



Best Practice No 158

Neuropathology

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Health and safety

It is essential that staff are aware of the health and safety issues.¹⁻¹⁶

The main danger of infection in neuropathology arises from the handling and processing of tissues from cases of AIDS, Creutzfeldt-Jakob disease (CJD), and tuberculosis.

All members of the department and mortuary staff should be immunised against hepatitis B. Because potentially dangerous cases can be encountered unexpectedly, technique should always be of a high standard and appropriate to the safety of all staff involved in the investigation. Any case of rapidly progressive dementia must be assumed to be a case of CJD until proved otherwise. The treatment of workplace, clothing, and instruments should be appropriate.

If a diagnosis of CJD is possible, samples of frozen brain, usually frontal lobe and cerebellum, should be retained for prion protein subtyping and prion protein gene sequencing. Advice is available from the CJD Surveillance Unit, Edinburgh.*

Samples from cases of CJD should be handled in a class 1 cabinet in a separate level 3 containment room. Blocks for sectioning need to be processed using an appropriate inactivating agent, currently immersion in neat formic acid for at least one hour.

Cases of human immunodeficiency virus (HIV) infection can be dealt with at containment level 2 with appropriate disinfection afterwards.

Brain biopsies

Although the general principles with regard to the processing of biopsy material in neuropathology are similar to those in general histopathology, there are several important differences. Biopsy of the central nervous system is potentially dangerous for the patient. To avoid a second biopsy it is important that the surgeon knows that representative tissue has been removed. Stereotactic techniques have greatly improved the accuracy of the technique, but the amount of material removed is usually small. Fortunately, most specimens smear easily, enabling a diagnosis to be made within a few minutes.

Small specimens dry out rapidly and should be delivered to the laboratory immediately in a sterile bottle containing damp gauze. What is done with the specimen will depend upon its size and consistency. Some tissues smear more easily than others and this will also affect the quality of fixation. Tougher specimens require frozen sections, which are particularly useful in assessing architectural pattern, but there is some loss of cytological detail.

The neuropathologist should be aware of clinical and radiological details, including age, sex, and anatomical site of the lesion. The request form should include the following information:

- Name, date of birth, address, registration number, and sex of patient.
- Laboratory identification number.
- Consultant, hospital, and ward.
- Site and size of lesion and nature of the specimen.
- Investigation required.
- Clinical history, including history of relevant previous surgery, immunosuppression, radiotherapy, dementia, or recent travel abroad.
- Radiological characteristics and diagnosis.
- Name (in capitals), signature, and bleep number of requesting clinician.
- A summary of Health and Safety and Data Protection Act requirements, including information on how to submit potentially dangerous specimens.

SMEAR TECHNIQUE

Pieces of tissue approximately 2 mm in diameter are smeared between glass slides taking care not to crush cells by applying too much pressure. A variety of techniques are used for fixation and staining, the latter usually being either haematoxylin and eosin or toluidine or methylene blue. Some laboratories use a mixture of stains. It is important that the pathologist is familiar with the stain used.

FROZEN SECTIONS

Frozen sectioning might be required if the tissue is too tough to smear, and is particularly useful in assessing cellular architecture, but the quality of cytology is poorer than in the case of smears.

In most cases, the smear or frozen section should provide a diagnosis and serve as a guide

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as to how the specimen should be processed. Samples might need to be taken for microbiology, electron microscopy, or for analysis after freezing in liquid nitrogen. Some immunohistochemical techniques require cryostat sections on fresh tissue. The remainder should be fixed in formaldehyde.

It is important that the surgeon realises that a report on a small sample might not be representative of the whole. For example, a biopsy from the edge of a highly malignant glioma might be "low grade". The neuropathologist must also be aware of the difficulty of distinguishing between reactive gliosis and neoplasm.

MACROSCOPIC DESCRIPTION

All specimens should be described, and in the case of larger specimens, weighed. After fixation, larger specimens (such as lobectomies) should be described, taking care to record the appearance of the surfaces. The specimen should then be sliced and any abnormalities on the cut surfaces described. Drawings and photography are useful in some cases. Blocks should be taken to include the deep edges and sides to assess the extent of involvement and adequacy of excision, and should include areas with different appearances and consistency, because these might show variations in pathology, such as evidence of dedifferentiation of a neoplasm. It is particularly important to include areas of necrosis and any brain tissue or meninges around the edge of a neoplasm to assess invasion.

MICROSCOPY REPORT¹⁷

The specimen might contain a variety of normal tissue components such as grey and white matter, cerebellum, meninges, or choroid plexus. Each of these should be recorded.

A variety of tissues might be present in congenitally abnormal lesions. All components and any anatomical or pathological changes within them, including the presence of a cyst or sac should be described.

If a neoplasm is present, the report should contain information on the cell type(s) present, the cellularity, growth pattern, and tumour grade. The most commonly used grading system in the case of gliomas is that of Daumas-Duport,¹⁸ which is based upon the presence of mitotic figures, pleomorphism, necrosis, and vascular proliferation. The WHO has devised a new grading system; however, this has not proved to be as popular as the former system.¹⁹ Information on the nature of the stroma such as gliosis, oedema, fibrous tissue, vascularity, evidence of haemorrhage (recent or old), thrombosis, changes in blood vessel walls, and the presence of mucin, calcification, or other abnormal substances should be mentioned. Finally, in the case of a neoplasm, the report should comment upon the extent of infiltration and the structures infiltrated.

In the case of inflammatory lesions, the report should include information on the cell types present, on their approximate proportions, on their location, and any associated vascular abnormalities. The presence of necrosis

might imply the formation of an abscess and some estimate of its stage of evolution might be acquired from the presence or absence of a capsule and its structure. Occasionally, microorganisms such as bacteria, fungi, protozoa, or metazoa might be identified, and the presence of inclusion bodies might be helpful in the diagnosis of viral infections. The presence of demyelination might raise the possibility of progressive multifocal leucoencephalopathy.

Biopsy is occasionally carried out on unusual cases of dementia. The report should include information on the cellularity, cell changes (particularly the presence of neurofibrillary tangles, Lewy bodies, and Pick bodies), the presence of gliosis, demyelination, microglial nodules, necrosis, abnormalities of blood vessels, and spongiform change.

Tissue removed from the spinal canal presents some special problems and the need for decompression and treatment might be urgent. The sample often contains bone and requires comment upon the trabeculae and marrow constituents. The range of tissues present and any abnormalities seen should be recorded. Full assessment might take a few days, and might include obtaining the opinion of other specialised pathologists such as an osteopathologist, but treatment for a compressing neoplastic lesion can often start before this process is completed, and can be modified later.

A variety of techniques have been used to provide some indication of prognosis, including flow cytometry to detect changes in DNA profile²⁰; the immunohistochemical demonstration of a thymidine analogue, bromodeoxyuridine,²¹ which is injected intravenously at the time of craniotomy; and the use of a variety of markers of cell proliferation, such as proliferating cell nuclear antigen (PCNA) and Ki67. There is general consensus that these techniques are of limited use in the individual patient and results must be interpreted with great caution.

Postmortem handling of brain and spinal cord²²

FIXATION AND DESCRIPTION

The brain should be fixed in 10% neutral formaldehyde by suspending it in a large plastic container by the basilar artery for one month. Adequate fixation requires immersion in at least 10 times the volume of fixative. It is usually not possible to provide containers of this size, so the formaldehyde should be changed after 48 hours and at weekly intervals for one month. Before examination, the brain should be washed in running water for at least two hours. It should have been weighed at the time of necropsy, but if this has not been done it should be weighed fixed. Its macroscopic features, including meninges, patency of the sinuses (if present) and other blood vessels, cranial nerves, and any abnormalities of surface anatomy should be described. A dictation machine with foot control is the most satisfactory method for description.

Features of the overall appearance that are particularly important include the presence of areas of softening, swelling, haemorrhage, and

herniation effect (movement of the cingulate gyri across the midline, the situation of grooves on the hippocampal unci, distortion of the midbrain, herniation of the cerebellar tonsils into the foramen magnum, compression or stretching of cranial nerves, particularly the third cranial nerve, and ischaemic changes in the territory of the posterior cerebral arteries).

The brainstem and cerebellum can be detached by a horizontal section through the upper pons, followed by detachment of the cerebellum by sectioning the cerebellar peduncles. The brainstem should be sectioned horizontally throughout its length and the cerebellum sectioned both horizontally and obliquely through the tonsils on each side. The presence of haemorrhages within the brainstem, their site and size, distortion of the midbrain and brainstem, and compression of the aqueduct should be noted.

The cerebral hemispheres are usually sectioned coronally, although horizontal slices are sometimes useful for comparison with computed tomography scans. The first coronal slice should be made at the level of the mamillary bodies and at 1 cm intervals backwards and forwards from this using a spacing device composed of two L shaped metal guides of that thickness. Thinner slices can be made by inserting a piece of plastic of the relevant thickness.

The appearances of the slices should be described and blocks taken. Line diagrams are useful for recording the site and size of abnormalities, and photographic records are helpful in some cases, particularly for teaching and medicolegal purposes, and for demonstrating cases at meetings.

The range of blocks taken will depend on the clinical history, pathological features, and possible diagnosis. Blocks should be laid out in sequence on a plastic sheet, labelled alphabetically, and described. It is important to record abnormalities seen within various blocks at this stage, so that the pathologist is reminded at the time of microscopy. The back of the block should be marked for identification of the face to be cut. This can be done with a groove cut in the back or with Indian ink, and one side can also be marked with a small groove. A record should be made of the range of stains to be carried out at this stage and smaller blocks for immunohistochemical and other complex stains should be taken.

The general principles involved in the reporting of some of the main groups of disorders are summarised below.

NEURODEGENERATIVE DISEASE

The most common cause of dementia is Alzheimer's disease. Because of the lack of unanimity on the clinical and pathological criteria required for a diagnosis, guidelines based upon an age related minimum number of plaques and neurofibrillary tangles in each low power microscopic field ($\times 100$) have been established. This is known as the CERAD protocol (consortium to establish a registry for Alzheimer's disease).²³ This takes a semi-quantitative approach to the assessment of plaque frequency and correlates this with the age of

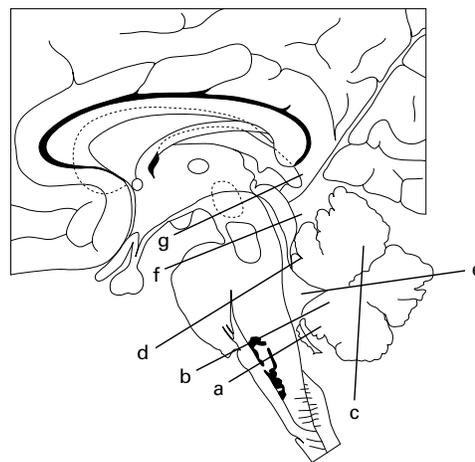


Figure 1 Sections of the brainstem that are useful in the investigation of neurodegenerative disease. (a) The medulla at the level of the olivary nuclei; (b) the medulla/pons transition; (c) the cerebellum to include the tonsil; (d) the midpons; (e) the cerebellum to include the vermis and dentate nuclei; (f) the midbrain at the level of the inferior colliculi; (g) the midbrain at the level of the superior colliculi.

the patient, resulting in an "age related plaque score". It is essential that the pathologist obtains a clinical history

Figures 1 and 2 illustrate a range of blocks that is adequate for most cases of dementia.

HEAD INJURY

The three main problems in cases of head and neck injury are:

- (1) Determining the cause of death.
- (2) Defining the mechanism of injury.
- (3) Determining the relation of haemorrhage to an episode of trauma. For example, an accident might be the result of spontaneous haemorrhage rather than the cause of it, and this can include haemorrhage into the meninges, including the subdural space, from a ruptured aneurysm.

Bony injuries to the skull and spinal column will have been described at the time of the postmortem examination. It is essential to remove the dura mater from the intracranial cavity because thin fracture lines can easily be hidden. Great care is required in assessing injuries to the spinal column; this will sometimes require removal of part of the spine for examination after fixation. Injuries to ligaments, joints, pedicles, spinous processes, intervertebral discs, vertebrae, vertebral arteries, meninges, and underlying spinal cord might provide important clues about the mechanism and severity of injury. Relevant x rays should be examined before dissection.

Many head injuries are associated with intracranial haemorrhage, which might or might not be associated with distortion of the brain. The size and site of haemorrhage, its relation to fractures and damage to the brain, and to the degree of cerebral swelling, distortion, and flattening should be noted. Examination of the cingulate gyri, hippocampal unci, parahippocampal gyri, corpus callosum, ventricles, midbrain, brainstem, cerebellar tonsils, and superior cerebellar peduncles is important in assessing shift effects.

Damage to the surface of the brain, including its site, should be described and measured. Lacerations differ from contusions in that the pia-arachnoid is torn in the former. It is important to realise that patients with head injuries can die without contusions. Herniation contusions occur in the parahippocampal gyri and the cerebellar tonsils when they come into contact with the tentorium and foramen magnum,

respectively. The presence of contusions in the parasagittal region and areas of haemorrhage within the corpus callosum are particularly useful in the assessment of diffuse brain damage.²⁴ Sections of the brain will demonstrate the depth of contusions and the presence of other areas of haemorrhage within the white matter or deeper structures. Haematomas that are not in contact with the surface of the brain

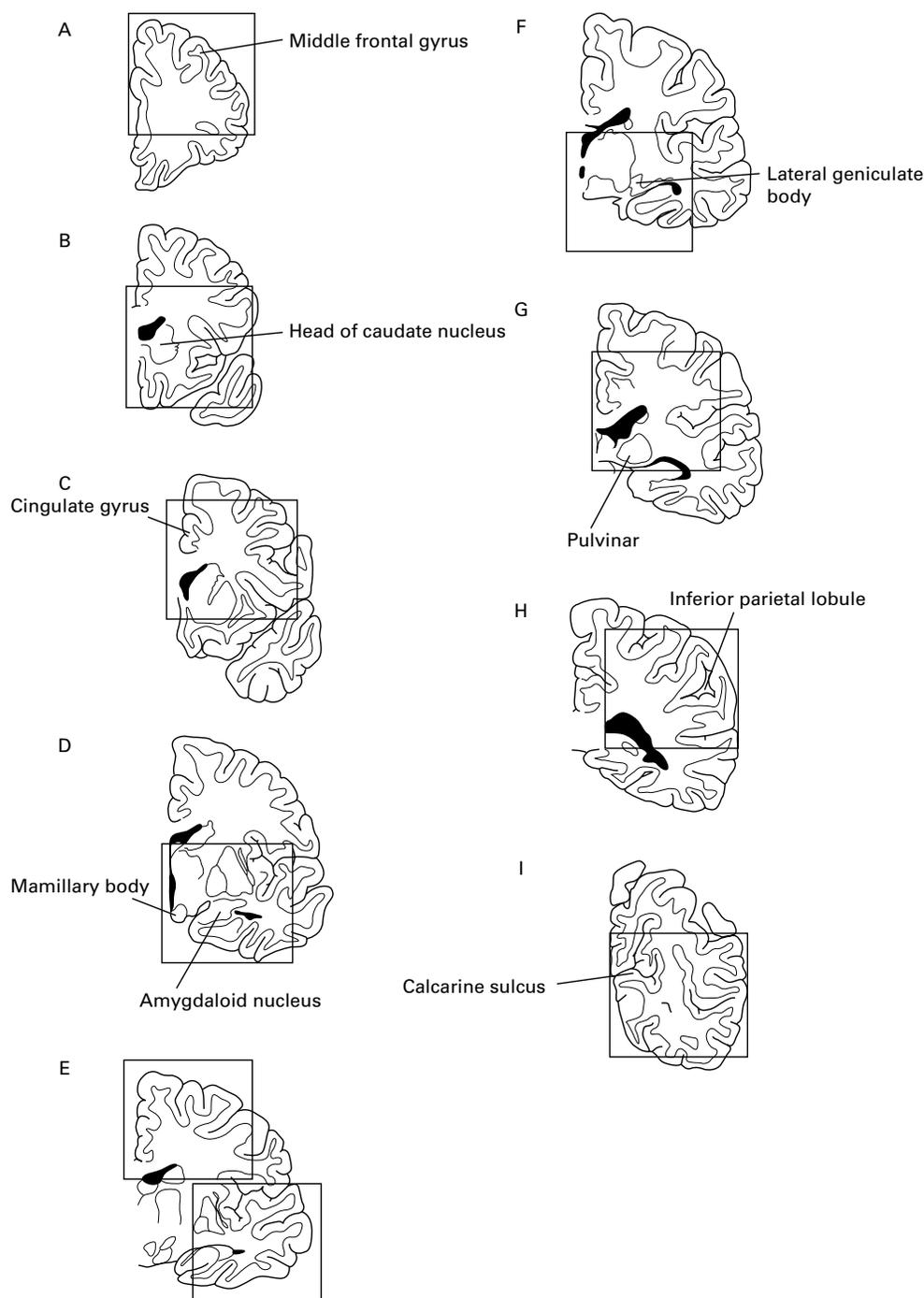


Figure 2 Sections of the brainstem that are useful in the investigation of neurodegenerative disease. (A) The frontal lobe to include the middle frontal gyrus. (B) The midfrontal lobe to include the head of the caudate nucleus and periventricular white matter. (C) The anterior cingulate gyrus. (D) The temporal lobe at the level of the mamillary body to include the amygdaloid and lentiform nuclei. (E) The temporal lobe to include the superior and middle temporal gyri, and the posterior frontal lobe to include the underlying cingulate gyrus. (F) The temporal lobe at the level of the lateral geniculate body, including the hippocampus. (G) The parietal lobe at the level of the pulvinar to include the periventricular white matter. (H) the midparietal lobe to include the inferior parietal lobule. (I) The occipital lobe to include the periventricular white matter and the calcarine cortex.

are common and may relate to trauma or indicate spontaneous haemorrhage. The anatomical site, size, and distribution are important. Even after the most thorough postmortem investigation it can be impossible to decide the cause of a haemorrhage, and much depends upon the circumstances of the episode of trauma and the statement of witnesses. Histological examination of areas of haemorrhage will sometimes demonstrate a cause, such as a vascular malformation or neoplasm.

Lesions that might be important in the assessment of acceleration injuries are tears and haemorrhages in midline structures such as the corpus callosum, septum pellucidum, internal capsule, cerebral peduncles, the dorso-lateral quadrant of the rostral brainstem, and the superior cerebellar peduncles. Blocks should be taken from these areas for histological examination, but great care is needed in the interpretation of the importance of axoplasmic bulbs. These can occur in similar areas of the brain in the absence of trauma, such as in hypoxia and after artificial ventilation.²⁵ More research needs to be carried out to determine the role of hypoxia, cerebral swelling with compression of axons, and artificial ventilation in their formation. A useful immunohistochemical stain for their detection uses an antibody to amyloid precursor protein. In addition, after two or more days small clusters of microglial cells may be present.

INFECTION

The main aims in the investigation of infection in the central nervous system (CNS) are to identify the cause, the route of the infection, and its effects on the nervous system. The potential source of infection will have been demonstrated at the time of the postmortem examination in most cases, but some will require further dissection of other structures such as sinuses, middle ear, skull bone, and spine. Congenital malformations, including those of the heart, and the presence of a shunt will sometimes be relevant.

It is important to remember that the brain might show no macroscopical abnormalities, particularly in the case of viral infection, and when organisms of low virulence that are often encountered in very young, elderly, debilitated, and immunosuppressed individuals are involved.

Specimens for microbiological investigations should be taken at the time of necropsy, and it is sometimes useful to keep samples of frozen cerebrospinal fluid (CSF) and serum for antibody studies. The advice of a microbiology department should be sought regarding the most appropriate transport medium. If a virus infection is suspected it is best to take samples for electron microscopy before fixing the brain and/or spinal cord. It is important to take samples from parts of the brain or spinal cord that are not necrotic, but show evidence of inflammation.

Blocks for histological examination should be taken from areas that appear normal, from areas that are obviously abnormal, and from transition areas between them. Routine blocks

should include each of the four main lobes, thalamus, basal ganglia, hippocampus, brainstem at several levels, cerebellum, and whenever possible the cervical, thoracic, and lumbar spinal cord, and dorsal root ganglia.

Histological identification of viruses has been greatly improved recently by immunohistochemistry and *in situ* hybridisation. The polymerase chain reaction (PCR) can be useful in the diagnosis of herpes simplex encephalitis and a variety of other agents such as JC virus, using a sample of CSF. Although more sensitive than serology, it might still be negative, depending upon the site of infection and its proximity to the CSF. A variety of sensitive molecular amplification techniques is available for use on frozen tissue samples, but sensitivity will be compromised if only paraffin wax embedded tissue is available.

Other techniques such as immunohistochemistry and hybridisation with DNA probes can be applied to frozen biopsy samples.

VASCULAR DISEASE

Postmortem assessment of vascular problems affecting the CNS requires examination of the carotid arteries and sometimes the vertebral arteries in the neck. This will often require relevant specimens to be retained for dissection after fixation. If the spinal cord is involved it might be necessary to remove the spinal column with the abdominal aorta attached.

The main aims in the assessment of bleeding within the intracranial cavity are to define the site, amount and extent of the bleeding, the source and cause of the bleeding, the age of the haemorrhage, whether bleeding has occurred on more than one occasion, and the role of haemorrhage in the cause of death.

If a ruptured aneurysm is likely to be the cause of haemorrhage it is advisable to look for aneurysms before the brain is fixed. Aneurysms are usually found at the point of bifurcation of the vessels of the circle of Willis, and bleeding is usually most intense at the site of an aneurysm. Blood should be removed by blunt dissection and gentle washing until the aneurysm and its point of rupture have been identified. It is important to realise that there might be more than one aneurysm.

Slicing of the brain after intracerebral haemorrhage might be difficult. It is sometimes preferable to remove as much of the blood clot as possible before fixation. Histological examination of the blood clot might reveal the cause of haemorrhage, particularly if the cause was a neoplasm, one of many haematological disorders that can be associated with an increased risk of haemorrhage, or a small vascular malformation. Larger vascular malformations and deeply situated aneurysms are usually recognisable macroscopically.

In some cases the cause of the haemorrhage might be venous. This will usually be apparent from the pattern of bleeding.

It is important to take blocks for histological examination from both abnormal and macroscopically normal parts of the brain and the meninges. Diseases of blood vessels that can cause haemorrhage or thrombosis include

many disorders that can only be recognised histologically, such as the various types of amyloidosis and vasculitis. Congophilic angiopathy, which accounts for up to 20% of strokes in elderly patients, is frequently seen in association with Alzheimer's disease, and a relevant range of blocks will need to be taken.

Examination of the brain is essential in assessing the effects of hypoxia and of problems that might have affected the uptake of oxygen by the brain, such as hypoglycaemia. Neurones show selective vulnerability, and there are also regional variations that might be the result of differences in blood supply. Some abnormalities, such as laminar necrosis within the cerebral cortex, might be recognisable macroscopically, but microscopical examination of a wide range of blocks including the cerebral cortex, hippocampus, cerebellum, caudate nucleus, putamen, globus pallidus, thalamus, hemispheric white matter, and brain stem is required. It is important that the pathologist is familiar with the changes associated with the "respirator brain".

TUMOURS

As in the case of any space occupying lesion, the effects of the mass on the brain and spinal cord should be described. These will vary according to the anatomical site of the lesion. It is also important to anticipate the effect of associated abnormalities such as epilepsy, chemotherapy, radiotherapy, immunosuppression, and oedema, and to take relevant blocks for histological examination. Examination of the spinal cord will be relevant in some cases, and the effects of metastatic neoplasm and of the non-metastatic effects of neoplasia will have implications in other parts of the nervous system and in other organs. Primary CNS neoplasms might have systemic effects, such as the endocrine effects of pituitary tumours, and CNS neoplasms rarely metastasise to other parts of the body. Some neoplasms associated with genetical abnormalities, such as neurofibromatosis and tuberous sclerosis, might be associated with neoplasms of various types, both within the nervous system and in other parts of the body. A few neoplasms also spread within the CSF pathways and involve the spinal cord and nerve roots.

DEMYELINATION

The most common demyelinating disease dealt with in most neuropathology departments is multiple sclerosis (disseminated sclerosis). The well demarcated plaques of demyelination are recognised as greyish areas situated mainly within the white matter, although they are also found within the grey matter. They are often particularly pronounced around the angles of the ventricles, and may be less well defined in the spinal cord than in the cerebral hemispheres. In most cases, the diagnosis will have been made during life. It is important that the pathologist removes the spinal cord and optic nerves in addition to the brain because there may be considerable variation in the distribution of plaques between different cases, and plaques might be limited to one area. There is

also considerable variation in the size and shape of different plaques and there are several varieties of the disease.

Other forms of demyelinating disease, including central pontine myelinolysis, progressive multifocal leucoencephalopathy, alcohol abuse, AIDS encephalopathy, perivenous encephalomyelitis, subacute sclerosing panencephalitis, the leucodystrophies, demyelination associated with head injuries or cerebral oedema, or vitamin B12 deficiency, might be less obvious macroscopically and require other information or investigations before a diagnosis can be made

METABOLIC DISEASES

An increasing number of diseases affecting the nervous system are known to be caused by biochemical and genetical abnormalities. Many of these can now be diagnosed during life. Retention of frozen tissue for analysis by a specialised laboratory is essential in these cases. It is also important to retain tissue for electron microscopy and histochemistry.

TOXIC DISORDERS

A wide variety of toxins affect the nervous system. These can be difficult to identify and might have medicolegal implications. If in doubt, samples of fresh brain and other organs (liver, kidney, hair, urine, and blood) should be retained for toxicological analysis if required. Histological assessment might require blocks from a wide variety of areas of the nervous system and should at least include cerebral grey and white matter, deep grey matter nuclei, midbrain, pons, medulla, cerebellum (cortex and vermis), spinal cord, peripheral nerve, and skeletal muscle.

SPINAL CORD

Many disorders that affect the brain also involve the spinal cord, but some disorders are specific to the spinal cord and its related structures, and these are also vulnerable to problems in the spinal column. The most common complication that the pathologist is likely to encounter is pressure exerted on the spinal cord by a variety of mechanisms from the spine and tissues that are adjacent to it. It is essential that the pathologist is familiar with all the clinical details and potential diagnoses before the postmortem examination is carried out. It is also essential that the pathologist is familiar with the relevant anatomy and what tissues might be required to establish a diagnosis. Full examination might require the examination of nerves, including autonomic nerves, dorsal root and autonomic ganglia, skeletal muscle, and specimens of bone and other tissues. The blood supply to the spinal cord might be important and is complicated; this might involve the assessment of both arteries and veins, and in some cases injection of part of the blood supply and radiological analysis will be required. The state of the aorta, vertebral, intercostal, and lumbar arteries will be particularly relevant.

The spinal cord will usually be received within its dural envelope. A convenient way of

fixing the spinal cord is to suspend it in formaldehyde in a two litre measuring cylinder with a small weight attached to the cauda equina. The external appearances are then described and the theca opened longitudinally, back and front. Midline grooves are present on both anterior and posterior surfaces (the posterior median sulcus and the anterior median fissure). The lateral surfaces contain the dorsolateral and ventrolateral sulci from which the dorsal and ventral nerve roots emerge, respectively, and which divide the white matter into posterior, lateral, and anterior funiculi. The dorsal root zone is situated between the posterior and lateral funiculi; and the ventral root zone is situated between the anterior and lateral funiculi. The grey matter contains the dorsal and ventral enlargements known as the posterior and anterior grey horns, respectively. In the thoracic and upper lumbar segments of the cord, small intermediolateral grey columns are also present. The anterior grey horns are the largest.

The arachnoid should normally be transparent, but might show areas of opacity and sometimes fatty plaques, which are of no pathological importance. After opening the dura mater, the anterior and posterior surfaces of the spinal cord can be identified from the vascular pattern. The posterior aspect contains two posterior spinal arteries and veins, whereas the ventral surface has only one anterior spinal artery and vein. Identifying segmental levels can be difficult because there are anatomical variations from individual to individual. The roots of the upper four cervical nerves are small and those of the lower four large. The dorsal roots of the cervical nerves are approximately three times thicker than the ventral roots, with the exception of the first cervical, where the dorsal root is thinner than the ventral. The cervical nerve roots increase in size from the first to the sixth, and the seventh and eighth and the first thoracic nerves are similar in size to that of

the sixth cervical nerve roots. The first thoracic segment is usually the last of the large nerve roots along the length of the cervical enlargement. A more reliable method is to identify the first nerve root to penetrate the dura mater after the lower end of the conus medullaris; this is the second lumbar root. The others can then be identified by counting above and below that.

In some cases, the spinal cord will be received within the spinal column and some pathologists prefer this method of removal. The spinal cord is easily damaged during removal and in the unfixed state, and the complete specimen includes many relevant structures such as nerve roots, ganglia, and some of the blood supply and drainage.

If the cord is received within the vertebral column, the first cervical nerve root emerges below the occipital bone and above the first cervical vertebra, the seventh nerve root above the seventh cervical vertebra, and the eighth nerve root above the first thoracic vertebra.

Gentle palpation of the cord might help to identify areas of softening and relevant pathology. The cord should then be sectioned segmentally. The back of each specimen required for histological examination should be marked with Indian ink. Blocks should be labelled from above downwards and a record made of the levels to be sectioned and of any macroscopical abnormalities. Blocks taken will depend upon the pathological process under investigation but should routinely include the cervical enlargement, upper, middle, and lower thoracic cord, and lumbosacral enlargement. Blocks should include dorsal nerve roots and ganglia when possible. It is often useful to include a bundle of nerve roots from around the end of the cauda equina.

Stains to be carried out should routinely include stains for myelin to detect degeneration of the long tracts, and for Nissl substance to detect abnormalities within neurones.

Table 1 Staining techniques useful in the practice of neuropathology

Cell or substance to be identified	Stain	Result
Nerve cells	Nissl stain (cresyl violet) Bielschowsky	Purple to dark blue
Axons	Palmgren Marsland, Glees, and Erikson Schofield: recommended for muscle and other peripheral tissues. Particularly useful for nerve endings	Brown/black Dark brown/black Dark brown
Myelin	Luxol fast blue: useful when combined with Nissl stain (Klüver-Barrera) Solochrome cyanin Gomori trichrome stain useful in peripheral nerves Osmic acid is useful in the Marchi technique for degenerating myelin and in staining teased nerve fibre preparations	Myelin stained blue/green Myelin stained blue Myelin stained red and connective tissue stained blue Degenerate myelin stained black and normal myelin stained light brown/brown
Glial fibres	Holzer PTAH Cajal's gold sublimate	Blue Dark blue Deep purple
Senile plaques and neurofibrillary tangles	Gallyas for neurofibrillary tangles Haga methenemine silver for plaques Modified Bielschowsky for plaques and tangles Thioflavine-S for plaques and tangles	Neurofibrillary tangles and plaque neurites stained black Amyloid plaques stained black, some tangles stained black (rare) Tangles and plaques stained black Tangles and plaques fluorescent under the fluorescent microscope
Inclusion bodies	Lendrum's phloxine tartrazine	Viral inclusion bodies stained bright red. Beware red cells that stain orange/red
Melanin	Masson-Fontana	Black
Fat	Oil red O: requires frozen sections but works on formaldehyde fixed tissue	Red
Yeasts and fungi	PAS Grocott	Magenta Black
Oligodendroglia and microglia	Weil and Davenport	Black

PAS, periodic acid Schiff; PTAH, phosphotungstic acid haematoxylin.

Table 2 Some immunohistochemical techniques currently available to assist in diagnostic problems in tumours of the central nervous system

Antibody	Molecular specificity	Diagnostic use
Gliofibrillary acidic protein (GFAP)	An intermediate filament protein of 52 kDa	Astrocytomas and ependymomas
S-100 protein	A family of proteins found in neuroectodermal tissues	Cells of neural crest origin and their neoplasms (gliomas, nerve sheath and choroid plexus tumours, melanomas, and granular cell myoblastomas)
Synaptophysin	A membrane glycoprotein. Occurs in presynaptic vesicles of neurones, adrenal medulla, and neuromuscular junctions	Tumours showing neurosecretory differentiation such as neuroblastoma, neurocytoma, medulloblastoma, paraganglioma, pituitary neoplasms, and a wide variety of neuroendocrine tumours
Chromogranin	An acidic protein widely distributed in neuronal tissues and in the secretory granules of endocrine cells	Neuroendocrine cells and their neoplasms
Protein gene product 9.5	A member of the ubiquitin C terminal hydroxylase family of proteins found in neurones and nerve fibres, in neuroendocrine cells, and in some non-neural cells	May be useful as a marker of neurones and the ubiquitinated inclusions found in several neurodegenerative diseases
Neurone specific enolase	Reacts with the gamma-gamma isoenzyme found in neurones, neuroendocrine cells, and a variety of non-neuronal cell types	Neuroendocrine cells and a wide variety of tumours of the nervous system (gliomas, meningiomas, ependymomas, choroid plexus papillomas, schwannomas, medulloblastomas, and some carcinomas (breast and lung))
Neurofilament antibodies	Several antibodies are available that react with the 68 kDa, 160 kDa, and 200 kDa components of neurofilaments	Tumours of neural lineage including ganglioneuromas, paragangliomas, neuroblastomas, medulloblastomas, and gangliogliomas
Epithelial membrane antigen	Found in the epithelial membrane of a variety of normal tissues	Identification of tumours of epithelial origin, chordomas, meningiomas, and choroid plexus neoplasms
Cytokeratins	There are numerous antigens expressed by different epithelia and mixtures of antibodies are available	Epithelial tumours, craniopharyngiomas, meningiomas, choroid plexus neoplasms, chordomas, and some germ cell tumours
Pituitary hormones	Prolactin, growth hormone, ACTH, FSH, LH, and TSH	Neoplasms of the pituitary gland
Embryonal cell markers: placental alkaline phosphatase, human chorionic gonadotrophin, α fetoprotein	The first two proteins are found in placenta and α fetoprotein is found in a variety of fetal tissues but not usually in the adult	Germ cell neoplasms
Vimentin	An intermediate filament protein of 57 kDa found mainly in cells of mesenchymal origin	Meningiomas, and nerve sheath tumours. Of limited use because it stains a very wide variety of cell types
S-antigen	This antigen is found in photoreceptors and pinealocytes. It is a cytosolic protein that regulates phototransduction in retinal rods. Its function in the pineal is unknown	Retinoblastomas and sometimes present in tumours of the pineal gland, and medulloblastoma
Peripherin	A 57 kDa intermediate filament found in the developing peripheral nervous system and also enriched in neuronal derivatives of the neural crest	Peripheral neurones including enteric ganglion cells. Also expressed in neuroblastomas and ganglioneuroblastomas but does not stain chromaffin cells
Ki67	The antigen is within the nucleolus during late G1, S, G2, and M phases of cell proliferation	There is a reasonable correlation with the grade of neoplasm but needs to be interpreted with caution. The Ki67 index does not always correlate with recurrence rate

ACTH, adrenocorticotropic hormone; FSH, follicle stimulating hormone; TSH, thyroid stimulating hormone.

Brain banks and research

Some diagnoses and research projects require the examination of unfixed material for biochemical and genetic analysis and need to be stored at -70°C for use in a relevant research project.

Staining techniques²⁶

A summary of some staining techniques relevant to the practice of neuropathology is included in table 1; tables 2 and 3 list some useful immunohistochemical techniques available at the present time. The list is not comprehensive and some techniques are rarely used. It is important that each laboratory selects a range of techniques that work reliably and that reagents are subject to strict quality controls.

Muscle biopsy

Muscle biopsies are received mainly from neurologists, physicians, rheumatologists, and paediatricians. Skeletal muscle is affected in a wide variety of diseases, both primary and secondary, and it is sometimes useful in genetic analysis. Most biopsies are taken from the quadriceps femoris, deltoid, or biceps brachii muscles, and it is important that the pathologist is familiar with the normal anatomy, including the range of histochemical fibre types. Muscles used for electromyography studies should not be used, and it is important to biopsy a muscle that is clinically involved, but not too severely so, because both neurogenic and myopathic change resemble each other in the late stages. Points of tenderness are likely to show changes in inflammatory muscle disease. The best site for the biopsy is the centre of the muscle and should avoid tendinous insertions. Open biopsy is carried out in an operating theatre, usually under local anaesthetic. The muscle should not be infiltrated by anaesthetic and the surgeon should avoid clamping, stretching, squeezing, or drying. Preservation is improved if the specimen is clamped at each end before removal to prevent contraction artefact. Needle biopsy is now the preferred method in many centres, particularly in children. It does not require access to an operating theatre and is more easily repeated. It is therefore useful in assessing disease progression or response to treatment. The specimen can, however, be difficult to orientate and there might be insufficient tissue for all investigations, such as biochemical analysis. So²⁷ describes an easy method for orientating small muscle biopsy tissue. Focal lesions can also be missed. Occasionally, a biopsy is taken from the motor point of innervation for the purpose of analysing end plate morphology.

Whenever possible, a sample of muscle should be frozen and stored. This is useful in the assessment of genetic, biochemical, or metabolic disorders, including those involving mitochondria.

FROZEN SECTIONS

All methods of preparing biopsies are subject to artefact. The maximum amount of useful

Table 3 Some immunohistochemical techniques currently available to assist in diagnostic problems in neurodegenerative disorders and trauma

Antibody	Molecular specificity	Diagnostic use
Amyloid precursor protein	A transmembrane precursor protein which, when mutated, can cause a familial form of Alzheimer's disease	Identification of axoplasmic bulbs
β A4 amyloid	An extracellular filamentous protein that forms part of the core of amyloid and neuritic plaques	Neurodegenerative disorders including Alzheimer's disease, Lewy body dementia, Down's syndrome, amyloidosis, and the Guam-Parkinson dementia complex
Tau protein	A microtubule associated protein	Neurodegenerative disorders. Particularly useful in the diagnosis of Alzheimer's disease, which is characterised by an abundance of neurofibrillary tangles, neuropil threads, and abnormal neurites
Ubiquitin	A polypeptide of ~ 8.5 kDa found in filamentous inclusions and cytosome related organelles in neurodegenerative diseases	Neurodegenerative disorders including Alzheimer's disease, Pick's disease, Lewy body dementia, and Parkinson's disease
α β Crystallin	A lens protein that has some homology with the small heat shock proteins	Detection of "swollen neurones" in non-Alzheimer's dementia, and in the diagnosis of corticobasal degeneration

information is obtained when sections are cut transversely.

A sample of muscle measuring no more than 1 cm³ is mounted on a cork disc with a small blob of Tissue-Tek on the surface. A dissecting microscope should be used to obtain correct orientation for transverse sections.

The specimen should then be snap frozen by immersion for 10–15 seconds in isopentane precooled in liquid nitrogen. Freezing should be done when the isopentane is just viscous. If immersion is too short, ice crystals might form, and if too prolonged the specimen may crack. After immersion for a few minutes, the specimen can be transferred to the cryostat for sectioning or stored in a refrigerator at -70° C, or in liquid nitrogen. It is essential that the cryostat knife is sharp and that the anti-roll bar is correctly aligned.

It is useful to retain a few unstained sections in a deep freeze for additional procedures or to send to other laboratories.

ELECTRON MICROSCOPY

Samples no larger than 1 mm³ should be fixed in glutaraldehyde for electron microscopy. All specimens should be embedded in resin, but because electron microscopy is expensive and time consuming it is only carried out in cases

where it is clinically indicated, or when relevant abnormalities are seen on light microscopy, such as the possibility of a mitochondrial myopathy.

PARAFFIN WAX EMBEDDED SECTIONS

The remainder should be fixed in 10% formaldehyde for 24 hours. After fixation, samples should be processed for both transverse and longitudinal sections. During processing some substances such as lipid and the basophilic granular material seen in inclusion body myositis are dissolved out.

MORPHOMETRY

Quantitative data can be useful in support of a clinicopathological report, in assessing disease progression or the effect of treatment, and in research. A variety of techniques is available, including the use of an eyepiece graticule or test lattice projected by drawing tube, photographs, camera lucida drawings, or computerised analysis systems.^{28, 29} Such data need to be interpreted with caution because they are vulnerable to variations of technique and sampling, and should be interpreted in comparison with suitable control material from individuals of the same sex and age, taken from the same site, using the same technique. Useful data

Table 4 Some immunohistochemical techniques currently available to assist in diagnostic problems in disorders of muscle

Antibody/Antigen	Molecular specificity	Diagnostic use
Dystrophin and spectrin (an essential control to monitor membrane integrity)	Antibodies are available to the rod domain, C-terminus, and N-terminus, and it is recommended that all three antibodies are used to avoid the possibility of occasional false negative results	Duchenne and Becker muscular dystrophy
Fetal myosin	Myosin is a contractile muscle specific protein composed of two heavy and two light chains. The heavy chain has many isoforms some of which are developmentally regulated	Muscle regeneration
DAGS (dystrophin associated glycoproteins). Spectrin is an essential control	The dystrophin-glycoprotein complex appears to link dystrophin to the plasma membrane and the laminin component of the extracellular matrix. Antibodies are available against the α , β , γ , and δ components of sarcoglycans of different molecular weight (50 kDa, 43 kDa, 35 kDa, and 35 kDa)	Expression of different members of the complex is altered in several types of muscular dystrophy
Merosin laminin. Spectrin is an essential control	An extracellular matrix component of the DAG complex	Congenital muscular dystrophy
CD 68	An intracellular glycoprotein primarily associated with cytoplasmic granules and to a lesser extent with cell membranes of macrophages, monocytes, neutrophils, basophils, and large lymphocytes	Macrophages
B and T cell markers	Numerous antibodies are available for the identification of lymphocyte types	Inflammatory disorders and lymphomas
Immunoglobulin heavy and light chains and complement	These are helpful in establishing whether a lesion is of lymphoid origin, reactive, or malignant	IgG, IgM, and complement are particularly useful in the diagnosis of dermatomyositis
HLA class I	Expressed on the surface of most human nucleated cells	Muscular dystrophy, inflammatory myopathy, and neuromuscular disorders

HLA, human major histocompatibility complex.

include assessment of muscle fibre size, fibre shape, relative frequency of fibre types, spatial distribution of fibre types, and relative occurrence of a variety of pathological features such as internal nuclei, vacuoles, necrosis, and regeneration.

ROUTINE STAINS

The following stains are sufficient for the screening of most disorders:

- haematoxylin and eosin
- nicotine adenine dinucleotide dehydrogenase tetrazolium reductase (NADH)
- myosin ATPase—preincubated at pH 9.4, pH 4.6, and pH 4.3
- modified Gomori trichrome (Engel's stain)
- succinic acid dehydrogenase
- cytochrome oxidase
- phosphorylase
- acid phosphatase
- PAS (periodic acid Schiff)
- oil red O
- Verhoeff-Van Gieson.

Immunohistochemical stains for dystrophin, dystrophin associated glycoproteins, merosin (using spectrin as a control in each case), fetal myosin, human major histocompatibility complex (HLA) class 1, and for lymphocyte subtypes (CD4 and CD8) are useful in selected cases. Table 4 lists several immunohistochemical stains that are currently useful in the diagnosis of muscle disorders.

Nerve biopsy

The most suitable nerve to assess is one that is moderately involved. This presents no problem in the case of necropsies, but scope for biopsy is limited. The most commonly biopsied nerve is the sural nerve because it is sensory and leaves minimum sensory loss and paraesthesia over the lateral aspect of the foot, which usually recovers within a few months. Removal of a motor nerve leaves permanent weakness in the relevant muscle; this can rarely be justified. Fascicular biopsy reduces the defect.

Usually, 3–4 cm of nerve are removed with as little handling as possible. Most artefacts result from crushing. The nerve should be placed on dental wax and not gauze to prevent drying out. The nerve should then be placed on a piece of card and samples taken for electron microscopy. Buffered formal saline is the best fixative for light microscopy and glutaraldehyde for electron microscopy. If possible, both transverse and longitudinal sections should be cut. Routine stains include haematoxylin and eosin, Van Gieson, and stains for myelin and axons. Many histochemical stains are available for assessing lipids, proteins polysaccharides, and enzymes.

Teased nerve fibre preparations are an excellent method for assessing demyelination and axonal degeneration. Lengths of nerve measuring 0.5 to 1 cm are fixed in 0.1 M phosphate buffered 3.6% glutaraldehyde for one hour and are post-fixed in 2% osmium tetroxide for four to six hours after washing twice in buffer. The nerve is then placed in 66% glycerin in water for at least 12 hours. It can be stored in pure glycerin for several months. Sharp needles are

used to separate the nerve fibres under a dissecting microscope. After teasing, the specimen can be mounted in glycerin and examined under the microscope. Alternatively, the nerve can be dehydrated in alcohol and propylene oxide after post-fixation in osmium tetroxide, and then soaked overnight in Araldite from which the accelerator has been omitted. After teasing, a coverslip is applied and the Araldite hardened at 60°C.

Motor nerve fibres are best studied in muscle biopsies taken at the motor point. Muscle end plates can be demonstrated with the cholinesterase stain. Intravital methylene blue can also be used.

Davidson and So have described an immunohistochemical technique for staining axons in frozen sections of nerve.³⁰

MORPHOMETRY

Quantitative data can be useful in support of a clinicopathological report and in research. A variety of techniques is available, including the use of an eyepiece graticule or test lattice projected by drawing tube, photographs, camera lucida drawings, and computerised analysis systems. Such data need to be interpreted with caution because they are vulnerable to variations of technique and sampling, and should be interpreted in comparison with suitable control material from individuals of the same sex and age, taken from the same site, using the same technique. Useful data include histograms of fibre diameters. When axon diameters of non-myelinated and myelinated fibres are compared, there is little overlap, and this can be useful in deciding whether an axon that has no myelin is truly non-myelinated or whether it has become demyelinated. There is a correlation between axonal diameter and myelin sheath thickness.

Measurements of internodal length in teased nerve fibre preparations can also be useful, but should only be interpreted by those with considerable experience with this technique. In general, the larger diameter fibres have longer internodal lengths within the same nerve but this correlation becomes less accurate with increasing age.

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