HISTOPATHOLOGY

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Introduction

In the guidelines for the performance of hospital autopsies, it is always stated that an autopsy is incomplete without histological examination of all major organs. It would not be realistic to claim that the same is true of the forensic autopsy. For example, the autopsy of a shotgun wound of the head or heart may not require further histological examination. With few exceptions, histology should be performed in every case. Because of the increasing scrutiny of media and lawyers in forensic issues, forensic histopathology becomes an essential link in the chain of a suspicious death investigation. Yet, in many jurisdictions, such comprehensive practice is limited by time, cost, and above all lack of experts in forensic histopathology. As a matter of fact, most textbooks of forensic medicine devote little attention, if any, to the microscopic features of forensic pathology, and textbooks of surgical pathology do not deal with forensic issues, such as asphyxia and dating of injuries.

Histology is essential:

1. to confirm the nature of lesions found by the naked eye, and to assess their extent and severity accurately
2. to identify those lesions not visible to the naked eye
3. to date injuries.

Moreover, histological slides and paraffin blocks preserve the evidence, because they are stored permanently.

In this article, recommendations for organ sampling and technical procedures are proposed. Some of the most common forensic situations, in which histology is essential, are examined. Limitations of microscopic examination are also discussed.

Technical Procedures

Autopsy techniques and methods of dissection of organs are beyond the scope of this article.

Ten percent formalin solution, a 4% solution of gaseous formaldehyde in water, is the most widely used fixative, and can be recommended for most purposes.

With the exception of the brain, which should be fixed whole before being sliced, and the heart, which is also fixed whole following dissection, it is emphasized that the smaller the specimen, the sooner the fixation will be completed. Specimens should not be thicker than 6–8 mm. The minimal acceptable volume of fixation fluid is about 5 times the volume of the specimen. Specimens must be rinsed before fixation to remove clots. After fixation, small samples are taken from the specimen, dehydrated, and embedded in paraffin. Three-μm thick sections are obtained from the paraffin blocks with a microtome, and stained with hematoxylin and eosin (H&E). Additional stains are often required. They should be mentioned in the pathology report. Perls's stain is used to detect iron-laden macrophages, especially in meningeal membranes, lungs, liver, and spleen, because iron deposits may be underestimated by H&E stain (Figures 1 and 2). Sirius red or Masson's trichrome stains are often useful in assessing fibrosis, which may also be underestimated by H&E stain. Reticulin fibers can be stained by Gordon–Sweet stain (Figure 3). This stain is essential in examining lungs for signs of asphyxia, or when the body is putrefied. In lungs, foreign bodies should be searched for with a polarized light microscope (Figure 4). Deposits of starch, talc, or other refractive materials are frequent in the lungs of long-standing drug users (Figure 4).

Since the 1990s, advances in histopathological techniques, such as enzyme histochemistry and immunohistochemistry, have been applied to forensic practice. These techniques, used mainly in dating wounds, are discussed later in this article. However, changes in tissues, whether due to disease or injury, are subject to numerous variables, and any conclusion drawn from such studies must be hedged about with qualifications.

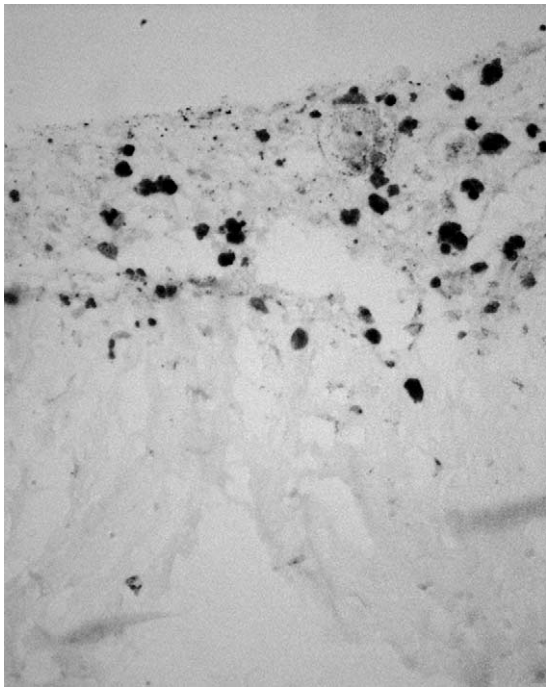

Figure 1 Iron-laden macrophages revealed by Perls's stain, indicating previous bleeding in dura. Original magnification ×400.

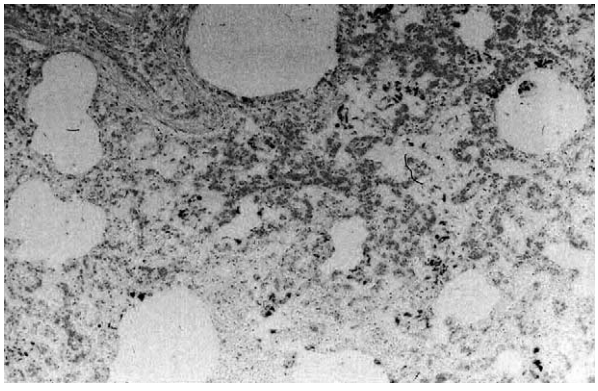

Figure 2 Siderophages revealed by Perls's stain in the lungs of an abused infant. They have been suggested to be a marker of previous repetitive hypoxic events. Original magnification ×100. Courtesy of A. Dorandeu, MD.

Organ Sampling: Practical Application

Brain, Dura, and Spinal Cord

The brain is weighed fresh, but should not be sliced before fixation. This unfailingly leads to distortion of the cut surface during subsequent fixation. Deleterious effects are particularly severe in the case of

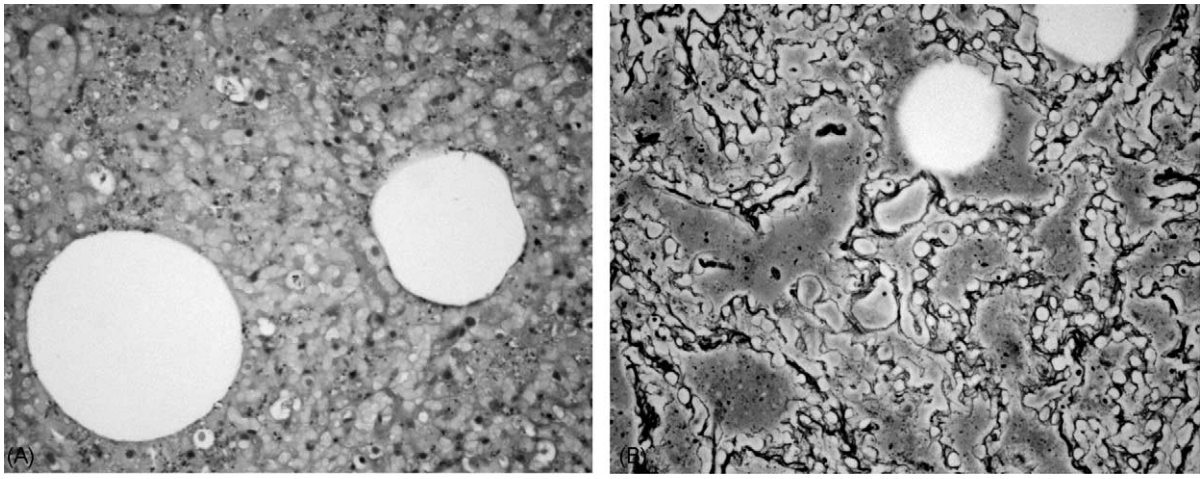

Figure 3 Alveolar architecture may not be analyzable in a putrefied body ((A), hematoxylin and eosin). Gordon-Sweet stain for reticulin fibers reveals basal membranes (B). Marked congestion is evident, as well as gas bubbles. There were no alveolar wall ruptures. Sudden death ensued. The male victim was found with high cocaine metabolite blood levels. Original magnification ×200.

abundant edema or large hematoma. The same rule applies to fetal and infantile brains, because of their pronounced softness.

The examination of the brain at the autopsy table should be confined to significant surface features, such as subarachnoidal hemorrhage. Blood in the subarachnoid space is best cleared away before fixation, after noting its apparent origin – a contused area, or a ruptured aneurysm.

Flattening of cerebral convolutions and marks of tentorial or foraminal herniation are indicative of cerebral edema. However, as experienced, pathologists tend to overestimate edema in the fresh brain. Because edema indicates a prolonged survival time, overestimation may have important unfortunate medicolegal implications. For this reason, we recommend edema is only assessed in the fixed brain and at histology.

Acute meningitis should only be assessed in the fresh brain when purulent exudate is readily visible in the subarachnoid space. Otherwise, one should be cautious not to misdiagnose turbid edema with meningitis. Histology is the gold standard for the diagnosis of meningitis, in addition to microbiology performed on the cerebrospinal fluid and/or small brain samples (Figure 5).

The brain is fixed whole in formalin solution. A 4-week fixation in formalin is recommended before dissection. The number and sites of samples taken for histology depend upon gross findings.

When the brain appears grossly normal, samples should be properly selected depending on suspected pathology: degenerative conditions, such as Alzheimer disease, metabolic/toxic encephalopathy, encephalitis, epilepsy, hypoxia, or traumatic axonal injury.

Beta-amyloid precursor protein (beta-APP) antibody is widely used as a marker for axonal injury with positivity 1.5–2 h after its occurrence (Figure 6). Recent work on infant head injury has indicated that the major type of brain damage seen is due to hypoxic–ischaemic damage with focal axonal injury at the craniocervical junction.

It must be borne in mind that axonal injury may not only be caused by trauma, but is also a marker of damage from other causes, and, in the context of head injury, occurs frequently in association with raised intracranial pressure and ischemic injury.

Perls's stain may reveal iron deposits indicating previous bleeding in dura, leptomeningeal membrane, or parenchyma (Figure 1). A trauma may have been involved; however, minimal repetitive bleedings may have been spontaneous due to certain vascular malformations, such as telangiectasia or angiomas.

The differential diagnosis of intracerebral hematoma depends on whether it is natural or traumatic; if it is natural, the relationship or otherwise to any trauma can be problematic. It is important in these cases to have other pathological evidence of hypertension and/or traumatic brain injury. Histology may also be of help in differentiating hypertensive hemorrhage from that associated with amyloid. Amyloid tends to have a lobar distribution and can be easily identified histologically with Congo red or thioflavin T stains.

Gliosis, macrophages, and neovessels may give an indication of the age of the injury. CD 68 antibody for microglial cells may aid in injury aging, as well as

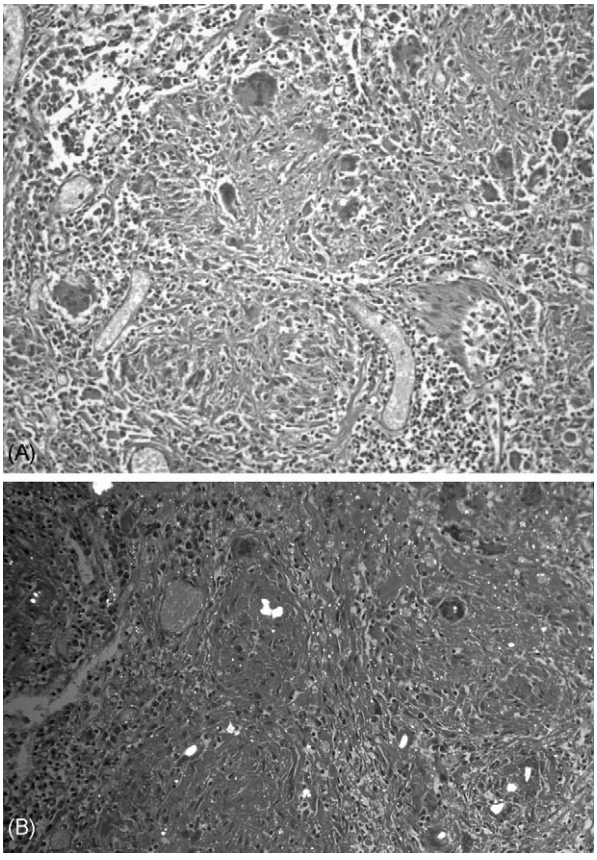

Figure 4 Sudden death in a 32-year-old male. Histology showed (A) pulmonary and hepatic massive sarcoid-like granulomatous inflammation. Original magnification ×200; stain: hematoxylin and eosin. Polarized light microscopy revealed numerous foreign bodies in giant cells (white spots), suggesting long-standing drug use (B); original magnification ×200; stain: hematoxylin and eosin. Toxic blood concentration of buprenorphine was found in combination with low levels of benzodiazepines and neuroleptics. The chronic drug-induced pulmonary condition associated with acute intoxication caused the death.

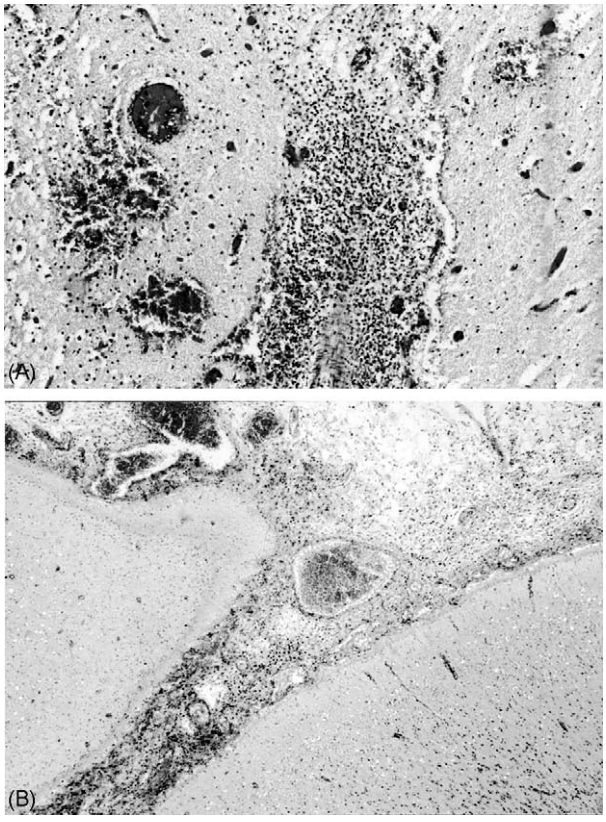

Figure 5 (A) Suspected child abuse in a 5-month-old infant with leptomeningeal hemorrhage associated with parenchymal hemorrhagic areas. Histology showed hemorrhagic meningoencephalitis. *Escherichia coli* was found in postmortem brain samples. Original magnification ×100; stain: hematoxylin and eosin. Courtesy of MD Piercecchi, MD. (B) Suspected child abuse in a 10-month-old infant with leptomeningeal hemorrhage. There was no skull fracture. Histology showed subacute leptomeningeal hemorrhage, parenchymal contusions, and edema. Original magnification ×100; stain: hematoxylin and eosin. Other autopsy findings, including bruises and history confirmed child abuse. Note the misleading apparent similarity between pathologic features.

highlight older injury. However, one must be cautious in assessing the age of a brain lesion. When there are several lesions, comparisons of histologic features may help in determining whether repetitive traumas have been inflicted. This issue is of paramount importance, especially in suspected child abuse cases.

In the absence of macroscopic lesions in an epileptic brain, gliosis and loss of neurons should be searched for in the hippocampus, neocortex, and cerebellum. Samples from hippocampus and cerebellum are systematically selected, because they are known to be vulnerable to hypoxia. The sites most likely to show cell loss and gliosis in the hippocampus are the Sommer sector of the pyramidal cell layer, and the end folium.

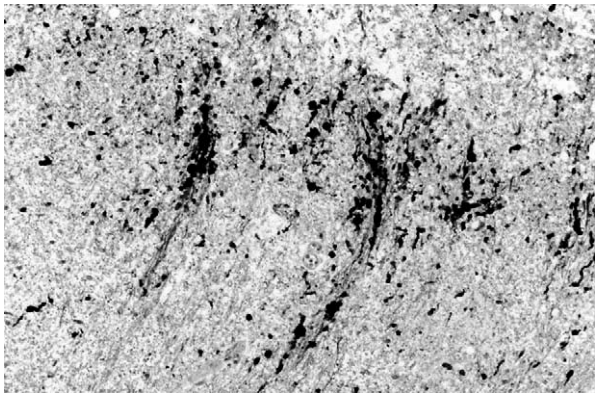

Figure 6 Traumatic axonal injury evidenced by beta-APP antibody, in the brain of a woman who died 3 h after a traffic accident. Original magnification ×200; stain: hematoxylin and eosin. Courtesy of MD Piercecchi, MD.

When the brain appears normal, both grossly and histologically, despite evidence of skull trauma, such as fracture or scalp hematoma, the pathologist may hypothesize that death occurred before lesions were visible. Dissection of a vertebral artery or spinal cord trauma should also be considered. The spinal cord is only kept for histological examination when traumatic lesions are suspected. Degenerative, tumoral, or inflammatory lesions are exceptional in forensic practice.

Dura should be systematically kept for histology, even when it appears normal to the naked eye. Although ochre areas are usually present in cases of previous trauma, Perls's stain may sometimes reveal small iron deposits invisible to the naked eye.

Eyes and Optic Nerves

Retinal hemorrhage, subdural hemorrhage, and cerebral edema have been considered diagnostic for a shaken-baby infant, since the syndrome was described in the 1970s. When nonaccidental injury in infants and young children is suspected, careful histological examination of retinal hemorrhages is of critical importance, although there remains debate about the significance of some findings. The question of whether retinal hemorrhages are of reliable diagnostic value in the controversial shaken-baby syndrome has not received a definite and clear-cut answer. Some authors have emphasized the diagnostic value of the distribution and pattern of these hemorrhages.

The International Retinal Hemorrhages Research Network has assembled a protocol for ocular examinations which provides detailed descriptions of technique and observations identified as having value in distinguishing between nonaccidental and accidental injuries.

Larynx

The organ block examined fresh should contain the floor of the mouth, tongue, soft palate, tonsils, trachea, larynx, cervical esophagus, neck muscles, carotid arteries, jugular veins, and the thyroid gland. The hyoid bone is examined for fracture and hemorrhages, and kept for histology. Soft tissues surrounding bone/cartilage are dissected and examined for hemorrhages, and also kept for histology. The larynx is opened along the posterior midline with scissors, and the lateral portions are pulled apart to expose the mucosa. This maneuver should be cautious, so as not to break an ossified laryngeal cartilage. The larynx should be kept for histology. As experienced, some lesions involving the mucosa, such as edema and inflammatory infiltrates, may not appear at gross examination. Hyperplastic inflamed tonsils, epiglottitis, or laryngitis may cause asphyxia (Figure 7). In forensic practice, interpretation of cervical hemorrhages and/or suspicious mobility of hyoid or thyroid horns is sometimes difficult. X-rays of the larynx and hyoid bone do not usually provide a clear-cut answer, when interpretation of gross findings has been impossible. Histology is the gold standard in demonstrating hemorrhagic fracture. Furthermore, as previously emphasized, histologic slides keep the evidence intact.

Heart and Aorta

Thorough examination of the heart, both grossly and histologically, is essential in forensic practice. Anatomy and detailed methods of dissection of

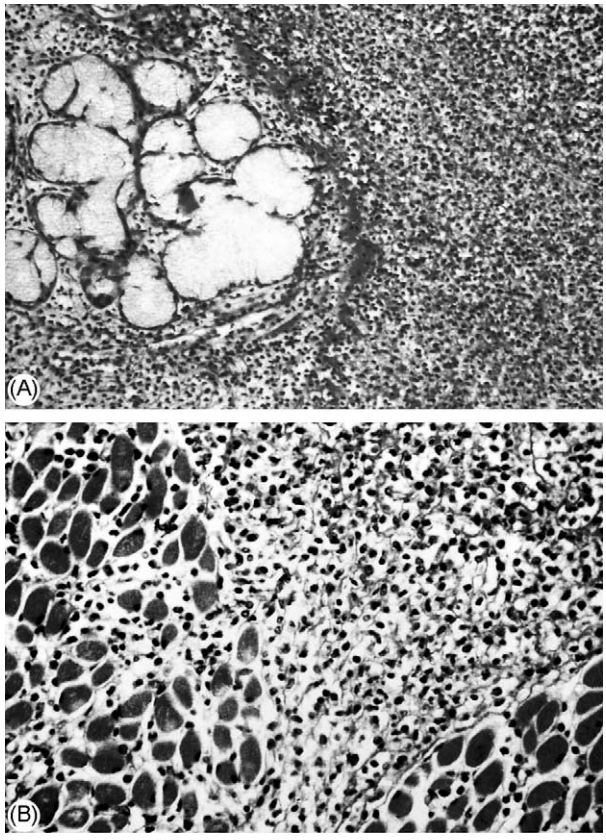

Figure 7 Homicide suspected in a 3-month-old infant found with rib fractures, bruises, and obstructive asphyxia. At gross examination, retropharyngeal hemorrhagic features suggested that a foreign body or fingers might have been used. The infant's father denied killing his child and said he attempted to resuscitate him. Histology showed that obstructive asphyxia had been caused by retropharyngeal and cervical infectious suppurative cellulitis. It was concluded that death was natural. Note the retrolingual salivary glands and cervical muscular inflammatory involvement ((A) and (B), respectively). Original magnification ×200; stain: hematoxylin and eosin.

the heart are beyond the scope of this article. Only general recommendations are proposed.

The heart should be dissected fresh. First, epicardial coronary arteries should be cut in cross-section at 3–5-mm intervals. Calcified vessels that cannot be readily cut with a scalpel should be stripped off the heart and decalcified for at least 24 h before cutting. A four-point system is applied, by 25% increments of narrowing in cross-sectional area. Stenosis of at least 75% is considered significant/severe. A 90%/pinpoint stenosis is considered critical. The narrowest segments and suspected thromboses are sampled for histology.

In our opinion, the short-axis method of cardiac dissection, the so-called bread slice method, is the method of choice. Cuts of 1.0 cm thick are made, parallel to the atrioventricular groove, starting from the apex. The basal third of the ventricles is left attached to the atria, in order to preserve the attachment of the chordae. The basal portion is then opened according to the inflow–outflow method.

The short-axis method, as opposed to the inflow–outflow method, allows accurate assessment of ventricular cavity dilatation and mapping of scar fibrosis and/or acute infarction.

The heart is weighed at the end of the dissection after removal of clots, and should be fixed whole for histological examination. A normal heart weight is less than 0.45% of body weight in the male, and less than 0.42% in the female.

Four samples are systematically taken from the left ventricle, in the anterior, lateral, posterior/inferior, and septal walls, as well as two samples from the right ventricle in the anterolateral and posterior walls. Additional samples are taken in pathological areas.

In a typical medical examiner practice, approximately 50% of deaths are natural, and most are sudden deaths. Clinicians and epidemiologists define sudden death as natural, unexpected, and occurring within 1 h of the onset of symptoms. For medicolegal purposes, this definition is recommended, because it includes only those deaths due to electrical disturbances, and therefore allows accurate diagnoses. Studies using a time interval of 6 h include other mechanisms of death such as acute heart or respiratory failure.

Coronary heart disease accounts for 80% of sudden cardiac deaths. Most sudden deaths related to myocardial infarction occur within the first hour of its onset.

Based on autopsy studies, it has been determined that greater or equal to 75% cross-sectional luminal narrowing is a useful figure for separating significant stenosis that may result in acute myocardial ischemia from noncritical stenoses. Myocardial infarction is almost always caused by thrombotic occlusion of an epicardial coronary artery upon a ruptured atherosclerotic plaque. The acute infarction may not be visible to the naked eye, due to the short time elapsing between onset of symptoms and death, even if the triphenyl tetrazolium chloride or other gross histochemical technique tests are employed. These techniques are not recommended in forensic practice, because many flaws may affect the results. Infarction is visible to the naked eye approximately 24 h after its onset. The histology of the myocardium in early infarction may also be negative as far as classical findings are considered, e.g., coagulative necrosis and accompanying inflammatory infiltrates. These histologic lesions are detectable approximately 12 h after infarction. Contraction band necrosis may be detectable a few hours earlier (Figure 8). However, one should be cautious in interpreting contraction band necrosis, since it may also be explained by reperfusion damage following resuscitation attempts. When histology is negative, C5b-9 complement compound may prove a helpful immunohistochemical marker in assessing and dating early necrosis (Figure 9).

Transient myocardial ischemia, occurring in the setting of stable/unstable and vasospastic angina pectoris, may also trigger fatal arrhythmias in the absence of overt myocardial damage.

In forensic practice, coronary artery thromboses are relatively rare. Lethal ventricular arrhythmias occur most frequently in patients with myocardial scars resulting from previous myocardial infarction, due to a slowing of conduction and the onset of reentry circuits at the border between normal and

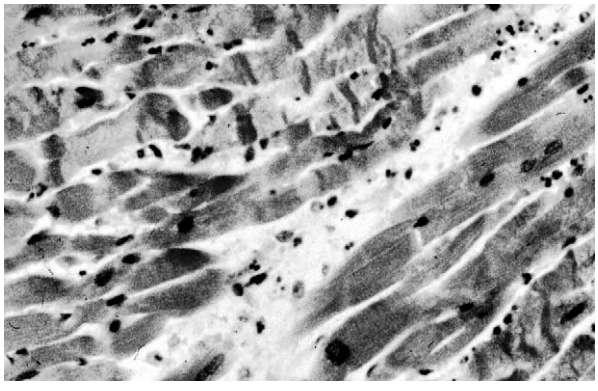

Figure 8 Myocyte necrosis with contraction bands in early myocardial infarction. There were no resuscitation attempts. Note the rare neutrophils in the interstitium. Original magnification ×400; stain: hematoxylin and eosin.

fibrotic myocardium. Neurovegetative influences, whether emotion or effort, may have a triggering role.

Histology of epicardial and intramyocardial coronary arteries may also reveal amyloidosis (Figure 10), necrotizing angiitis, Kawasaki disease, giant-cell arteritis, or thrombotic microangiopathy. Coronary artery dissection, either spontaneous or following invasive procedures, may be a cause of sudden death. A left ventricular mural thrombus, bacterial endocarditis with friable septic vegetations, tumors, such as myxoma or papilloma, and noninfective thrombotic endocarditis may be causes of coronary embolism and sudden death.

Myocarditis is defined, according to the Dallas Criteria, as an inflammatory infiltrate of the myocardium with necrosis and/or degeneration of myocytes not typical of the ischemic damage associated with coronary artery disease (Figure 11). According to the same criteria, the term borderline myocarditis is used, when the inflammatory infiltrate is too sparse or damage to the myocyte is not demonstrable by light microscopy, or both. A sparse population of lymphocytes up to a mean of fewer than five lymphocytes per field (×400) should be considered normal.

Myocarditis may be a cause of cardiac failure, but sudden death is likely to be a more frequent manifestation, albeit underestimated, particularly in the young. Sudden death may occur in both the active and healed phases as a consequence of ventricular arrhythmias. The gross appearance of the heart may be normal. In the acute phase, histology discloses a patchy inflammatory infiltrate, associated with myocardial necrosis. The inflammatory infiltrate is most often purely lymphocytic. Evidence of myocardial infection, whether viral, fungal, bacterial, or parasitic, is rarely found. Giant-cell myocarditis, sarcoidosis, and eosinophilic myocarditis are rare. When myocarditis is suspected in an otherwise grossly normal heart, at least 10 blocks should be selected in the left ventricle and five in the right, because patchy inflammatory infiltrates and necrosis may be overlooked.

Hypertrophic cardiomyopathy is a primary disease of cardiac muscle, which is usually genetically transmitted, and is characterized by a hypertrophied but nondilated left ventricle in the absence of another cardiac or systemic disease that may cause left ventricular hypertrophy, such as hypertension, coronary artery, or valve diseases. Genetic mutations involving, among others, the beta-myosin heavy chain, troponin T, alpha-tropomyosin, and myosin-binding protein C genes have been identified. The natural history of hypertrophic cardiomyopathy is often marked by sudden death.

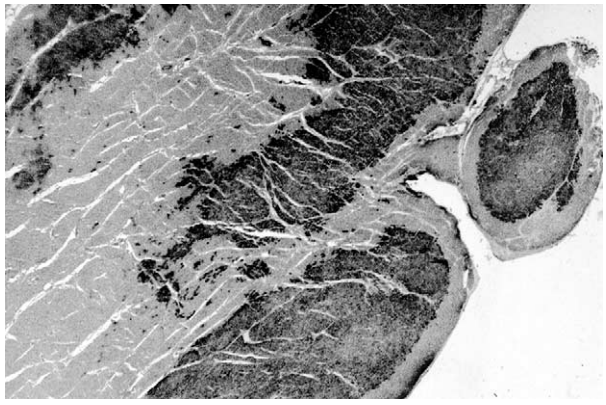

Figure 9 A subendocardial infarction a few hours old, evidenced by C5b-9 complement compound. Hematoxylin and eosin-stained slides did not show evidence of necrosis. Mapping of necrosis can also be of use in differentiating ischemic necrosis from myocarditis. Original magnification ×40.

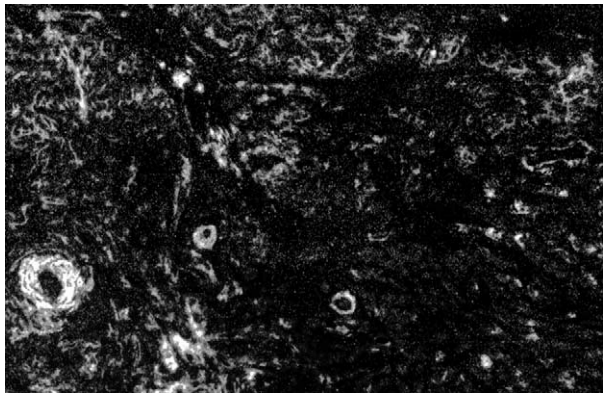

Figure 10 Cardiac amyloidosis evidenced by thioflavin T immunofluorescence in a 45-year-old woman, who died suddenly. A slightly increased heart weight was the only autopsy finding. There was no known medical history. Original magnification ×400; stain: hematoxylin and eosin.

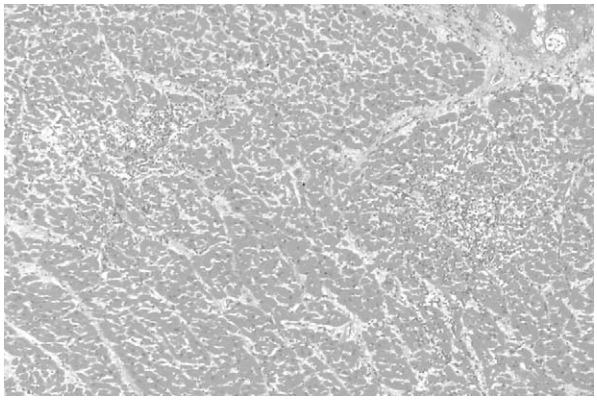

Figure 11 Sudden death. Patchy inflammatory infiltrates in an otherwise normal heart. Myocarditis may be overlooked if numerous ventricular samples are not examined histologically. Original magnification ×40; stain: hematoxylin and eosin.

Histologically, myocytes are spatially arranged in a chaotic manner (Figure 12). Myocyte disarray, interstitial and scar fibrosis cause intraventricular conduction instability. Postischemic scar fibrosis is caused by small-vessel disease, which is characterized by abnormal intramural coronary arteries with thickened walls and narrowed lumina, often associated with fibrosis. Myocyte disarray is sometimes the only marker of the disease; the heart weight and gross pattern are otherwise normal. In most cases, hypertrophy is asymmetrical, with predominant septal wall involvement. However, symmetrical forms of primary hypertrophic cardiomyopathy have been reported. Myocyte disarray is absent. Such forms should only be considered primary when any other cause of hypertrophy, including hypertension, has been ruled out.

Fat is a normal component of the right ventricle. However, in some hearts, the proportion of fat is dramatically increased, especially in obese and/or old women. As opposed to this pattern, arrhythmogenic right ventricular cardiomyopathy (ARVC), also known as right ventricular dysplasia, is characterized by fibrofatty replacement of the right ventricular myocardium (Figure 13). The left ventricle may also be affected. The patients are often young people, and sudden death is often the first manifestation of the disease. Clinical findings suggest a familial occurrence with autosomal dominant inheritance, various penetrance, and polymorphic phenotype expression. The intraventricular conduction delay, consequent to fibrofatty replacement, is a source of electrical instability, due to reentrant phenomena. At gross examination, heart weight is usually within the normal range. The pathological diagnosis of ARVC is assessed in the presence of gross and/or histological evidence of regional or diffuse transmural fibrofatty replacement of the right ventricular free wall. Rare sudden deaths have been reported in individuals with only extensive fatty replacement. This diagnosis should be assessed, when any other cause of death, including toxic death, has been ruled out.

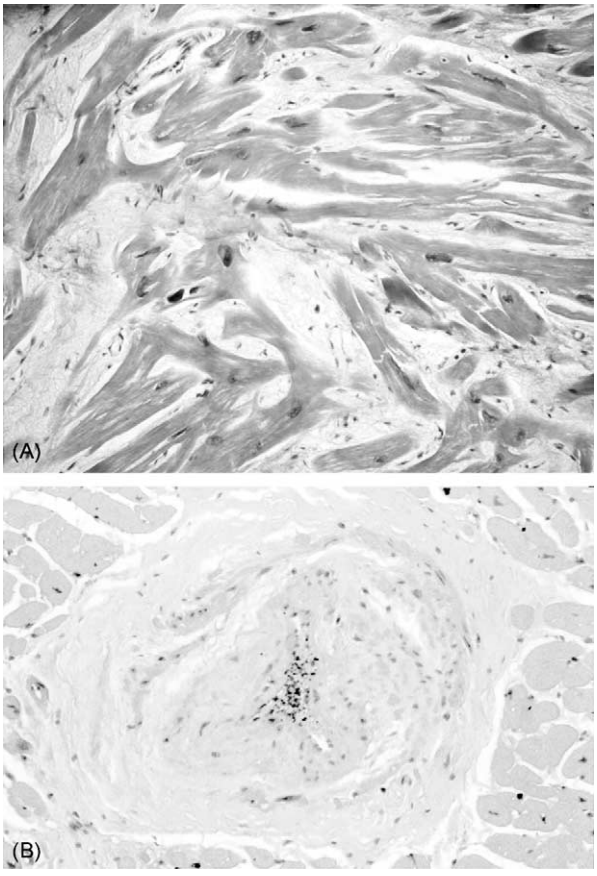

Figure 12 Myocyte disarray (A) and abnormal intramyocardial coronary artery (B) in hypertrophic cardiomyopathy. Original magnification ×200 (A), ×400 (B); stain: hematoxylin and eosin.

At least five samples from the right ventricle should be taken, especially in the anterior wall, as well as in the posteroinferior wall, at the apex and infundibulum (triangle of dysplasia). Focal myocarditis or lymphocytic infiltrate is sometimes observed.

In some cases, where the heart is both grossly and histologically normal, and circumstances of the death suggest an electrical event, conduction tissue should be histologically examined. Interpretation of findings is very difficult. It should be emphasized that the mere observation of histological changes involving the conduction tissue or an accessory atrioventricular (AV) connection does not allow any surmises about electrical or clinical significance, in the absence of an electrocardiogram (ECG) recording or electrophysiological data. Fibromuscular dysplasia of the AV node, tumors, fibrosis, and myocarditis, among others, may cause sudden death.

In some cases, sudden cardiac death remains unexplained even after a thorough macroscopic and microscopic examination, including the conduction system. The long QT syndrome, Brugada's syndrome, or polymorphic ventricular tachycardia are causes of sudden death with no structural lesions. However, these diagnoses cannot be assessed without ECG recording.

Pathologists should bear in mind that there is no close correlation between cardiac histological features and clinical events. In other words, sudden cardiac death may occur in persons with a heart that appears "too good to die," whereas a noncardiac death may occur in others with a heart "too bad to survive." Thorough histological examination of all organs and toxicological analyses are therefore required in assessing the cause of death.

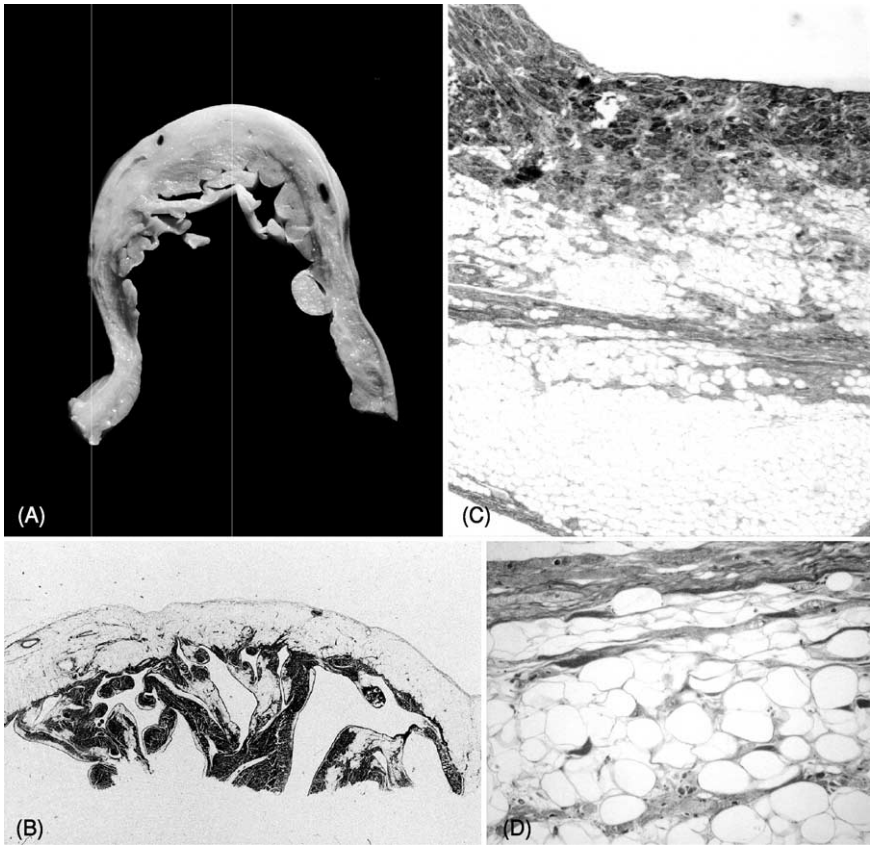

Figure 13 Right arrhythmogenic ventricular cardiomyopathy. (A) Gross features: massive fatty replacement of right ventricular myocardium. (B–D) Corresponding histologic features showing both diffuse transmural fat and fibrosis. Photograph of mounted slide (B) and original magnifications ×40 (C), ×400 (D). Stain: Masson's trichrome.

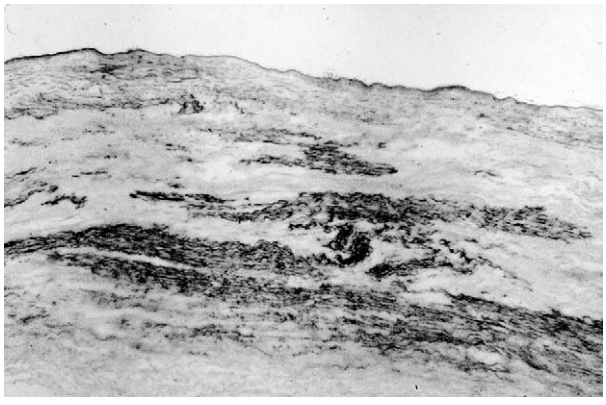

Figure 14 Marfan's syndrome. Massive elastic fiber loss and fragmentation in the aortic media. Original magnification ×200; stain: orcein.

Aortic dissection and aneurysm may be idiopathic or a complication of Marfan syndrome. The tunica media exhibits significant changes in terms of degenerative cysts, the so-called cystic medial necrosis, severe disruption of elastic fibers, and loss of smooth-muscle cells (Figure 14). The defective gene encodes fibrillin-1, which is the major constituent of microfibrils of the extracellular matrix.

Lungs

Interpretation of gross findings is often difficult. Pulmonary lesions are often patchy and histologic features are usually heterogeneous. Moreover, many lesions, such as granulomas, or related to chronic/acute asthma, are not visible to the naked eye. For these reasons, we recommend at least 12 samples are taken, including one in each proximal region near the hilus, one in the central region, and one at the periphery of every lobe. More samples should be taken when necessary, especially when obstructive asphyxia/drowning is suspected.

In obstructive asphyxiation, extensive hemorrhagic edema is present in the interstitium and alveolar spaces. The hemorrhagic feature and the interstitial edema are strong arguments against postmortem edema. However, although suggestive of obstructive asphyxia, lesions are not specific and should be interpreted in the light of autopsy findings and toxicological data.

Histologic investigation of bodies found in water should not be confined to the lungs, but requires complete histologic examination of all organs in order to differentiate between death by drowning and other causes of death. This task is difficult, because there are no pathognomonic gross or histologic findings on which to base the diagnosis. Varying amounts of water may have been aspirated. Thus, the length of time before irreversible hypoxia occurs might be different. The circumstances of the death also influence other pathophysiologic effects. One victim may die in water from physical exhaustion. Another may fall into the water, and die suddenly. Accidental drowning is likely to differ from suicidal drowning, in which victims may be intoxicated with drugs or alcohol. Moreover, histologic examination is often complicated by putrefaction. The diagnostic problem is further complicated by the heterogeneous distribution of changes in the lungs. Multiple sections must be examined, and stained for reticulin fibers. Examination of the reticulin network is particularly useful when the body is putrefied (Figure 3). The most important histologic findings in the lungs are interstitial congestion, edema, alveolar macrophages, alveolar hemorrhage, and emphysema aquosum (Figure 15). The latter is characterized by acute dilatation of the alveoli with extension, elongation, and thinning of the septa, and compression of the alveolar capillaries. It should be noted that some drowned victims die of laryngospasm without actually aspirating fluid.

In other organs – especially the brain, heart, and liver – there is marked acute congestion, associated with perivascular hemorrhage. These changes are indicative of hypoxia, but are not specific. Thorough examination of the heart is important to determine whether heart disease has been involved in the death.

Diatom analysis is one of the biologic tests used to assess the diagnosis of drowning. The forced entry of diatoms into the circulatory system results in embolization into internal organs. Diatom identification and quantification require long experience to avoid mistakes. Diatoms found in the lungs, bone marrow, and other organs are compared to those found in water where the body was found.

It has been suggested that the presence of siderophages in the lungs of infants who die unexpectedly should be considered a marker of a previous hypoxic event, or repetitive asphyxia, which may preclude a diagnosis of sudden infant death syndrome. Because iron-laden macrophages may be underestimated or overlooked on routine examination with H&E, Perls's stains should be used routinely in the investigation of unexplained infant deaths (Figure 2).

In asthma, airway obstruction is peripheral and diffuse. Histology discloses eosinophils, typical thickening of bronchial basal membrane and plugs in the bronchial lumen, due to hypersecretion of the mucinous glands (Figure 16).

Histology of the respiratory tract (trachea, bronchi, and lung tissue) in fire deaths is essential in determining whether the victims died before or during the fire. Vital lesions include swelling and superficial coagulation necrosis of the cylindrical epithelium, deposits of soot at the surface of the laryngeal and bronchial epithelium, and in alveolar spaces.

Genital Organs

The genital organs are kept for histology if findings at the death scene or autopsy findings raise the slightest doubt of a possible sexual assault. In addition, the perineum and the anorectal segment should also

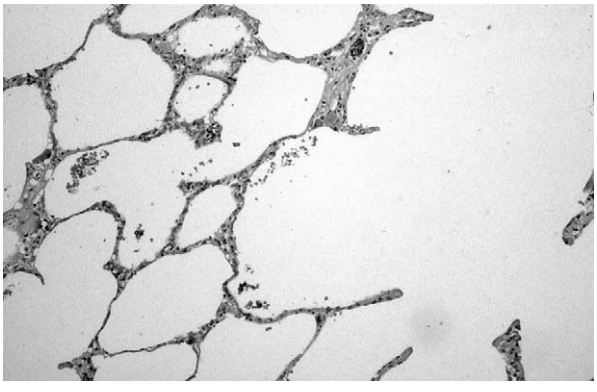

Figure 15 ''Emphysema aquosum'' in a drowning case. Elongated and ruptured alveolar walls and enlarged alveolar cavities. Original magnification ×200; stain: hematoxylin and eosin.

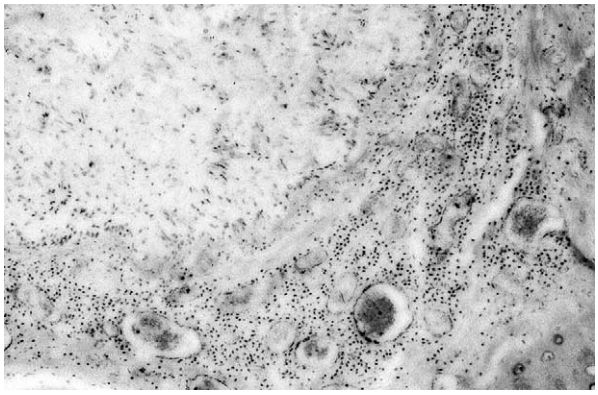

Figure 16 Death from asthma. The bronchial lumen is occluded by mucus containing desquamative epithelial cells. The basal membrane is thickened. The dense inflammatory infiltrate is almost exclusively eosinophilic. Original magnification ×400; stain: hematoxylin and eosin.

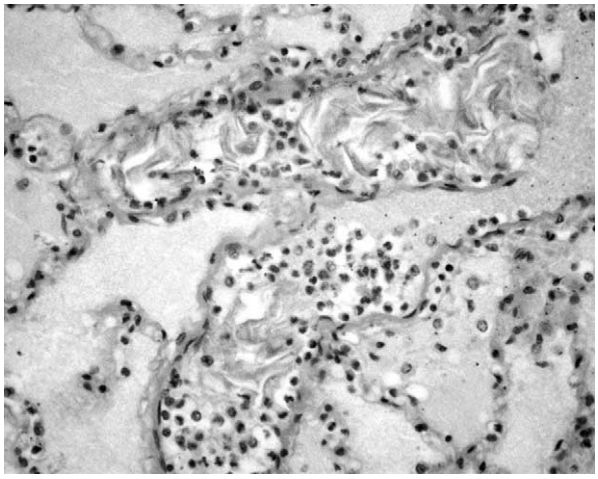

Figure 17 Maternal death shortly after delivery. Massive amniotic embolism. Original magnification ×400; stain: hematoxylin and eosin.

be kept for histology. Multiple sections should be examined for hemorrhage and ulcerative lesions.

Genital organs are also kept for histology in investigating a maternal death. Amniotic embolism is a possible cause of death in this context (Figure 17).

Bones

In infants/children, all acute or healed fractures should be histologically examined for aging.

Hemorrhage is present at the site of the fracture and extends into the surrounding muscles. The tissue damage stimulates an inflammatory response. Following neutrophilic infiltration, macrophages remove the fibrin, red cells, inflammatory exudates, and debris. Globules of fat and bone marrow are released and may enter disrupted vascular spaces and become embolic. The inflammatory phase lasts about 3 days. Following this phase, there is an ingrowth of capillary loops and fibroblasts forming granulation tissue, which appears by 4 days. By about 7 days, there are abundant fibroblasts. At this stage, there is usually a proliferation and infiltration of osteogenic cells in the periosteum at the periphery of, and not into, the fracture line. Within 1 week, small areas of new bone are visible. The growth of woven bone represents a temporary repair corresponding to union by primary callus formation at about 30 days. In the reorganization of remodeling phase, the trabeculae of woven bone gradually undergo resorption and are replaced by lamellae of bone, which are laid down in parallel plates corresponding to the lines of stress in the bone. This period of consolidation into mature lamellar bone may take as long as 1 year. It must be borne in mind that bone repair is faster in children than in adults.

Table 1 Main histological and immunohistochemical features in skin wounds

	Earliest appearance	*Regular appearance*
Neutrophils	15–30 min	>15 h
Macrophages	2–3 h	>3 days
Macrophages > neutrophils	20 h	>11 days
Siderophages/hemosiderin	3 days	
Granulation tissue	3 days	
Lymphocyte infiltrates	8 days	
P-selectin	minutes	7 h (latest appearance)
Fibronectin	10–20 min	>4 h
E-selectin	1 h	17 days (latest appearance)
ICAM-1	1.5 h	3.5 days (latest appearance)
Tenascin	2–3 days	>5 days
Collagen III	2–3 days	>6 days
Collagen V	3 days	>6 days
Collagen VI	3 days	>6 days
Collagen I	4–6 days	>6 days
Myofibroblasts (mf)	1.5 days	
Positive for laminin (mf) positive for alpha-actin	5 days	
Cytokeratin 5. Complete staining of the epidermis	13 days	>23 days

Adapted from Betz P (1995) Immunohistochemical parameters for the age estimation of human skin wounds. *American Journal of Forensic Medicine and Pathology* 16: 203–209.

Skin

The lesion surrounded by normal skin is sampled for histology.

Dating wounds and bruises is an essential but difficult task in forensic practice. The acute inflammatory reaction is characterized by sequential chronological events, which can be detected by routine histology and enzyme or immunohistochemical techniques. Various factors, including the age of the victim, site, size, or mechanism of injury, are to be taken into consideration in estimating the age of a lesion. Only published human autopsy series should be used as guidelines. Table 1 details the main histological features in skin wounds.

Morphological alterations are preceded by functional enzyme-related changes. Thus, it is to be expected that the demonstration of enzymes and other substances causing changes could reveal earlier reactions than could the visualization of the resulting histologically demonstrable alterations. Main developments in this aspect of vitality diagnosis are due to studies by Raekallio. Using enzyme histochemistry methods, he identified two clearly delineated zones of differing enzymatic activity around a vital wound: a central zone and a peripheral zone. The central

zone is an area 200–500 μm wide that is located at the edges of a vital wound and shows a gradual decrease in enzymatic activity. This decrease can be detected between 1 and 4 h after wound infliction. This enzymatic response has been called the "negative vital reaction." It is recognized as the maximum tissue destruction zone. The peripheral zone is an area 100–200 μm wide circumscribing the central zone. This area shows a remarkable increase in enzymatic activity 1 h after wound infliction. This response has been called the "positive vital reaction." The second important finding of Raekallio was the realization that the increase in enzymatic activity in the peripheral zone occurs over a specific time interval, and this time interval is different for each enzyme. Furthermore, these enzymatic activities possess good postmortem stability and can be seen up to 5 days after death. For these reasons, enzymatic activity is useful in determining the age of a wound. Both the central and the peripheral zones are not present in postmortem wounds, at least those inflicted 1 h or more after death. Table 2 details the main enzymatic histochemical features in skin wounds.

The immunohistochemical detection of antigens expressed during the early vascular phase of wound healing, in which cellular reactions are still absent, also provides important information on the intravital origin of lesions, i.e., that the wounds have been inflicted in life and not after death. However, the diagnostic value of immunohistochemistry for forensic purposes should be treated with caution. For example, it was shown in immunohistochemical studies that fibrin deposits can be demonstrated up to 6 h after death. Therefore, postmortem fibrin in bruises cannot be distinguished with certainty from antemortem fibrin.

Table 1 displays the sequential events detected by immunohistochemical techniques.

Table 2 Enzyme histochemical vital reactions in antemortem human skin wounds

	Increase in the peripheral zone (h)		*Decrease in the central zone (h)*	
Enzyme activity	*From*	*To*	*From*	*To*
Adenosine triphosphatases	1	2	1	2
Esterases	1	2	1	4
Aminopeptidases	2	4	2	8
Acid phosphatases	4	6	4	8
Alkaline phosphatases	8	12	4	8

Adapted from Raekallio J (1980) Histochemical and biochemical estimation of the age of injuries. In: Perper JA, Wecht CH (eds.) *Microscopic Diagnosis in Forensic Pathology*. Springfield, IL: Charles C. Thomas.

Although it is often difficult to assess accurate aging, comparisons of histologic features may help to determine whether repetitive traumas have been inflicted. This issue is of paramount importance in suspected child abuse.

Biochemical markers have also been investigated. Histamine and serotonin are both vasoactive compounds known to participate in the initial stages of the acute inflammatory reaction. Norepinephrine (noradrenaline), cathepsin D, and prostaglandins have also been investigated. It should however be borne in mind that these techniques require long experience to avoid errors.

Burns, whether thermal, chemical, or electrical, are sampled to determine vitality and mechanism. Enzyme histochemistry has also been applied to demonstrate earlier changes in antemortem burns than was possible with histological methods.

Histologically, at 4 h, a few neutrophils migrate outside the vessels. In 6-h burns there is obvious leukocytic infiltration of the peripheral zone. The concentration of neutrophils increases progressively and is highest at 48–72 h, when, in addition to the neutrophils, there are macrophages and fibroblasts. In the epidermis, by 12–24 h after burning, there is some necrosis and degeneration of the epidermal cells in the central zone located immediately beneath the heated area. In the peripheral zone, surrounding the central area, the epidermal cells start to stretch and their nuclei start to elongate, heralding the onset of epithelial migration. In burns 48–72 h old, migration of the epidermal cells is pronounced. The epithelial cells of the dermal appendages, such as the hair follicles, undergo changes similar to those of epidermal cells. As healing progresses there is increased proliferation of both epithelial and connective tissue elements, but these are not striking until 72 h or more after burning.

The depth and extent of burns are clinically estimated using the "rule of nines." The histological changes associated with the four degrees of burn severity are as follows.

First-degree burns There is dilatation of capillaries, condensation of nuclear chromatin, some necrotic epidermal cells, and edema of the subepidermal connective tissue.

Second-degree burns (blisters) There is subepidermal edema with blister formation, varying degrees of epidermal cell necrosis, and reduced staining reaction of epidermal cell nuclei. In the upper part of the dermis, there is hyperemia, edema, and a minimal perivascular accumulation of neutrophils,

macrophages, and occasional lymphocytes. Neutrophils have usually migrated into the dermis after 16 h.

Third-degree burns (complete destruction of skin) There is loss of epidermis and necrosis of the dermis (including the deeper layers); coagulation of collagenous fibers; necrosis of skin appendages; hyperemia of neighboring capillaries; along the edge of adjacent intact epidermis an elongation of cells and cell nuclei can be seen (palisade formation); the necrotic areas become demarcated by neutrophils after 6–24 h; dermal inflammation tends to lag by several days, although the subcutaneous tissue shows inflammatory cell infiltration by the second day.

Fourth-degree burns (charring) There is complete destruction of skin and subcutaneous tissue, sometimes with exposure of bone.

There are two forms of death from electrocution: (1) deaths caused through contact with electrical conductors and (2) those caused by lightning stroke. The pathological changes in both are similar. Histologically, the cells of the epidermal basal layer show marked nuclear elongation (Figure 18). Vacuolization can be seen in the cells of the spindle cell layer, and sometimes blisters. The typical elongation of the cell nuclei can be seen in the dermis. Metallic particles can be helpful in assessing an electrical burn.

In some cases, histology may be useful in distinguishing between entry and exit gunshot wounds. Attempts have been made to evaluate the presence or absence of soot associated with an entry wound by microscopic examination.

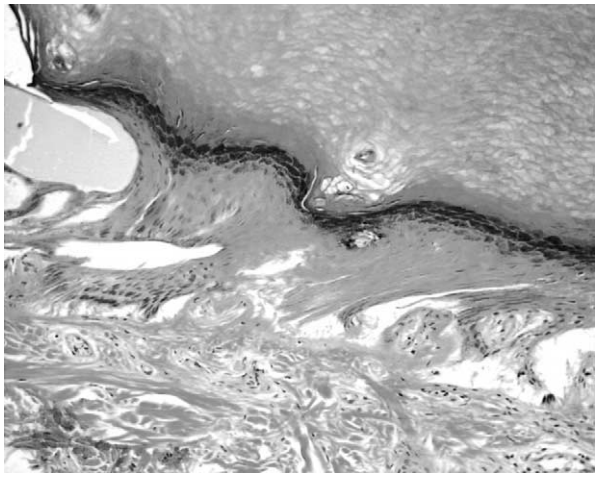

Figure 18 Electric burn (finger skin). The cells of the epidermal basal layer show marked nuclear elongation. The diagnosis should be systematically confirmed by the technical expertise of the suspected electrical equipment or device. Original magnification ×400; stain: hematoxylin and eosin.

Other Organs

One sample of the thymus is kept systematically in infants/children. One sample is taken from the liver, kidneys, spleen, and pancreas. More samples may be required by pathological gross findings. Adrenal glands are kept whole. Samples from esophagus, stomach, and intestines are only kept when gross examination shows abnormalities.

Ten Practical Key Points in Forensic Histology

1. The brain, heart, larynx, and samples of other organs should be systematically kept in formalin solution in all cases.
2. Histology is essential:
   a. to confirm the nature of lesions found by the naked eye, and to assess their extent and severity accurately
   b. to identify those lesions not visible to the naked eye
   c. to date injuries
   d. because histological slides can be stored permanently and therefore preserve the evidence.
3. The brain should not be sliced fresh. It should be kept whole. A 4-week fixation in formalin is recommended before dissection.
4. The heart should be dissected fresh, and fixed whole.
5. Multiple lung samples should be examined histologically, especially when asphyxia/drowning is suspected. Stains for reticulin fibers should be used in those cases.
6. Iron deposits may be missed or underestimated by H&E stain, and therefore should be searched for by Perls's stain.
7. In suspected infant head injury, with or without impact, histological examination of eyes is mandatory. Beta-APP is a useful immunohistochemical marker for traumatic axonal injury.
8. Enzyme and immunohistochemistry for dating wounds and ecchymoses require a long experience. Otherwise, we do not recommend these techniques.
9. Histology should not be performed in "blinded conditions," i.e., with no knowledge of the autopsy findings.
10. The cause of a death should never be based on histological findings only, but also on scene investigation data, medical records, autopsy findings, and laboratory analyses, especially toxicology.

See Also

Immunoassays, Forensic Applications; **Serology:** Overview; Blood Identification; Bloodstain Pattern Analysis

Further Reading

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