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CHAPTER 1

Introduction and Overview: Principles and Techniques

INTRODUCTION

This is a text of surgical neuropathology: a description and analysis of the pathology of the brain, the spinal cord, their coverings (meninges), their bony encasements (skull and spine), and adjacent tissues, including, to a limited extent, the cranial nerves and spinal nerve roots and nerves. In the following pages, the reader will find an attempt at a comprehensive description of the morphological changes wrought by disease processes in these organs and tissues, encompassing all of the descriptive and analytical techniques in current use in surgical neuropathology, including gross descriptions, histopathology using conventional stains, frozen sections, intraoperative cytological methods, immunohistochemistry, electron microscopy, and what is often termed "molecular pathology," including fluorescent in situ hybridization (FISH), and other types of molecular analyses. The bulk of these descriptions, of necessity, will be histopathological; this is foremost a guide to the histopathology of the nervous system. This chapter will largely deal with methods and approaches, rather than with specific pathological changes or findings. The opinions (for, of necessity, some of the following is opinion) are derived from regular practices of the faculty of the Division of Neuropathology of New York University School of Medicine, to which I belonged for more than twenty years and which I directed for more than thirteen years during that period.

At the outset, it must be stressed that an essential component of the clinical practice of surgical neuropathology is the correlation of clinical and imaging data with the findings of pathological analysis. Neuropathologists or surgical pathologists must not operate in an intellectual vacuum. Their diagnoses must be informed by the clinical history of the patient from whom a sample is obtained; by the appearance of one or more abnormalities in the increasingly sophisticated imaging techniques utilized by neuroradiologists, including magnetic resonance imaging (MRI) in all of its increasingly sophisticated different sequences and techniques, and not only computed tomography (CT) but also single photon emission CT (SPECT), positron emission tomography (PET), and other techniques occasionally of importance to the clinical interpretation of tissue samples; and the surgeon's impression of what the excised tissue was at the time of surgery. As will be seen, there are situations in which the knowledge of clinical history or of imaging will be critical to the correct interpretation of the histopathological data.

GROSS DESCRIPTIONS

Any interpretation of a neuropathological specimen must begin with a gross or macroscopic examination. This is too often neglected in teaching and writing about surgical neuropathology. Neurosurgical specimens may be obtained by open neurosurgical procedures in which relatively large pieces of tissue are obtained, or in which only small fragments are produced even if they are present in large amounts; the latter is particularly true when tumors or other lesions are aspirated either with the use of a device such as the Cavitron Ultrasonic Aspirator (CUSA) or solely by suction. Other specimens may be obtained by closed procedures, that is, needle or core biopsies, usually but not always taken with stereotactic guidance. An open procedure, which produces one or more large pieces of solid tissue, should be evaluated carefully (most often with photographic documentation). Questions about such specimens should include the following: Is the specimen recognizable as cerebral (or cerebellar) tissue? If so, can it be further identified? Hippocampus, for example, is readily recognized grossly and is a common tissue removed during surgery for temporal lobe epilepsy. If it is cerebral hemispheric tissue, is it covered by leptomeninges? Are they unremarkable, or is there a visible infiltrate that may represent inflammation (meningitis) or subarachnoid neoplasm? Is there fibrosis or hemosiderin, which may represent evidence of prior surgery? If the specimen is from an operation to treat epilepsy, is there evidence on the surface of placement of depth electrodes? Is the specimen attached to recognizable dura? Is it

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smooth surfaced, or is the natural surface (not the resection margin) granular? If the specimen is a mass, is it lobulated (as many meningiomas are)? Is it soft and friable, gelatinous, or firm and rubbery? All of these attributes may bear on diagnosis.

It is sometimes necessary to properly orient a specimen prior to gross cutting to examine its interior appearance. Surgical margins are not usually examined in glioma specimens in the same way as they are for excised cancers in other tissues because of the uniquely infiltrative nature of diffuse gliomas (as will be extensively discussed in later chapters) and of the potential effects of removing normal functioning brain or spinal cord with any margin around an intraparenchymal lesion. However, some lesions may be deliberately excised with a margin, either because their diagnosis has been previously established by prior biopsy or because their site permits a wider excision (such as an anterior temporal or frontal pole lesion) in the hope of obtaining clean margins. Proper communication with the surgeon to know about these needs and then appropriately orienting the specimen to examine the margins is key. Also, MRI now allows much knowledge about tumor tissues, which should dictate proper sampling with orientation. For example, a mostly non-enhancing tumor of the brain may have one focus with enhancement or there may be an area that, even though it does not enhance, has increased cerebral blood volume (often referred to as "perfusion" in the MRI lexicon) compared to other regions of the lesion; to properly sample these histopathologically requires that one knows the MRI data and the orientation of the specimen. Collaboration and communication with the neurosurgeon and neuroradiologist is essential in this task.

When there are no such specific concerns for orientation, I recommend, as a "best practice," that one sections such large specimens perpendicular to their longest axis. For dural-based mass lesions, it is important to cut through the mass and its dural base, so that some sections will demonstrate the relationship of the lesion to the dura. When cutting large specimens, it may be necessary to use a brain knife (which should be sharp) rather than a scalpel, if the thickness to be cut exceeds the length of the cutting edge of the scalpel in order, to obtain smooth-cut surfaces. Specimens should be sectioned at about 0.5 cm intervals, and the resulting slabs should be arranged on a cutting board from one end to the other to evaluate the internal structure of the specimen. Questions to answer then include "Is all of the tissue abnormal or is there any recognizable cerebral tissue (or very rarely, spinal cord tissue)?" "For abnormal mass lesions, is the lesion wholly solid or partially cystic?" "What is the thickness and nature of the cyst wall" Fibrous walls suggest an abscess, but some metastatic cancers can have a very firm texture at the rim around a cystic center and may mimic an abscess. This is very rarely true of gliomas, but examples do exist.

Are the tissues of an abnormal mass uniform, or is there variation in color or consistency? One should be wary of interpreting soft and friable tissue as necrotic, particularly in unfixed specimens; it becomes problematic when a gross description depicts necrosis but none is present in any of the histopathological sections. Is there recognizable brain tissue at any of the edges of an abnormal mass, and if so what is the nature of the boundary? Meningiomas and metastatic lesions tend to have sharply circumscribed boundaries, whereas intraparenchymal primary tumors often gradually blend with adjacent tissues. Is there recognizable cerebral cortex or white matter? What is the nature of the gray–white junctions? Note should also be taken of gross vascularity of the lesions and cerebral tissues; arteriovenous malformations and cavernous hemangiomas can be grossly recognized or suspected, guiding subsequent analysis. The answers to these and similar questions discussed earlier can have an important bearing on histopathological interpretation and the ultimate diagnosis.

Tissues from aspirates may be scanty or voluminous. I strongly encourage neurosurgeons to put collection traps on all suction lines and to submit the tissues therein in formalin. Sometimes, these are the best tissues one receives to evaluate certain lesions. Properly fixed and processed, they are suitable for immunohistochemical staining and for DNA extraction for molecular analysis.

Neurosurgeons have a variety of biopsy instruments available to them for sampling of deep lesions using stereotactic techniques. The best of these obtain cylindrical tissue cores of about 0.2 cm diameter and 1 cm length. Fine-needle aspirations (FNA) have been described for central nervous system (CNS) lesions, but it seems counterproductive as a hole must be drilled in the skull anyway to then obtain a less-than-optimal sample. FNA specimens allow only a few immunostains to be done and are much more limiting on the neuropathologist than core biopsies, and I do not advocate or encourage FNA procedures in the CNS.

Modern stereotaxy coupled to MRI, angiographic, and CT data allows a neurosurgeon to precisely place the biopsy instrument, and an excellent technique of such biopsies involves the "linear serial" method[1–4]. In these procedures an avascular trajectory is chosen from a surface point to a target point in the lesion as seen by MRI with three-dimensional planning; "avascular" means that the biopsy instrument will not go through any major arterial or venous structure on its way into the target. Biopsies are taken in a linear series along the trajectory, so that, end-to-end, they represent a counterpart of a geological core sample through many strata of rock. These are usually designated with their distance from the target point, (−) for distances closer to the surface than the target and (+) for distances beyond the target. Thus, a set of samples might be designated "−25 to −15," "−15 to −5," "−5 to +5," and "+5 to +15"; this series would sample all of the tissues along a line through tissues that might appear quite different by MRI, such as normal brain, brain with an abnormal T2 signal only, contrast-enhancing solid lesion, and necrotic center (Figure 1.1).

With this technique, the surgeon and the neuropathologist can be confident with a high probability that the tissues obtained will produce an accurate diagnosis, and there is no need to use part of such cores for frozen sections to verify that "lesional tissue" has been obtained. To reemphasize this point, frozen-section verification at the time of stereotactic biopsy that the lesion has been sampled is almost never necessary. It becomes necessary when only one or two cores can be obtained because of special circumstances related to the site of the lesion, its presumed vascularity based on imaging studies, and the attendant risks to the patient when more samples are taken.

Even with these small tissue samples, proper neuropathological examination must begin with an appropriate gross examination. If the cores are fragmented into many small pieces, it suggests that the sampled tissues were highly discohesive, perhaps necrotic; normal brain and many types of tumor tissue remain solidly cohesive in cores, but high-grade gliomas, lymphomas,

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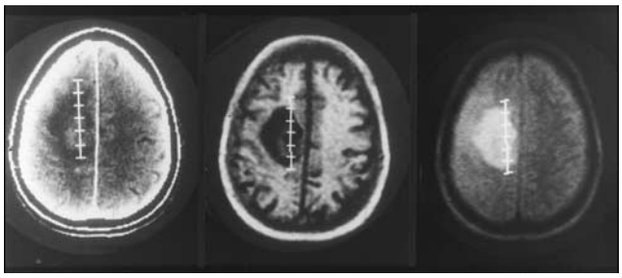

FIGURE 1.1: ***Diagram of linear stereotactic serial biopsy. Each of three axial MRI scan slices done with different magnetic resonance (MR) sequences have the trajectory of a proposed linear biopsy superimposed. The line of the trajectory is divided into 1 cm segments representing the individual 1-cm-long needle biopsies to be taken along the biopsy trajectory. As discussed in the text, this allows assessment of the different components of a lesion, including possible necrotic centers, areas of contrast enhancement, areas without contrast enhancement but marked T2 or fluid-attenuated*** **inversion recovery (FLAIR) abnormality, and adjacent normal-appearing brain, without a major resection, particularly of deep lesions not easily resected without risk of neurosurgically induced deficits. (With permission from Tumor Stereotaxis, Patrick J Kelly M.D., Saunders, 1991).**

and primitive neuroectodermal tumors (PNETs) may not. In some cores, gray matter and white matter are distinguishable, and the nature of the gray–white border should be discerned (discrete or indistinct). In some cores, white matter will be recognizable but discolored yellow, suggesting an infiltrating neoplasm. Cores should not be divided unless a small piece of clearly abnormal tissue needs to be processed for potential electron-microscopic examination. Embedded whole, they can be sectioned parallel to their long axis, and, handled carefully by the histotechnologists, provide plenty of tissue for immunohistochemistry as well as other special stains after routine hematoxylin and eosin (H&E) examination.

HISTOPATHOLOGICAL EXAMINATION

General Considerations

Routine diagnostic neuropathology is still best conducted with formalin-fixed tissues embedded in paraffin and cut at approximately 6 μm thickness. The standard histopathological stain in use in the overwhelming majority of laboratories is the combination of H&E, and it is from this starting point that all histopathological observations begin. There are advocates for other primary staining procedures incorporating dyes such as phloxine or saffranin, but, while conceding the occasional benefit of these, I have found that the disadvantages usually outweigh the virtues of such procedures. Surgical neuropathology is a subspecialty of surgical pathology, and with the large majority of surgical pathologists, in general, H&E is the preferred stain for routine initial analysis.

In processing tissues, there must be adequate fixation but not overfixation. Prolonged exposure to aldehyde fixatives, including formalin, can cross-link some antigens to an extent that immunohistochemical procedures become more difficult; some antigens may not be demonstrable. In recent years, the widespread use of antigen retrieval procedures has somewhat alleviated this problem, but overfixation should still be avoided. Synaptophysin, a brain protein for which immunohistochemical staining is very common, is particularly sensitive to overfixation, and even with antigen retrieval one may be misled by the appearance of synaptophysin immunostains in improperly processed tissues.

Alcohol-based "fixation" preserves the histological appearance of brain tissues well, and some immunostains work very well in sections of tissues primarily fixed in 70 percent ethanol; however, some do not work at all, and again synaptophysin is an important example, as it becomes essentially undetectable in ethanol-fixed tissues.

Other fixation methods, including some relatively new rapid processing systems, are not demonstrably better (although they may be faster). Preservation of tissue antigens for immunohistochemical stains and of nucleic acids for molecular analysis remains problematic for many of these alternative fixation and processing methods, despite some claims to the contrary. At New York University (NYU) Neuropathology, I had only limited experience with these methods, and we opted not to move away from routine formalin fixation.

H&E stains give the surgical neuropathologist most of the information needed for a precise diagnosis in most cases. Special stains including immunostains are often merely confirmatory or allow finer diagnosis within a broader category (such as high-grade glioma) established from the H&E stain alone. With an H&E stain, one can establish definitively in almost all cases diagnoses of most tumors commonly encountered in neuropathological practice, including schwannomas, neurofibromas, meningiomas, ependymomas, pilocytic astrocytomas, PNETs, non-Hodgkin's lymphomas (NHL), metastatic carcinomas, germinomas, yolk sac tumors, teratomas, pituitary adenomas, chordomas, and chondrosarcomas. One can identify those gliomas that have clear cell elements suggestive of a limited differential diagnosis of "oligodendroglioma," neurocytoma, or clear cell ependymoma. One may suggest the probability of sarcomatous components in high-grade gliomas and of embryonal carcinoma in germ cell tumors (GCTs). In non-neoplastic conditions, with H&E alone one can predict (but not prove) diagnoses of tumefactive demyelinating cases; definitively identify progressive multifocal leukoencephalopathy (PML); diagnose some viral diseases including herpes simplex encephalitis and cytomegalovirus encephalitis; diagnose cerebral vasculitis, including granulomatous vasculitis; and identify epileptogenic lesions such as contusions, malformations, heterotopias, tubers, and other hamartomas. These lists are not meant to be comprehensive; there will be more on this later

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in the chapters on individual entities. Otherwise, H&E stains guide further histopathological workup, including the choices for histochemical stains, immunostains, and electron microscopy.

Histochemical stains (special stains) are chosen for their ability to demonstrate specific features, which will help narrow or define a diagnosis based on the original H&E impression. Masson trichrome stains are in general use to demonstrate collagenous matrix material in various types of lesions; at NYU, we have preferred azocarmine (essentially, Heidenhain's aniline blue method as modified at NYU/Bellevue Neuropathology[5]) for the brilliance of its coloration and for its ability to stain fibrillar proteins, such as fibrin, a bright red. Stains for organisms [tissue Gram stains such as Brown & Brenn; stains for fungi including periodic acid Schiff (PAS) and the Grocott methenamine silver method; acid-fast stains such as Kinyoun's] are of obvious value in specific situations. Stains for elastic tissue (Voerhoeff–Van Gieson) and reticulin stains are of value to demonstrate particular extracellular matrix components in certain tumors or in vascular malformations. All of these are also in use in general surgical pathology. Congo Red and crystal violet stains are used to demonstrate amyloid. In surgical neuropathology, stains for myelin are of considerable importance, not only to demonstrate loss of myelin in suspected demyelinating diseases, but also (and, in fact, more frequently in my practice at NYU and currently) to delineate the microanatomy of tissues being examined, providing information, for example, on whether an infiltrative tumor is in white matter or gray matter or whether there are heterotopic gray matter elements in white matter in an epilepsy surgery specimen. Most commonly, Luxol Fast Blue (LFB) is used to demonstrate myelin. This can be combined with other stains, for example, many laboratories use an LFB/PAS combination; I much prefer an LFB/H&E combination because of the nuclear detail it provides in combination with the information on myelin. LFB also stains lipofuschin and the biochemical by-products, which accumulate in cells in many of the storage disorders, so it can be useful in brain biopsies for these disorders as well.

Immunostains have been part of the routine armamentarium of the surgical pathologist and neuropathologist for about thirty-six years at this writing. Neuropathology was one of the first subspecialties to significantly benefit from this technique; once isolation of the (relatively) specific astrocyte marker protein glial fibrillary acidic protein (GFAP) was reported by Eng, Bignami, and colleagues[6], subsequent widespread use of immunohistochemistry for GFAP transformed many aspects of surgical neuropathology[7–10] and, ultimately, largely banished to obscurity methods such as phosphotungstic acid hematoxylin (PTAH), gold sublimate, and Holzer stains, all formerly used to demonstrate astrocytes. Year after year, however, the list of available stains for specific antigens usefully identified in neuropathology grows, and the list is now quite extensive; it contains multiple markers for neuronal antigens, glial antigens, specific GCT markers, specific cytokeratins for identifying likely parent tissues in metastatic carcinomas, markers of gene products whose presence or absence may signal specific diagnoses (*e.g.*, the absence of INI-1 expression in atypical teratoid/rhabdoid tumors), markers of specific infectious agents (especially but not exclusively viruses), markers of endothelial cells, markers of muscle cells (including embryonal muscle markers), and markers of cellular proliferation. In muscle and peripheral nerve pathology, neither of which is within the scope of this book, immunofluorescent stains of snap-frozen tissue are of considerable benefit in selected areas of diagnosis. But in routine surgical pathology of the CNS, immunohistochemistry on fixed tissue using colorimetric demonstration of the antibody localization, with immunoperoxidase, avidin–biotin, or alkaline phosphatase or other similar reactions, is the rule. Immunohistochemical methods are increasingly being standardized, and the stains are often automated.

Electron-microscopic examination of tissues for diagnostic purposes has become much less frequently performed, as the range of diagnoses that can be established by immunostains has grown. There remain circumstances in which ultrastructural data are of considerable importance in either establishing a specific diagnosis or verifying one previously considered likely from light microscopic data, including immunostains. This is sometimes the case in poorly differentiated tumors, which have few distinctive characteristics in light microscopy and in which the results of immunostains are not definitive. Electron microscopy (EM) can also be useful to identify viral particles in certain infectious conditions. I have found that our patterns of use of EM have shifted; most of the EM we do now is in muscle or nerve pathology rather than in CNS pathology. If the potential need for EM examination is known when the tissue is freshly removed, based on a prior biopsy or on a frozen section, then small samples of presumably diagnostic tissue should be fixed in buffered glutaraldehyde. Freshly prepared paraformaldehyde is acceptable but is not as good. However, reserving small pieces of formalin-fixed tissue for possible EM examination during gross examination of tissues usually produces useful results, and so I routinely have just a small representative sample of the formalin-fixed tissue held while the routine light microscopy is done first; then, if EM is thought necessary, this small sample can be retrieved, postfixed in glutaraldehyde, and processed for ultrastructural examination.

Diagnostic medical genetics (molecular pathology), as applied in neuropathology, is a relatively new field, but a burgeoning one. Some of the techniques or assays of importance are actually cytogenetic. FISH is used for interphase cytogenetics to document the presence or absence of large portions of chromosomal arms, which are either characteristically deleted in certain types of tumor or otherwise provide useful prognostic data, or to demonstrate an increased copy number for certain genes. The best known and oldest of these assays is for deletion of the p arm of chromosome 1 and of the q arm of chromosome 19 in "oligodendrogliomas." When first described, this was conceived of as a diagnostic test; that is, the presence of such deletions established a diagnosis of "oligodendroglioma" or of a mixed glioma with a substantial oligodendrogliomatous component, whereas the absence of such deletions favored a diagnosis of astrocytoma. Longer experience has shown that some undoubted "oligodendrogliomas," defined histopathologically, lack 1p or 19q deletions, particularly in pediatric patients, and that some other tumors, notably some parenchymal neurocytomas and some mixed glial–neuronal tumors, have such deletions. It is now clear that the presence of these deletions establishes a strong probability of response by the tumor to certain chemotherapy regimens, a very important datum indeed, but not a way to determine a histological tumor

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classification. FISH can also be used to demonstrate extra copies of chromosomes or genes, for example, amplified genes for epidermal growth factor receptor (EGFR) in some high-grade gliomas.

A great deal of work has been done on the molecular genetic changes in high-grade gliomas, especially in purely astrocytic tumors called glioblastomas. Again, some prognostic information can be derived from the knowledge that such a tumor lacks or has a mutation in one *p53* gene with loss of heterozygosity of chromosome 17p, where that gene resides. A corresponding therapeutic decision cannot be made based on this information at the present time. Similarly, as noted earlier, many glioblastomas have amplification of the gene for EGFR. Now, there are small molecule drugs to block these receptors, and there is an expanding list of such drugs with specificity to variant or mutant receptors, so this is an area of molecular analysis on the threshold of considerable clinical importance. At this writing, however, it is fair to say that this area is still in its infancy, and routine or standard practice has yet to regularly incorporate such testing and guided treatment into standard or routine treatments.

APPROACHES TO READING SLIDES

This is a text of surgical neuropathology, and although the author (and publisher) hopes that it will be of interest to a wider audience of neurosurgeons, neurooncologists, and others who deal with patients with brain tumors or who research the biology of CNS neoplasms, the principal target audience is composed of pathologists. It may be presumptuous, then, to offer advice on how to read slides, but some comments on this necessarily acquired skill may be useful to readers who are still in training.

The most important consideration is that everything on a slide must be examined. To do this, the pathologist should begin by using the naked eye to look at the slide. One should verify that the slide's identifying label with the case number matches the numbers on the patient identifiers and gross descriptions; errors in reporting diagnoses due to examination of the wrong slide are not unknown and are likely to result in great unhappiness on the part of the clinicians who care for the patient given the wrong diagnosis (as well as potential medicolegal liability). Once that is done, an idea of the size of the tissue and its general characteristics (in H&E stains, whether it is largely eosinophilic or basophilic, and whether it has obvious normal tissue such as cerebral cortex or white matter) should be obtained. One should then proceed to examine all of the section with a low-power objective, one not higher than 4×, 2×, or 1× if available. One should strive to look at everything and resist the temptation to jump to higher power on an area of interest seen during the low-power scan, until everything has been seen. Then, one can move through progressively higher power objectives, through 40×. Neuropathologists should strive to train their "eye" to see mitotic figures, certain kinds of diagnostic inclusions, and other features at low to intermediate power and then confirm the observation at high power rather than search large areas, for example, for mitotic figures, at high power. Normal tissues and many lesions have architecture that can best (and often only) be appreciated at low power, and a pathologist who jumps too soon to high power may miss essential information. As just one example, there are ependymomas whose cells closely mimic "oligodendrogliomas," having round nuclei lying in cell bodies with clear cytoplasm (in H&E stains); on more than one occasion, cases called "oligodendroglioma" have, on review, been seen to be clear cell ependymomas, and this diagnostic information comes from a low-power scan of the slides showing multiple perivascular pseudorosettes, which were missed at high power (Figure 1.2). (This is discussed more in Chapters 5 and 7.) Similar considerations apply to examination of any other stain.

INTRAOPERATIVE CONSULTATION (FROZEN SECTIONS)

Intraoperative consultations are occasions when neuropathologists are called on to make at least preliminary diagnoses from tissues freshly removed from a patient in order to either guide the neurosurgical procedure or guide immediate postoperative care. It can be of immediate importance to a neurosurgeon to know if a lesion is a developing abscess or a neoplasm, and sometimes all of the extensive armamentarium of noninvasive diagnosis does not provide an answer. A smear or squash preparation of fresh tissue can show acute and chronic inflammation and the absence of frankly malignant cells; a frozen section may show the abscess wall as well as the inflammation. This can then suggest to the surgeon the need for different operative procedures and mandate that the tissue be sent for microbiological analysis, and it may change the choice of postoperative antibiotics pending results of cultures. Neurosurgeons will be more aggressive with spinal cord or brainstem sites when they learn that the tumor they are removing, is, as demonstrated by frozen section, an ependymoma, and correspondingly less aggressive with infiltrative diffuse gliomas. It can be very important for a neurosurgeon to have neuropathological support during surgery to identify likely tumefactive demyelinating disease and avoid resecting nonneoplastic demyelinated tissue. These are perfectly good reasons for intraoperative consultations, even if some of them pose difficult problems for the neuropathologist (such as the demyelination vs. tumor issue).

However, at many neurosurgical centers, all or most cases are done with frozen-section requests during the surgery. These requests may be unreasonable, especially if the resulting diagnosis will not change the surgeon's approach or the postoperative care. On the day of surgery, it does not matter if a tumor in the cerebellopontine angle is a meningioma or a schwannoma; a frozen section to determine which of these two entities a neurosurgeon is removing is unnecessary. In most circumstances, it is of no importance to the surgeon at the time of surgery whether a diffuse glioma is, based on the frozen sections, demonstrably low or high grade or purely astrocytic versus demonstrably at least partly "oligodendroglioma"-like.

Worse, it can actually be deleterious to the patient and medicolegally risky for both the neurosurgeon and the neuropathologist to make operative decisions based on frozen sections to distinguish between low- and high-grade gliomas, or lymphomas versus infections. In these and some other circumstances, wrong diagnoses can result from the errors introduced by limited sampling (first by the surgeon and then secondarily

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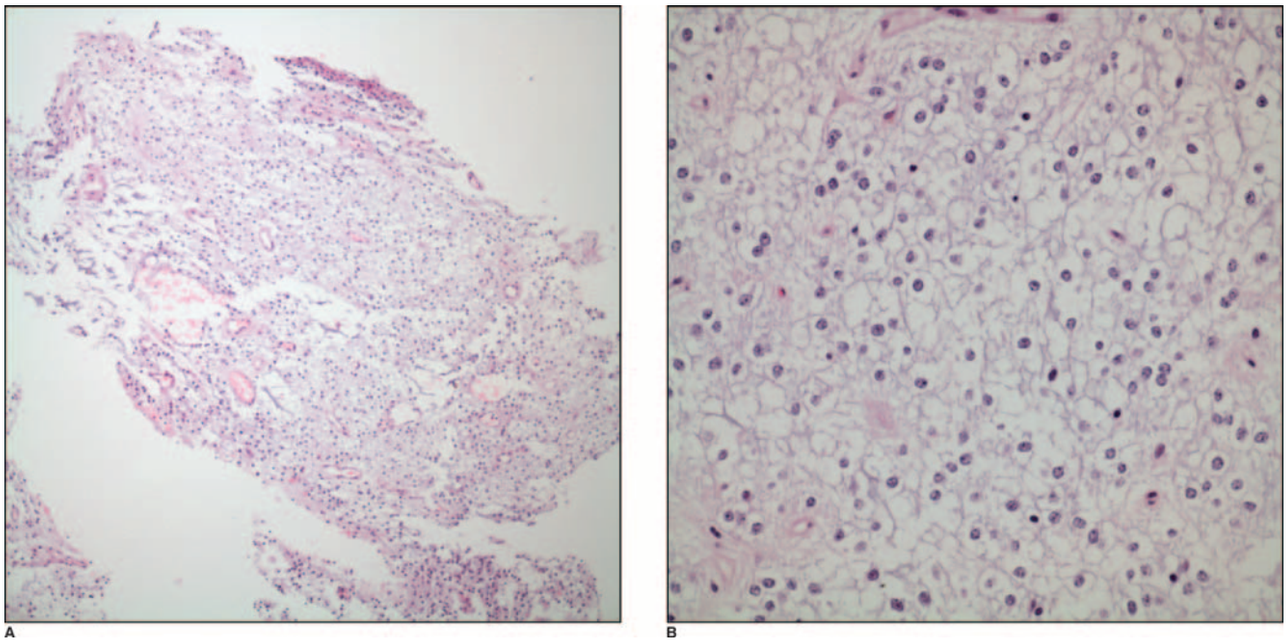

FIGURE 1.2: Clear cell ependymoma with pseudorosettes. (A) H&E-stained section showing medium-sized cells with small regular nuclei with some perivascular pseudorosettes (10×). (B) The tumor consists of cells with central round nuclei, clear surrounding cytoplasm, and distinct cell borders – oligodendroglioma-like cells. If a pathologist starts with the cells at high power, the architectural clue to the correct diagnosis of clear cell ependymoma – the pseudorosettes – can be missed and an incorrect diagnosis of oligodendroglioma will result (40×).

when the pathologist chooses what portion of the tissue sent to freeze) and by the artifacts intrinsic to the frozen section process. Added to this, the pathologist has few stains other than H&E that can be used to help arrive at an accurate diagnosis.

Thus, I have consistently and strongly advocated to our neurosurgeons that they should ask for frozen sections only in circumstances where the diagnosis so obtained will alter their surgical approach or the patient's care in the first 24 hours after surgery. We had good success with this at NYU over the last twenty-plus years, and I recommend that there be discussions of these issues among neuropathologists and neurosurgeons at all centers. These discussions ordinarily should not take place when the request for a frozen section is made; it is better to do this face to face and without the time pressure of a patient on the operating room (OR) table with an open craniotomy.

One reason that neuropathologists may be called to examine freshly removed tissue during a neurosurgical procedure is to triage it among the various needs for routine histopathological processing, special procedures such as EM, and research requirements for fresh or fresh-frozen tissue banking. These needs, especially the research needs, can be met without intraoperative consultations, that is, without a need to examine the tissues histologically with frozen sections, or by cytological methods (imprints, smears, and squash preparations) and without making a diagnosis as a pathologist during the surgical procedure. Indeed, we routinely have nonphysician personnel bank tissues as selected by the operating neurosurgeon.

Intraoperative consultations routinely should involve more than the performance and interpretation of a frozen section. First, there should be good communication between the neuropathologist and the neurosurgeon. A specimen should not be labeled, for example, "brain tumor" with no identification as to site, patient age, and clinical history, including any prior history of cancer, prior brain surgery, and MRI or other imaging data. It is not always possible to see the imaging data at the time of frozen section, although in more and more institutions digital image systems and libraries permit this, but at least the neuropathologist should have a description of what the preoperative imaging showed; seeing the images whenever possible is even better. Knowing the question the neurosurgeon needs to have answered during the intraoperative consultation is also important, as the question may not be limited to one of simple diagnosis.

When the specimen for intraoperative consultation arrives, several things need to be done with it. There should be a rapid gross description, keeping in mind the principles of gross examination laid out previously. These can help enormously in choosing what portion of a sample to freeze, what portion to use for cytopathological preparations, and what portion, if any, to reserve for fixation in formalin or glutaraldehyde for paraffin sections or ultrastructural examination.

Cytopathological methods useful at the time of intraoperative consultation include touch preparations, smears, and squash preparations. Brain tissue itself and neuroepithelial tumor tissue largely tend to be soft and often sticky, and one can lose significant amounts of small specimens in handling on cutting boards, paper towels, or gauze pads. At NYU and now at the University of Missouri, I have taught our residents to handle CNS specimens for intraoperative consultation only on glass. Thus, the pieces of tissue are removed from the container they

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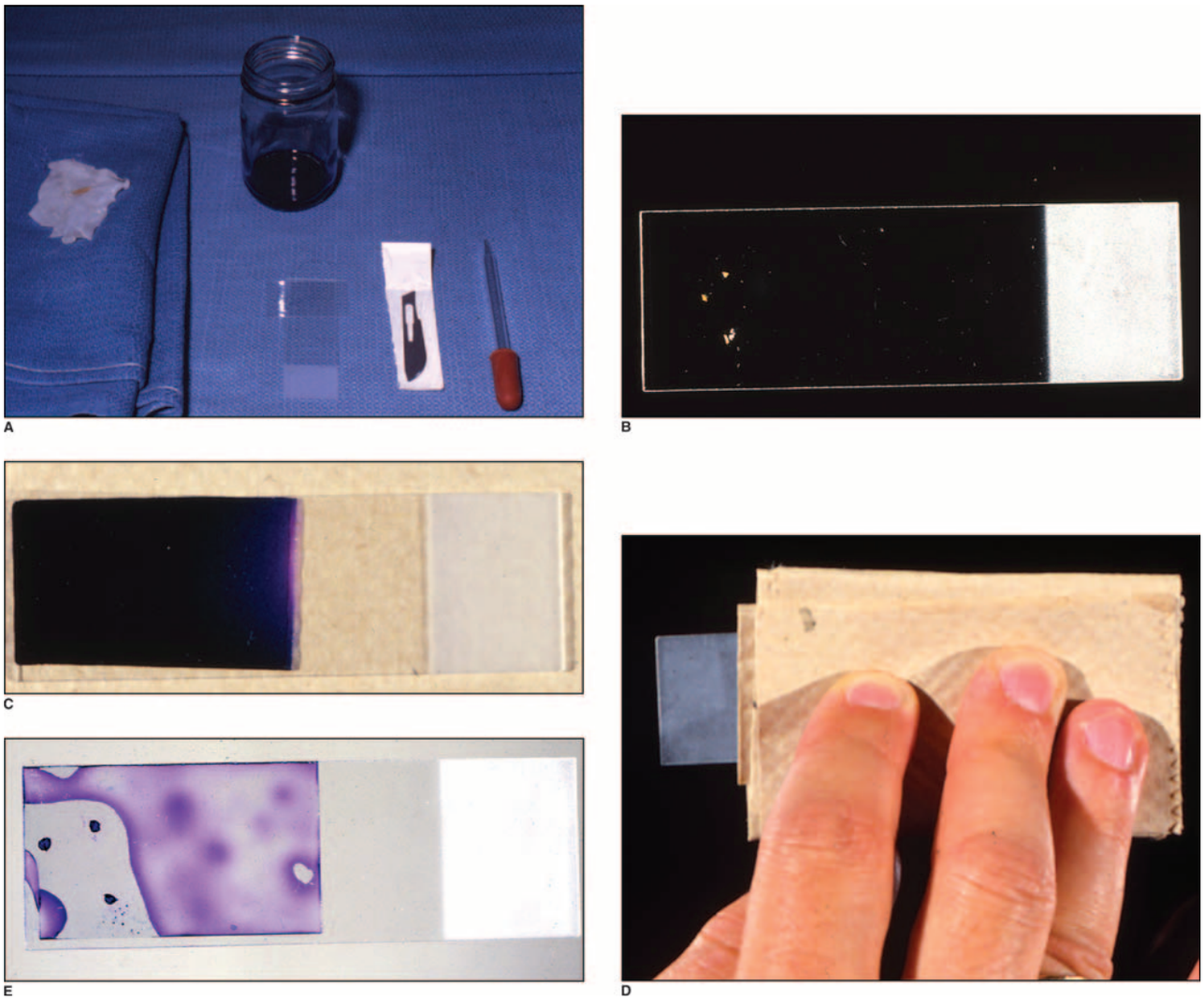

FIGURE 1.3: *Performance of a squash preparation. (A) Materials necessary include a standard glass slide, a large coverslip, a scalpel blade, a pipette, a supply of stain (usually Toluidine Blue or a similar metachromatic one-step aqueous stain), some paper towels, and the tissue (usually as submitted for intraoperative consultation – "frozen section"). (B) The tissue is placed on the slide and cut up into very small pieces with the scalpel. As it is moved around on the slide, cells are shed as in a "touch preparation," and the small pieces of minced tissue are left on the slide. Larger pieces submitted can be initially divided on the slide and the bulk removed for rapid freezing and cryostat sections – the standard frozen section procedure. (C) The slide is then placed on top of several paper towels (if not already on them) on a firm flat surface. The stain is placed on top of the slide using the pipette and allowed to sit for about fifteen to thirty seconds. It is then covered with the coverslip. (D) The edges of the paper towels are then wrapped around the slide, leaving it on the table. The slide is then firmly pressed with fingers, squashing the tissue between the coverslip and the slide within the towels. (If the surface is not flat the coverslip or slide may crack at this stage.) (E) The slide is then unwrapped and is ready to be viewed in a microscope. The example shown here had rather firmer tissue than is usually the case for brain and spinal cord tumors, and the solid pieces of tissue have not squashed out as much as most CNS specimens do.*

were sent in straight onto a glass slide; gross examination and any division of the tissue takes place using the slide as the cutting surface. In this way touch preparations are automatically made, and squash and smear preparations may also be facilitated.

It is often helpful to have two cytopathological preparations stained with different methods. An obviously useful stain is a rapid H&E, made the same way the frozen sections may be stained. This provides a familiar range of staining qualities and can be directly compared to the H&E-stained sections as they are made. There is also the advantage that these touch preparations or smears are permanent when they are rapidly fixed, dehydrated, and stained with H&E. However, other stains that can also be done rapidly are useful adjuncts. A very good technique is to stain a touch, smear, or squash preparation with Toluidine Blue or pinacyanol. Each of these stains is aqueous, and unfixed air-dried cytological slides are rapidly stained and then covered with a coverslip while still wet. Under these

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conditions, the stains are metachromatic; nuclei are as usual blue, and most cytoplasmic structures are not more than minimally stained, but both collagen and myelin stain shades of pink to red. In cases in which examination of H&E-stained frozen sections raises a probability of demyelinating disease, having a myelin stain at the time of frozen section is invaluable. Similarly, demonstration of infiltrating tumor cells or inflammation in white matter, proven to be that by a myelin stain, can also be quite important in reaching an appropriate intraoperative diagnosis. In demyelinating disease, as will be seen in a later chapter, macrophages filled with lipid are an important characteristic, and in these aqueous mounts, the refractile unstained lipid may also take on the tinctorial qualities of myelin if it represents fresh myelin debris. Also, in some instances, demonstrating unequivocally that certain tissues are collagen can greatly aid in diagnosis, delineating the borders of vessel walls in possible pseudorosettes, for example. Some neuropathologists use other rapid stains, such as Diff-Quik, which provide excellent cytological detail, but these sacrifice the metachromasia which, as described, can be very useful in reaching the proper diagnosis. A disadvantage of the rapid aqueous metachromatic stains is that they are not permanent; the metachromasia fades as the slide dries or is made permanent.

The techniques for touch, smear, and squash preparations are well described. Touch preparations involve literally gently touching a piece of tissue to a slide, often multiple times in adjacent loci to make multiple imprints. The slide is allowed to briefly air-dry and then can be stained with either H&E or an aqueous stain as described earlier. Smear preparations are best done by selecting small pieces of soft tissue and placing them just below the edge of the label or frosted portion of the slide. A second slide is gently placed over the tissue, oriented with its label end opposite to the first, and the two slides are pressed together and then drawn one over the other in a smearing motion. Ideally, the smears that result are not excessively thick. These two slides are then briefly air-dried and stained; an advantage of this technique is that two slides are automatically produced, allowing one to be used for H&E and the other for whatever other rapid stain that is chosen.

Squash preparations, which I use routinely, can give a modicum of "histological" information as well as cytological information. In these, small pieces of tissue are dissected off from the submitted specimen, again as described using a slide as the cutting surface. The slide is then flooded with a rapid aqueous stain such as Toluidine Blue, using a pipette (placing the slide on several paper towels to keep work surfaces clean). A coverslip is placed on top, directly on the stain, the slide is carefully wrapped in a few layers of paper towel, and then the coverslip is pressed hard onto the slide through the towels. The excess stain is squeezed out from under the coverslip and is absorbed by the towels, and the preparation is ready for examination (Figure 1.3).

Gliomas and similar soft tissues are flattened and spread out by this technique, giving pieces that are stained and thin enough to see through, allowing one to gain some sense of tissue architecture; additionally there are individual cells or small clusters in adjacent areas that are adherent to the slide, having come off during the cutting of the small samples from the main specimen, and these give excellent cytological detail identical to that in smears or touch preparations. Of note, firmer tissues, such as those of many meningiomas and schwannomas, and any tissue with significant mineralization squash poorly, which by itself provides some useful information. However, many of these types of tumors will squash satisfactorily, allowing recognition of cellular whorls and psammoma bodies in meningiomas, for example.

Tissues selected for frozen sections must be rapidly frozen. Artifacts due to ice crystals are minimized by rapid freezing, which can conveniently be accomplished with dry ice as a coolant. Liquid nitrogen is too cumbersome for routine use in most frozen-section suites, and it vaporizes too easily, forming a gas layer around tissue plunged into it, which insulates the tissue and slows the freezing process. Most cryomicrotomes will, if properly aligned and maintained, cut sections well at 10 μm thickness or lesser. The same stains used for intraoperative cytopathological examinations can also be used on sections; for Toluidine Blue or pinacyanol, air-dried frozen sections are directly stained without any fixation or dehydration, giving the same metachromasia for myelin and collagen seen in the squash or smear preparations. Another slide rapidly stained with H&E provides further information.

In many instances, an H&E frozen section is sufficient for a diagnosis; on some occasions a diagnosis may be obvious even from a touch or other cytologic preparation. In general, however, it is best to plan using all available techniques during an intraoperative consultation, as they are often complementary, and combined data leads one to the best rapid diagnosis. This must then be communicated to the neurosurgeon, by telephone, through an intercom system, or, often, in person if the surgeon comes out of the OR to review the slides with the neuropathologist. When this is feasible, it is best, as it allows discussion of the case directly with the operating surgeon; but this is not always possible. When communicating by intercom, it is essential to keep in mind that one must confirm that both sides clearly hear all of the communicated information. The neuropathologist must confirm that he is speaking to the correct OR and that he is delivering a diagnosis on the correct patient; he also should be aware if the patient is awake and aware, as is sometimes the case. The diagnosis, when delivered via telephone or intercom, should be read back to the neuropathologist by the person on the other end, whether it is a circulating nurse, a resident, or an attending neurosurgeon. These steps confirm that communication has been accurate and that no errors have been made in understanding what the neuropathologist is reporting.

Under most circumstances, the tissue used for frozen section should be thawed, fixed in formalin, and then submitted for routine histopathological processing as a "frozen section control." The actual frozen section and other (cytological preparation) slides should be retained, appropriately labeled, with the permanent sections from the case. Occasionally, all of the diagnostic findings are in the frozen tissue, and permanent sections do not contain similar tissues. Thus, it is in fact essential that the frozen section slides are not discarded or lost. If the diagnosis is one of probable or definite lymphoma, in some laboratories it is desirable to retain the frozen tissue cold for possible immunofluorescence or flow cytometric studies, but the wide array of lymphoid markers now available for use in paraffin sections as ordinary immunostains generally makes this unnecessary.

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PART ONE

NEOPLASMS

INTRODUCTION

Surgical neuropathology covers most of the gamut of nervous system diseases, but clearly the diseases of paramount importance for most pathologists and neuropathologists who practice in this specialty are tumors. Neurosurgeons remove tissues from the brain and spinal cord to obtain diagnoses as well as for therapeutic purposes, and most often the diagnoses concern the specific type and grade of neoplasms. The largest part (Part I) of this text is devoted, then, to this important task, the pathological diagnosis of tumors of the brain, the spinal cord, and the coverings and adjacent tissues thereof. The first several chapters are organized into Section I and deal with intrinsic tumors of the brain (Subsection 1), followed by chapters covering tumors within the cranial cavity but outside the brain (Subsection 2), and then there is a similar sequence of chapters on intrinsic and extrinsic tumors of the spinal cord (Section II, Subsections 1 and 2).

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