.

The ventral and dorsal surfaces of the spinal cord may be examined after cutting the dura longitudinally in the ventral and/or dorsal midline. The ventral surface of the spinal cord is identified easily by the single anterior spinal artery. Alternatively, especially in cases where pathologic material (eg, tumor, blood, pus, or herniated cerebellar tissue) fills the subdural or subarachnoid space, it may be preferable to leave the dura intact and, after fixation, take cross sections of spinal cord for histologic examination that include the unopened dura and nerve roots. The spinal cord may be fixed in a container long enough to allow it to lie straight, suspended in a cylinder of fixative, or it may be placed in the same container as the brain, if the dura has not been opened.

At brain cutting, the spinal cord traditionally is cut transversely, often at 0.5- to 2.0-cm intervals and more closely at levels where gross inspection or clinical history suggest the presence of a lesion. The cut surfaces are inspected for pathologic changes; these are described, measured, photographed, and sampled as appropriate.

Photography

When indicated, photography usually is performed after the brain is fixed and before taking samples for histology. Occasionally, photography of the unfixed or in situ brain may be appropriate at the time of autopsy (eg, in the case of an acute subdural hematoma unattached to dura or before washing off blood in a case of subarachnoid hemorrhage). Sometimes it may be important to take pictures of the surface of a fixed, abnormal brain before it is sectioned.

Photography of lesions in brain slices serves the important function of documenting the gross appearance and the precise location of a lesion for clinicopathologic correlation or for other purposes. Photographs usually include a metric rule as well as the identifying number. Occasionally, it may be desirable to include a section of a normal brain for comparison. A black or dark blue background usually provides optimal contrast.

Sampling for Histology

The choice of blocks from brain and spinal cord, if removed, will depend on the nature of the case and the goals of the autopsy. It is prudent to take blocks from the major regions of the central nervous system, as well as from significant lesions. A list of regions to be sampled might include the following:

- Cerebral cortex, including overlying meninges, such as the junction of superior and middle frontal gyri. (In de-

menting diseases, additional sections may be needed [see “Autopsy Guidelines for Alzheimer's Disease”].)
- Hippocampus with adjoining temporal cortex
- Basal ganglia, including striatum (caudate and putamen), globus pallidus, internal capsule, and hypothalamus
- Midbrain with substantia nigra and cerebral peduncle
- Pons with locus ceruleus and base of pons
- Medulla with inferior medullary olives and pyramids
- Cerebellar cortex and deep white matter with dentate nucleus
- Spinal cord, including lumbosacral enlargement with roots, if available. If the entire spinal cord is removed, representative sections of each major level (cervical, thoracic, and lumbar) may be included in one cassette.

This list of blocks would constitute less than 1% of the tissue of the central nervous system. Therefore, it is important to conduct a close and thorough inspection of the brain for macroscopic lesions, to have a thorough knowledge of areas typically affected in particular diseases, and to develop an awareness of areas likely to be involved with the expression of signs and symptoms as displayed by the patient.

After sampling, the sliced brain is returned to fixative until the case is signed out. When unexpected lesions are detected microscopically in routine sections of brain, further blocks may be taken. Moreover, additional tissue will be available should consultation with a neuropathologist become necessary.

Paraffin processing and special neurohistologic stains are described by Earle, Hirano, and Weller, and in texts on laboratory techniques.

Reporting

An autopsy is performed to gain information concerning a patient's illness, response to therapy, and mode of death. Thorough, accurate, and timely reporting of initial and final autopsy findings to the patient's physician and family is an essential component of the autopsy. Autopsy documents, in addition to photographs and histologic slides, also make up an important part of the permanent record of the patient.

Generally, the neuropathologic findings of an autopsy are reported in three parts: (1) gross autopsy findings, (2) findings made at the time of brain cutting, and (3) histologic findings described and integrated with the gross findings in a final report on the case. If indicated, negative (normal) and positive (abnormal) findings are included.

In addition to abnormal findings, the report usually includes the brain weight; the results of inspection of the intracranial cavity, spinal canal, dura, brain (including major vessels and cranial nerves), and spinal cord (including vessels, roots, and spinal ganglia); and relevant negative information that concerns issues raised by the clinical course.

Contents of Report

The report on the fixed dura, brain, and spinal cord usually includes the following:

- Brain weight after fixation if fresh brain weight was omitted
- Description of brain surface (eg, meninges; presence/absence of brain atrophy, edema, asymmetry, or focal softness on palpation and the location and size of ab-

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not given appropriate consideration. More recently, a practical standardized neuropathology protocol has been developed for the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD), a multicenter study supported by the National Institute on Aging. The CERAD protocol, used by many neuropathologists at Alzheimer’s disease research centers, establishes levels of diagnostic certainty, ie, “definite Alzheimer’s disease” or “probable Alzheimer’s disease,” and promotes common language among pathologists. An illustrated primer for pathologists, using these criteria, with sample cases and recipes for two commonly used stains (modified Bielschowsky and thioflavin S), is also available.

Following evaluation of the gross brain as detailed in this guideline, as in the CERAD publication, or as in the practical primer for pathologists, a minimum of seven sections are recommended for tissue sampling: four regions of neocortex (superior-middle temporal gyrus, middle frontal gyrus, inferior parietal lobule, and anterior cingulate gyrus); hippocampus; amygdala with entorhinal cortex; and midbrain, including the substantia nigra. These sections are embedded in paraffin. Recipes for recommended stains (Bielschowsky silver impregnation and/or thioflavin S fluorescent stain) and specific case examples are also provided in the primer or in standard sources. The silver- or thioflavin-stained neocortical sections are evaluated for the frequency of senile plaques of the neuritic type, as well as neurofibrillary tangles, and are scored using a semiquantitative rating system (eg, sparse, moderate, or frequent). This grade is then correlated with the patient’s age to arrive at an age-related plaque score, which is combined with the clinical history (presence or absence of dementia) to determine the diagnosis. If desired, a more detailed protocol is described in the CAP publication Autopsy Performance & Reporting.

The overwhelming majority of Alzheimer’s disease cases will fit not only the CERAD criteria for “definite Alzheimer’s disease,” but will also fulfill the quantitative guidelines proposed by the 1984 consensus panel. Cases lacking the classic changes of Alzheimer’s disease may be referred to a neuropathologist for consultation. Other conditions associated with dementia include multiple infarcts, Pick’s disease,Binswanger’s encephalopathy, posttraumatic or postanoxic encephalopathy, normal pressure hydrocephalus, Parkinson’s disease with dementia, Lewy-body disease, amyotrophic lateral sclerosis with dementia, Huntington’s disease, chronic cerebral infection, Creutzfeldt-Jakob disease of long duration, Wernicke’s disease, other alcohol-associated dementias, hippocampal sclerosis, and cases in which no specific neuropathologic lesions are identifiable.

Therefore, an autopsy of a patient diagnosed clinically as having Alzheimer’s disease may need to include appropriate steps for diagnosis of any of the conditions listed above. If Creutzfeldt-Jakob disease is a diagnostic consideration, the pathologist takes special precautions in the handling of the autopsy. This would be particularly appropriate in a patient with a rapid clinical course, early onset of myoclonus (shocklike muscle contractions), and characteristic changes on electroencephalogram. Recommendations for the handling of such tissue have been recently updated by the CAP Autopsy Committee. A simple and effective procedure in which formaldehyde-fixed tissue is disinfected by immersion in formic acid for 1 hour has been described.
AUTOPSY PROCEDURES FOR NEUROMUSCULAR DISORDERS

At postmortem examination it may be possible to diagnose some neuromuscular diseases by routine histologic sections; however, more often than not additional histopathologic stains, enzyme histochemistry, immunocytochemistry, or electron microscopy are required. These specialized studies may call for consultation with a neuropathologist. Adequate preservation and storage of tissue obtained at autopsy are the first steps toward securing the appropriate evaluation.

The Muscular System

While sampling muscle, specimens of nerve also are collected. Proper evaluation of neuromuscular disease usually requires both specimens. The prossector decides in advance which specimens are needed and how to preserve them. For assistance with this decision, the prossector may profit by referring to references 23 through 25.

It is best to sample muscle from both proximal and distal portions of the extremities, as well as the pertinent muscles and nerves determined to be clinically diseased. The proximal and distal leg muscles and nerves can be sampled without disfiguring the body. Two muscles that are very useful to examine are gastrocnemius and quadriceps. Not only are these easy to obtain, but they provide considerable data with respect to the muscle fiber types and size distributions from biopsy specimens, which permits comparison with normal and abnormal conditions. The upper extremities are more difficult to sample because the incisions might be visible. However, with the routine Y-incision, the deltoid muscle is accessible by extension and undermining of the main incision. It is inadvisable to sample muscles of the hands and forearms unless you receive specific consent to do so. Generally, these muscles are not examined unless the body is to be cremated or the funeral is to be held with a closed casket.

When sampling muscles, tendon insertion sites, muscles that are severely involved or end-stage, and sites of recent electromyography or biopsy are avoided. There are a number of standard references24,25 that describe the technique of obtaining muscle tissue. Each muscle sample, if feasible, consists of fixed tissue for paraffin sections, “flash” frozen tissue for histochemical or biochemical studies, and specially fixed tissue for electron microscopy.

Handling the Muscle Specimen

If the type of neuromuscular disease from which the patient suffered is uncertain, save enough tissue to permit all types of studies. One can always omit certain studies at a later time, but a necessary test cannot be performed without a preserved specimen.

In addition to paraffin sections that may include special stains, enzyme histochemical studies are performed on frozen sections of skeletal muscle. Cross sections are optimal for these histochemical studies. The tissue cylinder, which is approximately 1.0 cm long and 0.5 to 1.0 cm in diameter, is prepared for freezing. Methods for freezing are available in standard texts.24,25

Once the specimen has been frozen, it is kept frozen in an airtight container to prevent desiccation. The tissue is best stored in an ultralow freezer (−70°C). If only a cryostat is available for preserving the frozen specimen, one may need to override the defrost cycle manually.

Muscle, when frozen in the proper manner, can be used for routine histopathology: hematoxylin-eosin stain, periodic acid–Schiff for glycophen, and Sudan stains for neutral lipid, and for immunofluorescent studies. Enzymes can also be evaluated, especially if the autopsy has been performed promptly after death. The commonly studied enzymes are adenosinetriphosphatase, dehydrogenases, tetrazolium reductases, and acid and alkaline phosphatases. Immunocytochemistry and molecular studies, including in situ hybridization, can be performed on tissue preserved in this manner. After the appropriate frozen tissue sections have been cut, the remaining tissue can be thawed in formaldehyde solution, embedded in paraffin, sectioned, and stained in a routine fashion.

Techniques for processing specimens for electron microscopy are found in standard references.24,25 A common fixative is either 2% to 5% glutaraldehyde or paraformaldehyde/glutaraldehyde prepared in either 0.1 mol/L cacodylate buffer, pH 7.2, or 0.1 mol/L phosphate buffer, pH 7.2. Formaldehyde solution is not a preferred fixative for electron microscopy.

Tissue to be used for biochemical studies can be excised, snap frozen (liquid nitrogen, dry ice, isopentane/dry ice, or isopentane/liquid nitrogen), and stored in a −70°C freezer or on dry ice. The samples are frozen in small aliquots both to permit rapid freezing and to avoid repeated cycles of freezing and thawing, which might be necessary when performing multiple assays at different periods of time and in different laboratories. Muscle handled in this manner can be stored indefinitely without significant loss of enzymatic activity or substrate. Tissue stored at −20°C may be unsuitable for certain assays.

The Peripheral Nervous System

Lesions in the peripheral nervous system are often focal or regional; this necessitates directed sampling in accord with the clinical history. These lesions are difficult to document without using specialized techniques, such as 1.0-μm-thick (semithin) plastic section evaluation or teased nerve fiber analysis. The following protocol is suggested to document lesions such as vasculitis and amyloidosis using routine paraffin processing or to obtain samples for referral to centers where more specialized analyses are performed.

A limited portion of the peripheral nervous system is accessible through the usual Y-shaped incision; specific authorization in the autopsy permit may be necessary for additional incisions in the limbs. Available through the Y-shaped incision are the nerve roots in the spinal canal, dorsal root and sympathetic ganglia, nerve trunks, and lumbar as well as brachial plexuses. Somatic nerves may be found in proximal muscles, sensory nerves in the skin of the torso, and autonomic nerves in the paravertebral ganglia and in the nerves through various viscer. When the autopsy permit allows for the examination of nerves in the limbs, the pathologist may consider sampling long segments of nerves, such as the proximal and distal sciatic and particularly the sural nerve in the calf. Sampling proximal and distal nerves provides the large volume of tissue necessary for documenting diseases with localized diagnostic lesions. Distal nerves may show only the end-stage changes resulting from the accumulation of multiple proximally placed lesions.

Considerable data are available for the sural nerve since it is the preferred site for nerve biopsy. This nerve may be difficult to locate; consequently, we will discuss its acqui-
cision in some detail. It may be obtained by a 6.0- to 10.0-cm incision beginning halfway between the lateral malleolus and the Achilles tendon, and extending proximally parallel to the Achilles tendon. Dissection is continued down to the fascial plane overlying the peroneus muscle. Within the subcutaneous-deep dermal fat, the nerve can be identified and then carefully dissected from the surrounding fat. The sural nerve may be difficult to distinguish from a sclerotic lesser saphenous vein, which often runs parallel to the nerve. Pinching or bending the nerve is avoided. It is manipulated by grasping the fine epineurial connective tissue so as to avoid the nerve fascicles proper. At least 2.5 cm of nerve is desirable for the multiple procedures.

Nerve specimens can be slightly stretched at each end using forceps on the epineurium and allowed to adhere to a piece of stiff paper (such as an index card) for 10 seconds. The excess paper is cut away and the nerve specimen is then immersed in buffered glutaraldehyde or, if available, glutaraldehyde/paraformaldehyde at room temperature. Alternatively, the nerve may be suspended in fixative with a small-gauge needle (#25 or #27) inserted in the free end as a weight. Fixation in formaldehyde solution causes distortion of peripheral nerve and is avoided if possible. After several minutes of fixation, the nerve can be removed from the paper and it will maintain its straight, slightly stretched condition. The nerve remains in the room-temperature fixative for approximately 2 hours before fixation overnight, preferably at 4°C. Cross and longitudinal sections are taken by using a fresh razor blade with a drawing rather than pressing motion. This fixed nerve can then be used for embedding in epoxy for 1.0-μm-thick (semithin) plastic sections, for paraffin histology, and, following maceration in either glycerin or in epoxy resin, without a hardening agent for teased nerve fiber preparation.

At least 0.5 cm of nerve may be frozen at the time of autopsy for other special studies. For additional details, consult references 23 through 26.

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