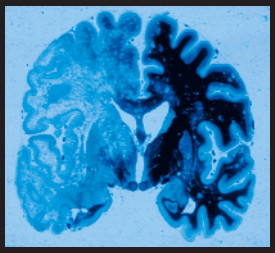

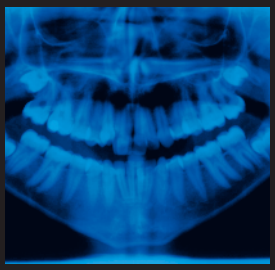

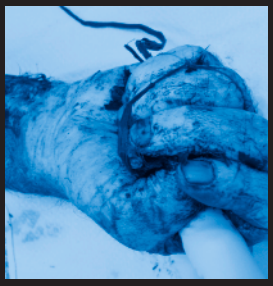

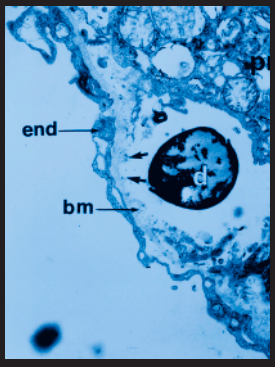

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Volume 3

Edited by

Michael Tsokos, MD

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Forensic Pathology Reviews

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Forensic Pathology Reviews

Volume 3

Edited by

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Series Introduction

Over the last decade, the field of forensic science has expanded enormously. The critical subfield of forensic pathology is essentially based on a transverse, multiorgan approach that includes autopsy, histology (comprising neuropathological examination), immunohistochemistry, bacteriology, DNA techniques, and toxicology to resolve obscure fatalities. The expansion of the field has not only contributed to the understanding and interpretation of many pathological findings, the recognition of injury causality, and the availability of new techniques in both autopsy room and laboratories, but also has produced specific new markers for many pathological conditions within the wide variety of traumatic and nontraumatic deaths with which the forensic pathologist deals.

The *Forensic Pathology Reviews* series reflects this expansion and provides up-to-date knowledge on special topics in the field, focusing closely on the dynamic and rapidly growing evolution of medical science and law. Individual chapters take a problem-oriented approach to a central issue of forensic pathology. A comprehensive review of the international literature that is otherwise difficult to assimilate is given in each chapter. Insights into new diagnostic techniques and their application, at a high level of evidential proof, will surely provide helpful guidance and stimulus to all those involved with death investigation.

It is hoped that this series will succeed in serving as a practical guide to daily forensic pathological and medicolegal routine, as well as provide encouragement and inspiration for future research projects. I wish to express my gratitude to Humana Press for the realization of *Forensic Pathology Reviews.*

***Michael Tsokos,* MD**

Preface

A 2003 editorial in the well-renowned journal *Science* was entitled "Forensic Science: Oxymoron?." An oxymoron is a rhetorical figure in which an epigrammatic effect is created by the conjunction of incongruous or contradictory terms. This short article questioned both the reliability and validity of forensic sciences, alleging a lack of such criteria as error rate, adequate testing, regular standards and techniques, as well as a general lack of acceptance within the field. The will of those involved in death investigation was also questioned, calling for an improvement in the quality of their work in a scientific setting by noticing "Both these public interests—security and justice—would be furthered by a more scientific and reliable technology for analyzing crimes. The mystery here is why the practitioners don't seem to want it!."

From this editor's point of view it is generally impossible to quantify the pain of tortured victims and, without a doubt, the violation of human rights cannot be measured by biostatistical methods. However, police investigators and forensic pathologists have evidenced and documented ethnic cleansing in war zones and thus testified against war criminals in order to continually protect human rights over the past decade. Physical evidence of torture is properly analyzed where based in a scientific setting. Following regular standards and techniques for the identification of human remains, forensic pathologists and anthropologists are able to identify those who are killed by terroristic acts as opposed to civilian deaths. The components of weapons of mass destructions are analyzed by means of modern forensic science techniques. A harmonization of autopsy rules has gained worldwide popularity, providing highly scientific international standards. However, it is probably relatively easy to doubt the methods used in forensic death investigation when one has no insight into real forensic casework. But to doubt the will of those practitioners doing the field work is beyond any serious discussion.

One year after the appearance of the first volume of *Forensic Pathology Reviews*, this series has gained considerable attention within the forensic and medicolegal scientific community worldwide, which is, among other things, reflected in the efforts of 25 researchers from nine different nations representing four continents who have contributed to this third volume of the series. Most of the authors are *the* leading authorities in their particular fields of research. The

chapters in this volume, once again, provide the reader with a profound scientific and practical knowledge on a broad variety of different topics.

Chapter 1 gives the reader a thorough insight into the medicolegal investigation of bodies found in water, focusing not only on victim identification, evaluation of postmortem submersion time, and determination of the cause and manner of death, but also in depth analysis of the pathophysiology of drowning. Chapter 2 devotes attention to human immunodeficiency virus (HIV)-1 infection of the central nervous system in the forensic pathological setting. The forensic pathologist is frequently confronted with HIV-1 infection, especially in the context of drug abuse. In particular, the sampling of specimens for histological examination during autopsy, the neuropathological examination, and the related findings of diagnostic relevance, including the macroscopic and microscopic appearance of opportunistic infections, cerebrovascular complications, and neoplasms associated with the disease, are emphasized. Chapter 3 deals with rare events such as deaths in a head-down position, which most often occur accidentally. The author examines the phenomenology and pathological features of such fatalities, providing new insight into the pathophysiology of inverse body position based on human and animal experiments under true and simulated microgravitational conditions.

Chapter 4 deals with forensic bitemark analysis, giving a comprehensive outlook on promising new areas of research in this field (e.g., the retention of DNA on skin over time and the newly described bacterial fingerprinting technique).

Chapters 5 and 6 are devoted to taphonomic changes of human bodies and their remains, namely the underlying biological processes and resultant postmortem changes that a corpse undergoes during the early postmortem interval. The broad range of variables influencing the morphological picture under which distinctive postmortem changes present, as well as elaborate findings that can serve as a basis for the macromorphological exclusion of a forensically relevant lay time of soil-embedded skeletal remains are provided.

Chapter 7 concerns arrhythmogenic ventricular dysplasia, a disease that plays a significant role that should not be underestimated in daily forensic pathological autopsy practice in cases of sudden death. The illness can lead to lethal cardiac arrhythmia and usually manifests during the third decade of life. Interestingly, regionally higher frequencies of the illness in some countries at least suggest a genetic disposition to the disease. Chapter 8 concerns the postmortem diagnosis of death in anaphylaxis. The authors provide the reader with an up-to-date overview concerning morphological, biochemical, and

immunological investigations toward the diagnosis of anaphylaxis and give helpful guidelines for practical casework.

Chapter 9 takes a comprehensive look at gross, microscopical, and genetic findings in the forensic pathological evaluation of fatal pulmonary thromboembolism and the potentially involved medicolegal issues. Chapters 10 and 11 cover aspects of suicide. A profound look at the trends of suicide in the United States during the twentieth century is given in Chapter 10. These trends have altered drastically, especially within the past century and most specifically in the United States. Chapter 11 addresses problems that may arise in the medicolegal investigation of murder–suicides, uncommon events that require careful investigation.

Chapter 12 deals with the investigation of iatrogenic deaths that constitute a substantial forensic contribution to injury prevention, medical audit, and continuing improvement in health care. Iatrogenic injuries such as perioperative hemorrhage, sepsis, trauma, embolic phenomena, cardiovascular and cerebrovascular events, complications associated with anesthesia, interventional radiology and radiotherapy, as well as adverse drug events and reactions are considered in detail. In Chapter 13, thorough information about the use of radiology in medicolegal investigations (e.g., for the location of foreign bodies within the body, documentation of mechanical injuries, identification purposes, or elucidation of child abuse) is provided.

Again, I owe great thanks to my contributors for making their practical and scientific knowledge available.

Michael Tsokos, *MD*

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Death From Environmental Conditions

1

Macroscopical, Microscopical, and Laboratory Findings in Drowning Victims

*A Comprehensive Review**

Philippe Lunetta, MD and Jerome H. Modell, MD, DSc (Hon)

CONTENTS

SUMMARY

The medicolegal investigation of bodies found in water focuses on victim identification, evaluation of postmortem submersion time, and determina-

* This chapter is based in part on the introductory section of the first author's doctoral dissertation entitled, "Bodies Found in Water–Epidermiological and Medico-Legal Aspects," Helsinki, 2005.

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tion of the cause and manner of death. In any given case the circumstances surrounding death, environmental factors, victim's preexisting diseases, and autopsy findings must be appropriately considered in reaching a diagnosis of the cause and manner of death. In addition to drowning, injuries, intoxications, or natural conditions are all among the potential causes of death in bodies found in water or the factor that may have contributed to the fatal outcome. The interpretation of autopsy findings in putative drowning requires a basic knowledge of the pathophysiology of drowning. Hypoxemia plays a primary role in death by drowning, whereas serum electrolyte changes may be observed in experimental models but have little or no clinical significance in humans. The volume of liquid inhaled depends on factors such as the duration of laryngospasm, the number and depth of respiratory movements before death, and the time of onset of cardiac arrest. Recent studies suggest that the actual incidence of drowning without liquid inhalation is much lower than previously estimated. The most important morphological changes associated with drowning are those related to liquid penetration into the airways: external foam, frothy liquid in airways, and lung overexpansion. However, these changes are not specific to drowning. The diagnostic value given to microscopic pulmonary changes varies significantly and is limited mostly by their heterogeneous distribution within the lung parenchyma. Laboratory methods for the diagnosis of drowning have their rationale in the shift of liquid and electrolytes across the pulmonary air–blood barrier, which may cause blood volume and electrolyte changes. Although some methods have been reappraised recently, their usefulness is greatly hampered by factors such as the variable volume of drowning liquid penetrating the airways, the differing duration of the drowning process, and postmortem biochemical instability. Contributions on the reliability of the diatom method for the diagnosis of drowning have yielded widely divergent opinions, of which the most critical often rely on studies lacking a rigorous methodology. Until standardized protocols and reliable separation values for diatoms between control and drowning cases are established, the diatom method cannot be accepted in definitively proving a diagnosis of drowning in the courtroom, but rather represents a useful supportive tool for the diagnosis of death by drowning.

Key Words: Bodies found in water; drowning; pathophysiology; laryngospasm; dry lungs, long QT syndrome; morphology; diatoms; electrolytes; manner of death; body disposal.

1. *Introduction*

The focus and aim of any medicolegal investigation concerning a body found in water is victim identification, evaluation of postmortem (PM) sub-

mersion time, and determination of cause and manner of death. Localization of the site of death, which can be close to the place where the body is found, a remote aquatic setting or, in the case of the cadaver's disposal, far away on dry land, represents an important element of these investigations.

The diagnosis of cause and manner of death relies on accurate assessment of autopsy findings, the victim's individual characteristics, the environment, and circumstances surrounding death. A wide range of possibilities must be considered. The sequence of events leading to death in water or to a body being found in water can be complex: drowning, injuries, intoxications, or natural conditions are all among the potential causes of death.

Pathological processes and traumatic lesions, even trivial, sustained before entering or while in water as well as toxicological findings must be thoroughly considered to reconstruct the events that led the victim into the water because any of them may have triggered or contributed to fatal outcome. Death can occur accidentally during recreational or occupational activities or be the result of an intentional action, for example, either suicide or homicide. Even if the cause of death is determined, the manner may, however, remain difficult to assess.

2. Historical Aspects

Medicolegal problems related to drowning were already mentioned in the Chinese *Hsi Yuan Chi Lu* (1247 AD), the oldest existing textbook of forensic medicine *(1)*. The chapter on drowning stresses the importance of determining the actual cause of death in bodies found in water. Although the chapter includes popular beliefs devoid of any scientific basis (e.g., position of the victim's hand, eyes, hair to determine the manner of death, different floating positions for males and females), it also stresses the value of frothy liquid in the victim's nose and mouth and of water in the stomach as evidence of in vivo submersion, as do modern studies.

In Europe, the first works of forensic medicine appeared during the Renaissance, after the *Bamberg Code* (1507) and the *Constitutio Criminalis Carolina* (1530) had highlighted the role of medical experts in the evaluation of injuries and causes of death in court. Textbooks by Paré, Fidelis, de Castro, Platter, Zacchia, Bohn, and Valentini are among the most representative and all contain passages concerning drowning.

Ambroise Paré´s *Les Oeuvres* (1575), in France, listed signs that prove the "vitality" of drowning as water in the stomach and abdomen, nasal secretions and foam protruding from the mouth, excoriations on the forehead and fingers owing to violent movement, and scraping against the bottom before

death *(2)*. Fortunatus Fidelis, in Italy, wrote in the *De Relationibus Medicorum* (1602) that investigation of the drowned is usually not difficult: the drowning victim has a swollen abdomen; mucous secretion appears from the nostrils, whereas the secretion that protrudes from the mouth is foamy; the fingertips are excoriated. The tumefaction of the victim's body is not caused by swallowed water but instead is derived from the steam produced by warming up of liquids during putrefaction *(3)*. Roderigo de Castro, in Portugal, also underlined in his *Medicus-Politicus* (1614) the dilatation of the abdomen, mucous secretion from nostrils, and foam from the mouth as signs of drowning, which are not present if the body is thrown into water after death. De Castro implicitly admits that excoriations on fingertips may be present even in bodies thrown into water after death. Moreover, the author maintains that the buoyancy of the body may be the result of its content in water or to PM gases *(4)*. In Switzerland, Felix Platter wrote on drowning in his *Observationes* (1614) and later in the *Quaestionum Medicarum Paradoxarum* (1625). In the former contribution, Platter describes four cases of women condemned to drowning for infanticide who were thrown from a bridge into the River Rhine, but who were recovered from the water after a variable interval of being still alive *(5)*. In the latter, he stresses that the stomach of drowning victims contains only a very limited volume of water and that the cause of death is asphyxia consequent to penetration of water into the airways *(6)*.

Paulus Zacchia's work, *Quaestiones Medico-legalis* (1726), contains two passages on drowning. In the Libri Quinti, Titulus II (De Vulneribus), Quaestio XI, Zacchia highlights the difficulties in distinguishing whether a person has drowned or was killed before submersion. He reminds us that this issue has been addressed by Paré, Fidelis, and de Castro (mentioned previously) who have unanimously agreed on the following signs: swollen abdomen full of water, mucous secretion protruding from the nostrils, and foamy secretion protruding from the mouth; the nasal secretion is mucous because the cerebral ventriculi are obstructed by water as a consequence of respiratory arrest, and the mouth secretion is foamy because air is violently extruded from the lungs and respiratory organs. Respiratory arrest is the cause of death rather than the swallowing of water. The third sign is represented by the excoriations on the fingertips because of attempts to avoid death by grasping stones and sand on the bottom of the sea *(7)*.

In Germany, Johannis Bohn in 1711 critically reviewed in his *De Renunciatione Vulnerum* the signs of the drowning mentioned by Paré, Fidelis, Castro, and Zacchia and stressed that these signs also may be absent in definite drowning and how, in some cases, the volume of water in the stomach or airways may be negligible (Fig. 1 *[8]*). Michaelis Bernhardt Valentini's *Cor-*

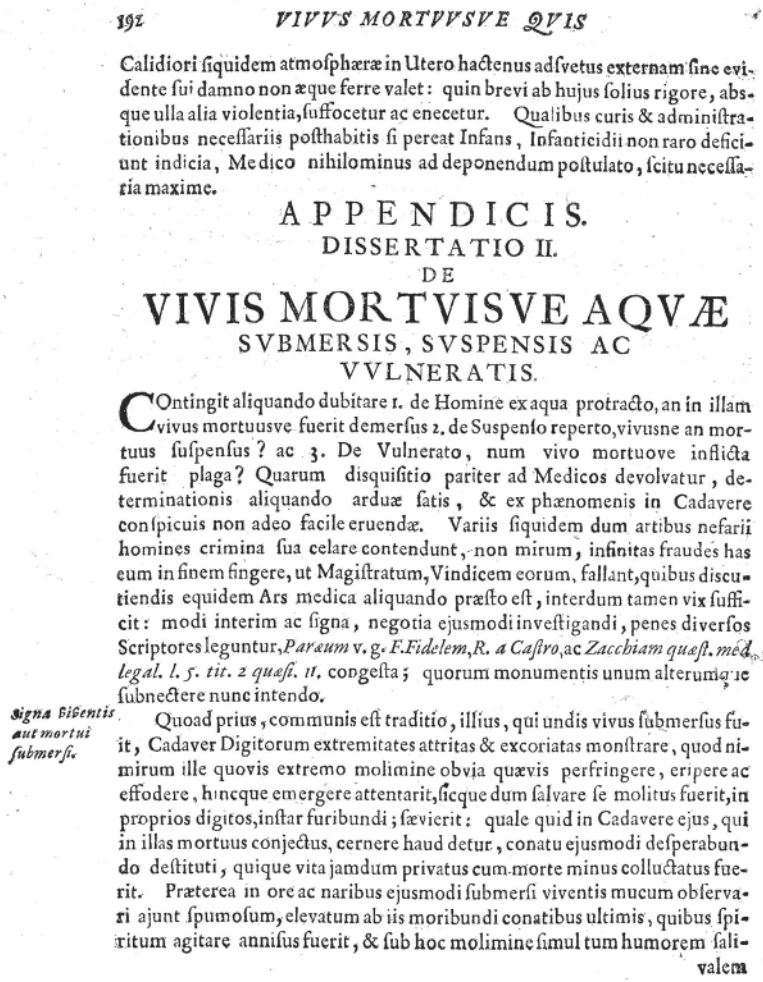
pus Iuris Medico-legalis (1722) also contains passages on drowning, including case reports on infanticide and suicide as well as correspondence between the author and Johannes Conrad Becker on drowning without inhalation or swallowing of water *(9)*.

3. Pathophysiology

During the 1940s and 1950s, Swann and associates performed experimental studies on the pathophysiology of drowning that have influenced modern views. Swann's works with dogs stressed the effects of drowning media of differing osmolarity on blood volume and serum electrolyte concentrations. In freshwater drowning, the hypotonic liquid penetrated into the circulation, causing hypervolemia, hemodilution with decrease of serum electrolytes, especially sodium (Na) and chloride (Cl), hemolysis with potassium (K) release from red blood cells, and death by ventricular fibrillation (VF) within 3 to 5 minutes. In seawater drowning, conversely, the hypertonic media pulled liquid from the circulation into the alveoli, causing hypovolemia and hemoconcentration with an increase in the concentration of serum Na, Cl, and magnesium (Mg), whereas no hemolysis and VF occurred, and dogs survived for 5 to 12 minutes *(10–12)*.

During the 1960s, studies demonstrated that liquid penetration into an organism may cause no clinically significant electrolyte imbalance because the volume of aspirate may be small and that in death by drowning hypoxemia plays a primary role. In 1966, Modell et al. *(13)* evaluated, in anesthetized canines, the effects of inhalation of varying volumes of freshwater, from 2.2 mL/kg to 66 mL/kg body weight. Volumes greater than 11 mL/kg were needed to cause a significant alteration in blood volume, greater than 22 mL/kg to observe significant electrolyte changes, and 44 mL/kg or greater to cause VF. The inhalation of such volumes is unlikely to occur in humans because, using the magnitude of serum electrolyte changes found in human drowning victims and comparing these with animal experiments that have a known quantity of water aspirated, it has been calculated that 85% of human drowning victims aspirate only 22 mL or less of water per kilogram body weight *(14)*. Accordingly, serum electrolyte concentrations of resuscitated drowning victims usually fail to reveal significant changes *(15)*.

It is generally agreed that although pathophysiological differences between drowning in freshwater or saltwater may be observed in experimental models, these have little or no clinical significance in human drowning *(16,17)*. The main physiological consequence of drowning is prolonged hypoxemia with resultant metabolic acidosis *(18,19)*. Yet, in peculiar envi-



192 VIVVS MORTVVSVE QVIS

Calidiori ſiquidem atmoſphæræ in Utero hactenus adſvetus externam ſine evidente ſui damno non æque ferre valet: quin brevi ab hujus ſolius rigore, absque ulla alia violentia, ſuffocetur ac enecetur. Qualibus curis & adminiſtrationibus neceſſariis poſthabitis ſi pereat Infans, Infanticidii non raro deficiunt indicia, Medico nihilominus ad deponendum poſtulato, ſcitu neceſſaria maxime.

APPENDICIS.
DISSERTATIO II.
DE
VIVIS MORTVISVE AQVÆ
SVBMERSIS, SVSPENSIS AC
VVLNERATIS.

CONtingit aliquando dubitare 1. de Homine ex aqua protracto, an in illam vivus mortuusve fuerit demerſus 2. de Suspenſo reperto, vivusne an mortuus ſuſpenſus? ac 3. De Vulnerato, num vivo mortuove inflicta fuerit plaga? Quarum disquiſitio pariter ad Medicos devolvatur, determinationis aliquando arduæ ſatis, & ex phænomenis in Cadavere conſpicuis non adeo facile eruendæ. Variis ſiquidem dum artibus nefarii homines crimina ſua celare contendunt, non mirum, infinitas fraudes hac eum in finem fingere, ut Magiſtratum, Vindicem eorum, fallant, quibus diſcutiendis equidem Ars medica aliquando præſto eſt, interdum tamen vix ſufficit: modi interim ac ſigna, negotia ejusmodi inveſtigandi, penes diverſos Scriptores leguntur, *Paræum* v. g. *F. Fidelem*, *R. a Caſtro*, ac *Zacchiam quæſt. med. legal. l. 5. tit. 2 quæſt. 11.* congeſta; quorum monumentis unum alterumque ſubnectere nunc intendo.

Signa Viventis aut mortui ſubmerſi.

Quoad prius, communis eſt traditio, illius, qui undis vivus ſubmerſus fuit, Cadaver Digitorum extremitates attritas & excoriatas monſtrare, quod nimirum ille quovis extremo molimine obvia quævis perfringere, eripere ac effodere, hincque emergere attentarit, ſicque dum ſalvare ſe molitus fuerit, in proprios digitos, inſtar furibundi; ſævierit: quale quid in Cadavere ejus, qui in illas mortuus conjectus, cernere haud detur, conatu ejusmodi deſperabundo deſtituti, quique vita jamdum privatus cum morte minus colluctatus fuerit. Præterea in ore ac naribus ejusmodi ſubmerſi viventis mucum obſervari ajunt ſpumoſum, elevatum ab iis moribundi conatibus ultimis, quibus ſpiritum agitare anniſus fuerit, & ſub hoc molimine ſimul tum humorem ſalivalem

Fig. 1. *De Renunciatione Vulnerum.* The book by Johannis Bohn (1711) includes a chapter on the differential diagnosis between drowning and post-mortem submersion.

ronments, significant electrolyte changes have been observed, for instance hypercalcemia and hypermagnesemia in the Dead Sea *(20)* and hypercalcemia in polluted water *(21)*.

3.1. Sequence of Events

In humans, the drowning process has been described as a continuum that begins when the victim's airways are located below the surface of the liquid,

which leads to voluntarily breath-holding and then laryngospasm triggered by the local effects of liquid on the upper airways *(22)*. During this period, the victim does not breathe, which causes hypoxemia, hypercapnia, and respiratory and metabolic acidosis. The victim also may swallow water into the stomach. In human volunteers, the breath-holding breaking point varies from 87 seconds at rest to up to 146 seconds when preceded by hyperventilation *(23)*. Once breath-holding breaks, the victim breathes and allows liquid to enter his or her airways. The respiratory efforts intensify, producing more intense negative airway pressure against a closed glottis, or the liquid column overdistends and ruptures lung alveoli. At this point, different authors believe one of two courses can occur. In 85 to 90% of the cases, as the arterial oxygen tension drops further, laryngospasm abates, and the victim actively inhales a variable volume of liquid (so-called "wet-drowning"). In the remaining 10 to 15%, the victim does not present evidence of water aspiration. Some attribute this to severe laryngospasm causing hypoxia, convulsions, and death before taking a breath (so-called "dry-drowning") *(16,24)*. These later cases led some researchers to question whether these victims actually die of drowning or of other causes *(25)*.

3.2. Respiratory System

The primary target organ for submersion injury is the lung. The respiratory disturbance depends more on the volume of water aspirated than on its osmolarity. In animal experiments, the aspiration of 2.2 mL of water per kilogram body weight decreases the arterial O_2 partial pressure to approx 60 mmHg within 3 minutes *(13)*. In humans, it seems that as little as 1 to 3 mL/kg produces profound alterations in pulmonary gas exchange and decreases pulmonary compliance by 10 to 40% *(13,26,27)*.

Freshwater, which moves rapidly across the alveolar–capillary membrane into the circulation, produces disruption and denaturation of surfactant, which leads to an increase in surface tension and a decrease in compliance, atelectasis, and intrapulmonary shunts with marked ventilation/perfusion mismatching *(26)*. In these conditions, as much as 70% of the cardiac output may be shunted past perfused but unventilated alveoli *(18)*. Because of the liquid shift across the alveolar–capillary interface, the freshwater drowning victim may develop acute hypervolemia. In saltwater drowning, the hypertonic liquid draws protein-rich liquid from the vascular space into the pulmonary alveoli, causing damage to the basement membrane, dilution and washout of surfactant, and reduction of compliance *(26)*. Pulmonary edema occurs rapidly, and usually within a few minutes the liquid-filled alveoli are incapable of normal gas

exchange, which leads to intrapulmonary shunting and a perfusion/ventilation mismatch *(28,29)*. The shift of liquid into the alveoli results in hypovolemia. Systemic hypoxemia, in fresh- and saltwater drowning, causes myocardial depression, reflex pulmonary vasoconstriction, and alteration of pulmonary capillary permeability, all of which contribute to pulmonary edema.

3.3. Cardiovascular System

The effects of liquid penetration on the circulation have been studied in detail in animal experiments. Significant hypervolemia occurs in dogs after aspiration of at least 11 mL/kg of freshwater; within 2 to 3 minutes, a linear relationship occurs between the volume of water aspirated and the increase in blood volume *(30)*. Blood volume increases by 1.4% for every milliliter of liquid/kg until 44 mL/kg of water is aspirated. At this value, the blood volume reaches a plateau, likely caused by the cessation of circulation *(31)*. The absorption of large quantities of freshwater can result in a dramatic decrease in blood density *(12)*. When the victim survives, the hypervolemia after aspiration of freshwater is transient, with blood volume returning to normal levels within 1 hour *(32)*. This readjustment in blood volume is likely the result of redistribution of the liquid into other body compartments and to plasma transudation into the lungs. When significant quantities of seawater are aspirated, the reverse is seen, with hypovolemia and elevated concentrations of serum Na and Cl (*28*).

Cardiac dysfunction during drowning is predominantly secondary to changes in arterial oxygen tension and acid–base balance. The acute hypoxemia results in catecholamine release, leading to tachycardia and hypertension, which are transient and are followed by bradycardia and hypotension as hypoxemia intensifies. In addition, hypoxemia may directly reduce myocardial contractility. Hypoxia and acidosis elevate the risk for arrhythmias, including ventricular tachycardia, fibrillation, and asystole. A variety of electrocardiographic abnormalities have been reported after drowning, such as a decrease in the amplitude of the P-wave, disappearance of the P-wave, widened PR interval, complete atrioventricular dissociation, depression of the ST segment, widening of the QRS complex, frequent premature ventricular contractions, increase in amplitude of the T-wave, auricular fibrillation, and VF, among others *(31)*. As previously discussed, early studies in the 1950s suggested that in freshwater death was caused by VF and in seawater by pulmonary edema *(12)*, but several studies since then have shown that VF as an immediate cause of death is uncommon in human drowning victims *(15)*.

3.4. Central Nervous System

Brain death is the common final pathway of fatal submersion, whether the pathophysiological mechanism is hypoxia attributable to liquid penetration into airways or to laryngospasm or anoxia from vagally mediated cardiac arrest *(33)*. When the brain is deprived of oxygen for more than approx 3 minutes, ischemic damage can occur. It is estimated that a window of up to 4 to 6 minutes may exist before irreversible neuronal damage occurs when the oxygen supply is completely interrupted under normothermic conditions. The central nervous system (CNS) has a selective vulnerability to hypoxic or anoxic events, involving, in decreasing order of vulnerability in adults, the hippocampus, cerebral neocortex, cerebellum, thalamus, basal ganglia, brainstem, and hypothalamus *(34)*. There is, however, no data as to exactly how long a drowning victim can remain submerged, receive cardiopulmonary resuscitation (CPR), and still recover with no sequelae. Among the factors that influence this interval perhaps the most important is the body temperature of the victim and the effectiveness of CPR applied. Generally, under normothermic temperatures, most researchers will agree that if a victim is rescued and effective CPR applied within 3 minutes, the vast majority of victims will successfully be resuscitated. By the time 5 minutes have passed, although return of an effective heartbeat is commonly observed, the majority of persons will show permanent hypoxic encephalopathic damage.

3.5. Other Organ Effects

Hypoxia secondary to drowning can affect various organs. Many reports point to acute renal and hepatic insufficiency, gastrointestinal injuries, and disseminated intravascular coagulation. Concerning abnormalities in blood-clotting factors, Modell et al. *(35)* described a child whose platelet count rose to 1.9 million/mm^3 after a submersion episode in excess of 20 minutes in cold water and who experienced a complete recovery.

3.6. Delayed Complications

Immediate complications of drowning include cardiac arrhythmias (VF, asystole) and cardiogenic shock caused by myocardial damage secondary to hypoxia and acidosis. At times, the drowning victim appears healthy in the emergency department but develops fulminant pulmonary edema as long as 12 hours after submersion owing to acute respiratory distress syndrome from the primary pulmonary damage by liquid, as a consequence of hypoxia and circulatory failure, or the drowning victim develops neurogenic pulmonary edema attributed to cerebral hypoxia. Acute respiratory distress syndrome also

may develop as a consequence of pulmonary injuries caused by aspiration of gastric contents. Common fatal sequelae of drowning in hospitalized drowning victims are brain death as the result of hypoxic encephalopathy, pneumonia (aspiration, chemical, bacterial), sepsis, and multiorgan failure. Posthypoxic encephalopathy may occur because of hypoxemia sustained during the drowning episode or secondarily to pulmonary damage or to increased intracranial pressure *(22)*.

3.7. Dry-Drowning

The sequence of events that follows the penetration of liquid into an organism has been the subject of considerable speculation that has focused on the volume of liquid penetrating the airways during the drowning process and the concept of drowning without aspiration of liquid ("dry-drowning") *(25,36)*. The volume of liquid aspirated varies considerably from one drowning victim to the next *(14)* and depends on factors such as the frequency and duration of laryngospasm, the number and depth of respiratory movements before death, and the time of onset of cardiac arrest. Experimental *(37)* and clinical *(15)* studies together with the autopsy finding of "dry lungs" in bodies found in water, suggest that death can occur with no significant aspiration of liquid into the lungs in approx 10 to 15% of alleged drowning victims *(38–40)*. Dry-drowning has been variously explained. In addition to laryngospasm, the role of mechanisms, such as vago-vagal cardiac inhibition triggered by contact of the liquid with the upper airways, sudden cardiac arrest, pulmonary reflexes, or absorption of aspirated liquid into the bloodstream after prolonged resuscitation, have been proposed *(30,40–42)*. Brinkmann *(43)* has listed different potentially life-threatening reflexes, which may occur in human beings during immersion or submersion.

The issue of dry-drowning has recently been reappraised, and the suggestion has been made that its actual incidence may be lower than previously estimated and that human bodies found in water with apparently normal lungs could conceal more natural deaths or body disposal in water than is actually recognized *(19,22,25,44)*. The "laryngospasm" hypothesis has its rationale in the complex innervation and reflexes of the upper airways under various stimuli *(45,46)*. However, no concrete evidence exists that prolonged laryngospasm until death occurs during submersion, whereas experimental evidence suggests that initial breath-holding and/or laryngospasm ceases within two minutes from the onset of submersion *(23,48)*.

3.8. Hypothermia

After immersion in cold water, hypothermia, defined as body temperature below the normal range of 36.8 to 37.7°C *(49)* can be, especially at high latitudes, a component of drowning by its effects on the heart, lungs, and CNS. When body temperature is less than 33ºC, hypoventilation occurs, and muscle rigidity ensues; between 28ºC and 30ºC, a decrease in heart rate and bradyarrhythmias occur, respiration becomes irregular, and apnea is a common feature; at temperatures less than 28ºC, VF, severe bradycardia or asystole can occur, and respiration may be difficult to detect *(24,36)*. As to the CNS, when body temperature decreases to less than 35ºC, victims may become confused and disoriented, and at less than 33ºC, they are semicomatose, with a substantial percentage of drowning occurring at this time. At temperatures less than 30ºC, it may be difficult to distinguish between hypothermia and death, as frank coma supervenes *(24,36)*. Cold-induced anaphylaxis in people with cold urticaria syndrome can be a rare cause of drowning *(50)*.

People who drown in water less than 5ºC generally have a better prognosis than those who drown in warm water because with metabolism diminution, O_2 consumption and CO_2 production decrease. For every 1ºC decrease in temperature, there occurs a 7–9% decrease in oxygen required *(25)*. Hypothermia is, however, a double-edged sword because it increases the risk for fatal arrhythmias especially below 28ºC *(51)*. The rapidity of the temperature fall in a body has a profound influence on the capacity of the brain to withstand hypoxia. In children, the large surface area-to-weight ratio associated with cold water aspiration often causes a rapid core cooling below 30ºC during submersion, which gives some degree of cerebral protection during hypoxia *(52)* and explains remarkable recoveries of children after even more than 30 minutes submersion with return to normal activity *(24,35,53,54)*.

4. Macroscopical Findings

The main macromorphological changes associated with drowning (external foam, frothy fluid in airways, lung overexpansion) are related to the penetration of drowning liquid into the airways. These changes can be valuable for the diagnosis of drowning when interpreted within an appropriate investigative context. However, they are not pathognomonic for drowning and are not always detected because they fade with the onset of putrefaction. Changes involving the body's systems other than the respiratory system will be briefly summarized in the next sections, although their relevance for the diagnosis of drowning is marginal.

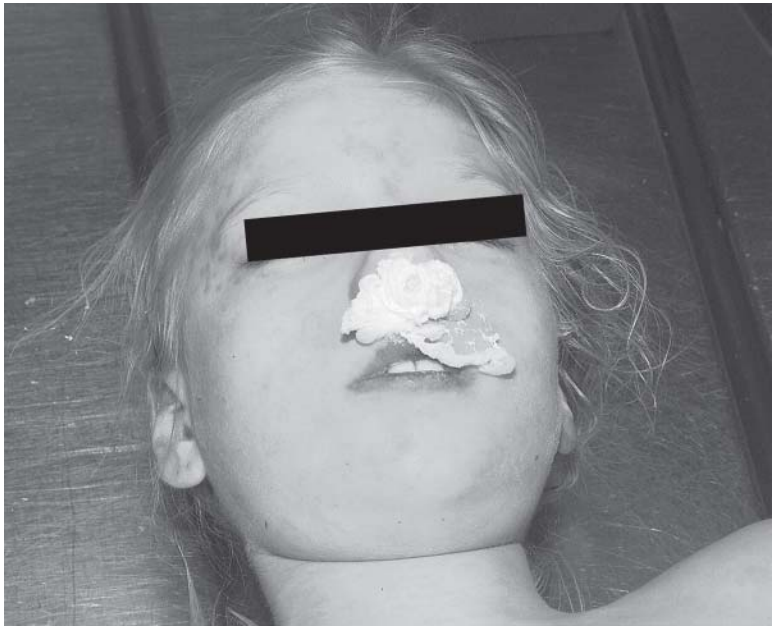


Fig. 2. Characteristic foam extruding from the mouth and nostrils of a drowned girl.

4.1. Upper Airways

The penetration of drowning media into the respiratory system increases airway pressure and causes a reactive pulmonary edema. The mixture of drowning liquid with edema liquid, bronchial secretions, and pulmonary surfactant produces a frothy fluid which, under respiratory efforts during drowning, can reach the upper airways and be extruded from the nostrils and mouth, at times as a mushroom-like foam (Figs. 2 and 3).

The external foam and internal frothy liquid (Fig. 4) are generally white or blood-tinged (especially when freshwater is aspirated) and consist of drowning and edema liquid, mucus, and fine air bubbles, which are relatively resistant to collapse because of surfactant content. Respiratory epithelial cells and $CD68^+$ alveolar macrophages have been isolated from the frothy fluid *(55)*. External foam and frothy fluid may persist up to several days and, after the onset of putrefaction, become red-brown, the fine air bubbles being replaced by larger gas bubbles.

This external foam is considered one of the most valuable findings for the diagnosis of drowning, yet it can be observed also in cardiogenic pulmonary edema, epilepsy, drug intoxication, and electrical shock. Moreover, it is

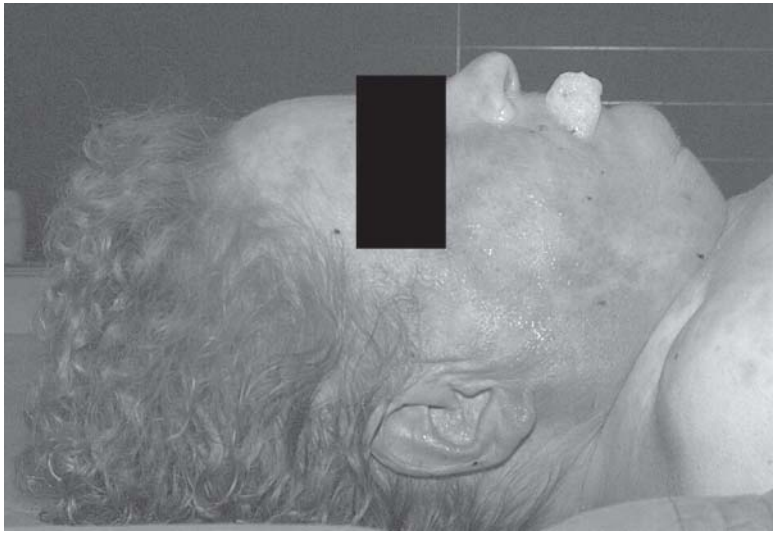


Fig. 3. Drowning victim. Characteristic foam extruding from the mouth and nostrils.

generally found in a minority of drowning victims *(43)*. In a series of 1590 bodies found in water, Lunetta et al. (2002) found external foam in 17.3% of the cases *(56)*. Some authors underline the greater quantity of frothy fluid in drowning compared with other causes of death *(42,57,58)*; however, no clear demarcation exists between different conditions. Great caution is necessary when interpreting the origin of frothy liquid in any suspicious death, in which the body may have been disposed of on land after homicidal drowning.

4.2. Lungs

The lungs of drowning victims with no putrefactive changes usually are waterlogged and overdistended ("emphysema aquosum"). Lungs occupy most of the pleural cavities with at times imprints of ribs on pleural surfaces and overlapping of the anterior margins on the mediastinal midline (Fig. 5). Lunetta et al. (2002) found overextension of lungs with overlap of the anterior margins in 42.1% of 1590 bodies found in water *(56)*. Pleural adhesions can mask these changes. The lung surfaces usually are pale and mottled, with red and grey areas displaying sometimes marked alveolar overdistension. After their removal from the pleural cavities, the lungs retain their shape and size, and cut sections ooze a variable quantity of foamy liquid. Subpleural hemorrhages (Paltauf's spots Fig. 6) are found in 5 to 60% of drownings *(43)*, and their blurring aspect is the result of hemolysis within intraalveolar hemorrhages *(42)*.

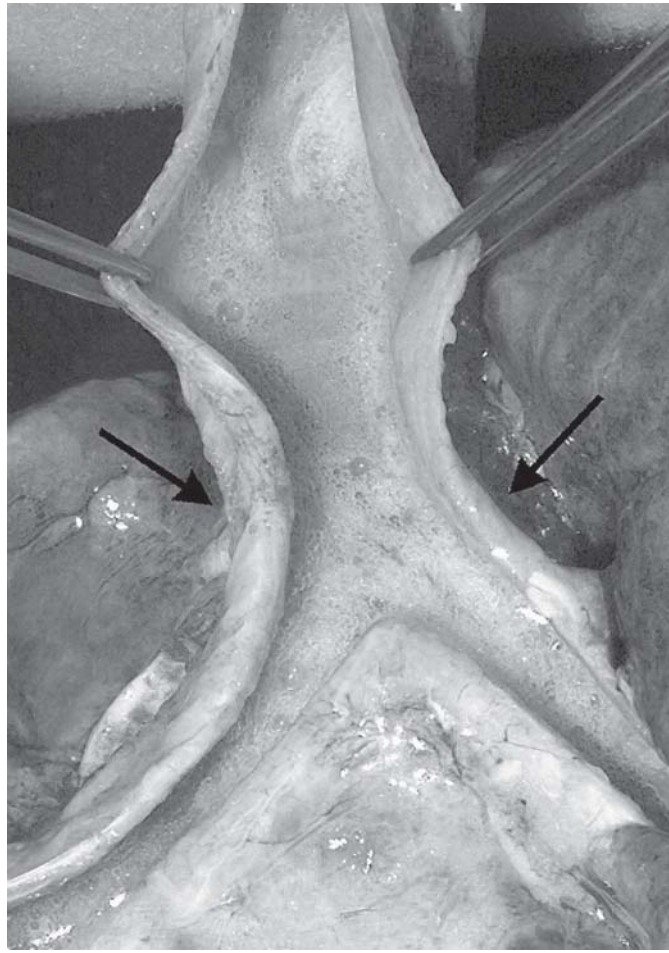


Fig. 4. Frothy fluid is seen in the trachea and bronchi of a drowning victim.

4.2.1. Lung Weight

Different studies have addressed the weight of lungs in freshwater and saltwater drowning. Lung weight alone has, however, little diagnostic significance because of frequent overlap between drowning and control values. Moreover, wide individual variations exist, as well as marked discrepancies as regards the normal range of lung weight *(59–63)*. De la Grandmaison et al. *(64)* reported the most detailed data on lung weight based on 684 healthy adults who died of injury after a survival time of less than 1 hour. In males, the right

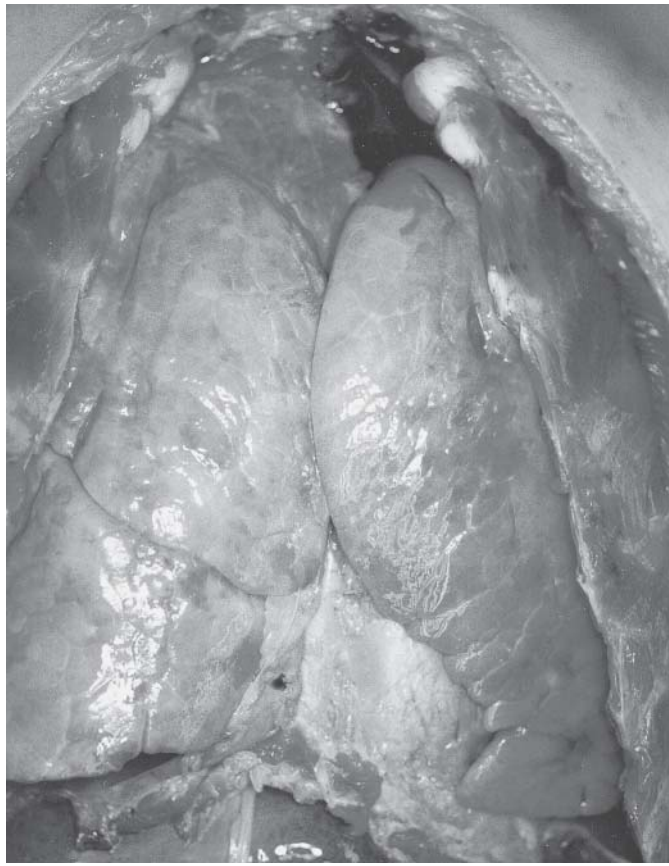


Fig. 5. Overdistension of the lungs with overlap of their anterior edges over the midline in a drowning victim.

lung weighed a mean 663 ± 239 g (SD) and the left 583 ± 216 g, whereas in females the corresponding values were 546 ± 207 g and 467 ± 174 g, respectively.

As for the values in drowning victims, Copeland *(65)* found a right lung weight of 744.9 ± 199.3 g (SD) and left 655.4 ± 184.2 g in saltwater drowning (n = 95), whereas the corresponding values for freshwater were 727.7 ± 210.6 g and 657 ± 206.3 g. Kringsholm et al. *(66)* reported a combined lung weight of 1411 ± 396.4 g in 91 adults with a PM submersion time of less than 24 hours *(66)*. Zhu et al. *(67)* suggest that differing body structure, pulmonary vital capacity, cardiac function, and survival time in water may account for differences in lung weight *(67)*.

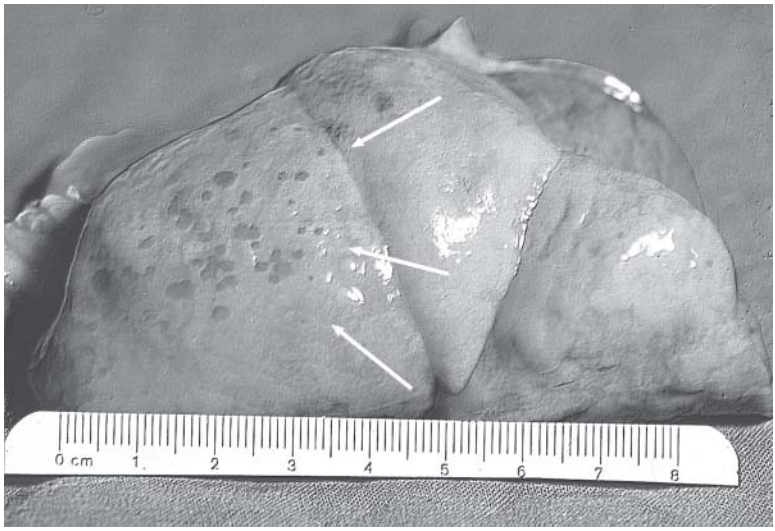


Fig. 6. Paltauf's spots (arrows) located in the upper lobe of the right lung. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

4.2.2. Dry Lungs

Apparently normal lungs with no signs of aqueous emphysema ("dry lungs") have been reported in approx 10 to 15% of all presumed drowning victims. Forensic pathologists have variably interpreted the PM finding of dry lungs. Spitz *(41)*, for instance, stressed the role of liquid reabsorption into the circulation, especially when resuscitative attempts are performed before death. Di Maio and Di Maio *(68)* mention the potential role of laryngeal spasm, and Saukko and Knight *(42)* name, in addition to the above mechanisms, also reflex cardiac arrest. Brinkmann *(43)* has listed several reflexes that may be triggered by contact of the body with water and result in death with no significant liquid inhalation.

Contributions on lung morphology in drowning have, at times, traced a direct correlation between dry lung and lung weight, most using 1000 g as a cut-off value for this definition *(65,66,69)*. This approach is misleading because, as mentioned previously, no consensus exists for normal lung weight, and low-weight lungs are not necessarily "normal" because they may be overdistended or show signs of liquid penetration. Lunetta et al. *(44)* observed the absence of overdistension or signs of liquid penetration in less than 2% of "low-weight lungs." Copeland *(65)*, using a cut-off value of 500 g per lung, found that in saltwater drowning the percentage of dry right and left lung was 11.5% and

18.9%, respectively, whereas the corresponding values for freshwater drowning were 10.4% and 16.8%. Kringsholm et al. *(66)* found that 7.7% of 91 drowning victims with a PM interval of less than 24 hours had a combined lung weight of less than 1000 g, but when considering a PM submersion time up to 1 week and the combined lung and pleural liquid weight, 13% of 131 drowning victims had a combined lung weight of less than 1000 g. Morild *(69)* found that only 6% of 133 drowning cases, most with a PM submersion time of less than 1 week, had a combined lung weight of less than 1000 g. In Copeland's *(65)* and Morild's *(69)* series, no significant differences are reported between salt- and freshwater drowning, whereas no data on the influence of water salinity are available in the study by Kringsholm et al. *(66)*.

4.2.3. Drowning Lung vs Postmortem Hydrostatic Lung

The PM penetration of drowning media into lungs of bodies submerged and having died from causes other than drowning has been addressed experimentally using high-pressure chambers or lowering human bodies under water to different depths. Reh *(70)* performed canine experiments in a baro-chamber and observed significant pulmonary overexpansion mimicking drowning lungs at 0.2 atm, whereas subpleural hemorrhages similar to Paltauf's spots appeared at 0.4 atm. Reh in the same year also published experiments on eight humans in hyperbaric chambers (atm: 0.3–1.35; duration 4 to 65 hours) who died of causes other than drowning *(71)*. Lung changes identical to those of drowning were observed in bodies kept at 0.3 atm for 65 hours, whereas those kept at more than 0.5 atm for at least 45 to 50 hours showed marked pleural exudates as well. Other investigators have reported, based on animal experiments, that active respiration must be present for significant quantities of water to enter the lungs of floating bodies *(38)*.

4.2.4. Pleural Effusion

Pleural effusion is a relatively common finding in bodies recovered from water, as the result of PM diffusion of pulmonary liquids into the thoracic cavity *(72)*. Morild *(69)* found pleural effusions (mean, 432 mL) in 53.3% of 133 drowning victims older than 16 years of age with no advanced putrefaction. Kringsholm et al. *(66)* reported an increase in the volume of pleural exudate during the PM interval. Yorulmaz et al. *(73)*, using univariate analysis, investigated the relationship between volume of pleural liquid, circumstances, and autopsy findings in 43 drowning victims and confirmed the link between PM submersion time and pleural effusion. The correlation between the volume of pleural liquid and lung weight varies among studies, with some authors describing a decrease in lung weight parallel to an increase in pleural liquid

(*66,67*) and others failing to demonstrate such a correlation *(69,73)*. Prolonged PM intervals and advanced putrefaction seem to be associated with a transthoracic leakage of pleural liquid *(67,73)*.

4.3. Temporal Bone

Gross hemorrhages occur in the petrous and mastoid regions of the temporal bone in drowning victims (Fig. 7). Niles *(74)* described hemorrhages, mostly bilateral, in the mastoid cells of 23 of 24 freshwater drowning victims. More recent contributions, based on a limited number of cases, also have drawn attention to the association of this finding with drowning *(75,76)*. Microscopically, the hemorrhages are localized in the mucosa of the middle ear or mastoid cells and are associated with submucosal edema and vascular congestion *(77–79)*. As to their pathogenesis, three mechanisms have been proposed: *(a)* barotrauma, *(b)* penetration into the middle ear of inhaled liquid via the eustachian tube, and *(c)* increased venous and capillary pressure owing to respiratory efforts against a closed glottis *(75,76,78)*. At present, broad agreement exists as to the nonspecificity of temporal bone hemorrhages, which have been found in as many as 80% of deaths other than drowning *(80,81)*.

4.4. Sinuses

The sinuses (frontal, ethmoidal, maxillary, sphenoidal) are air spaces associated with each nasal cavity and lined with a ciliated epithelium. Aqueous liquid in the sinuses is considered a sign of permanence of the body in water rather than a sign of drowning because liquid also may penetrate into the sinuses PM *(82)*. Hottmar *(83)* found liquid in sphenoid, maxillary, or paranasal sinuses in 75% of 387 freshwater drowning victims, whereas among 50 controls, only one case had liquid in the paranasal sinuses. Bohnert et al. *(84)* investigated the liquid content of the sphenoid sinuses in 60 drowning victims and in 157 deaths from other causes: 92% of drowning victims had 1 to 4 mL of liquid in the sphenoid sinuses, but positive results were also evident in 52% of the controls, although the average volume was lower than in the drowning group.

4.5. Other Organs

4.5.1. Spleen

The observation of a small spleen in victims of drowning dates back to the 19th century and, according to Reh *(82)*, this change occurs in approx 30% of all drowning cases. Haffner et al. *(85)* found in 42 victims of freshwater

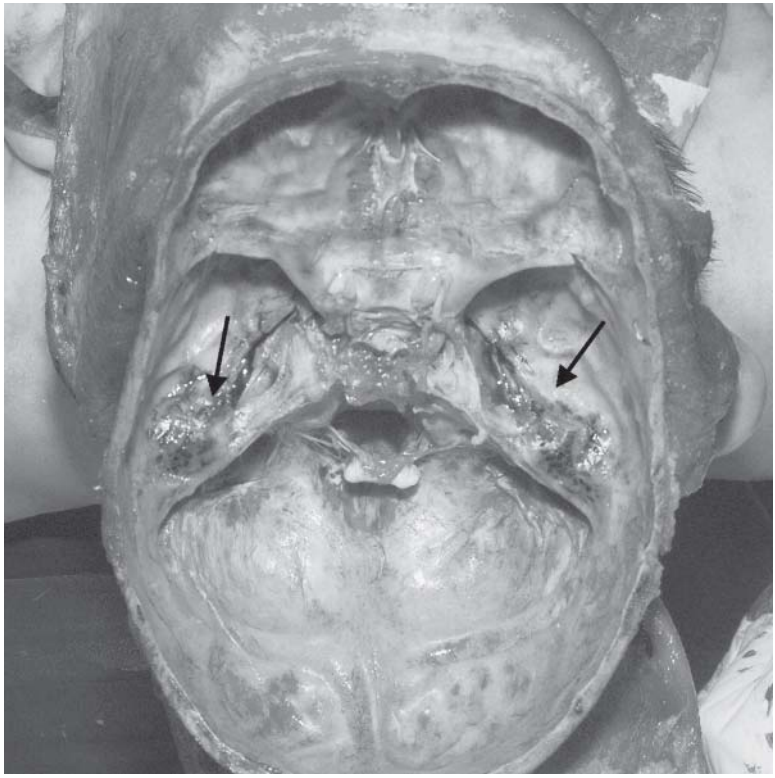


Fig. 7. Marked bilateral hemorrhages within the petrous region of the temporal bones (arrows) in a drowning victim.

drowning a spleen weight significantly lower (~18%) than in matched controls, with half the spleens being of a weight under the lower limit of controls. These authors estimate that a cut-off value set at 0.2% of body weight can be of considerable diagnostic value and speculate that spleen weight reduction may be caused by sympathetic stimulation with vasoconstriction and contraction of the spleen capsule and trabeculae. Hadley and Fowler *(86)* questioned these findings and suggested that low spleen weight may be the result of pure PM changes.

4.5.2. Muscles

Muscular hemorrhages have been reported as vital sequelae of agonal convulsions, hypercontraction, and overexertion of muscles during the drowning process. Carter et al. *(87)* found neck-muscle hemorrhages in 81% of drowning victims in their retrospective series. In a prospective study of 39 drowning

victims with dissection of the neck, trunk and upper extremity muscles, Püschel et al. *(88)* found at least one focus of intramuscular hemorrhages in 20 cases (51.3%). Overall, 93 hemorrhagic foci, unilateral in 50%, were found in descending order of frequency in respiratory and auxiliary respiratory muscles, musculature of the neck and back, and musculature of the shoulder girdle and upper arm. These authors corroborated the fact that muscular hemorrhages in drowning victims must be differentiated from those caused by other injuries and resuscitation procedures. Reh *(82)* described drowning-associated hemorrhages as more elongated than the more diffuse ones associated with strangulation. Histological examination may help in differentiating such vital hemorrhages from PM artifacts or local hypostasis *(82,87–89)*.

4.5.3. Gastroenteric Tract

Fagerlund's review *(90)* on drowning liquid in the stomach and bowel testifies to the range of studies focusing on this topic up to the 19th century, which reflect the early theories on swallowing of water as a cause of death in drowning. Reh *(82)* has attempted to reevaluate the presence of liquid in the esophagus, stomach, or intestine as a sign of drowning, based on a study in which 16 of 17 bodies submerged PM at 15 m for 65 hours showed no signs of liquid penetration into the gastroenteric (GE) system. However, standard medicolegal textbooks consider this finding unreliable for the diagnosis of drowning *(42)*. Laceration of the gastric mucosa caused by increased pressure in the stomach cavity during drowning is considered by some to be associated with drowning, but other authors stress the sporadic nature of this finding and difficulty in interpreting its origin *(43,82)*.

5. Microscopial Findings

5.1. Light Microscopy

5.1.1. Lung

The main light microscopic (LM) signs of drowning are represented by foci of acute lung emphysema with overdilation of alveoli, thinning and lacerations of septa, capillary congestion, interstitial and intraalveolar edema and hemorrhages and, sometimes, exogenous particles in the airways (Fig. 8). Reh *(82)* has proposed a classification in four stages of acute lung emphysema based on the degree of distension of the alveolar septa, changes in their reticular fibers (stained with Gomori), and compression of septal capillaries. The first stage is characterized by reduction in the normal thickness of the septa with capillaries still well recognizable and partial fiber ruptures; the second

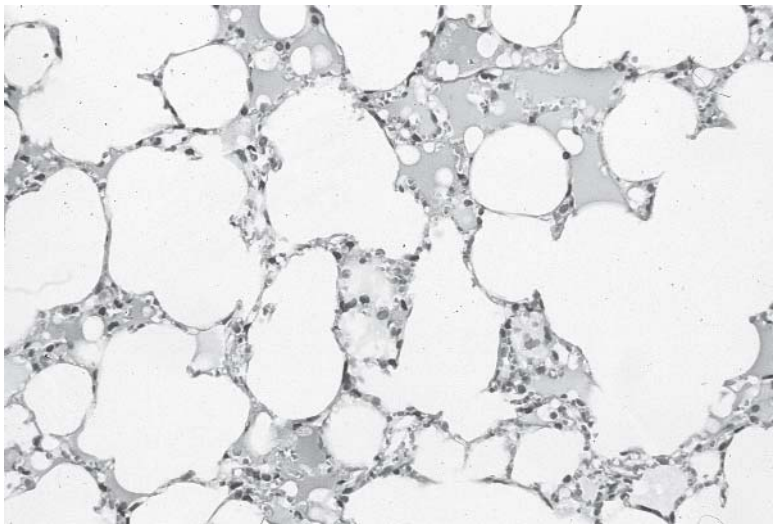


Fig. 8. Lung histology in drowning. Acute emphysema of the lung with edema liquid partially filling the alveoli (experimental conditions, mouse).

stage by increased distension of septa, capillary compression, and distinct fiber ruptures; the third stage by maximal distension of the alveolar wall with thread-like capillaries and distinct intraseptal fiber ruptures; and the fourth stage by ruptures of alveolar septa and retraction of fibers, which appear broad and wavy. The observation of reticular fiber changes in the nondrowned similar to that observed in drowning victims *(91)*, the wide variation in lung changes caused by factors such as the depth at which the body is submerged and the rapidity of the drowning process *(92)* as well as their fading during the PM interval limit the practical utility of Reh's classification.

The diagnostic weight given to these changes varies significantly, even in standard textbooks. Saukko and Knight *(42)*, for instance, state that "much has been written about both light and electron microscopy of the lungs in immersion deaths … the accounts are confusing … sometimes contradictory, the consensus of opinion being that such changes are inconstant and unreliable." Contrarily, especially German authors have traditionally emphasized the potential of microscopical and ultrastructural studies for the diagnosis of drowning *(43)*.

5.1.1.1. Surfactant

Pulmonary surfactant is a lipoprotein complex, mainly synthesized by the alveolar type II epithelial cells, which reduces the surface tension at the

air–liquid interface and contributes to host defense against infection and inflammation *(93)*. Surfactant is composed of approx 90% lipids (phospholipids and neutral lipids) and 10% proteins. Phospholipids and neutral lipids are stored in lamellar bodies of alveolar type II cells and released by exocytosis into alveoli *(94,95)*. There are four surfactant-specific proteins (SP): hydrophilic SP-A and SP-D, which have host defense functions, and hydrophobic SP-B and SP-C, which functional meaning is unknown *(96)*.

Giamonna and Modell *(26)* demonstrated in anesthetized dogs that freshwater significantly alters the surface tension characteristics of pulmonary surfactant, causing alveolar instability. Conversely, seawater washed out some of the surfactant but did not change its surface tension characteristics. Lorente et al. *(97)* studied, by using high-performance thin-layer chromatography, the modification of surfactant phospholipids (LSPs) from rabbits drowned in freshwater and saltwater, isolated from endobronchial lavage and lung tissue *(97)*. In the lavage samples the proportion of LSPs differed significantly in drowned and controls and between freshwater and saltwater drowning specimens, whereas in lung tissue the changes were less marked, likely because of a higher concentration of LSP stored in lung tissue. Lorente et al. *(98)* also have addressed the PM stability of the LSPs by using rats drowned in freshwater and saltwater in addition to controls. All LSPs were stable during the first 24 hours and significantly decreased after 48 hours.

Zhu et al. *(99)* assessed the distribution of pulmonary surfactant apoprotein A (SP-A) in 282 autopsy cases, 59 of which drowned. The most intense and dense granular immunostaining of intraalveolar SP-A was observed in drowning, in hyaline membrane syndrome, and in perinatal aspiration of amniotic fluid. Zhu et al. *(100)* also have evaluated the lung distribution of SP-A and the serum levels of SP-A in 53 saltwater and freshwater drowning specimens. In the lungs, SP-A immunostaining was classified into either a linear pattern on the alveolar interior surface and into an intraalveolar granular deposit pattern, each with a three-grade score. A high score for intraalveolar SP-A aggregate occurred more frequently in freshwater drowning, likely because of mechanical alteration or metabolic disturbance in the alveolar type II cells. The left/right ratios of cardiac blood serum SP-A was significantly high both in fresh- and saltwater drowning, but showed no relationship to aggregate SP-A scores *(100)*.

Ishida et al. *(101)* have investigated the difference in responsiveness between the SP-A1 and SP-A2 genes by quantitative reverse transcription polymerase chain reaction assay of SP-A1 and SP-A2 mRNA transcripts in lung tissue. The SP-A1/A2 ratio was markedly higher in drowning and other asphyxia deaths than in controls *(101)*. Maeda et al. *(102)* studied SP-A in

lung tissue by using immunostaining with monoclonal antibody anti-SP-A and a SP-A mRNA assay for SP-A1/A2 mRNA by quantitative reverse transcription polymerase chain reaction and in blood by using a serum SP-A assay kit containing two SP-A monoclonal antibodies. They found an increase in intra-alveolar granular SP-A and in the SP-A1/A2 mRNA ratio in more than 75% of freshwater drowning specimens, but only in some cases with a mild-to-moderate increase in serum SP-A level.

5.1.1.2. Alveolar Macrophages

Betz et al. *(55)* examined CD68+ alveolar macrophages in 40 randomly selected alveoli in each of 10 drowning cases and 15 controls and found no significant differences in the number of alveolar macrophages/alveoli. Excluding the influence of alveolar size, the drowning group showed fewer macrophages, which was interpreted as a wash out-effect of the drowning liquid. Brinkmann et al. *(103)* have investigated different myelomonocyte subtypes (MRP8, MRP14, and 27E10) in the intraalveolar, alveolar-interstitial, and alveolar-intracapillary compartments of drowning and control cases and demonstrated a higher number of all subtypes in all compartments in the former, but with wide variation from one case to another. Local (hypoxia, acidosis, catecholamine reactions, interstitial edema, osmotic cytotoxicity) or systemic factors (increase in pulmonary arterial and capillary pressure) may trigger activation of these cells. Some authors have reported the passage of alveolar macrophages into the left heart likely to be caused by the penetration of alveolar content into circulation after alveoli rupture *(104,105)*.

5.1.2. Other Organs

Gotohda et al. *(106)* observed in drowning deaths and other deaths by asphyxia a higher expression of heat shock protein-70 and c-Fos (both markers of stress- or damage-related events) in the hypoglossal nucleus, which innervates the upper respiratory tract. Quan et al. *(107)* investigated ubiquitin (heat shock protein) distribution in the midbrain of 16 drowning victims and of 18 other asphyxia victims and found a diffuse staining pattern in the nuclei of substantia nigra neurons, suggesting this finding could be a morphological equivalent of severe stress on the CNS.

5.2. Analytical Morphometry

Drowning-related pulmonary changes are distributed heterogeneously in the lung parenchyma. Only extensive investigation of an adequate number of samples can yield a representative picture of the overall changes.

Semiquantitative and computer-assisted morphometry of lung specimens has been tested more widely in chronic than in acute emphysema *(108,109)*. Computer-assisted morphometry has been used in drowning studies only occasionally. Fornes et al. *(110)* have analyzed two randomly selected formalin-fixed, reticulin-stained samples from each of 46 drowned subjects and 35 controls. For each sample, 15 microscopical fields were measured at 40-fold magnification using as variables the total length and mean thickness of the alveolar walls, and the number and mean area of the alveolar cavities. The authors demonstrated significant changes of these parameters in the drowned subjects. Kohlhase and Maxeiner *(111)*, based on a study of six drowned and seven nondrowned cadavers older than 70 years of age at the time of death, demonstrated the possibility of differentiating acute emphysema aquosum from senile chronic emphysema by computer-assisted morphometry when intrathoracic *in situ* fixation is performed to avoid PM collapse of alveoli. The main parameters used for analysis included total tissue area per image and percentage of interalveolar septa of the total area and per image. In this study lungs were fixed in formalin via pulmonary arteries through a funnel placed 1 m above the body for 30 minutes.

The main objective of histomorphometry is to yield reproducible data; for this reason, studies in this area should be based on standardized methods and parameters with regards to fixation procedures, sampling selection, and embedding procedures as well as parameters and programs used to analyze the results. For drowning, a correct perfusion pressure for fixative must be used to avoid PM collapse of alveoli or an artificially high degree of insufflation. The 25 cm H_2O normally used is insufficient to reproduce the overinflation of the lung consequent to drowning.

5.3. Electron Microscopy

LM studies are inadequate to demonstrate delicate alterations related to movement of liquid across the air–blood barrier in drowning. Ultrastructural studies, mostly performed on experimental animals drowned by immersion or tracheal intubation, have been performed both by scanning (SEM)(*112–115*) and transmission electron microscopy (TEM)(*116–121*).

Torre et al. *(114)* investigated the alveolar changes in experimental drowning by complete immersion using albino rats and sampling of the lung followed by fixation in glutaraldehyde. These authors have illustrated alveolar dilatation and atelectasis, lacerations, and disjunction of type I alveolar cells, and exposure of underlying capillaries, as well as disorganization, shortening, and reduction of villi in type II alveolar cells by using SEM. Torre and Varetto

(115) described similar SEM changes in human drowning victims with laceration of the alveolar wall, stretching of the alveoli, and flattening of capillaries, but more delicate variations were not detected, because of overlapping PM changes. Qu *(122)* has studied the tracheal cilia of drowned rats up to 96 hours PM showing their disorganization and structural damage.

In regard to TEM studies, Reidbord and Spitz *(117)* studied lung changes in rats drowned by tracheal intubation at 10 cm H_2O perfusion pressure. In freshwater drowning, the most striking feature was extensive interruption and fragmentation of plasma membranes of endothelial, septal, and alveolar cells. In the mostly damaged area the mitochondria and rough-surfaced endoplasmatic reticulum of the septal and endothelium, cells were swollen and the Golgi apparatus could not be identified. Within the vessels, numerous circular structures of various sizes were evident and limited by a single or double membrane. Conversely, in seawater drowning, the cell outlines and intracytoplasmatic organelles were better preserved. The most striking feature was the invagination of cell membranes, ranging from small elevations to blebs, projecting into alveoli or into the endothelial lumen, and continuous with the basement membrane as well as numerous circular and irregularly rounded structures layered by mostly single limiting membranes in the vascular lumina, septal areas, and alveolar spaces. These structures may be the morphological expression of liquid shifts across the alveolar wall, but they also have been described in lung capillaries of nondrowned individuals *(117)*.

Brinkmann and Butenuth *(119)* performed experimental drowning by tracheotomy on anesthetized rats using liquid of different osmolarities. In freshwater drowning there was edematous swelling of all compartments of the blood–gas barrier, cytolysis, karyolysis, hydropic alterations of the cell organelles, and dilation of the pinocytotic system, ending in endothelial and epithelial vesiculation. In saltwater drowning, there was compaction of the matrix, and the epithelium showed numerous finger-shaped protrusions, constrictions, and exposure of the basement membrane (villous transformation). Erythrocyte sludge and deformed erythrocytes were observable in the capillaries (*119*). Püschel et al. *(121)* detected similar findings in victims of drowning investigated within 24 hours PM.

Bajanowski et al. *(123)* used X-ray microanalysis and transmission SEM and fluorescence microscopy to study the penetration of tracers (gold particles, latex) through the alveolo-capillary barrier. Tracers with smaller diameters penetrated intercellular gaps in the alveolar epithelium and those larger were incorporated into the epithelial and endothelial cells by active pinocytosis, thus passing through the air–blood barrier and being detected in kidneys and lymph nodes.

6. Laboratory Findings

Physical, chemical, and biochemical blood changes have been investigated for more than 100 years to determine the pathophysiology of drowning and to find reliable criteria for its PM diagnosis. Laboratory methods to investigate drowning have their rationale in the shift of liquid and electrolytes through the pulmonary air–blood barrier, which may cause blood volume and electrolyte changes. The reliability of these methods is, however, hampered by factors such as the variable volume of drowning liquid penetrating the airways, the differing length of the drowning process, PM biochemical instability, and individual variations.

Extensive reviews of early studies on laboratory methods for the diagnosis of drowning are available from Gettler *(124)*, Moritz *(39)*, and Reh (*39*). Most, if not all, laboratory methods are at present considered of no practical utility for the diagnosis of drowning *(42,57,68)*. The most important early works and the most representative studies conducted since 1970 will be briefly addressed in this section.

6.1. Blood Properties and Composition

Through the end of the 19th century, hemodilution in drowning has been studied by measuring blood hemoglobin and red blood cell concentration *(125,126)*, specific gravity, freezing point, electrical conductivity *(127)*, and Cl content *(128)*. In 1921, Gettler *(124)*, investigating blood Cl in 18 drownings (15 in saltwater, 3 in freshwater) and 23 controls, found in saltwater drowning a higher Cl concentration in the left chamber of the heart than in the right chamber and the reverse for freshwater drowning, the differences between the two chambers varying from 19 mg to 294 mg, and control values being consistently less than 5 mg. He thus concluded that a biventricular difference of 25 mg Cl or more is a reliable criteria to diagnose drowning and to differentiate seawater from freshwater drowning.

Moritz *(39)* observed that Cl concentration diminishes in controls as early as 12 hours PM and that early works reporting reduction in Cl in freshwater drowning did not mention the PM interval. However, after determining biventricular Cl concentration from 34 freshwater and 32 saltwater drowning victims this author suggested that a difference of 17 meq/L or greater within 12 hours PM should be considered as presumptive evidence of drowning.

Swann and Spafford *(12)* in 1951 conducted experiments concerning the pathophysiology of freshwater and saltwater drowning. Their experimental model consisted of suddenly flooding a hood fastened tightly over the head of nonanesthetized dogs, serial sampling of arterial blood, and measurement of

whole blood O_2, CO_2, pH, serum electrolytes, proteins, globulins, and hemoglobin. Deuterium oxide was used to monitor the changes in body liquid. With freshwater, the drowning liquid penetrated into the circulation massively by the third minute, causing marked blood dilution and hemolysis, while fulminating edema caused a shift of protein-rich liquid and blood salts into the lungs. The increased ratio of K+/other cations caused by hemolysis and the concomitant decrease in other cations exposed the animals to VF. With seawater, conversely, the liquid, electrolyte, and protein shift from blood into the alveoli caused hemoconcentration.

Other studies have disputed the results of these early works by demonstrating no significant differences in biventricular Cl concentrations *(14,129,130)* or by showing the unreliability of hemoglobin and hematocrit determination for predicting blood volume changes *(28,30)*. Jeanmonod et al. *(131)* reassessed the value of biventricular hemodilution by using the freezing point as a measure of osmolarity and found a 15% false-positive rate.

Other studies have, conversely, reiterated the potential of these methods. Fisher *(132)*, for instance, reviewed the Cl content of 202 drownings (129 seawater, 73 freshwater) and showed this method to be a useful tool to diagnose drowning. Faroughi *(133)* reported a decrease in Na, Cl, and osmolarity in serum from the left heart blood of eight freshwater drowning victims, using for control femoral blood and cerebrospinal liquid. Rammer and Gerdin *(134)*, based on 38 freshwater drowning victims and 35 controls, concluded that a lower osmolarity or lower Na or K concentration in the left heart compared with that of cerebrospinal liquid is strongly suggestive of freshwater drowning.

6.2. Exogenous Substances

The drowning media penetrating into the airways contain numerous solutes, including electrolytes, inorganic debris, microorganisms, and zoo- and phytoplanktonic elements that may enter the bloodstream. The most widely studied markers of the drowning media are diatoms, which are considered in detail separately later.

Stockis suggested for the diagnosis of drowning a search for crystalline plankton in the cardiac cavities (*128*), and Icard *(135)* for blood electrolytes (i.e., strontium [Sr], bromide [Br], barium [Ba], fluorine [Fl]) present in large quantities in seawater. Mg concentration has been studied in serum, vitreous humor, and cerebrospinal liquid of drowning victims *(136–138)*, but the applicability of this method has been challenged by the PM increase of Mg serum concentration *(39)* and the passive diffusion of Mg in human and bovine eyeballs exenterated from cadavers and immersed in seawater *(139,140)*.

Aquatic organisms detectable in blood include chlorophyta, dinoflagellates, invertebrates, protozoan ciliates, and bacteria *(141,142)*. Lehmann and Beuthin *(143)* recommend searching for pollutants such as calcium lingosulfonate, and Chen et al. *(144)* recommend determining serum Fl in drowning occurring in water with a high Fl concentration. Mukaida et al. *(145)* demonstrated, by UV light and high-pressure liquid chromatography, fluorescent bath salts in the lung and kidney of a baby drowned in a bathtub.

Sr remains the most studied exogenous ionic tracer for drowning. The seawater and serum Sr concentration ratio (650:1) makes it a potential marker for drowning. Sr concentration, however, differs widely among geographic areas, being, for instance, higher in the Mediterranean Sea than in the Baltic Sea. Using flameless atomic absorption spectrophotometry, Piette et al. *(146)* measured Sr concentration in the serum of nondrowned living persons (n = 36), and individuals recovered from freshwater (n = 29), and from seawater (n = 33) and found significant biventricular differences in both freshwater and seawater drowning. These authors concluded that Sr can serve as valuable additional evidence for the diagnosis of seawater drowning, but cautioned about possible PM contamination in Sr-rich water.

Azparren et al. *(147)*, performing studies on human drowning victims, found biventricular Sr differences in "typical" drowning greater than 75 µg Sr/L, compared with less than 20 µg Sr/L in "atypical drowning." These results were confirmed in a further study that showed highly significant differences, especially when the drowning media had an Sr concentration higher than 800 µg/L *(148)*. Fornes et al. *(110)*, comparing 116 drowned subjects with 35 controls who died from causes other than drowning and 23 healthy living subjects, found mean Sr blood values much higher than in the controls with no overlap between drowned and nondrowned subjects. Azparren et al. *(149)* reported a study of 70 definite seawater drowning victims with a significant relation between Sr blood concentration and duration of the agonal period.

6.3. Artificial Tracers

Artificial tracers have been widely used in experimental models to study the absorption of drowning liquid into the organism. As early as 1752, Louis, in France, performed animal experiments with ink-traced liquid to investigate the passage of drowning media into lungs *(150)*. The penetration of the drowning medium into the respiratory tract, circulatory system, and peripheral organs has been studied qualitatively and quantitatively with a variety of tracers such as particulate matter, chromatic substances, radio-opaque media, and isotopes *(151–154)*. Whereas nonisotopic tracers furnish only qualitative data on this

penetration, many isotopic tracers are unreliable for quantitative studies because of their selective permeability at the pulmonary level and their affinity for tissues or compounds. Tritium (H_3) is the most reliable tracer of intravascular–extravascular liquid because it distributes uniformly into the H_2O molecule and has no selective permeability and/or tissue affinity. However, only one quantitative study on penetration of drowning media traced with H_3 has appeared *(154)*, whereas another study has addressed the time-dependent penetration of H_3-traced drowning media into the circulation *(155)*. More recently, the fine interaction between drowning media and the alveolo–capillary barrier has been studied ultrastructurally with latex particles, India ink, ferritin, myoglobin, and colloidal gold *(123,156)*.

7. The Diatom Method

7.1. General Remarks

The diatom test for the diagnosis of drowning is based on the assumption that diatoms, which are eukaryotic unicellular algae, reach the lung with inhalation of liquid and if effective cardio-circulatory activity exists, penetrate the pulmonary filter and disseminate to organs through the blood stream. Conversely, if a corpse is submerged PM, the diatoms may penetrate passively into the airways, but, owing to the lack of cardiac activity, will not be transported to other organs. Since the first description of diatoms in drowning victims toward the end of the 19th century *(157)*, more than 300 articles, several reviews, and 2 monographs *(158,159)* have addressed a wide range of issues on the diatom method for the diagnosis of drowning. Although a consensus of opinion exists on the greater potential of this method than of other laboratory tests, its utility and reliability remain very controversial.

7.2. Diatom Biology

Diatoms are eukaryotic unicellular or colonial algae, which are ubiquitous in water, air, and soil. The diatom's cell wall contains a high quantity of silica and comprises two units, called valves, bound by linking siliceous structures, the girdle elements. The whole siliceous part of the diatom wall (valve and girdles) is termed the frustule. A diatom size ranges from 2 µm to more than 500 µm, with most species being 10 to 80 µm in length or diameter.

The *valve structure*, which is specific for each class, represents the basis for taxonomic classification. The structure of the valve usually has either a pattern of radial symmetry or an elongated one, which provide the first distinction between centric and pinnate diatoms. Centric diatoms have silicate

ribs radiating from a center and pinnate ones have ribs extending from both sides of a longitudinal tick element. *Girdle bands* bind the valves together, have a protective function, and allow a volume increase in the diatoms during the cell cycle. The diatom *protoplast* consists of the plasmalemma, yellow-brown plastids containing DNA, chlorophylls, and carotenoids, as well as mitochondria, dictyosomes, vacuoles, and a nucleus with a double membrane and one or more nucleoli *(160)*.

Diatoms, which are found in all types of water and may live as single cells, paired cells, or in large colonies, can be classified according to their ecological properties. The classification based on salinity (oligophilic: salinity: <0.05%; mesohalophilic: brackish water; polyhalophilic: salinity >0.05%) is relevant in forensic medicine because it may assist in differentiating between freshwater and saltwater drowning. Diatoms can also be distinguished in planctonic (free in water), periphytic, or benthic (on bottom or immersed objects), and aerophilic or facultative aerophilic (in air or on soil and rocks)*(158)*.

7.3. Sample Preparation and Analysis

The whole procedure for the preparation of samples for diatom analysis includes water sampling from the putative drowning site, tissue sampling from victims at autopsy, tissue destruction to collect diatoms, diatom concentration, and microscopic analysis.

The collection of samples from putative drowning media should be performed during the recovery of the body, from the water surface and deeper, using 1 to 1.5 L sterile receptacles to be stored at 4°C, whereas samples from putative drowning victims are collected at autopsy sterilely, mostly from lungs, liver, kidney, brain, and bone marrow *(161)*.

The identification of diatom shells in lung and other internal organs requires the complete destruction of the organ tissues to be examined except for the diatom frustules. The most common extraction technique consists of chemical digestion by nitric or sulfuric acid *(158,162)*, solubilizers (e.g., soluene 350 *[163,164]*), or enzymes (e.g., proteinase K *[165,166]*). Incineration has been used for fatty-rich samples, the resulting ashes being treated by oxidizing acid.

Other methods for identification of diatoms in tissue include amplification of planktonic or diatom DNA and RNA in human tissues *(167–169)*, microscopic analysis in tissue sections *(170,171)*, diatom cultivation in appropriate media *(172)*, and spectrofluophotometry to quantify chlorophyll(a) of plankton in the lung *(173)*. Methods to detect diatoms in blood include direct observation of diatoms on a membrane filter *(174)*, after blood hemolysis by sodium

dodecyl sulfate *(175)*, or by combination of hemolysis, 5 mm pore-membrane filtering, digestion with nitric acid, and re-filtration *(176)*.

Once digestion is performed, diatoms can be isolated by centrifugation or membrane filtration *(176)*. Centrifugation cycles concentrate diatoms and remove all traces of acid by repeated washing, the supernatant being replaced each time with distilled water. The use of nitrocellulose filters is advocated for samples with low diatom content and is followed by LM analysis, eventually after acid treatment or incineration of filters *(176–178)*. Filters may, however, contain diatoms, and the deposition of other particles can obstruct pores and obscure diatoms.

For LM, a drop of suspension is dried onto a cover slip. Because cleaned diatoms are transparent and have a refraction index (r.i.) of 1.44, close to that of glass, mounting in water (r.i. = 1.33) or Canada balsam (r.i. = 1.55) will not reveal the fine structure of the cells, so synthetic resins with a high refraction index, such as Naphrax (r.i. = 1.74) should be used. LM analysis must be performed using 630- to 1000-fold magnification *(161)* and diatoms must be counted (diatom density), analyzed (species determination), and measured (morphometry).

7.4. Diatom Penetration in the Lung

Conflicting reports exist on the diagnostic value of diatoms in the lung. It is generally assumed that diatoms may enter the lungs by in vivo inhalation or during PM submersion. Tomonaga *(179)* found several diatoms in lungs of a cadaver kept for 30 minutes at a 23 m depth, and Nanikawa and Kotoku *(180)* reported up to 145 diatoms/g of lung in a nondrowned corpse submerged for 2 to 3 months at a depth of 120 m. Accordingly, Reh *(82)* considered diatoms in the lungs an unspecific finding, and Neidhart and Greendyke *(181)* stated that "… the demonstration of diatoms in lung tissue is of no value in determining whether a victim was alive before submersion…." However, Timperman *(182)* suggested that diatoms in the lung are strong evidence of rapid death in water, and Auer and Möttönen *(183)* that a diagnosis of drowning can be made when more than 20 diatoms per microscopic slide are found in lungs. According to Ludes and Coste *(158)*, when diatoms are found in the lungs but not in closed organs, the possibility of a passive penetration cannot be excluded.

Histological studies indicate that a different diatom distribution occurs in drowning and in PM submersion. In the former a generalized dissemination is observed with diatoms reaching the alveoli in the subpleural regions, whereas in PM submersion passive diffusion in interlobular and intralobular bronchi may occur, but they do not reach the bronchioli and alveoli *(151,184)*.

7.5. Diatoms in Peripheral Organs

Lunetta et al. *(185)* demonstrated, using SEM and TEM, the ultrastructural details of penetration of diatoms from the alveoli into the bloodstream during the experimental drowning process and their phagocytosis by alveolar macrophages. Diatoms passing through the pulmonary filter and extracted from closed organs represent a distinctive subset of the diatoms present in the drowning media. The selective filtration of diatoms depends in part on their size and shape as well as their possible aggregation in colonies. Figures 9 through 12 show different diatoms in diverse pulmonary compartments as seen in SEM and TEM.

Data concerning the size and shape of diatoms penetrating the alveolar capillary barrier are contradictory. Tomonaga *(179)*, for instance, fixed the maximum diameter and length of diatoms entering the bloodstream at 100 µm and 160 µm *(179)*. Most authors, however, set the limit at less than 60 µm. Hurlimann et al. (2000) found two-thirds of the valves as being less than 15 µm and more than 90% to be less than 40 µm *(161)*. Pachar and Cameron *(185)* and Giri et al. *(187)* have found only diatoms measuring less than 30 µm in closed organs.

The lack of standardized protocols for quantitative and qualitative diatom analysis makes any comparison between different studies virtually impossible. The diatom content of the drowning media, the aliquot of tissue analyzed, and the extraction procedures are all among the variables, details of which are not always available and which may substantially influence results. Most of the studies, thus, have a merely empirical value, with some older studies expressing their results only in terms of the positivity or negativity of given organs (e.g., ref. *188*) and some others not possessing the requisites for any quantitative assessment (e.g., ref. *189*).

Important differences exist concerning the number of positive cases within any given series. For instance in the Ludes and Coste *(158)* series of 40 drowning victims, 14 were lung positive and 11 both lung and internal organ (liver, kidney, or brain) positive, whereas 15 were completely negative *(158)*. Auer and Möttönen *(183)*, in 107 putative drowning victims, found 33 cases with diatoms only in the lung, 62 with diatoms in lung and closed organs, and 12 completely negative.

Striking differences also exist concerning the number of penetrating diatoms in each positive case. Some authors speak in terms of dozens or hundreds *(161,190)* and others of single diatoms *(191)*. Foged *(190)*, for instance, reports 6 to 221 valves/g lung, 5 to 68/g liver, and 9 to 127/g kidney whereas Giri et al. *(187)* report 40 diatoms/10 g lung, 25 in kidney, 20 in liver, and 10

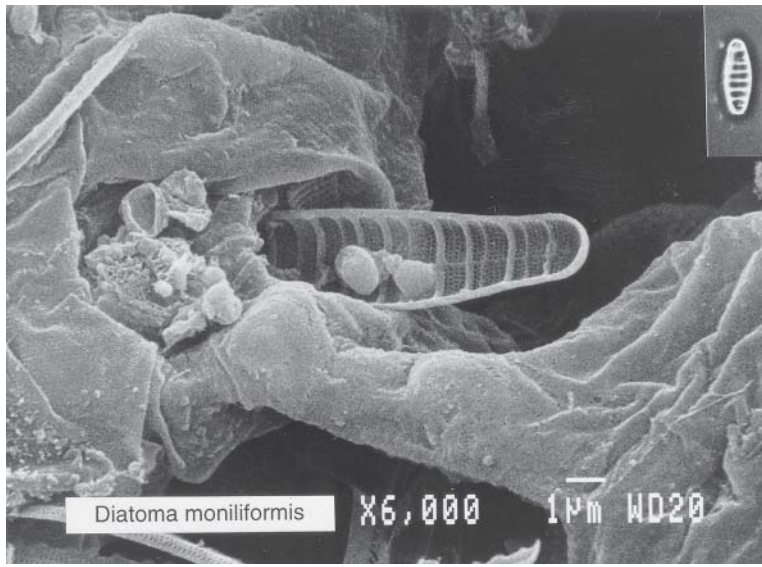


Fig. 9. *Diatoma moniliformis,* a diatom, penetrating the wall of a distal airway (scanning electron microscopy; experimental conditions, rat).

in brain. Hurlimann et al. *(161)* set the maximum diatom density at 54 to 108 diatoms/5 g in lung, 92 to 184 in liver, and 22 to 44 in kidney. Ludes and Coste *(158)* found more than 60 diatoms/10 g lung in 66% of 30 drowning cases, the maximum content in other organs (kidney, liver, brain) being 15 diatoms/10 g tissue. Few authors have established quantitative limits diagnostic for drowning. Ludes et al. *(192)*, for instance, set the limit at 20 diatoms per histological slide per 100 µL pellet for lungs and 5 diatoms per slide per 100 µL as a reliable criteria for the diagnosis of drowning. Hurlimann et al. *(161)* have proposed much higher separation values, for instance, up to 20 to 40 diatoms/5 g in bone marrow.

As to qualitative analysis, species composition may help in determining the site of drowning and excluding the source of contamination. Several indices can be used to compare diatom samples *(158)*. The species index (SI), which is defined as $SI_{1,2} = S_{1n2}/S_{1+2} * 100$ (%), where S_{1n2} is the number of species common to the two diatom communities, and S_{1+2} is the total number of species in the two communities, is a measure of the similarities between two diatom communities and ranges from 0 to 100%. An SI greater than 60% indicates that the two samples originate from the same diatom community. When calculating SI, the diatom samples must contain approximately the same number of diatoms (at least 500) with a difference no higher than 20% *(161)*.

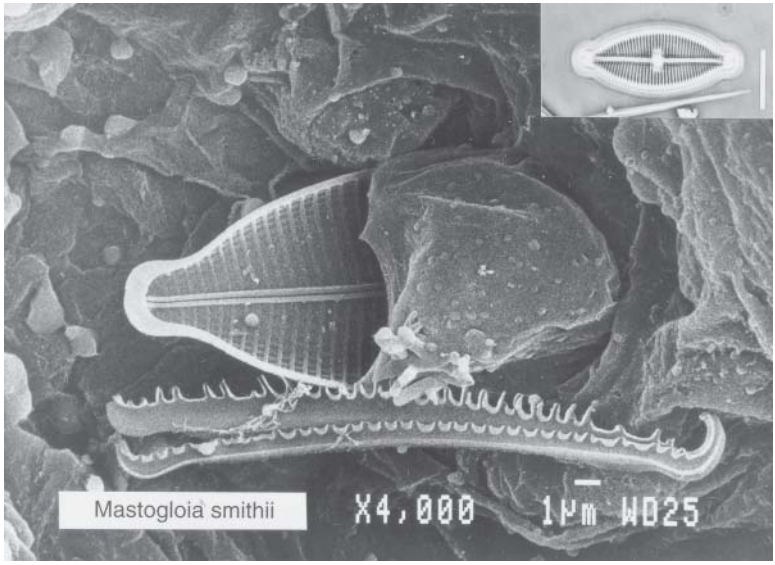


Fig. 10. *Mastogloja smithii*, a diatom, penetrating the alveolar wall through a clearly visible laceration (scanning electron microscopy; experimental conditions, rat).

Because this requirement generally is not fulfilled in drowning, the percentage of each species (<40 µm) present in organ samples and in the drowning medium must be calculated. Ludes et al. *(192)* maintain that when 60 diatoms/10 g lung are recovered, the determination of proportional abundance of taxa and its comparison with 300 frustules from the drowning media is possible.

Bone marrow, especially from the femur, is regarded as relatively protected from contamination even in cases of advanced putrefaction with adipocere representing an additional protective layer during prolonged submersion. Pollanen et al. *(193)* studied the femoral bone marrow in 771 presumed freshwater drowning victims and reported positive results in 205 cases (27%), the percentage being higher in April and July (40%) and in November (30%) likely because of the cyclic diatom blooms in freshwater occurring in early spring and autumn. As regards quantitative data, some authors have found no more than 10 diatoms per bone marrow sample *(177,187)* whereas other authors have reported much higher values. Foged *(190)*, for instance, has reported 1 to 230 diatoms/g bone marrow (vertebrae, femur) and Hurlimann et al. *(161)* 30 to 60 diatoms/5 g.

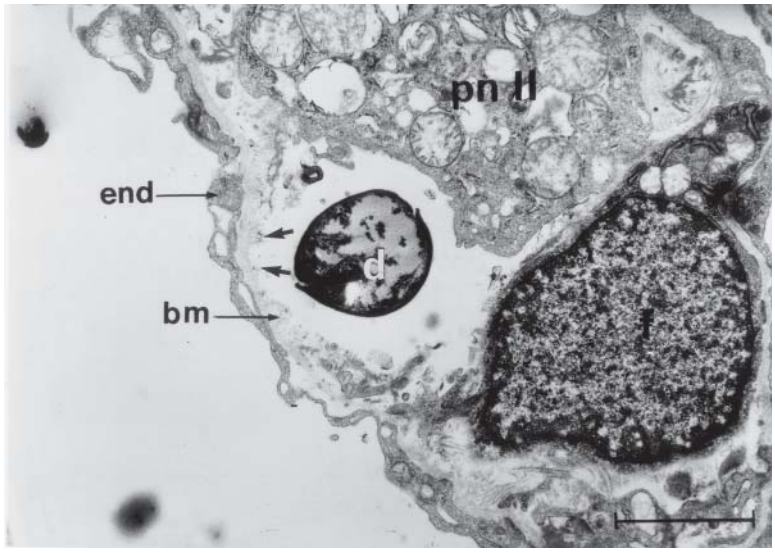


Fig. 11. *Phaeodactylum tricornitum*, a diatom, in the interstitial space with incipient ruptures of the endothelial membrane. End, endothelial lining; bm, basal membrane; pn II, pneumocyte type II; d, diatom (transmission electron microscopy; experimental conditions, rat).

7.6. False-Positive Results

The main criticism of the diatom test is the finding of diatoms in lungs and other organs in nondrowned human beings. Quantitative data on diatoms in organs of the nondrowned are, once more, contradictory. Foged *(190)* found in four nondrowned bodies up to 194 valves/cm^3 lung, in liver up to 54, in kidney up to 53, and in bone marrow (vertebrae, femur) up to 17. Pachar and Cameron *(186)* found 5 to 25 diatoms/100 g lung and up to 10/100 g in closed organs. Timperman *(182)* maintains that the number of diatoms in the nondrowned person does not exceed 10/100 g. Auer and Möttönen *(183)* found no diatoms from organs of 15 nondrowned individuals, nor did Ludes et al. *(166)* find diatoms in their controls.

Most studies on animals and humans report few diatoms in the peripheral organs of the nondrowned *(178,187,194)*. Mueller *(195)* found one Cyclotella-like diatom in 30 livers (30 g); Waltz *(196)* a single Cyclotella and two fragments in 40 livers (50 to 100 g); Timperman *(197)* one to four diatoms in 4 of 13 lungs (100 g), and Janitzki *(198)* one to three diatoms in two

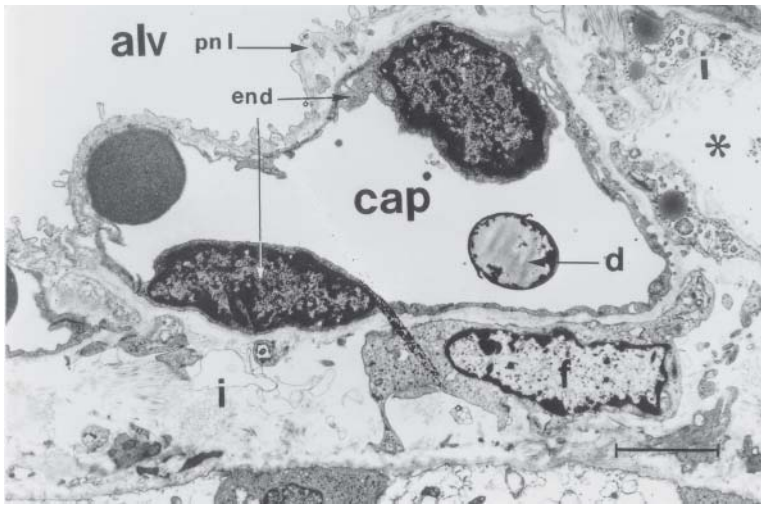


Fig. 12. *Phaeodactylum tricornitum,* a diatom, in a pulmonary capillary. Cap, capillary; alv, alveolar space; end, endothelial cell; pn I, pneumocyte type I; i, interstitial space (transmission electron microscopy; experimental conditions, rat).

lungs of eight (100 g). A diatom species found in tissue that does not match with any diatom species present in the water of the putative drowning site should be, according to Ludes and Coste *(158)*, considered a contaminating diatom.

7.6.1. Antemortem Contamination

GE absorption of diatoms may occur as a result of ingestion of diatom-laden food such as vegetables (e.g., salad, radish, watercress, celery) and shellfish (e.g., mussel, limpet, winkle, oyster) *(199)*. Beverages may contain diatoms in countries where natives drink ditch water and river water or where kieshelgur is used as a filtering material in breweries and wine factories *(177)*.

Researchers have investigated the GE absorption of diatoms. On one side, Spitz and Schneider *(191)* found diatoms in internal organs in 92% of rats fed with a diatom suspension and up to 111 diatoms/200 g liver in 21 of 22 nondrowned humans and suggested that GE absorption may occur via portal veins or lymphatics. Conversely, Mueller *(195)* found no diatoms in the liver or kidney of rats fed with diatoms, and Schneider *(200)* recovered no diatoms after also feeding rats with a suspension of Nitzchia (10×90 μm). Merli et al. *(201)*, after feeding five dogs diatom-rich food (3 million/100 mL), did not find diatoms in the lung, kidney, liver, or urine, nor in rats whose GE mucosa was chemically damaged before feeding.

Diatom inhalation may involve aerophilic species or kieshelgur, which is widely used as an inert chemical material, for instance, in the manufacture of building and insulating materials, in paint, paper, cosmetic powders, and safety matches *(199,202)*. Authors such as Spitz and Schneider *(190)*, Koseki *(191)*, and Foged *(194)* have demonstrated diatoms in air by using small beakers or filtration bands exposed to the open air. Diatoms also may be inhaled while smoking cigars since tobacco leaves contain diatoms *(203)*. Otto *(204)* reported a heavy diatom load in 23 of 28 lungs of workers exposed to silicate.

7.6.2. Postmortem Penetration

PM diatom penetration into an organism may occur during prolonged submersion at high hydrostatic pressure, through antemortem (AM) and PM wounds, or during reanimation procedures with artificial ventilation *(158)*.

De Bernardi et al. *(184)* submerged 36 dead rats for up to 30 days in a suspension containing up to 20,000 diatoms per milliliter (dimension: 5–90 μm), both at the surface and at 2 atm but found no diatoms in closed organs. Koseki *(194)* found a few diatoms in the long bones (humerus, femur) of rabbits and dogs submerged dead for 1 month into a pond but not in those with a shorter submersion period. When the bones were bleached and submerged in tap water, they contained up to 13 diatoms after 1 day, up to 93 after 1 week, and up to 276 after 1 month. In seawater, as many as 2550 diatoms were found after a 1-month submersion. Koseki concluded that "bones submerged for a long while and skeletonized bones are apt to produce errors in the determination of cause of death because of the intrusion of diatoms through foramen nutricium and other pores." Ludes and Coste *(158)* also stress the great risk of contamination in skeletonized bones. Kan *(205)* studied the relationship between water pressure and penetration of diatoms into bone marrow from bleached bones of rabbits kept in a kieshelgur suspension (35,000–40,000 diatoms per milliliter) at 0 to 4 atm for 30 minutes. He observed an increase in the number of bones yielding positive results and of the number of diatoms per bone with increasing pressure. At 0.5 m, there were three positive bones with up to four diatoms per bone (humerus), whereas at 40 m, all 11 bones were positive, with up to 11 diatoms per bone (right ulna). In similar experiments with unbleached bones, no diatoms were found even at 40 m depth.

Data on humans are more scant than for animals. Tamâska *(206)* found no diatoms in the bone marrow of seven persons shot dead before falling into water. Tomonaga *(179)* reported a "very small number" of diatoms in the heart and none in closed organs of a body submersed PM at 23 m depth for 30 minutes. Tomonaga *(207)* described diatoms in the liver and kidney of humans submerged at 130 m depth. Giri et al. *(187)* found as many as 20 diatoms in lung samples, 10 in heart, and 1 in kidney in nondrowned submersed bodies.

7.6.3. Contamination During Sample Preparation

Contamination may arise during the entire sequence of diatom preparation, from tissue sampling at autopsy to sample mounting onto the slide. At autopsy, careless sampling procedures may yield contamination from the victim's clothes and body surface during external examination or between different organs during internal examination. Moreover, during the whole sample-preparation process, air, instruments, gloves, paper, water supplies, reagents, and glassware represent potential contamination sources. Most studies concerning laboratory contamination have focused on tap water and reagents. It is generally accepted that tap water may contain some diatoms. As to distilled water, Porawski *(194)* and Koseki *(208)* found no diatoms, whereas Tabbara and Dérobert *(209)* reported a single diatom in 5 L but none in tri-distilled water. The presence of diatoms in reagents or glassware also varies from one study to another, but is usually extremely limited *(177)* if not completely absent *(183,186,210)*. To minimize risk of contamination, use of water should be limited to bi- or tri-distilled water and reagents and chemicals must be regularly tested for contaminating diatoms. Bottles and flasks used should be cleaned for 24 hours in a solution, and old flasks must be replaced regularly because glass irregularities may host diatoms. Paper material, such as ordinary laboratory filters, must be avoided because of their potentially high diatom content.

7.6.4. Other Sources of Contamination

Other potential sources of contamination include repeated swallowing or inhalation of water by divers *(211)* or swimmers *(212)*, transplacental passage *(158)*, and possible contamination of the pancreas and gallbladder by retrograde passage of duodenum contents *(179)*. PM contamination from sawdust in the coffin must be considered in exhumation cases *(177)*.

7.7. False-Negative Results

The low diatom concentration in drowning media, the low volume of inhaled liquid, and diatoms lost during sample preparation also may yield negative results in definite drowning. Devos et al. *(213)* and Funayama et al. *(214)* stressed that low diatom concentration may hamper the feasibility of the diatom test in tap water and open sea drowning. Some authors have attempted to determine the minimum number of diatoms in the drowning media required to produce diatoms in closed organs. Mueller *(215)* set this limit at 20,000/100 mL for rats and 13,500/100 mL for rabbits. Tomonaga *(179)*, defining the

diatom content in water as 100, found diatom values varying from 200 to 1300 in human lungs and from 1 to 25 in closed organs.

The number of diatom-negative cases in drowning series varies widely, depending also on whether the definition of a negative case includes instances with diatoms in neither lung nor closed organs or only those with no diatoms in closed organs. Angelini Rota *(188)* found in 48 drowned individuals 24.2% with no diatoms in the lungs and 32.6% with no diatoms in closed organs, whereas the respective values in Neidhart and Greendyke's series was 17% and 30.8% *(181)*. Timperman *(177)*, studying the whole lung in 40 drownings, found no diatoms in approx 10%. Auer and Möttönen *(183)* reported 11.2% having no diatoms in lungs and closed organs and Ludes and Coste *(158)* 37.5%.

In conclusion, studies on the reliability of the diatom test for the diagnosis of drowning have yielded widely divergent results and opinions. The latter rely, at least partially, on dated studies that have not been performed using rigorous and standardized methodologies and not by expert diatomologists.

Some concepts should be clear from this section on the diatom method for the diagnosis of drowning. Regarding "negative" cases, the absence of diatoms in a body should in no way allow for excluding drowning as the cause of death. On the other hand, the mere finding of a few diatoms in a human body does not establish a diagnosis of death by drowning. Any acceptable results which may satisfactorily resist the criticisms which have been leveled against the test depend on *(a)* the quantitative and qualitative taxonomical concordance between diatom content in the body and putative drowning media, *(b)* the adoption of a strict protocol to avoid contamination during sampling preparation, and *(c)* the exclusion from the results of any diatoms that potentially represent "contaminating" diatoms. Further studies are needed to establish standardized separation values between controls and definite drowning cases. Until then, the diatom method cannot be accepted in definitively proving a diagnosis of drowning in the courtroom, but rather represents a useful supportive evidence for diagnosing death by drowning.

8. Selected Issues

8.1. Identification

Bodies recovered from water are often, at least during the first phases of investigations, unidentified. Identification represents an important step in the early investigation of death in bodies found in water because personal history can furnish important clues to link circumstantial data and PM findings to the

actual death. The common medicolegal criteria for individual, sex, age, stature, and race identification apply also to bodies retrieved from aquatic environments and include visual characteristics and fingerprinting as well as odontological, anthropological, and DNA methods *(216)*.

Medicolegal identification may be required for fresh or decomposed bodies as well as for skeletonized remains found in water. During the early PM period, identification may be hampered because personal documents are washed out of the clothes, their features effaced by the effect of water, or because clothing and other personal effects are lost in the water. AM or PM trauma such as dismemberment by the perpetrator of a crime or by boat propellers or later PM lesions caused by the aquatic macrofauna or microfauna can hamper visual identification or fingerprinting. In individuals with advanced maceration changes suitable fingerprinting ridge impressions can be obtained by a variety of techniques *(217)*.

Identification of bodies found in water represents a recurrent problem, especially in jurisdictions operating in large coastal regions. In this context, the transient nature of the population and bodies drifting from one country's coast to another's are additional factors that may challenge prompt victim identification. The drifting of cadavers can occur either during the early phases of buoyancy and sinking or later during the decomposition process and depends on several factors including currents, tides, waves, and winds. In northern Europe, Kringsholm et al. *(218)* described a series of 80 initially unidentified bodies and 9 skulls found from 1992 to 1996 in Danish waters. Danish nationals constituted 57% of the 74 identified cases, whereas the remaining cases mostly involved victims from bordering countries.

Cadavers can be moved hundreds of miles away from their point of entry into water in a relatively short period of time. Giertsen and Morild *(219)* described two bodies that drifted for more than 500 km, following the Gulf Stream from Denmark and the east coast of Scotland to the Norwegian coast in 28 days and 4.5 months, respectively. Blanco Pampin and Lopez-Abajo Rodriguez *(220)* reported victims drowned in a river in Portugal who drifted by Atlantic Ocean currents to the Spanish coast 380 km away in only 60 hours. Carniel et al. *(221)* reported a body drifting in the Mediterranean Sea more than 300 km in 2 weeks.

Identification also is a primary issue in mass disasters occurring at sea or in internal waters and involving aircraft *(222)*, flooding *(223)*, and ships *(224)*. Identification also is a growing concern in connection with shipwrecks transporting clandestine immigrants.

8.2. Postmortem Changes

PM changes that occur in a corpse in an aquatic environment include early changes (e.g., skin maceration, goose flesh, hypostasis, rigor, and cooling) and late changes (e.g., putrefaction, adipocere formation, and skeletonization).

8.2.1. Early Postmortem Changes

Skin maceration ("washerwoman's skin," Fig. 13) is characterized by thickening, wrinkling, and whitening of the skin that occurs first on fingertips, palms and the backs of hands, soles and backs of feet, and later also on elbows and knees. Saukko and Knight *(42)* set the onset of maceration in warm water within minutes from exposure and in cold water after 4 to 5 hours, whereas Giertsen *(57)*, in Norway, states that maceration appears on fingertips in 2 to 4 hours and on the palm after approx 24 hours. Gee *(58)* maintains that "it takes nearly twice as long for the skin of a clothed foot to attain the same degree of maceration as the unprotected hand."

Prolonged skin exposure to water causes progressive loosening of the nails and skin peeling from hands (Fig. 14) and feet in a "glove and stocking-like" fashion. Wide variation exists as regards the time interval before this peeling occurs. Giertsen *(57)* stated that the skin may loosen from the hands after 2 weeks, but the skin slips off in a glove-fashion only after 3 to 4 weeks. Reh *(82)* observed that in a river during winter (water temperature: 3.2ºC), it takes 7 to 8 weeks before the nails loosen, whereas during summer (18.6ºC) this may occur after only 3 days. Püschel and Schneider *(225)* submitted macrophotographs of 48 hands with maceration to German and foreign medicolegal experts and reported a wide heterogeneity of interpretations, testifying to the difficulty of objectively grading these changes.

Histologically, early maceration changes have been characterized by detachment of the stratum corneum and perinuclear vacuolization of the cells of the Malpighian stratum; advanced lesions include homogenization of the stratum corneum, detachment of epidermis, necrosis of the granular and basal stratum, and fiber ruptures in hypoderma *(226)*. Weber *(227)* interpreted skin wrinkling as the result of repeated water input, which forms subepidermal water-filled collections, in contrast to in vivo conditions in which a dynamic balance exists between liquid uptake and its re-absorption into the bloodstream.

Goose flesh ("cutis anserina") occurs in bodies located in water but also is observed on those found on land. Spasm of the erector muscles of hair follicles seems to play an important role in this phenomenon, although its cause remains unclear. DiMaio and DiMaio *(68)* stress the association of goose flesh with rigor mortis, but Saukko and Knight *(42)* question the role of rigor in the etiology of this phenomenon.

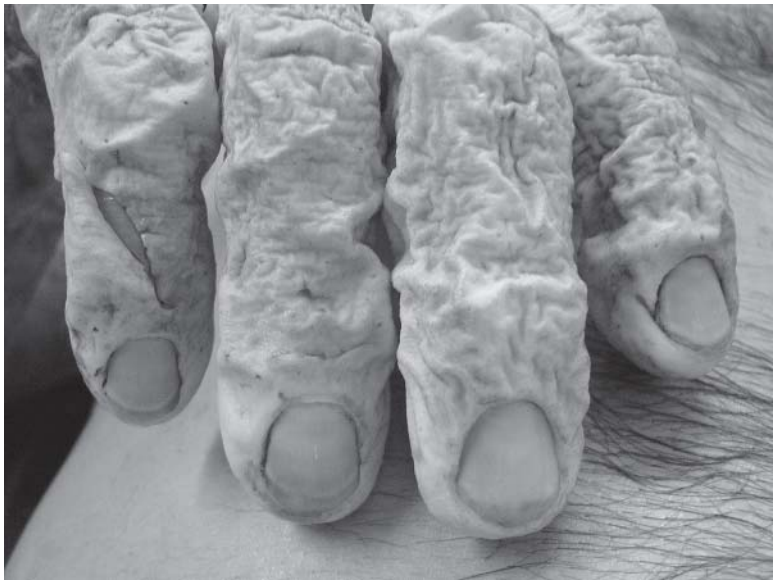


Fig. 13. Submersion. Typical maceration changes on the hand. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

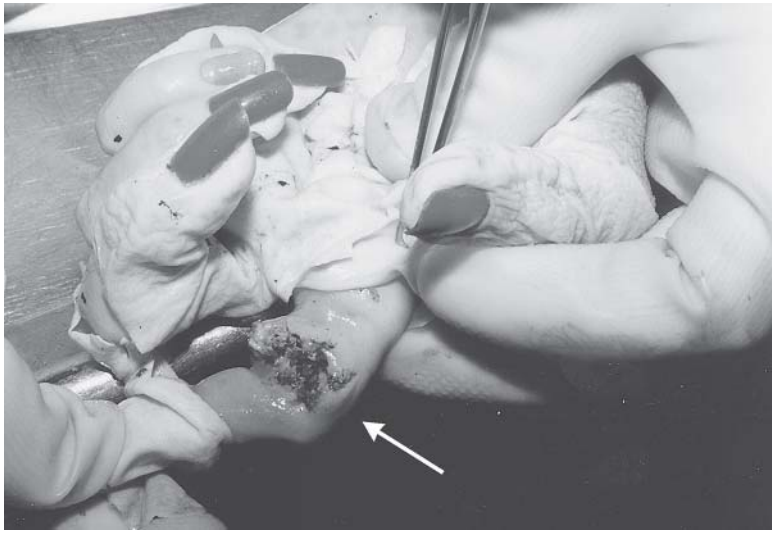


Fig. 14. Submersion. Maceration changes on right hand with partial peeling of skin and nails. Examination of the soft tissue under the peeled skin revealed small plastic fragments within irregular lacerations (arrow). Police investigations revealed that the victim was killed by firearm and that one bullet likely impacted a mobile phone kept on her right hand.

Hypostasis can be observed, as the result of the movements of the corpse in water, in any pattern around the body. Often, however, hypostasis is selectively located on the face, the upper parts of the chest, thighs, feet, and calves, because of the head- and leg-down position the body may assume after sinking. The pink-reddish color of hypostasis is likely attributed to unreduced oxyhemoglobin, the presence of which depends more on cold temperature than on water exposure *(42)*. Bonte et al. *(228)* have described a specific hypostasis pattern in victims of electrocution in the bathtub, with the upper margins of hypostasis along the water-surface line, whereas Wollenek et al. *(229)* have observed a pale and thin skin mark parallel to the water surface that was interpreted as a thermal phase-transition phenomenon independent from hypostasis.

The term *pink teeth* designates a characteristic pink discoloration of the crowns and roots of the teeth *(230)*. This change has been investigated histologically, biochemically, and ultrastructurally *(231)* and seems related to increased venous pressure in the pulp due to the head-down position of the corpse in water, followed by extravasation of erythrocytes, autolysis, and diffusion of hemoglobin and its breakdown products from pulp tissue into the dentin via dentinal tubules *(232,233)*. A moist environment has been suggested to accelerate hemolysis and the diffusion of blood in the dentin tubules. However, not all bodies found in water have pink teeth and pink teeth also have been found in many other types of death such as hanging, carbon monoxide poisoning, and barbiturate poisoning *(233)*. This phenomenon has no specific significance in regard to the PM interval *(234)*.

Rigor mortis seen in immersion deaths is influenced by environmental and individual factors similar to those acting on a body on land. Two factors may act specifically on corpses in an aquatic environment: a) The low environmental temperature, which usually retards the development of rigor and b) the victims' muscular contractions during the drowning process, which may lead to earlier onset, stronger development, and longer persistence of rigor mortis compared to that on land *(57)*. The rigor of hand articulations may facilitate grasping of seaweed and other marine material which may assist in the determination of the site of submersion.

The *cooling* of a body in water is faster than on land since the thermal conductivity of water is more than 20 times as high as that of air (0.60 vs 0.02). A naked corpse submerged in cold water cools approximately twice as fast as does a body on land *(58)* and, once brought ashore, body cooling is even more rapid because of liquid evaporation through the skin. The cooling rate in water depends on various factors such as water temperature, currents, the victim's clothing, and body temperature at the time of death.

Henssge et al. *(235)* measured the rectal temperature of 29 naked corpses suspended in a tub filled with water at 20ºC, 10ºC, and 0ºC, and by using Marshall and Hoare's body-cooling formula. They found that a body cools as quickly as an unclad corpse of half that body mass in calm air of the same temperature. Henssge *(236)* has discussed in detail the effects of water and wet environments on the constants of Marshall and Hoare's formula. The rapid cooling of a body in water implies that in this setting rectal temperature can be used to estimate the PM interval only within the first few hours (generally 12 hours) of submersion, an interval during which forensic pathologists only occasionally have the possibility to examine bodies retrieved from water.

8.2.2. Putrefaction, Adipocere, and Late Decomposition

Decomposition of a body in an aquatic environment (Fig. 15) occurs at a rate roughly half that in air because the cooler water temperature inhibits bacterial and insect activities. Once the body floats or is taken ashore, decomposition proceeds at an accelerated rate. Chromatic skin discoloration can appear first in the abdominal region, but at times, owing to the peculiar position of a corpse in water, also on the face, neck, and chest. In northern Europe, Giertsen *(57)* stated that at water temperatures of 5 to 6ºC no appreciable decomposition might be evident even after several weeks, whereas at 10 to 20ºC, the decomposition occurs after 3 to 5 days. In southern Europe, Gerin et al. *(226)* reported advanced chromatic changes occurring within 2 days PM and detachment of large epidermal areas and of hair after 5 to 6 days and 8 to 12 days. Adelson *(237)* states that during midsummer in temperate regions putrefactive gases may form and rise a body to the surface within 2 to 3 days in a lake or pond and after 2 to 3 weeks at sea. Several factors may influence the decomposition of corpses in an aquatic environment. These include water temperature and, to a lesser extent, the bacterial content of water and its salinity (which has influence on bacterial activity) as well as PM and AM injuries which create portals of entry for carrion insects and bacteria *(42,57,238)*.

Adipocere is a waxy decomposition product formed from bacterial hydrolysis and hydrogenation of adiposus tissue, which generally occurs in bodies under water or in moist soil, in a warm, damp, and anaerobic environment *(239,240)*. Adipocere provides good preservation of organs and tissues *(241)*. Adipocere biochemistry has been extensively investigated *(242–246)*. During the first steps of adipocere formation, triglycerides composed of neutral fats are degraded by endogenous lipases and then bacterial enzymes convert neutral fats into fatty acids. The latter are, in turn, converted to hydroxy-fatty acids, oleic acid being the primary source of 10-hydroxystearic acid which is the main component of adipocere. The low pH of fatty acid (between 4.5 and 5.5) inhibits bacterial growth and putrefaction.

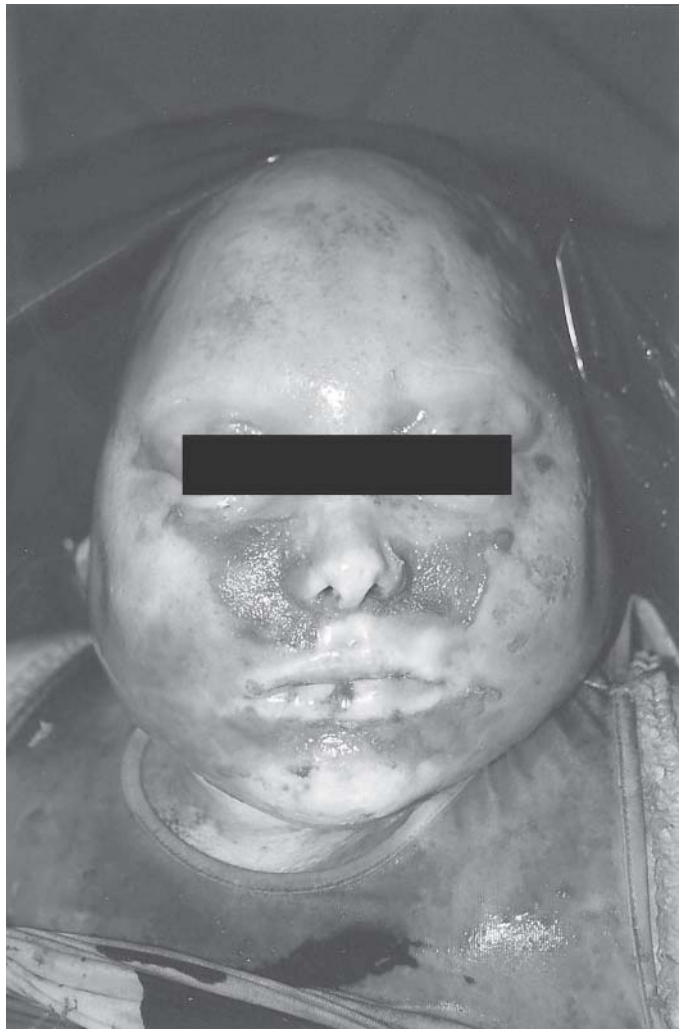


Fig. 15. Body found in water. Putrefactive and emphysematous changes on a young woman's face which hampered her visual identification. The dark grey changes on her cheek near the nose, arranged in a bilateral pattern, represent areas of algal colonization. Postmortem submersion time was 3 weeks.

The optimal temperature for adipocere formation has been related to the growth temperature of *Clostridium perfringens* (>21ºC), which has been suggested to be one of the main sources of enzymes for degradation of neutral fats *(241)*. Adipocere formation, however, also occurs at lower temperature.

Clostridium perfringens produces enzymes active below 21ºC *(247)*, and other bacteria are likely involved in the degradation of neutral fat *(246,248)*.

As to time of adipocere onset, wide variability exists because of climatic differences. Mellen et al. *(244)* performed experiments with human skin and subcutaneous tissues and observed adipocere formation after 2 to 3 months at 16 to 21ºC and after 12 to 18 months at 4 to 5ºC. Different workers in Europe and the United States mention that the minimum time required for adipocere formation is approx 3 months *(249)*, but some case studies show earlier adipocere formation within 3 to 4 weeks *(250–253)*.

When a body floats, tissue destruction may proceed differently in areas exposed to air, infested by terrestrial scavengers, and in those body parts submerged and exposed to marine life. Under specific circumstances, immersed body parts may show adipocere transformation and regions exposed to air may exhibit mummification changes. Marine scavenger organisms (e.g., worms, molluscs, arthropods, echinoderms, crustaceans, fishes) can colonize soft tissues and bones, produce PM artifacts, enhance bacterial penetration, and quicken the course of the body's disarticulation and skeletonization *(254,255)*. Crustaceans, for instance, cause crater-like pits of varying size in the face, small fishes destroy the soft tissues of the face, fingers, and the genital region, and smaller organisms may penetrate the respiratory or digestive tract. Colonization of skin and exposed soft tissues by algae gives them a greenish or blackish discoloration. Algae from specific habitats can be useful to link criminals to a crime scene in forensic investigations *(256)*.

Water environments produce peculiar patterns of a body's disarticulation. Although land provides a firm and static support for articulation, in water, the corpse's movements in three dimensions enhance soft tissue detachment and joint disarticulation. Synovial joints such as the shoulder are disconnected before fibrous joints such as intervertebral ones; limbs disarticulate first distally owing to the higher torsion forces than on those acting proximally *(257)*.

In a cold climate, bones which have been frozen, compressed within ice, ground between block ice and gravel bars, or crushed in ice floes may present with fractures, which should not be misinterpreted as AM trauma *(258)*.

8.3. Antemortem and Postmortem Injuries

The recognition and interpretation of injuries on a body found in water is essential for determining the actual sequence of events that led the victim into the water. Bodies found in water may present with a wide range of AM and PM injuries sustained before submersion, during the fall into the water, impact on the water surface or on the bottom, or while in water. These injuries may provide decisive insight into the cause and manner of death or can be unre-

lated to the actual terminal events. The differentiation between AM and PM injuries sustained before entering or while in water can be challenging and at times impossible. During the early PM interval, for instance, vital lacerations may be difficult to diagnose because bleeding can be washed away, and later advanced decomposition can mask a wider range of vital injuries such as bruises, gunshot or cutting wounds, and internal lesions.

The first group of AM injuries to be considered are injuries sustained before falling into water. These injuries can be the unique cause of death or can contribute to drowning. Indeed, accidental falling into water can be triggered by mechanisms such as air, boating or road traffic accidents, electrocution, or by more trivial injuries during recreational or occupational activities. In suicides special attention must be paid to self-inflicted injuries (e.g., stabbing, cutting, or shooting), which may reveal the mechanism of the victim's coming into water. In cases of homicide virtually all types of traumatic lesions can be inflicted in proximity to a body of water or, in the case of body disposal in water, far away from the water.

The pattern of injuries caused by falling into water, conversely, has no specificity for the manner of death. Whether the victim falls, jumps, or is thrown alive from a dock, bridge or ship, he or she can sustain any kind of injuries by striking fixed objects such as rock, cliffs, or parts of a bridge or boat before entering the water. These injuries may be responsible for death before the victim reaches the water or can contribute to drowning by rendering the victim unconscious or unable to swim once in the water. Injuries caused by impact on the water surface are generally caused by falls from a great height, for example, in suicide by jumping from a high bridge and depending on the velocity of the body, which is directly proportional to the height and to aerodynamic factors. Such injuries include skin lesions, muscle tears, bone fractures, and lacerations of internal organs. The impact of a victim on the bottom of a body of water with shallow water (e.g., swimming pool) is a well-recognized mechanism of severe head and neck injuries, and can lead to drowning by causing loss of consciousness or spinal cord paralysis *(259–261)*.

Once the victim has fallen into the water or while in the water, he or she can sustain further vital injuries by being washed by waves or currents against any material or the bottom, by being struck by a ship or boat (especially in harbors or other settings with high pleasure-boating traffic), or by being attacked by marine predators like sharks. Boat propellers can produce multiple parallel deep incision wounds especially in the head or trunk, amputation, or even dismemberment *(261–263)*. Electrocution (unintentional, self-inflicted, or homicidal) can occur also while the victim is in the water *(264)*. The linear marks of pallor on the water level, which have been interpreted as signs of lethal electric damage, may occur with no electric contact *(265)*.

Importantly, morphological changes to diagnose the cause of death may fade in water. The intensity and surface pattern of ligature marks in water may vanish and a strangulation furrow can disappear totally after exposure to water and treatment with ointments *(266)*. Betz et al. *(267)* showed experimentally that conjunctival petechiae may disappear in freshwater, likely because of hemolysis in a hyposmolar medium.

Different PM lesions and artifacts can be observed in submersed bodies. PM lesions include injuries produced before cadaver disposal (e.g., dismemberment) or by throwing the body into the water, by mechanical actions while the body is in water (e.g., squeezing between two ships, dashing against rocks or drifting along the bottom, propeller injuries; Figs. 16–18) as well as injuries produced during the decomposition process (e.g., by aquatic life depredation, "false" strangulation marks produced by tight clothes during putrefactive swelling of the body, fractures of the skull caused in cold climate by ice), or during search and retrieval procedures.

8.4. Manner of Death

Determination of the manner of death (accident, suicide, homicide, natural) for a body found in water requires a comprehensive approach based on analysis of PM findings, the victim's individual background, and circumstantial factors—a gathering that requires the coordinated action of the forensic pathologist and police investigators.

8.4.1. Unintentional Drowning

Most drowning deaths are unintentional. The wide range of settings and circumstances in which accidental drowning generally occurs (Fig. 19), together with the main individual risk factors, have been addressed in several epidemiological studies *(268)*. Witnessing and exclusion of other manners of death are generally the strongest factors on which the basis for the diagnosis of accidental drowning can be drawn, but are not constantly present *(56)*.

8.4.2. Suicide

Suicide by drowning is much less common than is unintentional drowning. Medicolegal studies on suicide by drowning have focused on littoral regions where rates appear higher than in noncoastal areas *(269,270)*. Byard et al. *(271)* reported the largest coastal series consisting of 123 suicidal drowning victims investigated in Adelaide (Australia) between 1980 and 2000. Wirthwein et al. *(270)* reported 52 suicidal drownings from the noncoastal area of Dallas, Texas, 1977 to 1996. Other studies on suicide by drowning have been performed in Florida *(272,273)*, in Canada *(274)*, and Finland *(275)*. In these

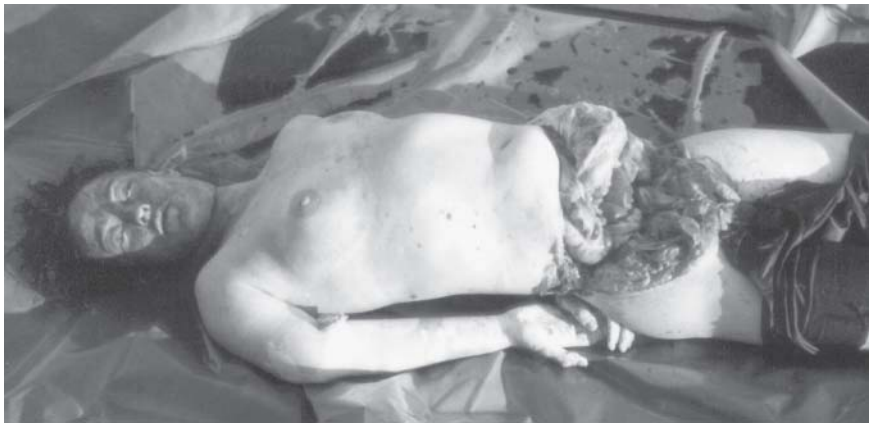


Fig. 16. Body found in water. Transversal postmortem propeller injury of the trunk with partial extrusion of abdominal viscera. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

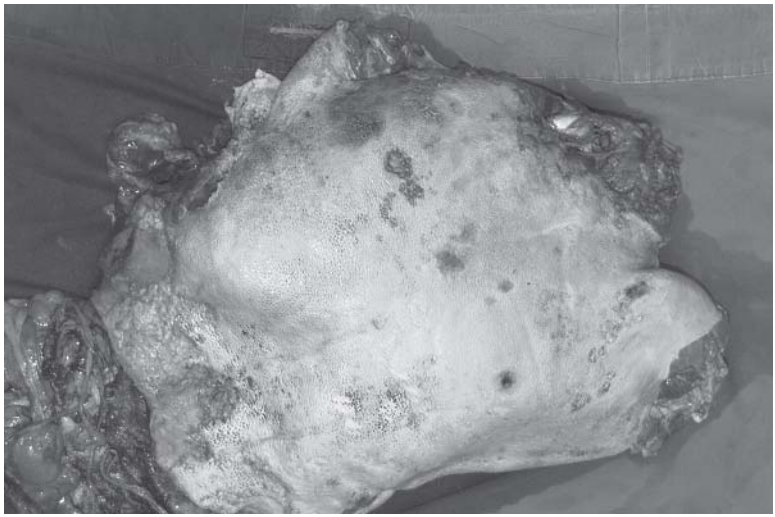


Fig. 17. Body found in water. Postmortem amputation of head and arms by motorboat propeller. Note the irregularity of tissue lacerations compared with the amputation surfaces shown in Fig. 18.

Fig. 18. Disposal of a body in water. Complete "defensive" dismemberment of the victim by the homicide perpetrator. Note the regular amputation surfaces compared with those seen in Fig. 17 and the lack of hemorrhages within the soft tissues.

series the percentage of suicide by drowning ranged from 0.85% *(271)* to 8.9% *(274)* of all suicides.

Some studies have addressed the epidemiological and injury patterns of suicide caused by jumping from high bridges in the United States *(276–279)*, Europe *(280,281)*, and Australia *(282)*. In these suicides, drowning is only one of the possible causes or is a contributing cause of death because death may also be due to trauma sustained before, during, or after the impact with the water surface.

Suicide notes, witnessing, and injuries related to combined suicide are among the more significant factors that may lead to a diagnosis of suicide by drowning. Factors such as previous suicide attempts, suicide ideation, and psychiatric history must be considered judiciously because they can occur also in victims of homicide and unintentional drowning. The percentage of suicides by drowning with farewell notes ranges from approx 14% *(271)* to 37%

Fig. 19. Drowning in a car: accident or suicide?

(270). Copeland *(216)* and Lucas et al. *(272)* have stressed that 25% and 6% of their cases, respectively, involved a verbal equivalent to a suicide note just prior to death. Among the Copeland *(270)* and Wirthwein et al. *(272)* series, 11% of the cases were witnessed whereas in the Li and Smialek *(278)* series of jumping from bridges the percentage was much higher, namely 57%.

Victims of suicidal drowning may present hesitation marks, for example, located on the wrists, and at times more extended wounds related to the combination of drowning with other suicide methods, for example, a deep cut on the wrist, antecubital region, or throat as well as gunshot wounds *(226,271,272)*. Gerling and Pribilla *(283)* have described a unique case of suicide in water by a hand grenade.

At times, suicide perpetrators, before entering the water, weight themselves down to ensure the success of their act by filling their pockets with stones or using heavy objects inside bags or by tightly binding their hands or feet with a rope. Byard et al. *(271)* reported nine such cases (a load of bricks, diving weight, rock, toolbox, dumbbell) and Wirthwein et al. *(270)* four cases (anchors, bucket, concrete block). Giersten *(57)* stressed that putrefactive gases may cause enough buoyancy to cause the body to ascend to the surface even if it is carrying a 25 kg extra-weight. When retrieving a weighted body from water, the differential diagnoses of homicide by drowning or body disposal in water must be appropriately considered: ascertaining the origin of weights

and whether the victim could have applied the weight or the ropes alone are important steps in this differential diagnostic approach.

Single case studies also demonstrate the difficulties that may arise in establishing the diagnosis of suicide by drowning. Schmidt et al. *(284)* reported a bathtub drowning compatible with homicide or suicide, likely associated with epileptic seizures. Nadjem et al. *(285)* described a drowning case in a young male boating with an inflatable on a lake. The man was found dressed in a knight's armor-like chain-mail coat and trousers: both suicide and accident during fantasy play were consistent with the scene and PM investigations. Petri et al. *(286)* reported the case of a diver found in an underwater cave with a knife wound in the thorax and signs of drowning. The death, first interpreted as homicide, after full investigation, was ruled as a suicide committed while running out of air to avoid the agony of drowning. Drowning with apparent suicide features has been described in the context of accidental autoerotic death during sexually oriented rituals under water *(287)*.

8.4.3. Homicide

Homicidal drowning is generally perpetrated by a physically stronger assailant against a weaker victim, generally a child or an incapacitated adult, often in a bathtub or shallow water.

Drowning as a form of fatal child abuse, especially in the bathtub, can be difficult to distinguish from unintentional drowning, sudden infant death syndrome, or other natural death *(289)*. Often, there is little or no evidence of foul play at drowning sites or on the victims' bodies themselves because the pressure required to keep a child under water is generally exerted by fingers and hand on the back of the head and torso and often does not leave any detectable signs of external violence. The diagnosis is thus generally based on characteristic features of child abuse, including physical signs, inconsistency of history, lack of resuscitation attempts, delay in seeking care, and a previous history of abuse *(289,290)*.

Concerning homicidal drowning in adults (Fig. 20), it is generally assumed that these involve situations with a physical or psychological disparity between the perpetrator and the victim. The victims are generally incapacitated by disease or alcohol and drug intoxication or are taken by surprise. Medicolegal textbooks mention the Smith cases where a husband drowned his three wives at different times by pulling their legs up in the air and placing their heads under water, causing no or minimal external violence *(41,58)*. Birkinshaw et al. *(291)* reported the homicide of a woman perpetrated by her husband first by injecting insulin and then by submersion. Gee *(58)* describes two other

Fig. 20. Body found in shallow water. Autopsy showed clear signs of liquid penetration into the lower airways of this male victim and a recent hemorrhage in the right basal ganglia; police investigations suggested also the possibility of a sexual homicide.

examples of adult homicidal drowning with minimal external signs of violence: a young girl thrown into a canal and another pushed into a ditch with the perpetrator holding her face under water. Glass and Robert *(292)* demonstrated the difficulties in proving a homicidal drowning in an 89-year-old woman with dementia found in a river and allegedly pushed in by a relative. Lau *(293)* reported a drowning case in a hotel bathtub that was initially classified as accidental and in which the body was cremated without an autopsy for which a late suspicion of homicide arose in connection with life insurance issues. Homicide by drowning or manslaughter by drowning can be the result of throwing a person into a body of water—only during play or as a joke with subsequent failure to rescue him or her—or throwing someone off of a boat or ship unobserved. Once more the scene investigation will reveal no indication of a fight or foul play and the victims will present no specific injuries.

The vast majority of reported drowning homicides remain those involving additional forms of violence such as strangulation, stabbing, or beating (Fig. 21)*(280,294)*. Missliwetz et al. *(295)* reported six murders of adults by drowning and distinguished those cases with premeditation where signs of injuries are usually missing from those where drowning is the final stage of an assault carried out by other means (e.g., strangulation).

8.4.4. Undetermined

Since the introduction of the International Classification of Diseases-8 (World Health Organization, 1967), drowning can be classified under the category "undetermined" when it is unclear whether it has been unintentional or purposely inflicted. The problem of "undetermined" drowning has only lately received attention in the medical literature. Smith *(296)* stressed how use of the "undetermined" code varies greatly between countries; this can lead to underestimation of unintentional drownings. Lunetta et al. *(297)* described the epidemiological profile of undetermined drowning in South Finland and stressed the factors leading to classification of drowning as undetermined. Various medicolegal reports exemplify the difficulties in determining the manner of death in drowning *(284–286,293,298)*.

8.4.5. Natural Deaths

A victim who has been found in water could have died as a result of sudden natural death. A preexisting disease itself may be responsible for death or may contribute to drowning while the victim is in water, for example, during swimming under the effect of physical exertion or cold, or by causing the victim to fall into the water from a boat or bank. PM investigation of a body found in water must thus not overlook any pathological lesion that may be responsible for death. In these cases, clear signs of drowning can be missing, but in some other cases the victims may also aspirate a significant volume of liquid before death.

Despite the fact that the potential of natural disease to cause death in water has been repeatedly stressed, few data and reports, with the exception of epilepsy *(296)*, are currently available on death in water associated with natural disease. Smith et al. *(299)* described two pediatric drownings, the first in an 8-year-old boy who suffered a subarachnoid hemorrhage caused by a cerebral arteriovenous malformation while swimming and the second in an 11-year-old boy who collapsed in a swimming pool and had marked hypoplasia of the right coronary artery. Schmidt and Madea *(300)* have reported a bathtub drowning in a child caused by severe heart failure resulting from Hurler´s syndrome.

Recently, increased interest has focused on potentially fatal arrhythmias, especially the long-QT syndrome (LQTS), which may be water-triggered and occur during swimming activities *(301–304)*. The LQTS has been indicated as a potentially overlooked cause of "dry lungs" *(25)*. Lunetta et al. (305), however, found in a series of 165 putative drowning only one victim carrying a LQTS founder mutation.

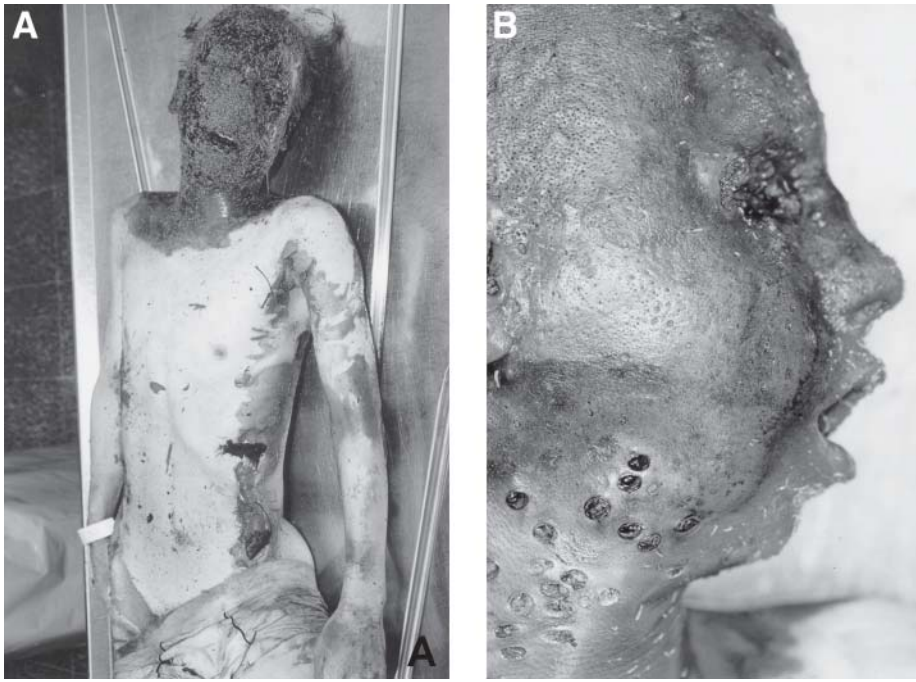


Fig. 21. Body found in shallow water. The young male victim's head and neck were submerged in a ditch and the corresponding regions showed advanced putrefaction and extensive fly maggots infestation (**A**,**B**). Police and medicolegal investigations ascertained the victim was killed by multiple stab wounds inflicted by a screw to the neck in combination with forceful head immersion in the ditch.

8.5. Disposal of Corpses in Water

Disposal of corpses in a water setting (Fig. 22) may reflect the following three aims:

1. Concealing the body with the expectation that it will remain under water or will be transported far away from the scene of crime;
2. Preventing or retarding the identification of the victim and of inflicted injuries because of artefacts and advanced PM changes;
3. Simulation of natural, accidental, or suicidal death in water.

The disposal of a victim in water after a crime perpetrated near a body of water does not require complex action and does not necessarily imply pre-

meditation. Conversely, when the murder is perpetrated far away from the site of concealment, disposal requires elaborate actions such as the body being weighted, hidden in a sack or other container, or even dismemberment.

The following examples of cadaver disposal in water stress how the prolonged interval between crime and body retrieval can hamper victim identification and obscure time of death as well as cause and manner of death. Schneider et al. *(306)* reported the examination of two legs and two arms belonging to the same person that were recovered at different times from water and showed signs of criminal "defensive" dismemberment and injuries from a ship's propellers. Dix *(252)* described four bodies disposed of in Missouri lakes and submerged between 3 weeks and 10 months. The bodies were discovered weighted down with concrete blocks (two cases), by a 34 kg barbell weight and by a combination of a cement anchor, tire wheel, and barbecue grill. In only two cases the cause of death could be determined (strangulation, blunt injury to head) and in only one was the assailant identified. Schumann et al. *(307)* described the case of a 32-year-old prison inmate whose body was retrieved embedded in a concrete block and a metal drum submerged in a river. The victim had been killed 1 year before by the blow of a forked crowbar to his head. Lew et al. *(308)* described the disposal of a male body for more than 15 years in a domestic septic tank after murder by multiple gunshot wounds. Rajs et al. *(309)* studied 22 cases of criminal body mutilation occurring in Sweden between 1961 and 1990 and found that in 4 out of 10 defensive dismemberments the body parts were dumped into the sea. Pollanen *(294)* reported two homicidal drownings with disposal of bodies on land, whereas Fanton et al. *(310)* described the case of a woman disposed of by her husband in a bathtub to simulate a natural death after a homicidal drowning in a marsh.

In certain regions of the world the possibility of burial at sea, for example, disposition of human remains in an aquatic environment, must be considered when investigating a body found in water. In the United States, most (90–95%) burials at sea are cremated remains, but the remaining 5 to 10%, accounting for approx 1000 cases per year, involve legal whole-body burial *(311)*.

8.6. Bathtub Deaths

Deaths in bathtubs frequently are encountered in medicolegal practice (Fig. 23). In addition to drowning, a wide range of causes of death must be considered. These include electrocution, drug or carbon monoxide intoxication, sharp or blunt violence, and strangulation as a consequence of accidents, suicides, or homicides *(213,312–317)*. Furthermore, natural deaths can occur in the bathtub, particularly as a consequence of epileptic seizure or cardiac

Fig. 22. Disposal of body. **(A,B)** A large bag that contained the corpse of a middle-aged woman was filled with stones and was submersed in the sea after a homicide by firearm (same case as Fig. 14).

attack *(316–318)*. In deaths other than drowning, the victim may aspirate little or a variable volume of water during the terminal events *(228,318)*. Geertinger and Voigt (1970) and Spitz (1973) believe that bathtub drowning occurs only when unconsciousness or weakened consciousness is brought about by a disease or by alcohol and drugs *(41,312)*.

Studies on bathtub deaths also have focused on children. Trübner and Püschel *(318)* have reported in Hamburg, Germany, 1971 to 1988, 24 out of

Fig. 23. Deceased found in bathtub: suicide by multiple stab wounds in a young male. At the time of the recovery of the body, the bathtub was partially filled with bloody water; autopsy revealed no signs of liquid inhalation in this subject.

245 bathtub deaths involving children or adolescents. Of these, 20 were accidents (of which 3 were drownings), 2 homicides (1 drowning, 1 strangulation), 1 natural death (seizure) and 1 undetermined. Schmidt and Madea *(300)* analyzed 12 bathtub deaths in children 9 months to 13 years old and found 7 accidents (of which 5 were drownings), 1 homicide (by stabbing), 2 natural deaths (epilepsy, Hurler's syndrome), and 2 undetermined deaths (seizure, subdural hematoma).

Medicolegal studies on bathtub deaths in adults also include homicide. In addition to drowning, homicides by strangulation, sharp instruments, and blunt force also have been reported *(213,318–320)*, as well as disposal of bodies in bathtubs aiming to disguise a homicide, to clean and remove the traces of violence, or to ensure death *(213,300)*. Bathtub homicide in adults can show a clear injury pattern or contrarily reveal only subtle changes. Schmidt and Madea *(300)* described 11 homicides in bathtubs. In 8 of the 11 cases, multiple injuries led to a strong suspicion of homicide, whereas in the remaining 3 cases the scene findings were subtle. Drowning was the cause of death in only 2 of these authors' cases.

9. *Conclusion*

We have presented a comprehensive review of factors and findings that have been reported to be compatible with, but not necessarily diagnostic of, death by drowning. At present, no single morphological or laboratory marker that conclusively identifies drowning as the cause of death and excludes all other possible causes or contributing factors does in fact exist. Research studies in various areas pertaining to such a classical forensic pathological topic as the diagnosis of cause of death in water once more need to be strengthened and implemented.

References

1. Sung Tz'u (1981) The washing away of wrongs (translated by McKnight BE). In Nathan Sivin, ed., Science, medicine, and technology in East Asia, Vol. 1. Center for Chinese Studies, The University of Michigan, Ann Arbor, pp. 114–120.
2. Paré A (1575) Les oevvres de M. Ambroife Paré. G. Baon, Paris, p. 1099.
3. Fidelis F (1602) De relationibus medicorum. A. de Francifcis, Panormi, p. 336.
4. de Castro R (1614) Medicus-politicus sive de oficiis medico-politicis tractatus Hamburgi, Ex Bibliopolio Frobeniano, p. 259.
5. Plateri F (1614) Observationum. Impensis Lvdovici König, Typis Conradi Waldkirchii, pp. 169–171.
6. Plateri F (1625) Questionum medicarum paradoxarum et endoxarum. Impensis Lvdovici Regis, Basileæ, pp. 144–145.
7. Zacchia P (1726) Quaestionum medico-legalium. Editio nova. Cura Joannis Danielis Horstii. Anisso & Poseul, Lugduni, pp. 394–395.
8. Bohn J (1721) De Renunciatione vulnerum seu vulnerum lethalium examen. T. Fritsch, Lipsiae, pp. 192–198.
9. Valentini MB (1722) Corpus juris medico-legale. Francof. ad Moen.
10. Swann HG, Brucer M, Moore C, Vezien BL (1947) Fresh water and sea water drowning: a study of the terminal cardiac and biochemical events. Tex Rep Biol Med 5, 423–437.
11. Swann HG, Brucer M (1949) The cardiorespiratory and biochemical events during rapid anoxic death. VI. Fresh water and sea water drowning. Tex Rep Biol Med 7, 604–618.
12. Swann HG, Spafford NR (1951) Body salt and water changes during fresh and sea water drowning. Tex Rep Biol Med 9, 356–362.
13. Modell JH, Gaub M, Moya F, Vestal B, Swarz H (1966) Physiologic effects of near drowning with chlorinated fresh water, distilled water and isotonic saline. Anesthesiology 27, 33–41.
14. Modell JH, Davis JH (1969) Electrolyte changes in human drowning victims. Anesthesiology 30, 414–420.
15. Modell JH, Graves SA, Ketover A (1976) Clinical course of 91 consecutive near-drowning victims. Chest 70, 231–238.

16. Zuckerman GB, Conway EE Jr (2000) Drowning and near drowning: a pediatric epidemic. Pediatr Ann 29, 360–366.
17. Ibsen LM, Koch T (2002) Submersion and asphyxial injury. Crit Care Med 30, Suppl, S402–S408.
18. Modell JH, Moya F, Williams HD, Weibley TC (1968) Changes in blood gases and A-aDO_2 during near-drowning. Anesthesiology 29, 456-465.
19. Orlowski JP, Szpilman D (2001) Drowning. Rescue, resuscitation, and reanimation. Pediatr Clin North Am 48, 627–646.
20. Yagil Y, Stalnikowicz R, Michaeli J, Mogle P (1985) Near drowning in the dead sea. Electrolyte imbalances and therapeutic implications. Arch Intern Med 145, 50–53.
21. Fromm RE Jr (1991) Hypercalcemia complicating an industrial near-drowning. Ann Emerg Med 20, 669–671.
22. Idris AH, Berg RA, Bierens J, et al. (2003) Recommended guidelines for uniform reporting of data from drowning: the "Utstein style." Circulation 108, 2565–2574.
23. Craig AB Jr (1961) Causes of loss of consciousness during underwater swimming. J Appl Physiol 16, 583–586.
24. Orlowski JP (1987) Drowning, near-drowning and ice-water submersion. Pediatr Clin North Am 34, 75–92.
25. Modell JH, Bellefleur M, Davis JH (1999) Drowning without aspiration: is this an appropriate diagnosis? J Forensic Sci 44, 1119–1123.
26. Giammona ST, Modell JH (1967) Drowning by total immersion. Effects on pulmonary surfactant of distilled water, isotonic saline, and sea water. Am J Dis Child 114, 612–616.
27. Orlowski JP, Abulleil MM, Phillips JM (1987) Effects of tonicities of saline solutions on pulmonary injury in drowning. Crit Care Med 15, 126–130.
28. Modell JH, Moya F, Newby EJ, Ruiz BC, Showers AV (1967) The effects of fluid volume in sea water drowning. Ann Intern Med 67, 68–80.
29. Modell JH, Calderwood HW, Ruiz BC, Downs JB, Chapman R Jr (1974) Effects of ventilatory patterns on arterial oxygenation after near-drowning in sea water. Anesthesiology 40, 376–384.
30. Modell JH, Moya F (1966) Effects of volume of aspirated fluid during chlorinated fresh water drowning. Anesthesiology 27, 662–672.
31. Modell JH (1971) The pathophysiology and treatment of drowning and near-drowning. Charles C. Thomas, Springfield, pp. 34–40.
32. Tabeling BB, Modell JH (1983) Fluid administration increases oxygen delivery during continuous positive pressure ventilation after freshwater near-drowning. Crit Care Med 11, 693–696.
33. Levin DL, Morriss FC, Toro LO, Brink LW, Turner GR (1993) Drowning and near-drowning. Pediatr Clin North Am 40, 321–336
34. Ellison D, Love S, Chimelli L, Harding BN, Lowe J, Vinters HV (2004) Neuropathology. A Reference Text of CNS Pathology, 2nd ed. Mosby, Edinburgh, pp. 163–171.
35. Modell JH, Idris AH, Pineda JA, Silverstein JH (2004) Survival after prolonged submersion in freshwater in Florida. Chest 125, 1948–1951.

36. Bohn D (1999) Drowning in a hypothermic environment. In Fletemeyer JR, Freas SJ, eds., Drowning. New Perspectives on Interventions and Preventions. CRC Press, Boca Raton, pp. 59–86.
37. Modell JH, Keck EJ, Ruiz BC, Heinitsh H (1972) Effect of intravenous vs aspirated distilled water on serum electrolytes and blood gas tensions. J Appl Physiol 32, 579–584.
38. Cot C (1931) Les asphyxies accidentelles (submersion, electrocution, intoxication oxycarbonique). Etude clinique, thérapeutique et preventive. Editions medicales N. Maloine, Paris.
39. Moritz AR (1944) Chemical methods for the determination of death by drowning. Physiol Rev 24, 70–88.
40. Swann HG (1962) Resuscitation in semi-drowning. In Whittenberger J, ed., Artificial respiration: therapy and application. Harper and Row, New York, pp. 202–224.
41. Spitz WU (1973) Drowning. In Spitz, ed. Medico-legal investigations of death, 3rd ed. Charles C. Thomas Publisher, Springfield, pp. 296–310.
42. Saukko P, Knight B (2004). Knight's Forensic Pathology. Arnold, London.
43. Brinkmann (2004) Tod im Wasser. In Brinkmann B, Madea B, eds., Handbuch Gerichtliche Medizin. Springer, Berlin, pp. 797–824.
44. Lunetta P, Modell JH, Sajantila A (2004) What is the incidence and significance of 'dry-lungs' in bodies found in water? Am J Forensic Med Pathol, 25, 291–301.
45. Rex MAE (1970) A review of the structural and functional basis of laryngospasm and a discussion of the nerve pathways involved in the reflex and its clinical significance in man and animals. Br J Anaesth 42, 891–899.
46. Widdicombe J (1998) Upper airway reflexes. Curr Opin Pulmon Med 4, 376–382.
47. Nishino T (2000) Physiological and pathophysiological implications of upper airway reflexes in humans. Jpn J Physiol 50, 3–14.
48. Longheed DW, Janes JM, Hall GE (1939) Physiological studies in experimental asphyxia and drowning. Can Med Assoc J 40, 423–428.
49. Mackowiak PA, Wasserman SS, Levine MM (1992) A critical appraisal of 98.6 degrees F, the upper limit of the normal body temperature, and other legacies of Carl Reinhold August Wunderlich. JAMA 268, 1578–1580.
50. Morris RJ, Schoenwetter WF (1993) Drowning. N Engl J Med 329, 64–65.
51. Modell J (1999) Etiology and treatment of drowning. In Fletemeyer JR, Freas SJ, eds., Drowning. New perspectives on interventions and preventions. CRC Press, Boca Raton, pp. 21–30.
52. Biggart MJ, Bohn DJ (1990) Effect of hypothermia and cardiac arrest on outcome of near-drowning accidents in children. J Pediatr 117, 179–183.
53. Siebke H, Rod T, Breivik H, Link B (1975) Survival after 40 minutes; submersion without cerebral sequelae. Lancet 1(7919), 1275–1277.
54. Nugent SK, Rogers MC (1980) Resuscitation and intensive care monitoring following immersion hypothermia. J Trauma 20, 814–815.
55. Betz P, Nerlich A, Penning R, Eisenmenger W (1993) Alveolar macrophages and the diagnosis of drowning. Forensic Sci Int 62, 217–224.

56. Lunetta P, Penttila A, Sajantila A (2002) Circumstances and macropathologic findings in 1590 consecutive cases of bodies found in water. Am J Forensic Med Pathol 23, 371–376.
57. Giertsen JC (1977) Drowning. In Tedeschi CG, Eckert WG, Tedeschi LG, eds., Forensic Medicine, vol. III. WB Saunders, Philadelphia, pp. 1317–1333.
58. Gee DJ (1985) Drowning. In Polson CJ, Gee DJ, Knight B, eds., The Essentials of Forensic Medicine, 4th ed. Pergamon Press, Oxford, pp. 421–448.
59. Whimster WF, MacFarlane AJ (1974) Normal lung weights in a white population. Am Rev Respir Dis 110, 478–483.
60. Joachim H, Riede UN, Mittermayer CH (1978) The weight of human lungs as a diagnostic criterium. Pathol Res Pract 162, 24–40.
61. Williams PL, Warwick R (1980) Gray's anatomy, 36th ed. Churchill Livingstone, London.
62. Cotran RS, Kumar V, Robbins SL (1989) Robbins pathologic basis of disease, 4th ed. W.B. Saunders Co., Philadelphia.
63. Murray JF, Nadel JA. Textbook of respiratory medicine. WB Saunders Company, Philadelphia, 1988, p. 13.
64. De la Grandmaison GL, Isabelle Clairand I, Durigon M (2001) Organ weight in 684 adult autopsies: new tables for a Caucasoid population. Forensic Sci Int 119, 149–154.
65. Copeland AR (1985) An assessment of lung weights in drowning cases. The Metro Dade County experience from 1978 to 1982. Am J Forensic Med Pathol 6, 301–304.
66. Kringsholm B, Filskov A, Kock K (1991) Autopsied cases of drowning in Denmark 1987–1989. Forensic Sci Int 52, 85–92.
67. Zhu BL, Quan L, Li DR, et al. (2003) Postmortem lung weight in drownings: a comparison with acute asphyxiation and cardiac death. Leg Med (Tokyo) 5, 20–26.
68. Di Maio DJ, Di Maio VJM (1993) Forensic Pathology. CRC Press, Boca Raton.
69. Morild I (1995) Pleural effusion in drowning. Am J Forensic Med Pathol 16, 253–256.
70. Reh H (1963) [Comparative animal experimental studies of the drowned lung] Acta Med Leg Soc 16, 61–67 [in German].
71. Reh H (1963) [On the specificity of the so-called drowning lung]. Dtsch Z ges Gerichtl Med 54, 45–48 [in German].
72. Terazawa K, Haga K (1996) The role of pleural effusion in drowning. Am J Forensic Med Pathol 17, 173–174.
73. Yorulmaz C, Arican N, Afacan I, Dokgoz H, Asirdizer M (2003) Pleural effusion in bodies recovered from water. Forensic Sci Int 136, 16–21.
74. Niles NR (1963) Hemorrhage in the middle-ear and mastoid in drowning. Am J Clin Pathol 40, 281–283.
75. Babin RW, Graves NN, Rose EF (1982) Temporal bone pathology in drowning. Am J Otol 3, 168–173.
76. Robbins RD, Sekhar HK, Siverls V (1988) Temporal bone histopathologic findings in drowning victims. Arch Otolaryngol Head Neck Surg 114, 1020–1023.

77. Kelemen G (1983) Temporal bone findings in cases of salt water drowning. Ann Otol Rhinol Laryngol 92, 134–136.
78. Liu C, Babin RW (1984) A histological comparison of the temporal bone in strangulation and drowning. J Otolaryngol 13, 44–46.
79. Kaga K, Nitou T, Suzuki JI, Tsuzuku T (1999) Temporal bone pathology findings due to drowning. Rev Laryngol Otol Rhinol (Bord) 120, 27–29.
80. Haarhoff K, Weiler G (1971) [The unspecificity of petrous bone hemorrhages]. Z Rechtsmed 69, 62–64 [in German].
81. Ito Y, Kimura H (1990) Histological examination of the temporal bone in medicolegal cases of asphyxia. Forensic Sci Int 44, 135–142.
82. Reh H (1970) Diagnostik des Ertrinkungstodes und Bestimmung der Wasserzeit. Mikael Triltsch Verlag, Düsseldorf.
83. Hottmar P (1996) [Detection of fluid in paranasal sinuses as a possible diagnostic sign of death by drowning]. Arch Kriminol 198, 89–94 [in German].
84. Bohnert M, Ropohl D, Pollak S (2002) [Forensic medicine significance of the fluid content of the sphenoid sinuses]. Arch Kriminol 205, 158–164 [in German].
85. Haffner HAT, Graw M, Erdelkamp J (1994) Spleen findings in drowning. Forensic Sci Int 66, 95–104.
86. Hadley JA, Fowler DR (2003) Organ weight effects of drowning and asphyxiation on the lungs, liver, brain, heart, kidneys, and spleen. Forensic Sci Int 133, 190–196.
87. Carter N, Ali F, Green MA (1998) Problems in the interpretation of hemorrhage into neck musculature in cases of drowning. Am J Forensic Med Pathol 19, 223–225.
88. Püschel K, Schulz F, Darrmann I, Tsokos M (1999) Macromorphology and histology of intramuscular hemorrhages in cases of drowning. Int J Legal Med 112, 101–106.
89. Sigrist T, Germann U (1989) [Homicide by asphyxia—yes or no? On the use of muscle histology]. Z Rechtsmed 102, 549–557 [in German].
90. Fagerlund LW (1888) Om drunknoingsvätskas inträngande i tarmarna. Helsingfors.
91. Heinen M, Dotzauer G (1973) [The lung of a drowned person] Beitr Gerichtl Med 30, 133–141 [in German].
92. Janssen W (1984) Forensic Histopathology. Springer, Berlin.
93. Wright JR (2003) Pulmonary surfactant: a front line of lung host defense. J Clin Invest 111, 1453–1455.
94. Goerke J (1998) Pulmonary surfactant: functions and molecular composition. Biochim Biophys Acta 1408, 79–89.
95. Veldhuizen R, Nag K, Orgeig S, Possmayer F (1998) The role of lipids in pulmonary surfactant. Biochim Biophys Acta 1408, 90–108.
96. Haagsman HP, Diemel RV (2001) Surfactant-associated proteins: functions and structural variation. Comp Biochem Physiol A Mol Integr Physiol 129, 91–108.
97. Lorente JA, Hernandez-Cueto C, Villanueva E, Luna JD (1990) The usefulness of lung surfactant phospholipids (LSPs) in the diagnosis of drowning. J Forensic Sci 35, 1367–1372.
98. Lorente JA, Lorente M, Villanueva E (1992) Postmortem stability of lung surfactant phospholipids. J Forensic Sci 37, 1341–1345.

99. Zhu BL, Ishida K, Quan L, Fujita MQ, Maeda H (2000) Immunohistochemistry of pulmonary surfactant apoprotein A in forensic autopsy: reassessment in relation to the causes of death. Forensic Sci Int 113, 193–197.
100. Zhu BL, Ishida K, Quan L, et al. (2002) Pulmonary immunohistochemistry and serum levels of a surfactant-associated protein A in fatal drowning. Legal Med (Tokyo) 4, 1–6.
101. Ishida K, Zhu BL, Maeda H (2002) A quantitative RT-PCR assay of surfactant-associated protein A1 and A2 mRNA transcripts as a diagnostic tool for acute asphyxial death. Legal Med (Tokyo) 4, 7–12.
102. Maeda H, Fujita MQ, Zhu BL, et al. (2003) Pulmonary surfactant-associated protein A as a marker of respiratory distress in forensic pathology: assessment of the immunohistochemical and biochemical findings. Legal Med (Tokyo) 5, Suppl 1: S318–S321.
103. Brinkmann B, Hernandez MA, Karger B, Ortmann C (1997) Pulmonary myelomonocyte subtypes in drowning and other causes of death. Int J Legal Med 110, 295–298.
104. Kärkolä K, Neittaanmäki H (1981) Diagnosis of drowning by investigation of left heart blood. Forensic Sci Int 18, 149–153.
105. Reiter C (1984) [Proof of death by drowning by smoker cells washed into the left heart blood]. Z Rechtsmed 93, 79–88 [in German].
106. Gotohda T, Kubo S, Kitamura O, et al. (2000) HSP70 and c-Fos expression of brain stem hypoglossal nucleus in drowning. J Med Invest 47, 76–79.
107. Quan L, Zhu BL, Ishida K, et al. (2001) Intranuclear ubiquitin immunoreactivity of the pigmented neurons of the substantia nigra in fatal acute mechanical asphyxiation and drowning. Int J Legal Med 115, 6–11.
108. Tschanz SA, Burri PH (2002) A new approach to detect structural differences in lung parenchyma using digital image analysis. Exp Lung Res 28, 457–471.
109. Robbesom AA, Versteeg EM, Veerkamp JH, et al. (2003) Morphological quantification of emphysema in small human lung specimens: comparison of methods and relation with clinical data. Mod Pathol 16, 1–7.
110. Fornes P, Pepin G, Heudes D, Lecomte D (1998) Diagnosis of drowning by combined computer-assisted histomorphometry of lungs with blood strontium determination. J Forensic Sci 43, 772–776.
111. Kohlhase C, Maxeiner H (2003) Morphometric investigation of emphysema aquosum in the elderly. Forensic Sci Int 134, 93–98.
112. Schneider V (1972) [Scanning electron microscopy of drowned lung]. Beitr Gerichtl Med 29, 266–274 [in German].
113. Böhm E (1973) [Scanning electron microscopy studies on pulmonary alveoli—demonstration on the example of lungs after drowning]. Beitr Gerichtl Med 30, 24–29 [in German].
114. Torre C, Varetto L, Tappi E (1983) Scanning electron microscopic ultrastructural alterations of the pulmonary alveolus in experimental drowning. J Forensic Sci 28, 1008–1012.
115. Torre C, Varetto L (1985) Scanning electron microscope study of the lung in drowning. J Forensic Sci 30, 456–461.

116. Montaldo S (1966) [Ultrastructural findings in experimental asphyxia by drowning. I. Pulmonary findings]. Minerva Medicoleg 86, 194–200 [in Italian].
117. Reidbord HE, Spitz WU (1966) Ultrastructural alterations in rat lungs. Changes after intratracheal perfusion with freshwater and seawater. Arch Pathol 81, 103–111.
118. Nopanitaya W, Gambill TG, Brinkhous KM (1974) Fresh water drowning. Pulmonary ultrastructure and systemic fibrinolysis. Arch Pathol 98, 361–366.
119. Brinkmann B, Butenuth W (1982) [Histology and ultrastructural pathology of the lung in experimental drowning]. Beitr Gerichtl Med 40, 95–98 [in German].
120. Brinkmann B, Fechner G, Püschel K (1983) Ultrastructural pathology of the alveolar apparatus in experimental drowning. Z Rechtsmed 91, 47–60.
121. Püschel K, Fechner G, Brinkmann B (1983) [Ultrastructure pathology of the lung following drowning in humans]. Beitr Gerichtl Med 41, 309–314 [in German].
122. Qu Y (1997) [Observation on postmortem tracheal cilia of drowning by scanning electron microscope (SEM)]. Fa Yi Xue Za Zhi 13, 140, 144, 190 inside back cover [in Chinese].
123. Bajanowski T, Brinkmann B, Stefanec AM, Barckhaus RH, Fechner G (1998) Detection and analysis of tracers in experimental drowning. Int J Legal Med 111, 57–61.
124. Gettler AO (1921) A method for the determination of death by drowning. JAMA 77, 1650–1652.
125. Paltauf A (1888) Über den Tod durch Ertrinken nach Studien an Menschen und Thieren. Urban u Schwarzenberg, Wien, Leipzig.
126. Brouardel P (1897) La pendaison, la strangulation, la suffocation, la submersion. Baillière et fils, Paris, pp. 419–578.
127. Carrara M (1902) Untersuchungen über den osmotischen Druck und die specifische elektrische Leitfähigkeit des Blutes bei der Fäulnis. Vjschr Gerichtl Med 24, 236–244.
128. Stockis E (1909) [Recherches sur le diagnostic medico-legal de la mort par submersion]. Ann Soc Med Leg Belg 20, 71–220.
129. Schwär TG (1972) Drowning—its chemical diagnosis: a review. Forensic Sci 1, 411–417.
130. Durlacher SH, Freimuth HC, Swan HE Jr (1953) Blood changes in man following death due to drowning, with comments on tests for drowning. Arch Pathol 56, 454–461.
131. Jeanmonod R, Staub C, Mermillod B (1992) The reliability of cardiac haemodilution as a diagnostic test of drowning. Forensic Sci Int 52, 171–180.
132. Fisher IL (1967) Chloride determination of heart blood. Its use for the identification of death caused by drowning. A short review of 202 cases in the last 7 years with reference to a few special cases. J Forensic Med 14, 108–112.
133. Faroughi E (1971) Serum changes in drowning. J Forensic Sci 16, 269–273.
134. Rammer L, Gerdin B (1976) Dilution of blood in fresh water drowning. Postmortem determination of osmolarity and electrolytes in blood, cerebrospinal fluid and vitreous humor. Forensic Sci 8, 229–234.
135. Icard S (1932) [La preuve de la mort par submersion suivant qu'elle a eu lieu dans une riviere ou dansl la mer]. Rev Path Comp Hyg Gen 32, 559–571 [in French].

136. Jetter WW, Moritz AR (1943) Changes in the magnesium and chloride contents of blood from drowning in fresh and sea water. Arch Pathol 35, 601–610.
137. Kot PA (1974) Drowning and near-drowning. Am Fam Phys 10, 72–76.
138. Coutselenis A, Boukis D (1976) The estimation of Mg2+ concentration in cerebrospinal fluid (C.S.F.) as a method of drowning diagnosis in seawater. Forensic Sci 7, 109–111.
139. Adjutantis G, Coutselinis A (1974) Changes in magnesium concentration of the vitreous humour of exenterated human eyeballs immersed in sea water. Forensic Sci 4, 63–65.
140. Sturner WQ, Balko A, Sullivan J (1976) Magnesium and other electrolytes in bovine eyeballs immersed in sea water and other fluids. Forensic Sci 8, 139–150.
141. Chardez D, Lambert J (1985) [Ciliated protozoa and thanatology]. Forensic Sci Int 28, 83–101 [in French].
142. Mishul'skii AM (1990) [The use of bacteriological analysis of the blood in the diagnosis of death by drowning]. Sud Med Ekspert 33, 26–28 [in Russian].
143. Lehmann K, Beuthin A (1971) [The detection of calciumlignosulfonate in blood and press-fluids of organs from drowned rats as evidence of death from drowning]. Z Rechtsmed 68, 11–16 [in German].
144. Chen YC, Deng ZK, Zhu JZ (1990) The significance of detecting serum fluorine level in the diagnosis of drowning. Forensic Sci Int 46, 289–294.
145. Mukaida M, Kimura H, Takada Y (1998) Detection of bathsalts in the lungs of a baby drowned in a bathtub: a case report. Forensic Sci Int 93, 5–11.
146. Piette M, Timperman J, Parisis N (1989) Serum strontium estimation as a medicolegal diagnostic indicator of drowning. Med Sci Law 29, 162–171.
147. Azparren J, de la Rosa I, Sancho M (1994) Biventricular measurement of blood strontium in real cases of drowning. Forensic Sci Int 69, 139–148.
148. Azparren JE, Vallejo G, Reyes E, Herranz A, Sancho M (1998) Study of the diagnostic value of strontium, chloride, haemoglobin and diatoms in immersion cases. Forensic Sci Int 91, 123–132.
149. Azparren JE, Ortega A, Bueno H, Andreu M (2000) Blood strontium concentration related to the length of the agonal period in seawater drowning cases. Forensic Sci Int 108, 51–60.
150. Louis A (1752) "Lettres sur la certitude des signes de la mort." Lambert M, Paris, pp. 221–299.
151. Holden HS, Crosfill JWL (1955) The significance of foreign bodies in the alveoli of apparently drowned. J Forensic Med 2, 141–150.
152. Funao T, Watanabe H, Yanagida J (1964) Experimental studies on the pigment penetration in a body during drowning. Jap J Legal Med 18, 238–242.
153. Recine H, Falchi Q (1964) Ricerche sperimentali sulla distribuzione dei liquidi radioopachi nell´annegamento rapido e lento. Minerva Medicoleg 84, 168–172.
154. Terazawa K, Takatori T (1979) On distribution of tritiated drowning water in mouse body. Hokkaido J Med Sci 54, 211–213.
155. Pierucci G (1964) Sulla diffusione nel sangue del liquido annegante, ricerche sperimentali mediante impiego di isotopi radioattivi. Minerva Medicoleg 84, 213–214.

156. Fechner G, Püschel K, Brinkmann B (1983) [Tracer studies in experimental drowning]. Beitr Z Gerichtl Med 16, 303–307 [in German].
157. Revenstorf V (1904) Der Nachweis der aspirierten Ertränkungsflüssigkeit als Kriterium des Todes durch Ertrinken. Vjschr Gerichtl Med 27, 274–299.
158. Ludes B, Coste M (1996) Diatomées et médecine légale. Technique et Documentation, Lavoisier, Paris.
159. Pollanen MS (1998) Forensic diatomology and drowning. Elsevier, Amsterdam.
160. Round FE, Crawford RM, Mann DG (1990) The Diatoms. Biology and Morphology of the Genera. Cambridge University Press, Cambridge.
161. Hurlimann J, Feer P, Elber F, Niederberger K, Dirnhofer R, Wyler D (2000) Diatom detection in the diagnosis of death by drowning. Int J Legal Med 114, 6–14.
162. Auer A (1991) Qualitative diatom analysis as a tool to diagnose drowning. Am J Forensic Med Pathol 12, 213–218.
163. Fukui Y, Hata M, Takahashi S, Matsubara K (1980) A new method for detecting diatoms in human organs. Forensic Sci Int 16, 67–74.
164. Sidari L, Di Nunno N, Costantinides F, Melato M (1999) Diatom test with Soluene-350 to diagnose drowning in sea water. Forensic Sci Int 103, 61–65.
165. Kobayashi M, Yamada Y, Zhang WD, Itakura Y, Nagao M, Takatori T (1993) Novel detection of plankton from lung tissue by enzymatic digestion method. Forensic Sci Int 60, 81–90.
166. Ludes B, Quantin S, Coste M, Mangin P (1994) Application of a simple enzymatic digestion method for diatom detection in the diagnosis of drowning in putrified corpses by diatom analysis. Int J Legal Med 107, 37–41.
167. Kane M, Fukunaga T, Maeda H, Nishi K (1996) The detection of picoplankton 16S rDNA in cases of drowning. Int J Legal Med 108, 323–326.
168. Abe S, Suto M, Nakamura H, et al. (2003) A novel PCR method for identifying plankton in cases of death by drowning. Med Sci Law 43, 23–30.
169. Suto M, Abe S, Nakamura H, et al. (2003) Phytoplankton gene detection in drowned rabbits. Leg Med (Tokyo) 5, Suppl 1, S142–S144.
170. Weinig E, Pfanz H (1951) [Diagnosis of death by drowning through demonstration of diatoms in optically negative tissue sections.] Dtsch Z ges Gerichtl Med 40, 664–668 [in German].
171. Jääskeläinen AJ (1967) [Diatom findings in bodies, found in water. A new method for quantative measurement of diatoms in the body]. Dtsch Z ges Gerichtl Med 61, 41–47 [in German].
172. Fallani M (1964) [The demonstration and significance of the presence of “algae” in the blood of the drowned]. Minerva Medicoleg 84, 131–135 [in Italian].
173. Qu J, Wang E (1992) A study on the diagnosis of drowning by examination of lung chlorophyll(a) of planktons with a spectrofluorophotometer. Forensic Sci Int 53, 149–155.
174. Möttönen M, Ravanko O (1971) [Foreign plant elements in the blood as evidence of death through drowning]. Z Rechtsmed 68, 261–266 [in German].
175. Yoshioka N, Takahashi K (1986) Determination of diatoms in heart blood. Res Pract Forens Med 29, 57–61.

176. Funayama M, Aoki Y, Sebetan IM, Sagisaka K (1987) Detection of diatoms in blood by a combination of membrane filtering and chemical digestion. Forensic Sci Int 34, 175–182.
177. Timperman J (1969) Medico-legal problems in death by drowning. Its diagnosis by the diatom method. A study based on investigations carried out in Ghent over a period of 10 years. J Forensic Med 16, 45–75.
178. Schellmann B, Sperl W (1979) [Detection of diatoms in bone marrow (femur) of nondrowned]. Z Rechtsmed 83, 319–324 [in German].
179. Tomonaga T (1960) Identification of death by drowning by the disorganization method (diatom method). Acta med Nagasaki 5, 116–125.
180. Nanikawa R, Kotoku S (1974) Medicolegal observation on a dead body drawn up from the sea bed, with special reference to ethanol and diatoms. Forensic Sci 3, 225–232.
181. Neidhart DA, Greendyke RM (1967) The significance of diatom demonstration in the diagnosis of death by drowning. Am J Clin Pathol 48, 377–382.
182. Timperman J (1972) The diagnosis of drowning. A review. Forensic Sci 1, 397–409.
183. Auer A, Möttönen M (1988) Diatoms and drowning. Z Rechtsmed 101, 87–98.
184. De Bernardi A, Tappero P, Tarditi P (1966) Sul reperto di diatomee nell'annegamento. Minerva medicoleg 86, 158–164.
185. Lunetta P, Penttilä A, Hällfors G (1998) Scanning and transmission electron microscopical evidence of the capacity of diatoms to penetrate the alveolo-capillary barrier in drowning. Int J Legal Med 111, 229–237.
186. Pachar JV, Cameron JM (1993) The diagnosis of drowning by quantitative and qualitative diatom analysis. Med Sci Law 33, 291–299.
187. Giri BS, Tripathi CB, Chowdary YB (1993) Characterization of drowning by diatom test. Indian J Med Res 98, 40–43.
188. Angelini Rota M (1960) Ulteriori osservazioni sul reperto di diatomee negli organi di annegati. Zacchia 23, 470–487.
189. Krstic S, Duma A, Janevska B, Levkov Z, Nikolova K, Noveska M (2002) Diatoms in forensic expertise of drowning—a Macedonian experience. Forensic Sci Int 127, 198–203.
190. Foged N (1983) Diatoms and drowning—once more. Forensic Sci Int 21, 153–159.
191. Spitz WU, Schneider V (1964) The significance of diatoms in the diagnosis of death by drowning. J Forensic Sci 9, 11–18.
192. Ludes B, Coste M, North N, Doray S, Tracqui A, Kintz P (1999) Diatom analysis in victim's tissues as an indicator of the site of drowning. Int J Legal Med 112, 163–166.
193. Pollanen MS, Cheung C, Chiasson DA (1997) The diagnostic value of the diatom test for drowning, I. Utility: a retrospective analysis of 771 cases of drowning in Ontario, Canada. J Forensic Sci 42, 281–285.
194. Koseki T (1968) Fundamental examinations of experimental materials and control animals on the diagnosis of death from drowning by the diatom method. Acta Med Biol 15, 207–219.
195. Mueller B (1963) [On the problem of the occurrence of diatoms in the organs of cadavers not having laid in the water] Dtsch Z ges Gerichtl Med 54, 267–272 [in German].

196. Waltz H (1965) Zur Beweiskraft von Diatomeenbefunden. Aktuelle Fragen der Gerichtlichen Medizin. Wiss Zschr Martin-Luther-Univ, Halle-Wittenberg (Sonderheft).
197. Timperman J (1968) [Observations on the diatom question] Dtsch Z ges Gerichtl Med 63, 127–128 [in German].
198. Janitzki V (1964) Zur Frage der Sicherheit des Diatomeen Nachweises. Arch Kriminol 134, 24–25.
199. Hendey NI (1973) The diagnostic value of diatoms in cases of drowning. Med Sci Law 13, 23–34.
200. Schneider V (1965) Über den Beweiswert von Diatomeen in den Organen des grossen Kreislaufs als Zeichen des Ertrinkungstodes. Med Thesis, Berlin.
20.1 Merli S, Durante F, Umani Ronchi GU (1964) Sul valore diagnostico del reperto di diatomee negli organi degli annegati. Zacchia 27, 516–532.
202. Peabody AJ (1977) Diatoms in forensic science. J Forensic Sci Soc 17, 81–87.
203. Langer AH, Mackler AD, Rubin I, Hammond EL, Selikoff IJ (1971) Inorganic particles in cigars and cigar smoke. Science 174, 585–587.
204. Otto H (1961) Über den Nachweis von Diatomeen in menschlichen Lungestauben. Frankf Z Pathol 71, 176–181.
205. Kan T (1973) Studies on the determination of drowning from bones. Jpn J Legal Med 27, 68–76.
206. Tamâska L (1949) [Von dem Diatomeengehalt der Knochenmarksubstanz von Wasserleichen]. Orv Hetil 16, 509–511 [in Hungarian].
207. Tomonaga T (1963) [On some questions in the practice of diatom method as the evidence of drowning and on the corpse under high water pressure]. J Legal Med 17, 188–189 [in Japanese].
208. Porawski R (1966) Investigations on the occurrence of diatoms in organs in death from various causes. J Forensic Med 13, 134–137.
209. Tabbara W, Derobert L (1962) [Technical note on the diatoms]. Ann Med Leg Criminol Police Sci Toxicol 42, 613–615 [in French].
210. Antonenko NE, Ferris JAJ (1987) Diatom analysis in the determination of death by drowning. J Canad Soc Forensic Sci 20, 1–11.
211. Calder IM (1984) An evaluation of the diatom test in deaths of professional divers. Med Sci Law 24, 41–46.
212. Taylor JJ (1994) Diatoms and drowning—a cautionary case note. Med Sci Law 34, 78–79.
213. Devos C, Timperman J, Piette M (1985) Deaths in the bath. Med Sci Law 25, 189–200.
214. Funayama M, Mimasaka S, Nata M, Hashiyada M, Yajima Y (2001) Diatom numbers around the continental shelf break. Am J Forensic Med Pathol 22, 236–238.
215. Mueller B (1959) [Im welchen Gewässern besteht die Möglichkeit der Diagnose des Ertrinkungstodes durch Diatomeennachweis?] Zacchia 34, 1–11 [in German].
216. Lucas J, Goldfeder LB, Gill JR (2002) Bodies found in the waterways of New York City. J Forensic Sci 47, 137–141.
217. Keating DM, Miller JJ (1993) A technique for developing and photographing ridge impressions on decomposed water-soaked fingers. J Forensic Sci 38, 197–202.

218. Kringsholm B, Jakobsen J, Sejrsen B, Gregersen M (2001) Unidentified bodies/skulls found in Danish waters in the period 1992–1996. Forensic Sci Int 123, 150–158.
219. Giertsen JC, Morild I (1989) Seafaring bodies. Am J Forensic Med Pathol 10, 25–27.
220. Blanco Pampin J, Lopez-Abajo Rodriguez BA (2001) Surprising drifting of bodies along the coast of Portugal and Spain. Legal Med (Tokyo) 3, 177–182.
221. Carniel S, Umgiesser G, Sclavo M, Kantha LH, Monti S (2002) Tracking the drift of a human body in the coastal ocean using numerical prediction models of the oceanic, atmospheric and wave conditions. Sci Justice 42, 143–151.
222. Gregersen M, Jensen S, Knudsen PJ (1995) The crash of the Partnair Convair 340/580 in the Skagerrak: identification of the deceased. Aviat Space Environ Med 66, 158–163.
223. Mannucci A, Casarino L, Bruni G, Lomi A, De Stefano F (1995) Individual identification of flood victims by DNA polymorphisms and autopsy findings. Int J Legal Med 107, 213–215.
224. Soomer H, Ranta H, Penttilä A (2001) Identification of victims from the M/S Estonia. Int J Legal Med 114, 259–262.
225. Püschel K, Schneider A (1985) [Development of immersion skin in fresh and salt water at different water temperatures]. Z Rechtsmed 95, 1–18 [in German].
226. Gerin C, Carella A, Merli S., Fucci P, Angelini Rota M (1965) I problemi medico-legali dell' annegament. Zacchia, XL, 3ª, pp. 1–288.
227. Weber W (1982) [Water uptake of dermatoglyphic skin]. Z Rechtsmed 88, 185–193 [in German].
228. Bonte W, Sprung R, Huckenbeck W (1986) [Problems in the evaluation of electrocution fatalities in the bathtub]. Z Rechtsmed 97, 7–19 [in German].
229. Wollenek G, Dietl H, Denk W, Laufer G (1989) [A transition zone phenomenon of the skin in cadavers lying in bath water]. Z Rechtsmed 102, 473–486.
230. Clark DH, Law M (1984) Post-mortem pink teeth. Med Sci Law 24, 130–134.
231. Ikeda N, Watanabe G, Harada A, Suzuki T (1988) A scanning electron microscopy and electron probe X-ray microanalysis (SEM-EPMA) of pink teeth. J Forensic Sci 33, 1328–1333.
232. Van Wyk CW (1987) Pink teeth of the dead: 1. A clinical and histological description. J Forensic Odontostomatol 5, 41–50.
233. Borrman H, Du Chesne A, Brinkmann B (1994) Medico-legal aspects of postmortem pink teeth. Int J Legal Med 106, 225–231.
234. Ortmann C, DuChesne A (1998) A partially mummified corpse with pink teeth and pink nails. Int J Legal Med 111, 35–37.
235. Henssge C, Brinkmann B, Püschel K (1984) [Determination of the time of death by measurement of rectal temperature of corpses suspended in water]. Z Rechtsmed 92, 255–276 [in German].
236. Henssge C (2002) Temperature-based methods II. In Henssge C, Knight B, Krompecher T, Madea B, Nokes L, eds., The Estimation of the Time Since Death in the Early Postmortem Period, 2nd ed. Arnold, London, pp. 43–103.
237. Adelson L (1974) The Pathology of Homicide. Charles C. Thomas, Springfield, pp. 557–575.

238. Rodriguez WC III (1997) Decomposition of buried and submerged bodies. In Haglund WD, Sorg MH, eds., Forensic Taphonomy. The Postmortem Fate of Human Remains. CRC Press, Boca Raton, pp. 459–467.
239. Mant AK, Furbank R (1957) Adipocere. A review. J Forensic Med 4, 18–35.
240. Yan F, McNally R, Kontanis EJ, Sadik OA (2001) Preliminary quantitative investigation of postmortem adipocere formation. J Forensic Sci 46; 609–614.
241. Cotton GE, Aufderheide AC, Goldschmidt VG (1987) Preservation of human tissue immersed for five years in fresh water of known temperature. J Forensic Sci 32, 1125–1130.
242. Takatori T, Yamaoka A (1977) The mechanism of adipocere formation I. Identification and chemical properties of hydroxy fatty acids in adipocere. Forensic Sci 9, 63–73.
243. Takatori T, Yamaoka A (1977) The mechanism of adipocere formation. II. Separation and identification of oxo fatty acids in adipocere. Forensic Sci 10, 117–125.
244. Mellen PF, Lowry MA, Micozzi MS (1993) Experimental observations on adipocere formation. J Forensic Sci 38, 91–93.
245. Takatori T (1996) Investigations on the mechanism of adipocere formation and its relation to other biochemical reactions. Forensic Sci Int 80, 49–61.
246. Takatori T (2001) The mechanism of human adipocere formation. Legal Med (Tokyo) 3, 193–204.
247. Tomita K (1984) On the production of hydroxy fatty acids and fatty acid oligomers in the course of adipocere formation. Nippon Hoigaku Zasshi 38, 257–272.
248. Gotouda H, Takatori T, Terazawa K, Nagao M, Tarao H (1988) The mechanism of experimental adipocere formation: hydration and dehydrogenation in microbial synthesis of hydroxy and oxo fatty acids. Forensic Sci Int 37, 249–257.
249. Reh H, Haarhoff K, Vogt CD (1977) [The estimation of the time of death of corpses recovered from water]. Z Rechtsmed 79, 261–266 [in German].
250. Simonsen J (1977) Early formation of adipocere in temperate climate. Med Sci Law 17, 53–55.
251. Jobba G, Foldes V (1978) [Premature postmortem adipocere formation]. Arch Kriminol 161, 82–84 [in German].
252. Dix JD (1987) Missouri's lakes and the disposal of homicide victims. J Forensic Sci 32, 806–809.
253. O'Brien TG (1997) Movement of bodies in Lake Ontario. In Haglund WD, Sorg MH, eds., Forensic Taphonomy. The Postmortem Fate of Human Remains. CRC Press, Boca Raton, pp. 559–565.
254. Sorg MH, Dearborn JH, Monahan EI, Ryan HF, Sweeney KG, David E (1997) Forensic taphonomy in a marine context. In Haglund WD, Sorg MH, eds., Forensic Taphonomy. The Postmortem Fate of Human Remains. CRC Press, Boca Raton, FL, pp. 567–604.
255. Haglund WD, Sorg MH (2002) Human remains in aquatic environment. In Haglund WD, Sorg MH, eds., Advances in Forensic Taphonomy. Method, Theory, and Archeological Perspectives. CRC Press, Boca Raton, pp. 201–218.
256. Hobischak NR, Anderson GS (2002) Time of submergence using aquatic invertebrate succession and decompositional changes. J Forensic Sci 47, 142–151.

257. Haglund WD (1993) Disappearance of soft tissue and the disarticulation of human remains from aqueous environments. J Forensic Sci 38, 806–815.
258. Nawrocki SP, Pless JE, Hawley DA, Wagner SA (1997) Fluvial transport of human crania. In Haglund WD, Sorg MH, eds., Forensic Taphonomy. The Postmortem Fate of Human Remains. CRC Press, Boca Raton, pp. 529–552.
259. Branche CM, Sniezek JE, Sattin RW, Mirkin IR (1991) Water recreation-related spinal injuries: risk factors in natural bodies of water. Accid Anal Prev 23, 13–17.
260. Watson RS, Cummings P, Quan L, Bratton S, Weiss NS (2001) Cervical spine injuries among submersion victims. J Trauma 51, 658–662.
261. Mann RJ (1976) Propeller injuries. South Med J 69, 567–569.
262. Mendez-Fernandez MA (1998) Motorboat propeller injuries. Ann Plast Surg 41, 113–118.
263. Di Nunno N, Di Nunno C (2000) Motorboat propeller injuries. J Forensic Sci 45, 917–919.
264. Di Nunno N, Vimercati L, Viola L, Vimercati F (2003) A case of electrocution during illegal fishing activities. Am J Forensic Med Pathol 24, 164–167.
265. Schroeder G, Windus G, Tröger HD (1989) [Experimental studies of the development of linear electric current marks]. Arch Kriminol 183, 21–28 [in German].
266. Madea B, Henssge C, Oehmichen M (1987) [Effect of water exposure on the recognizability of rope marks]. Arch Kriminol 180, 114–122 [in German].
267. Betz P, Penning R, Keil W (1994) The detection of petechial haemorrhages of the conjunctivae in dependency on the postmortem interval. Forensic Sci Int 64, 61–67.
268. Lunetta P, Smith GS, Penttilä A, Sajantila A (2004) Unintentional drowning in Finland 1970–2000: a population-based study. Int J Epidemiol, 33, 1053–1063.
269. Lester D (1993) Suicide by drowning and the extent of the nation's coastline. Percept Mot Skills 77, 1118.
270. Wirthwein DP, Barnard JJ, Prahlow JA (2002) Suicide by drowning: a 20-year review. J Forensic Sci 47, 131–136.
271. Byard RW, Houldsworth G, James RA, Gilbert JD (2001) Characteristic features of suicidal drownings: a 20-year study. Am J Forensic Med Pathol 22, 134–138.
272. Copeland AR (1987) Suicide by drowning. Am J Forensic Med Pathol 8, 18–22.
273. Davis LG (1999) Suicidal drowning in South Florida. J Forensic Sci 44, 902–905.
274. Avis SP (1993) Suicidal drowning. J Forensic Sci 38, 1422–1426.
275. Auer A (1990) Suicide by drowning in Uusimaa province in southern Finland. Med Sci Law 30, 175–179.
276. Snyder RG, Snow CC (1967) Fatal injuries resulting from extreme water impact. Aerosp Med 38, 779–783.
277. Lukas GM, Hutton JE Jr, Lim RC, Mathewson C Jr (1981) Injuries sustained from high velocity impact with water: an experience from the Golden Gate Bridge. J Trauma 21, 612–618.
278. Li L, Smialek JE (1994) The investigation of fatal falls and jumps from heights in Maryland (1987–1992). Am J Forensic Med Pathol 15, 295–299.
279. Lafave M, LaPorta AJ, Hutton J, Mallory PL II (1995) History of high-velocity impact water trauma at Letterman Army Medical Center: a 54-year experience with the Golden Gate Bridge. Mil Med 160, 197–199.

280. Simonsen J (1983) Injuries sustained from high-velocity impact with water after jumps from high bridges. A preliminary report of 10 cases. Am J Forensic Med Pathol 4, 139–142.
281. Blohm C, Püschel K (1998) [Epidemiologic and phenomenologic aspects of suicide caused by leaping from a high bridge]. Arch Kriminol 202, 129–139 [in German].
282. Coman M, Meyer AD, Cameron PA (2000) Jumping from the Westgate Bridge, Melbourne. Med J Aust 172, 67–69.
283. Gerling I, Pribilla O (1989) [An unusual death in water]. Arch Kriminol 183, 163–167 [in German].
284. Schmidt V, Guggolz M, du Bois R (1991) [Drowning in the bathtub after choking: Unusual death, accident or suicide? Reconstruction of an unusual fall]. Arch Kriminol 187, 163–172 [In German].
285. Nadjem H, Bohnert M, Logemann E, Pollak S (1997) [Suicide or fatal accident in fantasy play?] Arch Kriminol 199, 97–102 [in German].
286. Petri NM, Definis-Gojanovic M, Andric D (2003) Scuba diver with a knife in his chest: homicide or suicide? Croat Med J 44, 355–359.
287. Sivaloganathan S (1984) Aqua-eroticum—a case of auto-erotic drowning. Med Sci Law 24, 300–302.
288. Pearn JH, Nixon J (1979) An analysis of the causes of freshwater immersion accidents involving children. Accid Anal Prev 11, 173–178.
289. Griest KJ, Zumwalt RE (1989) Child abuse by drowning. Pediatrics 83, 41–46.
290. Gillenwater JM, Quan L, Feldman KW (1996) Inflicted submersion in childhood. Arch Pediatr Adolesc Med 150, 298–303.
291. Birkinshaw VJ, Gurd MR, Randall SS, Curry AS, Price DE, Wright PH (1958) Investigations in a case of murder by insulin poisoning. Br Med J 34, 463–468.
292. Glass A, Roberts J (1999) The case of R v. Sheila Bowler. Med Sci Law 39, 188–197.
293. Lau G (2002) Did he drown or was he murdered? Med Sci Law 42, 172–180.
294. Pollanen MS (1998) Diatoms and homicide. Forensic Sci Int 91, 29–34.
295. Missliwetz J, Stellwag-Carion C (1995) [6 cases of premeditated murder of adults by drowning]. Arch Kriminol 195, 75–84 [in German].
296. Smith GS and the Wet ICE Collaborative Group (2000) International Comparisons of Drowning Mortality: the value of multiple cause data. Proceedings of the International Collaborative Effort on Injury Statistics, Vol. III, Centers for Disease Control and Prevention, Hyattsville, MD, DHHS Publication No. PHS-00–1026–0, pp. 1–13.
297. Lunetta P, Smith GS, Penttilä A, Sajantila A (2003) Undetermined drowning. Med Sci Law 43, 207–214.
298. Kurihara K, Kuroda N, Murai T, Matsuo Y, Yanagida J, Watanabe H (1989) A case of suicide by drowning with hesitation marks on the back. Nippon Hoigaku Zasshi 43, 517–521.
299. Smith NM, Byard RW, Bourne AJ (1991) Death during immersion in water in childhood. Am J Forensic Med Pathol 12, 219–221.
300. Schmidt P, Madea B (1995) Death in the bathtub involving children. Forensic Sci Int 72, 147–155.

301. Ackerman MJ, Tester DJ, Porter CJ, Edwards WD (1999) Molecular diagnosis of the inherited long-QT syndrome in a women who died after near-drowning. N Engl J Med 341, 1121–1125.
302. Ackerman MJ, Tester DJ, Porter CJ (1999) Swimming, a gene-specific arrhythmogenic trigger for inherited long QT syndrome. Mayo Clin Proc 74, 1088–1094.
303. Yoshinaga M, Kamimura J, Fukushige T, et al. (1999) Face immersion in cold water induces prolongation of the QT-interval and T-wave changes in children with nonfamilial long QT syndrome. Am J Cardiol 83, 1494–1497.
304. Schwartz PJ, Priori SG, Spazzolini C (2001) Genotype-phenotype correlation in the long Q-T syndrome. Gene-specific triggers for life-treathening arrythmias. Circulation 103, 89–95.
305. Lunetta P, Levo A, Laitinen PJ, Fodstad H, Kontula K, Sajantila A (2003) Molecular screening of selected long QT syndrome (LQTS) mutations in 165 consecutive bodies found in water. Int J Legal Med 117, 115–117.
306. Schneider V, Bratzke H, Maxeiner H (1982) [Remarkable findings in the criminal dismemberment of a corpse]. Z Rechtsmed 89, 131–143 [in German].
307. Schumann M, Barz J, Bonte W (1995) [Disposal of the corpse by cement encapsulation and submersion in water]. Arch Kriminol 195, 18–26 [in German].
308. Lew EO, Bannach B, Rodriguez WC 3rd (1996) Septic tank burial: not just another skeleton in the closet. J Forensic Sci 41, 887–890.
309. Rajs J, Lundstrom M, Broberg M, Lidberg L, Lindquist O (1998) Criminal multilation of the human body in Sweden—a thirty-year medico-legal and forensic psychiatric study. J Forensic Sci 43, 563–580.
310. Fanton L, Miras A, Tilhet-Coartet S, Achache P, Malicier D (1998) The perfect crime: myth or reality? Am J Forensic Med Pathol 19, 290–293.
311. London MR, Krolikowski FJ, Davis JH (1997) Burials at sea. In Haglund WD, Sorg MH, eds., Forensic Taphonomy. The Postmortem Fate of Human Remains. CRC Press, Boca Raton, FL pp. 615–622.
312. Geertringer P, Voigt J (1970) Death in the bath. A survey of bathtub deaths in Copenhagen, Denmark, and Gothenburg, Sweden, from 1961 to 1969. J Forensic Med 17, 136–147.
313. Budnick L, Ross D (1984) Bathtub-related electrocutions in the United States 1979–1981. JAMA 252, 918–920.
314. Schmidt P, Madea B (1995) Homicide in the bathtub. Forensic Sci Int 72, 135–146.
315. Nowers M (1999) Suicide by drowning in the bath. Med Sci Law 39, 349–353.
316. Budnick LD, Ross DA (1985) Bathtub-related drownings in the United States, 1979–1981. Am J Public Health 75, 630–633.
317. Spitz M (1998) Injuries and death as a consequence of seizures in people with epilepsia. Epilepsia 39, 904–907.
318. Trübner K, Püschel K (1991) [Fatalities in the bathtub]. Arch Kriminol 188, 35–46 [in German].
319. Schneider V (1974) [Criminal submersion in bathtubs]. Med Leg Dommage Corpor 7, 397–399 [in French].

320. Lasczkowski G, Riepert T, Rittner C (1992) [Site of discovery in the bath tub. Evaluation of a fatality after four years using postmortem roentgen diagnosis]. Arch Kriminol 189, 25–32 [in German].

Forensic Neuropathology

2

HIV-1 Infection of the Central Nervous System

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CONTENTS

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Summary

The forensic pathologist frequently is confronted with human immunodeficiency virus (HIV)-1 infection, especially in the context of drug abuse. After involvement of the lung, the brain is the second most frequently affected organ in HIV-1 infection. Because HIV-1 rarely is the cause of focal macroscopical lesions, even in severely infected patients, the systematic sampling of specimens for histological examination is required. If focal lesions are present, they are almost always attributed to opportunistic infections, cerebrovascular complications, or neoplasms. Changes primarily attributed to HIV-1 include HIV-1 encephalitis, HIV-1 leukoencephalopathy, and HIV-1 myelitis. Early changes in the course of the infection are characterized by meningeal lymphocytic infiltration and perivascular lymphocytic infiltration. Changes probably attributed to HIV-1 include vacuolar myelopathy and vacuolar leukoencephalopathy. Opportunistic infections seen in the course of HIV-1 infection include a broad spectrum of viral, parasitic, fungal, and bacterial infections. Furthermore, ischemic stroke and intracranial hemorrhage, as well as lymphoma and Kaposi sarcoma, may be encountered. Despite the introduction of antiretroviral therapies with a greater life expectancy of HIV-1-infected individuals, epidemiological data suggest that involvement of the brain in acquired immunodeficiency syndrome subjects continues to be a frequent autopsy finding. In the brains of HIV-1-infected children, the most common findings are vascular mineralization/calcification, myelin pallor, and gliosis of the white matter as well as inflammatory infiltrates and/or multinucleated giant cells. In contrast to adults, opportunistic infections are comparatively uncommon. The pathogenetic mechanisms induced by HIV-1 infection and leading to the multiple facets of brain damage are not yet clearly understood. The development of brain lesions caused by opportunistic infections and lymphomas might be explained by the lack of a competent immunological defense system. In contrast, changes caused by direct or rather indirect effects of HIV-1 are more controversially discussed. HIV-1 enters the brain mainly by being passively carried by T lymphocytes and monocytes. Thereafter, perivascular macrophages spread productive HIV-1 infection to neighboring microglia. These are the major cell populations in the brain that are productively infected with HIV-1. They serve as a reservoir for persistent viral infection and replication, a vehicle for viral dissemination throughout the brain, and a major source of neurotoxic products that affect glial function, the blood–brain barrier and neuronal function and finally lead to cell death.

Key Words: Central nervous system; forensic pathology; HIV-1 infection; opportunistic infections; pathogenesis.

1. Introduction

Human immunodeficiency virus (HIV)-1 infection is a serious health problem worldwide, and the forensic pathologist frequently is confronted with this disease, especially in the context of drug abuse *(1–11)*. Intravenous drug abuse is a major risk factor for HIV-1 infection, and several drugs of abuse have been shown to enhance both the HIV-1 entry into the central nervous system (CNS) as well as the effects of HIV-1 on the CNS *(2,6,12–25)*. Similar to HIV-1, many abused drugs tend to affect neuronal function and enhance the microglial activation resulting from HIV-1 infection in some individuals *(26,27)*. Further paralleling HIV-1, some drugs seem to compromise immune function, which in turn may have secondary detrimental effects on the CNS *(28)*.

Neuropathological examinations show, in up to 95% of the brains, changes that may be attributed to the primary effect of HIV-1 or to opportunistic agents *(29–45)*.

In this chapter, the neuropathological features seen in the brains of patients infected with HIV-1 are reviewed briefly, and representative illustrations are shown. The reader also is referred to the multitude of articles and textbooks describing the neuropathological changes *(6,14,16,29–76)* as well as review articles on HIV-1 and the brain *(34,72,77–110)*. Moreover, changes especially seen in the early stages of HIV-1 infection in adults as well as those seen in the brains of children infected with HIV-1 are described briefly. In the following, the nomenclature and the neuropathology-based terminology as proposed in the consensus report of Budka et al. *(31)* is adopted. The definitions given in this report serve as guidelines for the neuropathological descriptions.

The term “AIDS dementia complex” (ADC) was coined in 1986 *(42,97)* to describe cognitive and motor disturbances frequently encountered in patients infected with HIV-1. The authors suggested that the pathogenesis of ADC was attributed to the changes found in the basal ganglia. Thus, ADC was considered to belong to the class of subcortical dementias. The name ADC was later changed into HIV-1-associated cognitive and motor complex with HIV-1-associated dementia complex (motor)/(behavior) (HAD) and HIV-1-associated myelopathy as its severe manifestations and HIV-1-associated minor cognitive/motor disorder as its mild manifestation *(111)*. It eventually affects up to 30% of untreated adult patients with acquired immunodeficiency syndrome (AIDS *[31,112,113]*). It is characterized by progressive cognitive decline, motor dysfunction, and behavioral abnormalities in 65% of patients *(97,98,101,112,114)*. HAD is the most devastating CNS consequence of AIDS because of its poor prognosis and functional impairment. In some persons

with HAD, the behavioral abnormalities can lead to violence and other criminal behavior *(115)*.

The pathogenesis of HIV-1-induced brain damage is uncertain, and there is evidence that multiple mechanisms leading to neurological injury occur (*see* Section 17). These mechanisms include the role of neurovirulent strains of HIV-1, the potential neurotoxicity of HIV proteins, macrophage/microglia-mediated CNS injury, and altered blood–brain barrier (BBB) permeability *(77,83,84,98,103,105,116–118)*.

Table 1 provides a survey of the neuropathological changes seen in the brains of patients infected with HIV-1. Table 2 summarizes the changes occurring in the peripheral nervous system and skeletal muscles of patients infected with HIV-1, but these are not dealt with in this review. The reader is directed to articles dealing in more detail with the neuropathology of peripheral nervous system and skeletal muscles *(119–127)*.

As a rule in the forensic setting, the HIV status of all persons undergoing autopsy should be determined ahead by enzyme-linked immunosorbent assay analysis of blood samples, which may be obtained from femoral vessels. The precautions in performing the autopsy of a patient who was infected with HIV, including the removal and fixation of the brain, are reviewed elsewhere and should be strictly practiced *(128–133)*. For a thorough neurohistopathological examination, infected cells may be identified by *in situ* hybridization or immunohistochemistry against HIV-1 proteins *(31,134–137)*.

2. Epidemiology

Worldwide, 33 million adults and 2 million children are infected with HIV-1. Despite preventive efforts, the epidemic continues to spread rapidly, and the socioeconomic consequences of the neurological dysfunction caused by HIV-1 infection are of enormous proportions. Most of the affected patients live in developing countries, where anti-retroviral medications are not available.

After involvement of the lung (75–85%), the brain is the second most frequently affected organ (60–80%) in HIV-1 infection *(16)*. The neurological complications of HIV-1 infection are highly stage-specific. Therefore, incidence rates depend on the stage, in which the individual is in, during the course of systemic HIV-1 infection *(70,99)*.

Neurological signs and symptoms are seen in up to 50% of patients infected with HIV-1 *(34,60,66,99,138,139)*. In approx 10% of the cases, neurological signs are the first presentation of the disease *(34,60,66,139)*. HIV-1-associated CNS complications vary with ethnicity *(140)* and geography *(40,47,141,142)*. The different findings likely reflect afflictions common to

Table 1
Survey of the More Common Neuropathological Changes Seen in the Brains of Patients Infected With HIV-1

Changes primarily attributable to HIV-1

1. HIV-1 encephalitis (HIVE)
2. HIV-1 leukoencephalopathy (HIVL)
3. HIV-1 myelitis
4. Lymphocytic meningitis (LM), meningeal lymphocytic infiltration (MLI), and perivascular lymphocytic infiltration (PLI)

Changes probably attributable to HIV-1

1. Vacuolar myelopathy (VM)
2. Vacuolar leukoencephalopathy (VL)

Opportunistic infections and neoplasias

1. Viral Infections
   - Cytomegalovirus infection (CMV)
   - Progressive multifocal leukoencephalopathy (PML)
   - Others including herpes simplex 1, herpes simplex 2, herpes zoster, HTLV-1
2. Parasitic infections
   - Protozoa: *Toxoplasma gondii*, Acanthamoeba, Leptomyxid amoeba, *Tryponosoma cruzi*
   - Metazoa: Strongyloides
3. Fungal infections
   - *Aspergillus fumigatus*
   - Candida albicans
   - Cryptococcus neoformans
   - Others including histoplasma, phycomyces, coccidioides, blastomyces, acremonium, cladosporium
4. Bacterial infections
   - Pyogenic: *Escherichia coli*, Listeria, Staphylococcus, Salmonella
   - Mycobacterial: *Mycobacterium tuberculosis, Mycobacterium avium intracellulare*
   - Spirochetal: *Treponema pallidum*
   - Filamentous: Nocardia
   - Miscellaneous: Whipple's disease
5. Neoplasia
   - Lymphoma (primary and secondary)
   - Kaposi sarcoma

Table 2
Neuropathological Changes Observed in the Peripheral Nervous System and Skeletal Muscles of Patients Infected With HIV-1

Peripheral nervous system
1. Acute inflammatory demyelinating (poly) (radiculo) neuropathy
2. Chronic inflammatory demyelinating (poly) (radiculo) neuropathy
3. Axonal neuropathy
4. Ganglionitis, ganglioradiculitis, (poly) (radiculo) neuritis
5. Necrotizing vasculitis, vasculitic neuropathy

Skeletal muscle
1. (Poly-)myositis
2. Necrotizing myopathy
3. Nemaline rod myopathy
4. Vesicular myopathy, mitochondrial myopathy
5. Necrotizing vasculitis

developing countries—a high prevalence of opportunistic infections and a high mortality rate. These conditions rarely lead to the development of complications such as lymphoma, which usually occurs later in the natural course of the HIV-1 infection. Death caused by systemic opportunistic infections may punctuate the course of HIV-1 encephalitis and prevent its full-blown morphological expression. However, larger autopsy studies of patients infected with HIV-1 over the course of longer time periods suggest that, despite the beneficial effects of modern antiretroviral combination therapy, involvement of the brain in AIDS subjects continues to be a frequent autopsy finding *(16,143–145)*.

3. Gross Anatomical Changes

It is important to emphasize the diffuse nature of the alterations that are likely to be present on gross examination. HIV-1 is rarely the cause of focal macroscopical lesions, even in heavily infected patients *(71)*. Therefore, systematic sampling of specimens for histological examination is required. If focal lesions are present, they are almost always caused by opportunistic infections, hemorrhages, or neoplasms.

Using imaging techniques (computed tomography, magnetic resonance imaging), it was claimed that brain atrophy is viewed in patients with HIV-1 infection and that this atrophy is already apparent in early stages of the infec-

tion *(146–148)*. Using autopsy brains and applying morphometrical techniques, one can assess whether signs of atrophy occur, which parts of the brain are affected, and to which extent these regions are affected.

The brain weight can be used as a rough indicator for atrophic changes occurring in the brain. Data on the weight of brains infected with HIV-1 are rarely published. The brain weight of 165 brains from HIV-1-positive patients and 155 age- and gender-matched controls was analyzed (Weis et al., unpublished data). No significant difference between controls and AIDS brains existed: the average brain weight of controls was 1434.94 g and that of HIV-1-positive patients was 1406.22 g. The degree of brain edema was rated using a three-point scale (0 = no brain edema; 1 = moderate brain edema; 2 = severe brain edema). There was no significant difference between the brains of controls and patients with AIDS. Furthermore, we used a three-point rating scale for assessing the widening of the ventricles (0 = no widening of lateral ventricles; 1 = moderate widening of lateral ventricles; 2 = marked widening of lateral ventricles). The widening of the lateral ventricles in the brains of patients with AIDS reached the level of statistical significance ($p = 0.04$; Weis et al., unpublished data).

Gelman and Guinto *(146)* measured cerebrospinal fluid (CSF) spaces in 64 consecutively autopsied patients with AIDS, which were compared with age-matched non-AIDS subjects (1992). Of the patients with AIDS, 37 (58%) had a CSF space index greater than 2 standard deviations above the mean of the age-matched control subjects. CSF spaces were expanded most in the frontal and temporal lobes; ventricular spaces were expanded more than the sulcal spaces. Patients with atrophy were much more likely to have HIV-1-associated histopathological changes in their brains, but the relationships were too weak to establish the microscopical cause of the atrophy.

Oster et al. *(147)* obtained stereological estimates of mean volumes, surface areas, and cortical thicknesses on formalin-fixed brains from 19 men with AIDS and 19 controls. In AIDS, the mean volume of the neocortex was reduced by 11%, and that of the central brain nuclei by 18%. Mean ventricular volume was increased by 55%. Mean neocortical thickness was reduced by 12%. The mean volume of the white matter was reduced by 13%. The findings in six clinically demented AIDS patients were not statistically different from the rest of the group.

The volumes of 20 cortical and 17 subcortical brain structures were estimated using Cavalieri's principle by Weis et al. *(149)*. Furthermore, the surface area and the mean cortical thickness of all cortical structures were measured. No significant changes were found in the cerebral cortex of patients

infected with HIV-1 as compared with age- and gender-matched controls. Only a significant reduction in volume was found in the internal capsule. The lack of significant changes in the brains of patients infected with HIV-1 might be attributed to the selection of the sample, which was composed of brains with the neuropathological diagnosis of HIV-1 encephalitis but that showed no remarkable gross anatomical changes.

Subbiah et al. *(148)* also used unbiased, stereological methods on post-mortem brain specimens to estimate volumes of different brain regions in patients prospectively diagnosed with and without HIV-associated dementia. A significant reduction in the mean neocortical volume (15%) was observed in the group with AIDS when compared with the seronegative controls, and this difference was accentuated when comparing only the group with HIV-associated dementia to the seronegatives (neocortex: 18%).

4. Changes Primarily Attributable to HIV-1

4.1. HIV-1 Encephalitis

HIV-1 encephalitis (HIVE) is characterized by multiple disseminated inflammatory foci composed of microglia, macrophages, and multinucleated giant cells (MGCs). The foci are predominantly located perivascularly in the cortex, deep gray matter, and the white matter. The MGCs serve as the hallmark for HIVE, representing an HIV-1-specific cytopathic effect and are derived from HIV-1-mediated fusion of infected microglia and macrophages (Fig. 1A *[31,71,103,150]*). In their absence, the presence of HIV antigen or HIV nucleic acids has to be demonstrated either by immunohistochemistry (Fig. 1B) or by *in situ* hybridization, respectively *(31)*. MGCs contain as many as 20 round or elongated, basophilic nuclei that usually are arranged at the periphery of the cell. There is no strong correlation between HIVE and the clinical stages of the HAD *(71)*. The cytoplasm of MGCs is eosinophilic and appears densely stained in the center and vacuolated at the periphery. The cells are of monocyte/histiocyte lineage and include microglia and macrophages, as shown by immunohistochemical studies *(32,37,150,151)*. The nucleic acids of HIV proteins are located within their cytoplasm *(116)*. Electron microscopic analysis reveals retroviral particles either free in the cytoplasm or in cytoplasmic cisternae (*32*). Microglia/macrophages and MGCs are capable of HIV synthesis and, thus, constitute the major reservoir and vehicle for the spread of the virus *(71,77,109,118,152)*.

Despite the introduction of highly active anti-retroviral therapy (HAART) with a greater life expectancy of infected individuals, epidemiological data

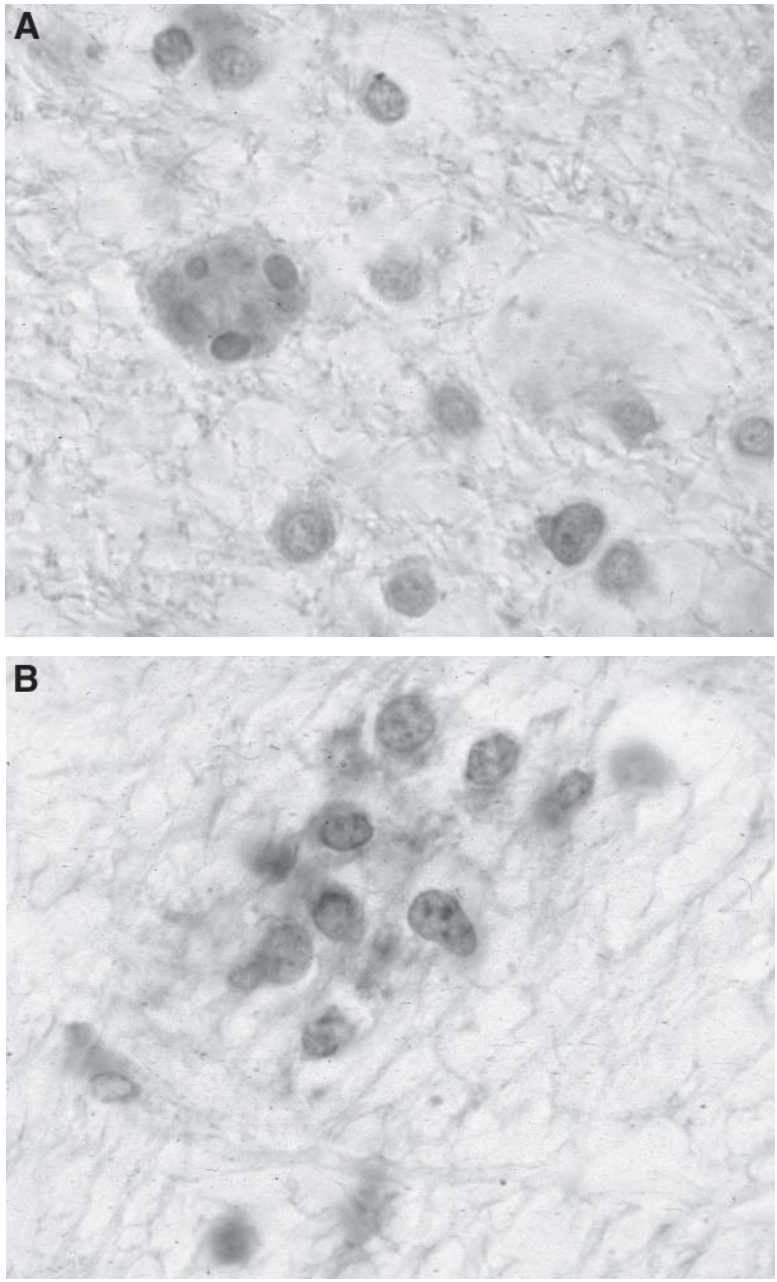

Fig. 1. (A), Multinucleated giant cell (cresyl violet). **(B),** HIV-1 antigen shown in a small gliomesenchymal nodule (immunohistochemistry for p24).

suggest that the prevalence of HIVE is on the rise *(153–155)*. Additionally, a new variant of HIVE has emerged in the era of HAART as a severe leukoencephalopathy with significant perivascular infiltration of macrophages and lymphocytes, which is assumed to be the result of an exaggerated response from a newly reconstituted immune system *(155)*. Synonyms previously used to describe HIVE include giant-cell encephalitis, multifocal giant-cell encephalitis, multinucleated cell encephalitis, and subacute encephalitis.

4.2. HIV-1 Leukoencephalopathy

HIV-1 leukoencephalopathy (HIVL) is characterized by diffuse damage to the white matter with myelin loss, reactive astrogliosis, macrophages, and MGCs. Only few, if any, inflammatory infiltrates are seen. In the absence of MGCs, the presence of HIV-1 antigen or HIV nucleic acids has to be demonstrated either by immunohistochemistry or by *in situ* hybridization *(31)*. Axonal damage can be demonstrated with immunohistochemistry for β-amyloid precursor protein *(156,157)*. *Progressive diffuse leukoencephalopathy* is another phrase previously used in describing HIVL.

HIVE and HIVL usually occur in the later stages of the AIDS infection. The differentiation between HIVE and HIVL can be confusing because in both cases the histological features have been described as MGCs in a focus with reactive astrocytes, microglia, and lymphocytes occurring in the white matter for HIVL and in both gray and white matter for HIVE. On the basis of our experience, the term HIVE should be used to describe the above-mentioned changes occurring in the gray matter only and HIVL to changes occurring in the white matter only. However, in most of the cases, myelin pallor usually is found around the gliomesenchymal nodule containing the MGCs. Furthermore, the analysis of 300 autopsy cases in our laboratory (Weis et al., unpublished observation) shows that the above-described changes (gliomesenchymal nodule with MGC) are found mainly in the cortex and deep gray matter but less frequently in the white matter, hence, necessitating a clear-cut delineation between HIVE and HIVL. Thus, the term HIVE should be reserved for changes in the gray matter and HIVL for changes in the white matter. If both gray and white matter are involved, the term HIVE/L should be used.

4.3. Lymphocytic Meningitis, Meningeal Lymphocytic Infiltrates, and Perivascular Lymphocytic Infiltration

Lymphocytic meningitis (LM) is characterized by a significant number of lymphocytic infiltrates in the leptomeninges (Fig. 2A). No opportunistic

pathogens are present in the meninges *(31)*. LM should be distinguished from meningeal lymphocytic infiltrates (MLI), which show lymphocytes in lesser quantity infiltrating the leptomeninges than LM (Fig. 2B). Perivascular lymphocytic infiltration (PLI) is characterized by a significant number of lymphocytic infiltrates in the perivascular spaces of the brain tissue (Fig. 2C). No opportunistic pathogens are present in the perivascular brain tissue *(31)*.

The relation of MLI and PLI to the HIV-1 infection is not yet clearly established, but it seems that lymphocytic infiltrates in the leptomeninges and in the perivascular spaces of the brain tissue constitute changes occurring in the early stages of the HIV-1 infection (*see* Section14).

5. Changes Probably Attributable to HIV-1

Vacuolar myelopathy (VM) and vacuolar leukoencephalopathy (VL) are changes that are probably attributable to HIV-1 *(31)*. However, it is not yet clear whether these changes are caused by a direct effect of HIV-1 or constitute secondary changes.

5.1. Vacuolar Myelopathy

VM is characterized by numerous vacuolar myelin swellings and macrophages in multiple areas of the spinal cord *(31,71,158–163)*. VM predominantly involves the dorsolateral spinal tracts (Fig. 3A,B). Some macrophages may be found in the vacuoles. The axon is at first unaffected, but it is damaged in the later stages of the disease *(71,164)*.

The changes of VM might not be specific for HIV-1 because they can occur in the absence of HIV-1. However, in several studies HIV-1 has been shown to be present in spinal cord tissue showing VM using immunohistochemistry or *in situ* hybridization *(38,163,165)*. Thus, a direct relationship between the presence of HIV-1 and the changes of VM can be drawn.

HIV-1 may rarely produce a MGC myelitis analogous to HIVE *(71,161,163)*. Opportunistic infections and lymphomas of the spinal cord are uncommon *(161,163)*.

5.2. Vacuolar Leukoencephalopathy

VL is characterized by numerous vacuolar myelin swellings in the central white matter. Some macrophages may be found in the vacuoles. VL is a rare condition *(166)*.

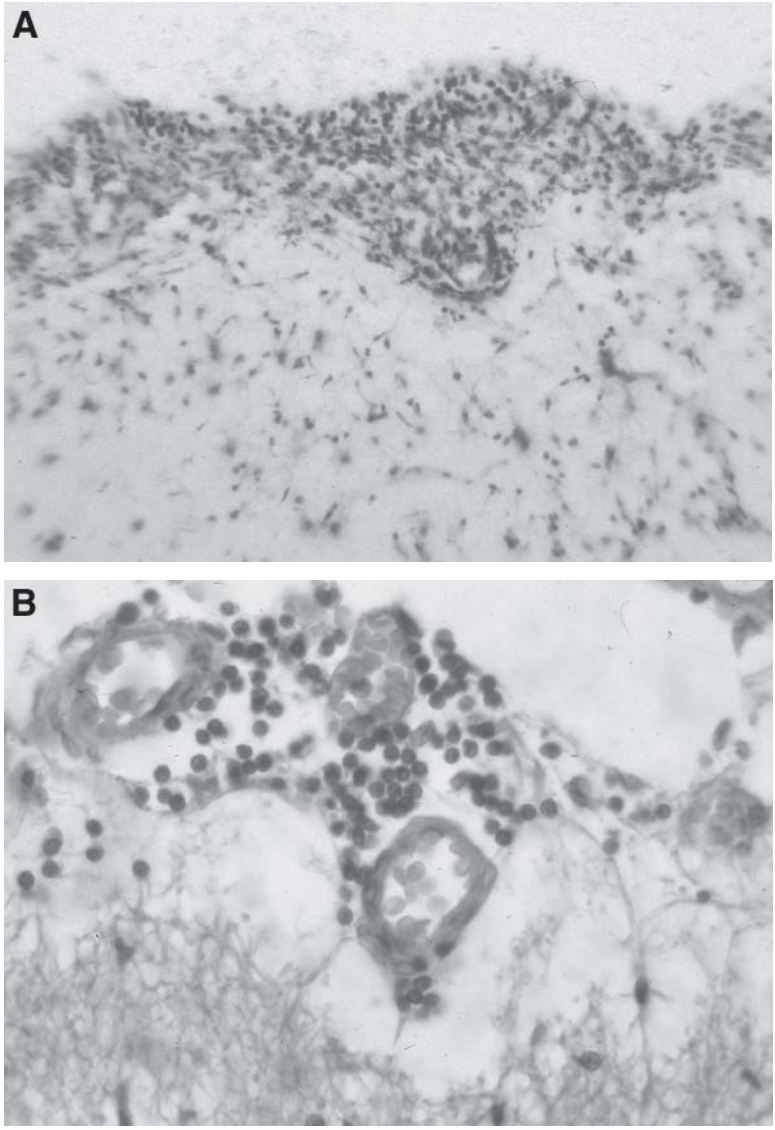

Fig. 2. (A), Lymphocytic meningitis (cresyl violet stain). **(B),** Meningeal lymphocytic infiltrates (Hematoxylin and eosin stain). **(C),** Perivascular lymphocytic infiltrates (Cresyl violet stain).

Fig. 2. *(continued)*

6. Viral Infections in the Course of HIV-1 Infection

6.1. Cytomegalovirus Infection

In general, there are no gross anatomical changes in the brains of patients with AIDS who have cytomegalovirus (CMV) infection *(167–171)*. In some cases, a necrotizing ependymitis with small areas of necrosis lining the ventricles is seen *(172)*. Histologically, microglial nodules are seen scattered throughout the nervous system. Within these microglial nodules, large cells containing inclusion bodies are found (Fig. 4A). The microglial nodules located in the gray and white matter usually are not surrounded by a necrotic area, whereas along the periventricular spaces, CMV-containing cells are found within the necrotic areas. Figure 4B shows the immunohistochemical staining of an inclusion body. Diagnostic difficulties encountered are described in Sec-

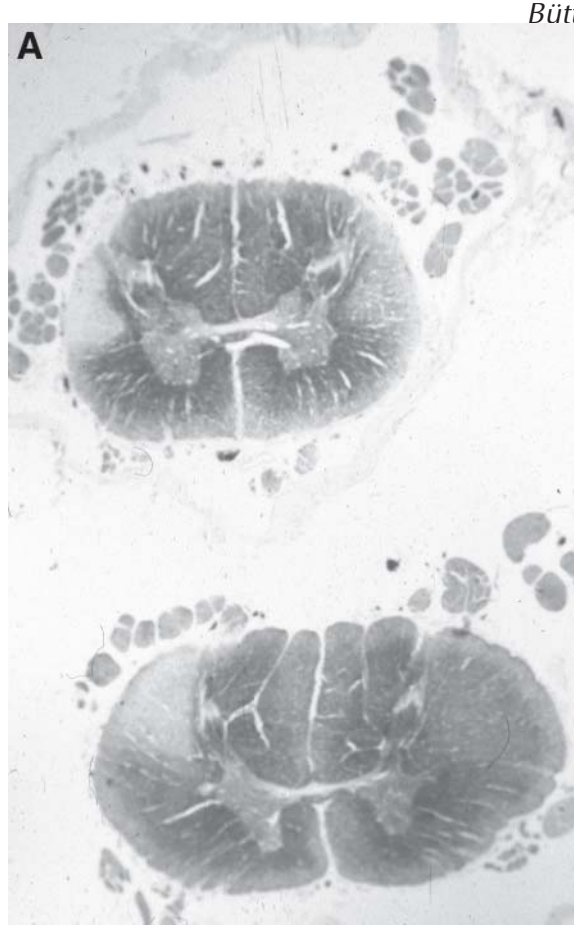

Fig. 3. Spinal cord showing vacuolar myelopathy of the dorsal tracts. **(A),** Woelcke's myelin stain; **(B),** Hematoxylin and eosin stain.

tion 12. The effects of CMV on the peripheral nervous system are reviewed elsewhere *(173,174)*.

6.2. Progressive Multifocal Leukoencephalopathy

The causative agent in progressive multifocal leukoencephalopathy (PML) is papovavirus. Grossly, multiple areas of discoloration of the white matter are quite characteristic of PML (Fig. 5). Sometimes, the white matter may appear softened and mottled. Histologically, the diagnostic features for the presence of PML are *(a)* multiple foci of demyelination seen in the white matter consisting of loss of myelin sheaths; *(b)* enlarged, bizarre astrocytes; and

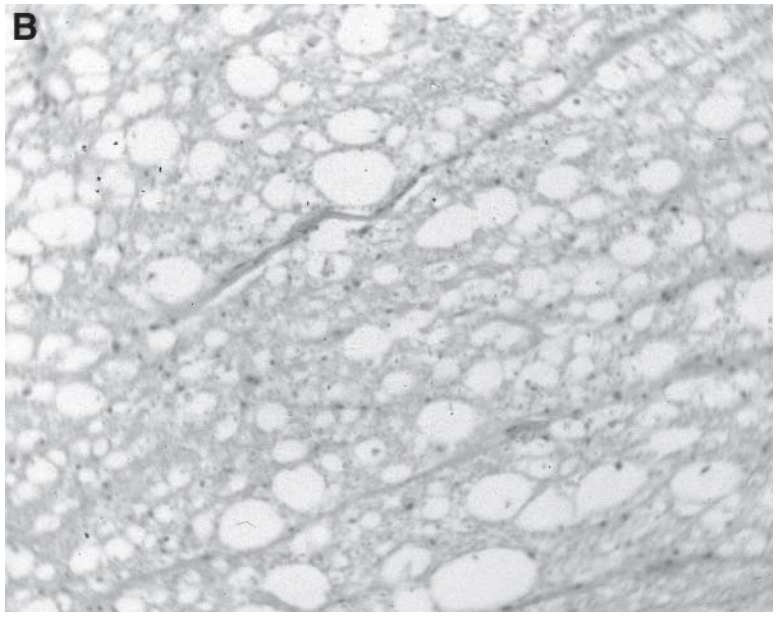

Fig. 3. *(continued)*

(c) intranuclear inclusions found within large swollen oligodendrocytes, the latter being the hallmark of PML *(175–178)*.

7. Parasitic Infections

7.1. Toxoplasma gondii

Macroscopically, lesions appear as zones of necrosis with an area of hyperemia and/or small hemorrhages surrounded by a poorly defined area of edema (Fig. 6A). Microscopically, large zones of necrosis are found in the brain tissue. Chronic inflammatory cell response may be seen in the area of necrosis, which might be intense and sometimes gives the appearance of a lymphoma. The infiltrates are composed of lymphocytes, plasma cells, and histiocytes. The necrotic lesions are surrounded by reactive astrocytes, activated microglia, and inflammatory cells. Toxoplasma may be found in two forms: encysted organisms (Fig. 6B) and tachyzoites found in small or larger collections diffusely distributed near the junctional area between necrosis and brain tissue *(179,180)*. Toxoplasmosis restricted to the CNS can be pathogenetically classified as reactivation of a latent infection, whereas acute, systemic toxoplasmosis involving other organs is seen in patients who prob-

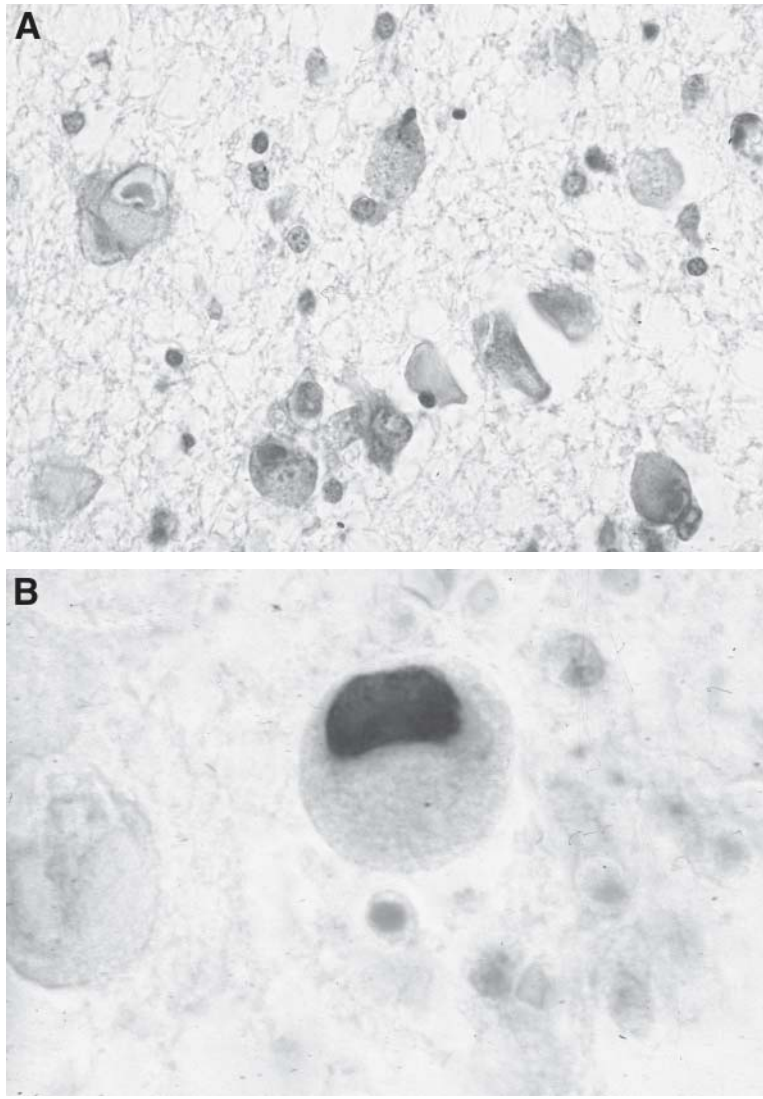

Fig. 4. Cytomegalovirus (CMV) infection. **(A),** Cells with inclusion bodies (Nissl stain). **(B),** Cytomegalic cell with inclusion body (immunohistochemistry for CMV).

ably acquired the infection during HIV-induced immunosuppression *(181)*. Diffuse, necrotizing toxoplasma encephalitis with widespread, confluent areas of necrosis was observed primarily during the early period of the AIDS epidemic and restricted to patients who did not receive chemotherapy. In subse-

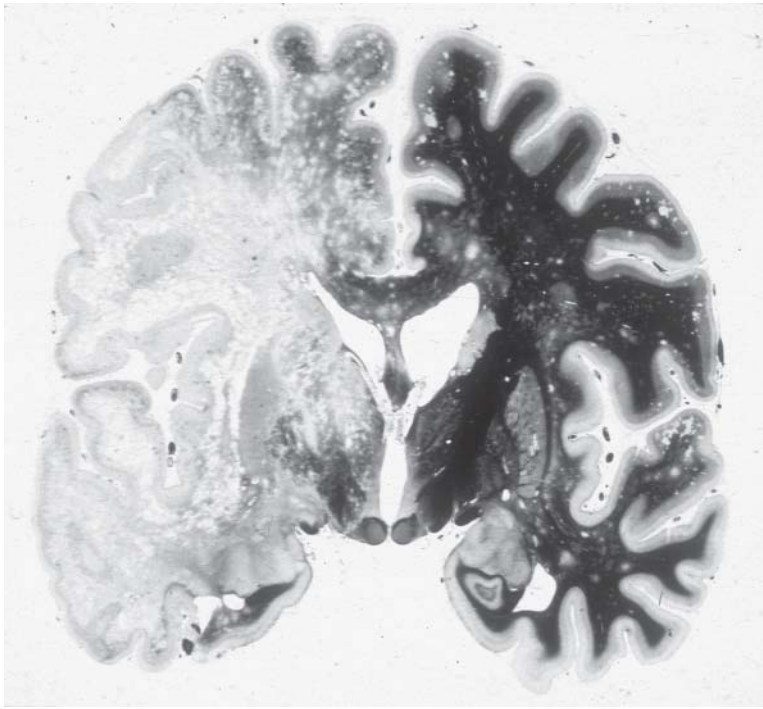

Fig. 5. Progressive multifocal leukoencephalopathy (Woelcke's myelin stain).

quent years, chronic, burnt-out lesions were observed. These are mainly composed of lipid-laden macrophages and immunocytochemistry for *Toxoplasma gondii* usually fails to detect the parasite *(181)*.

8. Fungal Infections

8.1. Aspergillus fumigatus

Macroscopically, necrotizing lesions in various regions of the brain might be seen in patients with Aspergillus infection. Aspergillus mainly involves large vessels. Thus, the lesions are usually associated with hemorrhage and hemorrhagic infarctions in large areas of the brain, but can be less pronounced. Microscopically, the vessel walls are invaded by septaled, branching hyphae. Thrombosis of the vessels and invasion of the tissue by the organisms are associated with necrosis *(64,76)*.

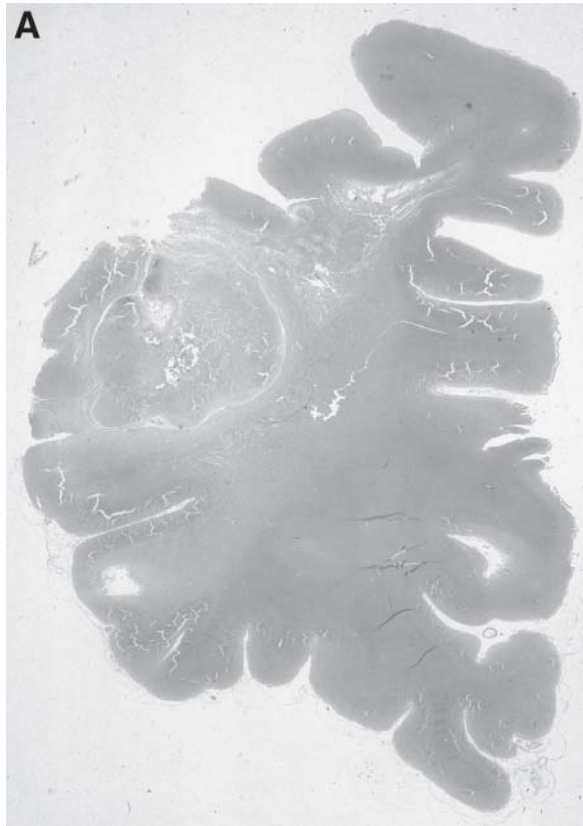

Fig. 6. *Toxoplasma gondii* infection. **(A),** Large area of necrosis in the frontal lobe (hematoxylin and eosin stain); **(B),** Encysted organisms in a gliomesenchymal nodule (cresyl violet stain).

8.2. Candida albicans

Nonspecific changes are found in the brain at gross anatomical examination. Occasionally, a mild to moderate edema with yellow softening of the brain tissue is noted. Histologically, multifocal microabscesses might be found containing polymorphonuclear, monocytic, and microglial cells sometimes surrounded by necrosis *(139)*.

8.3. Cryptococcus neoformans

Macroscopically, the appearance of the brain lesions attributable to cryptococcus are diverse *(11,182)*. Sometimes, no changes can be discerned, or

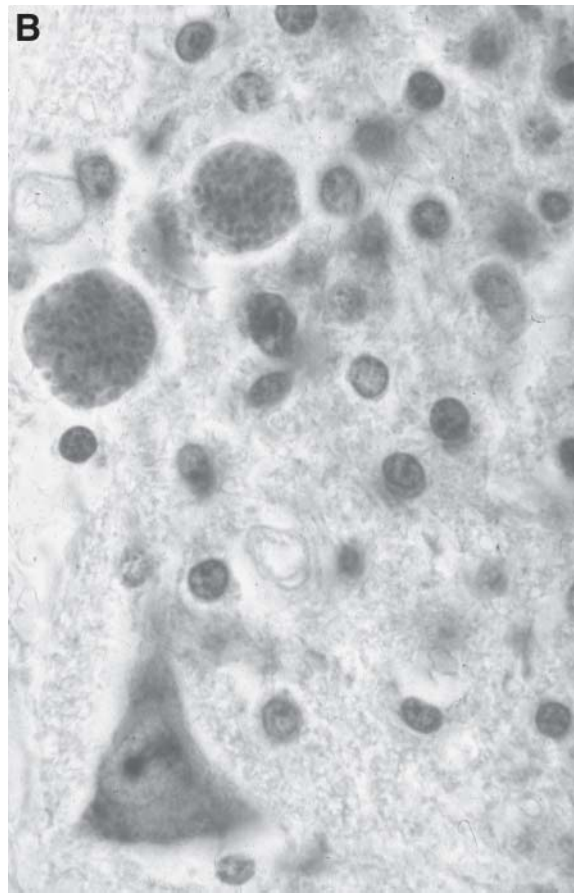

Fig. 6. *(continued)*

only small or large foci of gelatinous material may be seen either in the leptomeninges or within the brain parenchyma (Fig. 7). These gelatinous areas are histologically composed of thick mucin-positive capsules that contain the cryptococcus spores. There may be minimal inflammatory response in the neighborhood of the capsules *(182)*.

Furthermore, a spectrum of various opportunistic diseases, for example, varizella-zoster virus *(183,184)*, herpes simplex virus *(185)*, neurosyphilis *(186)*, tuberculosis *(40,187)*, rabies *(188)*, nocardiosis *(189)*, and coccidioidomycosis *(190)* has been described in patients infected with HIV-1, but these are rare conditions.

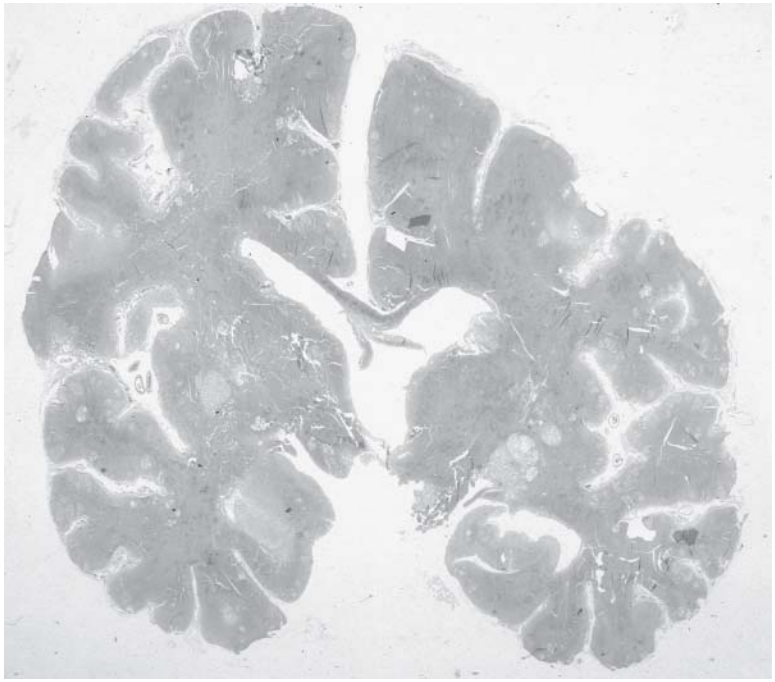

Fig. 7. Cryptococcus neoformans infection (Alcian blue stain).

9. Neoplasia in the Course of HIV-1 Infection

9.1. Lymphomas

Lymphomas may occur as primary or secondary lesions; primary lymphoma is most commonly seen in AIDS brains *(191,192)*. Multifocal mass lesions that may be partially necrotic, partially hemorrhagic can be macroscopically localized anywhere in the brain. The tumors are composed of a high number of large, pleomorphic malignant cells with cleaved and folded nuclei and a varying amount of cytoplasm (Fig. 8). Most of the lymphomas are non-Hodgkin's B-cell tumors *(193–196)*. The infiltration of the tumor consists either of single cells or of cells that diffusely infiltrate the adjacent tissue. Diffuse infiltration of the vessel walls can be noted.

Brain tumors (excluding lymphomas) occurring in patients infected with HIV-1 have only occasionally been described *(197)* but must be considered in the differential diagnosis of an intracranial space-occupying lesion in this population.

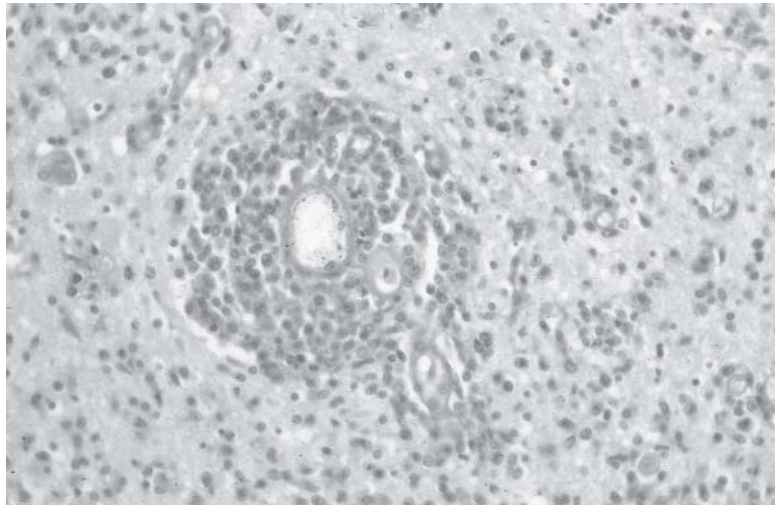

Fig. 8. Polymorphic non-Hodgkin's B-cell lymphoma (hematoxylin and eosin stain).

9.2. Kaposi Sarcoma

Intracerebral Kaposi sarcoma, which is frequently present in peripheral organs, has only been found in rare cases *(198)*.

10. Frequency and Topographical Distribution of Neuropathological Changes

10.1. Frequency of Neuropathological Changes

The frequency of neuropathological changes seen in the brains of patients infected with HIV-1 by different research groups is shown in Table 3 (Weis et al., unpublished data and refs. *6,8,14,16,29,32,33,35,37–43,47–49,51,52,55,57,60,61,63–65,69,70,74–76,80,139,141,143,151,194,199–202*).

Table 3 shows a high variation in the frequency of the different neuropathological entities varying among the different research groups. These differences in the frequency and the occurrence of the various neuropathological changes might be caused by several factors that are given in the next section.

10.1.1. Sample Size

Sample size was quite small in the early reports. In recent reports the sample size ranges between 150 and 200 cases. However, although the variability might be reduced by large sample sizes, there still exist intercontinen-

Table 3
Comparison of the Reported Frequencies of Neuropathological Changes in Brains of Patients Infected With HIV-1 (in %)

City/ Country	Year	*n*	HIVE	HIVL	CMV	PML	TOXO	LYM	Reference
Miami	1984	52	5.7	nip	3.8	3.8	30.7	1.9	*41*
NYCor	1984	40	nip	nip	37.5	2.5	12.5	7.5	*65*
New Jersey	1985	8	0.0	nip	12.5	12.5	50.0	0.0	*74*
UCSF	1985	128	27.3	nip	nip	1.6	14.1	8.6	*139*
UCLA	1986	89	[a]	nip	15.7	6.7	6.7	4.5	*29*
NYCor	1986	70	28.1	nip	24.3	nip	nip	nip	*42*
NYCor	1986	153	28.2	nip	26.1	2.0	10.5	5.9	*43*
Sao Paulo	1986	22	nip	nip	0.0	0.0	18.2	9.1	*141*
Bronx	1987	53	9.4	nip	11.3	nip	9.4	5.6	*37*
Boston	1987	30	90.0	nip	6.6	3.3	16.6	6.6	*151*
USC	1987	100	nip	nip	17.0	nip	5.0	13.0	*69*
AU/I	1987	100	26.0[b]	25.0[b]	18.0	5.0	17.0	6.0	*32*
Frankfurt	1987	28	60.7	nip	17.8	3.6	28.6	7.1	*199*
France	1988	40	37.5	nip	20.0	5.0	47.5	2.5	*55*
CH	1989	135	15.5	nip	10.4	6.6	25.9	6.6	*39*
Mu/I	1989	51	50.9[c]	nip	5.8	7.8	19.6	5.8	*35*
Denmark	1989	43	nip	nip	18.6	2.3	9.3	6.9	*57*
Oxf/Lon-hem	1989	11	9.1	nip	0.0	0.0	0.0	nip	*80*
Ox/Lon-nonhem		31	25.8	nip	9.7	9.7	22.6	nip	*80*
London	1989	26	23.0	nip	7.6	nip	7.6	11.5	*60*
Genoa (I)	1989	22	22.7	nip	13.6	18.2	31.8	13.6	*52*
UCLA	1989	158	10.1	nip	17.1	5.1	7.6	3.2	*75*
Japan	1991	15	26.6	nip	20.0	6.6	13.3	13.3	*51*
New York	1991	111	nip	nip	nip	nip	nip	13.5	*194*
Texas	1991	141	19.8	nip	16.3	2.8	3.5	6.4	*33*
Bronx	1991	221	25.8	nip	12.7	5.4	13.1	5.4	*38*
Sao Paulo	1992	252	10.7	nip	7.9	0.8	34.1	4.0	*47*
Vancouver	1992	39	43.5	nip	23.1	2.6	17.9	28.2	*48*
Milan	1993	202	27.7[d]	19.8	nip	nip	nip	nip	*200*
Untreated		120	29.2	27.5	nip	nip	nip	nip	*200*
Zidovudine-treated		82	25.6	8.5	nip	nip	nip	nip	*200*
USC	1993	400	35.7[e]	8.0[e]	7.7	nip	8.2	5.7	*70*
Düsseldorf	1993	70	17.1	2.8	8.5	2.8	21.4	8.6	*64*
Berlin	1995	200	33.5[f]	nip	13.0	8.0	34.0	14.0	*61*
Drug abusers		37	59.5	nip	8.0	16.0	43.0	11.0	*61*

Table 3
(continued)

City/ Country	Year	*n*	HIVE	HIVL	CMV	PML	TOXO	LYM	Reference
Homo/ bisexuals		150	28.0	nip	13.0	7.0	35.0	16.0	*61*
Hamburg	1996	28	10.7	nip	3.6	nip	3.6	nip	*8*
New York[g]	1996	471	10.6	nip	2.7	1.9	4.7	3.8	*6*
Asymptomatic		123	0.0	nip	0.0	0.0	0.0	0.0	*6*
Early stages		127	16.5	nip	0.0	0.0	0.0	0.0	*6*
Full-blown AIDS		221	13.1	nip	5.9	4.1	10.0	8.1	*6*
UK	1997[h]	349							
Homo/bisexual		238	21.4	–	20.2	6.7	8.8	13.9	*49*
Injecting drug users		53							
Blood recipient		40	17.5	–	12.5	2.5	5.0	5.0	*49*
Heterosexual		18							
Poland	1997	100	22.0	7.0	23.0	11.0	16.0	10.0	*63*
India	1998	85	0.0	–	7.0	–	13.0	–	*40*
Poland	1998	172	nip	nip	22.7	9.3	16.3	nip	*76*
International	1998	1087	26.4[i]	–	16.4	5.3	16.0	11.1	*14*
Baltimore		293	24.6	–	18.7	3.4	7.5	10.2	*14*
Newark		115	13.0	–	5.2	1.7	13.0	2.6	*14*
Edinburgh		81	44.4	–	13.6	3.7	8.6	16.0	*14*
London		283	22.3	–	18.4	4.9	9.5	11.3	*14*
Paris		243	30.0	–	16.5	10.7	38.7	13.6	*14*
Budapest		72	38.9	–	19.4	4.2	12.5	13.9	*14*
UCSD	2000	390	26.3	nip	22.3	2.9	2.5	8.4	*143*
Austria	2000	450	8.4	4.4	16.9	6.7	19.1	8.0	*16*
1984–1992		190	11.5	2.6	17.4	5.7	24.0	6.3	*16*
1993–1995		162	5.6	6.2	20.0	8.7	20.4	11.2	*16*
1996–1999		98	8.0	5.0	11.0	5.0	8.0	6.0	*16*
pre-HAART, <1996[j]		352	8.5	4.3	18.5	7.1	22.2	8.5	*16*
UCSD	2003	151	38.4	nip	16.5	5.9	2.6	13.2	*201*
Before 1995		62	25.8	nip	16.1	6.4	6.4	19.3	*201*
After 1995		89	43.8	nip	16.8	5.6	0.0	8.9	*201*
France	2003	23	17.4	nip	8.7	17.4	13.0	13.0	*202*
Munich		300	11.1	3.2	13.0	0.8	13.0	7.1	*Weis unpublished*

Table 3
(continued)

[a]Authors report microglial nodules present in 62.9% of the cases without further specification.

[b]13% of the cases with HIVE only, 12% of cases with HIVL only and 13% of cases with HIVE + HIVL.

[c]Subacute microglial encephalitis with and without multinucleated giant cells.

[d]The combination of HIVE + HIVL gave the following results: 6.4% of the total sample, 8.3% in the untreated group, and 3.6% of the zidovudine treated group.

[e]Nodular encephalitis defined as gliomesenchymal cell or microglial nodules and diffuse leukoencephalopathy defined as white matter pallor, demyelination, spongiform change, macrophages, and astrogliosis. Note that there is no specific mention of multinucleated giant cells.

[f]HIV-related encephalopathy defined as vacuolization or spongy changes and astrocytosis in subcortical white matter and, occasionally, in gray matter. Note that there is no specific mention of multinucleated giant cells. MGCs were seen in 17.5% of the cases.

[g]A total of 221 persons from this study were used in a paper published by Kure et al. (*38*).

[h]HIVE and HIVL given together as HIVE/L.

[i]Diagnoses were given as HIVE/L.

[j]This category was created by the authors of the present paper.

nip, no information provided; NYCor, New York Cornell University; UCSF, University of California, San Francisco; UCLA, University of California, Los Angeles; USC, University of Southern California; AU, Austria; I, Italy; CH, Switzerland; Mu, Munich; Oxf/Lon, Oxford/London; UCSD, University of California, San Diego.

tal differences (e.g., United States, European countries), differences between countries (e.g., Switzerland, Austria, Italy, Germany) and differences between various regions within one country.

10.1.2. Composition of the Sample

The composition of the sample according to age (only adults), race (blacks, Caucasians), ethnic origin (Haitians, Hispanics), and risk group (drug addicts, hemophiliacs, homosexuals) might have an influence on the observed differences in the frequency of changes. However, there exists, until now, no paper dealing systematically with this problem.

10.1.3. Source of the Brains Analyzed

In our experience, differences result if brains are derived from Institutes of Pathology or from Institutes of Forensic Medicine. Brains derived from Institutes of Pathology show changes mainly seen in the late stages of the disease. Brains derived from Institutes of Forensic Medicine (composed mainly of brains from autopsied persons who committed suicide or had lethal accidents or died from other non-natural causes) mainly show changes seen in the

Table 4
Frequency of Lesions Encountered in the Late Stages of HIV-1 Infection as Related to Their Topographical Localization in the Brain

Region	HIVE	HIVL	CMV	PML	TOX	CRY	ASP	LYM
Frontal	8.3	2.9	3.4	1.0	8.3	1.9	0.0	5.8
Parietal	3.2	1.6	3.2	1.1	7.4	2.1	0.0	1.6
Temporal	4.7	0.5	6.7	1.0	6.2	1.6	0.5	3.6
Occipital	2.0	1.5	3.9	1.0	7.1	1.5	0.5	4.1
Basal ganglia	5.7	–	7.8	–	8.8	1.6	0.5	4.7
Thalamus	4.9	–	7.4	–	2.5	1.8	0.0	3.1
Cerebellum	3.0	0.0	7.1	0.0	6.6	2.0	0.5	3.0
Mesencephalon	3.7	0.0	3.7	0.5	3.7	1.6	0.5	4.3
Pons	2.6	0.0	5.7	0.0	3.1	1.5	0.0	5.2
Medulla oblongata	2.6	0.0	6.7	0.0	3.1	1.5	0.5	4.1

HIVE, HIV-1 encephalitis; HIVL, HIV-1 leukoencephalopathy; CMV, cytomegalovirus encephalitis; PML, progressive multifocal leukoencephalopathy; TOX, *Toxoplasma gondii* encephalitis; CRY, *Cryptococcus encephalitis*; ASP, *Aspergillus fumigatus* encephalitis; LYM, lymphoma.

early stages of the disease. However, data regarding the time of seroconversion as well as the presence of clinical and neurological signs are often lacking.

10.1.4. Bias in Sampling

The number of patients infected with HIV-1 who undergo an autopsy after death might also have a considerable influence. Most of the time, clinicians are interested to see only the brains from interesting clinical cases to be analyzed by a pathologist/neuropathologist and insist for an autopsy, whereas "uninteresting cases" might not undergo autopsy.

10.1.5. Diagnostic Criteria

The diagnostic criteria may play a fundamental role. The use of immunohistochemistry or *in situ* hybridization to render the diagnosis of HIV encephalitis is not performed in all laboratories dealing with brains infected with HIV-1, thus limiting the reliability of this diagnosis.

10.2. Topographical Distribution of Neuropathological Changes

The topographical localization of the various neuropathological changes has not been described systematically until now. Table 4 shows the results of

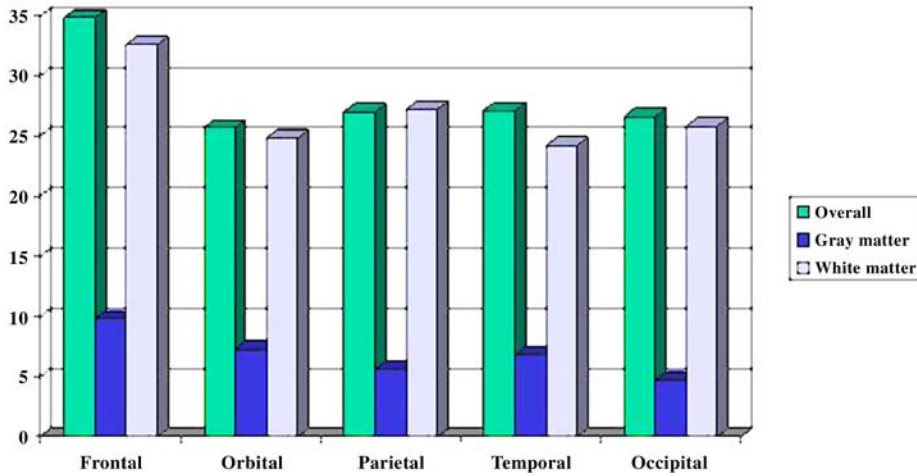

Fig. 9. Frequencies expressed as percent of cases of p24-immunopositive cells in various cortical regions.

a study in our laboratory of 300 cases. No consistent pattern for the distribution of the various neuropathological changes can be discerned (Weis et al., unpublished data).

11. Immunohistochemical Demonstration of HIV-1: Frequency and Topography

The incidence and distribution of HIV-1 antigen, as shown immunohistochemically with an antibody against the glycoprotein p24, is shown in Fig. 9 (Weis et al., unpublished data). Data derived from other systematic analyses are, thus far, lacking.

12. Diagnostic Difficulties: Unspecified Nodular Encephalitis

In many of the brains of patients infected with HIV-1, the only histological finding is the presence of gliomesenchymal nodules (GMNs; Fig. 10). GMNs, which are composed of microglia, macrophages, and reactive astrocytes, are found in the gray and white matter. The diagnostic problem is a result of the fact that there are neither MGCs present in the nodules nor large cells containing inclusion bodies. The differential diagnosis for GMNs includes HIV-1 encephalopathy/leukoencephalopathy, CMV encephalitis or *Toxoplasma*

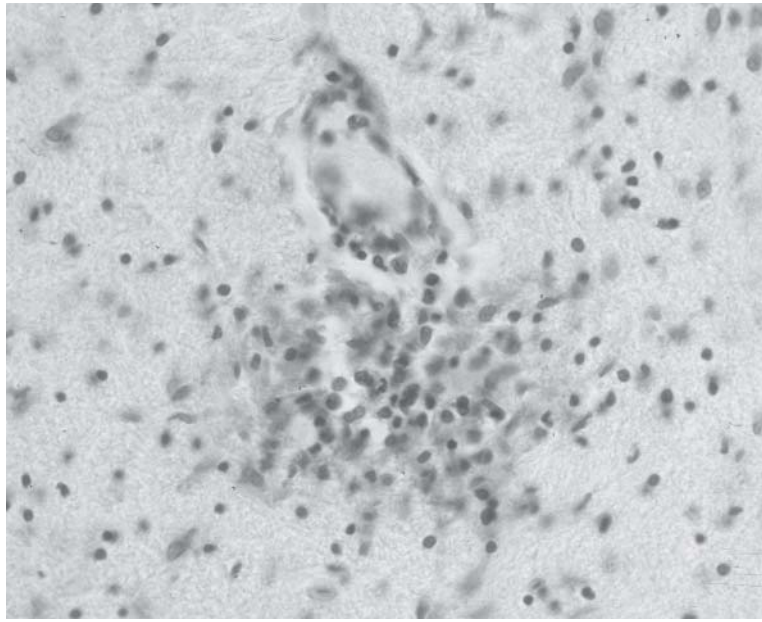

Fig. 10. Unspecified nodular encephalitis (cresyl violet stain).

gondii encephalitis. As proposed by Budka et al. *(32)*, these nodules are very suspicious to contain CMV when further serial sectioning is performed. In our experience, immunohistochemistry for CMV is of limited value. As described by Schmidbauer et al. (*169*), improvement may be provided by *in situ* hybridization, which might yield a positive result in cases with negative immunohistochemistry. In most of the cases, there is no immunohistochemical staining for gp41 or p24. Thus, the term "unspecified nodular encephalitis" is used in the diagnosis of these cases. Complete clarification is sometimes not possible.

13. Neuropathological Changes in Children Infected With HIV-1

Children born to mothers who are infected with HIV-1 also are infected by the virus 10 to 39% of the time *(203)*. These children develop symptoms before the age of 2 years. Approximately 30% of children infected with HIV-1 develop opportunistic infection or HIV-1 encephalopathy within the first year of life. Brain growth is impaired, leading to intellectual deficiency. The macroscopial analysis frequently shows brains that are too small for the child's age, either as microcephaly and/or brain atrophy *(203,204)*. The most com-

mon finding in the brains of children infected with HIV-1 is mineralization/calcification of predominantly small vessels in the basal ganglia found in 95% of the cases *(203,206)*. Myelin pallor and gliosis are noted changes of the white matter and occur in 78% of the cases *(203,205)*. Inflammatory infiltrates and/or MGCs are seen in 62% of children infected with HIV-1 *(203,205)*. HIV-1 infection in fetal brains usually is below the limits of detection of immunocytochemistry, but HIV-1 detection is usually successful with polymerase chain reaction (PCR *[38]*). In contrast to adults, opportunistic infections are comparatively uncommon in the pediatric population *(203)*. For details, the reader is referred to some articles and textbooks *(38,203,205–217)*.

14. Neuropathological Changes in Early Stages of HIV-1 Infection

Gray et al. described early brain changes in 11 HIV-1 seropositive, non-AIDS cases in comparison with 11 HIV-1 seronegative heroin addicts *(218)*. Cerebral vasculitis was significantly more frequent and marked in HIV seropositive cases and was often associated with lymphocytic meningitis. Granular ependymitis, myelin pallor with reactive astrocytosis, and microglial proliferation also were more frequent and more severe in HIV seropositive cases. Immunohistochemistry for HIV antigens was negative. Later, these authors interpreted their results as being related to the occurrence of a usually asymptomatic and transient immunopathological reaction coinciding with early HIV infection of the nervous system *(219)*. This immunological process includes an inflammatory T-cell reaction with vasculitis and leptomeningitis and immune activation of brain parenchyma with an increased number of microglial cells, upregulation of major histocompatibility complex class II antigens, and local production of cytokines *(220,221)*. Some co-authors of the Gray et al. *(220)* review articles reported separately their findings on 36 brains of asymptomatic individuals *(222)*. They detected HIV-1 DNA in 17 cases (47%), astrogliosis in 34 cases (94%), microgliosis in 31 cases (86%), and meningitis in 11 cases (31%). One has to stress that astrogliosis and microgliosis were only rated, but no quantitative data were provided. Furthermore, the authors demonstrated highly expressed cytokines (tumor necrosis factor-α, interleukin [IL]-1, -4, -6). They used these findings to explain neuropathologic changes and neuronal damage confirmed by the demonstration of apoptotic neurons by *in situ* end labeling *(222)*. Interestingly, it was stated in the review article by this group *(220)* that neuronal death is only observed in the late stages of the HIV-1 infection. The same group also reported having detected HIV-1 DNA by PCR in all HIV-1-positive asymptomatic cases and

by *in situ* PCR in astrocytes and endothelial cells, in addition to microglial cells, in 6 of 18 HIV-1-asymptomatic patients *(223)*. The validity of the studies reporting the presence of HIV-1 in various cell types other than macrophages was questioned recently *(224)*.

In an ongoing study of 44 males in our laboratory representing early stages of HIV-1 infection, PLIs or MLIs were found in 70% of the cases. PLI alone was observed in 61% of the cases, MLI alone in 43% of the cases, and the combination of PLI and MLI in 34% of the cases (Weis et al., unpublished data). Sometimes, the extent of lymphocytic infiltrates in the meninges is more than normal but not enough to render a diagnosis of LM. Therefore, we apply the term MLI to describe these changes. Both changes, PLI and MLI, may occur together or separately in the brains of patients with HIV-1 at the early stages of infection. The histopathological changes of vasculitis and leptomeningitis, as reported by Gray et al. *(218–220)*, are rarely seen in the late stages of full-blown AIDS. In our study of 300 cases, we could never identify these changes either in the pre-AIDS stages or the late stages of the infection. Our data are in accordance with findings published by Kibayashi et al. in 1996 on 123 asymptomatic carriers and 127 persons with the early stages of AIDS *(6)*. They found lymphocytic infiltration of the meninges in 24.4% and of the perivascular spaces in 28.5% of the asymptomatic carriers, whereas these respective changes were seen in 11.8% and 11% of the early stage patients.

15. Neuropathological Changes in the HAART Era

In 1995/1996, HAARTs that combine nucleoside reverse transcriptase inhibitors (NRTI) and protease inhibitors (PIs) were introduced in the treatment of patients infected with HIV-1. NRTIs specifically inhibit the viral reverse transcriptase enzyme necessary for DNA chain elongation of the virus. The non-nucleoside reverse transcriptase inhibitors (NNRTI) are similar to the NRTIs in their mechanism of action; however, their effects on reverse transcriptase are by noncompetitive means. The PIs prevent the production of active virus by interfering with the cleavage of proteins necessary for viral assembly *(225)*.

The frequency of HIV-1-related CNS diseases has been reduced by these combinations of antiviral agents, in part through the reduction of both viral load in the blood and continuous penetration of virus into the brain *(226)*, although this therapy will probably not be sufficient to completely abolish any neurological risk. HIV-1-related dementia also depends on the intensity of glial cell activation in the CNS, which induces reactivation of latent infection in these cells, and on the secretion of soluble inflammatory mediators

acting on nearby neurons. During the HAART era, patients who test positive for HIV live longer *(144)*. The median survival after AIDS increased from 19.6 months for those patients diagnosed with AIDS in 1993–1995 to 39.6 months for those diagnosed in 1996–2000 in an Australian patient group *(227)*. The proportion of deaths that followed an HIV-related disease decreased by 23% annually; in contrast, there was a 32% yearly increase in the proportion of deaths caused by known causes other than HIV-related or suicides *(144)*. The higher prevalences of cirrhosis and arteriosclerosis suggest that entities not targeted by antiretroviral reconstitution of immunity will play an increasingly important role in HIV-related mortality in the future *(140)*.

Maschke et al. *(228)* compared the incidence and prevalence in two groups of 563 patients seen between 1995–1996 and 1997–1998. Significantly, more patients received HAART in 1997/1998 and the mean $CD4^+$ cell count was significantly higher in this group. The prevalence of HIV-associated dementia and HIV-associated polyneuropathy were significantly lower in 1997–1998, and the incidence of toxoplasma encephalitis decreased from 5.7% in 1995–1996 to 2.2% in 1997–1998. In a prospective, single-center study of all consecutive patients infected with HIV who presented with focal brain lesions observed between January 1991 and December 1998 ($n = 281$), Ammassari et al. (*229*) reported the following results: during the HAART period, patients were less likely to be male, contracted HIV more often through heterosexual exposure, had fewer previous AIDS-defining events, received anti-toxoplasma prophylaxis less frequently, and had a $CD4^+$ lymphocyte count 2.5 times higher. They found a relevant decline of primary CNS lymphoma, whereas the frequency of toxoplasma encephalitis decreased during the pre-HAART period and was stable afterward. A slight increase was observed over time for PML. Focal white-matter lesions without mass effect or contrast enhancement became the focal brain lesions observed most frequently. In an Australian patient group of 4351 AIDS cases, the proportion of patients with ADC increased from 5.2% in 1993–1995 to 6.8% in 1996–2000. The median survival after ADC increased from 11.9 months in 1993–1995 to 48.2 in 1996–2000. Most striking was the increase in survival among those patients with ADC and a CD4 cell count below100×10^6 cells/L at diagnosis (5.1 months in 1993–1995 to 38.5 months in 1996–2000 *[227]*).

Larger cohort autopsy studies of HIV-infected patients over longer time periods suggest that, despite the beneficial effects of modern anti-retroviral combination therapy, involvement of the brain in AIDS subjects continues to be a frequent autopsy finding *(16,55,143,153,154,230)*.

Vago et al. *(230)* recently reviewed 1597 consecutive autopsies of HIV-1-positive patients performed between 1984 and 2000 and divided into four

Table 5
Incidence of HIVE/L and Opportunistic Infections Under Different Theraputic Regimes

Year infections	*n*	Therapy	HIVE/L	Opportunistic
1984–1987	119	None	53.8	40.3
1988–1994	1116	Mono	32.2	46.8
1995–1996	256	Dual	17.9	42.6
1997–2000	106	Triple	15.1	42.5

From ref. *230*.

time periods on the basis of the therapeutic regimens available: 1984–1987: no therapy; 1988–1994: monotherapy (zidovudine); 1995–1996: dual combination therapy with NRTIs; and 1997–2000: triple combination therapy, including two NRTIs and at least one PI or non-NRTI (*230*). They reported the results for HIV-related lesions (including HIVE and HIVL) and opportunistic infectious diseases (without specifying the causative agent) as shown in Table 5.

The results of other autopsy studies with more detailed information of neuropathological entities are listed in Table 6 *(16,55,143,202)*. The most consistent feature in it is its inconsistency of results. Thus, no consistent trend for the frequency of HIV-1-related changes is seen. In one study the frequency decreases, whereas in a second study it remains stable but increases dramatically in a third study. The frequency of CMV was reduced in two of three studies. PML and non-Hodgkin's lymphomas were increased in frequency in one study, whereas the frequency was unchanged in two studies. In all three studies, the frequency of toxoplasma encephalitis was significantly reduced.

Gray et al. speculated that the overall decrease in incidence of cerebral toxoplasmosis, CMV encephalitis, and HIVE was the result of successful treatment *(202)*. They also described a new variant of HIVE characterized by severe leukoencephalopathy with intense perivascular macrophage and lymphocytic infiltration, possibly the result of an exaggerated response from a newly reconstituted immune system. Furthermore, they detected chronic "burnt-out" forms of HIVE as well as varicella zoster virus encephalitis, toxoplasmosis, and PML, possibly associated with prolonged survival, in which neither inflammation nor organisms, could be detected.

In contrast, Langford et al. suggested that the increasing resistance of HIV strains to antiretrovirals led to the resurgence in the frequency of HIVE and HIVL *(201)*. HIVE and HIVL in AIDS patients failing HAART is charac-

Table 6
Frequencies of Various Neuropathological Changes in the Pre-HAART Era Compared With the HAART Era (in %; n = sample size)

Author (year) (Reference)	*n*	HIVE	HIVL	CMV	PML	TOX	LYM
Gray et al. (1988)							
(55) pre-HAART	40	37.5	nip	20.0	5.0	47.5	2.5
Gray et al. (2003)							
(202)[a] HAART	23	17.4	nip	8.7	17.4	13.0	13.0
Jellinger et al. (2000)							
(16) pre-HAART	352	8.5	4.3	18.5	7.1	22.2	8.5
HAART	98	8.0	5.0	11.0	5.0	8.0	6.0
Langford et al. (2003)							
(201) pre-HAART	62	25.8	nip	16.1	6.4	6.4	19.3
HAART	89	43.8	nip	16.8	5.6	0.0	8.9

[a]Frequencies for the pre-HAART could not be calculated because of the lack of original data in this publication.

HIVE, HIV-1 encephalitis; HIVL, HIV-1 leukoencephalopathy; CMV, cytomegalovirus encephalitis; PML, progressive multifocal leukoencephalopathy; TOX, *Toxoplasma gondii* encephalitis; LYM, primary non-Hodgkin's lymphoma; nip, no information provided.

terized by massive infiltration of HIV-infected monocytes/macrophages into the brain and extensive white matter destruction. This condition might be attributable to interactions of anti-retrovirals with cerebrovascular endothelium, astroglial cells, and white matter of the brain. These interactions might lead to cerebral ischemia, increased BBB permeability and demyelination. The authors postulate that potential mechanisms of such interactions include alterations in host cell signaling that may result in trophic factor dysregulation and mitochondrial injury *(201)*.

Future studies have to address the above-mentioned points by systematic investigation of the various cell systems (e.g., neurons, astrocytes, microglia, oligodendrocytes) in much larger samples. Before reaching final conclusions about the changed pattern of neuropathological changes in the HAART era, one has also to prove that these reported divergent results are not due to a sampling error, for example, the most interesting cases undergo autopsy, whereas the less interesting cases are not autopsied.

Table 7
Incidence of Cerebral Infarcts and Cerebral Hemorrhages in Larger Autopsy Series of Patients Infected With HIV-1

Authors (Year) (Reference)	Ischemic stroke	Intracranial hemorrhage
Anders et al. (1986) *(29)*	14.6% (13/89)	5,6% (5/89)
Berger et al. (1990) *(231)*	7.2 % (13/181)	nip
Budka et al. (1987) *(32)*	nip	11.0% (11/100)
Burns et al. (1991) *(33)*	7.8% (11/141)	5.7% (8/141)
Jellinger et al. (2000) *(16)*	5.6–6.3%	1.2–4.0%
Kieburtz et al. (1993) *(235)*	30% (14/70)	nip
Kure et al. (1991) *(38)*	19.5% (43/221)	5.4% (12/221)
Mizusawa et al. (1988) *(236)*	29% (24/83)	6% (5/83)
Moskowitz et al. (1984) *(41)*	3.9% (2/52)	8.0% (4/52)
Rhodes et al. (1993) *(70)*	9.0% (36/400)	5.8% (23/400)

nip, no information provided.

16. Cerebrovascular Complications

Several case reports and larger autopsy series described cerebrovascular complications in patients with HIV-1 infection *(16,29,32,33,38,41,47,70,231–237)*. Cerebrovascular disease has been reported to occur in about 10% of asymptomatic HIV-1 carriers, 7% of patients with early AIDS, and 5% of patients with fully developed AIDS *(6)*. Autopsy series of patients with AIDS have found a 4% to 30% prevalence of cerebral infarction and 1.2% to 11% of intracranial hemorrhage (Table 7). Cerebral infarcts were mostly caused by nonbacterial thrombotic endocarditis or concomitant opportunistic CNS infection, whereas intracerebral hemorrhages were usually associated with thrombocytopenia or primary CNS lymphoma. In a recent study it could be demonstrated that AIDS patients have an increased risk of stroke with an adjusted relative risk of 9.1 for ischemic stroke and 12.7 for intracerebral hemorrhage *(238)*. According to the authors, AIDS is strongly associated with both ischemic stroke and intracranial hemorrhage.

Besides these macromorphological changes, alterations of the BBB could be demonstrated by several research groups (*see* Section 17). Furthermore, an HIV-1-associated vasculopathy with small vessel thickening, rarefaction, perivascular pigment deposition, and vessel wall mineralization has been described in HIV-1-infected patients *(232,239)*, but a true cerebral vasculitis is exceptionally rare *(240)*.

17. Pathogenetic Mechanisms

The pathogenetic mechanisms induced by the HIV-1 infection and leading to the multiple facets of brain damage are not yet clearly understood. A complete review of the proposed mechanisms is not within the scope of the present chapter; rather, we elected to briefly describe some of the most probable aspects.

17.1. Mode of Entrance of HIV-1 to the Brain

HIV-1 is believed to enter the CNS by being passively carried by T-lymphocytes and monocytes, that is, the "Trojan Horse" hypothesis *(77,241,242)*. Recent evidence suggests that cell-free HIV-1 particles may also penetrate brain microvascular endothelial cells *(243)*. After crossing the BBB into the CNS, macrophages spread productive HIV-1 infection to neighboring microglia *(77,89,105,118,244)*. The potential role of the CSF or the choroid plexus as a means for HIV-1 entry in the brain is still unclear *(245)*. At the time of primary HIV-1 infection, an acute aseptic meningitis or encephalitis indicates CNS invasion *(103)*. Opportunistic infectious agents or drugs of abuse disturbing the BBB may further attract more HIV-1 infected T-lymphocytes and macrophages into the brain. The point in time when the migration of HIV-1-infected lymphocytes into the brain takes place is not known. It has been shown that, at the time of seroconversion, HIV-1 can be detected in the CSF; this is the time when, clinically, a subacute meningitis develops, thus, suggesting that HIV-1 enters the CNS at a very early stage of the disease *(96,246)*.

17.2. Target Cells of HIV-1

Although it has been claimed that HIV-1 is neurotrophic, the few articles describing the localization of HIV-1 in nerve cells have been subsequently disproved *(59,71,89,247)*. Despite the detection of HIV-1 proteins in endothelial cells *(248)*, it is now generally believed that productive HIV-1 infection of the cerebral endothelium does not occur *(103,137,247)*. Furthermore, there is only infection at low level in a subset of astrocytes *(71,103,249)*. Bissel and Wiley *(224)* reviewed the evidence of productive and nonproductive infection for each cell type (neurons, astrocytes, oligodendrocytes, microglia/macrophages, endothelial cells) in the brains of patients with HIV with and without HIV encephalitis. They concluded that despite the voluminous literature and substantial experimental effort during the past two decades, evidence for productive infection of any brain cell other than macrophages is weak.

The cells in the brain identified to contain HIV-1 are the microglia, macrophages, and MGCs *(59,71,84,89,109,116–118,137,152,247,250,251)*.

Microglia and monocyte-derived macrophages are the only cells in the CNS that express both the CD4 and chemokine coreceptors (CCR5, CXCR4), the prerequisite for HIV-1 to enter a cell *(77,252,253)*. The HIV-1 glycoprotein-mediated syncytia formation, which results from fusion of microglia or brain macrophages, may be observed as multinucleated giant cells (*see* Subheading 4.1.). It has been demonstrated that perivascular macrophages are a major CNS cell population that is productively infected with HIV-1. Such productive infection occurs with an accumulation of macrophages in perivascular cuffs, an event that correlates with entry of HIV-1 to the CNS *(241)*. Brain macrophages and microglia serve as a reservoir for persistent viral infection, a vehicle for viral dissemination throughout the brain, and a major source of neurotoxic products that, when produced in abundance, affect neuronal function and finally lead to cell death *(89,105,118,155)*. How HIV-1 evades the immune function characteristic for these cells as a first line of defense is still unclear.

In vitro test system by cell culture enables one to infect neuronal, astroglial, and endothelial cell lines, but does not seem to be the appropriate medium to test the infectivity of cell groups. Moreover, these results contradict the in vivo situation of the human brain.

17.3. Mechanisms of Brain Lesions

HIV-1 infection of the CNS occurs early after infection in the periphery, from hours to days, but neurological symptoms and HIV-associated dementia often occur years later, concomitant with or after the development of AIDS. Because productive infection reemerges with the development of AIDS, it is suggested that virus that enter the CNS early after infection can become latent and then reemerge with AIDS or that productive infection, which results with the development of AIDS, occurs from reseeding new virus from the periphery *(241)*.

The development of brain lesions from opportunistic infections and lymphomas might be explained by the lack of a competent immunological defense system. The clinical signs and symptoms can be correlated with the site of the lesion as well as the brain structures involved.

In contrast, changes caused by direct or rather indirect effects of HIV-1 are more controversially discussed. Data based on systematic morphometrical analyses of the various constituents of the brain give new insight into the damage of the brain caused by HIV-1 *(147–149,254–256)*. Neuronal loss was shown by independent research groups to occur in the frontal, parietal, and temporal cortex *(50,257,258)*. Ketzler et al. (*257*), Weis et al. (*258*), and Weis (*256*) showed that this neuronal dropout occurs in brain regions that are free from any neuropathological changes. Others showed that neuronal damage in AIDS

was, at least, partly caused by apoptosis *(259–262)*. However, no correlation was found between the presence and severity of neuronal loss or of neuronal apoptosis and a history of cognitive disorders.

Weis et al. *(263)* showed an increase of glial fibrillary acidic protein-positive astrocytes, a decrease of glial fibrillary acidic protein-negative astrocytes, but no changes in the total number of astroglial cells. The reactive astrogliosis was not correlated with the loss of nerve cells, indicating that this reaction pattern is rather a response to toxic factors secreted into the brain tissue.

In addition, significant structural and functional abnormalities in the microvasculature have been identified, including serum protein leakage *(264–266)*, alterations in endothelial cells and basement membranes *(267–269)*, as well as disruption of tight junctions *(252,270–272)*, suggestive for BBB damage in the course of HIV-1 infection. These alterations are believed to be the result of a complex cascade of events and molecules involving HIV-1 proteins as well as products of monocytes, microglia, astrocytes, and activated brain endothelial cells *(155,244,246,252,273–277)*.

One might assume that the above-described changes might result from infection with HIV-1. However, it has been shown that neither neurons, nor astrocytes, nor endothelial cells are significantly infected with HIV-1 *(103)*. Therefore, these changes are more probably the result of indirect toxic factors that are produced either by infected MGCs or by activated microglia, or neurotoxic viral proteins *(77,84,89,107–109,116,275)*.

The number of activated microglia/macrophages is significantly increased in all brain regions *(278)*. This activation of microglia is not correlated with the presence of HIV-1 antigen in the brain tissue. This result might give a hint that, most probably, the activated microglia/macrophages, rather than MGCs, secrete toxic factors. However, this hypothesis remains to be proven. How a finite number of infected macrophages/microglia, localized in particular areas of the brain, can lead to widespread neuronal injury is summarized below.

The neurotoxicity associated with HIV-1 infection is mediated, in part, through cytokines and arachidonic acid metabolites, produced during cell-to-cell interactions between HIV-1 infected brain macrophages and astrocytes *(84,89,103,105,118,155,279–281)*. The relationship between viral proteins, CA^{2+} channels, NMDA receptors, chemokines, and cytokines on one hand and cell damage on the other is summarized elsewhere *(84,89,93,94,103,155,282–286)*.

Briefly, the pathobiological events underlying the neurodegenerative processes in HIV-1-associated dementia are believed to begin with productive infection of monocytes/macrophages by HIV-1. Peripheral activation causes

the differentiation of macrophages to produce a variety of immune products that lead to the upregulation of adhesion molecules on brain microvascular endothelial cells and the expression of cytokines on the monocyte-macrophage cell surface. After penetration of the BBB, the differentiated brain macrophages and microglia can be vehicles for viral dissemination throughout the brain and focal reservoirs for productive HIV-1 replication. The neurotoxic events in the brain are caused by neurotoxins produced by these cells, which are primed by HIV-1 and secondarily activated by factors such as immune stimuli or by T-cells trafficking through the nervous system. The primed and immune-activated brain macrophages/microglia secrete a variety of factors that affect neural and glial function and eventually lead to CNS inflammation. A proinflammatory cytokine response from blood-derived monocytes/macrophages, microglia, and astrocytes is amplified and leads finally to neurodegeneration. Immune neurotoxic factors may contribute to the breakdown of the BBB and affect the generation of chemokines, leading to transendothelial migration of monocytes into the brain perpetuating the inflammatory cascade. As a result of the neurotoxic activities of activated macrophages/microglia, astrocytes may suppress or increase macrophages/microglia secretory functions and toxicity, depending on the astrocytic functional status. Cytolytic T-lymphocytes serve to eliminate infected cells, but are lost in late-stage HIV-1 disease, allowing the virus-induced, neurodegenerative response to continue unabated *(84,89,105,118,155,244)*.

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References

1. Appel PW, Joseph H, Richman BL (2000) Causes and rates of death among methadone maintenance patients before and after the onset of the HIV/AIDS epidemic. Mt Sinai J Med 67, 444–451.
2. Bell JE, Arango JC, Robertson R, Brettle RP, Leen C, Simmonds P (2002) HIV and drug misuse in the Edinburgh cohort. J Acquir Immune Defic Syndr 31, Suppl 2, S35–S42.
3. Farabee D, Prendergast M, Cartier JA (2002) Methamphetamine use and HIV risk among substance-abusing offenders in California. J Psychoactive Drugs 34, 295–300.
4. Fehlauer F, Koops A, Lockemann U, et al. (1999) Prävalenz von Hepatitis B, Hepatitis C und HIV-Infektionen bei Drogentodesfällen in Hamburg (1985 bis 1997) unter Berücksichtigung von epidemiologischen, forensischen und morphologischen Aspekten. Rechtsmedizin 9, 205–209.

5. Hagan H, Des Jarlais DC (2000) HIV and HCV infection among injecting drug users. Mt Sinai J Med 67, 423–428.
6. Kibayashi K, Mastri AR, Hirsch CS (1996) Neuropathology of human immunodeficiency virus infection of different disease stages. Hum Pathol 27, 637–642.
7. Lockemann U, Wischhusen F, Püschel K (1997) Entwicklung der HIV-1-Prävalenz bei Drogentodesfällen in Deutschland und Europa—Analyse des vergangenen Jahrzehnts (1985–1994). Rechtsmedizin 7, 117–120.
8. Makrigeorgi-Butera M, Hagel C, Laas R, Püschel K, Stavrou D (1996) Comparative brain pathology of HIV-seronegative and HIV-infected drug addicts. Clin Neuropathol 15, 324–329.
9. Penning R, Tutsch-Bauer E, Beer G, Gürtler L, Spann W (1989) HIV-Infektion bei gerichtlichen Leichenöffnungen. Beitr Gerichtl Med 47, 23–29.
10. Püschel K (1993) HIV-1-Prävalenz bei Drogentoten in der Bundesrepublik Deutschland sowie im internationalen Vergleich (Stand 31.12.1991). Rechtsmedizin 3, 40–43.
11. Zappi E, Zappi M, Zugibe FT (1995) Disseminated cryptococcosis and sudden death. Report of an autopsy case. Am J Forensic Med Pathol 16, 38–41.
12. Bouwman FH, Skolasky R, Hes D, et al. (1998) Variable progression of HIV-associated dementia. Neurology 50, 1814–1820.
13. Chao CC, Gekker G, Sheng WS, Hu S, Tsang M, Peterson PK (1994) Priming effect of morphine on the production of tumor necrosis factor-alpha by microglia: implications in respiratory burst activity and human immunodeficiency virus-1 expression. J Pharmacol Exp Ther 269, 198–203.
14. Davies J, Everall IP, Weich S, et al. (1998) HIV-associated brain pathology: a comparative international study. Neuropathol Appl Neurobiol 24, 118–124.
15. Goodkin K, Shapshak P (1998) Cocaine abuse and HIV-1 infection: epidemiology and neuropathogenesis. J Neuroimmunol 83, 88–101.
16. Jellinger K, Setinek U, Drlicek M, Böhm G, Steurer A, Lintner F (2000) Neuropathology and general autopsy findings in AIDS during the last 15 years. Acta Neuropathol 100, 213–220.
17. Li Y, Wang X, Tian S, Guo CJ, Douglas SD, Ho W-Z (2002) Methadone enhances human immunodeficiency virus infection of human immune cells. J Infect Dis 185, 118–122.
18. Li Y, Merrill JE, Mooney K, et al. (2003) Morphine enhances HIV infection of neonatal macrophages. Pediatr Res 54, 282–288.
19. Maragos WF, Young KL, Turchan JT, et al. (2002) Human immunodeficiency virus-1 Tat protein and methamphetamine interact synergistically to impair striatal dopaminergic function. J Neurochem 83, 955–963.
20. Marder K, Liu X, Stern Y, et al. (1995) Risk of human immunodeficiency virus type 1-related neurologic disease in a cohort of intravenous drug users. Arch Neurol 52, 1174–1182.
21. Nath A, Maragos WF, Avison MJ, Schmitt FA, Berger JR (2001) Acceleration of HIV dementia with methamphetamine and cocaine. J Neurovirol 7, 66–71.
22. Nath A, Hauser KF, Wojna V, et al. (2002) Molecular basis for interactions of HIV and drugs of abuse. J Acquir Immune Defic Syndr 31, Suppl 2, S62–S69.

23. Shapshak P (1996) HIV-1 neuropathogenesis and abused drugs. Current views, problems, and solutions. Adv Exp Med Biol 402, 171–186.
24. Tyor WR, Middaugh LD (1999) Do alcohol and cocaine abuse alter the course of HIV-associated dementia complex? J Leukoc Biol 65, 475–481.
25. Zhang L, Looney D, Taub D, et al. (1998) Cocaine opens the blood-brain barrier to HIV-1 invasion. J Neurovirol 4, 619–626.
26. Arango JC, Simmonds P, Brettle RP, Bell JE (2004) Does drug abuse influence the microglial response in AIDS and HIV encephalitis? AIDS 18, Suppl 1, S69–S74.
27. Tomlinson GS, Simmonds P, Busuttil A, Chiswick A, Bell JE (1999) Upregulation of microglia in drug users with and without pre-symptomatic HIV infection. Neuropathol Appl Neurobiol 25, 369–379.
28. Basso M, Bornstein RA (2000) Neurobehavioural consequences of substance abuse and HIV infection. J Psychopharmacol 14, 228–237.
29. Anders KH, Guerra WF, Tomiyasu U, Verity MA, Vinters HV (1986) The neuropathology of AIDS. UCLA experience and review. Am J Pathol 124, 537–558.
30. Budka H (1989) Human immunodeficiency virus (HIV)-induced disease of the central nervous system: pathology and implications for pathogenesis. Acta Neuropathol 77, 225–236.
31. Budka H, Wiley CA, Kleihues P, et al. (1991) HIV-associated disease of the nervous system: review of nomenclature and proposal for neuropathology-based terminology. Brain Pathol 1, 143–152.
32. Budka H, Costanzi G, Cristina S, et al. (1987) Brain pathology induced by infection with the human immunodeficiency virus (HIV). A histological, immunocytochemical, and electron microscopical study of 100 autopsy cases. Acta Neuropathol 75, 185–198.
33. Burns DK, Risser RC, White CL (1991) The neuropathology of human immunodeficicency virus infection. The Dallas, Texas, Experience. Arch Pathol Lab Med 115, 1112–1124.
34. De Girolami U, Smith TW, Hénin D, Hauw JJ (1990) Neuropathology of the acquired immunodeficiency syndrome. Arch Pathol Lab Med 114, 643–655.
35. Gullotta F, Kuchelmeister K, Masini T Ghidoni P, Cappricci E (1989) Zur Morphologie der HIV-Enzephalopathie. Zentralbl allg Pathol pathol Anat 135, 5–13.
36. Kanzer MD (1990) Neuropathology of AIDS. Crit Rev Neurobiol 5, 313–362.
37. Kato T, Hirano A, Llena JF, Dembitzer HM (1987) Neuropathology of acquired immune deficiency syndrome (AIDS) in 53 autopsy cases with particular emphasis on microglial nodules and multinucleated giant cells. Acta Neuropathol 73, 287–294.
38. Kure K, Llena JF, Lyman WD, et al. (1991) Human immunodeficiency virus-1 infection of the nervous system: an autopsy study of 268 adult, pediatric, and fetal brains. Hum Pathol 22, 700–710.
39. Lang W, Miklossy J, Deruaz JP, et al. (1989) Neuropathology of the acquired immune deficiency syndrome (AIDS): a report of 135 consecutive autopsy cases from Switzerland. Acta Neuropathol 77, 379–390.
40. Lanjewar DN, Jain PP, Shetty CR (1998) Profile of central nervous system pathology in patients with AIDS: an autopsy study from India. AIDS 12, 309–313.

41. Moskowitz LB, Hensley GT, Chan JC, Gregorios J, Conley FK (1984) The neuropathology of acquired immune deficiency syndrome. Arch Pathol Lab Med 108, 867–872.
42. Navia BA, Cho EK, Petito CK, Price RW (1986) The AIDS dementia complex: II. Neuropathology. Ann Neurol 19, 525–535.
43. Petito CK, Cho EK, Lemann W, Navia BA, Price RW (1986) Neuropathology of acquired immunodeficiency syndrome (AIDS): an autopsy review. J Neuropathol Exp Neurol 45, 635–646.
44. Sharer LR (1992) Pathology of HIV-1 infection of the central nervous system. A review. J Neuropathol Exp Neurol 51, 3–11.
45. Vinters HV, Anders KH (1990) Neuropathology of AIDS. CRC Press, Boca Raton.
46. Artigas J, Grosse G, Niedobitek F (1993) The Central Nervous System in AIDS. Neurology, Radiology, Pathology, Ophthalmology. Springer, Berlin.
47. Chimelli L, Rosemberg S, Hahn MD, Lopes MBS, Barretto Netto M (1992) Pathology of the central nervous system in patients infected with the human immunodeficiency virus (HIV): a report of 252 autopsy cases from Brazil. Neuropathol Appl Neurobiol 18, 478–488.
48. Cornford ME, Holden JK, Boyd MC, Berry K, Vinters HV (1992) Neuropathology of the acquired immune deficiency syndrome (AIDS): report of 39 autopsies from Vancouver, British Columbia. Can J Neurol Sci 19, 442–452.
49. Davies J, Everall IP, Weich S, McLaughlin J, Scaravilli F, Lantos PL (1997) HIV-associated brain pathology in the United Kingdom: an epidemiological study. AIDS 11, 1145–1150.
50. Everall IP, Luthert PJ, Lantos PL (1993) A review of neuronal damage in human immunodeficiency virus infection: its assessment, possible mechanism and relationship to dementia. J Neuropathol Exp Neurol 52, 561–566.
51. Funata N, Maeda Y, Koike M, Okeda R (1991) Neuropathology of the central nervous system in acquired immune deficiency syndrome (AIDS) in Japan. With special reference to human immunodeficiency virus-induced encephalomyelopathies. Acta Pathol Jpn 41, 206–211.
52. Giampalmo A, Pesce C, Ardoino S, Provaggi MA, Quaglia AC (1989) Neuropathological findings in an autopsy series of Italian subjects with AIDS. Clin Neuropathol 8, 120–125.
53. Gonzales MF, Davis RL (1988) Neuropathology of acquired immunodeficiency syndrome. J Neuropathol Exp Neurol 14, 345–363.
54. Gray F (1993) Atlas of the Neuropathology of HIV Infection. Oxford University Press, Oxford.
55. Gray F, Gherardi R, Scaravilli F (1988) The neuropathology of the acquired immune deficiency syndrome (AIDS). A review. Brain 111, 245–266.
56. Hall WW, Farmer PM, Takahashi H, Tanaka S, Furuta Y, Nagashima K (1991) Pathological features of virus infections of the central nervous system (CNS) in the acquired immunodeficiency syndrome (AIDS). Acta Pathol Jpn 41, 172–181.
57. Jensen OA, Klinken L (1989) Pathology of brain and eye in the acquired immune deficiency syndrome (AIDS). APMIS 97, 325–333.

58. Kibayashi K, Ng'walali PM, Mbonde M, et al. (1999) Neuropathology of human immunodeficiency virus 1 infection. Significance of studying in forensic autopsy cases at Dar es Salaam, Tanzania. Arch Pathol Lab Med 123, 519–523.
59. Koenig S, Gendelman HE, Orenstein JM, et al. (1986) Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. Science 233, 1089–1093.
60. Lantos PL, McLaughlin JE, Schoitz CL, Berry CL, Tighe JR (1989) Neuropathology of the brain in HIV infection. Lancet 1, 309–311.
61. Martínez AJ, Sell M, Mitrovics T, et al. (1995) The neuropathology and epidemiology of AIDS. A Berlin experience. A review of 200 cases. Pathol Res Pract 191, 427–443.
62. Matthiessen L, Marche C, Labrousse F, Trophilme D, Fontaine C, Vedrenne C (1992) Etude neuropathologique de l'encéphale de 174 patients morts du SIDA dans un hôpital parisien, de 1982 à 1988. Ann Med Interne (Paris) 143, 43–49.
63. Mossakowski MJ, Zelman IB (1997) Neuropathological syndromes in the course of full blown acquired immune deficiency syndrome (AIDS) in adults in Poland (1987–1995). Folia Neuropathol 35, 133–143.
64. Neuen-Jacob E, Figge C, Arendt G, Wendtland B, Jacob B, Wechsler W (1993) Neuropathological studies in the brains of AIDS patients with opportunistic diseases. Int J Legal Med 105, 339–350.
65. Nielsen SL, Petito CK, Urmacher CD, Posner JB (1984) Subacute encephalitis in acquired immune deficiency syndrome: a postmortem study. Am J Clin Pathol 82, 678–682.
66. Nielsen SL, Davis RL (1988) Neuropathology of acquired immunodeficiency syndrome. In Rosenblum ML, Levy RM, Bredesen DE, eds., AIDS and the Nervous System. Raven Press, New York, pp. 155–181.
67. Petito CK (1988) Review of central nervous system pathology in human immunodeficiency virus infection. Ann Neurol 23, Suppl, 54–57.
68. Rao C, Anzil AP, Hollenberg Sher J (1993) The neuropathology of AIDS: a review. Adv Neuroimmunol 3, 1–15.
69. Rhodes RH (1987) Histopathology of the central nervous system in the acquired immunodeficiency syndrome. Hum Pathol 18, 636–643.
70. Rhodes RH (1993) Histopathologic features in the central nervous system of 400 acquired immunodeficiency syndrome cases: implications of rates of occurrence. Hum Pathol 24, 1189–1198.
71. Rosenblum MK (1990) Infection of the central nervous sytem by the human immunodeficiency virus type 1. Morphology and relation to syndromes of progressive encephalopathy and myelopathy in patients with AIDS. Pathol Annu 25, 117–169.
72. Rosenblum ML, Levy RM, Bredesen DE (1988) AIDS and the Nervous System. Raven Press, New York.
73. Scaravilli F (1993) The Neuropathology of HIV Infection. Springer, London.
74. Sharer LR, Kapila R (1985) Neuropathologic observations in acquired immunodeficiency syndrome (AIDS). Acta Neuropathol 66, 188–198.

75. Vinters HV, Tomiyasu U, Anders KH (1989) Neuropathologic complications of infection with the human immunodeficiency virus (HIV). In Rotterdam H, Sommers SP, Raes P, Meyer PR, eds., Progress in AIDS Pathology. Field & Wood, New York, NY, pp. 101–130.
76. Zelman IB, Mossakowski MJ (1998) Opportunistic infections of the central nervous system in the course of acquired immune deficiency syndrome (AIDS). Morphological analysis of 172 cases. Folia Neuropathol 36, 129–144.
77. Albright AV, Soldan SS, González-Scarano F (2003) Pathogenesis of human immunodeficiency virus-induced neurological disease. J Neurovirol 9, 222–227.
78. Brew BJ (1994) The clinical spectrum and pathogenesis of HIV encephalopathy, myelopathy, and peripheral neuropathy. Curr Opin Neurol 7, 209–216.
79. Epstein LG, Gendelman HE (1993) Human immunodeficiency virus type I infection of the nervous system: pathogenetic mechanisms. Ann Neurol 33, 429–436.
80. Esiri MM, Scaravilli F, Millard PR, Harcourt-Webster JN (1989) Neuropathology of HIV infection in haemophiliacs: comparative necropsy study. Br Med J 299, 1312–1315.
81. Fauci AS (1988) The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. Science 239, 617–622.
82. Gabuzda DH, Johnson RT (1990) Nervous system infection with human immunodeficiency virus: biology and pathogenesis. Curr Asp Neurosci 1, 285–309.
83. Gartner S (2000) HIV infection and dementia. Science 287, 602–604.
84. Gendelman HE, Lipton SA, Tardieu M, Bukrinsky MI, Nottet HSLM (1994) The neuropathogenesis of HIV-1 infection. J Leukoc Biol 56, 389–398.
85. Glass JD, Johnson RT (1996) Human immunodeficiency virus and the brain. Annu Rev Neurosci 19, 1–26.
86. Ho DD, Pomerantz RJ, Kaplan JC (1987) Pathogenesis of infection with human immunodeficiency virus. N Engl J Med 317, 278–286.
87. Johnson RT (1995) The pathogenesis of HIV infections of the brain. Curr Top Microbiol Immunol 202, 1–10.
88. Johnson RT, McArthur JC, Narayan O (1988) The neurobiology of human immunodeficiency virus infections. FASEB J 2, 2970–2981.
89. Kaul M, Garden GA, Lipton SA (2001) Pathways to neuronal injury and apoptosis in HIV-associated dementia. Nature 410, 988–994.
90. Kolson DL (2002) Neuropathogenesis of central nervous system HIV-1 infection. Clin Lab Med 22, 703–717.
91. Kolson DL, Lavi E, González-Scarano F (1998) The effects of human immunodeficiency virus in the central nervous system. Adv Virus Res 50, 1–47.
92. Levy JA (1993) Pathogenesis of human immunodeficiency virus infection. Microbiol Rev 57, 183–289.
93. Lipton SA (1991) HIV-related neurotoxicity. Brain Pathol 1, 193–199.
94. Lipton SA, Yeh M, Dreyer EB (1994) Update on current models of HIV-related neuronal injury: platelet-activating factor, arachidonic acid and nitric oxide. Adv Neuroimmunol 4, 181–188.
95. Masliah E, Ge N, Mucke L (1996) Pathogenesis of HIV-1 associated neurodegeneration. Crit Rev Neurobiol 10, 57–67.

96. Michaels J, Sharer LR, Epstein LG (1988) Human immunodeficiency virus type 1 (HIV-1) infection of the nervous system: a review. Immunodef Rev 1, 71–104.
97. Navia BA, Jordan BD, Price RW (1986) The AIDS dementia complex: I. Clinical features. Ann Neurol 19, 517–524.
98. Power C, Johnson RT (1995) HIV-1 associated dementia: clinical features and pathogenesis. Can J Neurol Sci 22, 92–100.
99. Price RW (1996) Neurological complications of HIV infection. Lancet 348, 445–452.
100. Price RW, Perry SW (1992) HIV, AIDS and the brain. J Neurol Sci 113, 125–132.
101. Price RW, Brew BJ, Sidtis J, Rosenblum M, Scheck AC, Cleary P (1988) The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. Science 239, 586–592.
102. Rafalowska J (1998) HIV-1 infection in the CNS. A pathogenesis of some neurological syndromes in the light of recent investigations. Folia Neuropathol 36, 211–216.
103. Sotrel A, Dal Canto MC (2000) HIV-1 and its causal relationship to immunosuppression and nervous system disease in AIDS: a review. Hum Pathol 31, 1274–1298.
104. Spencer DC, Price RW (1992) Human immunodeficiency virus and the central nervous system. Annu Rev Microbiol 46, 655–693.
105. Swindells S, Zheng J, Gendelman HE (1999) HIV-associated dementia: new insights into disease pathogenesis and therapeutic interventions. AIDS Patient Care STDs 13, 153–163.
106. Tucker T (1989) Central nervous system AIDS. J Neurol Sci 89, 119–133.
107. Tyor WR, Wesselingh SL, Griffin JW, McArthur JC, Griffin DE (1995) Unifying hypothesis for the pathogenesis of HIV-associated dementia complex, vacuolar myelopathy, and sensory neuropathy. J Acquir Immune Defic Syndr Hum Retrovirol 9, 379–388.
108. van de Bovenkamp M, Nottet HSLM, Pereira CF (2002) Interactions of human immunodeficiency virus-1 proteins with neurons: possible role in the development of human immunodeficiency virus-1-associated dementia. Eur J Clin Invest 32, 619–627.
109. Williams KC, Hickey WF (2002) Central nervous system damage, monocytes and macrophages, and neurological disorders in AIDS. Annu Rev Neurosci 25, 537–562.
110. Zink WE, Zheng J, Persidsky Y, Poluektova L, Gendelman HE (1999) The neuropathogenesis of HIV-1 infection. FEMS Immunol Med Microbiol 26, 233–241.
111. No authors listed (1991) Nomenclature and research case definitions for neurologic manifestations of human immunodeficiency virus-type 1 (HIV-1) infection. Report of a Working Group of the American Academy of Neurology AIDS Task Force. Neurology 41, 778–785.
112. Masliah E, Achim CL, Ge N, DeTeresa R, Terry RD, Wiley CA (1992) Spectrum of human immunodeficiency virus-associated neocortical damage. Ann Neurol 32, 321–329.
113. McArthur JC, Hoover DR, Bacellar H, et al. (1993) Dementia in AIDS patients: incidence and risk factors. Neurology 43, 2245–2252.

114. Ho DD, Bredesen DE, Vinters HV, Daar ES (1989) The acquired immunodeficiency syndrome (AIDS) dementia complex. Ann Intern Med 111, 400–410.
115. Tselis A, Booss J (2003) Behavioral consequences of infections of the central nervous system: with emphasis on viral infections. J Am Acad Psychiatry Law 31, 289–298.
116. Brew BJ, Rosenblum M, Cronin K, Price RW (1995) AIDS dementia complex and HIV-1 brain infection: clinical-virological correlations. Ann Neurol 38, 563–570.
117. Glass JD, Fedor H, Wesselingh SL, McArthur JC (1995) Immunocytochemical quantitation of human immunodeficiency virus in the brain: correlations with dementia. Ann Neurol 38, 755–762.
118. Perry VH, Lawson LJ, Reid DM (1994) Biology of the mononuclear phagocyte system of the central nervous system and HIV infection. J Leukoc Biol 56, 399–406.
119. Dalakas MC, Pezeshkpour GH (1988) Neuromuscular diseases associated with human immunodeficiency virus infection. Ann Neurol 23, Suppl, S38–S48.
120. Fuller GN, Jacobs JM (1993) Peripheral nerve and muscle disease in HIV infection. In Scaravilli F, ed., The Neuropathology of HIV Infection. Springer, London, pp. 215–233.
121. Fuller GN, Jacobs JM, Guiloff RJ (1993) Nature and incidence of peripheral nerve syndromes in HIV infection. J Neurol Neurosurg Psychiatry 56, 372–381.
122. Gherardi R (1994) Skeletal muscle involvement in HIV-infected patients. Neuropathol Appl Neurobiol 20, 232–237.
123. Hantai D, Fournier J-G, Vazeux R, Collin H, Baudrimont M, Fardeau M (1991) Skeletal muscle involvement in human immunodeficiency virus infection. Acta Neuropathol 81, 496–502.
124. Lange DJ, Britton CB, Younger DS, Hays AP (1988) The neuromuscular manifestations of human immunodeficiency virus infections. Arch Neurol 45, 1084–1088.
125. Miller RG, Kiprov DD, Parry G, Bredesen DE (1988) Peripheral nervous system dysfunction in acquired immunodeficiency syndrome. In Rosenblum ML, Levy RM, Bredesen DE, eds., AIDS and the Nervous System. Raven Press, New York, pp. 65–78.
126. Pardo CA, McArthur JC, Griffin JW (2001) HIV neuropathy: insights in the pathology of HIV peripheral nerve disease. J Periph Nerv Syst 6, 21–27.
127. Rizzuto R, Cavallero T, Monaco S, et al. (1995) Role of HIV in the pathogenesis of distal symmetrical peripheral neuropathy. Acta Neuropathol 90, 244–250.
128. Demiryürek D, Bayramoglu A, Ustaçelebi S (2002) Infective agents in fixed human cadavers: a brief review and suggested guidelines. Anat Rec 269, 194–197.
129. Geller SA (1990) The autopsy in acquired immunodeficiency syndrome. How and why. Arch Pathol Lab Med 114, 324–329.
130. Ironside JW, Bell JE (1996) The 'high-risk' neuropathological autopsy in AIDS and Creutzfeldt-Jakob disease: principles and practice. Neuropathol Appl Neurobiol 22, 388–393.
131. Klatt EC, Noguchi TT (1990) AIDS and infection control in forensic investigation. Am J Forensic Med Pathol 11, 44–49.
132. Lucas SB (1993) HIV and the necropsy. J Clin Pathol 46, 1071–1075.

133. Reichert CM (1992) New safety considerations for the acquired immunodeficiency syndrome autopsy. Arch Pathol Lab Med 116, 1109–1110.
134. Achim CL, Wang R, Miners DK, Wiley CA (1994) Brain viral burden in HIV infection. J Neuropathol Exp Neurol 53, 284–294.
135. Morrison HL, Neal JW, Parkes AB, Jasani B (1998) Immunohistochemical retrieval of the principal HIV antigens p24, gp41, and gp120 in formalin fixed tissue: an investigation using HIV infected lymphoblasts and postmortem brain tissue from AIDS cases. Mol Pathol 51, 227–231.
136. Shapshak P, Yoshioka M, Sun NC, et al. (1992) HIV-1 in postmortem brain tissue from patients with AIDS: a comparison of different detection techniques. AIDS 6, 915–923.
137. Takahashi K, Wesselingh SL, Griffin DE, McArthur JC, Johnson RT, Glass JD (1996) Localization of HIV-1 in human brain using polymerase chain reaction/in situ hybridization and immunocytochemistry. Ann Neurol 39, 705–711.
138. de Gans J, Portegies P (1989) Neurological complications of infection with human immunodeficiency virus type 1. A review of literature and 241 cases. Clin Neurol Neurosurg 91, 199–219.
139. Levy RM, Bredesen DE, Rosenblum ML (1985) Neurological manifestations of the acquired immunodeficiency syndrome (AIDS): experience at UCSF and review of the literature. J Neurosurg 62, 475–495.
140. Morgello S, Mahboob R, Yakoushina T, Khan S, Hague K (2002) Autopsy findings in a human immunodeficiency virus-infected population over 2 decades. Arch Pathol Lab Med 126, 182–190.
141. Rosemberg S, Lopes MBS, Tsanaclis AM (1986) Neuropathology of acquired immunodeficiency syndrome (AIDS). Analysis of 22 Brazilian cases. J Neurol Sci 76, 187–198.
142. Trujillo JR, Garcia-Ramos G, Novak IS, Rivera VM, Huerta E, Essex M (1995) Neurologic manifestations of AIDS: a comparative study of two populations from Mexico and the United States. J Acquir Immune Defic Syndr Hum Retrovirol 8, 23–29.
143. Masliah E, DeTeresa R, Mallory M, Hansen LA (2000) Changes in pathological findings at autopsy in AIDS cases for the last 15 years. AIDS 14, 69–74.
144. Mocroft A, Brettle RP, Kirk O, et al. (2002) Changes in the cause of death among HIV positive subjects across Europe: results from the EuroSIDA study. AIDS 16, 1663–1671.
145. Sacktor N, Lyles RH, Skolasky R, et al. (2001) HIV-associated neurologic disease incidence changes: multicenter AIDS cohort study, 1990–1998. Neurology 56, 257–260.
146. Gelman BB, Guinto FC (1992) Morphometry, histopathology, and tomography of cerebral atrophy in the acquired immunodeficiency syndrome. Ann Neurol 32, 31–40.
147. Oster S, Christofferson P, Gundersen HJG, Nielson JO, Pakkenberg B, Pedersen C (1993) Cerebral atrophy in AIDS: a stereological study. Acta Neuropathol 85, 617–622.
148. Subbiah P, Mouton P, Fedor H, McArthur JC, Glass JD (1996) Stereological analysis of cerebral atrophy in human immunodeficiency virus-associated dementia. J Neuropathol Exp Neurol 55, 1032–1037.

149. Weis S, Llenos IC, Büttner A, Rebhan A, Soreth D, Mehraein P (1993) Macroscopic morphometry of human brains in neurodegeneration. Acta Stereol 12, 299–304.
150. Budka H (1986) Multinucleated giant cells in brain: a hallmark of the acquired immune deficiency syndrome (AIDS). Acta Neuropathol 69, 253–258.
151. De la Monte SM, Ho DD, Schooley RT, Hirsch MS, Richardson EP Jr (1987) Subacute encephalomyelitis of AIDS and its relation to HTLV-III infection. Neurology 37, 562–569.
152. Cosenza MA, Zhao ML, Si Q, Lee SC (2002) Human brain parenchymal microglia express CD14 and CD45 and are productively infected by HIV-1 in HIV-1 encephalitis. Brain Pathol 12, 442–455.
153. Brew BJ (2004) Evidence for a change in AIDS dementia complex in the era of highly active antiretroviral therapy and the possibility of new forms of AIDS dementia complex. AIDS 18, Suppl 1, S75–S78.
154. Neuenburg JK, Brodt HR, Herndier BG, et al. (2002) HIV-related neuropathology, 1985 to 1999: rising prevalence of HIV encephalopathy in the era of highly active antiretroviral therapy. J Acquir Immune Defic Syndr 31, 171–177.
155. Persidsky Y, Gendelman HE (2003) Mononuclear phagocyte immunity and the neuropathogenesis of HIV-1 infection. J Leukoc Biol 74, 691–701.
156. Giometto B, An SF, Groves M, et al. (1997) Accumulation of β-amyloid precursor protein in HIV encephalitis: relationship with neuropsychological abnormalities. Ann Neurol 42, 34–40.
157. Raja F, Sherriff FE, Morris CS, Bridges LR, Esiri MM (1997) Cerebral white matter damage in HIV infection demonstrated using β-amyloid precursor protein immunoreactivity. Acta Neuropathol 93, 184–189.
158. Artigas J, Grosse G, Niedobitek F (1990) Vacuolar myelopathy in AIDS. A morphological analysis. Pathol Res Pract 186, 228–237.
159. Bergmann M, Gullotta F, Kuchelmeister K, Masini T, Angeli G (1993) AIDS myelopathy. A neuropathological study. Pathol Res Pract 189, 58–65.
160. Goldstick L, Mandybur TI, Bode R (1985) Spinal cord degeneration in AIDS. Neurology 35, 103–106.
161. Hénin D, Smith TW, DeGirolami U, Sughayer M, Hauw JJ (1992) Neuropathology of the spinal cord in the acquired immunodeficiency syndrome. Hum Pathol 23, 1106–1114.
162. Maier H, Budka H, Lassmann H, Pohl P (1989) Vacuolar myelopathy with multinucleated giant cells in the acquired immune deficiency syndrome (AIDS). Light and electron microscopic distribution of human immunodeficiency virus (HIV) antigens. Acta Neuropathol 78, 497–503.
163. Shepherd EJ, Brettle RP, Liberski PP, et al. (1999) Spinal cord pathology and viral burden in homosexuals and drug users with AIDS. Neuropathol Appl Neurobiol 25, 2–10.
164. Rottnek M, Di Rocco A, Laudier D, Morgello S (2002) Axonal damage is a late component of vacuolar myelopathy. Neurology 58, 479–481.
165. Rhodes RH, Ward JM, Cowan RP, Moore PT (1989) Immunohistochemical localization of human immunodeficiency viral antigens in formalin-fixed spinal cords with AIDS myelopathy. Clin Neuropathol 8, 22–27.

166. Schmidbauer M, Budka H, Okeda R, Cristina S, Lechi A, Trabattoni R (1990) Multifocal vacuolar leukoencephalopathy: a distinct HIV-associated lesion of the brain. Neuropathol Appl Neurobiol 16, 437–443.
167. Cinque P, Marenzi R, Ceresa D (1997) Cytomegalovirus infections of the nervous system. Intervirology 40, 85–97.
168. Holland NR, Power C, Mathews VP, Glass JD, Forman M, McArthur JC (1994) Cytomegalovirus encephalitis in acquired immunodeficiency syndrome (AIDS). Neurology 44, 507–514.
169. Schmidbauer M, Budka H, Ulrich W, Ambros P (1989) Cytomegalovirus (CMV) disease of the brain in AIDS and connatal infection: a comparative study by histology, immunocytochemistry and in situ DNA hybridization. Acta Neuropathol 79, 286–293.
170. Setinek U, Wondrusch E, Jellinger K, et al. (1995) Cytomegalovirus infection of the brain in AIDS: a clinicopathologic study. Acta Neuropathol 90, 511–515.
171. Vinters HV, Kwok MK, Ho HW, et al. (1989) Cytomegalovirus in the nervous system of patients with the acquired immune deficiency syndrome. Brain 112, 245–268.
172. Kalayjian RC, Cohen ML, Bonomo RA, Flanigan TP (1993) Cytomegalovirus ventriculoencephalitis in AIDS. A syndrome with distinct clinical and pathologic features. Medicine 72, 67–77.
173. Morgello S, Simpson DM (1994) Multifocal cytomegalovirus demyelinative polyneuropathy associated with AIDS. Muscle Nerve 17, 176–182.
174. Roullet E, Assuerus V, Gozlan J, et al. (1994) Cytomegalovirus multifocal neuropathy in AIDS: analysis of 15 consecutive cases. Neurology 44, 2174–2182.
175. Berger JR, Concha M (1995) Progressive multifocal leukoencephalopathy: the evolution of a disease once considered rare. J Neurovirol 1, 5–18.
176. Chaisson RE, Griffin DE (1990) Progressive multifocal leukoencephalopathy in AIDS. JAMA 264, 79–82.
177. Kuchelmeister K, Gullotta F, Bergmann M, Angeli G, Masini T (1993) Progressive multifocal leukoencephalopathy (PML) in the acquired immunodeficiency syndrome (AIDS). A neuropathological autopsy study of 21 cases. Pathol Res Pract 189, 163–173.
178. von Einsiedel RW, Fife TD, Aksamit AJ, et al. (1993) Progressive multifocal leukoencephalopathy in AIDS: a clinicopathologic study and review of the literature. J Neurol 240, 391–406.
179. Bertoli F, Espino M, Arosemena JR, Fishback JL, Frenkel JK (1995) A spectrum in the pathology of toxoplasmosis in patients with acquired immunodeficiency syndrome. Arch Pathol Lab Med 119, 214–224.
180. Falangola MF, Reichler BS, Petito CK (1994) Histopathology of cerebral toxoplasmosis in human immunodeficiency virus infection: a comparison between patients with early-onset and late-onset acquired immunodeficiency syndrome. Hum Pathol 25, 1091–1097.
181. Strittmatter C, Lang W, Wiestler OD, Kleihues P (1992) The changing pattern of human immunodeficiency virus-associated cerebral toxoplasmosis: a study of 46 postmortem cases. Acta Neuropathol 83, 475–481.

182. Lee SC, Dickson DW, Casadevall A (1996) Pathology of cryptococcal meningoencephalitis: analysis of 27 patients with pathogenetic implications. Hum Pathol 27, 839–847.
183. Gray F, Bélec L, Lescs MC, et al. (1994) Varizella-zoster virus infection of the central nervous system in the acquired immune deficiency syndrome. Brain 117, 987–999.
184. Kleinschmidt-DeMasters BK, Amlie-Lefond C, Gilden DH (1996) The patterns of varicella zoster virus encephalitis. Hum Pathol 27, 927–938.
185. Chrétien F, Bélec L, Hilton BA, et al. (1996) Herpes simplex virus type 1 encephalitis in acquired immunodeficiency syndrome. Neuropathol Appl Neurobiol 22, 394–404.
186. Katz DA, Berger JR (1989) Neurosyphilis in acquired immunodeficiency syndrome. Arch Neurol 46, 895–898.
187. Farrar DJ, Flanigan TP, Gordon NM, Gold RL, Rich JD (1997) Tuberculous brain abscess in a patient with HIV infection: case report and review. Am J Med 102, 297–301.
188. Adle-Biassette H, Bourhy H, Gisselbrecht M, et al. (1996) Rabies encephalitis in a patient with AIDS: a clinicopathologic study. Acta Neuropathol 92, 415–420.
189. LeBlang SD, Whiteman MLH, Post MJD, Uttamchandani RB, Bell MD, Smirniotopoulos JG (1995) CNS nocardia in AIDS patients: CT and MRI with pathologic correlation. J Comput Assist Tomogr 19, 15–22.
190. Mischel PS, Vinters HV (1995) Coccidiodomycosis of the central nervous system: neuropathological and vasculopathic manifestations and clinical correlates. Clin Infect Dis 20, 400–405.
191. Straus DJ (1997) Human immunodeficiency virus-associated lymphomas. Med Clin N Am 81, 495–510.
192. Zelman IB, Mossakowski MJ, Niewwiadomska H (1998) Cerebral lymphomas in AIDS. Neuropathological study. Folia Neuropathol 36, 65–79.
193. Iglesias-Rozas JR, Bantz B, Adler T, et al. (1991) Cerebral lymphoma in AIDS. Clinical, radiological, neuropathological and immunopathological study. Clin Neuropathol 10, 65–72.
194. Ioachim HL, Dorsett B, Cronin W, Maya M, Wahl S (1991) Acquired immunodeficiency syndrome associated lymphomas: clinical pathologic, immunologic, and viral characteristics of 111 cases. Hum Pathol 22, 659–673.
195. Morgello S, Petito CK, Mouradian JA (1990) Central nervous system lymphoma in the acquired immunodeficiency syndrome. Clin Neuropathol 9, 205–215.
196. So YT, Beckstead JH, Davis RL (1986) Primary central nervous system lymphoma in acquired immune deficiency syndrome: a clinical and pathological study. Ann Neurol 20, 566–572.
197. Büttner A, Weis S (1999) Non-lymphomatous brain tumors in HIV-1 infection: a review. J Neurooncol 41, 81–88.
198. Büttner A, Marquart KH, Mehraein P, Weis S (1997) Kaposi's sarcoma in the cerebellum of a patient with AIDS. Clin Neuropathol 16, 185–189.
199. Schlote W, Gräfin Vitzthum H, Thomas E (1987) Neuropathologische Beobachtungen in 28 Fällen von erworbenem Immundefektsystem (AIDS). In

Fischer PA, Schlote W, eds., AIDS und Nervensystem. Springer, Berlin, pp. 85–116.
200. Vago L, Castagna A, Lazzarin A, Trabattoni G, Cinque P, Costanzi G (1993) Reduced frequency of HIV-induced brain lesions in AIDS patients treated with zidovudine. J Acquir Immune Defic Syndr 6, 42–45.
201. Langford TD, Letendre SL, Larrea GJ, Masliah E (2003) Changing patterns in the neuropathogenesis of HIV during the HAART era. Brain Pathol 13, 195–210.
202. Gray F, Chrétien F, Vallat-Decouvelaere AV, Scaravilli F (2003) The changing pattern of HIV Neuropathology in the HAART era. J Neuropathol Exp Neurol 62, 429–440.
203. Burns DK (1992) The neuropathology of pediatric acquired immunodeficicency syndrome. J Child Neurol 7, 332–346.
204. Kozlowski PB, Brudkowska J, Kraszpulski M, et al. (1997) Microencephaly in children congenitally infected with human immunodeficiency virus—a gross-anatomical morphometric study. Acta Neuropathol 93, 136–145.
205. Kozlowski PB, Snider DA, Vietze PM, Wisniewski HM (1990) Brain in pediatric AIDS. Karger, Basel.
206. Dickson DW, Llena JF, Nelson SJ, Weidenheim KM (1993) Central nervous system pathology in pediatric AIDS. Ann NY Acad Sci 693, 93–106.
207. Bell JE, Lowrie S, Koffi K, et al. (1997) The neuropathology of HIV-infected African children in Abidjan, Cote d'Ivoire. J Neuropathol Exp Neurol 56, 686–692.
208. Belman AL (1992) Acquired immunodeficiency syndrome and the child's central nervous system. Pediatr Clin North Am 39, 691–714.
209. Belman AL, Ultmann MH, Horoupian D, et al. (1985) Neurological complications in infants and children with acquired immune deficiency syndrome. Ann Neurol 18, 560–565.
210. Brenneman DE, McCune SK, Gozes I (1990) Acquired immune deficiency syndrome and the developing nervous system. Int Rev Neurobiol 32, 305–353.
211. Epstein LG, Gelbard HA (1999) HIV-1-induced neuronal injury in the developing brain. J Leukoc Biol 65, 453–457.
212. Epstein LG, Sharer LR, Goudsmit J (1988) Neurological and neuropathological features of human immunodeficiency virus infection in children. Ann Neurol 23, Suppl, S19–S23.
213. Gavin P, Yogev R (1999) Central nervous system abnormalities in pediatric human immunodeficiency virus infection. Pediatr Neurosurg 31, 115–123.
214. Joshi VV (1993) Pathology of pediatric AIDS. Overview, update, and future direction. Ann NY Acad Sci 693, 71–92.
215. Masini T, Chinaglia D, Ghidoni P, Gullotta F (1994) Autoptic findings in HIV-1 positive children. Klin Pädiatr 206, 45–49.
216. Sharer LR, Dowling PC, Michaels J, et al. (1990) Spinal cord disease in children with HIV-1 infection: a combined molecular biological and neuropathological study. Neuropathol Appl Neurobiol 16, 317–331.
217. Sharer LR, Epstein LG, Cho ES, et al. (1986) Pathologic features of AIDS encephalopathy in children: evidence for LAV/HTLV-III infection of brain. Hum Pathol 17, 271–284.

218. Gray F, Lescs MC, Keohane C, et al. (1992) Early brain changes in HIV infection: neuropathological study of 11 seropositive, non-AIDS cases. J Neuropathol Exp Neurol 51, 177–185.
219. Gray F, Hurtrel M, Hurtrel B (1993) Early central nervous system changes in human immunodeficiency virus (HIV)-infection. Neuropathol Appl Neurobiol 19, 3–9.
220. Gray F, Scaravilli F, Everall IP, et al. (1996) Neuropathology of early HIV-1 infection. Brain Pathol 6, 1–15.
221. Wingertsmann L, Chrétien F, Authier FJ, Paraire F, Durigon M, Gray A (1997) Les lésions du système nerveux central au stade précoce de l'infection par le VIH. Arch Anat Cytol Pathol 45, 106–117.
222. An SF, Scaravilli F (1997) Early HIV-infection of the central nervous system. Arch Anat Cytol Pathol 45, 94–105.
223 An S, Groves M, Gray F, Scaravilli F (1999) Early entry and widespread cellular involvement of HIV-1 DNA in brains of HIV-1 positive asymptomatic individuals. J Neuropathol Exp Neurol 58, 1156–1162.
224. Bissell SJ, Wiley CA (2004) Human immunodeficiency virus infection of the brain: pitfalls in evaluating infected/affected cell populations. Brain Pathol 14, 97–108.
225. Clifford DB (1998) Antiretroviral therapies for HIV infection implications for treatment of neurologic manifestations: an overview. In Gendelman HE, Lipton SA, Epstein L, Swindells S, eds., The Neurology of AIDS. Chapman and Hall, New York, NY, pp. 353–363.
226. Tardieu M (1999) HIV-1-related central nervous system diseases. Curr Opin Neurol 12, 377–381.
227. Dore GJ, McDonald A, Li Y, Kaldor JM, Brew BJ (2003) Marked improvement in survival following AIDS dementia complex in the era of highly active antiretroviral therapy. AIDS 17, 1539–1545.
228. Maschke M, Kastrup O, Esser S, Ross B, Hengge U, Hufnagel A (2000) Incidence and prevalence of neurological disorders associated with HIV since the introduction of highly active antiretroviral therapy (HAART). J Neurol Neurosurg Psychiatry 69, 376–380.
229. Ammassari A, Cingolani A, Pezzotti P, et al. (2000) AIDS-related focal brain lesions in the era of highly active antiretroviral therapy. Neurology 55, 1194–1200.
230. Vago L, Bonetto S, Nebuloni M, et al. (2002) Pathological findings in the central nervous system of AIDS patients on assumed antiretroviral therapeutic regimens: retrospective study of 1597 autopsies. AIDS 16, 1925–1928.
231. Berger JR, Harris JO, Gregorios J, Norenberg MD (1990) Cerebrovascular disease in AIDS: a case-control study. AIDS 4, 239–244.
232. Connor MD, Lammie GA, Bell JE, Warlow CP, Simmonds P, Brettle RP (2000) Cerebral infarction in adult AIDS patients: observations from the Edinburgh HIV autopsy cohort. Stroke 31, 2117–2126.
233. Evers S, Nabavi D, Rahmann A, Heese C, Reichelt D, Husstedt IW (2003) Ischaemic cerebrovascular events in HIV infection: a cohort study. Cerebrovasc Dis 15, 199–205.

234. Gillams AR, Allen E, Hrieb K, Venna D, Craven D, Carter AP (1997) Cerebral infarction in patients with AIDS. AJNR Am J Neuroradiol 18, 1581–1585.
235. Kieburtz KD, Eskin TA, Ketonen L, Tuite MJ (1993) Opportunistic cerebral vasculopathy and stroke in patients with the acquired immunodeficiency syndrome. Arch Neurol 50, 430–432.
236. Mizusawa H, Hirano A, Llena JF, Shintaku M (1988) Cerebrovascular lesions in acquired immune deficiency syndrome (AIDS). Acta Neuropathol 76, 451–457.
237. Pinto AN (1996) AIDS and cerebrovascular disease. Stroke 27, 538–543.
238. Cole JW, Pinto AN, Hebel JR, et al. (2004) Acquired immunodeficiency syndrome and the risk of stroke. Stroke 35, 51–56.
239. Smith TW, DeGirolami U, Hénin D, Bolgert F, Hauw JJ (1990) Human immunodeficiency virus (HIV) leucoencephalopathy and the microcirculation. J Neuropathol Exp Neurol 49, 357–370.
240. Büttner A, Pfister HW, Quintern J, Itoh K, Mehraein P, Weis S (1997) Vasculitis with predominantly leptomeningeal involvement in an AIDS patient. Neuropathology 17, 238–242.
241. Kim WK, Corey S, Alvarez X, Williams K (2003) Monocyte/macrophage traffic in HIV and SIV encephalitis. J Leukoc Biol 74, 650–656.
242. Peluso R, Haase A, Stowring L, Edwards M, Ventura P (1985) A Trojan Horse mechanism for the spread of visna virus in monocytes. Virology 147, 231–236.
243. Liu NQ, Lossinsky AS, Popik W, et al. (2002) Human immunodeficiency virus type 1 enters brain microvascular endothelia by macropinocytosis dependent on lipid rafts and the mitogen-activated protein kinase signaling pathway. J Virol 76, 6689–6700.
244. Nottet HSLM (1999) Interactions between macrophages and brain microvascular endothelial cells: role in pathogenesis of HIV-1 infection and blood-brain barrier function. J Neurovirol 527, 659–669.
245. Falangola MF, Hanly A, Galvao-Castro B, Petito CK (1995) HIV infection of human choroid plexus: a possible mechanism for viral entry into the CNS. J Neuropathol Exp Neurol 54, 497–503.
246. Annunziata P (2003) Blood-brain barrier changes during invasion of the central nervous system by HIV-1. Old and new insights into the mechanism. J Neurol 250, 901–906.
247. Budka H (1991) Neuropathology of human immunodeficiency virus infection. Brain Pathol 1, 163–175.
248. Poland SD, Rice GPA, Dekaban GA (1995) HIV-1 infection of human brain-derived microvascular endothelial cells in vitro. J Acquir Immune Defic Syndr Hum Retrovirol 8, 437–445.
249. Ranki A, Nyberg M, Ovod V, et al. (1995) Abundant expression of HIV Nef and Rev proteins in brain astrocytes in vivo is associated with dementia. AIDS 9, 1001–1008.
250. Dickson DW, Mattiace LA, Kure K, Hutchins K, Lyman WD, Brosnan CF (1991) Microglia in human disease, with an emphasis on acquired immune deficiency syndrome. Lab Invest 64, 135–156.
251. Gosztonyi G, Artigas J, Lamperth L, Webster HD (1994) Human immunodeficiency virus (HIV) distribution in HIV encephalitis: study of 19 cases with com-

bined use of in situ hybridization and immunocytochemistry. J Neuropathol Exp Neurol 53, 521–534.
252. Persidsky Y, Zheng J, Miller D, Gendelman HE (2000) Mononuclear phagocytes mediate blood-brain barrier compromise and neuronal injury during HIV-1-associated dementia. J Leukoc Biol 68, 413–422.
253. Albright AV, Shieh JTC, Itoh T, et al. (1999) Microglia express CCR5, CXCR4, and CCR3, but of these, CCR5 is the principal coreceptor for human immunodeficiency virus type 1 dementia. J Virol 73, 205–213.
254. Oster S, Christoffersen P, Gundersen HJG, Nielsen JO, Pedersen C, Pakkenberg B (1995) Six billion neurons lost in AIDS. A stereological study of the neocortex. APMIS 103, 525–529.
255. Weis S (1992) Morphometric aspects of the brain in HIV-1 infection. In Weis S, Hippius H, eds., HIV-1 Infection of the Central Nervous System. Clinical, Pathological, and Molecular Aspects. Hogrefe & Huber Publishers, Seattle, pp. 199–220.
256. Weis S (1994) Neuronal loss in HIV [letter]. Neurology 44, 1365–1366.
257. Ketzler S, Weis S, Haug H, Budka H (1990) Loss of neurons in the frontal cortex in AIDS brains. Acta Neuropathol 80, 92–94.
258. Weis S, Haug H, Budka H (1993) Neuronal damage in the cerebral cortex of AIDS brains: a morphometric study. Acta Neuropathol 85, 185–189.
259. Adle-Biassette H, Chrétien F, Wingertsmann L, et al. (1999) Neuronal apoptosis does not correlate with dementia in HIV infection but is related to microglial activation and axonal damage. Neuropathol Appl Neurobiol 25, 123–133.
260. Adle-Biassette H, Levy Y, Colombel M, et al. (1995) Neuronal apoptosis in HIV infection in adults. Neuropathol Appl Neurobiol 21, 218–227.
261. An SF, Giometto B, Scaravilli T, Tavolato B, Gray F, Scaravilli F (1996) Programmed cell death in brains of HIV-1 positive AIDS and pre-AIDS patients. Acta Neuropathol 91, 169–173.
262. Petito CK, Roberts B (1995) Evidence of apoptotic cell death in HIV encephalitis. Am J Pathol 146, 1121–1130.
263. Weis S, Budka H, Haug H (1993) Astroglial changes in the cerebral cortex of AIDS brains: a morphometric and immunohistochemical investigation. Neuropathol Appl Neurobiol 19, 329–335.
264. Petito CK, Cash KS (1992) Blood-brain barrier abnormalities in the acquired immunodeficiency syndrome: immunohistochemical localization of serum proteins in postmortem brain. Ann Neurol 32, 658–666.
265. Power C, Kong PA, Crawford TO, et al. (1993) Cerebral white matter changes in acquired immunodeficiency syndrome dementia: alterations of the blood-brain barrier. Ann Neurol 34, 339–350.
266. Rhodes RH (1991) Evidence of serum-protein leakage across the blood-brain barrier in the acquired immunodeficiency syndrome. J Neuropathol Exp Neurol 50, 171–183.
267. Büttner A, Mehraein P, Weis S (1996) Vascular changes in the cerebral cortex of HIV-1 infected brains: an immunohistochemical and lectinhistochemical analysis. Acta Neuropathol 92, 35–41.

268. Weis S, Haug H, Budka H (1996) Vascular changes in the cerebral cortex in HIV-1 infection: I. A morphometric investigation by light and electron microscopy. Clin Neuropathol 15, 361–366.
269. Weis S, Haug H, Budka H (1988) Stereological investigation of the microvasculature of cerebral cortex in AIDS-demented patients. Clin Neuropathol 7, 221.
270. András IE, Pu H, Deli MA, Nath A, Hennig B, Toborek M (2003) HIV-1 Tat protein alters tight junction protein expression and distribution in cultured brain endothelial cells. J Neurosci Res 74, 255–265.
271. Boven LA, Middel J, Verhoef J, De Groot CJA, Nottet HSLM (2000) Monocyte infiltration is highly associated with loss of tight junction protein zonula occludens in HIV-1-associated dementia. Neuropathol Appl Neurobiol 26, 356–360.
272. Dallasta LM, Pisarov LA, Esplen JE, et al. (1999) Blood-brain barrier tight junction disruption in human immunodeficiency virus-1 encephalitis. Am J Pathol 155, 1915–1927.
273. Banks WA, Akerstrom V, Kastin AJ (1998) Adsorptive endocytosis mediates the passage of HIV-1 across the blood-brain barrier: evidence for a post-internalization coreceptor. J Cell Sci 111, 533–540.
274. Hurwitz AA, Berman JW, Lyman WD (1994) The role of the blood-brain barrier in HIV infection of the central nervous system. Adv Neuroimmunol 4, 249–256.
275. Kanmogne GD, Kennedy RC, Grammas P (2002) HIV-1 gp120 proteins and gp160 peptides are toxic to brain endothelial cells and neurons: possible pathways for HIV entry into the brain and HIV-associated dementia. J Neuropathol Exp Neurol 61, 992–1000.
276. Kim TA, Avraham HK, Koh YH, Jiang S, Park IW, Avraham S (2003) HIV-1 tat-mediated apoptosis in human brain microvascular endothelial cells. J Immunol 170, 2629–2637.
277. Persidsky Y, Ghorpade A, Rasmussen J, et al. (1999) Microglial and astrocyte chemokines regulate monocyte migration through the blood-brain barrier in human immunodeficiency virus-1 encephalitis. Am J Pathol 155, 1599–1611.
278. Weis S, Neuhaus B, Mehraein P (1994) Activation of microglia in HIV-1 infected brains is not dependent on the presence of HIV-1 antigens. Neuroreport 5, 1514–1516.
279. Genis P, Jett M, Bernton EW, et al. (1992) Cytokines and arachidonic metabolites produced during human immunodeficiency virus (HIV)-infected macrophage-astroglia interactions: implications for the neuropathogenesis of HIV disease. J Exp Med 176, 1703–1718.
280. Giulian D, Vaca K, Noonan CA (1990) Secretion of neurotoxins by mononuclear phagocytes infected with HIV-1. Science 250, 1593–1596.
281. Sabri F, Titanji K, de Milito A, Chiodi F (2003) Astrocyte activation and apoptosis: their roles in the neuropathology of HIV infection. Brain Pathol 12, 84–94.
282. Garden GA (2002) Microglia in human immunodeficiency virus-associated neurodegeneration. Glia 40, 240–251.
283. Merrill JE, Chen ISY (1991) HIV-1, macrophages, glial cells, and cytokines in AIDS nervous system disease. FASEB J 5, 2391–2397.
284. Mrak RE, Griffin WST (1997) The role of chronic self-propagating glial responses in neurodegeneration: implications for long-lived survivors of human immunodeficiency virus. J Neurovirol 3, 241–246.

285. Ryan LA, Cotter RL, Zink WEI, Gendelman HE, Zheng J (2002) Macrophages, chemokines and neuronal injury in HIV-1-associated dementia. Cell Mol Biol 48, 137–150.
286. Seilhean D, Kobayashi K, He Y, et al. (1997) Tumor necrosis factor-α, microglia and astrocytes in AIDS dementia complex. Acta Neuropathol 93, 508–517.

Forensic Pathophysiology

3

Death in a Head-Down Position

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CONTENTS

SUMMARY

Although deaths of persons in a head-down position are rare events, there can be no doubt that they occur from time to time, most often accidentally. The prolonged head-down position itself may lead to fatal outcome. The common features of such cases are as follows: *(a)* the finding of a body in an inverted or head-down tilted position; *(b)* marked ("monstrous") congestion of face, scalp, neck, and other dependent parts of the body (e.g., hands, shoulders); *(c)* accompanying effects of internal congestion with swelling of and petechial bleedings at the affected parts as well as edema of the brain and lungs; and *(d)* lack of a definite pathoanatomical cause of death. In some cases, one may find traces of self-rescuing attempts on the deceased's body as well. Because postmortem examinations are unlikely to reveal the cause of death in such cases, additional pathophysiological considerations are required to make this determination. This chapter examines 10 cases in which the deceased was found in a head-down position. Based on these cases, it is observed that eld-

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erly people, and in particular elderly with preexisting cardiovascular diseases, seem to be more prone to death in a head-down position than others. This suggests that final heart failure is the cause of death rather than cerebral or pulmonary dysfunction. Results from human and animal experiments and observations under true and simulated microgravitational conditions confirm this assumption, suggesting that a prolonged, markedly elevated burden of work for the heart because of increased volume load in an inverted body position eventually leads to death by heart failure. Other mechanisms, such as suffocation ("positional asphyxia"), reduced blood reflux to the heart attributable to vanishing of blood in the venous system, decreased oxygen supply to the brain after reduced arteriovenous pressure difference, and carotid sinus or baroreceptor reflexes as well as other factors seem to play only a minor role, if any, in deaths in head-down position.

Key Words: Head-down position; positional asphyxia; inverted suspension; cardiovascular dysfunction; heart failure; blood distribution; congestion.

1. *Introduction*

In August 1997, in an amusement park in Belgium, a roller coaster failed, and a group of visitors was trapped in the wagon in a head-down position for 90 minutes before rescue could be achieved. It appears that none of the victims suffered harm resulting from this accident, indicating that human beings can survive at least 1.5 hours in such an uncomfortable position.

There are, nevertheless, some rare reports of fatalities from an inverted body position. Most of these reports describe cases in which the deceased were accidentally trapped by their feet and left to hang, head-down, for a prolonged period of time before being found dead. Normally, postmortem examination reveals no obvious pathoanatomical cause of death in such cases, aside from signs of severe congestion in the region of the head and upper torso. Such observations prove that an inverse body position may have a fatal outcome, particularly if endured for a considerable period of time.

Some authors ascribe the term "inverse (or reverse) suspension" to such deaths. The word "suspension," however, includes an element of "hanging" or "being fixed" in some way. Although this may be the most frequent underlying mechanism in such fatalities, other mechanisms have been observed as well. For example, one may fall head-down into a narrow, deep hole, get stuck there, and die. In other cases, the victims have been found lying on a kind of declined plane, for example, on a stairwell with their head facing down on the half-landing, rather than in an inverted vertical position. Because "suspension" is not a necessary condition for this kind of accident, this author prefers

as the more neutral expression "head-down (or inverted) position." Similarly, the expression "positional asphyxia" is sometimes used to describe the cause of death in such cases. By applying this term, it is suggested that "asphyxia" (suffocation) is the cause of death, which is, as evidenced from the review of the following 10 cases, most doubtful in the most cases. If real asphyxia is not obvious, for example, by compression of the thorax, this term should be avoided as well.

A number of pathophysiological considerations and even experimental approaches have been published to theoretically assess the cause of death in head-down position. Here, a survey of the results already known is provided, including some modern insights from space medicine, for example, the examination of physiology under microgravitational conditions.

2. Ten Case Reports

In the following, 10 well-documented case reports where the deceased was found in a head-down position are portrayed. Excluded from this review are certain cases from the earlier literature, which, although interesting, lack relevant information such as autopsy results; these are:

- The case of a 77-year-old man who was found dead hanging over a window sill, with his head and arms hanging down outside, after having called a doctor because of an asthma attack (case 356 in ref. *1*).
- The case of an elderly woman who was found hanging head-down on the outside house wall with her left foot caught between the window and its frame (Fig. 184 in ref. *2*).
- The case of a 73-year-old man who was hanging head-down, naked, with his feet fixed by ropes at the uppermost rung of a ladder (of what appeared to be a self-constructed "slaughtering bench"). He had a self-inscribed writing on his abdominal region: "Schlachtsau, Handelsklasse II" (pig for slaughtering, grade II) (case 13 in ref. *3*).

Some better documented cases are discussed here.

2.1. Case 1

The body of a middle-aged man was found on a staircase, with his legs and nearly his entire body laying lengthwise on the stair. His head was located at the lowest position on a half-landing. Because of acute alcohol intoxication (blood alcohol level [BAL] = 346 mg/dL), he must have lost his balance and fallen into this position, where he stayed for an undetermined period of time until he died. Autopsy revealed no signs of external violence and no definite

pathoanatomical cause of death. Marked congestion of the head and upper parts of the trunk, brain edema, and hemorrhagic edema of the upper parts of the lungs were the most striking findings (author's own case).

2.2. Case 2

An elderly man was at the pub one night. The next morning, he was found dead, sticking head-down in a narrow hole on the ground of a building site. He must have fallen into the hole at night on his way home from the pub. Unable to rescue himself, he died during the night and was found about 6 hours later. Postmortem examination showed no external injuries or any internal cause of death. A marked congestion of the head, edema of the brain, and hemorrhagic edema of the upper parts of the lungs were the most prominent findings. His BAL was 341 mg/dL (author's own case).

2.3. Case 3

A similar case is described by Yoshida et al. *(4)*, in which a 74-year-old man lived in a house where one room was filled with trash, the latter forming 1 to 1.8 m high "mountains." While climbing over this trash, the man fell head-over-heels into a hole and got stuck with his body nearly perpendicular to the floor. He was found dead between 20 to 48 hours later. Upon autopsy, the man had a pressure mark on the top of his head that was caused by a pan that was situated at the bottom of the hole. No other signs of external injuries and no definite pathoanatomical cause of death could be identified. The body showed severe congestion of the head and the upper part of the trunk, petechial hemorrhages in the oral and bronchial mucosae, brain edema, and congestion of cerebral vessels.

2.4. Case 4

Purdue *(5)* reported the case of a 48-year-old man who tried to climb over a 3-meter-high security fence to steal some goods from a warehouse yard. Approximately 2 hours after he was last seen alive, a yell for help was heard, but a police officer who investigated the area neither heard nor saw anything out of the ordinary. The man was found dead the next morning, hanging perpendicular to the ground by his right foot attached to the fence. His foot had been caught between two spikes of the fence and the body hung freely downward without any support. Some blood-stained liquid had issued from his mouth and nostrils. Upon autopsy, diverse superficial bruises and abrasions were found, but no signs of severe trauma or underlying internal diseases were identified. Soft tissues of neck and face showed marked swelling and congestion,

as did the lungs and brain. The gastric mucosa exhibited numerous tiny (stress) hemorrhages and the blood from mouth and nose derived from the stomach. BAL was 129 mg/dL.

2.5. Case 5

An 11-month-old baby was found dead one morning hanging from its crib with one foot caught between the crib's rods. The body was hanging freely, without head contact to the floor. Autopsy revealed congestion of the head, petechial hemorrhages in the soft tissues, and some atelectatic alveoli in the lungs (from ref. *6*).

2.6. Case 6

On the way home from the pub, a 67-year-old man fell over a thorn hedge that was separating the road from a ditch. He was caught there, hanging in a head-down position over the ditch. The man was stuck in this position by the thorns and was unable to rescue himself. He was found dead the next morning. Autopsy showed severe swelling and congestion of neck and head with marked edema of the conjunctivae, diverse non-lethal underlying diseases, and a BAL of 230 mg/dL (from ref. *6*).

2.7. Case 7

An 85-year-old woman was admitted to a hospital with vague abdominal pain. A barium enema was applied for diagnostic radiography and, after this, the woman was positioned in a 30° head-down position for better distribution of the medium, when she suffered sudden cardiac arrest. At autopsy, advanced general atherosclerosis was found with "calcified aortic stenosis with aortic insufficiency, hypertrophy and dilatation of left ventricle and insufficiency of the mitral valve." In addition, dilation of the atrium of the left heart and a pulmonary edema was noted. Left heart failure was determined as cause of death (from ref. *7*).

2.8. Case 8

After not having been seen for 2 days, a 56-year-old man was found dead hanging head-down, perpendicular to the floor in a sack. The sack itself was fixed by ropes to a rod that was extended over a door and a cupboard. Beside the sack, there were two chairs, one of them overturned. The man must have been standing on the chairs, preparing some autoerotic act with the sack pulled over his head and trunk. He must have lost balance and fallen head-over-heels into the sack, which then turned around, resulting in the head-down position.

Fig. 1. Case 9. **(A)** Death scene. The body of a 64-year-old man is hanging head-down from a railing that separates the yard of a house from a staircase leading down to the cellar. **(B)** Death scene. Closer view of the man's right knee jammed into a gap between the railing and the wall. Note waste bin, apparently used as a step stool, and the bleeding wound seen on the left calf. **(C)** Hemorrhagic congestion of conjunctiva and sclera. **(D)** Marked congestion of lips and bloody fluid seen in the oral cavity. (Courtesy of Dr. Wolfgang Huckenbeck, Düsseldorf, Germany.)

He held a pair of scissors in his hand, obviously prepared to rescue himself in case of an accident, but he had only managed to cut a small hole into the sack. Postmortem examination showed severe congestion of the skin of the head and arms and of the internal organs and structures of head, neck, and thorax. In addition to brain and lung edema, subcutaneous hemorrhages in the arms, microscopically accompanied by a polymorphonuclear leukocyte infiltration,

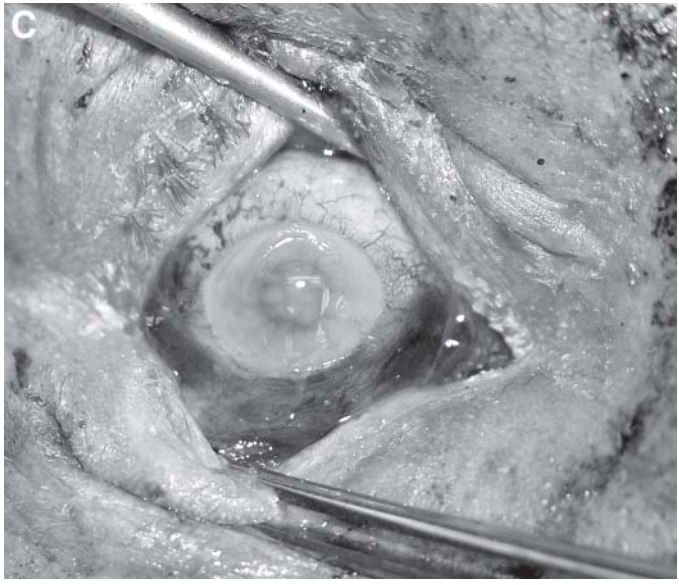

Fig. 1. *(Continued)*

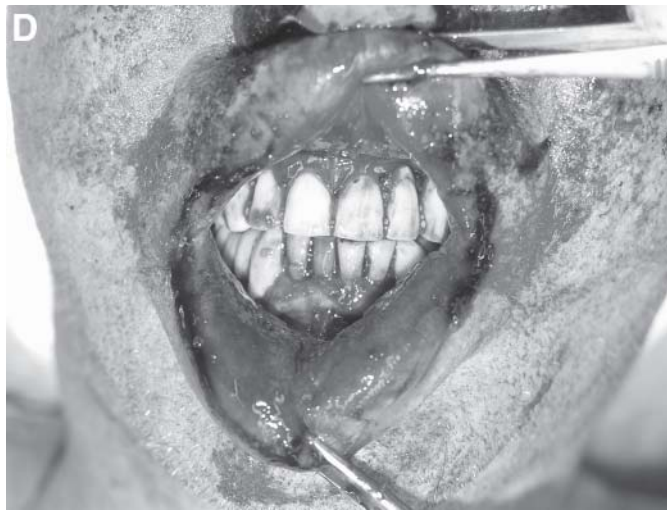

Fig. 1. *(Continued)*

were observed. A hypoxic vacuolization of liver cells was detected microscopically (from ref. *7*).

Cases 9 and 10 represent the most recent cases.

2.9. Case 9

A 64-year-old man was found dead, hanging head-down from a railing that separated the yard of his house from the staircase leading down to the cellar (Fig. 1A). His right knee was jammed into the gap between the end of the staircase railing and the wall of the house (Fig. 1B). A fresh wound was found on his left calf. Next to the wall, nearly under the kitchen's window, stood a waste bin. It was concluded that the victim had forgotten his key when leaving the house and that he tried to enter the house through the window, using the dustbin as a step stool. Doing so, he lost balance and fell backward over the railing, accidentally jamming his knee into the gap between the end of the staircase railing and the wall of the house. The period of time that he had remained hanging in this position could not be estimated. Autopsy showed congestion of head (Fig. 1C), neck and arms, and also of the brain and upper parts of the lungs. The mucous membranes of the upper airways were severely congested, with some bloody fluid coming out of his mouth and nostrils (Fig. 1D). Further findings were superficial lacerations of the skin, numerous petechial hemorrhages of the gastric mucosa, fresh bleeding into the abdominal wall muscles, general atherosclerosis, biatrial dilatation of the heart, and fatty

degeneration of liver cells (ref. *8* and W. Huckenbeck, Düsseldorf, Germany, personal communication, 2004).

2.10. Case 10

A 77-year-old man was found dead in his home, laying completely naked in a supine position on his kitchen table, head and lower legs hanging down over the table's edges. He had last been seen alive 24 hours before. The man was known to be a chronic alcoholic, but an autopsy blood sample was found to be free of alcohol. Besides severe congestion of the dependent parts of the head accompanied by numerous, partly confluent, petechial skin bleedings (Fig. 2A,B) and brain edema, marked general atherosclerosis and narrowing of the coronary vessels due to sclerosis were found, as well as signs of older myocardial infarctions. No signs of external violence were detected at autopsy. A fresh myocardial infarction was ruled out by routine histological examination as well as immunohistochemistry. It remained unclear whether the man may have believed himself to be in bed, possibly following delirious disorientation. It was concluded that the cause of death most probably was cerebral hypoxia as a sequel of insufficient cerebral blood flow due to head-down position (M. Tsokos, Hamburg, Germany, personal communication, 2004).

3. Discussion

The most obvious observation derived from the case reports presented here is that men seem to be more prone to death in a head-down position than are women (of the 10 cases, 8 were males, 1 was a female, gender of the infant is unknown) and that in some cases high BALs constitute an additional relevant factor. This may be explained by different behavioral patterns of men and women. Women are generally more cautious; they are less likely to fall into holes (case 2) or over hedges (case 6) on their way home from pubs or drinking halls. They rarely climb over fences to steal goods (case 4). Women typically do not construct strange devices for autoerotic practices (case 8). Men, particularly after drinking, tend to be more daring and thus are more prone to accidents, including the rare case of being caught in a head-down position.

More striking is the observation that elderly people seem to have an increased risk of dying in a head-down position when compared with younger persons (at least six of the victims were older than 50, and among these were three persons who were older than 70 years [in two cases, the exact age remained unknown]); this could be owing to two different reasons. First, younger people are more agile and may be able to rescue themselves more easily if they acci-

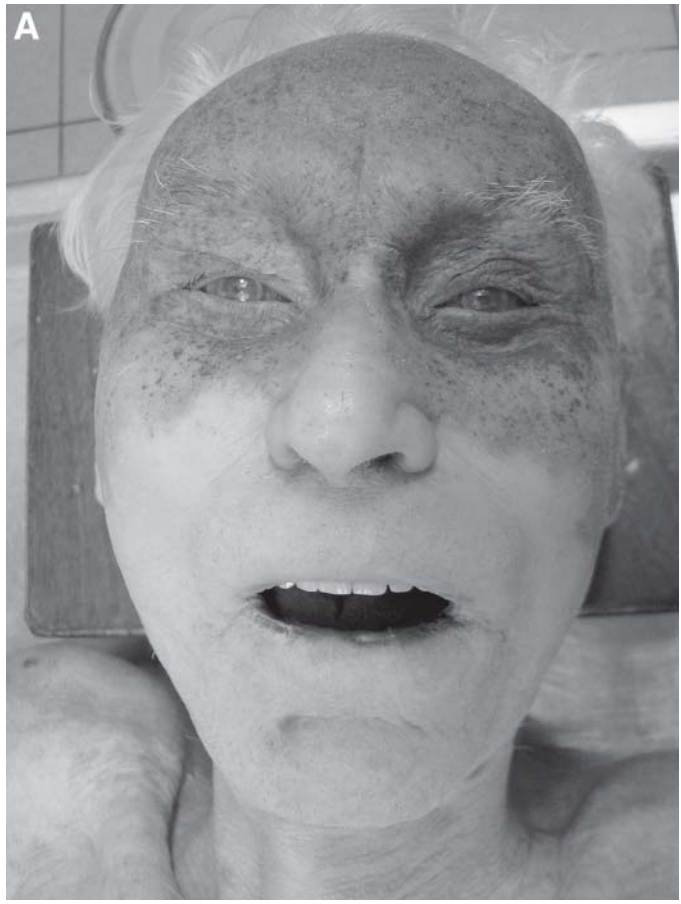

Fig. 2. Case 10. Autopsy features. **(A)** Enormous congestion of the dependent (upper) parts of the head due to the hanging down of this 77-year-old man's head over the edge of a table. **(B)** A sharp demarcation is seen between dependent and nondependent parts of the face. Note the numerous, partly confluent, petechial skin bleedings. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

dentally come into such a body position. Second, the cardiovascular system of elderly people is less resistant to unfamiliar stress and may fail earlier than that of younger persons. In other words, the elderly appear to die before they can be found and rescued, whereas younger persons may survive for a longer period of time and thus have a greater chance to be saved. However, case 5 (the infant) shows that head-down death is possible in any age group.

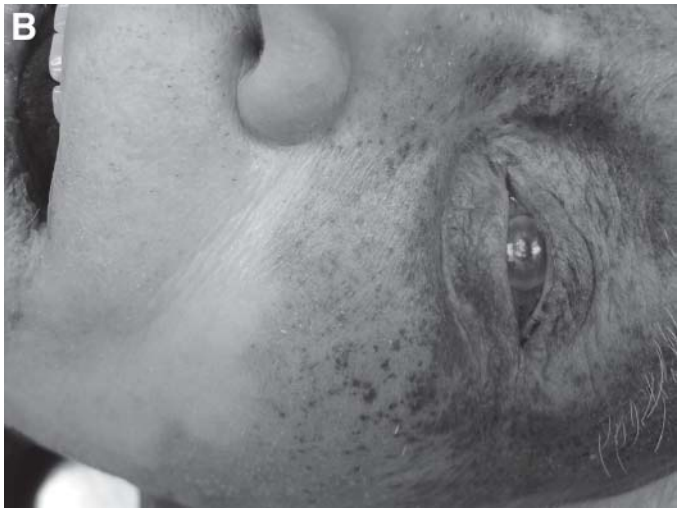

Fig. 2. *(Continued)*

Some of the cases reported here show that a strictly vertical body position is not necessarily needed to die in a head-down position. A declivity of 45° (estimated in case 1), 30° (case 7), or the isolated hanging down of the head may lead to death, particularly if, as demonstrated in cases 7 and 10, there is severe preexisting cardiovascular insufficiency. This observation suggests that the cardiovascular system may play a major role among the mechanisms of death in head-down position, an aspect that is further elaborated below.

All head-down victims exhibited some common, characteristic features among which are the severe congestion of head, neck and, depending on their posture, arms and upper part of the trunk is the most striking finding. Such congestion has been described by some authors as "hypostasis" or "livores," and it may well appear as such after death. But because this congestion already begins while the affected persons are still alive, it should not be confused with livores. The polymorphonuclear leukocyte infiltration of subcutaneous bleedings in the arms in case 8 could have developed only while the victim was still alive and thus indicates that the man must have survived the formation of the bruises for at least some time.

The grade of congestion in such cases is severe, sometimes even "monstrous," and leads to a bluish-violaceous discoloration of face, neck, and scalp, combined with marked swelling of the soft tissues, mucous membranes, and anatomical structures of the eyes. Additional phenomena after such congestion are petechial bleedings in skin, conjunctivae, and mucous membranes of

mouth and nose (sometimes presenting as confluent hemorrhages), brain edema, congestion of brain vessels, and pulmonary edema, particularly in the upper parts of the lungs, which also may appear hemorrhagic. If arms and shoulders constituting dependent body parts in a given case, the same phenomena may be observed in these body parts as well. Such congestion is easily understood to be a consequence of increased hydrostatic blood pressure in the affected body parts owing to the victim's inverse body position.

In some cases, petechial hemorrhages of the gastric mucosa were observed, too (cases 4 and 9). These may be interpreted as being induced by stress, as similar effects have been identified in victims of severe blunt trauma, infection, or burns. This indicates a prolonged phase of agony in head-down position. Whether histopathological changes of liver cells (hypoxic vacuolization as seen in case 8, fatty degeneration as found in case 9) belong to the postural effects in inverse position or represent preexisting pathological conditions remains unclear.

More typical are bruises, skin lacerations, wounds, and similar aftereffects of falling and self-rescue attempts. Such were the abrasions and bruises observed in case 4, diverse scratches found in case 8, and the wounds and lacerations detected in case 9. The latter case also is remarkable because of the bleeding into the victim's abdominal wall muscles, which probably was caused by the victim's attempts to reach some support with the hands. Broken fingernails and similar traces may be observed in such cases as well. Such findings indicate that the victims were not unconscious immediately after they came into the head-down position, but that they rather were conscious for a considerable period of time and were obviously aware of their situation. In other cases, however, no such traces can be found, be it due to alcohol intoxication (cases 1 and 2), to rapid death (case 7), or to loss of consciousness by unknown causes (case 10).

4. Pathophysiological Considerations

Definite pathoanatomical causes of death, such as myocardial infarction, pulmonary embolism, airway obstruction, and cerebral bleeding, normally are not observed in head-down deaths, although in some of the cases presented here severe cardiovascular diseases (cases 7 and 10) or alcohol intoxication (cases 1 and 2) may have contributed to fatal outcome. In other cases, the head-down position itself must have been the only determining factor of death. The question is, how can this abnormal body position lead to death and what are the underlying pathophysiological mechanisms?

Earlier animal experiments *(9)* with rabbits, dogs, frogs, and even snakes that have been fixed lengthwise on a board and then brought into different

body positions show that abnormal postures lead to a decrease of arterial blood pressure and to marked circulatory disturbances. More recent experiments by Uchigasaki et al. *(10)* revealed that 14 rabbits that were subjected to head-down position died after 26 *(17–44)* hours. Prior to death, a slight increase of respiratory frequency and a gradual decrease of amplitude of respiratory movements, of arterial oxygen saturation, and of blood pressure were observed. The authors interpret their observations as "resulting from hindered respiratory movements" and conclude therefore that "positional asphyxia" was the cause of death.

Besides the many problems of transferring results of animal experiments to humans, observations and experiments involving humans have revealed further possible mechanisms of death induced by the inverted body position. As early as 1968, Marshall *(6)* listed the three most likely mechanisms. In addition to real "positional asphyxia," Marshall noted *(a)* insufficient oxygen supply of the brain as a result of reduced cerebral blood flow, following diminished arteriovenous pressure difference (venous pressure is elevated by the additional hydrostatic pressure of blood in an inverted body position), and *(b)* cardiac insufficiency and eventual cardiac arrest resulting from increased blood flow to the heart and, therefore, increased volume load, and increased burden of work that cannot be tolerated by the victim for a prolonged period of time.

Is one of these the dominant cause of death in head-down cases?

Wilkins et al. *(11)* subjected 42 healthy volunteers to a short period of being positioned head-down. Besides general muscular tension and discomfort, the volunteers reported feelings of congestion of head and face, impediment of respiration, swelling of nasal mucosa and nasal mucous flow, ocular tearing, and sweating of the face and neck. Objectively, jugular venous pressure was increased and femoral arterial pressure diminished. Right atrial pressure, ventricular stroke volume, and cardiac output were increased (indicating increased burden of work for the heart). In another experiment, Deklunder et al. compared –70° head-down position to +70° head-up position (all angles measured from the horizontal) in 12 healthy male volunteers *(12)*. They observed in the head-down posture an increased passive filling of the left ventricle and increased cardiac output, increased arterial pressure, and tachycardia. These authors concluded that "in man, the cardiac responses to the changes in posture appear to be related more to the passive changes in ventricular filling due to the blood shift than to the nervous regulation by the arterial baroreflexes…."

Experiments with centrifuges may mimic a broad range of gravitational influences by extent and by direction, respectively. Such experiments simu-

lating head-down positions showed complex cardiovascular responses to this altered circulatory situation *(13,14)*. Both venous and arterial pressure in the head region increase, but the arteriovenous pressure difference decreases, resulting in an inadequate cerebral blood flow. The venous system reacts to the increased internal pressure with a reflex vasodilation whereas, in contrast, the arteries react by reflex vasoconstriction (the latter representing the so-called "Bayliss effect"). The intrathoracic pressure and the volume load of the heart are increased and so are the cardiac stroke and output volumes. Furthermore, such situations provoke dramatic carotid sinus reflexes; bradycardia and even asystole may occur. Thus, the cardiovascular reaction to a head-down position seems to be governed by a series of different, interacting reflexes and reactions, all of which are induced by an increased blood volume in the upper half of the body.

Experiments under microgravity conditions, be they real or simulated, provide further insight into the effect of altered blood distribution in the human body. Preliminary investigations have shown that a head-down tilt of –5° to –6° is equivalent to the microgravity conditions in space. Although more rare, water immersion is also used for simulating weightlessness *(15)*. Under such conditions, a redistribution of body fluids from the lower to the upper parts of the body and from blood vessels into the interstitial spaces, which can be observed as swelling of facial tissue and shrinking of soft tissues of lower limbs, is found *(16)*. Under simulated hypoxic conditions, this effect is even more pronounced *(17)*, but the healthy human seems to be able to adapt to such conditions *(18)*. For space missions, a trouser-like device has been developed that applies negative pressure to the lower body parts in order to reduce or prevent such fluid redistribution *(19)*.

Other effects are identified in conjunction with the shift of body fluids. The intraocular pressure rises *(20)* after only 20 seconds of microgravity exposure *(21)*. Diuresis is forced and humans under prolonged microgravity conditions loose body weight (7.7% in one case *[16]*), at least partially by water loss. This effect seems to be forced by an increase of serum atrial natriuretic peptide that could be observed in six subjects (*17*), indicating an elevated volume load to the heart which, just under such a condition, emits atrial natriuretic peptide. Other researchers, however, could not confirm the aforementioned findings *(22)*. Increase of serum dopamine, reduction of blood velocity in the middle cerebral artery, and changes to the arterial baroreflex control of heart rate, all with complex but limited influence on the cardiovascular function, have been observed as well *(23–26)*. The more subtle effects of microgravity conditions include reduced glucose tolerance *(27)* and difficulties of circadian rhythm adaptation *(28)*.

It is well known from long-term observations in space and from simulation experiments that the human organism may adapt to microgravity conditions and does not suffer severe health defects from them *(29)*. However, the pathophysiological changes as well as long-term effects induced by microgravity seem to be of minor interest for our reflection on the lethal mechanisms of the head-down position. Nevertheless, it should be mentioned that the first and most prominent effect of microgravity is a redistribution of body fluids from the lower to the upper parts of the body. A similar but far more substantial fluid shift to the head is caused by the head-down position. This fluid shift is therefore to be discussed as the leading lethal mechanism in such cases.

The observation that persons with cardiovascular diseases may die rapidly in a head-down position (case 7) and even under moderate head-down tilt (case 10) supports the opinion that disturbed cardiac function plays an important role as cause of death in inverted body positions. The increased blood flow to the heart and the increased volume load require considerable more work from the heart, which it, eventually, cannot withstand. This understanding of the cardiac role in such deaths is compatible with all experiments and observations mentioned above and helps to explain as well the fact that elderly people, whose hearts may be less resistant to unfamiliar stresses, seem to be more prone to death in head-down position than are younger persons.

It has been claimed that quite the contrary effect, namely a reduced blood reflux to the heart following a "vanishing" of blood in the veins of the head and upper torso (which do not support blood flow owing to the absence of a muscle pump), contributes to heart failure in a head-down position *(30)*. Such an assumption, however, does not fit the experimental results cited above that show an increase in passive filling of the heart and of atrial pressure in head-down position *(11,12)*. Whether slowing down of the heart beat rate following carotid sinus reflex to elevated hydrostatic pressure in the arterial system has an influence on lethal outcome remains unclear. As to this author's opinion, this effect cannot be too marked; otherwise, the victims could not survive some hours in an inverted body position.

Reduced oxygen supply to the brain as the result of reduced arteriovenous pressure difference and hindrance of respiration by the pressure of abdominal intestina on the lungs may play additional, but less important, roles. If the brain was insufficiently supplied with oxygen, rapid loss of consciousness should be expected, but marked traces of self-rescuing efforts in some of the cases presented previously in this chapter (cases 4, 8, and 9) show that this is not necessarily the case. Moreover, if the thorax and lungs, respectively, are

compressed over a considerable period of time, cries for help (as observed in case 4) would be impossible.

Thus, it appears that death in a head-down position is a death of gradual (occasionally sudden) heart failure and not a kind of cerebral death or death by suffocation ("asphyxia"). Of course, asphyxia should be discussed as an additionally factor influencing lethal outcome in cases where the victim is caught in very tight openings or similar structures that prevent proper breathing by thorax compression. However, asphyxia should not generally be assumed in all cases of head-down deaths. In one of the most recent contributions to the literature, Glatter and Karch (2004) correctly warn against the application of the term "positional asphyxia" to any otherwise unexplained death *(31)*.

5. Conclusion

Although head-down fatalities are rare events and are infrequently reported in the literature, some authors *(6,7)* mention that in the past inverted suspension was a common torture and death penalty method. St. Peter is said to have been crucified in a head-down position because he himself desired not to die in the same manner as Jesus (crucifixion in upright body position). Even until World War II, such torture was applied from time to time and probably still takes place in some countries nowadays. As the forensic examiner must, on occasion, examine victims of torture, it may be useful to remember the after-effects and objective traces, if any, of such a torture method. To this author's knowledge, detailed descriptions on this point are missing in literature.

Another point of uncertainty is the survival time after coming into an inverted body position. Reports in the earlier literature (cited in ref. *7*) claim that agony after head-down hanging may last many hours up to 1 day. The cases described in this chapter suggest a similar survival time, at least of some hours in most cases. Apparently, the survival time depends on the strength and endurance of the victim's cardiovascular system. The period of time needed after that sudden death of a person with preexisting severe cardiovascular disorders (as in case 7) has to be expected after this person has been tilted down remains entirely unclear.

References

1. Schwarz F (1970) Der außergewöhnliche Todesfall. Enke, Stuttgart.
2. Prokop O, Radam G (1987) Atlas der gerichtlichen Medizin. Karger, Basel.
3. Hilgermann R, Richter O (1973) Einige besondere Fälle aus dem rechtsmedizinischen Obduktionsgut. Beitr Gerichtl Med 30, 163–174.

4. Yoshida K, Harada K, Sorimachi Y, Makisumi T (1995) Death in head-down position: An autopsy report with reference to physiological mechanism. Jpn J Legal Med 49, 33–36.
5. Purdue B (1992) An unusual accidental death from reverse suspension. Am J Forensic Med Pathol 13, 108–111.
6. Marshall TK (1968) Inverted suspension. Med Sci Law 8, 49–50.
7. Madea B (1993) Death in a head-down position. Forensic Sci Int 61, 119–132.
8. Thiel I, Huckenbeck W (2003) Der kalte Tod an der Treppe. SeroNews 8, 32–34.
9. Kauffmann F (1927) Einfluss des hydrostatischen Drucks auf die Blutbewegung, Anpassung der Gefäße. In Bethe A, Bergmann G v, Embden G, Ellinger A, eds., Handbuch der normalen und pathologischen Physiologie, Vol. VII, part 2. Springer, Berlin, pp. 1414–1439.
10. Uchigasaki S, Takahashi H, Suzuki T (1999) An experimental study of death in a reverse suspension. Am J Forensic Med Pathol 20, 116–119.
11. Wilkins RW, Bradley SE, Friedland CK (1950) The acute circulatory effects of the head-down position (negative G) in normal man, with a note on some measures designed to relieve cranial congestion in this position. J Clin Invest 29, 940–949.
12. Deklunder G, Lecroart JL, Chammas E, Goullard L, Houdas Y (1993) Intracardiac hemodynamics in man during short periods of head-down and head-up tilt. Aviat Space Environ Med 64, 43–49.
13. Folkow B, Heymans C, Neill E (1965) Integrated aspects of cardiovascular regulation. In Hamilton WF, Dow P, eds., Handbook of Physiology, Sect. 2, Vol. III. American Physiological Society, Washington DC, pp. 1787–1823.
14. Gauer OH, Thron HL (1965) Postural changes in the circulation. In Hamilton WF, Dow P, eds., Handbook of Physiology, Sect. 2, Vol. III. American Physiological Society, Washington DC, pp. 2409–2439.
15. Shulzhenko EB, Panfilov VE, Gogolev KI, Aleksandrova EA (1979) Comparison of physiological effects of head-down tilting and immersion on the human body. Aviat Space Environ Med 50, 1020–1022.
16. Kirsch KA, Baartz FJ, Gunga HC, Röcker L, Wicke HJ, Bünsch B (1993) Fluid shifts into and out of superficial tissues under microgravity and terrestrial conditions. Clin Invest 71, 687–689.
17. Loeppky JA, Roach RC, Selland MA, Scotto P, Luft FC, Luft UC (1993) Body fluid alterations during head-down bed rest in men at moderate altitude. Aviat Space Environ Med 64, 265–274.
18. Loeppky JA, Roach RC, Selland MA, Scotto P, Greene ER, Luft UC (1993) Effects of prolonged head-down bed rest on physiological responses to moderate hypoxia. Aviat Space Environ Med 64, 275–286.
19. Baisch FJ, Petrat G (1993) Body fluid distribution in man in space and effect of lower body negative pressure treatment. Clin Invest 71, 690–699.
20. Draeger J, Schwartz R, Groenhoff S, Stern C (1993) Self-tonometry under microgravity conditions. Clin Invest 71, 700–703.
21. Mader TH, Gibson CR, Caputo M, Hunter N, Taylor G, Charles J, et al. (1993) Intraocular pressure and retinal vascular changes during transient exposure to microgravity. Am J Ophthalm 115, 347–350.

22. Drummer C, Heer M, Dressendörfer RA, Strasburger CJ, Gerzer R (1993) Reduced natriuresis during weightlessness. Clin Invest 71, 678–686.
23. Lacolley PJ, Pannier BM, Cuche JL, et al. (1993) Microgravity and orthostatic intolerance: carotid hemodynamics and peripheral responses. Am J Physiol 264, H588–H594.
24. Frey MAB, Mader TH, Bagian JP, Charles JB, Meehan RT (1993) Cerebral blood velocity and other cardiovascular responses to 2 days of head-down tilt. J Appl Physiol 74, 319–325.
25. Harrison MH, Rittenhouse D, Greenleaf JE (1986) Effect of posture on arterial baroreflex control of heart rate in humans. Eur J Appl Physiol 55, 367–373.
26. Yamazaki F, Matsumura N, Nagata J, Ando A, Imura T (2001) Spontaneous arterial baroreflex control of the heart rate during head-down tilt in heat-stressed humans. Eur J Appl Physiol 85, 208–213.
27. Trumbach S (1988) Glukose-Toleranz und Insulin-Sekretion unter simulierter Schwerelosigkeit. Medical Thesis, RWTH Aachen, Germany.
28. Allmers H (1992) Zirkadiane Rhythmik bei der Simulation eines D-2 Weltraumfluges. Medical Thesis, RWTH Aachen, Germany.
29. Lathers CM, Diamandis PH, Riddle JM, Mukai C, Elton KF, Bungo MW, et al. (1990) Acute and intermediate cardiovascular responses to zero gravity and to fractional gravity levels induced by head-down or head-up tilt. J Clin Pharmacol 30, 494–523.
30. Schmidt P, Madea B (2004) Tod in abnormer Körperposition—Physical restraint. In Madea B, ed., Praxis Rechtsmedizin. Springer, Berlin, pp. 204–208.
31. Glatter K, Karch SB (2004) Positional asphyxia: inadequate oxygen, or inadequate theory? Forensic Sci Int 141, 201–202.

Forensic Odontology

4

Bitemarks

Presentation, Analysis, and Evidential Reliability

Jules Kieser, BDS, PhD, Geoffrey Tompkins, PhD, Donna Buckingham, BA, LLB, Norman A. Firth, MDSc, and Michael Swain, PhD

CONTENTS

SUMMARY

A bitemark is the physical end product of a complex set of events that occur when human or animal teeth are applied to skin or foodstuff. Despite its long history of admission as evidence, there remains a number of significant challenges to its evidential usefulness. Principal among these are the presumed uniqueness of the human dentition, the variable visco-elastic properties of

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human skin, and the qualitative nature of bitemark analysis. In this chapter, we outline these and other issues and we review the newly described bacterial fingerprinting technique.

Key Words: Bitemark; evidence; human dentition; digital evaluation; animal bites; postmortem damage; salivary analysis; *Streptococcus*; Daubert; Frye.

1. Introduction

A bitemark may be defined as the physical alteration on a surface such as skin or food that is caused by the dentition of a human or animal. A bitemark is the end product of a highly complex set of events that includes the closure of the mouth, movements of the victim, the angle of approach, and the force exerted by the biter. This is further complicated by factors such as the positioning and state of the biting teeth, the biter's temporomandibular joint and occlusion, and the visco-elastic properties of the material bitten. The analysis of bitemarks has a long history, dating back to the trial of the Reverend George Burrows, who was accused of torturing and soliciting two young women into witchcraft in 1692 *(1)*. Other early cases include *Ohio vs Robinson* of 1870, in which Dr. Robert Taft testified that he had examined marks left on the murdered Mary Lunsford's arm and found that they were toothmarks, and the 1906 case at the Cumberland Assizes at Carlisle, where a burglar was convicted on the basis of a comparison of his teeth and the marks left on a piece of cheese *(2)*. The recent revival of interest in bitemark analysis followed two technological advances. First, the use of polymerase chain reaction (PCR)-based typing of DNA present in the saliva either on human skin or on foodstuff, which is then compared with the DNA profile of the suspect *(3,4)* and, second, the use of digital imaging techniques to facilitate bitemark analysis *(5,6)*. It has been argued that bitemark analysis may not be as accurate as it has been claimed *(7)*. This argument was supported by those who claimed that the human dentition was in fact not as unique as was previously supposed *(8)*, that it was impossible to determine who else in the broader population could have produced a bite similar to the one under investigation *(9)*, or that some overlay methods of analysis were inaccurate and subjective *(7,10)*. However, it has recently been suggested that genotypic comparison of oral streptococci might be used in bitemark analysis *(11)* and also that if it is acknowledged that bitemark analysis is qualitative that this evidence might be used for selecting a perpetrator from a small group of suspects *(12)*.

This review is divided into six parts. First, we consider the presentation of human and animal bitemarks; second, we look at bitemarks in food; third,

we review the visco-elastic properties of human skin as well as the individuality of teeth; fourth, we critically evaluate the current ways in which bitemarks are evaluated; fifth, we introduce the novel technique of bacterial genotyping (DNA "fingerprinting"); and finally, the evidential reliability of bitemark analysis.

2. Prevalence and Presentation

2.1. Human Bitemarks

Bite wounds are responsible for about 1% of emergency department visits in the United States and, of these, bites by dogs and cats are the most common, followed by human bites *(13,14)*. There are two types of human bites; occlusional bites, which result from the teeth being sunk into the skin, and tooth marks sustained when a body part (e.g., a fist) strikes the dentition *(15)*. Their prevalence peaks between the ages of 10 and 34 years and during spring, early summer and on weekends *(16)*. The prevalence of human bites in children is given as 1 per 600 emergency department visits *(17,18)*. Human bites occur most frequently on the hands and upper extremities (60–75%) but can also involve the neck, nipples, and face (15–20%) and the lower extremities (5% *[19]*). Traumatic love bites have been recorded mostly from the head, neck, and genital areas *(20,21)*.

Occlusional bitemarks are described as oval- to circular-patterned injuries that typically consist of two opposing U-shaped arches, each representing a maxillary and mandibular arch, separated to some degree at their bases (Fig. 1). A central contusion or ecchymosis is often recorded and is thought to result either from negative pressure produced by the biter at the time of the bite or by pressure of the teeth, which rupture small vessels or capillaries in that area *(22,23)*. Lacerations, linear abrasions (drag marks), and areas of punctured skin caused by the movement of teeth over the skin or indentations caused by imprinting of the palatal surfaces of the teeth against the skin may also be present. Although individual arches are usually produced by the anterior dentition, markings from as far back as the molars have been recorded *(24)*. Clearly, the physical appearance of a bite is determined by a number of factors, including the bite force, the presence or absence of a struggle, the anatomical position of the bite, the age and ethnicity of the victim, and the presence or absence of clothing over the skin *(25)*. The appearances of bitemarks also vary with time. Recently, Avon and her co-workers *(26)* presented the first qualitative experimental analysis of differences between bites made antemortem and postmortem but were unable to show that it was possible to determine whether the bite was made before or after death.

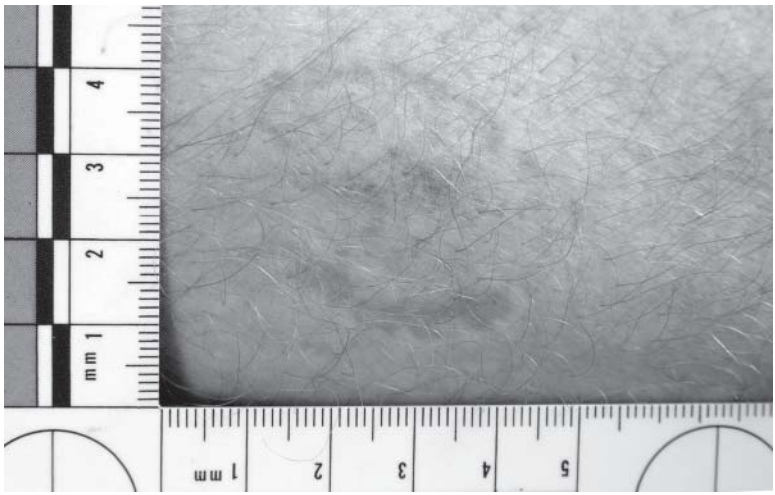

Fig. 1. Classic appearance of a human bitemark on skin. Note the two opposing arches and central contusion.

2.2. Animal Bitemarks

Probably the earliest recorded bitemarks are those attributed to *Tyrannosaurus rex*. Carpenter *(27)* interpreted healed wounds on an *Edmontosaurus* caudal vertebra as evidence of a failed *T. rex* predation bite. More recently, a number of *Triceratops* bones exhibiting large *T. rex* bite marks have been described *(28)*. Figure 2 shows predatory bite marks on a pilosaur's humerus.

Animal bites show a massive spectrum of variation, related to the animal involved, the circumstances of the attack and the age, gender, or ethnicity of the victim. Given the close association between humans and dogs, it is probably not surprising that dog bites are the most common animal bites found in humans, with a reported prevalence of 0.3% to 1.1% of all emergency department visits *(13,29,30)*. It has been suggested that almost half of all children will have been bitten by a dog at some point in their lives *(31)*. Among children, more than 50% of documented bites have been to the face, neck, and head *(32)*. Canine bitemarks are usually described as puncture or tear marks, sometimes resembling stab wounds, occurring singly or in pairs (Fig. 3) and differ from those of wolves in that the latter usually attack the gluteal regions *(33)*. Classically, wolves hunt in packs and leave only after all edible parts have been consumed, unless interrupted by human intervention. Bitemark analysis has been shown to be useful in distinguishing between wolf and dog attacks *(33)* and, more recently, in a number of large cat attacks *(34,35)*.

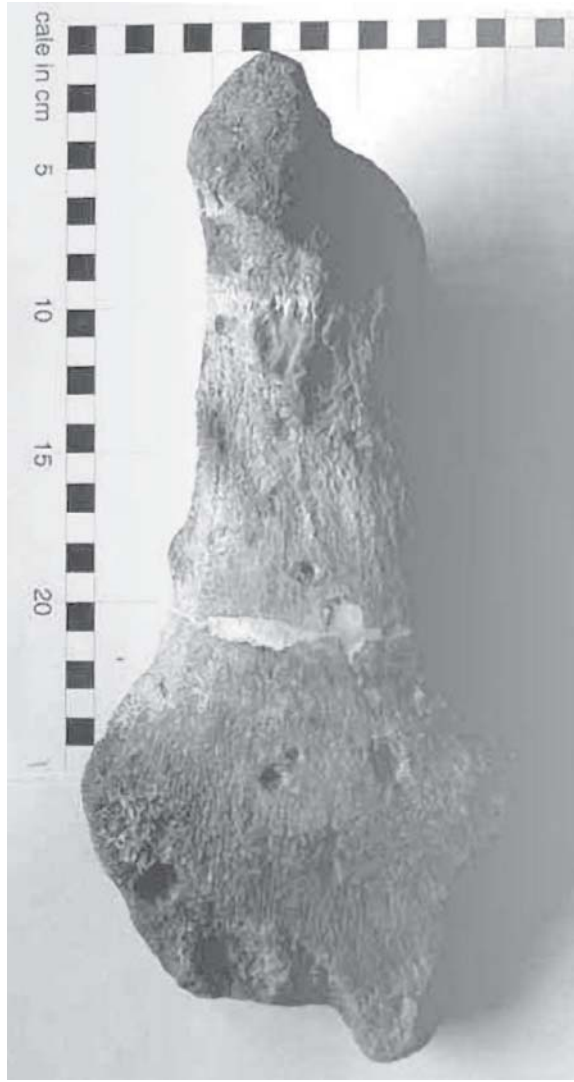

Fig. 2. Predatory bite marks on the left humerus of *Peloneustes*, a small pliosaur. (Courtesy of Dr. Richard Forrest, Leicester, United Kingdom.)

Postmortem injuries inflicted on human corpses by domestic animals have been reported repeatedly in the literature. Large soft tissue defects inflicted postmortem by dogs usually display rounded wound margins (Fig. 4). In contrast, bite injuries inflicted by domestic cats exhibit both sharp and crenated

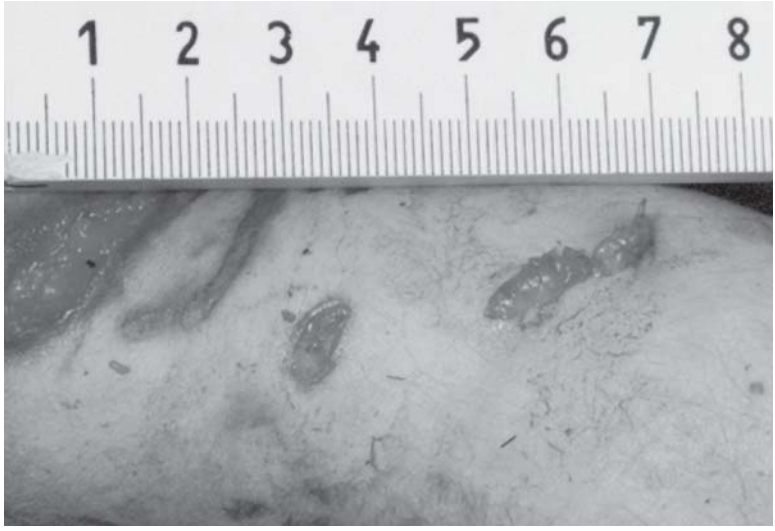

Fig. 3. Classic appearance of a dog bite: oval, stab wound-like bitemarks caused by the canine teeth of an American Staffordshire Terrier. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

wound margins as well as adjacent punctured wounds, the latter corresponding to canine teeth marks (Fig. 5A,B).

Mostly, animal depredation is seen as postmortem damage by small carnivores or rodents. Although the differentiation between postmortem and antemortem bites usually presents no problems, species attribution can be a challenge. Tsokos and his co-workers *(36,37)* described postmortem bites caused by rodent activity to fresh skin as circular, with finely serrated wound margins. Parallel cutaneous lacerations caused by rodent incisive chewing is considered to be diagnostic when present (Figs. 6 and 7A,B). Generally, the areas involved are those exposed and unprotected by clothing: eyelids, mouth and nose, and the hands.

In an unusual animal bitemark case, the recent deaths of a highly endangered species from Australia, the Brush-tailed Bettong (*Bettongia penicillata),* was attributed to feral cats by examining the intercanine distances left by predator teeth on radio collars worn by the Bettongs *(38).*

3. Bitemarks in Food

As noted earlier, one of the earliest bitemark cases relates to the conviction of a burglar who had bitten the side of a cheese in 1906 *(1)*. Although

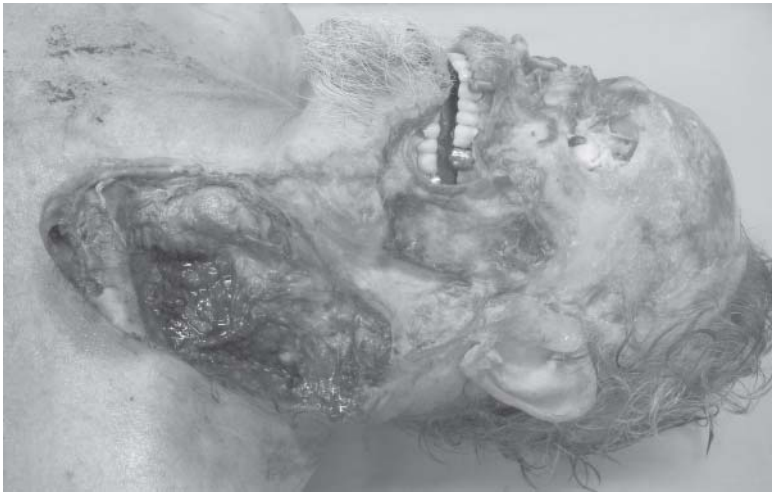

Fig. 4. Partly seletonization of the head and a large soft tissue defect on the left side of the neck owing to postmortem animal interference by a dog. (Courtesy of Dr. Seisaku Uchigasaki, Hamburg, Germany.)

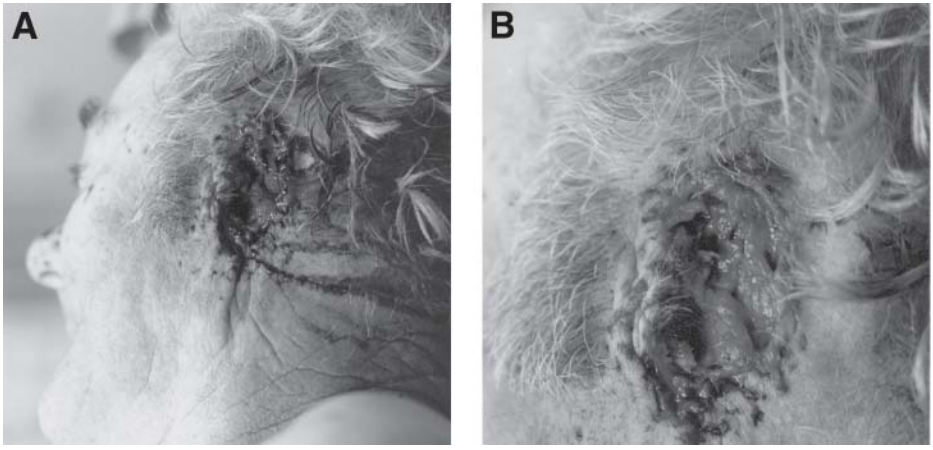

Fig. 5. (A,B) Postmortem bite injuries caused by a domestic cat (*Felis silvestris f. catus*) with complete loss of the decedent's left ear. Both sharp and crenated wound margins are seen around the removed ear as well as adjacent punctured wounds. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

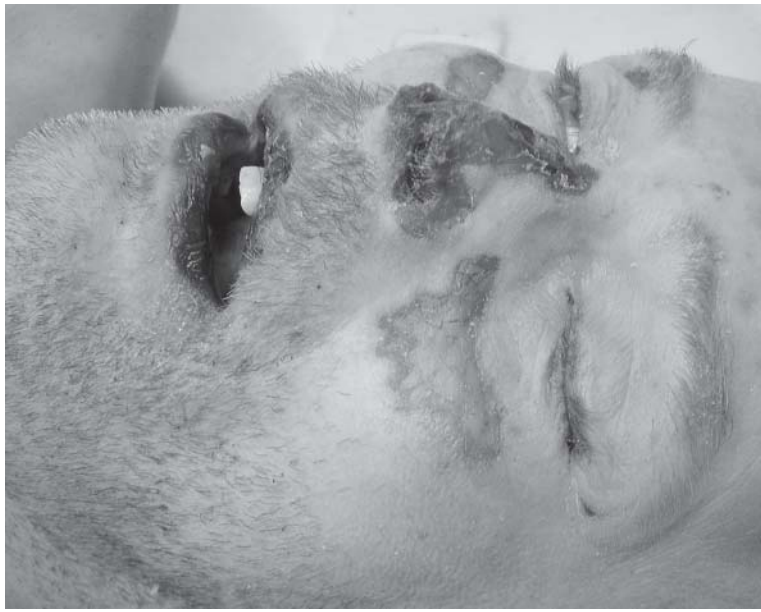

Fig. 6. Superficial postmortem bitemarks over the cheek bones and deeper soft tissue loss of the nose caused by rodents. Note the serrated wound margins and parallel cutaneous lacerations. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

there have been a number of early cases in which bitemarks on foodstuffs have been analyzed *(39–42)*, there has been little experimental or empirical work directed toward understanding biting mechanics in food. An exception was Stoddart *(43)*, who described a method of producing permanent models of bitten perishable substances, but unfortunately no analysis of reliability or accuracy was included in the study. Rudland *(44)* investigated the dimensional stability of bitemarks in apples after long-term (10 years) storage in a fixative consisting of formaldehyde, glacial acetic acid, and ethanol and found that the apples retained their dimensions as compared with a stone model. In 1994, Aboshi et al. *(45)* empirically demonstrated the usefulness of computer imaging when applied to bites on various foods, including sponge cake, chocolate, and coconut slices. Further experimental analysis by this group attempted to quantify the comparison between biter's teeth and bitemarks produced on foodstuffs by means of a computer-based shape analysis program *(46)*. Interestingly, the group failed to use this method in subsequently reported cases of bitemarks left in chocolate and chewing gum *(47,48)*.

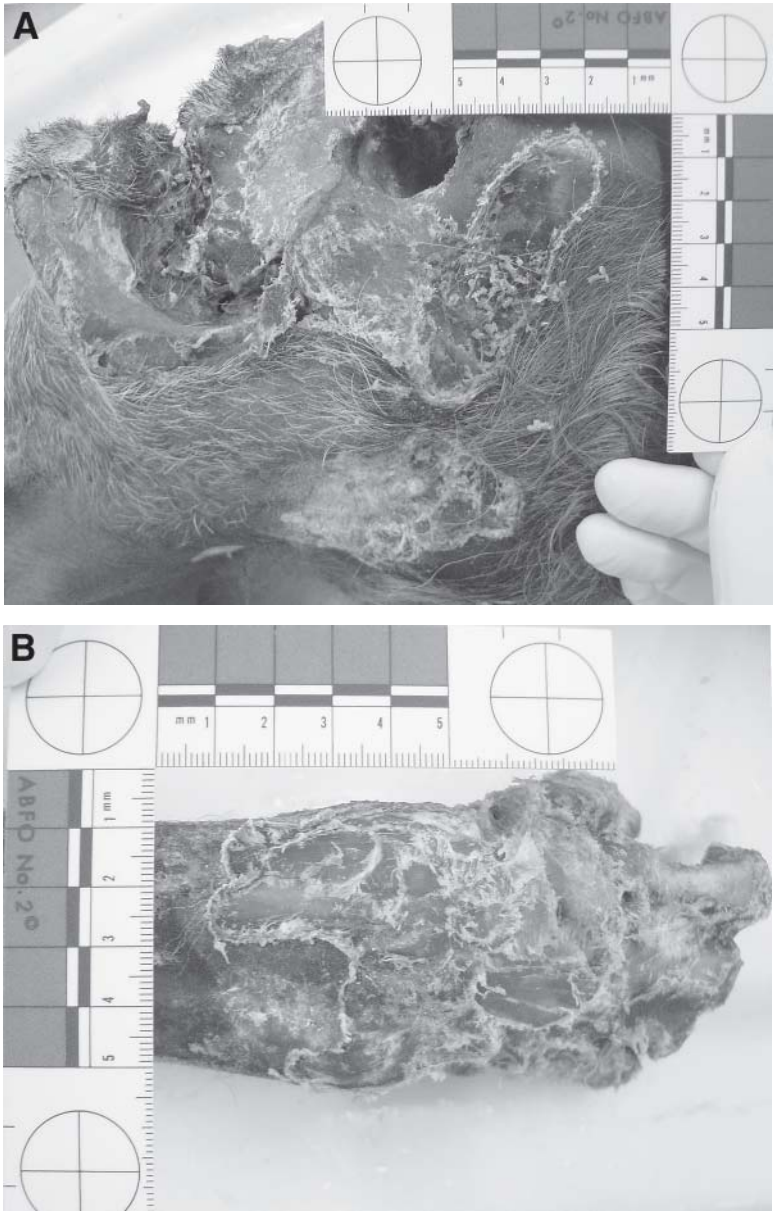

Fig. 7. Mummified body with extensive skin and soft tissue artefacts as the result of postmortem damage caused by rodents. Parallel cutaneous lacerations are seen in the desiccated tissue. **(A)** Face. **(B)** Back of the right hand. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

A recent double-blind experiment highlighted the ability of forensic odontologists to use pattern-associated comparisons in the positive identification of bitemarks in a number of foodstuffs *(49)*. Subsequently, this method was successfully used by the same unit in the case of a bitemark left in cheese in which there were limited numbers of concordant features *(50)*. Interestingly, the comparison leaned heavily on a centrally positioned defect on the incisal edge of the upper central incisor as reproduced in Fig. 8.

4. Properties of Skin and Teeth

4.1. Visco-Elastic Behavior of Skin

The visco-elastic properties of skin are anisotropic or directionally dependent *(51)*. Its biomechanical properties are primarily related to three of its constituents and their spatial arrangement, namely collagen, elastin, and proteoglycans. Collagen fibers are randomly aligned parallel to the surface of the skin in a somewhat loose fashion, especially when compared with tendon. Elastin fibers vary in their density through the dermis with the amount increasing with depth *(52)*. The behavior of the collagen fibers is highly dependent on their prealignment and state of strain; tendinous collagen fibers are prestrained and well aligned, but those in skin tend to be loose and randomly coiled, and hence have a low stiffness upon loading. Increased tension results in greater fiber alignment and greater stiffness. The stress-strain response of collagen fibres in skin typically consists of three regions; first, a low modulus region typically for extensions of 20 to 40%, followed by a transitional region over the next 5 to 50% strain and, third, a region where the fibers are completely aligned and the stiffness of tendon is achieved. A scanning electron microscopy examination of human abdominal skin also showed that with increasing strain the alignment of the collagen fibres gradually increased with depth within the skin *(53)*.

The visco-elastic properties of collagen have been investigated by Fung *(54)* and more recently by Purslow et al. *(55)*. It was initially thought that the visco-elastic behavior was an intrinsic attribute of collagen, but Purslow et al. showed that relaxation within the fiber was associated with creep of the surrounding tissue *(55)*. This has more recently been confirmed by Puxkandl et al. *(56)*, who showed that the proteoglycan-rich matrix surrounding the collagen was the source of the relaxation.

Although elastin is only a minor component of skin (typically less than 2% by weight of the dermis), it has an almost entirely linear stress–strain response, with a far lower effective elastic modulus than collagen, namely 0.5

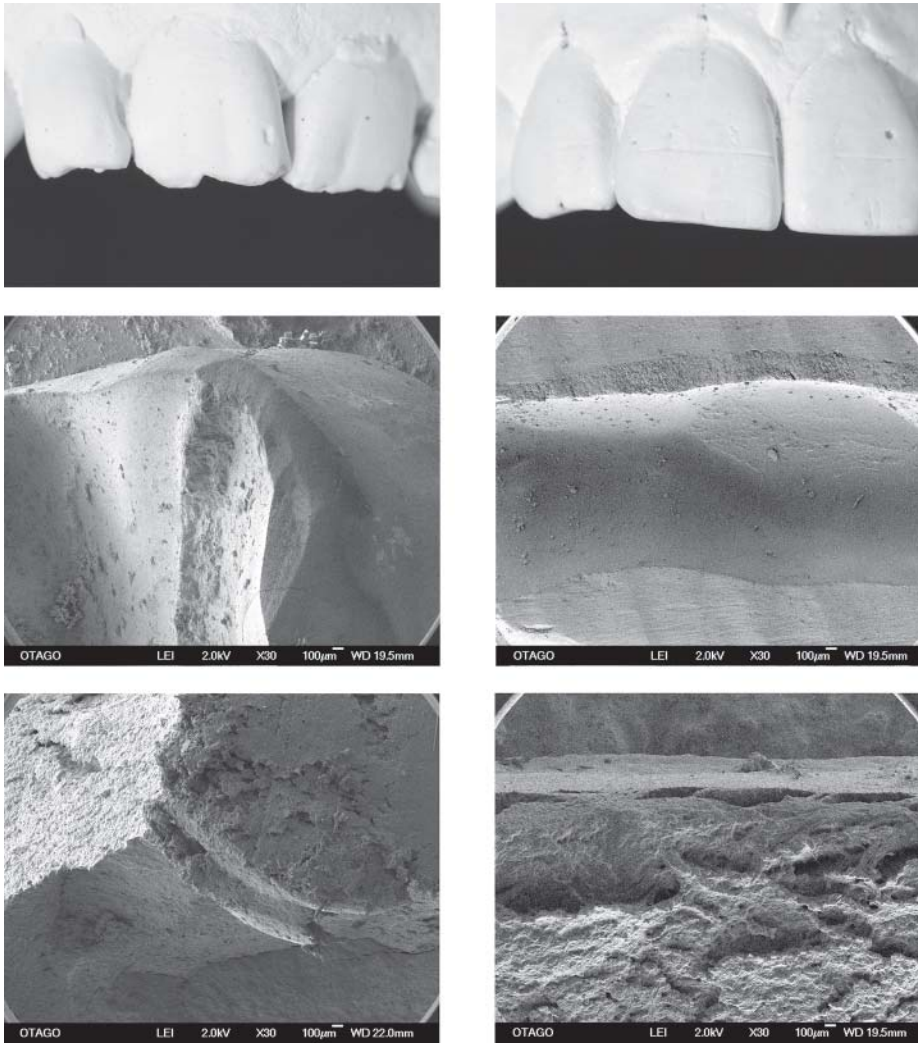

Fig. 8. Incisor notching and its effect on a bitemark in cheese. Left column shows a cast of a notched upper right incisor (top), a scanning electron microscopy (SEM) view of the incisal edge (middle), and an SEM of an incisal bite into cheese. Right column shows the same sequence for a smooth incisor.

to 0.6 MPa *(57)*. It also is capable of entirely elastic (reversible) extension of 100% of its original length, well beyond that of collagen. Proteoglycans are considered to be primarily responsible for the visco-elastic behavior of skin especially at small strains and this is associated with the presence of hyalu-

ronic acid, which demonstrates highly visco-elastic behavior that is strongly dependent on strain rate.

The anisotropic behavior of skin was first described by Langer in 1861 *(58)* and was subsequently shown by Cox *(59)* to be associated with the orientation of its constituent collagen and elastin fibres. This behaviour is associated with the initial deformation of the skin and the fact that the transition from region 1 to region 3 of the stress–strain curve, which is reached earlier in directions more aligned with the collagen and elastin fibers than in the orthogonal directions. Skin also exhibits significant lossy behavior, as do all visco-elastic materials (i.e., energy is dissipated during the loading and unloading cycle). This lossy behavior is demonstrated by the angle between the maximum stress and maximum strain during small strain cyclic deformation of visco-elastic materials. In the case of skin, there is typically a 15 to 20° loss angle. In comparison, hyaluronic acid varies between almost 90° to less than 20° as the strain rate increases over the range from 0.1 to 10 Hz *(60)*.

Bite marks of skin are manifestations of large strain deformations, well beyond their reversible range. Teeth can be considered rigid indenter-like structures that generate a complex deformation state, even for isotropic elastic materials. High compressive stresses together with superimposed shear stresses are generated directly beneath the points of contact between the teeth and skin. However, as the skin surface is extended by the biting action of the teeth, high tensile strain is developed near the surface immediately next to the point of contact *(61)*. This process may be further complicated by the presence of bone, muscle, fascia or tendons beneath the contact area and of course by permanent rupture when critical strain is exceeded.

In terms of bitemark analysis, it means that not only are skin marks dependent on factors such as angle and force of attack, presence or absence of movement, sweat or clothing on the skin, age, gender, and ethnicity of the victim but also on the complex visco-elastic properties of skin. These are largely determined by the spatial arrangement of collagen, elastin and proteoglycans, which vary from location to location, and have not systematically been investigated from a forensic point of view.

4.2. Uniqueness of the Human Dentition

Although it is common knowledge that the human body is a developmentally and functionally integrated whole, individualization requires the decomposition of the organismic whole to isolate characters that are useful for comparative forensic analysis. Such characters cannot be any observable feature of the body but rather a feature that captures distinguishing peculiarities

of the individual concerned. No investigation has directly focused on the decision-making criteria used by forensic odontologists to include or exclude characters in bitemark analysis. Yet, there is evidence of widely differing opinions among odontologists as to where the dividing line is between a marginally acceptable or unacceptable character. Clearly, a meaningful character must be one that can be described and critically evaluated, tested and potentially be rejected, to conform to Popperian criteria of falsification *(62)*. Because individualization analysis depends on parsimony, meaningful characters for use in forensic identification must be independent from one another, in other words they must have a demonstrable lack of co-variation. Meaningful characters must also be well defined. Badly defined characters will provide no severity of test, no matter how many of them are involved in the analysis.

The reliability of bitemark evidence rests on the assumption that no two humans have identical dentitions in respect to the size, shape, or arrangement of the teeth *(63–65)*. Yet, this assumption of uniqueness rests on the results of a single paper, that of Rawson and co-workers, published in 1984, who referred only to the uniqueness of the position of the teeth *(66)*. Interestingly, this is probably the least stable of the three characters, especially if one considers the large number of persons of all ages undergoing orthodontic treatment that actively seeks to change relative tooth positions. Hence, the question remains: how unique is the dentition? Kieser *(67,68)* has argued that although consideration of odontometric data has centred largely on discrimination between sexes and ethnic groups, little attention has been paid to the allocation or assignment of individuals on the basis of tooth size. These two processes are independent, although often confused, and require different statistical analyses. Their results showed that using tooth size, correct discrimination between male and female Negroes, Caucasoids, and Amerindians could only be expected in 66 to 78% of cases. Moreover, a lower proportion (12–55%) of individuals could be allocated with a high degree of confidence, even if their original population was known *a priori*. Harris *(69)* powerfully argued that because the greatest component of tooth-size variation is within population groups rather than between them, these data are insensitive for forensic purposes. The genetic covariance of tooth morphologies and sizes within tooth fields (incisors, canines, postcanine teeth) renders many tooth crown dimensions statistically redundant. He added that measuring more teeth or more dimensions does not increase the ability to discriminate between genders or ethnic groups.

5. Digital Evaluation of Bitemarks

The use of image editing and computer-assisted bitemark analysis has been documented for more than 10 years. The efficacy of such techniques has been tested among forensic odontologists and general dentists *(5,70,71)*. Although conventional photographs have continuous tonal qualities derived from the small crystals embedded in the photographic emulsion, digital images are derived from multiple pixels (picture elements) each being a square or rectangle of assigned colour. The density of pixels is expressed as pixels per square inch and the highest possible resolution of a piece of equipment (e.g., a camera or scanner) is expressed as the maximum number of pixels per square inch for width and height (e.g., 1020×720). A photo-quality print expressed as dots per inch (dpi) is considered to be 300×300 dpi. The maximum size of an acceptable image without significant distortion is therefore derived by 1020 divided by 300 and 720 divided by 300 ($\sim 3 \times 2$ inches). If the image was enlarged to 8×10 inches, a jagged edge between pixels (pixelation) would be noticeable to the observer of the image. The price of high digital resolution is file size (10–20 megabytes) and demands on the computers random access memory, which should be three times the size of images being processed. The major advantage of digital photography lies in the direct control over the image (e.g., focus, angulation, area of field). Moreover, image-processing software can be used to help control image processing, for example, adjusting the image, sharpening out of focus areas, correction of photographic distortion, adjusting contrast, brightness and color balance, removing (cropping) unwanted distracting areas away from the field of interest, and other editing functions. Comparison of images can be done at either 1:1 image sizing or by magnification, and independent adjustments in both *x*- and *y*-axes is possible.

The most commonly used image processing software is Adobe Photoshop (Adobe Systems, Seattle, WA). This program records each step of image manipulation, which is extremely important for presentation of evidence. Reproducibility and accuracy can be demonstrated and has been reported by Naru and Dykes *(5)* and Sweet et al. *(72)*.

Three disadvantages of digital imaging techniques (print quality, original image quality, and image file storage) have now largely been resolved. Photographic quality prints are useful for the presentation of evidence to a jury. In recent years, affordable domestic printers with photographic quality printing have become the norm. Images can also be presented in court with digital projectors. Images saved in a lossless format (e.g., tagged image format file) are big files. In recent years, image-storage technology has largely addressed the problems of multiple large files. Both image-storage devices

and operating system changes (e.g., Macintosh OS X) have been developed to satisfy domestic computer users.

Sweet and co-workers *(72)* have demonstrated that a computer-overlay method is superior to hand-traced overlays and recommend that the latter technique should be discontinued.

Recording of a three-dimensional object such as a bitemark as a two-dimensional image requires a scale in the same plane as the bitemark and the camera positioned at right angles to this plane directly above the centre of the field of interest. An ABFO no. 2 scale (Lighting Powder Co. Inc., Jacksonville, FL) is ideal for this. However, distortion arises if the camera is not over and at right angles to the plane of the bitemark and the scale, or if the scale is not in the same plane as the bitemark. Distortion will also result if the scale is bent during the taking of the photograph.

A flatbed scanner is required to scan study models from suspects who have given consent to have impressions taken. The study models cast in dental stone together with an ABFO no. 2 scale should be placed on the scanner. Not all the cusps and incisal edges will be in contact with the glass. A sharper edge to the biting surfaces of the teeth is obtained if the direction of the scan is from the lingual aspect through to the buccal aspect if one side has features of interest. Ambient light can be eliminated by placing a box over the scanner. Once scanned, the image can be rotated to a desired position. Rotation can be selected at 90 or 180 degrees or "arbitrary" clockwise or counterclockwise. In order to resize the image 1:1 the ABFO scale should be aligned to coincide with *x–y* axes on the computer screen. It is possible to move the image of the maxillary and mandibular study models independently of each other. Selection of biting surfaces is made with the "magic wand" tool (also holding down the shift key if multiple selections are desired). This is the most subjective part of the process, but with practise consistent results can be achieved by all examiners, as shown by Naru and Dykes *(2)*. Adjusted images need to be saved to layers, each clearly labeled and preferably with some text that, besides providing information, prevents accidental transposition. The scale also needs to be added to each layer. The overlayer is then ready for comparison with the bitemark. This comparison can be done digitally or by comparing photographs, etc. Superimposition of images is performed with commands to drag and rotate images.

6. *Salivary Analysis*

Until recently, saliva stains could provide only supportive evidence linking an aggressor to a bitemark. This evidence was provided as serological

comparison of the saliva stain with a specimen from a suspect. Generally, serological comparisons have low discriminatory capability, although, significantly, they are able to unequivocally discount a non-matching suspect. Advancements in DNA technology have had great impact on forensic science, but as recently as 1994 it was widely held that an aggressor's DNA is not recoverable from a bitemark or other saliva stains because the nucleases present in saliva rapidly degrade DNA *(73)*. The half-life of DNA incubated in human saliva has been estimated at about 1 minute *(74)*.

Sweet and collaborators have devised a method aimed at overcoming this difficulty. The technique centers on the recovery of intact epithelial cells (derived from the biter) that have been deposited in the bitemark *(75)*. The aggressor's DNA (within the epithelial cells) is protected from the salivary nucleases and can be recovered once the cells have been washed free of the nuclease-containing saliva. The purified DNA can then be compared to that of a suspect by established molecular techniques such as analysis of short tandem repeat sequences. The success of this method relies on careful double-swabbing of the bitemarks, first with a moist swab to loosen the cells and then with a dry swab to lift the cells from the skin surface. Under controlled experimental conditions, in which drops of saliva were placed on the skin of cadavers, the approach had a success rate of approx 80% *(75)*. However, under diverse and variable forensic conditions, the success rate is likely to be lower and anecdotal reports suggest that this is indeed the case. Nevertheless, when successful, this approach can provide compelling evidence either to convict or to exonerate a suspect. The recovery of the biter's DNA should certainly be attempted because of the discriminative power provided by analysis of human DNA. Undoubtedly, with time, the method will be refined to a point that it will be confidently applied in all appropriate circumstances. Until such a time, however, alternative approaches should be considered.

The mouth is home to a myriad of bacteria; recent analyses suggest that as many as 500 distinct species may be found in the human oral cavity *(76)*. The teeth themselves provide a unique niche for a specialised microbial community that can develop into macroscopical aggregations called dental plaque. Even in the absence of visible plaque, a bacterial biofilm coats the teeth and this community will start to develop within minutes of professional dental prophylaxis *(77,78)*. The most abundant bacterial genus harboured within the human mouth is *Streptococcus (79)*. Some streptococcal species cause dental caries, whereas others can lead to more threatening conditions such as rheumatic fever. However, most oral streptococci are relatively benign and are present in large numbers on the teeth of virtually all healthy humans. Thus, essentially every bite will deposit large numbers of streptococci on the bitten

surface. Although it has its own microbial community, the skin is not normally colonised by streptococci. Recovery of these bacteria from the skin therefore will generally imply oral contact. In our own studies involving experimental bitemarks self-inflicted on human skin, oral streptococci were recovered from all sites (using selective culture media) for up to 24 hours *(80)*. Because the victims of violent sexual crimes generally seek medical assistance well within this time frame *(81)*, it should be possible to isolate oral streptococci derived from the biter provided the bitemark has not been washed.

The taxonomy of the oral streptococci has always been somewhat confusing and investigations aimed at defining species by molecular (DNA) techniques have not generally helped because the common nonpathogenic species such as *S. sanguinis*, *S. oralis*, *S. mitis*, and *S. gordonii* are genotypically highly diverse *(82–84)*. In fact, it has been very difficult to find two identical genotypes (strains) within a species *(84)*.

The problem of extreme genotypic diversity may have delayed taxonomic advances but could be of value to forensic scientists. By comparing the DNA profiles (genotypes) of streptococci isolated from the teeth of eight unrelated adults, each individual could be characterised by specific bacterial genotypes *(80)*. Furthermore, no isolated streptococcal genotypes were shared between individuals *(80)*. In a refinement of this approach, using a randomly primed PCR method to compare oral bacterial genotypes with those recovered from an experimental bitemark, an investigator was able to unambiguously identify, from a group of eight individuals, the perpetrator of the bitemark *(85)*. The same eight participants were examined 12 months later and each found to retain at least 20% of the predominant streptococcal genotypes on their teeth (Rahimi M, Heng NCK, Kieser JA, Tompkins GR, unpublished data). An example of arbitrarily primed-PCR-generated streptococcal genotypic profiles is shown in Fig. 9. The frequency at which streptococcal genotypes are shared between unrelated individuals has yet to be determined but is expected to be less than 10^{-2} (based on previous studies *[82–84]*). Assuming that oral streptococcal genotypes are transmitted and established independently of one another, then the chance of two familially unrelated individuals harbouring the same four streptococcal genotypes would be of the order of 10^{-8}, which is a compelling statistic.

7. Evidential Reliability of Bitemark Analysis

From the foregoing, it is clear that bitemark analysis has had a long history of admission as evidence and that there are a number of promising new areas of research in this field. However, significant and unanswered questions

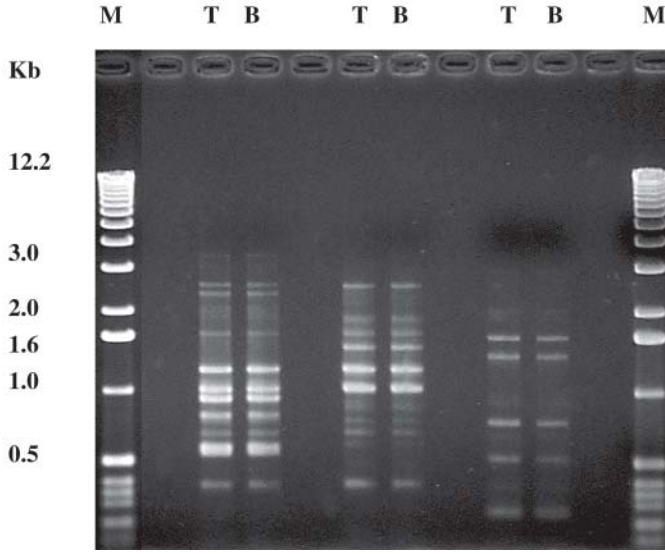

Fig. 9. Arbitrarily primed-polymerase chain reaction-generated genotype profiles of oral streptococci isolated from lower incisors (T) matching those recovered from an experimentally induced bitemark (B). Molecular mass calibration standards (M) are run in the extreme wells on either side (Courtesy of Dr. Mehdi Rahimi, Sydney, Australia.)

remain. Principal among these are the presumed uniqueness of the human dentition, the retention of DNA on skin over time and the variability of viscoelastic properties of skin. The question now remains, what is the evidential reliability of bitemark analysis? The answer lies in two issues that seem different, but are in fact closely allied at a deeper level: the qualitative nature of bitemark analysis and the rules of admission of scientific evidence. Bitemark analysis is inherently qualitative. Kittelson et al. *(12)* stressed that although some characteristics, such as tooth dimensions, angulations, and intercanine distances, may be measurable, the ability to discern these depends on impressions left on skin, which is a poor impression material. The second issue relates to the rules of evidence in science and law. Restriction of space precludes a discussion of *Frye* and *Daubert (86,87)*, but essentially the underlying theme is the continuing clash of two evidentiary values; the desirability of presenting as complete an evidentiary record as possible (including expert assistance) and the protection of the fact-finding process against distortion. The latter value often is expressed as the fear that such assistance will demand a surren-

der of judgement by the trier of fact to the expert (the "mystic infallibility" risk).

Although *Frye* is engineered to protect against the "mystic infallibility" effect by requiring "general acceptance" in the relevant scientific community, *Daubert* (and its sister cases) are premised on a court-conducted reliability inquiry as the proper prophylactic against the same risk. As *Daubert* observes, evidentiary rules are "not designed to seek cosmic understanding but to resolve legal disputes." But where scientific evidence is offered in a legal forum, the strength of the scientific foundation should be commensurate with the effect of admitting to the results of that science. To ensure this congruence (that the probative value of the evidence outweighs its prejudicial effect), the better path would be to assess reliability in a gate-keeping inquiry. Yet, although scientists and academics continue to battle it out on that front, bitemark identification evidence currently seems immune to such admissibility challenges. Put positively, this may demonstrate a trust in the trier of fact to accord this kind of evidence the appropriate weight, no matter how "scientific" the science. Put negatively, it may reflect a trial strategy devoted to the advantages of unhelpfulness (confusion by the trier of fact and the according of inappropriate weight).

References

1. Humble BH (1933) Identification by means of teeth. Brit Dent J 54, 528–536.
2. Pierce LJ, Strickland DJ, Smith ES (1990) The case of Ohio vs Robinson. Am J Forensic Med Pathol 11, 171–177.
3. Sweet D , Lorente JA, Lorente M, Valenzuela A, Villanueva E (1997) PCR-based typing of DNA from saliva recovered from human skin. J Forensic Sci 42, 447–451.
4. Sweet D, Hildebrand DP (1999) Saliva from cheese bite yields DNA profile of burglar: a case report. Int J Legal Med 112, 201–203.
5. Naru AS, Dykes E (1996) The use of digital imaging technique to aid bite mark analysis. Sci Justice 36, 47–50.
6. Sweet D, Parhar M, Wood RE (1998) Computer based production of bitemark overlays. J Forensic Sci 43, 1046–1051.
7. Sweet D, Bowers CM (1998) Accuracy of bite mark overlays: a comparison of five common methods to produce exemplars from a suspect's dentition. J Forensic Sci 43, 362–367.
8. *State v. Garrison*, 120 Arizona 255,585 P.2d 563.
9. Rothwell B (1995) Bite marks in forensic dentistry: a review of legal, scientific issues. J Dent Assoc Am 126, 223–232.
10. Whittaker DK (1975) Some laboratory tests on the accuracy of bitemark comparison. Int Dent J 25, 166–170.

11. Borgula LM, Robinson FG, Rahimi M, et al. (2003) Isolation and genotypic comparison of oral streptococci from experimental bitemarks. J Foren Odontostomatol 21, 23–30.
12. Kittelson JM, Kieser JA, Buckingham DM, Herbison GP (2002) Weighing the evidence: quantitative measures of the importance of bitemark evidence. J Foren Odontostomatol 20, 31–37.
13. Douglas LG (1975) Bite wounds. Am Fam Phys 11, 93–99.
14. Edwards MS (1987) Infections due to human and animal bites. In Feigin RD, Cherry JD, eds., Textbook of Paediatric Infectious Diseases. Saunders, Philadelphia, pp. 2362–2372.
15. Liston PN, Tong DC, Firth NA, Kieser JA (2001) Bite injuries: pathophysiology, forensic analysis and management. N Z Dent J 97, 58–63.
16. Bunzli WF, Wright DH, Hoang AD, et al. (1998) Current management of human bites. Pharmacotherapy 18, 227–234.
17. Baker MD, Moore SE (1987) Human bites in children; a six year experience. Am J Dis Child 141, 1285–1290.
18. Leung AK, Robson WL (1992) Human bites in children. Paediatr Emerg Care 8, 255–257.
19. Epstein JB, Scully C (1992) Mammalian bites: risk and management. Am J Dent 5, 167–171.
20. Fallouji MA (1990) Traumatic love bites. Br J Surg 77, 100–101.
21. Wolf JS, Gomez R, McAninch JW (1992) Human bites on the penis. J Urol 147, 1265–1267.
22. Stimson PG, Mertz CA (1997) Bite mark techniques and terminology. In Stimson PG, Mertz CA, eds., Forensic Dentistry. CRC Press, Boca Raton, pp. 137–159.
23. Wright FD, Dailey JC (2001) Human bite marks in forensic dentistry. Dent Clin N Am 45, 365–379.
24. Sweet D (1997) Human bitemarks: examination, recovery, and analysis. In Bowers CM, Bell GL, eds., Manual of Forensic Odontology, 3rd ed. Am Soc Foren Odontol, pp. 148–169.
25. Payne-James J, Busuttil A, Smock W (2003) Forensic Medicine. Clinical and Pathological Aspects. Medical Media, Greenwich.
26. Avon SL, Wood RE, Blenkinsop B (2003) A porcine model of contusive bitemark injuries in human bitemark analysis. Paper delivered at the American Academy of Forensic Sciences.
27. Carpenter K (1988) Evidence for predatory behaviour by *Tyrannosaurus*. In Hunter JR, ed., International Symposium on Vertebrate Behaviour as Derived from the Fossil Record. Montana State University (unpaginated).
28. Erickson GM, Olson KH (1996) Bite marks attributable to *Tyrannosaurus rex*. J Vert Paleontol 16, 175–178.
29. Aghababian RV, Conte JE (1980) Mammalian bite wounds. Ann Emerg Med 9, 79–83.
30. Avner JR, Baker MD (1991) Dog bites in urban children. Pediatrics 88, 55–57.
31. Beck AM, Jones BA (1985) Unreported dog bites in children. Public Health Rep 100, 315–321.
32. Weiss HB, Friedman DI, Coben JH (1998) Incidence of dog bite injuries treated in emergency departments. JAMA 279, 51–53.

33. Wong JK, Blenkinsop B, Sweet DJ, Wood RE (1999) A comparison of bitemark injuries between fatal wolf and domestic dog attacks. J Foren Odontostomatol 17, 10–15.
34. Cohle SD, Harlan CW, Harlan G (1990) Fatal big cat attacks. Am J Forensic Med Pathol 11, 208–212.
35. Vogel SJ, Parker JR, Jordan FB (2000) Persian leopard (*Panthera pardus)* attack in Oklahoma. Am J Forensic Med Pathol 21, 264–269.
36. Tsokos M, Matschke J, Gehl A, Koops E, Püschel K (1999) Skin and soft tissue damage caused by rodents. Forensic Sci Int 104, 47–57.
37. Tsokos M, Schulz F (1999) Indoor postmortem animal interference by carnivores and rodents: report of two cases and review of the literature. Int J Legal Med 112, 115–119.
38. James H, Acharya AB, Taylor JA, Freak MJ (2002) A case of bitten Bettongs. J Forensic Odontostomatol 20, 10–12.
39. Layton JJ (1969) Identification from a bitemark in cheese. Austral Police J April, 116–125.
40. Kerr NW (1974) Apple bitemark identification of a suspect. Int J Forensic Dent 4, 20–23.
41. Simon A, Jordan H, Pforte K (1974) Successful identification of a bitemark in a sandwich. Int J Forensic Dent 2, 12–21.
42. Dinkel EH (1974) The use of bitemark evidence as an investigative aid. J Forensic Sci 19, 535–547.
43. Stoddart TJ (1973) Bite marks in perishable substances. Brit Dent J 135, 285–287.
44. Rudland M (1982) The dimensional stability of bite marks in apples after long-term storage in a fixative. Med Sci Law 22, 47–50.
45. Aboshi H, Taylor JA, Takei T, Brown KA (1994) Comparison of bitemarks in foodstuffs by computer imaging: a case report. J Forensic Odontostomatol 12, 41–44.
46. Nambiar P, Bridges TE, Brown KA (1995) Quantitative forensic evaluation of bite marks with the aid of a shape analysis program, part 2: SCIP and bite marks in skin and foodstuffs. J Forensic Odontostomatol 13, 26–32.
47. McKenna CJ, Haron MI, Brown KA, Jones AJ (2000) Bitemarks in chocolate: a case report. J Forensic Odontostomatol 18, 10–14.
48. Nambiar P, Carson G, Taylor JA, Brown KA (2001) Identification from a bitemark in a wad of chewing gum. J Forensic Odontostomatol 19, 5–8.
49. Bernitz H, Piper SE, Solheim T, VanNiekerk PJ, Swart TJP (2000) Comparison of bitemarks left in foodstuffs with models of the suspect's dentitions as a means of identifying a perpetrator. J Forensic Odontostomatol 18, 27–31.
50. Bernitz H, Kloppers BA (2002) Comparison microscope identification of a cheese bitemark: a case report. J Forensic Odontostomatol 20, 13–16.
51. Dawes-Higgs EK, Swain MV, Higgs RJ, Appleyard RC (2004) Accuracy and reliability of a dynamic biomechanical skin measurement probe for the analysis of stiffness and viscoelasticity. Physiol Meas 25, 97–105.
52. Dunn MG, Silver FH (1983) Viscoelastic behaviour of human connective tissues: relative contribution of viscous and elastic components. Connect Tissues Res 12, 59–70.
53. Brown IA (1973) A scanning electron microscope study of the effects of uniaxial tension on human skin. Br J Dermatol 89, 383–393.
54. Fung YC (1993) Biomechanics, Mechanical Properties of Living Tissues, 2nd ed. Springer, New York.

55. Purslow PP, Wess TJ, Hubkins DW (1998) Collagen orientation and molecular spaing during creep and stress-relaxation in soft tissues. J Exp Biol 201, 135–142.
56. Puxkandl R, Zizak I, Paris O, Keckes J (2002) Visco-elastic properties of collagen: synchroton radiation investigations and structural model. Phil Trans R Soc Lond B Biol Sci 357, 191–197.
57. Oxlund H, Manschot J, Viidik A (1988) The role of elastin in the mechanical properties of skin. J Biomech 21, 213–218.
58. Langer K (1861) Zur Anatomie und Physiologie der Haut: I. Über die Spaltbarkeit der Cutis. Sitzungber Akad Wiss Wien 44, 19–46.
59. Cox HT (1942) The cleavage lines of the skin. Br J Surg 29, 234–240.
60. Dawes-Higgs ED, Swain MV, Higgs RJ, Appleyard RC, Kossard S (2004) Accuracy and reliability of a dynamic biomechanical skin measurement probe for the analysis of stiffness and viscoelasticity. Physiol Measure 25, 97–105.
61. Johnson KL (1985) Contact Mechanics. Cambridge University Press, Cambridge.
62. Popper KR (1992) The Logic of Scientific Discovery. Routledge, London.
63. Aksu MN, Gobetti JP (1996) The past and present legal weight of bite marks as evidence. Am J Forensic Med Pathol 17, 136–140.
64. Naru AS (1995) Methods for the analysis of human bite marks. Forensic Sci Rev 9, 123–139.
65. Sweet D, Pretty IA (2001) A look at forensic dentistry. Part 2: Teeth as weapons of violence—identification of bitemark perpetrators. Br Dent J 190, 415–418.
66. Rawson RD, Ommen RK, Kinard G, Johnson J, Yfantis A (1984) Statistical evidence for the individuality of the human dentition. J Forensic Sci 29, 245–253.
67. Kieser JA, Groeneveld HT (1989) Allocation and discrimination based on human odontometric data. Am J Phys Anthropol 79, 331–337.
68. Kieser JA (1990) Human Adult Odontometrics. Cambridge University Press, Cambridge.
69. Harris EF (2003) Where's the variation? Variance components in tooth sizes of the permanent dentition. Dent Anthropol 16, 84–94.
70. Pretty IA, Sweet D (2001) Digital bite mark overlays—an analysis of effectiveness. J Forensic Sci 46, 1385–1391.
71. Bowers CM, Johansen RJ (2001) Digital analysis of bite marks and human identification. Dent Clin N A 45, 327–342.
72. Sweet D, Parhar M, Wood RE (1998) Computer-based production of bite mark comparison overlays. J Forensic Sci 43, 1050–1055.
73. Blumenthal I (1994) Child Abuse: A Handbook for Health Care Practitioners. Edward Arnold, London.
74. Mercer DK, Scott KP, Bruce-Johnson WA, Glover LA, Flint HJ (1999) Fate of free DNA and transformation of the oral bacterium *Streptococcus gordonii* DL1 by plasmid DNA in human saliva. Appl Environ Microbiol 65, 6–10.
75. Sweet D, Lorente JA, Valenzuela A, Lorente M, Villanueva E (1997) PCR based DNA typing of saliva stains recovered from human skin. J Forensic Sci 42, 447–451.
76. Paster BJ, Boches SK, Galvin JL, et al. (2001) Bacterial diversity in human subgingival plaque. J Bacteriol 183, 3770–3783.

77. Socransky SS, Manganiello AD, Propas D, Oram V, van Houte J (1977) Bacteriological studies of developing supragingival dental plaque. J Periodont Res 12, 90–106.
78. van Houte J, Gibbons RJ, Banghart SB (1970) Adherence as a determinant of the presence of *Streptococcus salivarius* and *Streptococcus sanguis* on the human tooth surface. Archs Oral Biol 15, 1025–1034.
79. Krasse B (1954) The proportional distribution of *Streptococcus salivarius* and other streptococci in various parts of the mouth. Odontol Revy 5, 203–211.
80. Borgula LM, Robinson FG, Rahimi M, et al. (2003) Isolation and genotypic comparison of oral bacteria from experimental bite marks. J Forensic Odonto-Stomatol 21, 23–30.
81. Peipert JF, Domagalski LR (1994) Epidemiology of adolescent sexual assault. Obstet Gynecol 84, 867–871.
82. Rudney JD, Neuvar EK, Soberay AH (1992) Restriction endonuclease-fragment polymorphisms of oral viridans streptococci, compared by conventional and field-inversion gel electrophoresis. J Dent Res 71, 1182–1188.
83. Alam S, Brailsford SR, Whiley RA, Beighton D (1999) PCR-based methods for genotyping viridans group streptococci. J Clin Microbiol 37, 2772–2776.
84. Wisplinghoff H, Reinert RR, Cornely O, Seifert H (1999) Molecular relationships and antimicrobial susceptibilities of viridans group streptococci isolated from blood of neutropenic cancer patients. J Clin Microbiol 37, 1876–1880.
85. Pretty IA, Sweet DA (2000) Comprehensive examination of bitemark evidence in the American legal system. Proc Am Acad Forensic Sci 6, 146.
86. Foster KR, Huber PW (1999) Judging Science—Scientific Knowledge and the Federal Courts. MIT Press, Cambridge

Taphonomy

5

Postmortem Changes and Artifacts Occurring During the Early Postmortem Interval

Michael Tsokos, MD

CONTENTS

SUMMARY

The underlying biological processes that a human body or its remains undergoes after death are complex and, as with other biological phenomena, there is a broad range of variables influencing postmortem changes by the

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alteration of the underlying progress of tissue destruction. The understanding of the resultant postmortem changes is of great importance for the forensic pathologist and medical examiner. As a general rule, changes in ambient temperature tend to alter the rate but do not change the underlying biological mechanisms of postmortem changes. The manifestation of putrefaction may cause interpretational problems and, accordingly, a death may seem suspicious in a given case. Putrefaction may mask traumatic injuries an invidual sustained before death. However, purging of putrefaction fluid from the mouth and nostrils is frequently confused with blood, for example, deriving from antemortem facial injuries, by those investigators unfamiliar with the phenomenon. When tight clothing is worn by the deceased, putrefactive bloating of the neck region may lead to cutaneous alterations mimicking strangulation marks. In contrast to livor mortis, vibices, rigor mortis, autolysis, and putrefaction, all of which are known as postmortem phenomena that are frequently observed in the death investigator´s daily practice, more uncommon postmortem changes that do only occur occasionally and under specific intra-individual or environmental conditions may be interpreted falsely by the inexperienced. Abrasions and lacerations on the skin may be produced by manipulation of the body during postmortem handling, transportation, and storage. Urine may cause extensive skin damage postmortem to an infant on the perigenital skin areas that were in contact with a urine-soaked diaper postmortem. One has to be aware to differentiate such postmortem skin changes from vitally acquired alterations and not to interpret them uncritically as signs of neglect prior to death. Postmortem hypostasis in the muscles located in the lateral submalleolar region and the thenar eminence may mimic antemortem bruising. It generally is impossible to draw any definite conclusions concerning the time of death by the appearance of a single postmortem change, or conversely, to predict what postmortem changes are to be expected in a given case after a particular postmortem interval has elapsed. Nevertheless, in some distinct cases, particularly the presence and picture of several postmortem changes may, when analyzed combined with the rectal temperature of the deceased, give the death investigator valuable hints concerning the time frame in wich the subject most probably has died.

Key Words: Taphonomy; livor mortis; rigor mortis; algor mortis; postmortem cooling; vibices; autolysis; imbibition; maceration; putrefaction; venous marbling; freeze-drying; pink teeth; animal depredation; washerwoman's skin; blistering; postmortem injuries; skin lesions; differential diagnoses.

1. INTRODUCTION

The understanding of the underlying biological processes leading to particular postmortem changes is of great importance for the forensic pathologist and medical examiner, concerning the differentiation between artifactual changes of the body surface or internal organs vs underlying (true) pathological conditions. Just to give a few examples: the inexperienced may interpret livor mortis in the myocardium as circumscribed hemorrhage accompanying fresh myocardial infarction or, when livor mortis is found in the lungs, as edema or pneumonia. Vibices ("postmortem ecchymoses") may be confused by the unwary with petechial bleedings caused by mechanical asphyxia or with hematomas. Some postmortem changes may render a careful external examination of the body difficult, for example, putrefactive skin changes may conceal cutaneous injuries. When found in curious death scene scenarios or in fatalities with additional signs of external violence preceding death, uncommon postmortem changes such as marks caused by animal depredation, injuries following handling and transportation of the body, or skin changes caused postmortem by leaking out of urine may be interpreted falsely. In children, a dilated anal orificium (from the loss of muscle tone immediately after death, with anal dilation occurring as a result of rigor mortis) may be erroneously mistaken as a sign of penetrating anal abuse prior to death. In such cases hastened conclusions may lead the investigative inquiries in a wrong direction or, in the worst case, to the miscarriage of justice.

In this chapter, death is defined as the irreversible cessation of blood circulation. After death, a complex cascade of cellular events and biological phenomena begins, starting with cellular oxygen depravation. With the progression of the postmortem interval, autolytic changes and putrefaction take over. Death is a process rather than an event. The underlying biological processes that a human body or its remains undergo after death are complex and, as with other biological phenomena, there is a broad range of variables influencing postmortem changes by the alteration of the underlying progress of tissue destruction. As a general rule, changes in ambient (environmental) temperature tend to alter the rate but do not change the underlying biological mechanisms leading to a particular postmortem change. A summary of the main intrinsic and extrinsic factors accelerating or decelerating, respectively, the onset and extent of postmortem changes is presented in Table 1.

This chapter does not focus in detail on aspects of the estimation of the time elapsed since death; for this, one should refer to the well-renowned textbook edited by Knight *(1)* and the recent publications by Henssge et al. *(2,3)*. The following review rather concentrates on the morphological pictures under

Table 1
Intrinsic and Extrinsic Factors Influencing the Onset and Extent of Postmortem Changes

Acceleration of onset and extent of postmortem changes
Death occurring in a hot, moist environment/under high ambient temperatures
Body surface insulation by warm clothing or other covering
Considerable time interval elapsed after death until artifactual cooling of the body
Subject was overweight/had a high fat content
Subject suffered/died from underlying infection or sepsis
Subject was intoxicated (e.g., with illicit drugs such as heroin)
Subject suffered/died from open wounds (perforating/penetrating traumatic injuries such as stab wounds, gunshot wounds, impalement injuries) or during surgical procedures
Deceleration of onset and extent of postmortem changes[a]
Death occurred in a cold, dry environment/under low ambient temperatures
Subject was scantily dressed/naked/undressed shortly after death
Subject was stored in a cooling apparatus shortly after death

[a]These factors slow the rate of postmortem changes but do in general not alter the underlying postmortem biological processes.

which different postmortem changes may present. Apart from giving a synopsis of common and uncommon postmortem changes seen in the death investigator's daily practice, it is also the aim of this chapter to draw the reader's attention to potential differential diagnoses between postmortem changes and vitally acquired body alterations and the pitfalls the similarities between some of them may contain.

2. Livor Mortis (Livores, Postmortem Lividity, Postmortem Hypostasis)

Livor mortis is visible as a usually bluish-violaceous to purple coloration appearing on the lower (dependent) parts of the body within 30 minutes to 3 hours after death.

After the cardiovascular system has ceased to function, under the influence of gravity, movement of blood into the dependent parts of the body occurs (Fig. 1). Livores correspond to hypostasis (gravitational pooling of blood postmortem) into the capillaries within the dermis (consisting of the papillary and reticular layer) in the dependent parts of the body. Therefore, when a subject

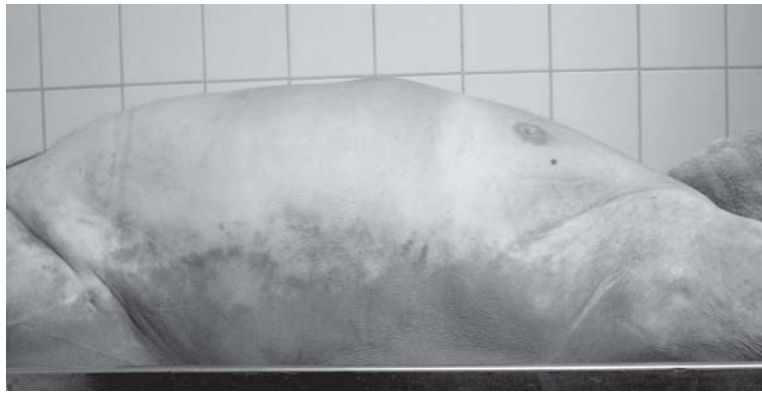

Fig. 1. Livor mortis in dependent areas of the body.

has died in a prone position, livores will spread over the front of the body and when death took place in a supine position, livores will spread over the back of the body.

2.1. Patterned Appearance and Contact Blanching of Livor Mortis

Livor mortis frequently is patterned because the appearance of livores is hindered when the vessels in dependent parts of the body are obstructed as the result of outer body surface compression. The resulting contact blanching of livor mortis is seen where prominent parts of the body surface, for example, areas over bony structures, are firmly adhering to rigid surfaces because of the weight of the body. In such areas, livor mortis is absent, with the skin in such parts appearing pale to white. When livor mortis formation is present on the back of the body, this contact blanching has a typical “butterfly appearance” over the upper half of the body (Fig. 2). The same phenomenon of a patterned appearance of livores occurs when tight clothing compresses the involved vessel lumina (Fig. 3). One has to be well aware of the fact that creasing of the skin or tight clothing may produce a kind of contact blanching on the neck that may resemble a ligature mark (Fig. 4). Therefore, the knowledge of the position of the head and neck as well as the clothing worn at the time of death is occasionally highly important to the death investigator.

Contact blanching also may image the exterior of objects that were in contact with the dependent parts of the body surface during livor mortis formation, and occasionally the distinctive morphological appearance of contact blanching may give the death investigator valuable hints toward the case in question (Fig. 5).

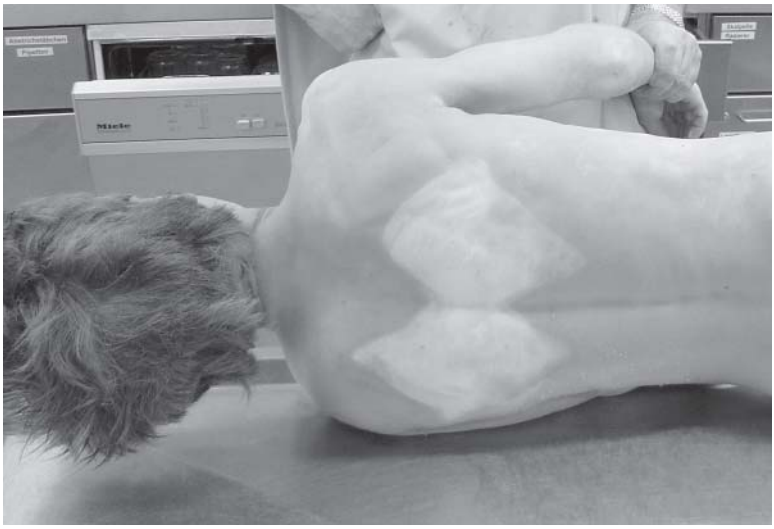

Fig. 2. Intense livor mortis formation on the posterior aspect of a body with typical "butterfly-like"contact blanching over the scapular region.

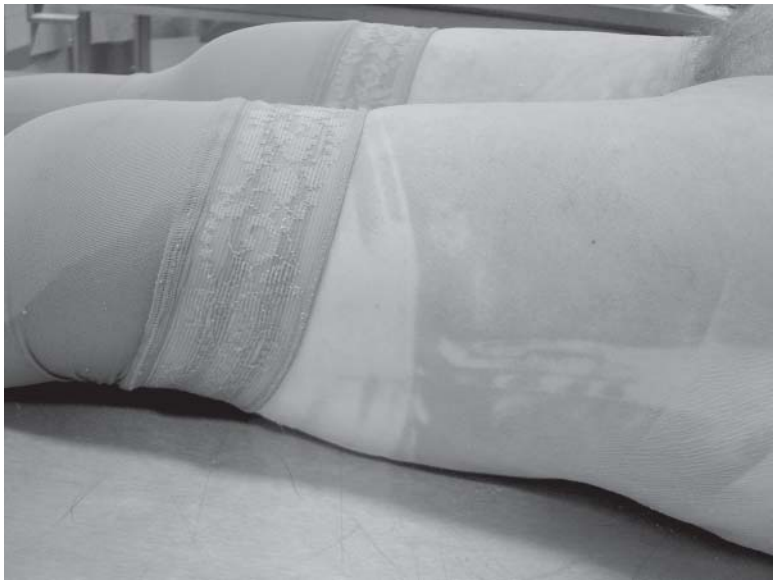

Fig. 3. Contact blanching of livor mortis induced by a tight suspender.

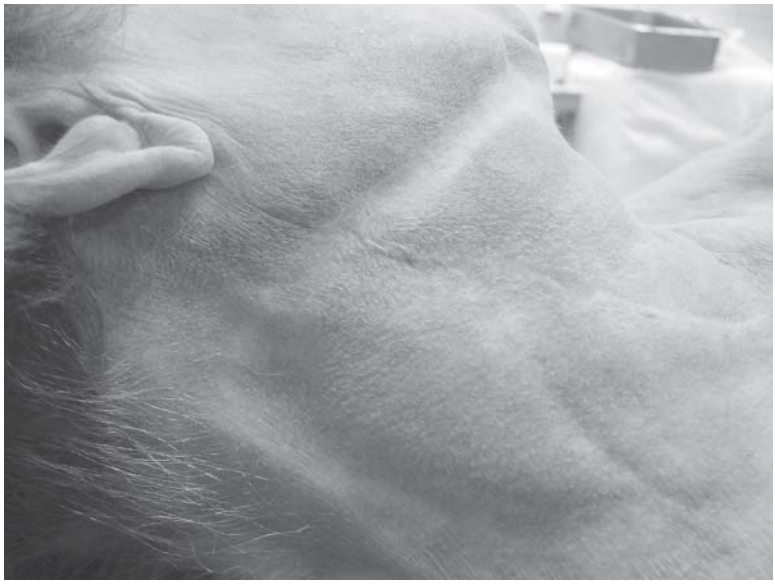

Fig. 4. Contact blanching of livores on the skin of the anterior neck caused by creasing of the skin. This postmortem phenomenon is easily confused with a ligature mark by the inexperienced.

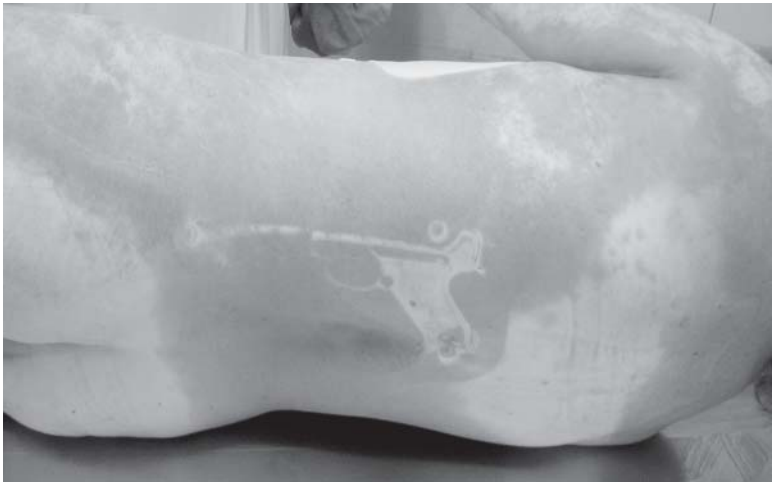

Fig. 5. Contact blanching of livores on the back of the body outlining a pistol that was located under the body when death occured.

Table 2
Sequential Order of Usual Appearance of the Different States of Livor Mortis Under Moderate to Cool Climatic Conditions

Observation	Earliest appearance	General appearance	Latest appearance
Patchy beginning of development	0.5 hours PM	2 hours PM	3 hours PM
Full development, confluence	4 hours PM	6 hours PM	8 hours PM
Reaching its maximum of intensity	6 hours PM	10 hours PM	16 hours PM

PM, postmortem.

2.2. Chronological Sequence of Livor Mortis Formation

After a first patchy development of livor mortis within 30 minutes to 3 hours after death, livores become confluent. Under moderate to cool climatic conditions, livores are usually fully developed within 4 to 8 hours postmortem, reaching their maximum intensity after an average of 10 hours postmortem (Table 2). Livor mortis is most intense in cases of sudden death with a short agonal period and a great circulating blood volume.

In the early postmortem interval, roughly until 12 to 18 hours after death, livor mortis is not yet fixed. Nonfixation of livor mortis means that livores can be blanched when a blunt object such as a finger, the hand, or an instrument is pressed against the skin in areas of livores formation (Fig. 6 A,B). This selective pressure forces the blood from the engorged dermal capillaries, which results in a pale to white blanching that quickly refills. The principally same phenomenon results if the body is moved into a new posture. Livor mortis will then shift to the dependent parts of the body brought about after body movement. The capability of livores to shift as a result of the gravitational movement of blood is assumed to depend on a prevailing number of intact erythrocytes within the vascular system: Selective pressure moves the intact blood cells within the vessels. However, this assumption has been questioned more than once by different authorities.

After approx 18 to 24 hours, livor mortis becomes fixed, which relates to the observation that livores can not be blanched by selective pressure on the outer body surface and the incapacity of gravity to bring livores about to shift. The time of onset of fixation of livores depends mostly on the ambient temperature the body has been exposed to, that is, high ambient temperatures are positively correlated with an early onset of fixation of livor mortis and conversely. When livor mortis is fixed, a change in body position will have no effect on the original pattern of livores formation.

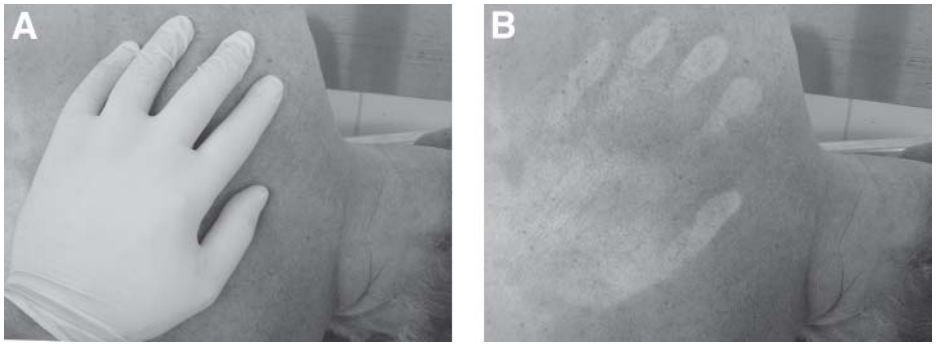

Fig. 6. Contact blanching of livor mortis within the state of nonfixation of postmortem lividity. Selective pressure **(A)** with the hand leads to a pale to white area of blanching **(B)**.

Fixation of livor mortis is considered a result of hemolysis of the blood serum. With the breakdown of erythrocyte membranes during autolysis, the erythrocytes become pervious for hemoglobin and its derivates with subsequent diffusion of hemolytic blood serum through the walls of the vessels involved in livores formation. In this case, selective pressure over an area of livor mortis will have no noticeable effect on the movement of blood cells within the vessels or on the hemolytic coloration of the surrounding tissue. This theory has also been doubted, but the underlying pathophysiological mechanisms decisive for nonfixation or fixation of livores have no real practical value anyway.

It has to be mentioned that cases have been reported where shifting of livores was observed even after 48 hours or more, but such observations will be mostly restricted to cases where cold ambient temperatures prevail.

2.3. Color of Livor Mortis

In the early phase of their formation, livores have a reddish color, which results from the prevailing number of erythrocytes carrying oxygenated hemoglobin. As the postmortem interval increases, livores intensify and become darker. The normal color of livor mortis is bluish-violaceous to purple. This bluish-violaceous to purple color is the result of oxygen dissociation from the hemoglobin of erythrocytes postmortem and continuous oxygen consumption from cells that initially survive the cessation of cardiovascular function (e.g., liver cells survive cessation of the cardiovascular system for approx 40 minutes and skeletal muscle cells between 2 and 8 hours). The resulting product is deoxyhemoglobin that has typically a bluish-violaceous to purple color.

2.3.1. Light Reddish-Pink Livores

Light reddish or pink livores are seen in carbon monoxide poisoning, fatal hypothermia, cyanide poisoning, or after disposal of a body postmortem under cold ambient temperatures.

2.3.1.1. Carbon Monoxide Poisoning

A light reddish or pink, sometimes described as "cherry red," coloration of livores is classically found in carbon monoxide poisoning as a result of carboxyhemoglobin formation. The assumption often brought forward that a bluish-violaceous color of the matrix of the nails when found together with a light reddish color of livores refutes carbon monoxide poisoning as the underlying cause of the light reddish coloration of livores must be contradicted vehemently. This author, as well as others, have seen cases of fatal carbon monoxide poisoning where the matrix of the nails showed a bluish-violaceous color despite carbon monoxide hemoglobin concentrations of 50% and more. In such doubtful cases, laboratory testing of heart blood samples must be performed immediately to avoid exposing other persons to danger at the scene of death.

2.3.1.2. Hypothermia

Light reddish or pink livores also are seen frequently in fatal hypothermia and bodies stored postmortem in a cooling apparatus since cold ambient temperatures inhibit dissociation of oxygen from the hemoglobin. Oxygenated hemoglobin has a lighter red color than deoxyhemoglobin. Under cold ambient temperatures (roughly below 15°C), reoxygenation of hemoglobin does slowly occur postmortem, which is the explanation for the light red color of livores seen in bodies after storage in a cooling apparatus postmortem. This reoxygenation of hemoglobin does not occur in livores located in the matrix of the nails owing to their protection from the gases (and especially oxygen) of the outside environment by the fingernails. This phenomenon enables the death investigator to differentiate between the presence of light reddish livores as a result of postmortem reoxygenation of hemoglobin owing to cold ambient temperatures and underlying carboxyhemoglobin formation.

2.3.1.3. Cyanide Poisoning

In cyanide poisoning, the cyanide inhibits dissociation of oxygen from the hemoglobin by blocking the cytochromoxydase activity thus equivalently leading to a light reddish (or pink) coloration of livor mortis.

Fig. 7. Internal livores seen in the myocardium on the lower cutting edge.

2.3.2. Brownish Color of Livores

A brownish, sometimes described as "chocolate," color of livor mortis is seen in poisoning with nitrates, nitrites, or sodium chloride. The reason for this brown coloration of livores is the formation of methemoglobin.

2.3.3. Greenish Color of Livores

Livores often partially turn to green under the influence of putrefaction processes due to hemoglobin conversion into sulfhemoglobin.

2.4. Livor Mortis in Internal Organs

Comparable with the development and their location of appearance on the outer body surface, livores also are found in dependent parts of internal organs such as the lungs, heart, liver, and kidneys.

In the lungs, internal livores corresponding to accumulation of blood and transudation of hemolytic serum caused by hypostasis may be occasionally confused with edema of the lungs or pneumonia.

In the heart, internal livores as reflected by a reddish-violaceous discoloration zone in the myocardium may mimick fresh infarction (Fig. 7). The exact location of internal livores in the myocardium is depending on the posture of the body after death.

Fig. 8. Internal livores seen on the surface of the right lobe of the liver with contact blanching deriving from a rib. Note the indentation of Glisson's capsule and the underlying liver parenchyma above as a sequel of chronic lung emphysema.

In the liver, when livores have developed in a right-sided position of the body, contact blanching deriving from the ribs may be observed (Fig. 8).

Difficulties arising from the presence of livores in internal organs and their differentiation from underlying diseases are easily solved by microscopic examination.

2.5. Absence of Livor Mortis

Livores may be sparse or even absent in fatalities with a considerable loss of blood prior to death, be it from internal sources (e.g., gastrointestinal bleeding) or as a result of external hemorrhage (e.g., stab wounds or traumatic amputation of limbs). In most of these cases, the external examination of the body will already give the explanation for inconspicuous or absent livores by revealing a possible source of bleeding (e.g., blood smears on the face, especially around the mouth, resulting from hematemesis or melena; external injuries).

Total absence of livores necessitates at least a blood loss of approx 65% of the circulating blood volume in adolescents and 45% in infants and children. In cases with antemortem anemia (e.g., aplastic anemia, autoimmune hemolytic anemia, anemia secondary to malignancy, malnutrition, or infec-

tion) livor mortis may be occasionally hardly noticeable, depending on the amount of the circulating blood volume, thus determining the total hemoglobin count within the circulation before death. In suntanned or dark-skinned individuals, livores may be difficult to establish or unnoticeable at all.

In drowning deaths, depending on the submersion time and depth the body was located underwater, livores may not develop because the vessels beneath the outer surface of the body (that are normally involved in livor mortis formation) are obstructed as a result of compression by the surrounding hydrostatic water pressure. If the body is recovered from the water within roughly 24 hours, livores will often still develop in the then dependent parts of the body, but whether this occurs or not is highly depending on the water temperature, which will be a determining factor in whether livor mortis is already fixed or not. In bodies recovered from very cold water, the appearance of livores may be observed even after a postmortem interval of 48 to 72 hours.

2.6. Criminal Aspects of Livor Mortis Formation and Appearance

When a subject has died in a supine position, livores will spread over the posterior parts of the body, and when death took place in a prone position, livores will spread over the anterior parts of the body. However, the reverse assumption that a deceased who has livores on the back of his body has died in a supine position or that livores found on the front of the body indicate that this person has died in a prone position may be misleading because, as mentioned previously, livores have the ability to shift when the body is moved into a new position before livor mortis has become fixed.

In hanging deaths, livor mortis will be apparent likewise in the dependent parts of the body, for example, glove-like and stocking-like appearance of livores on the lower parts of the arms and legs, respectively, as shown in Fig. 9. However, the finding of livores in the dependent parts of the body corresponding to hanging does not unequivocally exclude that this body has not been transferred into this posture postmortem, for example, to conceal a homicide and pretend a suicidal mode of death.

If a body is found for instance in a supine position, but livores are present on the anterior parts of the body, this does imply that the body has been moved a considerable time span after death, namely after fixation of livor mortis.

2.7. Differential Diagnoses

Although rarely, livores, especially when appearing in a circumscribed pattern, may be confused with subcutaneous hematomas by the inexperienced.

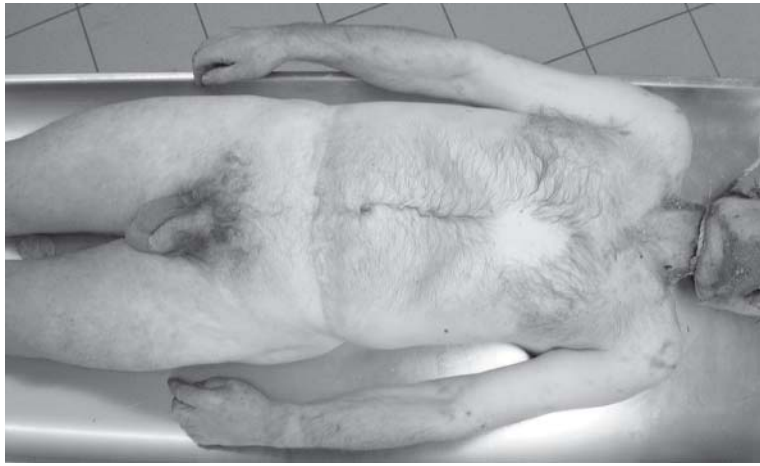

Fig. 9. Hanging: glove-like appearance of livores on the lower parts of the arms and on the dependent parts of the legs.

Within the first 18 to 24 hours after death, proof of contact blanching caused by selective pressure to the outer body surface will help to differentiate between hematomas and livores because application of surface compression to an area of bruising will not cause any blanching. In the later postmortem interval, the incision of a doubtful skin area will make a clear distinction since no hemorrhage will be apparent in the soft tissue beneath livores formation. Livores occasionally resemble confluent cutaneous bleedings owing to septic disseminated intravascular coagulation *(4)*.

3. *Vibices (Postmortem Ecchymoses, Death Spots)*

Vibices are tiny, most often spot-like, sometimes confluent, oval-to-round, bluish-blackish hemorrhages of postmortem origin exclusively limited to areas of livor mortis (Fig. 10). Vibices result from postmortem mechanical rupture of subcutaneous capillaries and smaller vessels (predominantly veins) resulting from an increase in intravascular pressure coming from pooling of erythrocytes in this vascular compartments under the influence of gravity during livor mortis formation. Histologically, erythrocytes appear intact within vibices in the early postmortem interval (Fig. 11 A,B). With increasing length of the postmortem interval, under the influence of autolysis and putrefaction, vibices diminish in number and intensity due to the breakdown of erythrocyte membranes with subsequent hemolysis and diffusion of hemoglobin and its derivates into the surrounding tissue.

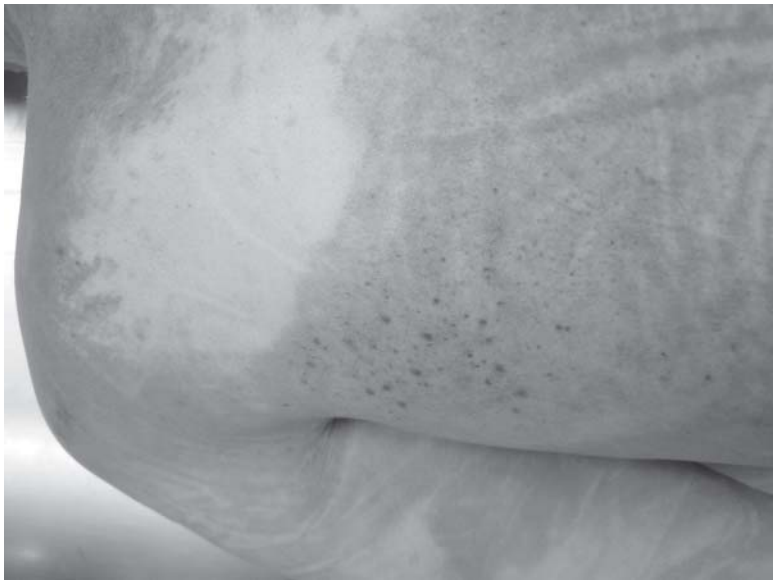

Fig. 10. Vibices strictly limited to livor mortis formation on the back of a deceased.

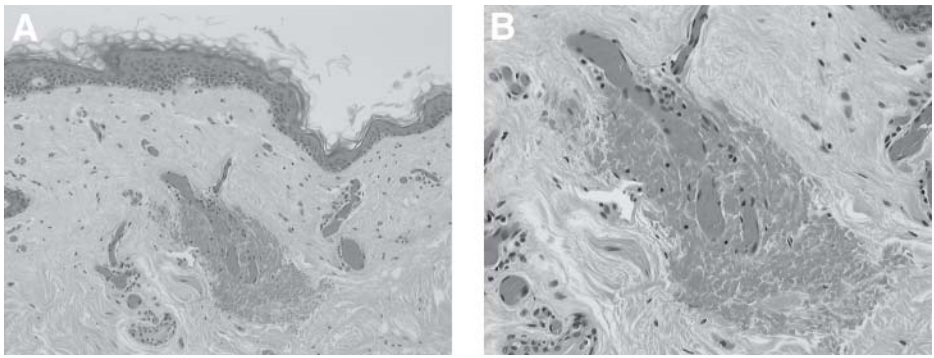

Fig. 11. Histology of vibices. Vibices are seen next to two subcutaneous venules with intact erythrocytes lacking any surrounding inflammatory cellular response. **(A)** Panoramic view. **(B)** Higher magnification.

When livores are sparse, vibices usually are absent. The formation of vibices is dependent on the hydrostatic pressure within the vessels, which is defined by both the total amount of the circulating blood volume and the posture of the body after death. Accordingly, vibices are seen more commonly in those corpses that are obese rather than in those that are underweight. Vibices

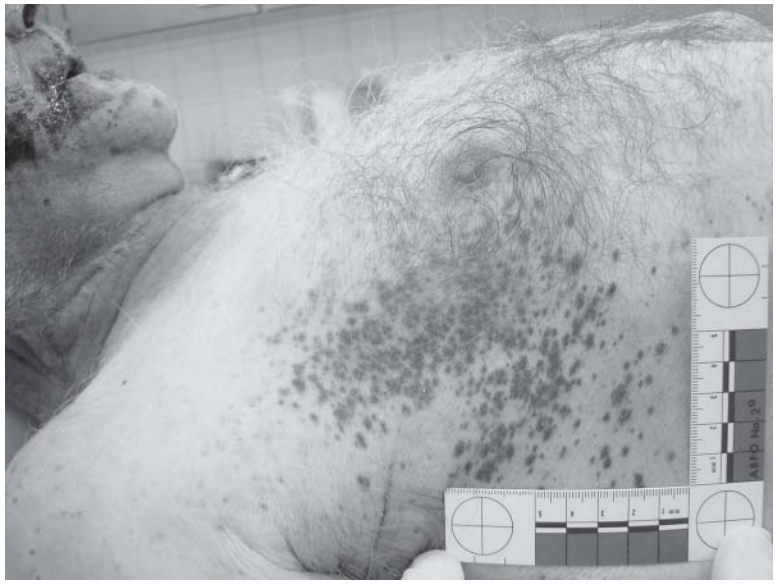

Fig. 12. Intense vibices formation on the lateral aspect of the chest. In this case death occurred in a partly right-sided body position and was caused by acute right heart failure.

are a frequent finding in the dependent parts of the body in hanging deaths, especially in the lower parts of the legs. The duration of the agonal period seems to have no influence whether vibices manifest or not.

3.1. Differential Diagnoses

Vibices should not be confused with petechial bleedings as a result of strangulation or traumatic asphyxia or spot-like or more confluent cutaneous bleedings due to septic disseminated intravascular coagulation. As a general rule and most helpful for the differential diagnosis is the fact that the appearance, intensity, and extent of vibices is positively correlated with that of livor mortis and therefore, vibices are strictly limited to the body parts where livores are existent. However, a clear distinction between conjunctival and facial petechial bleedings and vibices may be difficult or even impossible in a face-down or head-down position of the body after death.

From time to time, vibices develop heavily on the body of the deceased and have a more confluent rather than spot-like appearance. In such cases one may mistake these postmortem bleedings with vital hematomas (Figs. 12 and 13). When located on the anterior aspect of the neck, vibices occasionally may be erroneously taken for signs of strangulation, especially when appear-

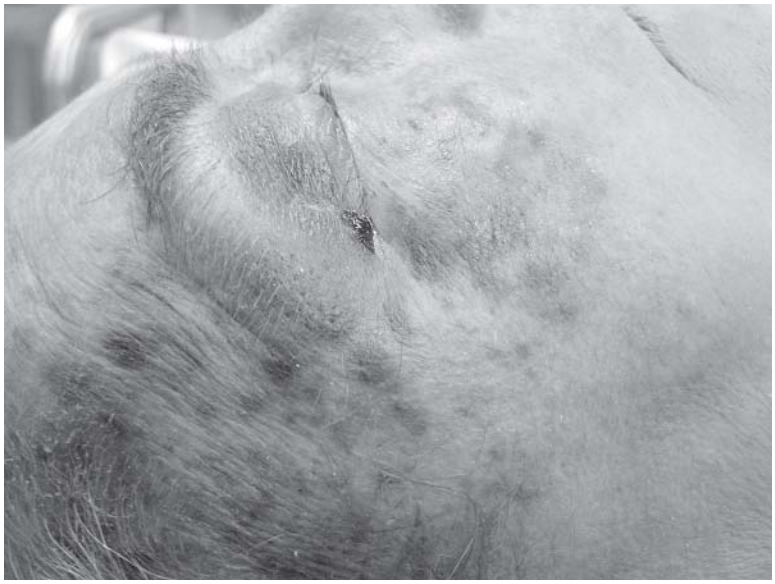

Fig. 13. Heavily developed, confluent vibices on the face that are easily mistaken for vital hematomas.

ing together with creasing of the skin resulting in contact blanching of livor mortis (Fig. 14).

4. Rigor Mortis (Postmortem Rigidity, Postmortem Stiffening)

Rigor mortis is the stiffening of muscles after death. Rigor mortis is preceded by a total (primary) relaxation of the musculature immediately after death. Rigor mortis begins to appear in the muscles of the eyelids and the jaw (at earliest approx 20 minutes postmortem), the latter becoming tightened resulting from the stiffening of the masticatory muscles. After that, postmortem rigidity begins to affect larger muscle groups with stiffening of elbow and knee joints approx 2 to 6 hours after death. However, the rate of onset and time of full development of rigor mortis is highly variable and, as with all postmortem changes, for the most part depending especially on the ambient temperature: High ambient temperatures accelerate the onset and intensity of rigor mortis, although extremes of cold have been observed to produce a rapid onset of rigor mortis as well.

In forensic pathological practice, the intensity of rigor mortis is assessed on a pure subjective basis, meaning whether skeletal muscles offer resistance

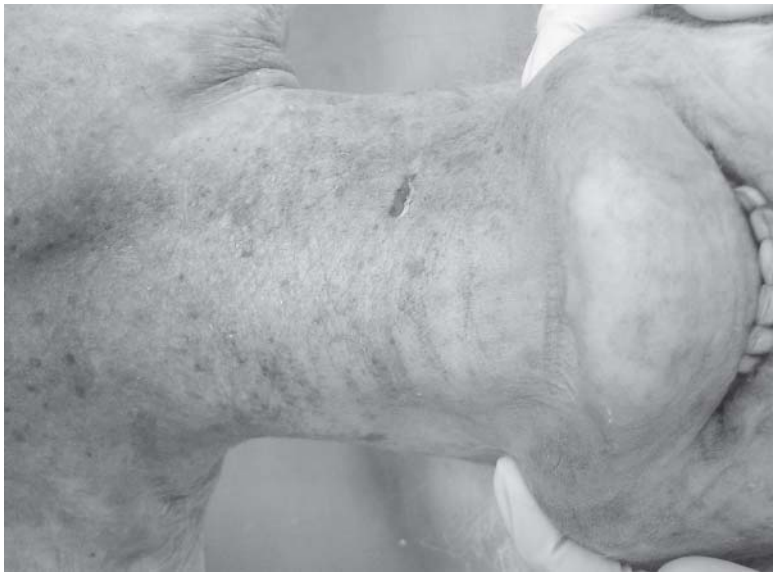

Fig. 14. Vibices formation located on the anterior aspect of the neck in conjunction with creasing of the skin that resulted in partial contact blanching of livor mortis. Such findings may mimick signs of strangulation. A dried-up postmortem skin abrasion is additionally present on the right side of the anterior neck.

when joints are moved (flexion/extension). As a result, the determination of the state of rigor mortis highly varies from one investigator to the other. In addition, numerous intrinsic and extrinsic factors affect the development of rigor mortis (see following sections) and, therefore, using the state of rigor mortis to estimate the postmortem interval is, in general, of no real value. In mechanical terms, postmortem rigidity is characterized by a loss of muscle elasticity and plasticity, an increase in stiffness, and shortening of muscle length.

4.1. Pathophysiology of Rigor Mortis Formation

Muscles are composed of myofibrils, which are again composed of myofilaments. Two types of myofilaments can be distinguished: actin and myosin. Under the influence of adenosine triphosphate (ATP), actin and myosin form a contractile compound, actomyosin, which is the basic source of energy for muscle contraction. After death, ATP formation terminates, and ATP is continuously consumed (strictly speaking, some ATP is still generated by anaerobic glycolysis for a short period of time postmortem, but this can be neglected in the present context). The theory most often brought forward concerning the

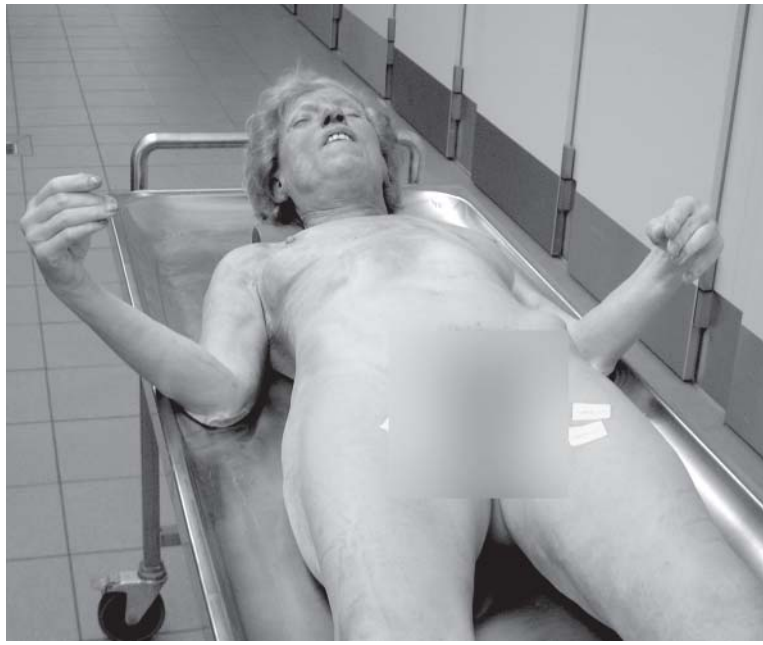

Fig. 15. Fully developed rigor mortis in the upper limbs.

underlying pathophysiological musculature changes that lead to postmortem stiffening of muscles is that with decrease of ATP levels postmortem, actin and myosin enter into a nonshiftable and rigid state of adhesion until, under the influence of autolysis and putrefaction, protein disintegration of the myofibrils leads to loosening of rigor mortis (secondary relaxation).

Experimental investigations revealed that the onset of rigor mortis is earlier and more rapidly progressing in red muscles than in white muscles, which has been attributed to ATP levels falling more rapidly after death in red muscles than in white muscles *(5)*. However, this observation is highly academic and of no real value for practical forensic casework.

4.2. Chronological Sequel of the Development and Disappearance of Rigor Mortis

In cool and temperate climate zones, rigor mortis is usually fully developed (Fig. 15) after about 6 to 18 hours. Under high ambient temperatures, the onset of rigor mortis is accelerated and postmortem rigidity may be even fully developed as early as 1 to 2 hours after death.

Any forceful physical exertion before death will lead to a decrease of ATP levels within the musculature. It is often mentioned that this ATP decrease

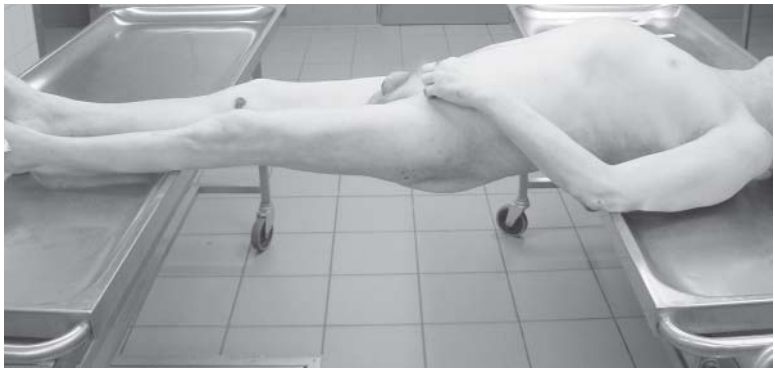

Fig. 16. When fully developed, rigor mortis may be capable of supporting the whole body weight.

is the explanation for an accelerated onset of rigor mortis in cases with physical exhaustion prior to death, for example, in manual strangulation deaths with violent struggling during the strangulation process. The onset of rigor mortis also may be very rapid in children or in deaths caused by electrocution. When the onset of rigor mortis is rapid, its duration is usually shorter than in cases with delayed onset under equal ambient temperatures.

Rigor mortis develops in all muscles at the same time and at the same speed. However, because of the different diameters of the muscles involved, postmortem rigidity becomes noticeable at first in smaller muscle groups.

When fully developed, rigor mortis may lead to such a rigidity of the body that it may be capable of supporting the whole body weight (Fig. 16). In such cases, even the most forceful efforts to break down rigor mortis may be fruitless.

The development of postmortem rigidity usually descends from the head to the shoulder girdle and arms and then to the legs, a phenomenon that is known since 1811 as "Nysten's rule." This finding can be explained by the greater diameter of the muscle groups located at joints that are located farther downwards the body. But no rule is without exception: after forceful physical exertion of the musculature of the legs (e.g., following a flight on foot) rigor mortis will develop earlier in the inferior parts of the body than in the superior parts (most probably a result of muscle activity in the legs with resulting decrease of ATP levels).

When rigor mortis is broken, which means when the joints are stretched forcefully against their fixation by rigor mortis, rigor mortis may develop again when it was not fully developed at this point of time. When it had already been fully developed, rigor mortis will not return after its breaking.

Table 3
Intrinsic and Extrinsic Factors Influencing the Onset of Rigor Mortis

Factors accelerating the time of onset of rigor mortis
Physical exhaustion prior to death (e.g., forceful muscular exertion during a fight or violent struggle during drowning)
High body temperature/fever at the time of death (e.g., due to hyperthyroidism, infection, or intoxication)
Convulsions prior to death (e.g., due to underlying epilepsy or drug-induced)
High ambient temperatures
Factors delaying the time of onset of rigor mortis
Debilitating diseases
Cachexia
Cool/cold ambient temperatures
Death after a short agonal period

In cool and temperate climate zones loosening of rigor mortis, reflected by a secondary relaxation of the muscles (meaning a decrease in tension after full development of postmortem muscle stiffening) begins approx 24 to 36 hours postmortem, a finding that is again highly dependent on the ambient temperature: high ambient temperatures accelerate the time of onset of loosening of rigor mortis and therefore shorten the duration of rigor mortis. Usually, rigor mortis disappears in the order in which it has appeared, but the finger joints remain stiff usually for the longest time.

In putrefied bodies, rigor mortis is absent. Rigor mortis may be weak or even unnoticeable in subjects who suffered from debilitating illnesses prior to death, in cachectic individuals, or those who died in advanced states of multiple sclerosis, amyotrophic lateral sclerosis, or Duchenne muscular atrophy. Table 3 gives an overview on intrinsic and extrinsic factors influencing the onset of postmortem rigidity.

4.3. "Cadaveric Spasm"

Reports of an instantaneous appearance of fully developed rigor mortis of the whole body musculature immediately after death, the so-called "cadaveric spasm," appear from time to time in the literature. The true existence of cadaveric spasm is more than doubtful from the academic point of view, and this author rejects its true existence completely because a satisfactory patho-

Fig. 17. A syringe with attached needle found clutched in the hand of a deceased with known prior intravenous drug abuse. Actually, this individual died in a prone position and the syringe was located between palm and abdomen when rigor mortis set in (no "cadaveric spasm"!).

physiological explanation for this phenomenon is absolutely lacking. When personal belongings or leaves are found clutched in the hands postmortem (Figs. 17 and 18), the logical explanation for this finding is that they were actually located under the palms when postmortem rigidity set in.

Most reports of cases of cadaveric spasm derive from observations made on the European battlefields of World Wars I and II. War correspondents as well as military personnel reported cadavers found in body postures that seemed illogical when death would have occurred in this particular body position, for example, bodies lying in a supine position with their arms and hands stretched out upward. Because it was already established in those days on a scientific basis that "normal" rigor mortis would not develop in the observed manner in such a body position (because of primary relaxation of the muscles after death and gravity-induced falling down of the limbs), the myth of "cadaveric spasm" was raised. In these cases, the phenomenon of cadaveric spasm is easily explained by a postmortem movement of the affected bodies, for example, because of blast waves from explosives that hit the battlefield and flung the bodies around in which rigor mortis was already fully developed. After this

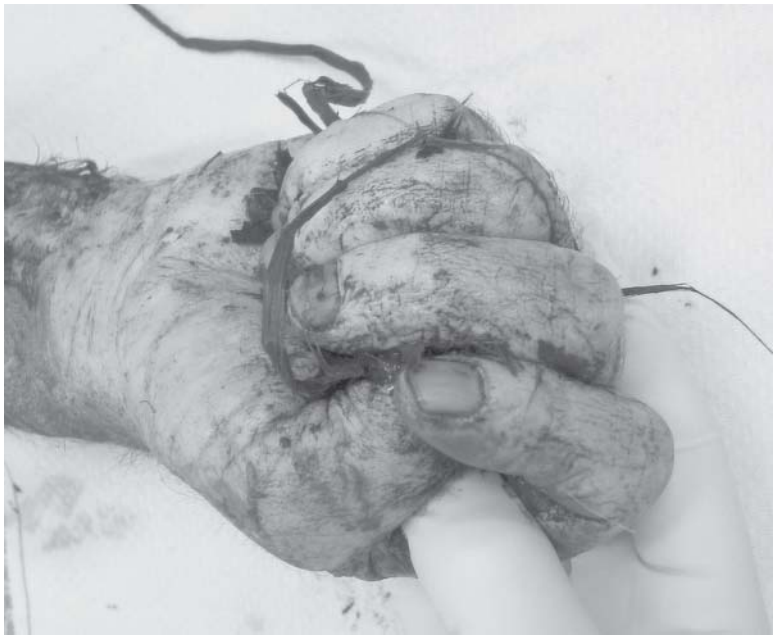

Fig. 18. Leaves and blades of grass found clutched in the hand of a body recovered from water (no "cadaveric spasm"!).

blast-wave induced movement of the bodies, the cadavers were found in unreasonable positions because rigor mortis maintained. Personal communications to this author from survivors of World War II who as children searched killed soldiers for valuables, thereby turning the bodies in whom rigor mortis was, in most cases, already fully developed around, confirm the aforementioned opinion that the observation of "cadaveric spasm" is in reality the result of manipulation of body posture postmortem. Another logical explanation may be the fixation of tetanic convulsion in rigor mortis, which has been observed in rare cases.

4.4. Rigor Mortis in Internal Organs

Postmortem rigidity does not only affect the skeletal muscles. Rigor mortis also is found in internal organs such as the myocardium, uterus, gall bladder, and urinary bladder. As with the skeletal musculature, rigor mortis also is preceded by a total primary relaxation of all muscles of internal organs. This muscle relaxation immediately after death explains the finding of leaking out of urine or seminal fluid from the orificium of the urethra owing to flaccidity of the urinary bladder and the pelvic diaphragm.

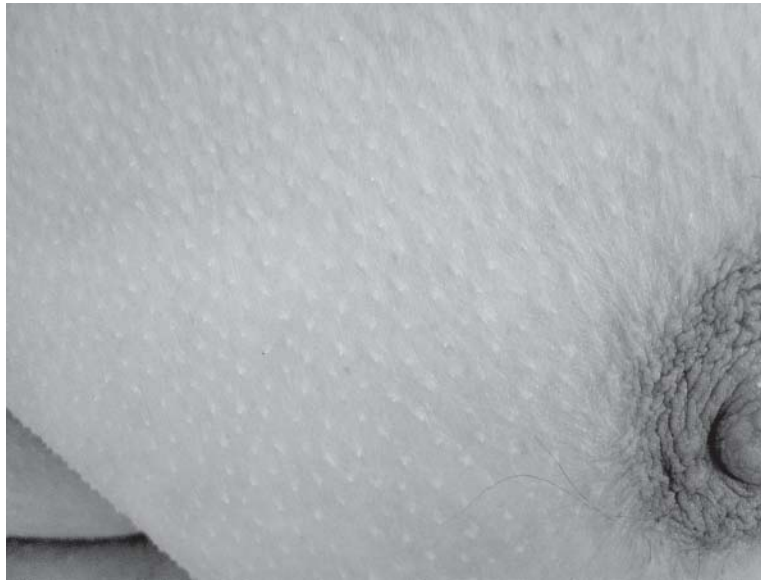

Fig. 19. "Goose-skin" postmortem.

The finding of "goose-skin" ("cutis anserina") after death (Fig. 19) is the result of postmortem contraction of the Musculi erector pilae in the sequel of rigor mortis formation.

4.5. Criminal Aspects

Rigor mortis occasionally is helpful in determining whether a body has been moved after death. If a body is found in an illogical posture, this means a body position that would not have been maintained under the influence of gravity (primary relaxation of the muscles after death), this position implies that the body has been moved after the development of rigor mortis.

Rigor mortis may make the examination of the palms and the inside aspects of the fingers difficult in such a way that current marks or defense injuries located here may be overlooked.

Particularly in infants and children, a marked dilation of the anal orificium may be observed postmortem (Fig. 20). As mentioned before, immediately as death occurs and preceding the onset of rigor mortis, the whole body musculature loses its tone. In children a dilatated anal orificium (because of the loss of tone of the musculature immediately as death occurs) may be fixed by rigor mortis, and this finding may even persist after rigor mortis has faded. Therefore, anal dilation alone is not a sufficient marker for penetrative anal abuse of children prior to death *(6)*.

Fig. 20. Marked dilatation of the anal orificium in an infant postmortem. Anal dilatation alone is not a sufficient marker for penetrative anal abuse prior to death.

Muscle relaxation immediately after death with opening of the eyes and the mouth with subsequent fixation in rigor mortis may give the face a somehow grimace-like look postmortem. However, one cannot predict from the face of a deceased, as often assumed by the unaware, whether this individual's last moments were of fear or fright.

5. *Algor Mortis (Postmortem Cooling)*

Algor mortis is the normal cooling of a body after death as a result of equilibration of the body with the ambient (environmental) temperature. The normal rectal temperature in the living is 36.9°C (range, 34.2–37.6°C), but the assumption that a "healthy" person had a "normal" body (rectal) temperature at the time of death is often erroneous because many factors influence body temperature at the time of death (Table 4).

The heat exchange between the body and the surroundings is mediated by conduction, convection, radiation, and evaporation. The main factors influencing the fall in body temperature after death are conduction and con-

Table 4
Individual Factors Potentially Influencing Body Temperature at the Time of Death

Raise of body temperature at the time of death owing to …
Infectious diseases (e.g., pneumonia, sepsis)[a,b]
Psychic (“emotional”) stress
Physical activity (e.g., sports, fight, escape)
Central fever (e.g., stroke, intracranial hemorrhage, intoxication with illicit drugs)
Hyperthyroidism
Malignant hyperthermia
Exsiccosis
Administration of neuroleptic medication
Lowering of body temperature at the time of death owing to …
Hypothermia (e.g., prolonged exposure of the subject to a natural cold environment, artefactual hibernation)
Hypothyroidism
Peripheral arterial occlusive disease
Administration of muscle relaxants

[a]Note that in elderly people infectious diseases may present without fever.

[b]When estimating the time since death using temperature-based methods, the error owing to fever is greatest during the first hours postmortem and decreases with the progression of the postmortem interval.

vection. Radiation can be usually neglected, but evaporation may become important if the body itself or the clothing worn by the deceased are wet.

The pace of cooling of a body after death depends, for instance, on the following:

1. Body weight in relation to surface area (the higher the body weight, the slower the cooling).
2. Temperature gradient between the core temperature of the deceased and the ambient temperature (the higher this temperature gradient, the faster the cooling; exchange of heat between the core and surface of the body is mediated exclusively by conduction).
3. Gender (female individuals have a higher fat content than male individuals, which results in a slower postmortem cooling of females when compared with males of identical weight).
4. Environmental conditions and surrounding mediums (e.g., still or flowing water,

draft, wind, sun radiation on the body [with potential re-warming of the body days after death]),
5. Surface insulation of the body by clothing or other covering such as blankets.
6. Wet clothing.
7. Body posture (faster cooling in a stretched out body position than in a crouched down position).

After death, the body temperature stays relatively constant, which is referred to as the "postmortem temperature plateau." In moderate to cool climates, this temperature plateau lasts about 1 to 3 hours and is then followed by a linear rate of cooling of 0.5 to 1.5°C per hour for the next 10 to 16 hours. Then, as body temperature nears the ambient temperature, the hourly cooling rate slows down. Water immersion cools a body much more quickly by convection than does exposure to air of the same temperature.

Estimation of the time since death based only on the body (rectal) temperature is often not accurate because the actual length of the temperature plateau is generally unknown and such an assessment is useless when the body temperature has approached the ambient temperature.

5.1. Criminal Aspects

Temperature-based nomogram methods for estimation of the time since death are founded on measurement of rectal temperature and mean ambient temperature at the death scene, determination of body weight, and the use of an empirical corrective factor. These factors are considered the most reliable methods by the leading authorities in the field *(1)*. Such temperature-based methods and their related formulas are most useful in temperate and cool climate zones in industrialized countries in which the majority of people dies indoors with structures of indoor heating but are often useless in warm or tropical climate zones and outdoor deaths.

6. Autolysis

Autolysis is "self-digestion" of tissue resulting from the breakdown of cell function postmortem. After death, when continuous oxygen supply stops and the cytoplasmatic pH decreases, loss of cell membrane integrity results. As a result, lysosomes and their digestive enzymes (mainly hydrolases) are released from the cells. Self-protective mechanisms of cells and tissues from endogenous noxae break down. Lysosome-rich organs, such as the pancreas, spleen, and lungs, express signs of autolysis earlier than do organs with a lesser hydrolytic enzyme content. Autolysis develops faster under warm and hot ambient temperatures than under cool or cold conditions. Autolysis is

Fig. 21. Autolytic imbibition as a result of a hemolytic discoloration seen in the inner vessel layer of the aorta.

accelerated by high body temperatures, for example, if the deceased had fever at the time of death.

At external examination of the body, the earliest sign of autolysis is detectable as a whitish, cloudy appearance of the cornea. At autopsy, autolytic changes manifest for instance as a doughy consistency of the parenchyma of the pancreas with loss of its normal macroscopic architecture on its cut surfaces. Liquefaction of the splenic pulp is another early phenomenon of autolysis that may be confused with the softening of the spleen as a sequel of acute splenitis ("septic spleen"). The lung parenchyma contains a large number of macrophages whose lysosomes release hydrolytic enzymes during autolysis, thus leading to a dim appearance of the outlines of cellular structures under the microscope. The adrenals normally retain their macroscopic appearance for several days postmortem, but appear flabby with loss of cohesion of the medulla as a sequel of autolysis. In the stomach, where the secretion of mucus has stopped after death, gastric acid affects the mucosal surface with resultant loss of the relief of the gastric mucosa. Postmortem leakage of gastric juice within the peritoneal cavity as a result of autolytic self-digestion of the gastric wall has been reported to take place in rare cases. As a result of the breakdown of erythrocyte membranes, hemolysis of the blood serum occurs. The intima of larger and smaller arteries becomes a reddish to light brownish, muddy appearance that is referred to as "imbibition" (Fig. 21). This imbibition is a result of a hemolytic discoloration of the inner vessel layer.

Fig. 22. Maceration in a stillbirth manifesting as skin slippage with underlying brownish-blackish discoloration of tissue.

By definition, autolysis is a pure result of endogenous enzyme activity and bacterial processes play no role. However, destruction of tissue by autolysis and by bacterial processes runs a parallel course and their products are overlapping. Therefore, it is more an academic question than a matter of practical relevance whether, for instance, superficial skin slippage is a result of pure autolysis (as considered by some authors) or if putrefaction processes play the major part in its development.

Histology is most useful to distinguish whether organ changes seen at autopsy are a sequel of shock or a manifestation of autolysis; however, this task can be extremely difficult. For a detailed overview of histological changes occurring as a result of autolysis, it is referred to the comprehensive review by Rutty *(7)*.

7. Maceration

Maceration is sterile autolysis of an unborn fetus who has died in utero enclosed within the amniotic cavity. The most prominent finding at the external examination is skin slippage with underlying brownish-blackish discoloration of tissue (Fig. 22). If the amniotic cavity has been opened prior to the delivery of a stillborn fetus, bacterial putrefaction will alter the morphological picture of maceration. The presence of maceration without any putrefactive changes in a recently delivered child is indicative of stillbirth *(8)*.

8. PUTREFACTION

Putrefaction is bacterial degradation of soft tissue. After death, when homeostasis ceases, anaerobic bacteria (mostly *Clostridia* and *Proteus* species) migrate from the gut into blood vessels and into tissue where they multiply and spread throughout the whole body.

The terms *putrefaction* and *autolysis* are often used synonymous in the Anglo-American literature, but these processes have to be strictly differentiated because, by definition, bacterial processes play no role in the development of autolytic changes. On the other hand, autolysis paves the way for bacteria to spread throughout the body by the breakdown of cell integrity of the gut mucosa. The term *decomposition* is often used synonymous with putrefaction, too. By strict definition, decomposition is the product of soft tissue degradation by aerobic bacteria (originating from outside environmental sources), but for practical purposes this differentiation seems far too academic since both processes can, for the most part, not be distinguished adequately.

8.1. Factors Accelerating the Onset of Putrefactive Tissue Changes

Many variables affect the onset, extent, and time course of putrefaction, but temperature is the most decisive factor. Putrefactive tissue changes develop faster under warm and hot ambient temperatures than under cool or cold conditions. Putrefaction is accelerated in subjects who died of infectious diseases (e.g., sepsis, gas gangrene) because blood and organs have already been invaded by bacteria before death on the one hand and on the other hand body temperature is usually raised in such fatalities at the time of death.

The administration of antibiotics before death often slows down the putrefaction process. Because open wounds are a portal of entry for microorganisms from the outside environment, those subjects who die with or from wounds that are wide open and extending far down within the subcutaneous tissue show accelerated rates of putrefaction. Obesity accelerates the onset of putrefaction, too.

Putrefaction is delayed in individuals with a considerable loss of blood prior to death since hemoglobin as well as other proteins from blood cells are a main source of energy for bacteria involved in putrefaction processes.

8.2. Pathophysiological Processes and Chemistry of Putrefactive Organ Changes

The process of putrefaction is catalyzed by autolysis-induced breakdown products of proteins, carbohydrates, and lipids that serve bacteria as a source of energy.

Hydrogen sulfide (H_2S) is the main product of reductive catalysis by endogenous bacteria. The compound of H_2S with hemoglobin that is released from autolytic erythrocytes leads to the formation of sulfhemoglobin, which is responsible for the characteristic greenish discoloration of putrefied human bodies.

Venous marbling, or the outlining of superficial epidermal blood vessels, is the result of a combination of autolysis of erythrocytes (postmortem hemolysis) and intravascular multiplication and growth of intestinal bacteria that use blood vessels as "through roads" to spread over the entire body. Whether marbling manifests with a greenish or a more violaceous to muddy-brownish color is depending on the total amount of sulfhemoglobin formation within the affected vessels.

The characteristic bloating of a putrefied body is reflected by the swelling of the face, distension of the abdomen and, in males, scrotal swelling as a result of bacterial gas formation. On palpation, crepitus is noticed. Putrefactive gas has an offensive foul odor and is the volatile final product of bacterial reductive catalysis. Putrefactive gas is mainly composed of methane, H_2S, CO_2, ammonia, mercaptans, and primary amines. The purging of putrefactive fluid from mouth and nostrils as well as eversion of the lips and protrusion of the tongue is the result of an increase of intrathoracic pressure owing to bacterial gas formation within the thoracic cavities.

8.3. Morphology of Putrefaction

The exact chronological order of appearance of putrefactive changes is highly variable and depending on a broad variety of individual as well as environmental conditions. Therefore, it is far beyond the scope of this chapter to give a nearly satisfactory overview on all morphological findings and the time frames in which they may have developed in different seasons and climate zones. However, putrefactive body changes usually follow a sequential order.

The earliest sign of putrefaction is a greenish skin discoloration of the abdomen, which usually is first visible in the right lower abdominal quadrant. As this greenish skin discoloration becomes more prominent and spreads over the whole body, skin slippage with glove-like peeling of the horny skin layer of the hands, formation of gaseous or putrefactive fluid-filled skin blisters

Fig. 23. Putrefactive skin blistering. "Strawberry-like" appearance.

(Fig. 23), venous marbling (Fig. 24A–C), purging of putrefaction fluid from mouth and nostrils (Fig. 25), swelling of the face with bulging of the eyes, eversion of the lips, and protrusion of the tongue between the teeth and lips, bloating of the abdomen under tension and, in males, gaseous swelling of the scrotum and penis (Fig. 26) develops. Hair and nails become loose and can be easily dislodged. In advanced states, the skin has a brownish-blackish appearance.

Changes of internal organs as a result of bacterial gas formation are, for example, dilation of the renal pelvis and the ventricles and vestibules of the heart. Muddy-brown putrefaction fluid is found within the pleural and peritoneal cavities. So-called "putrefaction crystals," yellowish particles composed of tyrosine and leucine, are found on the surface of internal organs, especially on the surface and bottomside of the liver, adhering loosened to Glisson's capsule, as well as on the capsule of the spleen. The liver shows a spongy consistency giving both cut sections and histological sections in more advanced stages a foamy (so-called "swiss cheese-like") appearance (Fig. 27). The intestinal loops are distended as a result of gas formation. The myocardium appears in a muddy brownish to blackish color and hence, myocardial infarction

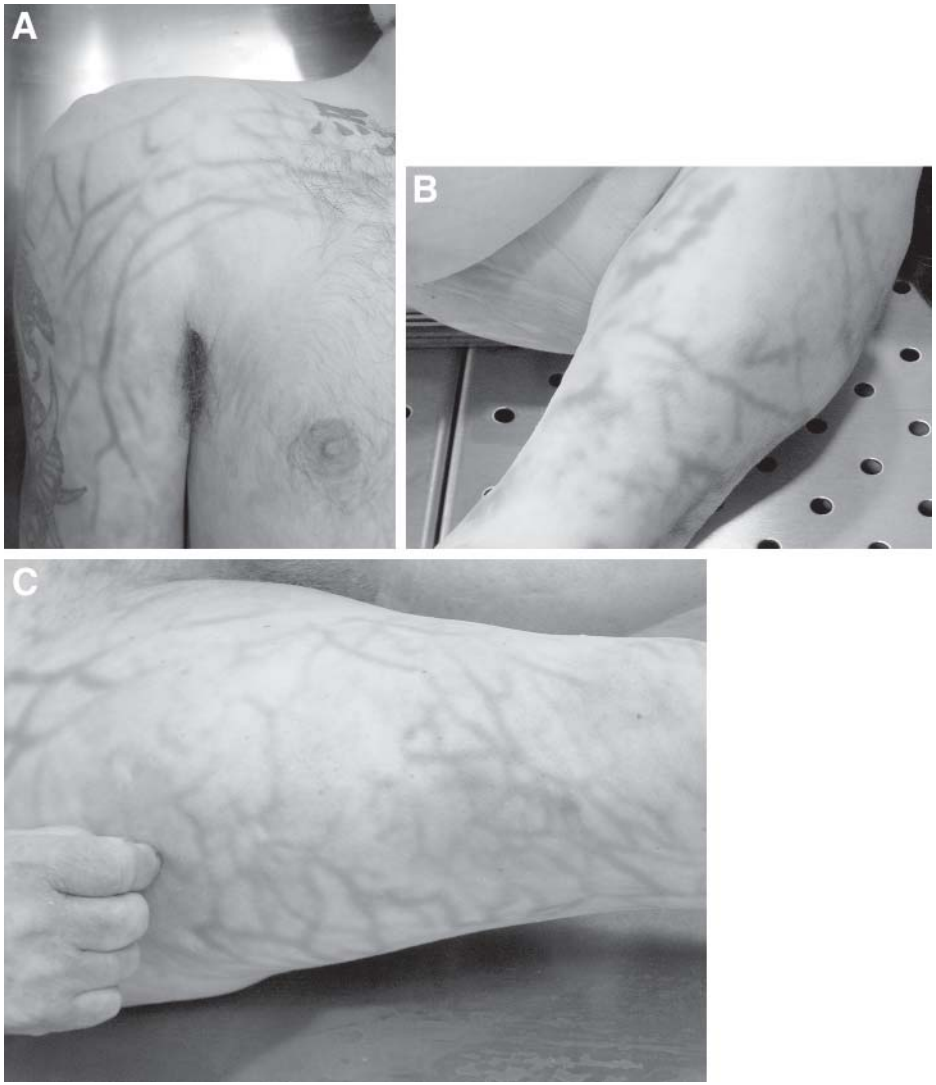

Fig. 24. (A–C) Venous marbling in putrefied corpses.

often escapes macroscopial detection. The brain appears soft to liquified with loss of the cortical surface structures and shows a dark grayish to green discoloration of cortex, caudate nucleus, trabs, and putamen on cut sections. Gaseous bubble formation is seen under mucosal surfaces of internal organs especially in the stomach and urinary bladder.

Venous marbling is not strictly limited to the outer surface of the body, this phenomenon is also observed under mucosal or serous surfaces of hollow

Fig. 25. Purging of putrefaction fluid from mouth and nostrils.

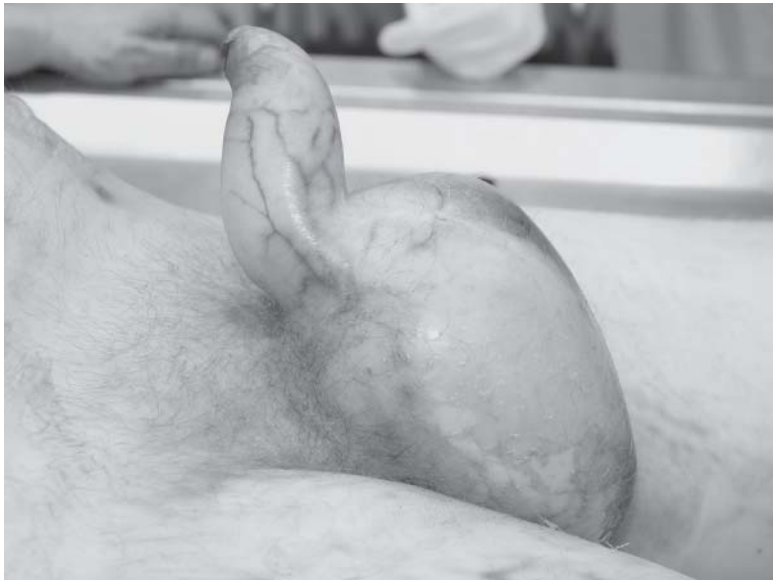

Fig. 26. Gaseous swelling of the scrotum and penis in a putrefied body.

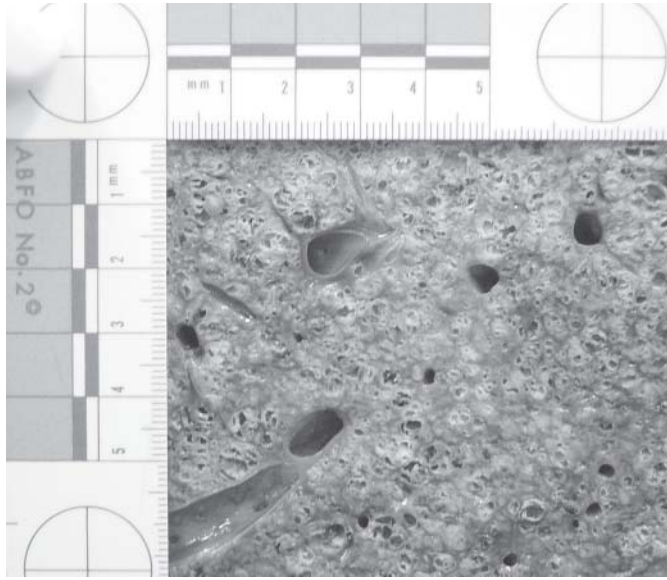

Fig. 27. Cut surface of putrefied liver parenchyma exhibiting a foamy appearance ("swiss cheese pattern") surrounding opened vessels' lumina.

organs (Fig. 28). In advanced stages of putrefaction, the volume of bacterial gases produced is usually enough to float solid organs like liver, kidneys, or spleen when brought into a water bowl at autopsy. The prostate gland is usually the organ offering most resistance toward putrefaction and may occasionally be used to determine the gender of otherwise totally putrefied human remains.

8.4. Criminal Aspects

The manifestation of putrefaction can cause interpretational problems and, accordingly, a death may seem suspicious in a given case. Putrefaction may mask traumatic injuries that a person sustained before death. On the other hand, purging of putrefaction fluid from the mouth and nostrils is frequently confused with blood, for example, deriving from antemortem facial injuries or originating from a gastrointestinal source of bleeding, by those investigators unfamiliar with the phenomenon. When tight clothing is worn by the deceased, putrefactive bloating of the neck region may lead to cutaneous alterations mimicking strangulation marks. Putrefactive tissue changes of head and face often make visual identification difficult or impossible at all.

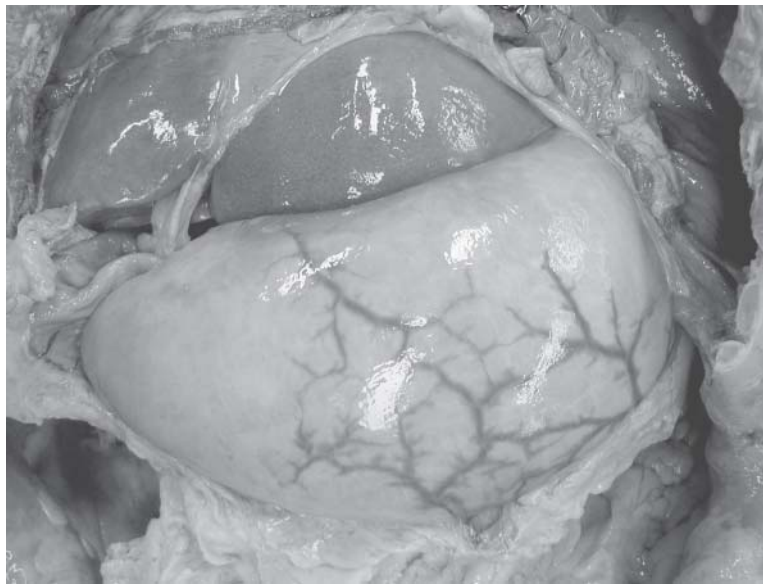

Fig. 28. After opening of the abdominal cavity, venous marbling of the gastric mucosa is shining through underneath the serous surface of the (still intact) stomach.

8.5. Differential Diagnoses

A major problem in forensic autopsy practice is the broad variety of a number of artifacts produced in more advanced states of putrefaction as well as the possible overlapping of putrefactive tissue changes with preexisting diseases, the latter being missed at gross inspection of the affected organ. Examples are the presence of froth in the heart in a putrefied body that must not be misinterpreted for air embolism, a flabby appearance of the heart caused by bacterial gas production that may mimic dilatation of the ventricles and atria of the heart, venous marbling seen under the mucosa of the esophagus mistaken for esophageal varicosis, or putrefactive liquefaction of the lung parenchyma misdiagnosed as edema. Putrefaction fluid within the pleural cavity may be mistaken for hemothorax or pleural effusions.

However, the prominence of congested subcutaneous venous vessels (Fig. 29), as especially seen in older, cachectic individuals may be mistaken for venous marbling in otherwise fresh bodies. The documentation of such a false interpretation in the protocol of external examination in a given case may later lead to false conclusions when roughly estimating the time since death purely based on such descriptions in addition to other criteria.

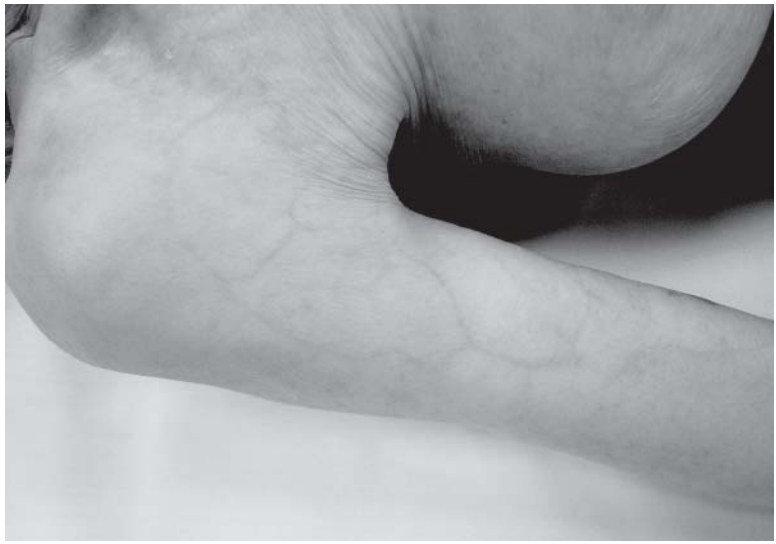

Fig. 29. Prominence of congested subcutaneous venous vessels seen on the anterior aspect of the upper arm of a cachectic 86-year-old-woman. This finding should not be mistaken for venous marbling as a sequel of putrefaction.

Coma blisters (Fig. 30), that are bullous cutaneous lesions occasionally found in the setting of coma and caused by intoxication with barbiturates, benzodiazepines, theophylline, or in carbon monoxide poisoning, may be mistaken for skin blistering as a result of putrefaction and, conversely, coma blisters may be overlooked when putrefactive changes of the body have occurred. Concerning the differential diagnosis, coma blisters are most often located at sites of dermal compression and appear histologically as subepidermal blister formation without any epidermal necrosis, but with eosinophilic necrosis of the eccrine sweat gland coils *(9)*.

Very early onset of a rapid progressive course of putrefactive skin and tissue changes especially when seen adjacent to wounds, surgical incisions, or necrotic skin areas, and when found in a circumscribed pattern and accompanied by tissue emphysema (as indicated by crepitus on palpitation), is highly suspicious for a vitally acquired infection with clostridae (gas gangrene).

9. *Postmortem Preservation by Freezing*

If a body has been frozen immediately after death, for instance, in a freezer or another type of cooling apparatus with the purpose to hide the corpse, the rate of postmortem changes slows virtually to zero. Homicides in which the victim's body had been hidden in deep-freezes or similar devices have been

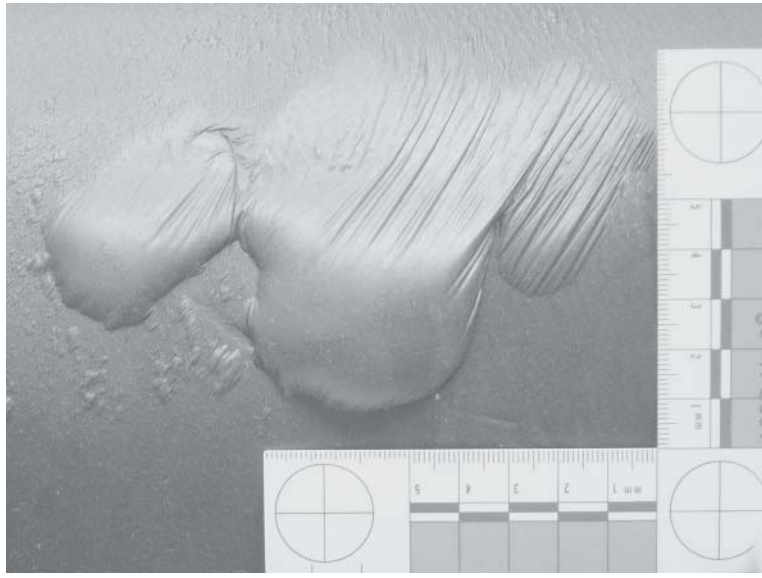

Fig. 30. Coma blisters on the back of the body of an African male who died of mixed intoxication with pentobarbital, thiopental, midazolam, and propofol.

reported *(10)*. In such cases the estimation of the time since death is impossible based solely on the forensic pathological findings. When a corpse that was frozen prior to its dumping is thawed again, more advanced putrefaction is usually observed on the outer body surface than in internal organs. The reason for this phenomenon is that the enteric flora has in such cases been most often put to death before any relevant putrefaction of the viscera could occur. Accordingly, anaerobic bacteria from the outside will have greater effects on the course and manifestation of putrefaction of the outer body surface than on internal organs.

Freezing of tissues leads to the formation of ice crystals in extracellular spaces, for example, in the myocardium or liver. When the tissue is rewarmed, the ice crystals melt. Therefore, the forensic pathologist will only see the after-effects of freezing of tissue in the extracellular spaces under the microscope since the specimens were thawed and prepared for histology. These ice crystal artifacts are described as long, parallel areas that resemble the shape of ice crystals and expand the extracellular spaces of liver and myocardium *(11)*.

9.1. Freeze-Drying

Freeze-drying, the process of body preservation mediated by sublimation, is predominately seen in bodies recovered from polar regions and permafrost zones in Siberia or the Middle East. Such bodies are usually well preserved externally

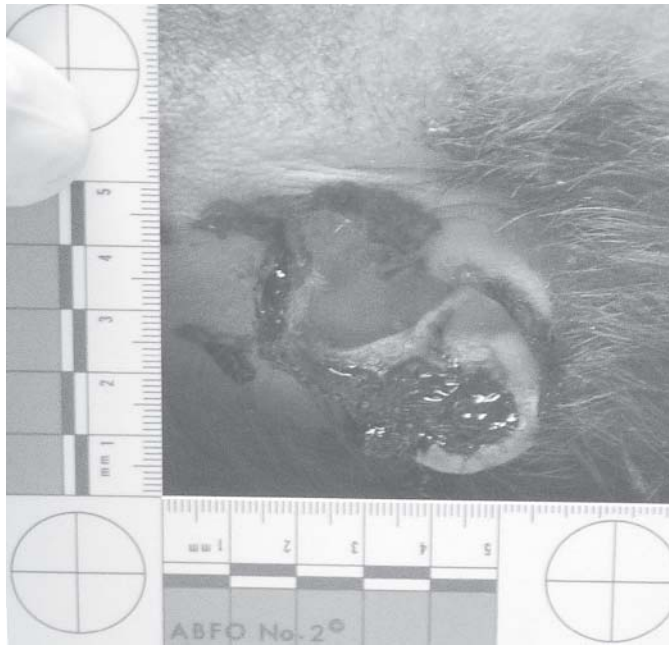

Fig. 31. Tissue defects of the left auricle showing serrated wound margins with irregular edges. These injuries were caused postmortem by rodents (most probably mice according to the scene findings).

and internally or may show mummification. When freeze-dried bodies are mummified, the internal organs are usually better preserved than in those cases where mummification occurred under hot environmental conditions.

10. Postmortem Animal Depredation

Postmortem animal depredation is a substantial part of the taphonomic processes a body undergoes after death *(12,13)*. Although the occurrence and presentation of such injuries are well known to forensic pathologists, the morphological appearance of these injuries can be misinterpreted by the consulted medical practitioner or police officers and other investigating authorities.

Postmortem injuries may be inflicted by all kinds of animals irrespective their size or environmental origin either from land, sea, or air *(14–20)*. The discrimination between antemortem injury vs postmortem artifacts generally presents no difficulties because of the total absence of hemorrhage and reddening in the tissue adjacent to the wound margins as well as the lack of any vital reaction under the microscope in the latter cases. Low ambient tempera-

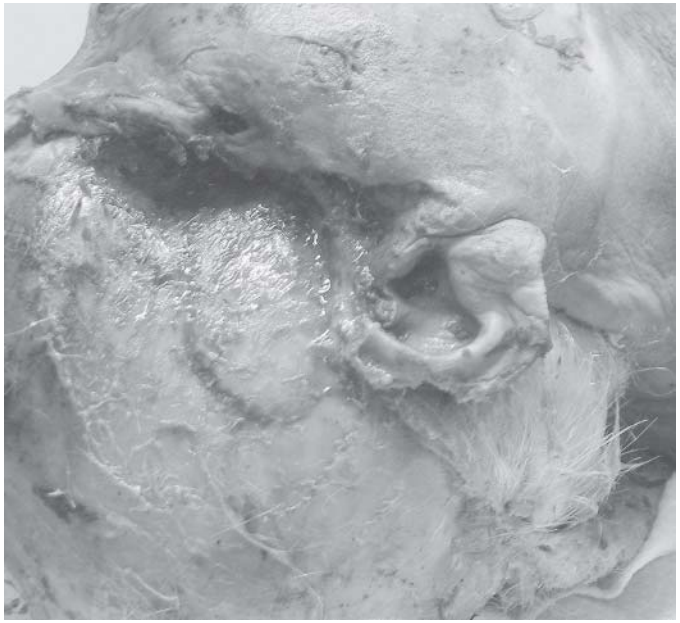

Fig. 32. This large tissue defect of parts of the face, right auricle, and scalp was caused postmortem by water rats.

ture has, in addition to slowing the onset and extent of postmortem changes, considerable impact on a delayed manifestation of odor of the body, thus making the human remains less olfactory absorbing for carnivores and rodents.

10.1. Injuries Caused by Land Animals

The most effective tissue removers are insects and rodents *(21–23)*. Cutaneous holes and soft tissue defects, for example, made by maggots, especially when overlapped by tissue shrinkage caused by mummification with resulting enlargement of the defects, can mimic gunshot wounds or other mechanical tissue defects that were sustained before death, for example, as a result of stabbing with sharp-pointed instruments such as knives or scissors.

Skin and soft tissue artifacts caused by rodents may occur as early as within the first hour postmortem *(23)*. In the majority of injuries inflicted postmortem by rodents, the wounds have a circular appearance and the wound margins are finely serrated showing irregular edges (Figs. 31 and 32). Distinct parallel series of cutaneous lacerations deriving from the biting action of the upper and lower pairs of the rodent incisors are diagnostic for rodent activity *(23,24)*. However, the determination of a distinct rodent species (e.g., rats, mice) solely based on the morphological appearance of the damage to skin

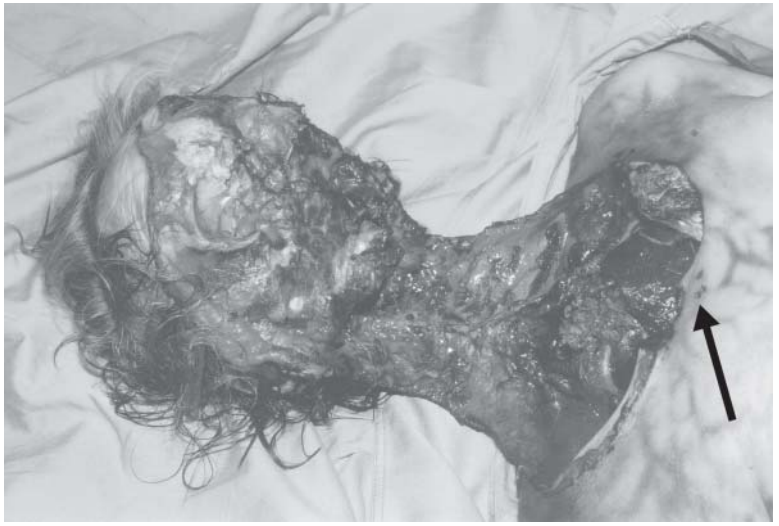

Fig. 33. Rounded, regular wound margins caused by a German shepherd postmortem. Note the two punctured wounds on the intact skin in the immediate vicinity to the actual wound margins (arrow) originating from the canine teeth of the dog.

Fig. 34. Skin lesions caused postmortem by ants. (Courtesy of Dr. Wolfgang Huckenbeck, Düsseldorf, Germany.)

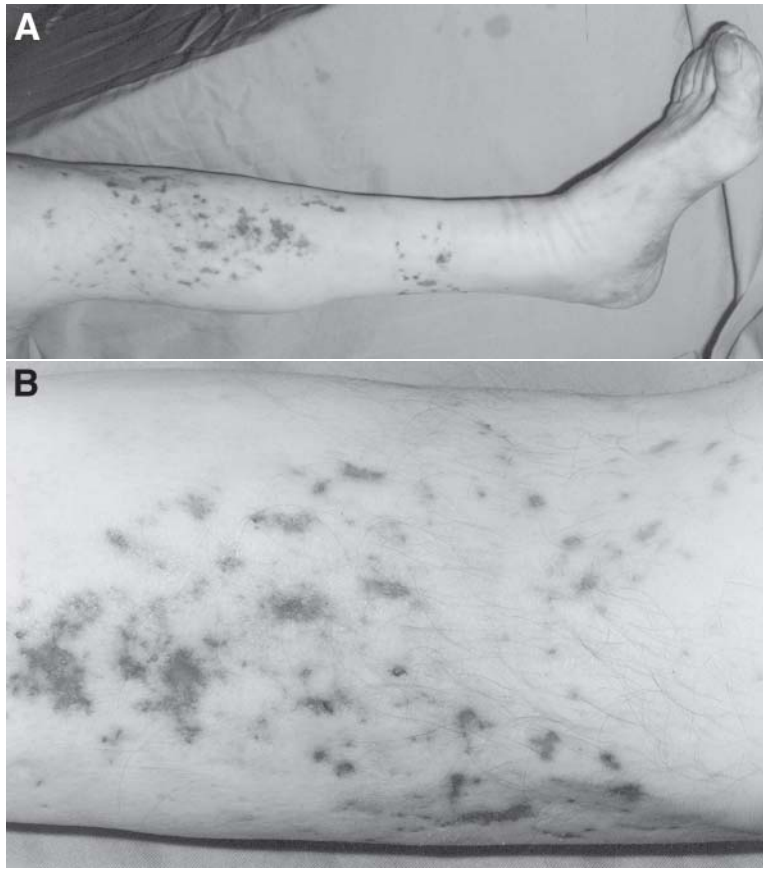

Fig. 35. (A) Dermal abrasions caused postmortem by cockroaches. **(B)** Detailed view.

and soft tissue often is unconvincing. The finding of rodent excrements as the connecting link for the diagnosis of rodent interference to human remains has been proposed, thus providing the possibility of distinguishing DNA sequences of the animal itself from those of food residues of human origin *(18,25,26)*.

A broad range of carnivores can be involved in the postmortem destruction of corpses located in open spaces or indoors (e.g., wild animals such as foxes and big cats or domestic animals such as dogs and cats). The wound margins caused by carnivores often appear more regular than those caused by rodents and V-shaped or rhomboid punctured wounds are often seen on the intact skin in the immediate vicinity to the actual wound margins (Fig. 33). Such stab wound-like defects represent canine tooth marks of carnivores' origin. An additional criterion for animal depredation by carnivores is the pres-

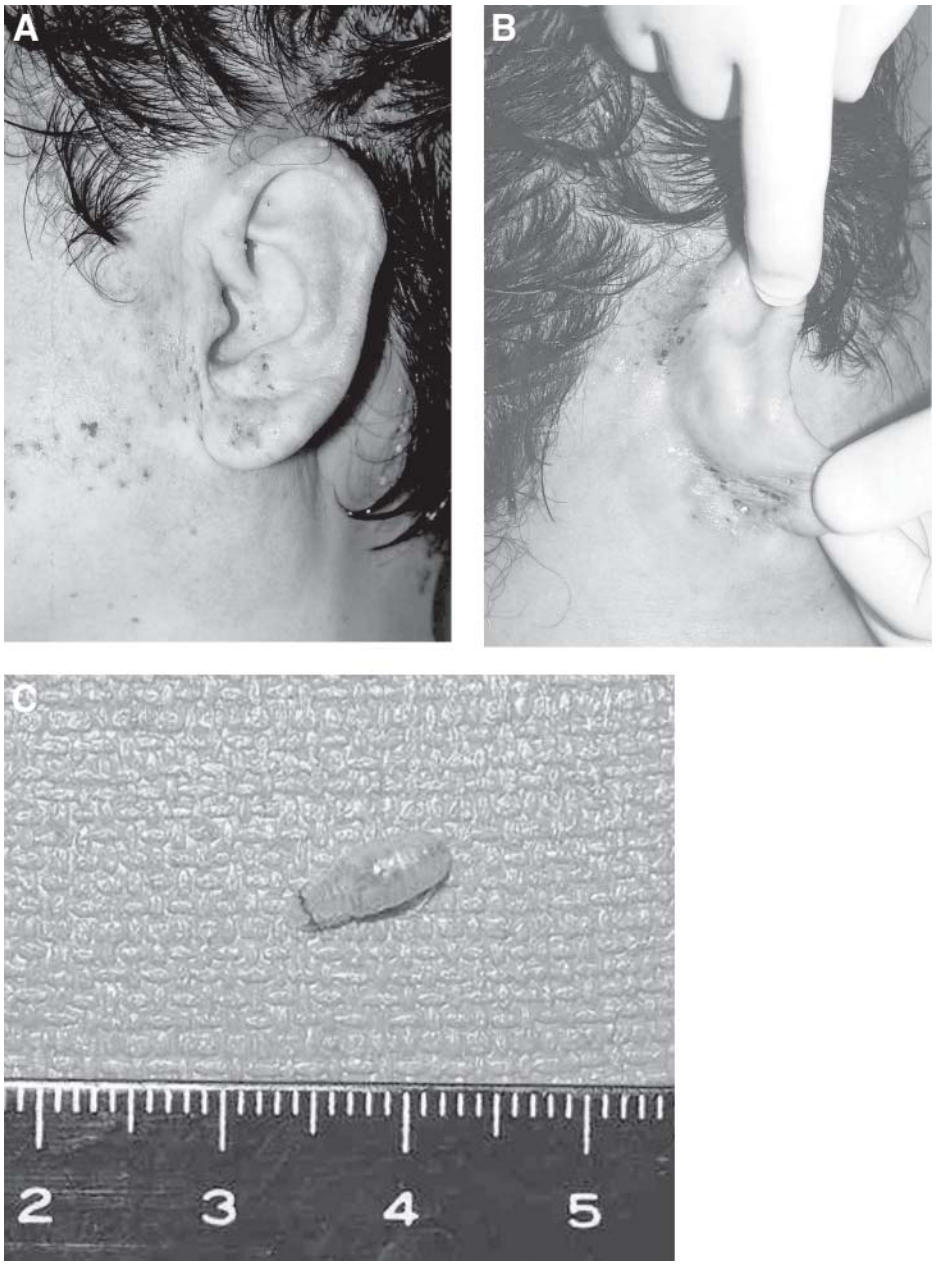

Fig. 36. (A,B) Punctured round to oval cutaneous defects caused by *Excirolana japonica*, a marine isopod. **(C)** This specimen of *Excirolana japonica* was recovered from the subcutaneous tissue behind the right auricle after dissection during autopsy. (Courtesy of Prof. Satoru Miyaishi, Okayama, Japan.)

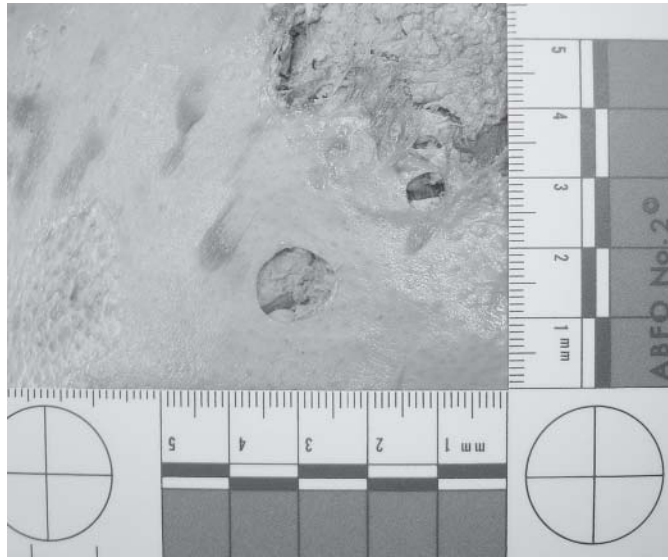

Fig. 37. Round, crater-like dermal lesions caused by crustaceans postmortem.

ence of claw-induced linear scratch-type abrasions in the vicinity of the damaged skin areas *(24)*.

Insects producing corrosive secretions, for example ants, may cause postmortem skin lesions (Fig. 34) that, when located on the neck, may look like cutaneous marks resulting from manual strangulation. Cockroaches produce superficial dermal abrasions that may mimick skin diseases. Injuries of cockroaches' origin are typically observed on body regions formerly protected by tunnel-like layers of clothing such as the arms and legs (Fig. 35A,B).

10.2. Injuries Caused by Marine Animals

In drowning victims or persons whose death led to their deposition in water or when a body has been dumped in an aquatic environment, postmortem artifacts on the body surface owing to aquatic living structures are often observed *(19,27)*.

Depending on the given aquatic environment, a broad variety of marine living structures is involved in tissue destruction of bodies recovered from water (Fig. 36A–C). Crustaceans are considered the most effective tissue removers in water, typically leaving oval to round, crater-like dermal lesions of varying size (Fig. 37).

Sea lice (*Natatolana woodjonesi*), which are approx 2.5 cm long isopods that live on the surface of sand in shallow water, are found in aquatic environ-

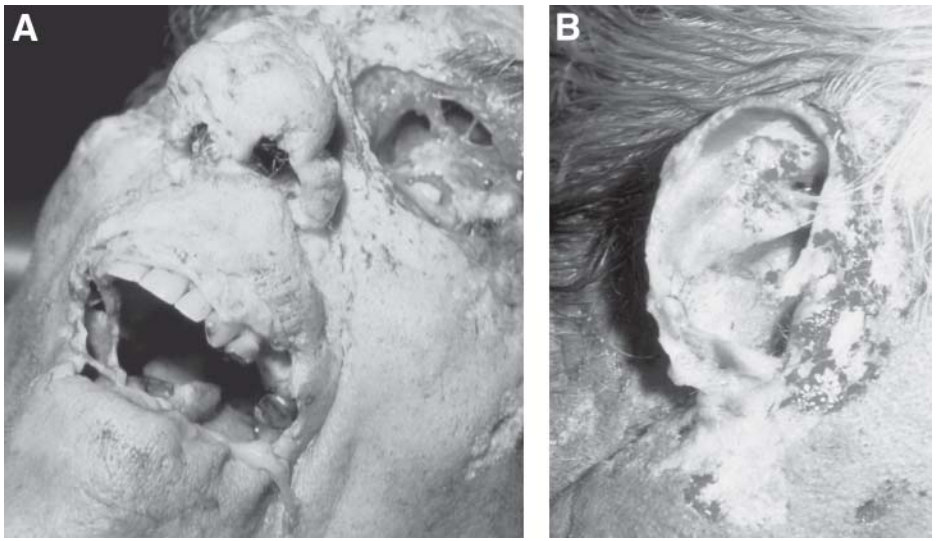

Fig. 38. Facial **(A)** and auricular defects **(B)** caused by sea lice (*Natatolana woodjonesi*). This body was recovered from the sea within less than 12 hours submersion time. (Courtesy of Prof. Jules Kieser, Otago, New Zealand.)

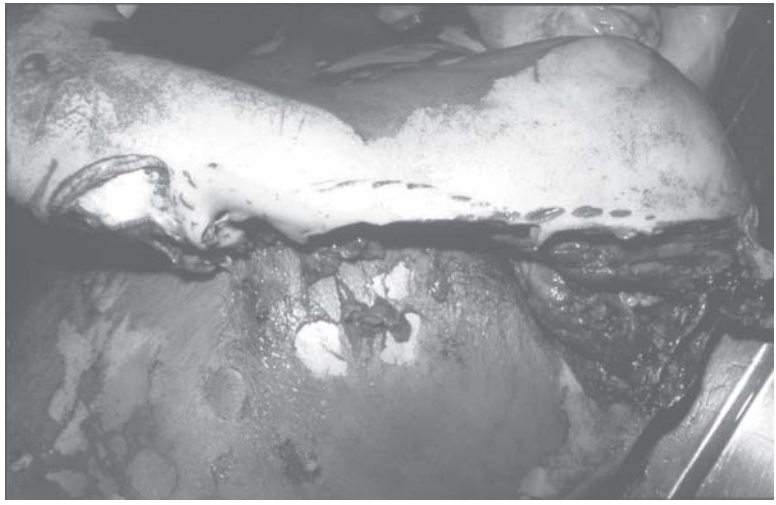

Fig. 39. Large shark bite defect on the left arm of a body recovered from water with significant loss of skin and soft tissue. Note the arch of characteristic shark tooth marks adjacent to the cleanly incised wound margin. (Courtesy of Prof. Jules Kieser, Otago, New Zealand.)

ments worldwide. They are active swimmers when searching for food. Sea lice can reduce a dead fish to a skeleton in a few hours. Sea lice may cause bizarre mutilation of submerged bodies as shown in Fig. 38; however, sometimes the animals leave more defined, oval punctate defects, occasionally resembling shotgun pellet wounds *(19)*.

Starfish cause dermal hematomas by their peculiar mechanism of feeding, namely sucking. When inflicted in the early postmortem interval, these hematomas have been reported to be easily mistaken for vitally sustained hematomas *(28)*. When such starfish-induced injuries are present upon a body that was recovered from water or when starfishes themselves are found adhering to such a corpse, this finding indicates that this body must have been, at least for a short period of time, located at the bottom of a body of water because starfishes are not able to swim and are only found at the bottom of their particular aquatic living space.

Shark bites occur every so often in intact and fragmented bodies found at sea. Shark bites tend to have cleanly incised edges with significant loss of skin and underlying soft tissue. The pattern of typical shark bites corresponding to the triangular shape of their teeth can often be clearly identified (Fig. 39). If the attack was not witnessed and only parts of the body recovered, it is usually impossible to determine from the injuries whether death was a result of the shark attack or if the injuries merely represent postmortem feeding on human remains *(19)*.

11. Miscellaneous

In contrast to livor mortis, vibices, rigor mortis, algor mortis, autolysis, and putrefaction, all of which are postmortem phenomena that frequently are observed in the death investigator's daily practice, the following postmortem changes occur only occasionally and under specific intraindividual or environmental conditions.

11.1. Postmortem Changes and Injuries to the Skin

11.1.1. Injuries Caused by Handling, Transportation, and Storage of the Body

Abrasions and lacerations on the skin may be produced by manipulation of the body during postmortem handling, transportation, and storage *(29)*. When arising in the early postmortem interval, these postmortem injuries are relatively easily distinguishable from vital injuries by their light yellowish-brownish to golden, shiny appearance in cases were the epidermis is still intact and

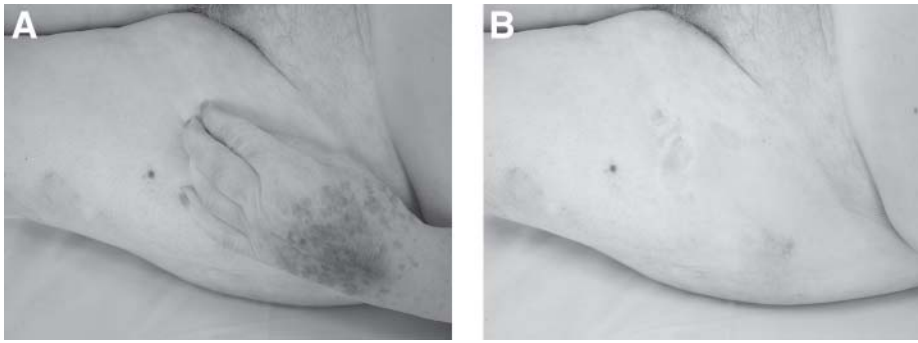

Fig. 40. (A,B) Marks on the skin of the left thigh caused by pressure from fingertips and nails of the deceased.

no postmortem skin slippage has occurred as a result of autolysis and/or putrefaction. These shiny postmortem skin alterations result from loss of the barrier function of the epidermal layer of the skin with subsequent evaporation of tissue fluid. In doubtful cases, incision of cutaneous injuries of postmortem origin will reveal a hardened, slightly flattened area on cut sections without any hemorrhage in the underlying soft tissue. A similar phenomenon is observed at pressure points of the body surface without any injuries or loss of superficial skin layers, respectively. When caused by the fingertips and nails of the deceased him or herself, typically these skin alterations appear dried up, yellowish to light brownish with blanching in central portions corresponding to the points of most pressure (Fig. 40).

In skin areas where the epidermis is very thin (e.g., the tip of the nose and the scrotum), drying up as a result of postmortem evaporation of tissue fluids occurs. The result is a hardened, light brownish appearance of the affected epidermal surfaces (sometimes referred to as the earliest stage of mummification).

11.1.2. Skin Changes Produced by Corrosives

Corrosives such as acids or alkalis may lead to loss of the epidermal layer of the skin. Regurgitation of gastric juice may produce skin lesions resembling to those caused by chemical agents containing corrosives *(30)*. The running out of gastric juice during the handling or transportation of the body may produce pale, sometimes band-like, cutaneous alterations that should not be confused with ligature marks when located on the neck *(29)*.

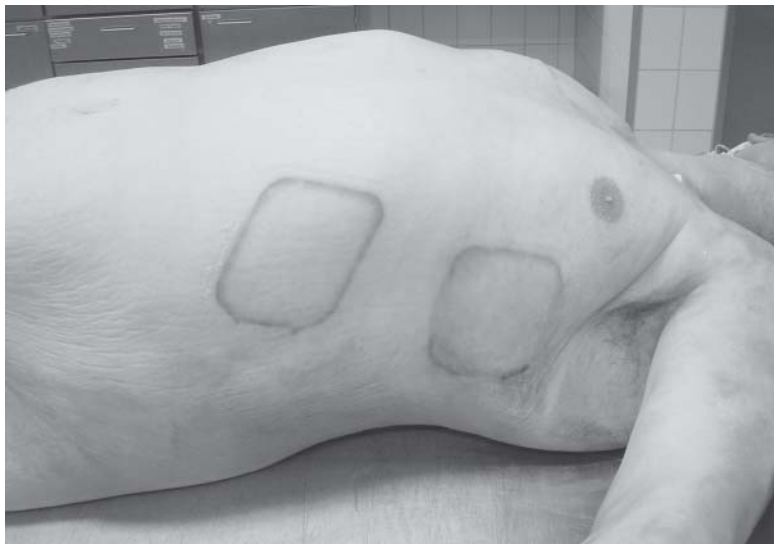

Fig. 41. Defibrillation marks.

11.1.3. Skin Changes Produced by Postmortem Urine Leakage

Leaking of urine postmortem may cause extensive skin damage to an infant on the perigenital skin areas. The same is the case when the infant's skin was in contact with a urine-soaked diaper postmortem. One has to be aware to differentiate these postmortem skin changes from vitally acquired alterations and not to interpret them uncritically as signs of neglect prior to death *(31)*. Histologically, no vital reaction will be detected in such skin alterations.

11.1.4. Skin Changes Caused by Resuscitation Procedures

Resuscitation procedures may leave a broad pattern of "injuries" on a deceased person's skin. The most common signs corresponding to external heart massage performed before death are superficial dermal abrasions above the sternum stemming from the hands of the person performing cardiac massage *(32)*. As a general rule, these dermal abrasions are better observed at later time points postmortem than in earlier stages of the postmortem interval because of postmortem evaporation of tissue fluids out of the superficially injured epidermis and subsequent drying out of the skin with further progression of the postmortem interval. Defibrillation leaves typical rectangular marks on the body surface as shown in Fig. 41.

Fig. 42. Washer-woman's skin formation on the hand in a drowning victim.

11.1.5. Washer-Woman's Skin and Heat-Mediated Changes Mimicking Washer-Woman's Skin

In bodies recovered from water or moist environments, the skin of the palms of the hands and the feet becomes a whitish discoloration of the epidermis associated with swelling, wrinkling, and vesicular detachment up to glove-like peeling, mainly as a result of soaking of the horny layer of the epidermis. This finding, referred to as washer-woman's skin, which is seen particularly after prolonged exposure to water in drowning deaths (Fig. 42), should not be confused with another special form of skin changes seen on the hands and feet that are caused by heat (Fig. 43). In the latter, histological examination shows fluid-filled blisters in the stratum germinativum, hyperchromasia and palisade arrangement of the nuclei, as well as clumping of the erythrocytes corresponding to a morphological variation of a second-degree burn caused by the special anatomy of skin friction *(33,34)*.

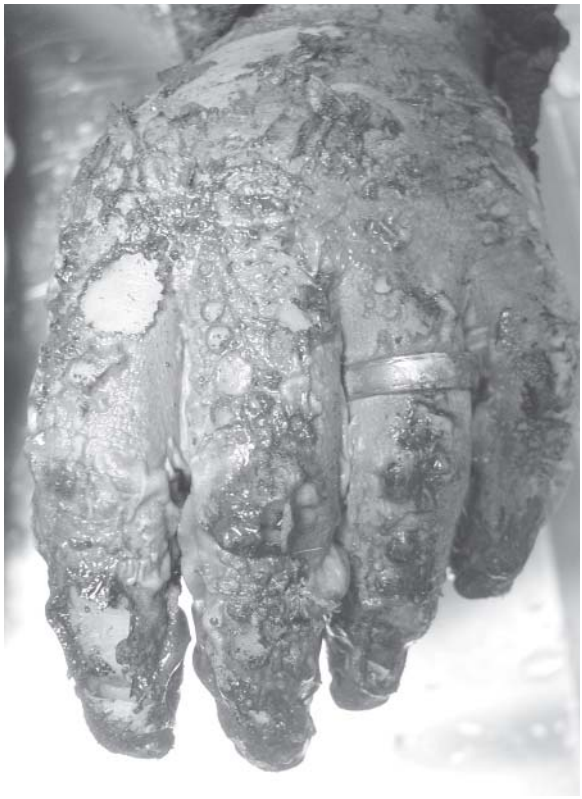

Fig. 43. Heat-mediated changes to the hand mimicking washer-woman´s skin.

11.2. Drying of Mucosal Surfaces

Postmortem evaporation of tissue fluids and hypostasis after cessation of circulation leads to drying up of mucosal surfaces, for example, of the lips, the tip of the tongue, the glans of penis, the glans of clitoris, or the pudendal lips, thus resulting in a hardened, light-to-dark brown appearance of the affected mucosal surfaces. Especially a dark brown, sometimes black appearance of parts of the tip of the tongue should not be confused with an epileptic tongue bite.

11.3. External Changes of the Eye After Death

As with most postmortem changes, alterations of the eye after death are accelerated in their onset and intensity under warm ambient temperatures and

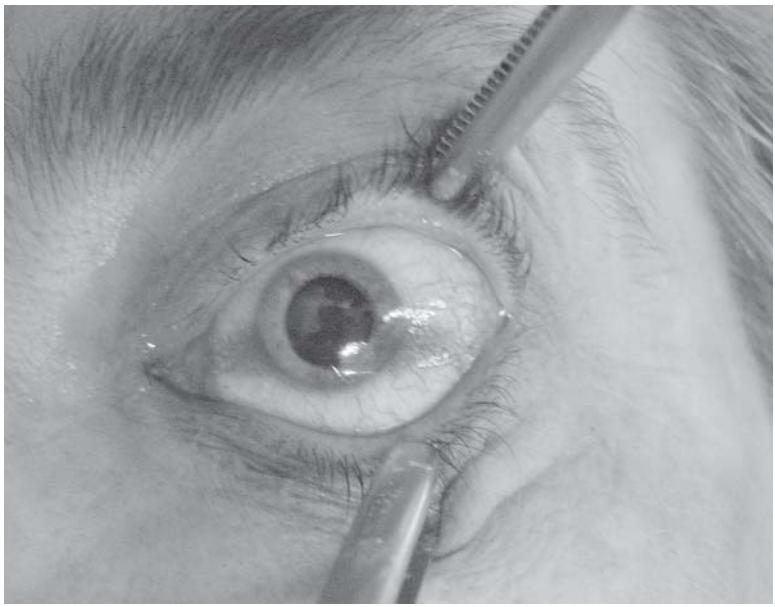

Fig. 44. Tache noir (postmortem interval 7 to 8 hours; opened eyes after death).

in dry climates. Under moderate to cool ambient temperatures, approx 3 to 9 hours after death, the cornea becomes a whitish, cloudy appearance that is a result of autolysis. With further increase of the postmortem interval, the cornea looses its turgor.

If the eyes remain open after death, the areas of the sclera exposed to air dry out, which results in a first yellowish then brownish-blackish band-like discoloration zone called "tache noire" (Fig. 44).

The conjunctivae soften and become a light grayish color. In states of advanced putrefaction, conjunctival petechiae, for example, as a result of asphyxia, may not only be masked because of sulfhemoglobin production, giving the conjunctivae a homogenous grayish to light-greenish appearance, but these petechiae may vanish completely because of postmortem hemolysis in the course of autolysis.

11.4. Pink Teeth and Nails Phenomenon

Pink discoloration of teeth and nails is a rare postmortem finding that is thought to derive from hemolysis after exudation of hemoglobin and hemoglobin derivates. This phenomenon seems to depend on the anatomical presence of porous structures, an anatomical feature that is found in the dentine

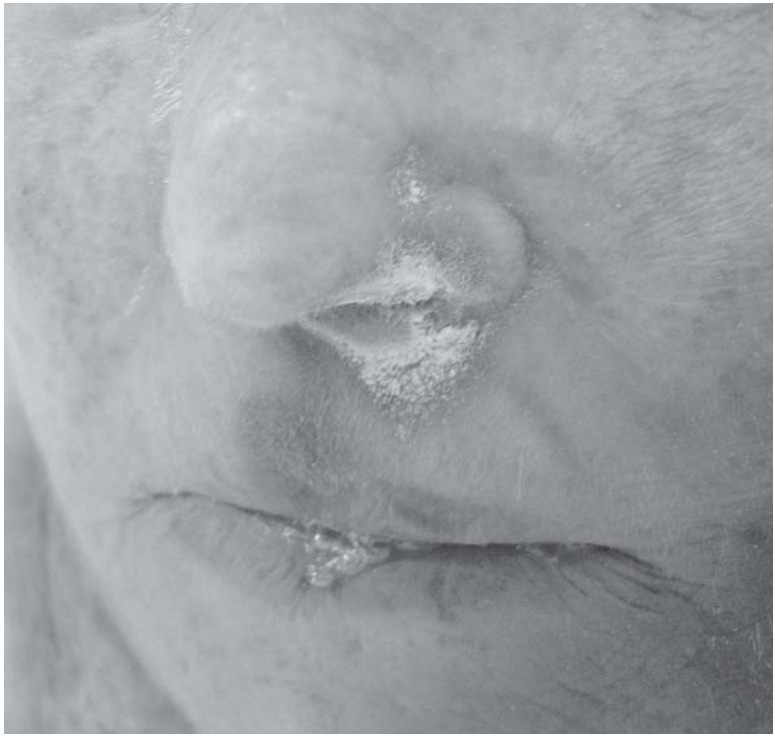

Fig. 45. Fungi colonization around left nostril and mouth (postmortem interval 2 days; moist environmental conditions).

tubules of the crowns and roots of the teeth (but not in the enamel which is more compact and therefore not colored) as well as in the fingernails and toenails *(35)*. Most observations of this phenomenon have been described in association with antemortem cranial blood congestion especially in asphyxial deaths, such as strangulation, death in a head-down position, and drowning. The presence of a pink discoloration of teeth and nails has been reported to be apparent even after postmortem intervals of several months.

11.5. Submalleolar and Thenar Eminence Hypostasis

Postmortem hypostasis in the muscles located in the lateral submalleolar region and the thenar eminence may mimick antemortem bruising *(29)*. An incision will show lack of hemorrhage within the muscle tissue.

11.6. Fungi Colonization

Fungi may colonize the body in every possible location and at each time during the postmortem interval. However, the eyes are more often affected by fungi colonization under dry conditions and the mouth and nose are more often colonized with fungi in moist to wet environments (Fig. 45). This author has more than once investigated cases where inexperienced physicians mistook postmortem fungi colonization around mouth and nostrils for dried up edema fluid as often seen in cases of failure of the left heart.

12. Conclusion

It generally is impossible to draw any definite conclusion concerning the time of death by the appearance of a single postmortem change, or conversely, to predict what postmortem changes are to be expected in a given case after a particular postmortem interval has elapsed. Nevertheless, in the very early postmortem interval (approx within 24 hours after death), in some distinct cases, particularly the presence and picture of several postmortem changes may, when analyzed combined with the rectal temperature of the deceased, give the death investigator valuable hints concerning the time frame in which the subject most probably has died. The presented tables summarizing the chronologic order of appearance and sequence of events of distinctive postmortem changes such as livor mortis and rigor mortis as well as the comments on algor mortis ("postmortem cooling") will prove useful in forensic casework to give an approximate estimation of the time since death.

A detailed knowledge of the changes as well as the underlying biological processes that a human body undergoes after death is essential for every forensic pathologist and medical examiner, respectively. Especially a good survey of potential differential diagnoses of the multitude of possible skin alterations and lesions occurring postmortem is crucial for every death investigator.

References

1. Knight B (2002) The Estimation of the Time Since Death in the Early Postmortem Period, 2nd ed. London, Arnold.
2. Henssge C, Althaus L, Bolt J, et al. (2000) Experiences with a compound method for estimating the time since death. I. Rectal temperature nomogram for time since death. Int J Legal Med 113, 303–319.
3. Henssge C, Althaus L, Bolt J, et al. (2000) Experiences with a compound method for estimating the time since death. II. Integration of non-temperature-based methods. Int J Legal Med 113, 320–331.

4. Sperhake JP, Tsokos M (2004) Pathological features of Waterhouse-Friderichsen syndrome in infancy and childhood. In Tsokos M, ed., Forensic Pathology Reviews, Vol. 1. Humana Press Inc., Totowa, NJ, pp. 219–231.
5. Kobayashi M, Takatori T, Nakajima M, Sakurada K, Hatanaka K, Ikegaya H, et al. (2000) Onset of rigor mortis is earlier in red muscle than in white muscle. Int J Legal Med 113, 240–243.
6. McCann J, Reay D, Siebert J, Stephens BG, Wirtz S (1996) Postmortem perianal findings in children. Am J Forensic Med Pathol 17, 289–298.
7. Rutty GN (2004) The pathology of shock versus post-mortem change. In Rutty GN, ed., Essentials of Autopsy Practice, Vol. 2. Springer, London, Berlin, Heidelberg, pp. 93–127.
8. Byard RW (2004) Medicolegal problems associated with neonaticide. In Tsokos M, ed., Forensic Pathology Reviews, Vol. 1. Humana Press Inc., Totowa, NJ, pp. 171–185.
9. Tsokos M, Sperhake JP (2002) Coma blisters in a case of fatal theophylline intoxication. Am J Forensic Med Pathol 23, 292–294.
10. Zugibe FT, Costello JT (1993) The Iceman murder: one of a series of contract murders. J Forensic Sci 38, 1404–1408.
11. Schäfer AT, Kaufmann JD (1999) What happens in freezing bodies? Experimental study of histological tissue change caused by freezing injuries. Forensic Sci Int 102, 149–158.
12. Saukko P, Knight B (2004) Knight's Forensic Pathology, 3rd ed. Arnold, London.
13. Mason JK (1993) Forensic Medicine. Chapman and Hall Medical, London.
14. Weiler G (1978) Leichenzerstörung durch Hunde- und Löwenfraß. Arch Kriminol 162, 108–114.
15. Strauch N (1927) Über Anfressen von Leichen durch Hauskatzen. Dtsch Z Ges Gerichtl Med 10, 457–469.
16. Pollak S, Reiter C (1988) Vortäuschung von Schußverletzungen durch postmortalen Madenfraß. Arch Kriminol 181, 146–154.
17. Rossi ML, Shahrom AW, Chapman RC, Vanezis P (1994) Postmortem injuries by indoor pets. Am J Forensic Med Pathol 15, 105–109.
18. Ropohl D, Scheithauer R, Pollak S (1995) Postmortem injuries inflicted by domestic golden hamster: morphological aspects and evidence by DNA typing. Forensic Sci Int 72, 81–90.
19. Byard RW, James RA, Gilbert JD (2002) Diagnostic problems associated with cadaveric trauma from animal activity. Am J Forensic Med Pathol 23, 238–244.
20. Rothschild MA, Schneider V (1997) On the temporal onset of postmortem animal scavenging. "Motivation" of the animal. Forensic Sci Int 89, 57–64.
21. Benecke M (2004) Arthropods and corpses. In Tsokos M, ed., Forensic Pathology Reviews, Vol. 2. Humana Press Inc., Totowa, NJ, pp. 207–240.
22. Patel F (1994) Artefact in forensic medicine: postmortem rodent activity. J Forensic Sci 39, 257–260.
23. Tsokos M, Matschke J, Gehl A, Koops E, Püschel K (1999) Skin and soft tissue artifacts due to postmortem damage caused by rodents. Forensic Sci Int 104, 47–57.
24. Tsokos M, Schulz F (1999) Indoor postmortem animal interference by carnivores and rodents: report of two cases and review of the literature. Int J Legal Med 112, 115–119.

25. Höss M, Kohn M, Pääbo S, Knauer F, Schröder W (1992) Excrement analysis by PCR. Nature 359, 199.
26. Hopwood AJ, Mannucci A, Sullivan KM (1996) DNA typing from human faeces. Int J Legal Med 108, 237–243.
27. Lunetta P, Modell JH (2005) Macroscopical, microscopical, and laboratory findings in drowning victims: A comprehensive review. In Tsokos M, ed., Forensic Pathology Reviews, Vol. 3. Humana Press Inc., Totowa, NJ, pp. 3–77.
28. Ziemke H (1913) Zur Entstehung von Verletzungen an Leichen durch Tierbisse. Vierteljahrsschr Gerichtl Med Öffentl Sanitätswesen 45, 53–58.
29. Rutty GN (2001) Postmortem changes and artefacts. In Rutty GN, ed., Essentials of Autopsy Practice, Vol. 1. Springer, London, Berlin, Heidelberg, pp. 63–95.
30. Klotzbach H, von den Driesch P, Schulz F (2003) Perimortale Hautläsionen durch Regurgitation von Magensaft. Arch Kriminol 212, 30–40.
31. Evans MJ (2001) Mimics of non-accidental injury in children. In Rutty GN, ed., Essentials of Autopsy Practice, Vol. 1. Springer, London, Berlin, Heidelberg, pp. 121–142.
32. Darok M (2004) Injuries resulting from resuscitation procedures. In Tsokos M, ed., Forensic Pathology Reviews, Vol. 1. Humana Press Inc., Totowa, NJ, pp. 293–303.
33. Bohnert M, Pollak S (2003) Heat-mediated changes to the hand mimicking washerwoman's skin. Int J Legal Med 117, 102–105.
34. Bohnert M (2004) Morphological findings in burned bodies. In Tsokos M, ed., Forensic Pathology Reviews, Vol. 1. Humana Press Inc., Totowa, NJ, pp. 3–27.
35. Ortmann C, DuChesne A (1998) A partially mummified corpse with pink teeth and pink nails. Int J Legal Med 111, 35–37.

6

Macroscopical Findings on Soil-Embedded Skeletal Remains Allowing the Exclusion of a Forensically Relevant Lay Time

Marcel A. Verhoff, MD and Kerstin Kreutz, PhD

CONTENTS

SUMMARY

So far, a reliable determination of the postmortem interval (PMI) of human bones or skeletal remains is possible neither by morphological examinations nor by extensive technical investigations. Concerning forensic osteological practice, in most cases, the question of the length of the PMI can be restricted to the exclusion of a forensically relevant lay time (in the present context corresponding to a PMI of >50 years). We reviewed 21 original publications from the literature and surveyed the results on the basis of our own experience with macroscopical findings of soil-embedded bones with known lay times. A

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total of 1259 bones and the respective findings were evaluated. Eleven macromorphological findings are presented that are of relevance when present and, in addition, five findings that are relevant through their absence for the determination of a PMI of more than 50 years. In none of the reviewed publications were these criteria described in soil-embedded bones with a lay time of less than 50 years. These elaborated findings are a basis for the macromorphological exclusion of a forensically relevant lay time of soil-embedded skeletal remains in a given case.

Key Words: Forensic osteology; decomposition; postmortem interval (PMI); forensically relevant lay time; taphonomy.

1. Introduction

The find of skeletal remains will always lead to the question of whether they are of forensic relevance. First of all, the forensic pathologist or anthropologist has to determine whether the bones are of human origin *(1,2)*. If a nonhuman provenance can be affirmed, forensic interests are limited. If the bones turn out to be of human origin, on principle three major topics need to be discussed: *(a)* The length of the postmortem interval (PMI) elapsed *(3)*, *(b)* the presence of signs of perimortem trauma *(4)*, and *(c)* the identification of the individual the remains belong to *(5,6)*. In the United States, inquiries directed to forensic anthropologists by official authorities concerning the length of the PMI obviously are increasing *(7)*. Experts' opinions on skeletal remains suggesting that one is dealing with historical (ancient) material usually allow one to stop preliminary proceedings for prosecution.

2. Lay Time Analysis of Skeletal Remains

Giving an appropriate estimation of the length of the PMI is one of the most difficult problems dealt with in forensic osteological practice *(8–11)*. Attention is always called to the necessity of the investigator's experience *(12,13)*. In most cases, the circumstances of the finding of the skeletal remains, their location, as well as other accompanying finds (e.g., burial goods) may give evidence with respect to the lay time or indicate a historical find *(14)*. Old maps should be checked for the former existence of graveyards in the respective area. The orientation or alignment of the excavated skeleton in the ground, the imprint of a faded coffin, remains of metal coffin trimmings and fastenings, as well as personal belongings representing burial goods may point to an official burial.

In finds of bones from modern times, clothing, newspapers, or plastic bags will allow one to narrow the time frame of the time elapsed since death or the disposal of the body, respectively. Marks left by vegetation, such as the

growth of roots from nearby plants and the marks they produce *(15)*, as well as entomological investigations *(16)* or pollen analyses *(17)* are additional relevant factors for a determination of the PMI.

The fields of forensic pathology and anthropology have for a long time striven to develop adequate methods to obtain objective and reliable markers for the determination of the PMI of skeletal remains. Basically, there are morphological studies at hand, both macro- and microscopical, the latter using different staining methods *(18)*. Scanning electron microscopical investigations also have been performed *(19)*. In addition, there exist physical, chemical, and extensive technical investigative techniques *(20)*, such as ultrasonic examinations *(21)*, analysis of amino acid spectrums *(12,22)*, determination of atomic absorption spectrums of anorganic substances *(9)*, complex analysis of ions, lipids, and proteins *(23)*, and the analysis of the quantity of nitrogen *(24)*.

2.1. Decomposition and Other Thaphonomic Influences

The above-mentioned methods detect changes of bony tissue caused by decomposition. In addition to the pure effects of lay time on the morphological appearance of skeletal remains, there is a broad variety of factors involved, each influencing the other, which are difficult to estimate in their specific effect *(25)*. Lay conditions are determined mainly by soil composition *(8,26–28)*. Detailed listings of the effect of soil composition on decomposition can be found in Hunger's textbook *(26)*. A rough overview of different soils and their effect on bones was presented by Kunter (*[27]* Table 1).

Apart from the specific type of soil, there are many other factors influencing lay settings in different dimensions. In the early stages of the PMI, coffin material and seasons have to be considered. In later stages, factors like burial depth, seasonal humidity, and soil warming also are relevant *(26,27,29)*. However, skeletons with identical PMIs from the same graveyard that were obviously buried in soil of the same composition can show different qualitative and quantitative signs of decomposition *(14,27,30)*.

2.2. Radionuclide Methods

Because of the high variability of the factors that influence lay conditions and the difficulty in assessing these influences on decomposition of skeletal remains, the goal is to establish absolute indicators or to develop investigative methods that depend solely on the PMI. A number of such methods are based on radionuclide measurements. However, these methods are expensive, time-consuming, and squander material. The most popular method is the radiocarbon (C-14) analysis *(31)*. With a half-life of 5730 years for C-

Table 1
Soil Types and Their Effect on the State of Preservation

Soil type		State of preservation
Loess		Good preservation; mostly complete bones
Clayey soil		Bones mostly softened; excavation difficult
Rocky soil		Bones dispersed or destroyed by stones
Lime resp. chalk containing soil		Bones are often very fragile; smashed and friable; soft
Lime and minerals containing soil		Good preservation of the bones; fossilization if enough iron and manganese are present
Turf (moor)		"Moor corpses": Quick decalcification of the bones, but preservation of organic structures; preservation of form and height
Sour soil		Worst state of preservation of bones; in most cases no skeletal remains, only imprints
Sand	Chalky	Good preservation; heavy fossilization
	Dry	Good preservation; mummified tissue remains

Modified according to ref. *27*.

14, this method is, however, insufficient for a PMI of less than 100 years *(32)*. For skeletal remains with shorter PMIs, recent research with strontium-90 *(33)* and plutonium *(34)* provided promising results.

2.3. Forensically Relevant Lay Time

Apparently, there are no technical methods available so far that allow a precise determination of the PMI of skeletal remains for forensic purposes. For practical casework, it would be most useful that the investigator is able to exclude cases of nonforensic relevance immediately. First, it has to be determined on a national and international level, respectively, which PMI has to be considered as forensically relevant. There are different statute-barred prosecution times to be considered. A period of less than 50 years appears to be, in most jurisdictions, the most forensically relevant period of time *(1,12,35,36)*.

Diagnostic criteria that are appropriate to exclude a PMI of less than 50 years are therefore highly desirable. Only changes of a given bone that could under no circumstances appear in lay times more than 50 years would have to be considered.

2.4. Selection of Appropriate Methods

For practical purposes, methods giving the investigator valuable criteria for a possible exclusion of a forensically relevant lay time at hand should require low technical efforts. To evolve exclusion criteria for forensically relevant lay times, tests with various materials under different specified conditions would be necessary. However, a number of reports concerning findings on recent and historical (ancient) bone material from systematic investigations or case studies already exist.

2.4.1. Literature Review

A detailed analysis based on the observations and data given in the literature was conducted to scrutinize whether there exist any macromorphological findings in soil-embedded bones that have not been described for a PMI of less than 50 years. In this, the most extensive literature review performed by our study group to date *(37)*, all relevant journals and textbooks as well as the Medline and other online resources were searched. In our analysis, only original publications were included where *(a)* the results were based on the respective authors' own macroscopical investigations of soil-embedded bones and skeletons, *(b)* the PMI was known, and *(c)* the results were well documented. All results were collected, sorted by PMI, and listed by diagnosis and description, independent of soil composition.

2.4.2. Results

Twenty-one original publications fulfilling the above-mentioned criteria were identified; with 1259 individuals examined in total. The highest PMI was approx 6000 years, the shortest only a few years.

Sixteen criteria were described for soil-embedded bones with a PMI more than 50 years, 11 of which were relevant through their presence and 5 through their absence. None of the publications described these criteria for a soil-embedded lay time of less than 50 years. Figures 1 through 5 display some of the relevant findings and the results are listed in Tables 2 and 3.

3. Discussion

For the daily routine of estimating the PMI of soil-embedded skeletal remains (e.g., bones found during construction work), extensive technical methods, including radionuclide measurements, are too expensive and time-consuming.

For a precise determination of the lay time of bones and skeletal findings, there is no morphological or technical method available that can be considered adequate to determine a PMI of 100 years or less. With the exception of the radionuclide methods, all analyses are highly dependent on soil compo-

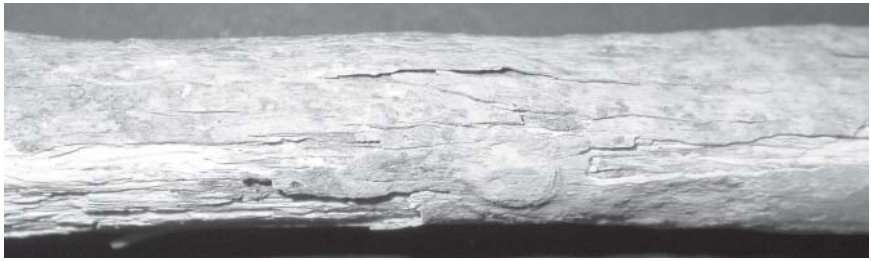

Fig. 1. Diaphysis of a right human humerus. The surface of the bone shows cuttings and exfoliation (Middle Ages).

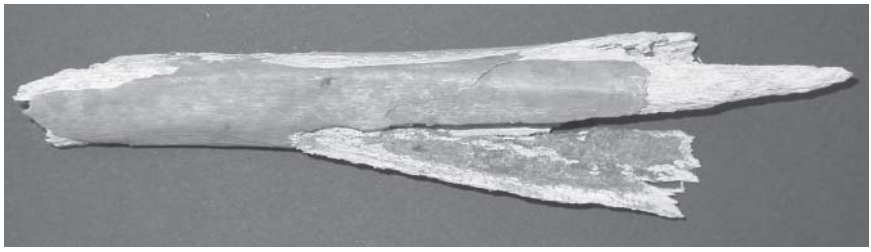

Fig. 2. Fragment of a left human tibia. Frayed cortical area up to the bone marrow region with lifting, heavy decomposition, breaking, and deep chunking (Middle Ages).

Fig. 3. Diaphysis of a right human humerus. Enlarged exfoliation of the outer cortical layer and partly darkish stains due to bacterial interference (Middle Ages).

sition and lay conditions. Even when in a given case soil samples, geological, and metereological data, and the like, are available or can be clearly reconstructed, there still is the possibility that the given skeletal finding could perhaps not have laid at the location of excavation throughout the whole PMI.

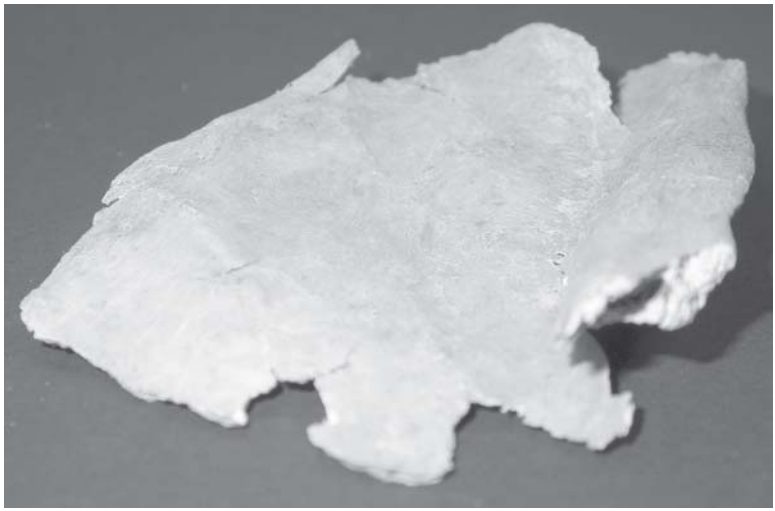

Fig. 4. Fragment of a right human scapula, dorsolateral aspect. Note partly deep cuttings and torsion of the spina scapulae towards the corpus scapulae (Middle Ages).

Fig. 5. Phalanx of a human finger. Palmar aspect showing enlarged powdery-layered brushite development (16th century, sarkophagus).

If a forensically relevant lay time can be denied irrespective of the precise determination of the PMI, the question of the precise lay time is irrelevant to the official authorities. Such an exclusion would be given when a historical finding is at hand or the PMI is definitely more than 50 years. Therefore, the elaborated findings derived from the literature concerning an exclusion of a forensically relevant lay time seem to be valid and reliable. The question

Table 2
Macromorphological Bone Findings: Earliest Described Phenomena of Soil-Embedded Bones According to the Literature (Starting With a PMI of 50 Years)

Findings	Earliest PMI (Reference)
No soft tissue coloring left	56 years *(30)*
Macroscopically no traces of adipocere on outer surface, compact bone or bone marrow	>50 years *(26)*
Noticeable incisions of humerus head and femur condyles	52 years *(30)*
Compact bone opening in the direction of the incisions, partly polygonally arranged	155 years *(38)*
Longitudinal and transversal incisions of compact bone	200 years *(39)*
Fraying of outer lamellar system	155 years *(38)*
Lifting of cortical bone	200 years *(39)*
Enlarged defects of outer surface	>50 years *(26)*
Torsion of tissue	200 years *(39)*
Dark brown, earthy color	155 years *(38)*
Intensive blackish-brownish discoloration due to micro-organisms on outer surface	155 years *(38)*
Brushite: White efflorescence on the compacta	155 years *(38)*
Bone manually breakable	1200 years *(30)*
Reduced UV-fluorescence of freshly incised surface of limb bones (“sandwich,” “centralization”)	100 years *(35)*
Reduced UV-fluorescence of freshly incised surface of smaller bones and skull (“sandwich,” “centralization”)	50 years *(35)*
No UV-fluorescence on freshly incised surface	200 years *(35)*

PMI, postmortem interval; UV, ultraviolet. (Modified according to ref. *37*.)

remains whether an expert opinion that is based purely on morphological criteria is acceptable and justifiable or if additional investigations will be necessary and requested by the investigating public authorities.

Apart from the radionuclide methods, which are not precise enough at all to exclude a forensically relevant lay time, all physical, chemical, and other technically extensive methods, as well as histological methods, have no basic advantage when compared with macroscopical methods because all parameters that can be measured also are dependent on the lay conditions as well as on the PMI. Consequently, it must be critically asked whether any technical effort undertaken to exclude a forensically relevant lay time after a thorough

Table 3
Macromorphological Findings Allowing the Exclusion of a Forensically Relevant Lay Time of Soil-Embedded Bones (PMI Up to 50 Years)

Outer Surface Appearance
• Macroscopically no adipocere
• Deep incisions of outer compact layer
• Extensive defects on outer surface
• Intense brownish-blackish discoloration due to interference of microorganisms
• Fraying of outer lamellar system
• Lifting of cortical area
• Torsion of tissue
• Attached brushite
• Bone manually breakable
Appearance on freshly cut cross-sections
• Lack of adipocere
• Brushite in bone marrow region
• Reduced UV-fluorescence or total lack of UV-fluorescence

PMI, postmortem interval; UV, ultraviolet.

macroscopical analysis will have any benefit in providing additional information.

The results of our literature evaluation (Table 2) must be verified by further investigations including, for example, analyses of graveyard excavations and case studies of skeletons with a known PMI and an exclusively soil-embedded lay time. It must be examined if one of the listed findings shown in Table 2 is also found in cases with soil-embedded lay times of less than 50 years and under which particular conditions this may occur.

4. Practical Advice

An important precondition for the practical application of the elaborated findings given in Table 2 is that there are no implications for an open-air lay condition, such as signs of outdoor weathering. The reported findings are restricted to soil-embedded lay conditions. Influences of open-air lay conditions cannot be estimated yet.

If one or more of the listed criteria (Table 2) can be applied to a bone or skeleton examined, under the assumption that there are no signs of an open-air lay condition, then the forensic anthropological expert judgment would exclude

a forensically relevant soil-embedded lay time of less than 50 years. The more of these criteria can be documented for a skeletal finding, the more precise this statement will be. However, it must be considered that some findings cannot appear combined because they follow each other in different stages of decomposition or they are subject to different lay conditions, respectively. The following examples illustrate this: former deep cuttings on the outer surface of a bone with already frayed outer lamellar systems will not be identified as they are no longer apparent for the investigator. Under humid lay conditions, the formation of adipocere is promoted and its presence is therefore prolonged *(26,40)*; when adipocere formation has already disappeared, there will be no brushite development because of the lack of a dry environment *(38)*.

Some of the reported findings given in Table 2 may be difficult to differentiate between each other. Furthermore, it has been reported that soft tissue stains were not detectable on a skull after a PMI of only approx 40 years in a humid clayey soil from a graveyard in Middle Hesse (Germany). According to observations derived from excavations at a graveyard in Puglia (Italy), nearly all examined bones had a deep brown color after a PMI of 30 years; this color correlated with the soil color itself *(37)*.

Based on the results of our review of the literature, we propose to keep to the macromorphological criteria given in Table 3 that will, if present, allow the exclusion of a forensically relevant lay time (in the present context corresponding to a PMI of >50 years) of soil-embedded bones *(37)*.

References

1. Byers SN (2002) Introduction to Forensic Anthropology—A Textbook. Allyn & Bacon, Boston.
2. Verhoff MA, Heidorn F, Kreutz K (2002) Die interindividuelle morphologische Variabilität als Ursache von Fehldeutungen in der forensischen Osteologie am Beispiel einer Rippe. Arch Kriminol 210, 112–120.
3. Kreutz K, Verhoff MA (2002) Forensische Anthropologie. Lehmanns Media - LOB.de, Berlin
4. Verhoff MA, Kreutz K (2003) Verletzungsspuren an Knochenfunden–Analyse und Beurteilung. Arch Kriminol 212, 41–52.
5. Rathburn TA, Buikstra JE (1984) Human Identification—Case Studies in Forensic Anthropology. Charles C. Thomas, Springfield.
6. Leopold D (1998) Identifikation unbekannter Toter. Schmidt-Römhild, Lübeck.
7. Nafte N (2002) Flesh and Bone. Carolina Academic Press, Durham.
8. Hunger H (1978) Methoden der Liegezeitbestimmung menschlicher Knochen. In Hunger H, Leopold D, eds., Identifikation. Johann Ambrosius Barth, Leipzig, pp. 63–99.

9. Földes V, Kósa F, Virágos-Kis E, Rengei B, Ferke A (1980) Atomabsorptionsspektrometrische Untersuchung des Gehaltes an anorganischen Substanzen von Skelettfunden zur Ermittlung der Dauer des Begrabenseins in der Erde. Arch Kriminol 166, 105–111.
10. Bass WM (1984) Time interval since death—a difficult decision. In Rathburn TA, Buikstra JE, eds., Human Identification—Case Studies in Forensic Anthropology. Charles C. Thomas, Springfield, pp. 136–147.
11. Penning R, Riepert T (2003) Identifikation und forensische Osteologie. In Madea B, Brinkmann B, eds., Handbuch gerichtliche Medizin, Vol. 2. Springer, Berlin, Heidelberg, pp. 1117–1270.
12. Bonte W, Johansson J, Garbe G, Berg S (1976) Die Bestimmung des Aminosäurenspektrums als Hilfsmittel bei der Datierung von Skelettfunden. Arch Kriminol 158, 163–174.
13. Krause D, Jachau K (2003) Identifizierung/Osteologie. In Madea B, ed., Praxis Rechtsmedizin. Springer, Berlin, Heidelberg, New York, pp. 72–76.
14. Berg S, Rolle R, Seemann H (1981) Der Archäologe und der Tod—Archäologie und Gerichtsmedizin. C.J. Bucher, Munich, Luzern, pp. 94–97.
15. Haglund WD (2003) Forensic Taphonomy. In James SH, Nordby JJ, eds., Forensic Science. CRC Press, Boca Raton, pp. 99–112.
16. Benecke M (2001) A brief history of forensic entomology. Forensic Sci Int 120, 2–14.
17. Szibor R, Schubert C, Schöning R, Krause D, Wendt U (1998) Pollen analysis reveals murder season. Nature 395, 449–450.
18. Berg S, Specht W (1958) Untersuchungen zur Bestimmung der Liegezeit von Skeletteilen. Dtsch Z Gerichtl Med 47, 209–241.
19. Bell LS, Skinner MF, Jones SL (1996) The speed of post mortem change to the human skeleton and its taphonomic significance. Forensic Sci Int 82, 129–140.
20. Berg S, Protsch von Zieten R (1998) Die Datierung von Skelettfunden. In Leopold, D, ed., Identifikation unbekannter Toter. Schmidt-Römhild, Lübeck, pp. 107–128.
21. Berg S, Specht W (1958) Eine neue Technik als naturwissenschaftlicher Beitrag zur Altersbestimmung von Knochenfunden. Arch Kriminol 122, 43–65.
22. Armstrong WG, Tarlo LBH (1966) Amino acid components in fossil calcified tissues. Nature 210, 481–482.
23. Castellano M, Villanueva EC, von Frenckel R (1984) Estimating the date of bone remains. A multivariate study. J Forensic Sci 29, 527–534.
24. Jarvis DR (1997) Nitrogen levels in long bones from coffin burials interred for periods of 26–90 years. Forensic Sci Int 85, 199–208.
25. Haglund WD, Sorg M (1997) Method and theory of forensic taphonomy research. In Haglund WD, Sorg M, eds., Forensic Taphonomy. CRC Press, Boca Raton, pp. 13–26.
26. Hunger H (1967) Untersuchungen zum Problem der Liegezeitbestimmung an menschlichen Skeletten. Med Thesis, Karl-Marx-University Leipzig, Germany.
27. Kunter M (1988) Rekonstruktion, Konservierung und Reproduktion. In Knussmann R, ed., Anthropologie, Vol. I/1. Gustav Fischer, Stuttgart, pp. 551–615.

28. Herrmann B, Gruppe G, Hummel S, Piepenbrink H, Schutkowski H (1990) Prähistorische Anthropologie. Springer, Berlin, Heidelberg.
29. Sledzik P (1998) Forensic taphonomy: postmortem decomposition and decay. In Reichs KJ, ed., Forensic Osteology—Advances in the Identification of Human Remains, 2nd ed. Charles C. Thomas, Springfield, pp. 109–119.
30. Berg S (1962) Zur Todeszeitbestimmung bei Skelettfunden. Beitr Gerichtl Med 22, 18–30.
31. Münnich KO (1960) Die C_{14}-Methode. Geologische Rundschau 49, 237–244.
32. Taylor RE, Suchery JM, Payen CA, Slota PJ Jr. (1989) The use of radiocarbon (C-14) to identify skeletal materials of forensic science interest. J Forensic Sci 34, 1196–1205.
33. Neis P, Hille R, Paschke M, Pilwat G, Schnabel A, Niess C, Bratzke H (1999) Strontium-90 for determination of time since death. Forensic Sci Int 99, 47–51.
34. Swift B, Lauder I, Black S, Norris J (2001) An estimation of the post-mortem interval in human skeletal remains: a radionuclide and trace element approach. Forensic Sci Int 117, 73–87.
35. Knight B (1969) Methods of dating skeletal remains. Med Sci Law 9, 247–252.
36. Knight B, Lauder I (1969) Methods of dating skeletal remains. Hum Biol 41, 322–341.
37. Verhoff MA, Wiesbrock UO, Kreutz K (2004) Makroskopische Befunde zum Ausschluss einer forensisch relevanten Erdliegezeit bei Knochenfunden—eine Literaturauswertung. Arch Kriminol 213, 1–14.
38. Herrmann B, Newesely H (1982) Dekompositionsvorgänge des Knochens unter langer Liegezeit—1. Die mineralische Phase. Anthrop Anz 40, 19–31.
39. Piepenbrink H (1984) Beispiele biogener Dekompositionserscheinungen an Knochen unter längerer Liegezeit. Anthropol Anz 42, 241–251.
40. Pfeiffer S, Milne S, Stevenson RM (1998) The natural decomposition of adipocere. J Forensic Sci 43, 368–370.

Death From Natural Causes

7

Right and Left Ventricular Arrhythmogenic Dysplasia

Pathological Features and Medicolegal Significance

Wolfgang Huckenbeck, MD, and Adonios Papadomanolakis, MD, PhD

Contents

Summary

Arrhythmogenic right ventricular cardiomyopathy is a heart muscle disease of unknown cause that is characterized by a gradual loss of myocytes and replacement by adipose and fibroadipose tissue. Although this condition is believed to be a selective disorder involving the right ventricle, there have been reports of concomitant "minor" abnormalities of the left ventricle. Clas-

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sically, left ventricular involvement is considered much less profound than that of the right ventricle. Nevertheless, right, left, and biventricular involvement and the similarities of pathological lesions might suggest that varying localizations do not represent separate entities, but instead different expressions of the same disease. Arrhythmogenic ventricular dysplasia plays a role that should not be underestimated in cases of sudden death, particularly of younger adolescents. Thus, the question of a correct *ex ante* diagnosis may arise in specific cases.

Key Words: Sudden death; arrhythmogenic ventricular dysplasia; arrhythmogenic cardiomyopathy; genetic aspects; forensic pathology; medicolegal aspects.

1. Introduction

Arrythmogenic right ventricular dysplasia (ARVD) is by far the most frequent variant of ventricular arrhythmogenic disease. It is a heart muscle disease that is characterized by a progressive muscular dystrophy of the right ventricle and an associated transmural deposit of adipose or fibroadipose tissue. The pathological process originates from the subendocardium and progresses to the endocardium. Because of the different types of replacement tissue found, various synonyms have been coined, such as "fatty infiltration of the right ventricle," "parchment heart," or earlier, "Uhl's anomaly" *(1)*. This type of cardiomyopathy (whose cause is not clearly understood) and the consequent life-threatening arrhythmias can be histomorphologically distinguished from cardiac lipomatosis.

The term "arrhythmogenic dysplasia of the right ventricle" was used for the first time by Abe and co-workers in 1973 (*2*). This term was also used by Fontaine et al. and Marcus et al. *(3,4)*, and by the beginning of the 1980s had been adopted by most investigators *(4–14)*. Whereas the majority of reports were of clinical and pathological relevance, only a few publications on this topic can be found in the medicolegal literature. In 1988, Sigrist et al. *(15)* were the first to refer to this possible cause of natural death, and their work has been followed more recently by a set of casuistic reports *(16–18)*. For the sake of completeness, the German review article of Zack and Wegener *(19)* should also be mentioned here.

Typically, histopathological changes are found in the right ventricle. In recent years, however, several investigations have reported left ventricular fibrofatty alterations that occurred either exclusively or in addition to myocardial changes of the right ventricle *(8,18,20–23)*.

2. Clinical Findings

Clinically, arrhythmogenic ventricular dysplasia manifests itself in stress-dependent precordialgia, palpitations, or recurrent syncopes, which occur typically after physical exertion. In most cases, the patient's electrocardiogram (ECG) at rest does not show any abnormalities. The typical polytopic ventricular tachycardia showing a left bundle branch block is observed only under stress. Allmann et al. *(20)* reported that ventricular tachycardias could be reproduced in affected patients by programmed stimulation or a dose of isoprenaline. Whereas no abnormalities usually are displayed by radiological investigations, echocardiographic methods often give indications of akinetic or dyskinetic myocardial areas at an early stage. The most sensitive imaging method is ECG-triggered magnetic resonance tomography, which is believed to be capable of demonstrating both adipose tissue infiltration as well as muscular motility disorders.

The underlying cause of arrhythmogenic dysplasia is still unclear. In the literature, the following three possible causative processes are discussed: dysontogenetic factors (congenital aplasia), chronic inflammation (viral infection), or a genetically regulated degenerative process.

3. Genetic Aspects

Although the report of Uhl *(24)* referred to an 8-month-old child, the illness generally manifests during the third decade of life *(6,20,25)*. Published case reports show that gender distribution appears more or less even, with a slight tendency to the male gender. Regionally higher frequencies of the illness, for example, in the Veneto region of Italy *(1,26)* or on the Greek island of Naxos *(27)*, suggest at least a genetic disposition to the disease. Genetic investigations have thus far implicated the involvement of six gene loci, two of which are located on chromosome *14* and one each on chromosomes *1, 2, 3*, and *17 (28–32)*. The inheritance of the condition is described as autosomal dominant. The variant described on Naxos, however, is believed to follow recessive inheritance with a penetrance of approx 90% *(29)*. In this case, the affected gene locus is *17q21*.

4. Gross Pathology

The gross pathological hallmark of ARVD is a muscular degradation of the right ventricle of varying extent (Figs. 1 and 2). In extreme cases, complete replacement by either adipose or adipose and connective tissue can occur. Some reports described an extremely thin ventricle wall with substitution of

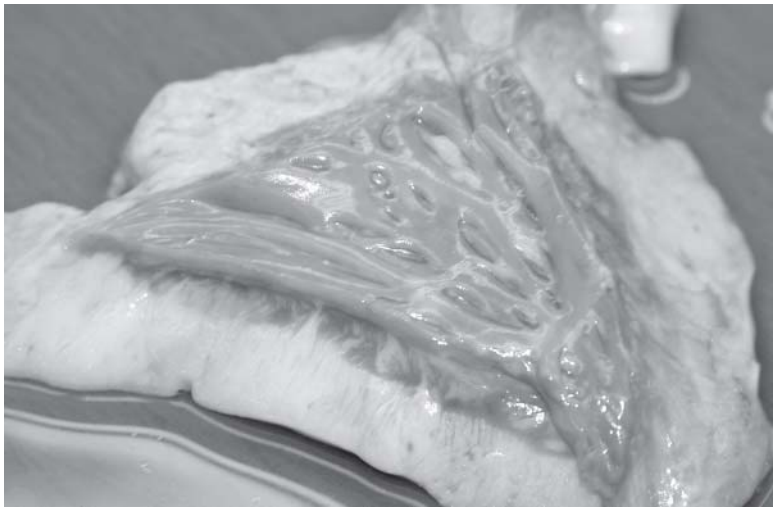

Fig. 1. Gross appearance of arrythmogenic right ventricular dysplasia at autopsy.

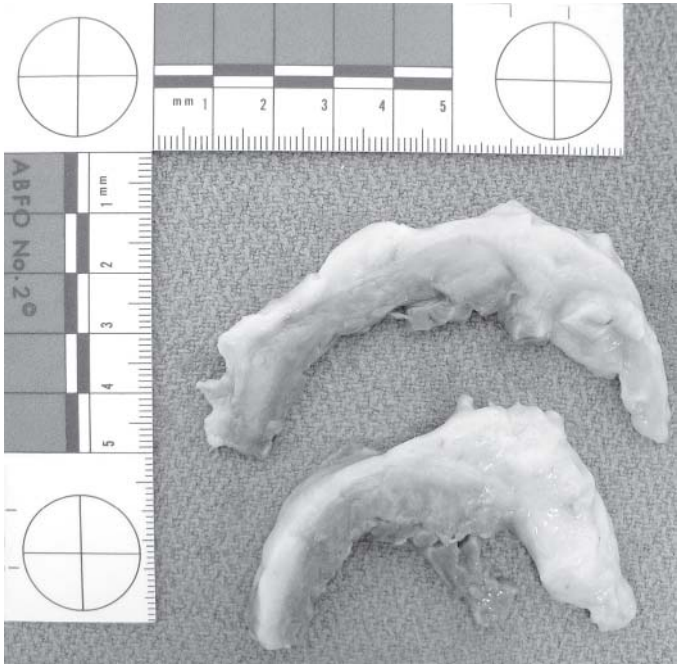

Fig. 2. Cut sections through the ventricle of the right heart showing adipose tissue replacing the myocardium. The pathological process progresses to the endocardium.

muscular by connective tissue ("parchment heart" *[33,34]*). More generally, morphological abnormalities are caused by a substitution of muscular tissue by either adipose tissue, or a mixture of adipose and connective tissue also termed "Uhl's anomaly" *(24)*.

5. *Histopathology*

Fibrofatty infiltration of the myocardium has been recognized as a distinct pathomorphological correlative and a possible cause of sudden heart death since the end of the 1970s. Nevertheless, uncertainties as to its etiology and localization and terminological inconsistencies remain. The most commonly described findings essentially consist of pathological–anatomical evidence of infiltration and scattered or extensive replacement of the right ventricular myocardium tissue by adipose tissue, frequently concomitant with fibrous connective tissue and, more rarely, with inflammatory leukocytic infiltrates.

ARVD is histomorphologically distinguishable from cardiac lipomatosis, which is characterized by adipose tissue deposits between muscle fiber bundles, which themselves exhibit no fat deposits. In contrast, in the case of ARVD, sharp delimitations between adipose and remaining myocardial tissue are observed. The remaining myocardium often is partially hypertrophied (Fig. 3) but may appear atrophic as well (Fig. 4A,B) and is embedded in the predominant adipose tissue either as single fibers or groups of fibers. Myocytes can appear vacuolated, thus exhibiting fatty degeneration (Fig. 5) or may show coagulation necroses (Fig. 6). Sometimes they are surrounded by inflammatory, mostly lymphocytic, infiltrates *(6,26)*, which, in contrast to myocarditis, are not lined up on the surface of the muscle cells (Fig. 7).

The number of reports of cases of left ventricular functional disorders concomitant with diagnosed right ventricular dysplasia is increasing *(2,17,25,27,34–44)*. Furthermore, in some published casuistries, histologically detectable adipose and fibrous infiltrations of the left ventricular myocardium have been described. However, in all cases a concomitant infiltration of the right ventricle was present *(45–47)*. The additional infiltration of the left ventricle was regarded as a supplementary diagnostic finding.

A similar situation was found in cases where fatty fibrous myocardial infiltrations of the left ventricle—limited to the subepicardial layers and not amounting to the complete substitution of the myocardium—also were discovered *(7,47)*. If the inner layer of the cardiac wall also was affected (Fig. 8), simultaneous infiltration of the right cardiac wall was always found *(10,41)*. Only Shrapnel and co-workers have *(18)* reported a case with a predominant left ventricular fatty degeneration. The casuistry of Allmann et al. *(20)* also

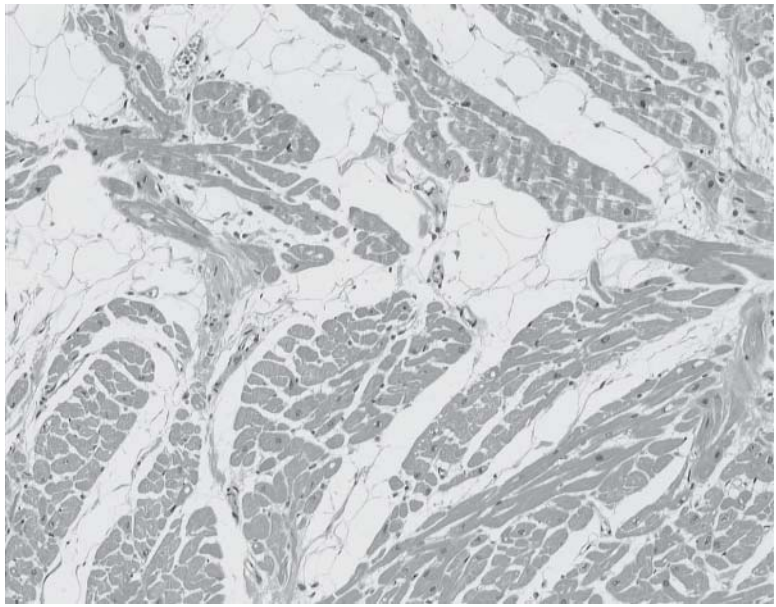

Fig. 3. Histological appearance of arrhythmogenic right ventricular dysplasia with adipose tissue infiltration and hypertrophied myocardial cells.

reported on a mainly left ventricular dysplasia concomitant with some detectable fatty fibrous centers in the inner layer of the right ventricle.

So far, only one report on isolated localizations of fatty fibrous infiltrations in the myocardium of the left ventricle has been published *(22)*. In an unpublished observation, the authors examined five cases of ventricular dysplasia that were discovered at medicolegal autopsies performed at Heraklion (Greece) and surroundings (Crete). Two cases deviated from the usual findings. In one case, a nearly equal-sized adipose myocardial infiltration of the right and left ventricle was found, whereas the second case revealed an extensive, purely left ventricular localization of adipose infiltration. The latter case displayed extensively developed fibrous connective tissue both in the endocardiac as well as in the subendocardiac layer—an image corresponding to that of right ventricular dysplasia described in the literature. An autopsy series from northern Italy showed that ARVD was implicated in 20% of cases of the sudden death of adolescents younger than 20 years of age *(1,26)*.

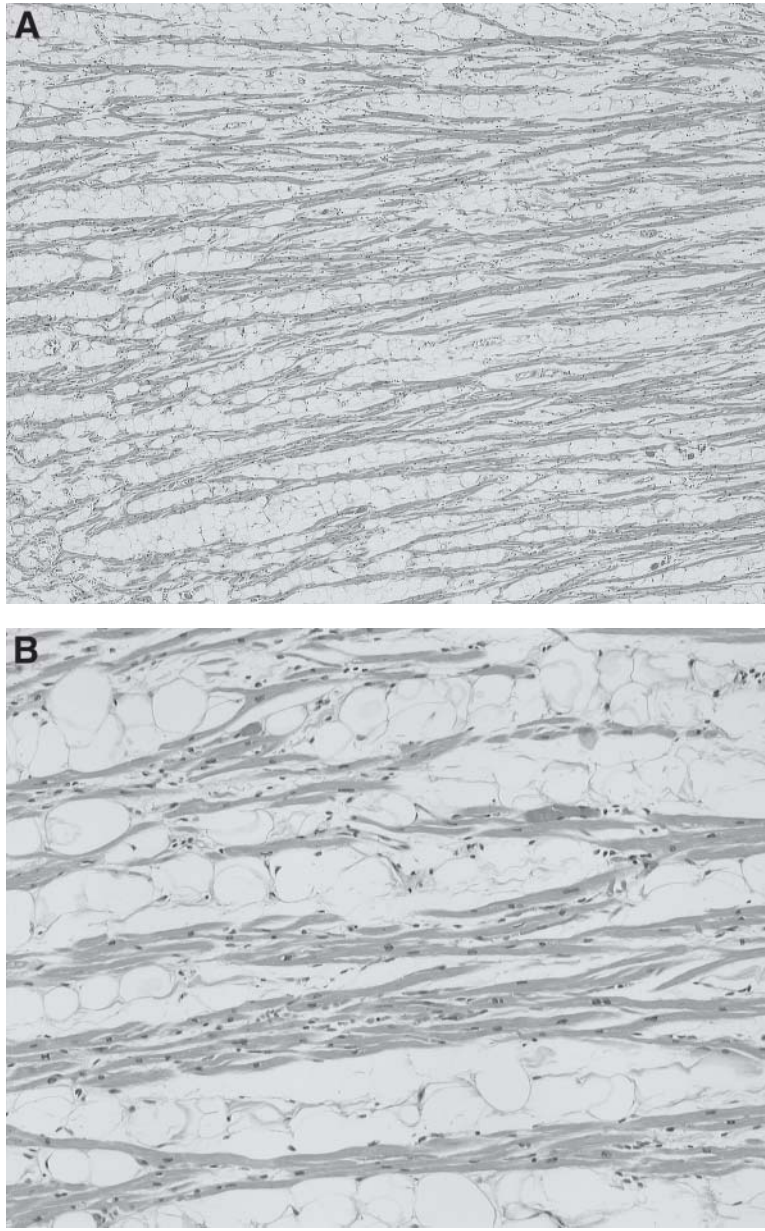

Fig. 4. Histological appearance of fatty replacement of cardiac muscle in arrythmogenic right ventricular dysplasia. (**A,B**) Adipose tissue infiltration is associated with atrophic myocardial cells.

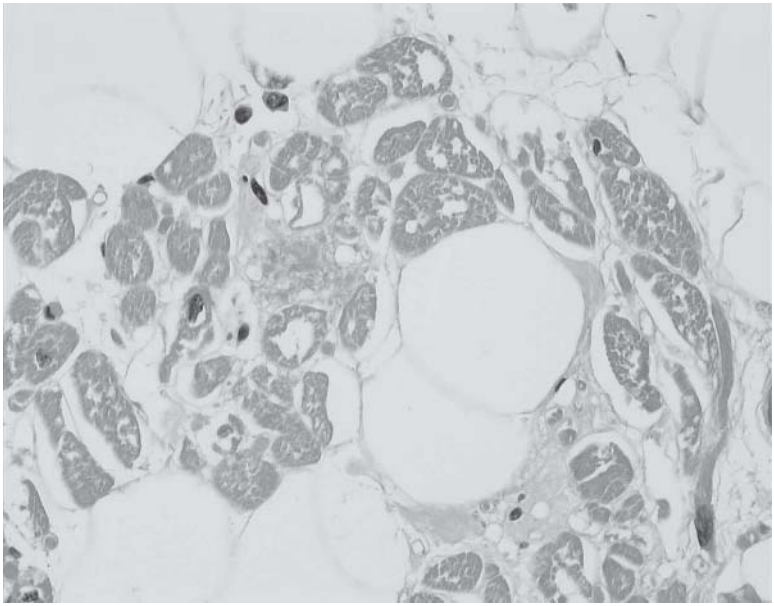

Fig. 5. Fatty replacement of cardiac muscle within the right ventricular wall. High-power view of myocardial cells showing fatty degeneration as displayed by large vacuoles.

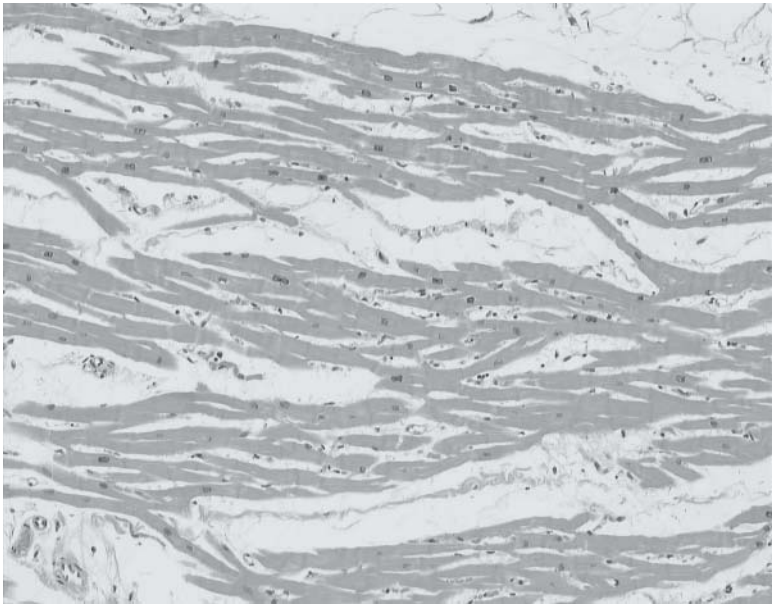

Fig. 6. Right ventricular dysplasia: coagulation necroses of myocytes not accompanied by any inflammatory reaction.

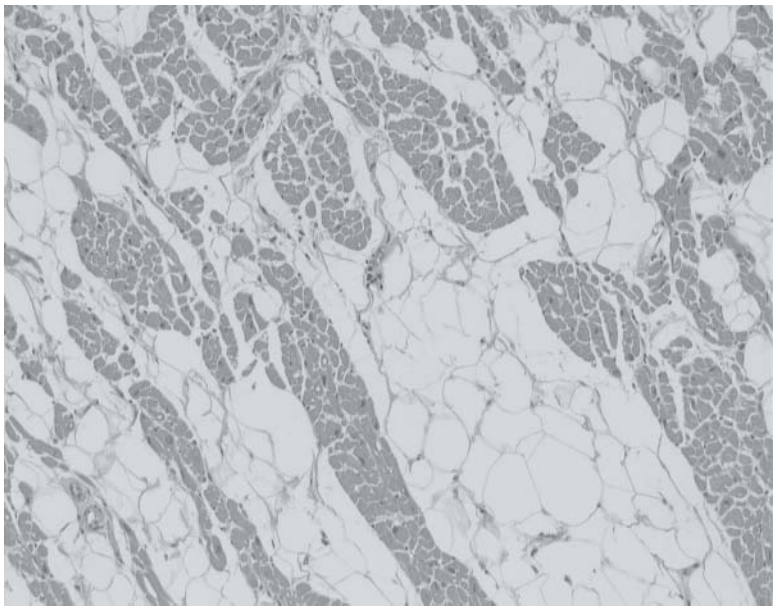

Fig. 7. Fatty replacement of cardiac muscle in arrythmogenic right ventricular dysplasia infiltrated by rare isolated lymphocytes.

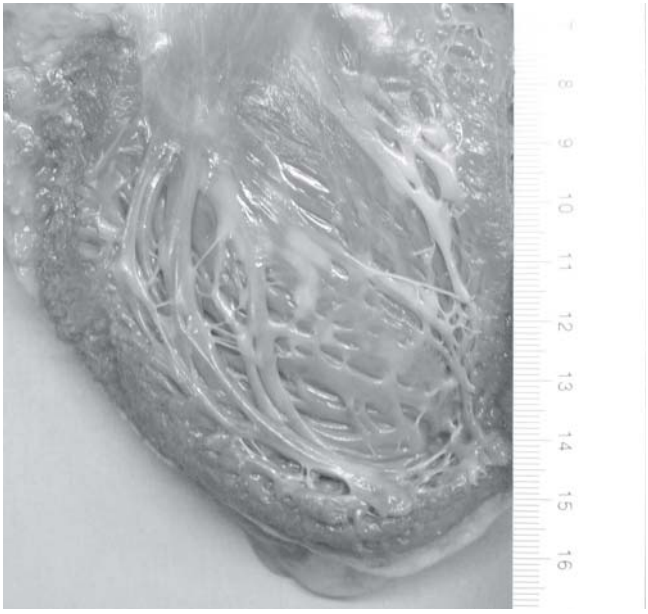

Fig. 8. Gross pathological appearance of left ventricular fatty degeneration with adipose tissue replacing the subendocardium.

6. Medicolegal Significance

As ever, the proof of lethal cardiac arrhythmia by morphological correlatives represents a medicolegal challenge in cases of sudden, unexpected deaths, in particular of young individuals. A possible cause that was most often overlooked previously would be a ventricular dysplasia that occurs mainly in the right chamber. This could explain the occurrence of ventricular arrhythmias on a pathogenetic basis.

Apart from recognizing the actual cause of death, further questions can arise in medicolegal practice. The compilation of several studies by Schmidt et al. *(34)* revealed that in 34 cases of sudden death of young adolescents, only 12 displayed initial manifestations of the disease. Thus, the question of a correct *ex ante* diagnosis for the remaining 22 cases exists. According to the clinical literature, a thorough clinical examination is recommended if palpitations, syncopes, and T-wave inversions in precordial ECG derivations or ventricular arrhythmias with left bundle branch blocks are detected for otherwise symptomless patients. The examination process should commence with two-dimensional echocardiography. For further clarification, the application of invasive methods such as angiography and ventriculography is advised. Today, biopsy of the endocardium is considered to be a diagnostic measure of last resort *(48)*.

7. Outlook

Arrhythmogenic ventricular dysplasia plays a role that should not be underestimated in cases of sudden death, particularly in younger people. Autopsy diagnosis of the actual cause of death should generally not present a problem: histology can be used to provide evidence of diagnosis. Because of the possibility of both the right and left ventricle being affected, we recommend that the term *arrhythmogenic right ventricular dysplasia* should not be used in the future. A further subdivision of terms is required, using additional terms such as right- or left-predominant, right- or left-limited, or bilateral ventricular dysplasia. Investigations into genetic disposition promise a decisive improvement in future clinical diagnoses.

References

1. Thiene G, Basso C (2000) Pathology of arrhythmic right ventricular cardiomyopathia. Herz 25, 210–215.
2. Abe T, Kuribayashi R, Sato M, Nieda S, Abe S (1973) Congenital hypoplasia of the right ventricular myocardium (Uhl's anomaly). J Cardiovasc Surg 14, 431–438.

3. Fontaine G, Guiraudon G, Frank R, et al. (1982) Arrhythmogenic right ventricular dysplasia and Uhl's disease. Arch Mal Coeur 75, 361–371.
4. Marcus F, Fontaine G, Guiraudon G, Frank R (1982) Right ventricular dysplasia: A report of 24 adult cases. Circulation 65, 384–398.
5. Aguilera B, Suarez Mier M, Morentin B (1999) Arrhythmogenic cardiomyopathy as cause of sudden death in Spain. Report of 21 cases. Rev Esp Cardiol 52, 656–662.
6. Bierhoff E, Jung W, Manz M (1992) Zur arrhythmogenen rechtsventrikulären Dysplasie. Pathologe 13, 141–145.
7. Fornes P, Ratel S, Lecomte D (1998) Pathology of arrhythmogenic right ventricular cardiomyopathy/dysplasia—an autopsy study of 20 forensic cases. J Forensic Sci 43, 777–783.
8. Gallo P, D'Amati G, Pellicia F (1992) Pathologic evidence of extensive left ventricular involvement in arrhythmogenic right ventricular cardiomyopathy. Hum Pathol 23, 948–952.
9. Gemayel C, Pelliccia A, Thompson PD (2001) Arrhythmogenic right ventricular cardiomyopathia. J Am Coll Cardiol 38, 1773–1781.
10. Kulbertus H (2001) Sudden death in athletics. Rev Med Liege 56, 318–325.
11. Li D, Ahmad F, Gardner M, et al. (2000) The locus of a novel gene responsible for arrhythmogenic right ventricular dysplasia characterized by early onset and high penetrance maps to chromosome 10p12-p14. Am J Hum Genet 66, 148–156.
12. Miani D, Pinamonti B, Bussani R, Silvestri F, Sinagra G, Camerini H (1993) Right ventricular dysplasia: A clinical and pathological study of two families with left ventricular involvement. Br Heart J 69, 151–157.
13. Michalodimitrakis E, Tsiftsis D, Tsatsakis A, Stiakakis I (2001) Sudden cardiac death and right ventricular dysplasia. Am J Forensic Med Pathol 22, 19–22.
14. Nava A, Folino A, Bauce B, et al. (2000) Signal-averages electrocardiogram in patients with arrhythmogenic right ventricular cardiomyopathy and ventricular arrhythmias. Eur Heart J 21, 58–65.
15. Sigrist T, Bär W, Zink P (1988) Myokardiale Dysplasie der rechten Herzkammer (Uhl'sche Anomalie) als mögliche Ursache eines plötzlichen, unerwarteten Todes. Z Rechtsmed 100, 177–189.
16. Mund MT, Hailemariam S, Cathomas G (2002) Die arrhythmogene rechtsventrikuläre Kardiomyopathie als Ursache des plötzlichen Herztodes. Rechtsmed 12, 371–374.
17. Reeve R, McDonald D (1964) Partial absence of the right ventricular musculature—partial parchment heart. Am J Cardiol 14, 415–419.
18. Shrapnel M, Gilbert JD, Byard RW (2001) Arrhythmogenic left ventricular dysplasia and sudden death. Med Sci Law 41, 159–162.
19. Zack F, Wegener R (1994) Zur Problematik der Diagnose „rhythmogener Herztod" durch histologische Untersuchungen des Erregungsbildungs- und -leitungssystems. Z Rechtsmed 5, 1–5.
20. Allmann J, Keiser G, Schneider J (1993) Linksventrikuläre arrhythmogene Dysplasie. Schweiz Med Wochenschr 123, 1615–1619.
21. Nava A, Thiene G (1990) Displasia o cardiomiopatia aritmogena. G Hal Cardiol 20, 562–563.

22. Tabib A, Loire R, Chalabreysse L, et al. (2003) Circumstances of death and gross and microscopic observations in a series of 200 cases of sudden death associated with arrhythmogenic right ventricular cardiomyopathy and/or dysplasia. Circulation 108, 3000–3005.
23. Pinamonti B, Sinagra G, Salvi A, et al. (1992) Left ventricular involvement in right ventricular dysplasia. Am Heart J 123, 711–724.
24. Uhl H (1952) A previously undescribed congenital malformation of the heart: almost total absence of myocardium of the right ventricle. Bull Johns Hopkins Hospital 91, 197–209.
25. Silver MM, Silver MD (1991) Arrhythmogenic right ventricular dysplasia. In Silver MM, ed., Cardiovascular Pathology, Vol 1, 2nd ed. Churchill Livingstone, New York, pp. 787–789.
26. Thiene G, Nava A, Corrado D, Rossi L, Pennelli N (1988) Right ventricular cardiomyopathy and sudden death in young people. N Engl J Med 318, 129–133.
27. McKoy G, Protonotarios N, Crosby A, et al. (2000) Identification of a deletion in plakoglobin in arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease). Lancet 355, 2119–2124.
28. Ahmad F, Duanxiang L, Karibe A, et al. (1998) Localization of a gene responsible for arrhythmic right ventricular dysplasia to chromosome 3p23. Circulation 98, 2791–2795.
29. Coonar A, Protonotarios N, Tsatsopoulou A, et al. (1998) Gene for arrhythmogenic right ventricular cardiomyopathy with diffuse nonepidermolytic keratoderma and wooly hair (Naxos disease). Circulation 97, 2049–2058.
30. Rampazzo A, Nava A, Danieli GA, et al. (1994) The gene for arrhythomogenic right ventricular cardiomyopathy maps to chromosome 14q23-q24. Hum Mol Genet 3, 959–962.
31. Rampazzo A, Nava A, Miorin M (1997) ARVD4, a new locus for arrythmogenic right ventricular cardiomyopathy, maps to chromosome 2 long arm. Genomics 45, 259–263.
32. Segall HN (1950) Parchment heart (Osler). Am Heart J 40, 948–950.
33. Osler W (1905) Dilatation of the heart. In Osler W, ed., The Principles and Practice in Medicine, 6th ed. Appleton, New York, p. 820.
34. Schmidt P, Arnold G, Glenewinkel F, Madea B (1996) Plötzlicher Tod bei arrhythmogener rechtsventrikulärer Dysplasie unter Berücksichtigung arztrechtlicher Gesichtspunkte. Rechtsmedizin 6, 92–96.
35. Bayer HP, Ostermeyer J (1974) Ein Fall von konnataler subtotaler Myokardreduktion im Bereich beider Herzvorhöfe. Virchows Arch 363, 63–72.
36. Castleman B, Towne V (1952) Case records of the Massachusetts General Hospital No 38201. N Engl J Med 246, 785–790.
37. Cumming GR, Bowman JM, Whytehead L (1965) Congenital aplasia of the myocardium of the right ventricle (Uhl's anomaly). Am Heart J 70, 671–676.
38. Diaz LP, Jimenez MQ, Granados FM, Martinez VP, Batres GM (1973) Congenital absence of myocardium of right ventricle: Uhl's anomaly. Br Heart J 35, 570–572.
39. French JW, Baum D, Popp RL (1975) Echocardiographic findings in Uhl's anomaly. Am J Cardiol 36, 349–353.

40. Gasul BM, Lendrum BL, Arcilla RA (1960) Congenital aplasia or marked hypoplasia of the myocardium of the right ventricle (Uhl's anomaly). Circulation 22, 752–753.
41. Gould L, Guttman AB, Carrasco J, Lyon AF (1967) Partial absence of the right ventricular musculature. Am J Med 42, 636–641.
42. Novak G, Szanto PB, Gasul B, Dillon E (1957) Congenital aplasia of the myocardium of the right ventricle. Proc Inst Med Chicago 21, 334–335.
43. Ostermeyer J (1974) Uhl's disease: partial parchment right ventricle. Virchows Arch 362, 185–194.
44. Virmani R, Robinowitz M, Clark MA, McAllister HA (1982) Sudden death and partial absence of the myocardium of the right ventricle. Arch Pathol Lab Med 106, 163–167.
45. Corrado D, Basso C, Thiene G, et al. (1997) Spectrum of clinicopathologic manifestations of arrhythmogenic right ventricular cardiomyopathy/dysplasia: a multicenter study. J Am Coll Cardiol 30, 1512–1530.
46. Nemec J, Edwards B, Osborn M, et al. (1999) Arrhythmogenic right ventricular dysplasia masquerading as dilated cardiomyopathy. Am J Cardiol 84, 237–239.
47. Nishikawa H, Kasai A, Ono N, et al. (1991) Two cases of bi-ventricular dysplasia associated with ventricular tachycardia and familial occurrence of sudden death. J Cardiol 21, 735–747.
48. Severini GM, Krajinovic M, Pinamonti B, et al. (1996) A new locus for arrhythmogenic right ventricular dysplasia on the long arm of chromosome 14. Genomics 31, 193–200.

8

Postmortem Diagnosis of Anaphylaxis

Erik Edston, MD, PhD
and Marianne van Hage-Hamsten, MD, PhD

Contents

Summary

Death in anaphylaxis often occurs suddenly and outside hospitals. The incidence of fatal outcome is not known with certainty but has been estimated to be about 1% of the total incidence of anaphylaxis per year. The morphology of anaphylaxis is nonspecific. In rapidly occurring deaths, the only finding might be visceral congestion. In more prolonged agony, the main findings are airway obstruction caused by edema and mucous plugging. Microscopical

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examination occasionally reveals edema in the respiratory mucosa, discrete inflammation with eosinophilia, and epithelial desquamation. The number of mast cells in different organs and tissues in anaphylactic deaths has hitherto not been properly evaluated. Furthermore, mast cells that have degranulated are difficult to identify in postmortem tissues. The development of immunological methods to detect and quantify mast cell proteases, especially tryptase, has made it easier to diagnose or confirm anaphylaxis postmortem in quite a few cases. But tryptase may not be elevated when death occurs very suddenly and in deaths caused by food anaphylaxis. Moreover, it has been found that tryptase can be elevated in a few cases believed not to be caused by allergy. An artifactual increase of tryptase caused by postmortem diffusion from tissues into blood might also occur. Knowledge of the patient's history and circumstances of death is of major importance when investigating suspected anaphylaxis. Tryptase measurements should always be accompanied with analysis of allergen-specific immunoglobulin E antibodies or, if no allergen is known, a panel of common airborne and food allergens.

Key Words: Anaphylaxis; mast cell; tryptase; IgE; forensic pathology; sudden death.

1. INTRODUCTION

Sudden anaphylactic deaths that occur outside hospital, sometimes in the emergency room, or during medical treatment usually are subject to forensic autopsies. These cases are a source of frustration to the forensic pathologist because the diagnosis of anaphylactic shock is one of exclusion and has hitherto been based solely on circumstantial evidence, for example, in deaths immediately after ingestion of certain foods or medications known to cause allergy or after stings (e.g., wasp or honey bee stings). The forensic literature on anaphylactic deaths typically comprises numerous case studies and a few population-based studies. In unclear accidental or out-of-hospital deaths, reliable diagnostic methods to confirm or exclude anaphylaxis would be of great value. Death resulting from anaphylaxis occurs, as reported by witnesses, suddenly in most of the cases. With the increasing incidence of allergies worldwide, it is conceivable that also anaphylactic deaths might show an upward trend. In a 10-year forensic autopsy material from Sweden, the incidence of diagnosed fatal anaphylaxis was about 0.2 per 1000 autopsies (source: database of the Swedish National Board of Forensic Medicine). The total incidence of death in anaphylaxis worldwide, may it be in huge countries such the United States, or even in small countries like Sweden, is largely unknown. This ignorance can be explained, on the one hand, by the fact that reliable

methods for diagnosing anaphylaxis postmortem have not been developed until recently and that unwitnessed deaths caused by anaphylaxis may not be subject to a careful death investigation on the other. In different epidemiological studies of anaphylaxis as registered at hospitals and emergency departments, the percentage of fatal anaphylaxis from the total number of incidents is about 1% *(1–3)*. A retrospective study of unexplained sudden deaths revealed that 13% of the cases might actually have been caused by unrecognized anaphylaxis *(4)*.

This review focuses on the postmortem diagnosis of suspected anaphylactic deaths using morphological, biochemical, and immunological methods.

2. Anaphylaxis

Anaphylaxis involves a constellation of symptoms that can be mild or severe. Fatal outcome may have been preceded by vascular collapse, angioedema, airway obstruction caused by laryngeal edema, asthma, nausea and vomiting, diarrhea, palpitations, lightheadedness, itching of palate and pharynx, pruritus, and urticaria *(5)*. The allergic anaphylactic reaction is an immunoglobulin (Ig)E-mediated, systemic reaction that is triggered by allergens ingested, injected, or inhaled, in an allergic individual who is sensitized (an individual who has allergen-specific IgE antibodies) to the allergen involved *(6)*. The IgE antibodies are bound to high-affinity receptors on mast cells and basophils and crosslinking of receptors by allergen-bound IgE induces the release of inflammatory mediators (e.g., histamine), which in turn causes the immediate reaction.

In some instances, anaphylactoid reactions can occur when mast cells release their content without mediation of IgE antibodies in response to endogenic substances, for example, complement factors *(7)*, or in response to exogenic causes such as opiates *(8)* or physical exertion *(9)*. In quite a few cases, the factors that triggered the anaphylactic or anaphylactoid reactions are never established *(10)*.

3. Mast Cells

Mast cells, the principal effector cells of anaphylaxis, are found throughout the body, but mast cells are especially abundant in connective tissue in the skin and around blood vessels, in the respiratory and intestinal mucosa, and connective tissue. Mast cells contain metachromatic granules that empty their contents when stimulated, for example via IgE receptors on the cell surface. In these granules, histamine and neutral proteases such as tryptase, chymase, and carboxypeptidase are stored. Mast cells also produce membrane lipid

mediators, cyclooxygenase and lipoxygenase, metabolites of arachidonic acid, and cytokines *(11,12)*. Mast cell function and the physiological effects of the neutral peptidases have been subjected to intense investigation during the last two decades *(13)*. Two main types of mast cells have been identified: connective tissue mast cells rich in tryptase and chymase (MC_{TC}) and mucosal mast cells containing tryptase and less chymase (MC_T; *[14]*).

4. Mast Cell Mediators

Histamine, serotonin, and heparin are substances stored in mast cells and also are present in other cells *(11,12)*. Their physiological and biochemical effects have been known for a long time. In vivo, histamine has been used to indicate mast cell activation *(7)*. However, histamine is rapidly metabolized postmortem *(15)*. The more recently characterized neutral proteases are more stable and have been reported to be located exclusively in mast cells and basophils *(16)*. One of the neutral protease, tryptase, is the most abundant protein product produced by human mast cells and principally derives from two genes on chromosome *16p13.3*, α-tryptase and β-tryptase *(17)*. Mature β-tryptase is stored in secretory granules as an enzymatically active tetramer in a complex with heparin proteoglycan until the cells are activated to degranulate and release the protease-proteoglycan complex. α-Tryptase is the predominant form of tryptase detected in normal serum and is markedly elevated in patients with systemic mastocytosis *(18)*. The release of β-tryptase into the circulation serves as a clinical marker of mast cell activation *(19)*.

5. Morphological Investigations Toward the Diagnosis of Anaphylactic Deaths

5.1. Autopsy Findings

External signs of anaphylactic deaths are rare and, when present, a skin rash, petechial hemorrhages, and distinct marks after insect stings are usually unobtrusive. James and Austen *(20)*, who reported autopsy findings in six cases, demonstrated that the main findings in five of the six cases were located in the airways and lungs (e.g., laryngeal edema and acute pulmonary emphysema). The sixth person had only unspecific changes of a preceding cardiovascular collapse. In a study analyzing 50 insect sting fatalities *(21)*, Barnard argued that there existed four types of pathology. The first comprises findings in the respiratory tract, such as laryngeal edema, mucous bronchial secretion, and acute pulmonary emphysema. The second group exhibits vascular changes. The third group is defined as anaphylaxis, and the last group applies when the

principal lesions were found in the nervous system. In the first group, which includes prominent respiratory findings, the mechanism of death might have been a local reaction rather than a systemic shock. The group defined as anaphylaxis showed no specific anatomical lesions, and five of six individuals died within 30 minutes. The individuals included in the other groups had a longer survival time after the insect sting and the corresponding lesions. Vascular congestion and brain edema, meningeal edema, perivascular hemorrhage, and encephalomalacia could be interpreted as secondary to circulatory shock.

Delage and Irey *(22)* studied 43 cases of verified fatal anaphylactic reaction caused by drugs or therapeutic agents. An autopsy was performed in 40 of the 43 cases, and 36 individuals showed pulmonary congestion, 20 a pulmonary edema, 18 intraalveolar bleedings or tracheobronchial secretions, 15 had a laryngeal edema, and 11 an acute emphysema.

Pumphrey and Roberts *(23)* studied postmortem findings in 56 cases of probable anaphylactic deaths classified into deaths caused by venom, food, and drugs. Upper airway edema was found to be most common in food-related deaths (62.5%) and was seen in about 30% of the cases included in the other two groups. In contrast, pulmonary congestion and edema were observed in 73.7% of cases in the venom group and in 85.7% in the drug group but only in 56.3% in the food group. Mucous plugging and acute emphysema were uncommon, as were petechial hemorrhages. Cutaneous erythema and edema were seen in only three cases (5.4%).

Thus, autopsy findings in suspected anaphylactic deaths are variable and appear to depend on the type of allergen and the route of administration as well as the time between initiation of the allergic reaction and death. The question is whether anaphylactic death should be defined in its strictest meaning, namely that of a sudden vascular collapse, or in a wider sense also including individuals dying from respiratory failure resulting from upper and/or lower airway edema *(24)*. However, it seems that rapid anaphylactic deaths (sudden cardiovascular collapse) may show nothing more than visceral congestion at autopsy.

5.2. Histology

No case–control or prospective histological postmortem study on anaphylaxis is found in the medical literature. In his retrospective study on insect sting fatalities, Barnard noted that no particular inflammatory cell was seen histologically in airway edema or at the site of the sting *(21)*. However, Barnard did not use any specific mast cell stain. Delage and Irey *(22)* used histological sections from 40 cases stained with only hematoxylin and eosin. They demon-

strated an increased number of eosinophils in the splenic red pulp, but no reference to control cases or statistical calculations were presented. In early reports on fatal anaphylaxis, similar aggregations of eosinophils in the spleen and liver were observed *(25,26)*. In another study, Delage et al., using the hematoxylin-basic fuchsin-picric acid method, found myocardial lesions in the form of discreet myocyte damage in 80% of 30 cases of anaphylactic deaths *(27)*. In the retrospective study by Pumphrey and Roberts *(23)*, in 20 of 56 cases histology showed a pronounced eosinophilia in the upper airway edema and inflammation with epithelial sloughing in cases of mucous plugging.

Counting mast cells in different organs and tissues is one possible postmortem approach toward the diagnosis of anaphylactic deaths. Mast cell stains are based on the metachromatic properties of their cytoplasmic granules, as first described by Ehrlich in 1879. Several stains for mast cells have been developed using the metachromatic reaction *(28)* among which the toluidine blue stain has been the most commonly used. However, the ability of mast cells to stain positively seems to vary with the fixatives and staining techniques used *(29)*. The staining time and pH of the buffer can also influence the outcome *(30)*.

Because mast cells degranulate promptly in anaphylaxis, the number of visible mast cells might be substantially reduced in postmortem tissues in anaphylactic fatalities. The baseline amount of mast cells also may vary considerably between different individuals. No systematic evaluation of mast cell numbers in the lung or other tissues and organs in anaphylactic deaths has been performed so far. In one study using the long toluidine blue stain *(30)*, an increased number of mast cells were found in the lungs in patients with allergic alveolitis and a decreased number was found in deaths from asthma when compared to lung biopsies from controls *(31)*. However, the number of cases in this study was too small to allow any statistical calculations. In a few studies, mast cell counts in the bronchi and lungs have been compared to serum tryptase- and serum IgE levels, but no correlations were found *(32–34)*. Occasionally, mast cell granules can be observed in the vicinity of a mast cell. Whether this is caused by recent degranulation or is an artifactual result of preparation is difficult to evaluate, especially in postmortem tissue samples *(34,35)*. Experimental studies using electron microscopy can visualize mast cell degranulation *(36)*, but electron microscopy obviously is not suitable for postmortem studies.

The basophil granulocyte is a discrete participant in the anaphylactic reaction, but its contribution in the process is not known *(37)*. The low numbers of basophils in the tissues and their similarity to the mast cells in ability

to take up metachromatic dyes render them less useful than mast cells for a micromorphological diagnosis with histochemical methods.

5.3. Immunohistochemistry

The earliest attempts to produce antibodies to identify mast cells in tissue samples did not address any specific epitope *(38)*. Antibodies against histamine were found to be effective *(39)*, but because of postmortem instability of histamine and a lack of specificity for mast cells, they have not been investigated systematically on postmortem material. Identification of neutral proteases as constituents of the mast cell granules *(40,41)*, and the development of monoclonal antibodies against mast cell tryptase and chymase *(42,43)* have facilitated the identification and typing of mast cells in histological sections. The potential advantages of immunohistochemistry vs histochemical stains have been evaluated in a few studies *(44)* but not on postmortem tissue. These studies found that tryptase antibodies stained far more cells (morphologically corresponding to mast cells) than did conventional stains. Antibodies against tryptase for use in immunohistochemistry have been developed commercially and can be applied on paraffin-embedded formaldehyde-fixed tissue and are therefore suitable for routine pathology and retrospective studies. Apparently, tryptase antibodies identify far more mast cells than for example does the toluidine blue stain *(45)*.

Chymase antibodies are also available commercially, but until recently they were not suitable for formaldehyde-fixed tissue and have not been widely applied to postmortem material. By using a sandwich technique applying both tryptase and chymase simultaneously, it is now possible to estimate the relative number of the two subtypes (MC_{TC} and MC_{T}) of mast cells in the same tissue section *(46)*.

Antibodies to identify basophilic granulocytes are those directed against basogranulin, which could be useful to differentiate between mast cells and basophils in tissue sections *(47)*.

In summary, increased numbers of cells potentially participating in anaphylaxis, for example, mast cells and eosinophils, have been observed in intravasal locations or within the bronchial mucosa and the parenchyma of the lungs in cases of anaphylactic death. However, no serious attempts to confirm and statistically evaluate those findings have been performed so far. Moreover, the potential of immunohistochemistry to diagnose anaphylactic deaths has not been fully investigated to date.

6. Biochemical and Immunological Methods

6.1. Histamine

The earliest attempts to measure mast cell degranulation involved measurements of histamine levels in body fluids *(48)*. Although not specific for mast cells, histamine has been a successful marker for mast cell activation in vivo *(49,50)*, and the time course of its release and disappearance has been well documented. For postmortem studies, the catabolism of histamine (half-life: 2 minutes) is too rapid to make analysis of histamine in serum useful *(51)*. It would be possible to measure its degradation product methylhistamine *(52)*, which is more stable in urine, but again in rapid anaphylactic deaths significant amounts of methylhistamine might not have had time to appear in urine. Passive diffusion of methylhistamine into extravasal fluids, for example vitreous humor, might occur postmortem, but to our knowledge such measurements have not been performed.

6.2. Tryptase

Commercial kits for determination of mast cell tryptase, based on the work of Schwartz et al. *(53,54)* have been developed (Pharmacia Diagnostics AB, Uppsala, Sweden). The first version measured only the free form of active tryptase (β) in serum. A more sensitive method was subsequently developed *(55)* that measures both the protein-bound form of protryptase α and its free form β. The usefulness of tryptase in diagnosing anaphylaxis postmortem was first demonstrated in 1991 by Yunginger et al. *(56)*. In anaphylactic deaths, the authors showed differences between food-induced anaphylaxis, in which tryptase usually was slightly to moderately elevated (the normal concentration of β-tryptase in healthy, living subjects is 0), and anaphylactoid reactions caused by radio-contrast media, where tryptase concentrations of as many as 6000 μg/mL were detected. Anaphylactic fatalities due to venom (except for one case) fell in between these extremes.

In previous studies by Schwartz et al., tryptase was shown to have its peak concentration in serum between 1 and 2 hours, with a half-life of 2 hours *(51)*. It could, therefore, be a marker of ongoing allergic reactions and, moreover, tryptase was found to be rather stable in blood samples kept under room temperature for several hours *(51)*.

The study of Yunginger et al. *(56)* has been followed by some case studies making use of tryptase as an indicator of anaphylactic deaths *(57–60)*. However, in a study that included control individuals who had died from various well defined causes, Randall et al. *(61)* found that tryptase was sometimes

elevated in deaths not clearly related to anaphylaxis. We as well as other investigators have shown that tryptase is elevated in sudden infant death syndrome *(63–66)*, in traumatic deaths *(67)*, and heroin-related fatalities among drug abusers, among whom 30 to 40% had elevated values of tryptase *(34,68)*.

The reasons for variations of tryptase concentrations postmortem in nonallergic fatalities are not clear, but one probable factor might be postmortem diffusion of tryptase from lysed tissue mast cells into the blood vessels, which could explain the consistently higher values in blood samples postmortem, especially from the heart chambers vs femoral blood *(69,70)*. Therefore, sampling of blood from the femoral vein should be preferred. Because of these variations, the reference values for tryptase in clinical cases cannot readily be applied on serum obtained postmortem.

6.3. Measurements of Total Serum IgE and Specific IgE

IgE, as shown by numerous studies, appears to be stable in postmortem blood samples and experimentally it can be demonstrated even in putrefied corpses *(71)*. Measuring total IgE in anaphylactic deaths might, however, be of limited value as elevated values are not synonymous with allergic disease. Moreover, IgE shows a seasonal variation *(72)* and may even increase with the length of the postmortem interval *(70)*. To confirm high postmortem values of tryptase, measurement of allergen-specific IgE in serum should be performed (e.g., with the Pharmacia CAP system, Pharmacia Diagnostics AB *[73]*). If the allergen causing the anaphylactic reaction is known or can be suspected, IgE analysis against the allergen should be carried out. If the allergen is totally unknown, the serum could be tested against a panel of common food or airborne allergens *(74)*. However, these tests are less sensitive than IgE analysis against a single allergen.

6.4. Other Markers of Anaphylaxis

Measurements of mast cell chymase and carboxypeptidase have so far not been carried out in cases of anaphylactic death. Elevated levels of eosinophil cationic protein have been demonstrated together with tryptase in asthma fatalities and in heroin-related deaths that were believed to have been caused by anaphylaxis or an anaphylactoid reaction *(34)*. Whether eosinophils play any role in acute anaphylactic reactions is not known with certainty, but it is known that eosinophil cationic protein can induce mast cell degranulation *(75)*.

Other indicators of mast cell degranulation and anaphylaxis are angiotensin I and II measured in plasma or urine *(76)*, cytokines *(77)*, and anaphylatoxins (complement split products that can be catalyzed by tryptase)

in serum *(78)*. However, these are non-specific indirect tests that might possibly be performed postmortem as an adjunct to direct measurements of mast cell proteases.

7. Conclusions and Recommendations

So far, no specific morphology has been found in anaphylactic deaths. Findings such as visceral congestion, edema and eosinophilia of the laryngeal and bronchial mucosa, increased numbers of mast cells in the lungs and connective tissue, as well as conspicuous amounts of eosinophils in the capillaries of the liver and spleen are only consistent with the diagnosis.

During the last decade, the knowledge of mast cells, their mediators, and their role in anaphylaxis has increased, and there are now reliable laboratory methods to support the diagnosis. However, a definite conclusion that anaphylaxis was the cause of death cannot be made without a thorough penetration of the patient's history and the circumstances of death. If food-induced anaphylaxis is suspected, gastric contents could be collected to confirm that a certain food allergen was ingested prior to death. However, identification of food allergens in gastric contents has seldom been successful *(23)*.

Tissue biopsies from the larynx, bronchi, lungs, heart, and spleen should be stained for mast cells and eosinophils, preferably by immunohistochemistry. At autopsy, samples of blood should be collected from the femoral vein for analysis of tryptase, for example, α- and β-tryptase. As stated earlier, in postmortem serum the concentration of tryptase is sometimes slightly to moderately elevated in control samples. If an elevated value of tryptase is found and a probable or possible allergen has been identified, a test for allergen-specific IgE should follow. Otherwise, IgE analysis against a panel of common food and airborne allergens could be valuable. If the tests render a high tryptase value in combination with a negative IgE analysis, an anaphylactoid reaction vs artifactually elevated tryptase concentrations must be further evaluated. The circumstances of death (e.g., physical exertion, intake of antibiotics, antiinflammatory compounds, and other medications, signs of recent drug abuse) should be taken into account as well as factors related to the autopsy (e.g., method of sampling, hemolysis, length of the postmortem interval).

Systematic studies of postmortem changes in anaphylactic fatalities vs controls and the access to new immunohistochemical antibodies against various mast cell mediators might reveal better micromorphological indicators. Along with continuing validation of tryptase and chymase assays in postmortem fluids and tissues, a more reliable diagnosis of anaphylactic sudden death could be reached in the future.

References

1. Yocum MW, Butterfield JH, Klein JS, Volcheck GW, Schroeder DR, Silverstein MD (1999) Epidemiology of anaphylaxis in Olmstead County: a population based study. J Allergy Clin Immunol 104, 271–273.
2. Neugut AI, Ghatak AT, Miller RL (2003) Epidemiology of anaphylaxis in the United States. Curr Allergy Asthma Rep 3, 30–35.
3. Helbling A, Hurni T, Mueller UR, Pichler WJ (2004) Incidence of anaphylaxis with circulatory symptoms: a study over a 3-year period comprising 940,000 inhabitants of the Swiss canton Bern. Clin Exp Allergy 34, 285–290.
4. Schwartz HJ, Yunginger JW, Schwartz LB (1995) Is unrecognized anaphylaxis a cause of sudden unexpected death? Clin Exp Allery 25, 866–870.
5. Ring J, Brockow K, Behrendt H (2004) History and classification of anaphylaxis. Novartis Found Symp 257, 6–16.
6. Ewan PW (1998) Anaphylaxis. BMJ 316, 1442–1445.
7. Smith PL, Kagey-Sobotka A, Bleecker EB, et al. (1980) Physiologic manifestations of human anaphylaxis. J Clin Invest 66, 1072–1080.
8. Barke KE, Hough B (1993) Opiates, mast cells and histamine release. Life Sci 53, 1391–1399.
9. Sheffer AL, Austen KF (1980) Exercise-induced anaphylaxis. J Allergy Clin Immunol 66, 106–111.
10. Wiggins CA, Dykewicz MS, Patterson R (1988) Idiopathic anaphylaxis: Classification, evaluation, and treatment of 123 patients. J Allergy Clin Immunol 82, 849–855.
11. Williams CM, Galli SJ (2000) The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. J Allergy Clin Immunol 105, 847–859.
12. Nilsson G, Costa JJ, Metcalf DD (1999) Mast cells and basophils. In Gallin JI, Snyderman R, eds., Inflammation: Basic Principles and Clinical Correlates. Lippincott-Raven Publications, Philadelphia, pp. 97–117.
13. Schwartz LB (1990) Neutral Proteases of Mast Cells. Karger, Basel.
14. Irani AA, Schechter NM, Craig SS, DeBlois G, Schwartz LB (1986) Two types of human mast cells that have distinct neutral protease compositions. Proc Natl Acad Sci USA 83, 4464–4468.
15. Kapeller-Adler R (1965) Histamine catabolism in vitro and in vivo. Fed Proc 24, 757–765.
16. Schwartz LB (1985) The Mast Cell. In Kaplan AP, ed., Allergy. Churchill Livingstone, New York, pp. 53–92.
17. Schwartz LB (1994) Tryptase: a mast cell serine protease. Methods Enzymol 244, 88–100.
18. Schwartz LB, Sakai K, Bradford TR, et al. (1995) The alpha form of human tryptase is the predominant type present in blood at base line in normal subjects and is elevated in those with systemic mastocytosis. J Clin Invest 96, 2702–2710.
19. Schwartz LB, Metcalfe DD, Miller JS, Earl H, Sullivan T (1987) Tryptase levels as an indicator of mast cell activation on systemic anaphylaxis and mastocytosis. N Engl J Med 316, 1622–1626.
20. James LP, Austen KF (1964) Fatal systemic anaphylaxis in man. N Engl J Med 270, 597–603.

21. Barnard JH (1967) Allergic and pathologic findings in fifty insect-sting fatalities. J Allergy 40, 107–114.
22. Delage C, Irey NS (1972) Anaphylactic deaths. A clinicopathologic study of 43 cases. J Forensic Sci 17, 525–540.
23. Pumphrey RSH, Roberts ISD (2000) Postmortem findings after fatal anaphylactic reactions. J Clin Pathol 53, 273–276.
24. Roberts ISD, Pumphrey RSH (2001) The autopsy in fatal anaphylaxis. Rec Adv Pathol 19, 145–162.
25. Dean HR (1922) Histology of a case of anaphylactic shock. J Path Bact 25, 305–315.
26. Vance BM, Strassman G (1941) Sudden death following injection af foreign protein. Arch Pathol 34, 849–865.
27. Delage C, Mullick FG, Irey NS (1973) Myocardial lesions in anaphylaxis. A histochemical study. Arch Pathol 95, 185–189.
28. Bancroft JD, Stevens A (1990) Cytoplasmic granules, organelles and special tissues. In Bancroft JD, Stevens A, Turner DR, eds., The Theory and Practice of Histological Techniques. Churchill Livingstone, Edinburgh, pp. 638–639.
29. Strobel S, Miller HRP, Ferguson A (1981) Human intestinal mucosal mast cells: evaluation of fixation and staining techniques. J Clin Pathol 34, 851–858.
30. Wingren U, Enerbäck L (1983) Mucosal mast cells of the rat intestine: a re-evaluation of fixation and staining properties, with special reference to protein blocking and solubility of the granular glycosaminoglycan. Histochem J 15, 571–582.
31. Heard BE, NunnAJ, Kay AB (1989) Mast cells in human lungs. J Pathol 157, 59–63.
32. Edston E, Gidlund E, Wickman M, Ribbing H, van Hage-Hamsten M (1999) Increased mast cell tryptase in sudden infant death—anaphylaxis, hypoxia or artefact? Clin Exp Allergy 29, 1648–1654.
33. Platt MS, Yunginger JW, Sekula-Perlmann A, Irani A-MA, Smialek J, Mirchandani HG, et al. (1994) Involvement of mast cells in sudden infant syndrome. J Allergy Clin Immunol 94, 250–256.
34. Fineschi V, Cecchi R, Centini F, Paglicci Reattelli L, Turillazzi E (2001) Immunohistochemical quantification of pulmonary mast cells and post-mortem blood dosages of tryptase and eosinophil cationic protein in 48 heroin-related deaths. Forensic Sci Int 120, 189–194.
35. Craig SS, De Blois G, Schwartz LB (1986) Mast cells in human keloid, small intestine, and lung by an immunoperoxidase technique using a murine monoclonal antibody against tryptase. Am J Pathol 124, 427–435.
36. Dvorak AM, Schulman ES, Peters SP, et al. (1985) Immunoglobulin E-mediated degranulation of isolated human lung mast cells. Lab Invest 53, 45–56.
37. Schroeder JT, MacGlashan DW, Lichtenstein LM (2001) Human basophils: mediator release and cytokine production. Adv Immunol 77, 93–122.
38. Rimmer EF, Turberville C, Horton MA (1984) Human mast cells detected by monoclonal antibodies. J Clin Pathol 37, 1249–1255.
39. Johansson O, Virtanen M, Hilliges M, Yang Q (1992) Histamine immunohistochemistry: a new and highly sensitive method for studying cutaneous mast cells. Histochem J 24, 283–287.
40. Glenner GG, Cohen LA (1960) Histochemical demonstration of species-specific trypsin-like enzyme in mast cells. Nature 105, 846–847.

41. Schwartz LB, Lewis RA, Austen KF (1981) Tryptase from human pulmonary mast cells: purification and characterization. J Biol Chem 256, 11939–11943.
42. Irani AMA, Bradford TR, Kepley CL, Schechter NM, Schwartz LB (1989) Detection of MC_T and MC_{TC} types of human mast cells by immunohistochemistry using new monoclonal anti-tryptase and anti-chymase antibodies. J Histochem Cytochem 37, 1509–1515.
43. Walls AF, Bennett AR, McBride HM, Glennie MJ, Holgate ST, Church MK (1990) Production and characterization of monoclonal antibodies specific for human mast cell tryptase. Clin Exp Allergy 20, 581–589.
44. Horny HP, Sillaber C, Menke D, et al. (1998) Diagnostic value of immunostaining for tryptase in patients with mastocytosis. Am J Surg Pathol 22, 1132–1140.
45. Walls AF, Jones DB, Williams JH, Church MK, Holgate ST (1990) Immunohistochemical identification of mast cells in formaldehyde-fixed tissue using monoclonal antibodies specific for tryptase. J Pathol 162, 119–126.
46. Buckley MG, McEuen AR, Walls AF (1999) The detection of mast cell subpopulations in formalin-fixed human tissues using a new monoclonal antibody specific for chymase. J Pathol 189, 138–143.
47. McEuen AR, Buckley MG, Compton SJ, Walls AF (1999) Development and characterization of a monoclonal antibody specific for human basophils and the identification of a unique secretory product of basophil activation. Lab Invest 79, 27–38.
48. Roberts M, Adam HM (1950) New methods for the quantitative estimation of free and conjugated histamine in body fluids. Br J Pharmacol 5, 526–541.
49. Ind A, Miyatake PW, Heavey DJ, Dollery CT (1984) Local histamine release after immunological and non-immunological mast cell degranulation in vivo. Agents Actions 14, 417–419.
50. van der Linden PWG, Hack CE, Poortman J, Vivié-Kipp YC, Struyvenberg A, van der Zwan JK (1992) Insect-sting challenge in 138 patients: relation between clinical severity of anaphylaxis and mast cell activation. J Allergy Clin Immunol 90, 110–118.
51. Schwartz LB, Yunginger JW, Miller J, Bokhari R, Dull D (1989) Time course of appearance and disappearance of human mast cell tryptase in the circulation after anaphylaxis. J Clin Invest 83, 1551–1555.
52. Keyzer JJ, Breukelman H, Wolthers BG, Richardson FJ, de Monchy JG (1985) Measurement of N tau-methylhistamine concentrations in plasma and urine as a parameter for histamine release during anaphylactoid reactions. Agents Actions 16, 76–79.
53. Wenzel S, Irani AM, Sanders JM, Bradford TR, Schwartz LB (1986) Immunoassay of tryptase from human mast cells. J Immunol Methods 86, 139–142.
54. Enander I, Matson P, Nystrand J, Andersson AS, Bradford TR, Schwartz LB (1991) A new radioimmunoassay for human mast cell tryptase using monoclonal antibodies. J Immunol Methods 138, 39–46.
55. Schwartz LB, Bradford TR, Rouse C, et al. (1994) Development of a new, more sensitive immunoassay for human tryptase: use in systemic anaphylaxis. J Clin Immunol 14, 190–204.
56. Yunginger JW, Nelson DR, Squillace DL, et al. (1991) Laboratory investigation of deaths due to anaphylaxis. J Forensic Sci 36, 857–865.

57. Ansari MQ, Zamora JL, Lipscomb MF (1993) Postmortem diagnosis of acute anaphylaxis by serum tryptase analysis. Am J Clin Pathol 99, 101–103.
58. Prahlow JA, Barnard JJ (1998) Fatal anaphylaxis due to fire ant stings. Am J Forensic Med Pathol 19, 137–142.
59. Fineschi V, Monasterolo G, Rosi R, Turillazzi E (1999) Fatal anaphylactic shock during a fluorescein angiography. Forensic Sci Int 100, 137–142.
60. Edston E, van Hage-Hamsten M (2003) Death in anaphylaxis in a man with house dust mite allergy. Int J Legal Med 117, 299–301.
61. Randall B, Butts J, Halsey JF (1995) Elevated postmortem tryptase in the absence of anaphylaxis. J Forensic Sci 40, 208–211.
62. Platt MS, Yunginger JW, Sekula-Perlman A, et al. (1994) Involvement of mast cells in sudden infant death syndrome. J Allergy Clin Immunol 94, 250–256.
63. Holgate S, Walters C, Walls AF, et al. (1994) The anaphylaxis hypothesis of sudden infant death syndrome (SIDS): mast cell degranulation in cot death revealed by elevated concentrations of tryptase in serum. Clin Exp Allergy 24, 1115–1122.
64. Edston E, Gidlund E, Wickman M, Ribbing H, van Hage-Hamsten M (1999) Increased mast cell tryptase in sudden infant death syndrome—anaphylaxis, hypoxia or artefact? Clin Exp Allergy 29, 1648–1654.
65. Buckley MG, Variend S, Walls AF (2001) Elevated serum concentrations of beta-tryptase, but not alpha-tryptase in Sudden Infant Death Syndrome: investigation of anaphylactic mechanisms. Clin Exp Allergy 31, 1696–1704.
66. Nishio H, Suzuki K (2004) Serum tryptase levels in sudden infant death syndrome in forensic autopsy cases. Forensic Sci Int 139, 57–60.
67. Edston E, van Hage-Hamsten M (2003) Mast cell tryptase and hemolysis after trauma. Forensic Sci Int 131, 8–13.
68. Edston E, van Hage-Hamsten M (1997) Anaphylactoid shock—a common cause of death in heroin addicts? Allergy 52, 950–954.
69. Horn KD, Halsey JF, Zumwalt RE (2004) Utilization of serum tryptase and immunoglobulin E assay in the postmortem diagnosis of anaphylaxis. Am J Forensic Med Pathol 25, 37–43.
70. Edston E, van Hage-Hamsten M (1998) β-Tryptase measurements post-mortem in anaphylactic deaths and in controls. Forensic Sci Int 93, 135–142.
71. Hieda Y, Kageura M, Hara K, Kashimura S (1991) Postmortem changes in hapten-specific IgE antibody responses in mice. Int J Legal Med 104, 133–135.
72. Yunginger JW, Gleich GJ (1973) Seasonal changes in serum and nassal IgE concentrations. J Allergy Clin Immunol 51, 174–186.
73. Kelso JM, Sohdi N, Gosselin VA, Yunginger JW (1991) Diagnostic performance characteristics of the standard Phadebas RAST, modified RAST, and pharmacia CAP system versus skin testing. Ann Allergy 67, 511–514.
74. Matricardi PM, Fattorossi A, Nisini R, Le Moli S, Castagliuolo PP, D'Amelio R (1989) A new test for specific IgE to inhalant allergens (Phadiatop) in the screening of immediate respiratory hypersensitivity states. Ann Allergy 63, 532–535.
75. Zheutlin LM, Ackerman SJ, Gleich GJ, Thomas LL (1984) Stimulation of rat mast cell histamine release by eosinophil granule-derived cationic proteins. J Immunol 133, 2180–2185.

76. Hermann K, Rittweger R, Ring J (1992) Urinary excretion of angiotensin I, II, arginine, vasopressin and oxytocin in patients with anaphylactoid reactions. Clin Exp Allergy 22, 845–853.
77. Strait R, Morris SC, Finkelman FD (2004) Cytokine enhancement of anaphylaxis. Novartis Found Symp 257, 80–91.
78. Schwartz LB, Kawahara MS, Hugli TE, Vik D, Fearon DT, Austen KF (1983) Generation of C3a anaphylatoxin from human C3 by human mast cell tryptase. J Immunol 130, 1891–1895.

Vascular Conditions

9

The Medicolegal Evaluation of Fatal Pulmonary Thromboembolism

James R. Gill, MD

CONTENTS

SUMMARY

Deaths resulting from pulmonary emboli are common. The autopsy dissection, documentation, and ancillary studies pertaining to pulmonary emboli are important components of evaluating such fatalities. The detection of a saddle embolism at autopsy does not signify the end of the investigation because the underlying risk factors still may need to be determined. The gross, microscopical, and genetic findings can distinguish various thrombotic risk factors and etiologies. Because deaths caused by pulmonary emboli may involve medicolegal issues, a dependable protocol is needed for their investigation. In particular, the timing of a pulmonary embolism may have important medicolegal consequences. Because of the pathophysiology and propagation of a thrombus, one may see a broad histological range of thrombosis and organization.

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Histological examination of residual deep vein thrombus provides the best opportunity to properly age the thrombus. Understanding the pathogenesis and pitfalls of venous thromboembolism allows the pathologist to properly certify the proximate cause of death. Currently, there are DNA techniques that allow for the postmortem diagnosis of some hereditary thrombophilias. These include factor V, prothrombin, and methylenetetrahydrofolate reductase mutations. Decedents who are candidates for these tests include those who are younger than 45 years of age; those whose deaths were related to pregnancy; those with a history of recurrent or unexplained stillbirths, oral contraceptive pill use, hormone replacement, or treatment with chemotherapy; those with weak risk factors (long car ride, flights, obesity); or those with deep venous thrombosis of undetermined etiology. These tests benefit the investigator, who is attempting to discern the proximate cause, and potentially the surviving family members.

Key Words: Forensic pathology; pulmonary embolism; thrombus; hereditary thrombophilia; autopsy; postmortem; venous thromboembolism.

1. INTRODUCTION

Venous thromboembolism (VTE) encompasses pulmonary thromboemboli (PE) and deep venous thrombosis (DVT), typically of the pelvic and lower-extremity veins (1). The incidences of pulmonary embolism and DVT are difficult to calculate and are commonly underdiagnosed *(2–8)*. Not all pulmonary emboli are fatal (9). Pulmonary emboli have been estimated to occur in more than 600,000 patients per year in the United States and result in 50,000 to 200,000 deaths annually *(2,6,10)*. Approximately 11% of patients with an acute PE die within 1 hour; this accounts for two-thirds of the fatalities caused by PE *(6,10)*. Most people who die of a PE were never appropriately treated because the diagnosis was not made *(10)*. The mortality rate of an acute untreated PE is 30–35% *(11)*.

VTE is a common immediate cause of death diagnosed by the forensic and hospital pathologist. Because of the sudden and unexpected presentation, out-of-hospital deaths caused by pulmonary embolism are investigated by the medical examiner/coroner. Hospital deaths caused by complications of therapy or diagnostic studies also are usually reportable. Fatal pulmonary emboli that complicate a proximate injury are under the jurisdiction of the medical examiner/coroner. The prosector must recognize that the detection of a saddle embolism at autopsy does not signify the end of the investigation. Although there is a clear immediate cause of death, the underlying risk factors must be investigated.

Virchow described three risk factors for thrombosis, which bear his eponym, *Virchow's Triad*. These include endothelial injury, stasis, and hypercoagulability *(12–14)*. With the advent of genetic tests, inheritable thrombophilias may be definitively diagnosed. The majority of idiopathic VTE are caused by specific coagulation disorders *(15)*. For deaths caused by PE, postmortem blood samples may be analyzed for the most common causes of thrombophilic coagulation disorders: mutations in factor V, prothrombin, and methylenetetrahydrofolate reductase (MTHFR) *(16)*. The benefits of these tests as clinical screening tools have been debated; however, their role in the investigation of death is useful *(15,17–20)*.

Because of medical, legal, and familial issues, deaths caused by pulmonary emboli need to be properly investigated and documented *(21)*. The medicolegal investigation of fatal PE commonly includes photographic, written, and microscopical documentation of the embolism and DVT. In addition, DNA testing for hereditary thrombophilias is a new option.

2. Pathogenesis and Pathophysiology

A thrombus may form in the deep leg veins because of endothelial injury, stasis, and/or a hypercoagulable state. Once the thrombus forms, it can gradually enlarge as more fibrin and blood are layered (the so-called "lines of Zahn"). There are three possible fates of the thrombus: lysis, organization with recanalization and resorption, or embolism. Before resolution, the thrombus may extend further in the veins. Proximal propagation is the most dangerous because the risk of embolism is greatest from thrombi in the inguinal/proximal leg veins *(22)*. Because of the pathophysiology of propagation, this proximal thrombus is the most recent and therefore least adherent to the vessel wall. In fact, treatment of a DVT with anticoagulation therapy is aimed to stop the progression of the thrombus to allow time for the body to naturally lyse or organize and resorb the thrombus. Heparin and warfarin have no direct thrombolytic action.

Kakkar et al. *(22)* examined the natural history of VTE in patients undergoing surgery without anticoagulant prophylaxis. DVT began in the lower leg veins and had three outcomes: one-third spontaneously lysed, one-third remained in the calf veins, and one-third extended to the popliteal or femoral veins. PE occurred only in the group with proximal extension.

Once the thrombus dislodges and travels to the lung, the embolism, depending on its size and coexisting cardiovascular diseases, interferes with hemodynamics *(6)*. Increased pulmonary vascular resistance will cause right heart strain/failure. Inadequate gas exchange occurs because of decreased per-

fusion, which results in increased dead space and ventilation–perfusion mismatches. This, combined with decreased cardiac output, leads to global hypoxia *(23)*. Because of the dual blood supply of the lungs (bronchial arteries) and the rapidity of death, infarction of the lung is uncommon in these sudden deaths. Pulmonary infarcts may occur, particularly in patients with primary heart disease (congestive heart failure etiologies) and protracted clinical courses. Even grossly appearing infarcts, however, may be pulmonary hemorrhage and edema caused by ischemia with inflammation and not true tissue necrosis when viewed microscopically *(24)*.

The initial causes of the thrombus and propagation are the risk factors related to endothelial injury, stasis, and/or hypercoagulability. Endothelial injury is typically secondary to trauma of the lower extremity. Blunt injury that causes a fracture of the leg can result in endothelial injury in addition to stasis from subsequent inactivity. Hypercoagulability may be due to various hereditary or acquired (e.g., malignancy, pregnancy) thrombophilias.

Strong risk factors for DVT include lower extremity fractures, major general surgery (especially lower extremity orthopedic operations), or spinal cord injury *(25)*. Moderate risk factors include hormone therapy *(26)*, paralytic stroke, malignancy/chemotherapy *(27,28)*, history of venous thrombosis, congestive heart failure etiologies, and the postpartum period *(12,29,30)*. Weak risk factors include inactivity as the result of prolonged sitting *(31,32)*, obesity *(33)*, varicose veins, and the prepartum period *(12)*.

Thrombophlebitis and phlebothrombosis are two designations for venous thrombosis. Classically, thrombophlebitis is defined as inflammation of a vein with secondary thrombosis. Phlebothrombosis is simply a thrombus in a vein. Because venous thrombosis may lead to inflammatory changes within the vein wall, thrombophlebitis and phlebothrombosis are considered, by some, as two designations for a single entity *(34)*. Morphologically, the terms, however, continue to serve a purpose. For example, there are some fatalities in which thrombophlebitis is detected in one leg and phlebothrombosis in the other leg.

Although residual thrombus is not always detected, dissection of the leg veins may provide important information *(21)*. The detection of the thrombus depends upon where and how extensively the dissection is done. The vast majority of pulmonary emboli (90%) arise from thrombi of the veins of the lower extremities and pelvis; however, there are other locations as well. Thrombi arising from indwelling central venous catheters, upper extremity veins, and the right heart have been described *(24)*. Once these locations have been excluded, one may conclude that the entire thrombus embolized to the lung. Often the gross morphology (diameter, length) of the embolism will allow one to conclude that it originated in a deep leg vein.

Because the vast majority of pulmonary emboli arise from the veins of the lower extremities and pelvis, the rareness of upper-extremity DVT (compared with lower leg) raises interesting questions of pathophysiology. Fractures of bones in the arm can potentially cause as much vascular injury as an injured leg and the arm is equally immobilized. The frequency of death from a pulmonary embolism after an isolated upper extremity injury, however, is minuscule compared with those after leg injuries. This disparity may be related to several factors, including the ability to ambulate with an upper extremity injury that would stimulate cardiac output. Prolonged inactivity/immobility, for any reason, has major health implications, particularly for the cardiovascular system. Inactivity even may play a major role in some of the other risk factors for VTE (e.g., obesity and increasing age). Cardiovascular deconditioning occurs in astronauts in space because of microgravity *(35–37)*. Models on earth that study this deconditioning use various bed rest regimens that demonstrate the extent of cardiovascular compromise from prolonged inactivity/immobility *(36–38)*. Another factor to consider is the smaller diameter and shorter length of the deep veins in the arm compared with the leg veins *(24)*. Thrombi may form in the arm veins, but any subsequent emboli may be too small to cause pulmonary symptoms *(24)*.

3. Dissection and Morphology

PE and DVT have gross and microscopical features that may be important in the evaluation of these deaths. The autopsy investigation of fatal PE commonly includes photographic, written, and microscopical documentation of the VTE. Bilateral dissection of the deep veins of the pelvis and legs in VTE deaths can provide valuable information regarding the proximate cause and manner of death. Even if there is no grossly apparent thrombus, microscopical examination of the veins can be of benefit. One may see microscopical thrombus or signs of a recently departed thrombus (e.g., phlebitis).

3.1. Gross Pathology

Gross features may allow the differentiation of thromboemboli from postmortem clot. These features include the shape, color, size, and the extent of attachment to the blood vessel. None of these findings is absolute, and one must be careful of pitfalls. The gross appearance of the classic saddle pulmonary embolism would include a tangled embolism bulging from the proximal pulmonary arteries that is slightly adherent to the blood vessel and has a heterogeneous red–blue–tan appearance (Fig. 1A,B). A typical postmortem clot will slip out of the blood vessel, has distinct purple (red clot) and tan compo-

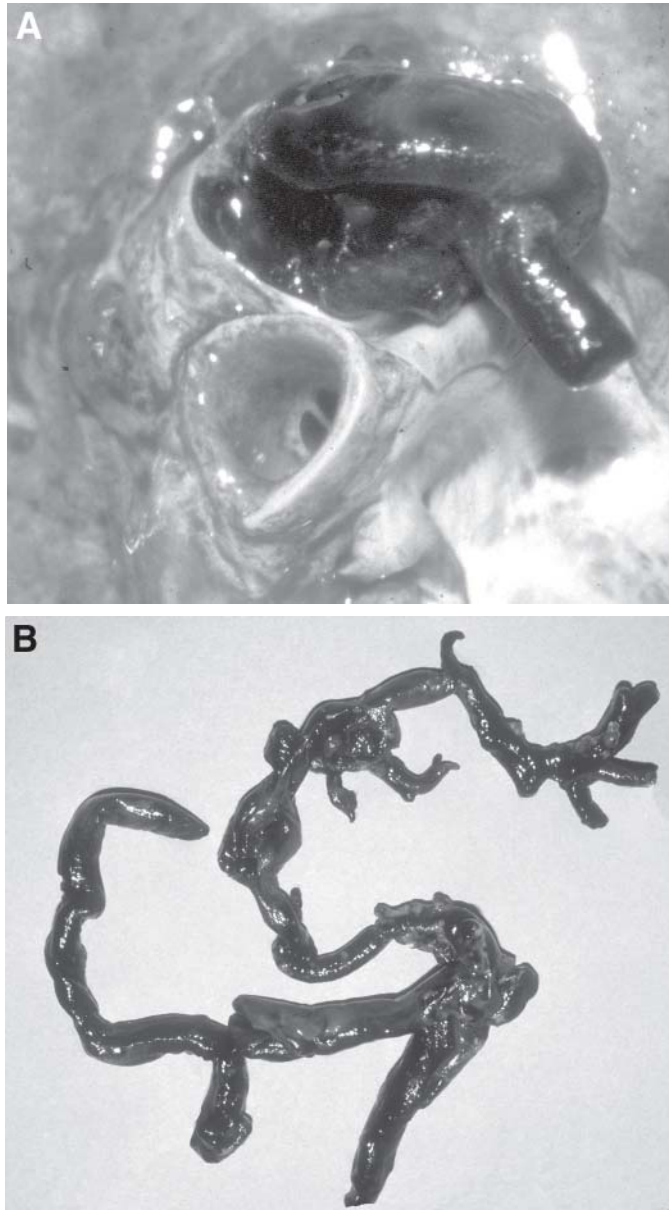

Fig. 1. Gross appearance of pulmonary embolism. **(A)** *In situ* pulmonary embolism. **(B)** Untangled embolism.

nents (fibrin/serum), and has the shape of the blood vessel in which it is found. Pulmonary emboli, however, may also take the shape of the blood vessel in which they have lodged, particularly if postmortem clot has formed a sheath surrounding the embolism in the blood vessel. Emboli that arise from very recent DVT may have a more homogeneous appearance. Because of their pathogenesis, acute thrombi are more likely to embolize than organizing thrombi, which have become adherent to the blood vessel wall from the ingrowths of granulation tissue. Microscopical examination is particularly helpful with grossly equivocal emboli.

At autopsy, the pulmonary arteries should be incised during the routine removal of the lung and close attention to the cut surfaces of the blood vessels (including the vena cava) should be given. Some emboli may lodge in the right heart and can be missed if they drop out of the vena cava during removal of the heart *(39)*. Typically, adherent embolism will bulge from the incised pulmonary arteries. Even after removal of the lungs from the thorax, the thromboemboli usually will remain lodged in the hilar pulmonary arteries. The hilum and then the removed and unraveled emboli may now conveniently be photographed. The pulmonary artery tree should be opened from the hilum toward the periphery to search for smaller emboli in the lobar and segmental arteries *(24)*. Finally, transverse incisions of the pulmonary parenchyma may disclose emboli in the small peripheral vessels, which tend to protrude above the cut surface.

Dissection of the leg veins is useful in deaths from PE. Simply "milking" the upper leg veins is inadequate to include or exclude a DVT. Dissection of the upper leg veins may be performed with the body in the supine position. Starting with the already exposed iliac vein, continue the dissection with a pair of scissors along the medial thigh to expose the femoral veins. Adherent thrombus may be documented with photographs and microscopical sections. Microscopical sections of the thrombus should include the adjacent vein wall and muscle. The popliteal and posterior tibial veins are dissected with the body in the prone position. Removal and transverse sectioning of the muscles of the posterior tibial compartment (soleus and gastrocnemius) may reveal thrombi in leg veins with the characteristic protruding morphology (Fig. 2A,B). Often the anterior tibial and peroneal veins between the tibia and fibula will contain thrombi. Even if a thrombus is found on dissection of the first leg, the contralateral leg has to be dissected as well.

Therapeutic attempts can modify autopsy findings in deaths caused by PE. These include vigorous resuscitation and use of thrombolytics, which may partially disrupt or displace the embolism. In these deaths, the absence of a

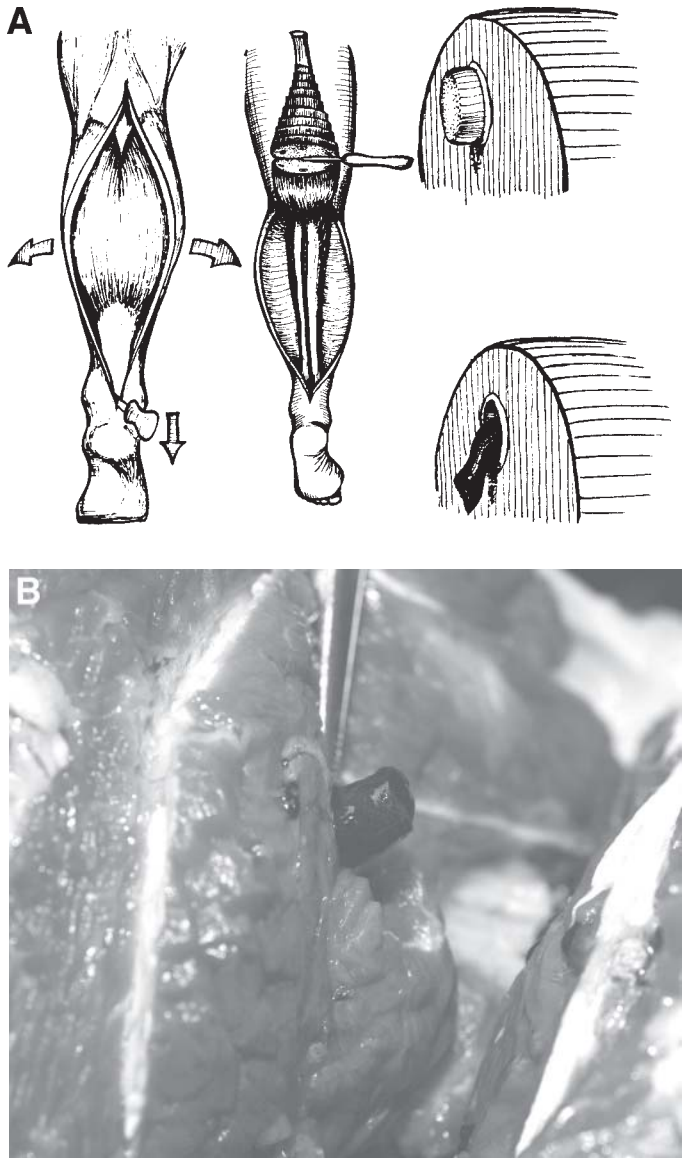

Fig. 2. (A) Technique of DVT leg dissection with thrombus (upper cross-section) and postmortem clot (lower cross-section). **(B)** DVT as seen at dissection of the leg. (Fig. 2A reprinted with permission from Spitz and Fisher's Medicolegal Investigation of Death, 3rd ed., Figure XXI, page 779; 1993.)

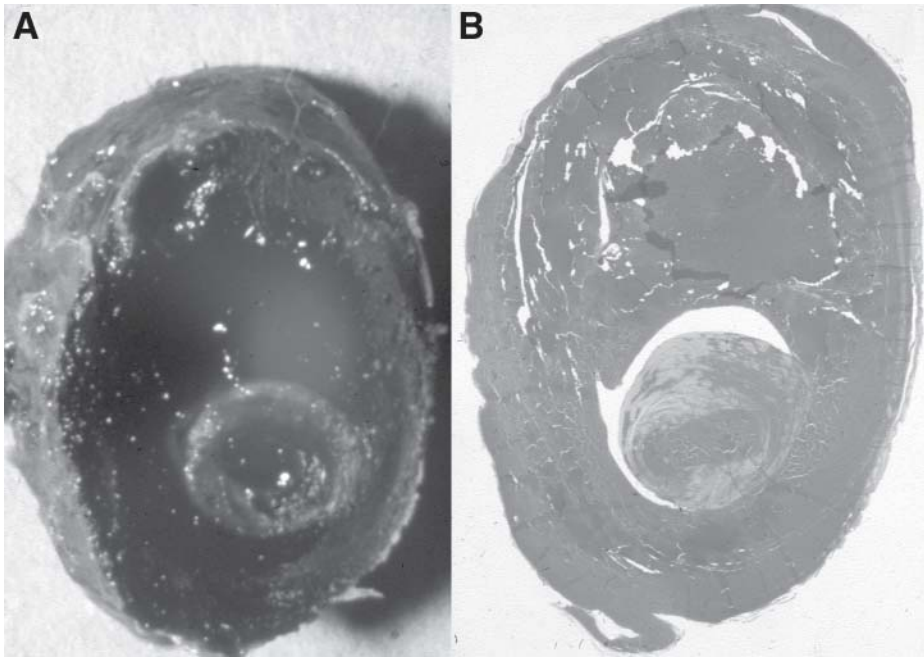

Fig. 3. (A) Gross section of embolism encased in postmortem clot. **(B)** Microscopical section of the same embolism (hematoxylin and eosin stain). (Courtesy of Dr. Mark Flomenbaum, Office of Chief Medical Examiner, New York City, New York.)

saddle embolism at autopsy does not exclude a pulmonary embolism. Histological examination of the lung will typically demonstrate thromboemboli in small blood vessels. Removal of the saddle embolism by a tissue harvesting team who has removed the heart for valve tissue donation also should be considered. In one such death, the embolism was found undisturbed in the pericardial sac with a chart notation by the recovery team of its discovery at harvesting.

Postmortem artifacts, particularly related to embalming, should be considered. Arterial embalming may dislodge postmortem clots in veins that then may "embolize" toward the heart. Performing a microscopical examination can distinguish these false emboli. Postmortem clot surrounding a thrombus in the main pulmonary artery may mask the typical tangled gross appearance of the PE. Performing serial sectioning of the "clot" and a histological examination will confirm these true emboli (Fig. 3A,B).

3.2. Histopathology

The age of a DVT may have medicolegal importance. Various microscopical criteria may be used to estimate the age of a thrombus *(40)*. Typically, one may determine whether a thrombus is acute, organizing, or remote. Because of individual variability, it is not possible to determine a precise age. The histological appearance, however, can be compared with the clinical information (*see* Section 5). An opinion may be given on whether the age of a thrombus is or is not consistent with a known history. Because of the pathophysiology of thrombus formation, there may be thrombi of various ages in the same vein. Because for medicolegal purposes, the maximum age usually is most important, adequate sampling of the thrombus is needed. The veins of both legs, including segments with and without thrombus, may be sampled with appropriate "right" and "left" designations.

In one case, there were deep-red, slightly granular emboli in the proximal pulmonary arteries. Microscopically, there were lines of Zahn and fibrin thromboemboli scattered in distal small pulmonary vessels. Partial recanalized, gray–tan thrombi of the deep veins of the right lower leg extended to the right popliteal fossa. At the proximal and distal aspects, the thrombus was deep red. Microscopically, sections of the deep veins showed thrombi that ranged from acute to organized. Portions of the thrombus showed fibroblast proliferation with recanalization, and hemosiderin-laden macrophages (Fig. 4A,B).

Histological examination of the lungs may demonstrate thrombotic lesions in small arteries. These typically are prior small emboli but also may be seen with acute larger emboli that have been treated with thrombolytics. Widespread primary thrombosis in the pulmonary artery, though rare, does occur, particularly with primary pulmonary hypertension. Morphologically, it is difficult to distinguish emboli from primary thrombi in the small vessels of the lung. Other findings (e.g., a proximal PE, DVT, or plexogenic pulmonary arteriopathy) may clarify the pathology *(24)*.

4. Thrombophilias

There is a subgroup of patients who die of PE without known major risk factors *(19,20,29,30,41)*. The role of previously unrecognized hypercoagulable states in these deaths can now be further investigated. With the use of DNA techniques (polymerase chain reaction), new diagnoses of certain genetic thrombophilias can be made *(42,43)*. In some deaths, the medicolegal investigation of PE may include genetic analysis for particular mutations that increase the risk of DVT. These include factor V, prothrombin, and MTHFR muta-

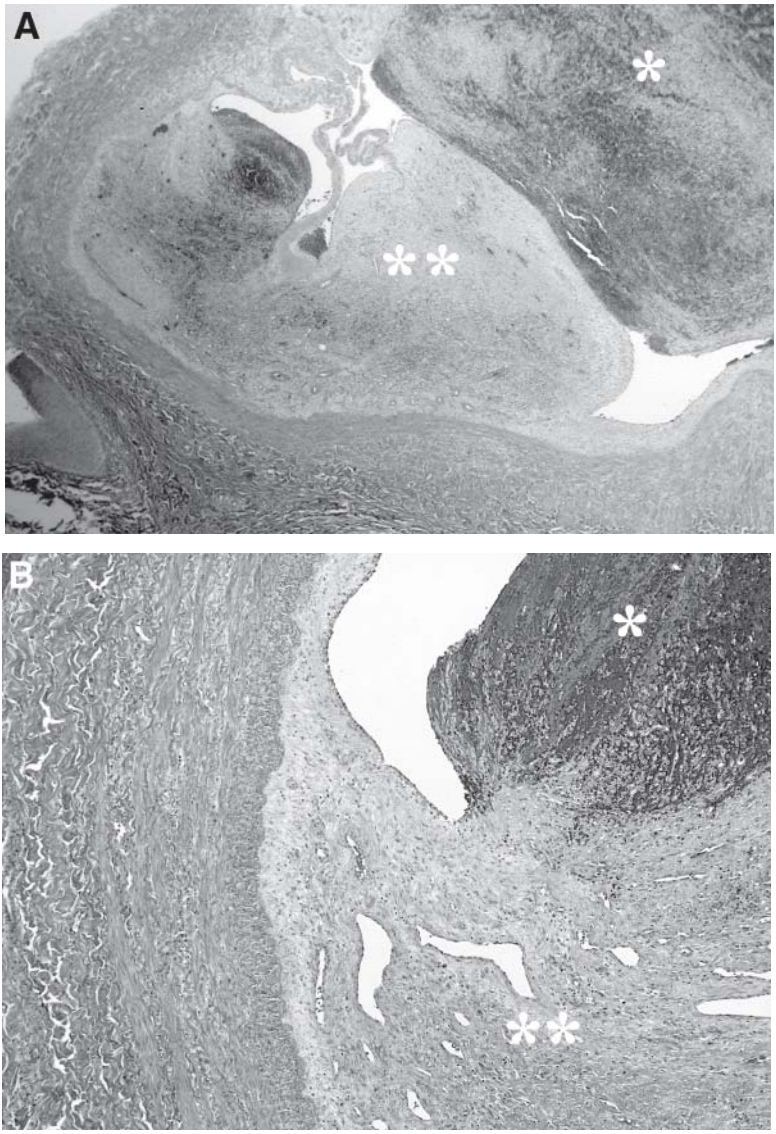

Fig. 4. (A,B) Sections of a DVT from the same leg vein with acute (*) and organizing (**) morphology (hematoxylin and eosin stain). The acute component (*) is a layered mixture of blood and fibrin. The organizing component shows granulation tissue, neovascularization, and hemosiderin deposition.

tions. Functional coagulation studies, including factor concentration assays, are useless on postmortem specimens and of questionable benefit antemortem during an acute thrombotic event *(44)*. Because the genetic tests involve DNA analysis, they are well suited for postmortem analysis.

The DNA tests are costly, and the clinical benefits for relatives have been debated *(14,15,17,18)*. In selected cases, these results may be helpful in fully elucidating the cause of death. Decedents who are candidates for these tests include those who are younger than 45 years of age; those whose deaths were related to pregnancy; those with a history of recurrent or unexplained stillbirths, oral contraceptive pill use, hormone replacement, or treatment with chemotherapy (methotrexate or tamoxifen), particularly if cancer-free at autopsy; those with weak risk factors (long car ride, flights, obesity); or those with DVT of undetermined etiology.

4.1. Activated Protein C Resistance (Factor V Leiden Mutation)

Resistance to activated protein C, the most potent endogenous anticoagulant, is the result of a mutation of the factor V gene (e.g., the Leiden mutation), which results in decreased control of thrombin generation. Normally, activated factor V augments factor X which leads to the production of thrombin. Downregulation of thrombin generation is controlled by activated protein C, which inactivates factor Va. The Leiden mutation (Arg506Gln) of factor V makes it more difficult for protein C to inactivate factor V. This leads to prolonged thrombin generation.

In North America, the heterozygous factor V Leiden is most common in Caucasians (up to 7% incidence) and causes an increased risk for VTE of up to sevenfold *(45–47)*. The homozygous mutation has been reported to result in an 80-fold increase in relative risk for DVT *(45,48,49)*. The hereditary nature of the mutation was confirmed by Simioni et al. in 1997, who identified the defect in at least one first-degree relative of all 41 of their patients who were diagnosed with factor V Leiden *(47)*.

4.2. Prothrombin Mutation

The G20210A autosomal dominant mutation in the prothrombin gene is associated with an increased amount of prothrombin, which promotes the formation of thrombin. The prothrombin mutation is highest in Caucasians (1–3%) and has an up to threefold increase in relative risk for DVT *(21,43,50)*. Patients with mutations in both the prothrombin gene and factor V are at an even higher risk *(49)*.

4.3. Hyperhomocystinemia

Hyperhomocystinemia (plasma homocystinemia concentration >15 μmol/L) is a risk factor for venous (and arterial) thrombosis. The mechanism of thrombosis is unknown; however, hyperhomocystinemia is an independent risk factor for VTE *(12,51)*. The relative risk for VTE in patients with elevated homocysteine concentrations (>95th percentile) has been demonstrated to be twice that of the patients with lower concentrations *(15,51,52)*. The risk of VTE in patients with both hyperhomocystinemia and factor V mutations is greater than the sum of the individual risks *(52)*.

Increased concentration of homocysteine is determined partly by enzymes involved in its metabolism. Mutations in MTHFR and cystathionine-B-reductase are associated with elevated concentrations of homocysteine. Currently, there are no clinical tests available for the cystathionine-B-reductase mutations. Hyperhomocystinemia also may be owing to acquired defects. Deficiency in folic acid or vitamins B_{12} or B_6, increasing age, hypothyroidism, and renal failure can all affect homocysteine metabolism. Postmortem testing of homocysteine concentrations is unreliable *(19)*.

4.4. Other Thrombophilias

Other hereditary thrombophilic disorders (autosomal dominant) include antithrombin, protein C, and protein S deficiencies. Antithrombin inhibits thrombin and other clotting factors. Proteins C and S promote anticoagulation through inactivation of factors Va and VIIIa. Mutations in any of these three factors can result in deficiencies and thereby increase the risk of thrombosis. Assays on postmortem blood samples for antithrombin and proteins C and S are not reliable. In addition, their concentrations may be depressed during an acute thrombotic event, which can result in misleading results *(44)*. Because these three factors are produced by hepatocytes, liver disease may cause an acquired deficiency.

The antiphospholipid syndrome is an acquired thrombophilia, which involves antibodies directed at phospholipid-protein complexes. The cause of antiphospholipid syndrome may be idiopathic or associated with autoimmune diseases (e.g., systemic lupus erythematosus), medications, or malignancies. It increases the risk of thrombosis through inhibition of endothelial activation of protein C. Clinically, tests for the lupus anticoagulant are performed because the result may affect anticoagulation management *(44)*.

5. Medicolegal Aspects of Pulmonary Thromboembolism

The timing of VTE may have important medicolegal consequences *(53)*. DVT may form in hours, and the resultant embolism often occurs after a sudden onset of movement after inactivity. Although immobility because of prolonged sitting has been found to be a weak risk factor *(12)*, there has been interest in the lay press and studies have examined the risk *(31,32,54)*. A review of nonfatal PE after airplane travel found that the incidence of PE was markedly higher among passengers who traveled by air for more than 5000 km *(31)*. None of the initial presentations of the PE occurred beyond the jetway in passengers arriving after long flights *(31)*. Because of study limitations, symptomatic cases that occurred beyond the airport facility and fatalities were not included.

Most surgical teaching is that a DVT starts "on the table." The use of preoperative, operative, and postoperative prophylaxis usually is dictated by individual patient risk factors and the type and anticipated length of the surgical procedure *(11,12,55)*. Studies on postoperative PE have found that PE may present up to 1 month after surgery *(25,44)*. Bergqvist and Lindblad *(56)* found that 25% of postoperative PE occurred 15 to 30 days following surgery and 15% occurred more than 30 days after surgery. Some have described intervals of up to 90 days after an injury or surgery for the occurrence of a pulmonary embolism *(57)*. For the medicolegal investigator, it becomes more difficult to find or establish an initiating link when the interval increases beyond several weeks.

Because of the pathophysiology and propagation of a thrombus, one may see a broad histological range of thrombosis and organization. Histological examination of residual DVT gives the best opportunity to properly age the thrombus. The embolism will typically appear recent and offers little help in dating the initiating thrombosis. Thorough sampling of the deep leg veins, with particular attention to the thrombo-endothelial junction, provides the most information (58). Endothelial proliferation, collagen deposition, hemosiderin, and neovascularization are common features to note *(58)*.

Depending upon the particulars of an investigation, one may offer an opinion of the age (and therefore possibly the etiology) of a thrombus. For example, a woman presents to the emergency department with a swollen ankle. She has had decreased activity because she injured her ankle several days ago. A lower fibula fracture is diagnosed, and a surgical pinning is performed. One day after surgery, she dies of a pulmonary embolism. At autopsy, there is organizing thrombus in both leg veins. These findings are consistent with the initiation and development of the thrombus caused by inactivity prior to medi-

cal attention. The detection of thrombi in both legs is consistent with risk factors from stasis and decreased mobility as opposed to a direct injury of one leg. The proximate cause (the blunt injury of the ankle) would be the same; however, the role of the surgery and subsequent immobility would be reduced. Medicolegally, this may be important in certain cases of minor injury in which ambulation from the injury was or was not impaired.

6. *Conclusion*

Despite clinical recognition of risk factors and various prophylactic therapies, VTE remains an unexpected cause of death that cannot be completely prevented. Because of its association with injury and its sudden appearance, the forensic pathologist commonly investigates it. The autopsy evaluation of a pulmonary embolism is enhanced by dissection of the leg veins with microscopical examination. As the availability increases and the cost decreases, genetic testing will become a common component of the evaluation of these deaths. Clinicians still need to fully define how this information may alter patient management; postmortem testing will provide valuable mortality data and have a positive impact on the living.

References

1. Consensus-Conference (2002) College of American Pathologists Consensus Conference XXXVI: Diagnostic Issues in Thrombophilia. Arch Pathol Lab Med 126, 1277–1433.
2. Anderson FA, Wheeler B, Goldberg RJ, et al. (1991) A population-based perspective of the hospital incidence and case-fatality rates of deep venous thrombosis and pulmonary embolism. Arch Intern Med 151, 933–938.
3. Dalen JE, Alpert JS (1975) Natural history of pulmonary embolism. Pro Card Dis 17, 259–270.
4. Gillum RF (1987) Pulmonary embolism and thrombophlebitis in the US, 1971–85. Am Heart J 114, 1262–1264.
5. Rossman I (1974) True incidence of pulmonary embolization and vital statistics. JAMA 230, 1677–1679.
6. Wood KE (2002) Major pulmonary embolism: review of a pathophysiologic approach to the golden hour of hemodynamically significant pulmonary embolism. Chest 121, 877–905.
7. White RH (2003) The epidemiology of venous thromboembolism. Circulation 107, I4–I8.
8. PIOPED-Investigators (1990) Value of the ventilation/perfusion scan in acute pulmonary embolism. Results of the prospective investigation of pulmonary embolism diagnosis (PIOPED). JAMA 263, 2753–2759.
9. Carson JL, Kelley MA, Duff A, Weg JG, Fulkerson WJ, Palevsky HI, et al. (1992) Clinical course of pulmonary embolism. N Engl J Med 326, 1240–1245.

10. Dalen JE (2002) Pulmonary embolism: what have we learned since Virchow? Natural history, pathophysiology, and diagnosis. Chest 122, 1440–1456.
11. Dalen JE (2002) Pulmonary embolism: what have we learned since Virchow? Treatment and prevention. Chest 122, 1801–1817.
12. Anderson FA Jr, Spencer FA (2003) Risk factors for venous thromboembolism. Circulation 107, Suppl 1, I9–I16.
13. Becattini C, Agnelli G (2002) Pathogenesis of venous thromboembolism. Curr Opin Pulm Med 8, 360–364.
14. Greaves M (2001) Thrombophilia. Clin Med 1, 432–435.
15. Locke CF, Evans NC (2003) Evaluating idiopathic venous thromboembolism: what is necessary, what is not. J Fam Pract 52, 770–777.
16. Bertina RM, Koeleman BP, Koster T, et al. (1994) Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 369, 64–67.
17. Vandenbroucke JP, van der Meer FJ, Helmerhorst FM, Rosendaal FR (1996) Factor V Leiden: should we screen oral contraceptive users and pregnant women? BMJ 313, 1127–1130.
18. Bauer KA (2001) The thrombophilias: well-defined risk factors with uncertain therapeutic implications. Ann Intern Med 135, 367–373.
19. Miller EJ, Marques MB, Simmons GT (2003) Etiology of pulmonary thromboembolism in the absence of commonly recognized risk factors. Am J Forensic Med Pathol 24, 329–333.
20. Andrew TA, Fairweather R (2003) Prothrombin G20210A mutation and sudden death. Am J Forensic Med Pathol 24, 377–380.
21. Knight B, Zaini MRS (1980) Pulmonary embolism and venous thrombosis. Am J Forensic Med Pathol 1(3), 227–232.
22. Kakkar VV, Howe CT, Flanc C, Clarke MB (1969) Natural history of postoperative deep-vein thrombosis. Lancet 6, 230–232.
23. Goldhaber SZ, Elliott CG (2003) Acute pulmonary embolism: part I: epidemiology, pathophysiology, and diagnosis. Circulation 108, 2726–2729.
24. Wagenvoort CA (1995) Pathology of pulmonary thromboembolism. Chest 107, Suppl, 10S-17S.
25. Lindblad B, Eriksson A, Bergqvist D (1991) Autopsy verified pulmonary embolism in a surgical department 1951–88. Br J Surg 78, 849–852.
26. DiSiena MR, Intres R, Carter DJ (1998) Factor V Leiden and pulmonary embolism in a young woman taking an oral contraceptive. Am J Forensic Med Pathol 19, 362–367.
27. Svendsen E, Karwinski B (1989) Prevalence of pulmonary embolism at necropsy in patients with cancer. J Clin Pathol 42, 805–809.
28. Otten HM, Mathijssen J, ten Cate H, et al. (2004) Symptomatic venous thromboembolism in cancer patients treated with chemotherapy: an underestimated phenomenon. Arch Intern Med 164, 190–194.
29. Zimmerman T, Adelson L, Ratnoff O (1971) Pulmonary embolism and unexpected death in supposedly normal persons. N Engl J Med 283, 1504–1505.
30. Breckenridge RT, Ratnoff OD (1964) Pulmonary embolism and unexpected death in supposedly normal persons. N Engl J Med 270, 298–299.

31. Lapostolle F, Surget V, Borron SW, et al. (2001) Severe pulmonary embolism associated with air travel. N Engl J Med 345, 779–783.
32. Gallus AS, Goghlan DC (2002) Travel and venous thrombosis. Curr Opin Pulm Med 8, 372–378.
33. Hunsaker D, Hunsaker JC 3rd (2005) Obesity Epidemic in the United States. In Tsokos M, ed., Forensic Pathology Reviews, Vol. 2. Humana Press Inc., Totowa, NJ, pp. 61–98.
34. Cotran RS, Kumar V, Collins T (1994) Robbin's Pathologic Basis of Disease. W.B. Saunders Company, Philadelphia.
35. Sekiguchi C (1994) Issues of health care under weightlessness. Acta Physiol Scand Suppl 616, 89–97.
36. Hargens AR (1994) Recent bed rest results and countermeasure development at NASA. Acta Physiol Scand Suppl 616, 103–114.
37. Natelson BH, Goldwater DJ, De Roshia C, Levin BE (1985) Visceral predictors of cardiovascular deconditioning in late middle-aged men. Aviat Space Environ Med 56, 199–203.
38. Takenaka K, Suzuki Y, Kawakubo K, et al. (1994) Cardiovascular effects of 20 days bed rest in healthy young subjects. Acta Physiol Scand Suppl 616, 59–63.
39. Adams VI, Hirsch CS (1993) Sudden and unexpected death from natural causes in adults. In Spitz WU, ed., Spitz and Fisher's Medicolegal Investigation of Death. Charles C Thomas, Springfield, p. 164.
40. Knight B (1984) The dating of pulmonary emboli. Acta Med Leg Soc (Liege) 34, 190–192.
41. Butler JM, Schoske R, Vallone PM, Redman JW, Kline MC (2003) Allele frequencies for 15 autosomal STR loci on U.S. Caucasian, African American, and Hispanic populations. J Forensic Sci 48, 908–911.
42. Seligsohn U, Lubetsky A (2001) Genetic susceptibility to venous thrombosis. N Engl J Med 344, 1222–1231.
43. Laffan M (1998) Genetics and pulmonary medicine. 4. Pulmonary embolism. Thorax 53, 698–702.
44. Goldhaber S (1998) Pulmonary emboli. N Engl J Med 339, 93–104.
45. Juul K, Tybjaerg-Hansen A, Schnohr P, Nordestgaard BG (2004) Factor V Leiden and the risk for venous thromboembolism in the adult Danish population. Ann Intern Med 140, 330–337.
46. Rees DC (1996) The population genetics of factor V Leiden (Arg506Gln). Br J Haematol 95, 579–586.
47. Simioni P, Prandoni P, Lensing AW, Scudeller A, Sardella C, Prins MH, et al. (1997) The risk of recurrent venous thromboembolism in patients with an Arg506—>Gln mutation in the gene for factor V (factor V Leiden). N Engl J Med 336, 399–403.
48. Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH (1995) High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). Blood 85, 1504–1508.
49. De Stefano V, Martinelli I, Mannucci PM, et al. (1999) The risk of recurrent deep venous thrombosis among heterozygous carriers of both factor V Leiden and the G20210A prothrombin mutation. N Engl J Med 341, 801–806.

50. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM (1996) A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 88, 3698–3703.
51. Ray JG (1998) Meta-analysis of hyperhomocystinemia as a risk factor for venous thromboembolic disease. Arch Intern Med 158, 2101–2106.
52. Ridker PM, Hennekens CH, Selhub J, Miletich JP, Malinow MR, Stampfer MJ (1997) Interrelation of hyperhomocyst(e)inemia, factor V Leiden, and risk of future venous thromboembolism. Circulation 95, 1777–1782.
53. Copeland A (1987) Sudden natural death due to pulmonary embolism in the medical examiners jurisdiction. Med Sci Law 27, 288–293.
54. Ansell JE (2001) Air travel and venous thromboembolism—is the evidence in? N Engl J Med 345, 828–829.
55. Goldhaber SZ, Elliott CG (2003) Acute pulmonary embolism: part II: risk stratification, treatment, and prevention. Circulation 108, 2834–2838.
56. Bergqvist D, Lindblad B (1985) A 30-year survey of pulmonary embolism verified at autopsy: an analysis of 1274 surgical patients. Br J Surg 72, 105–108.
57. Knight B (1966) Fatal pulmonary embolism: Factors of forensic interest in 400 cases. Med Sci Law 6, 150–154.
58. Knight B (1996) Forensic Pathology. Arnold, London.

Suicide

10

Trends of Suicide in the United States During the 20th Century

Lisa B. E. Shields, MD, Donna M. Hunsaker, MD, and John C. Hunsaker III, MD, JD

CONTENTS

SUMMARY

Suicide represents a global health concern that encompasses a myriad of biological, psychological, and social factors. Suicide, strictly speaking a manner of death, currently ranks epidemiologically as the 11th leading cause of death in the United States, accounting for approx 30,000 deaths annually. Although reports of suicide have existed since the Greek and Roman times, the trends of suicide specifically in the United States have drastically changed, especially within the past century. The most striking finding is the significant increase in suicide rate for both men and women in the second and third decade of life. The highest suicide rate throughout the 20th century was recorded for

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Caucasian males and increased with advancing age. In the United States, the national suicide rate decreased by 14% during the last decade of the 20th century, dropping suicide from the 8th to the 11th leading contributory cause of death. Methods of suicide have changed over the century. In 1860, the most common cause of suicide was hanging, which was supplanted by poisoning in 1900. By 1910, and every decade thereafter, death by firearms has prevailed as the primary method of choice. Numerous risk factors have been associated with an increased risk for suicide, such as being divorced or unemployed or suffering from a physical or mental illness. Individuals with a disease of the central nervous system have the highest risk of suicide and may commit suicide within a short time period after diagnosis, or in the late stages of the disease, when the pain and burden have become debilitating. Three mental illnesses most frequently linked to an increased risk of suicide, namely major affective disorder, schizophrenia, and neuropsychological sequelae of chronic alcoholism, are highlighted. The forensic pathologist plays a pivotal role in classifying the manner of death as homicide, suicide, accident, or natural. Both an extensive scene investigation and a thorough postmortem examination with complete toxicological study are warranted in the determination of a suicide. Although "classic" findings at the scene and at autopsy characterize a suicidal death, investigators should be astute to features that may have been intentionally altered to conceal the accurate manner of death. The psychodynamics of the suicide autopsy, which must be realistically evaluated in each case, are discussed in this review. This chapter also addresses causes of suicidal death, including firearm injuries, hanging, stabbing, self-immolation, and overdose. Particular attention to findings at autopsy is addressed. Suicide in the United States is the primary focus of this review because of the wealth of literature and because it is the authors' country of residence. Nevertheless, an international perspective on suicide with specific consideration of gender differences, adolescent suicide, and self-inflicted death by firearms is presented that correlates well with United States findings. In summary, this review encompasses numerous aspects of suicide, including statistics and trends in the United States, psychosocial characteristics, risk factors, and autopsy findings.

Key Words: Suicide; trends; risk factors; firearms; autopsy; forensic pathology.

1. *Introduction*

The determination of a suicidal death ultimately rests on the reasonable inferences establishing the self-inflicted nature of the act and the underlying intent *(1)*. Table 1 indicates certain well-known risk factors. The self-inflicted

Table 1
Risk Factors for Suicide

- Male
- Caucasian
- Elderly
- Divorced
- Unemployed
- Physical disease
- Mental illness
- Spring and summer seasons

basis of the death may be inferred from the pathological and toxicological evidence obtained from a postmortem examination. Certain features apparent on discovery of the victim, such as multiple gunshot wounds of the head, initially may be construed as homicidal. This presumption may well be overcome by a thorough autopsy and collection of pertinent historical information about the decedent. Such investigational evidence may include a detailed scene analysis, police reports, and the victim's medical records. Suicidal intent refers to the decedent's wish to end his or her life and the knowledge that a particular act would result in death *(1)*. Explicit expression of intent in the form of the suicide note occurs in only between 20 and 30% of suicides *(2,3)*. A suicide note may offer explanations as to the motivations behind a suicide and provide a healing or blaming role for the survivors. Ho et al. analyzed the suicide notes of 154 suicides in 1992, accounting for 20% of all suicide victims *(2)*. The majority of authors of the notes were young females of nonwidowed marital status, with no history of previous suicide attempts or psychiatric illness, who held religious beliefs and characteristically wrote passionate letters. Elderly victims composed notes that were more succinct, less emotional, and included detailed instructions pertaining to funeral arrangements and allocations of possessions.

Implicit evidence of intent conveying suicidal ideation may include premature preparations for death, exclamations of farewell in anticipation of an impending death, and a previous suicide attempt. Failed suicide attempts are significant in the psychosocial history of a deceased individual and it is well known that these attempts occur in greater numbers in females. The determination of intent may be obscured by a decedent's history of ethanol intoxication or mental illness *(1)*; however, these conditions do not necessarily preclude an individual from forming intent. Other self-inflicted mortal acts, such as Russian roulette, have been hotly debated without resolution as to whether the underlying motivation is intentional *(4,5)*.

The self-destructive behavior inherent in a suicide may be divided as a triad of intrapersonal and interpersonal characteristics, and implementation *(6)*. The intrapersonal features include both conscious and unconscious intention, specifically, the awareness of the effects of an individual's self-destructive behavior and associated suicidal ideation on others, devices for rescue, and the psychodynamic factors of anxiety, hostility, and dependency. The intrapersonal component also includes "ego organization" in the form of impulsivity versus control, rigidity vs flexibility, and isolation vs relatedness *(6)*. The interpersonal aspect of self-destructive behavior refers to an individual's communicative abilities with others and his or her role in society. Finally, implementation incorporates the lethality of the suicidal method selected, taking into account the reversibility and rescue capability of the action. The contemplation and completion of a suicide encompass a host of biopsychosocial factors. By delving into the underlying motivations behind a suicide and addressing the historical trends of suicide, investigators marshal well-founded support to conclude that the manner of death was suicide.

2. Incidence and Prevalence

The World Health Organization estimates an incidence of 1 million suicides and 10 to 20 times as many attempted suicides worldwide in 2000, amounting to one death every 40 seconds and one attempt every 3 seconds *(7)*. Women were three times more likely than men to attempt suicide *(8)*.

Approximately 30,000 individuals committed suicide in the United States in 2001 *(9)*. The manner of suicide ranked as the 11th leading associated causes of death in the United States in 2001 (Table 2*[9]*). Combining both genders and all ages and races, New Mexico and Montana had the highest suicide rates in 2001 with age-adjusted rates of 20.2 and 19.15 per 100,000, respectively *(9)*. New York and Massachusetts had the lowest suicides rates with age-adjusted rates of 6.45 and 6.43 per 100,000, respectively. Between 1990 and 1994, crude suicide rates in the United States were highest in the west (14.1 per 100,000), followed by the south (13.1), midwest (11.4), and northeast (9.3; ref. *10*). The most popular suicide site worldwide is the Golden Gate Bridge in San Francisco, which more than 800 suicide victims have chosen since its opening in 1937 *(11)*.

3. Trends

The foregoing analysis of distinctive trends of suicide in the United States offers a unique perspective on the methods used to commit suicide, especially

Table 2
The 12 Leading Causes of Death in the United States in 2001

1.	Heart disease
2.	Malignant neoplasms
3.	Cerebrovascular disease
4.	Chronic respiratory disease
5.	Unintentional injury
6.	Diabetes mellitus
7.	Influenza and pneumonia
8.	Alzheimer's disease
9.	Nephritis
10.	Septicemia
11.	**Suicide** (manner; from all types of causes)
12.	Liver disease

when viewed in combination with a relative mix of gender, race, and age of the victims.

3.1. Gender, Age, and Race Trends

Suicide rates have exhibited several striking trends during the past century in the United States with regard to gender, age, and race of victims. The most startling change during this period is the increasing youth suicide rate of both males and females in the 15 to 24 age range. Between 1940 and 1980, the rate for white males aged 15 to 19 years more than tripled, representing a 231% increase, and doubled for white males aged 20 to 24, a 116% increase *(12)*. During this same time span, the rate increased by 262% for non-white males aged 15 to 19 and increased by 182% for non-white males aged 20 to 34. The suicide rate of men older than the age of 65 gradually decreased during this period. The rates for white and non-white women also increased between 1940 and 1980, although not as drastically as seen in the male counterpart.

Between 1980 and 1992, suicide rates in the United States increased for persons aged 10 to 19 years and older than 75 years of age *(13,14)*. The rate increased by 120% (from 0.8 to 1.7) in persons aged 10 to 14 years for both males and females and in all racial groups *(13)*. Furthermore, the rate increased by 28.3% among those aged 15 to 19 years (from 8.5 to 10.9), with a 165.3% increased rate for black males. The rate also significantly increased in the elderly population: 11% between 75 and 79 years, 35% between 80 and 84 years, and 15% 85 years and older *(14)*. Men constituted 81% of suicides of people aged 65 years or older.

Suicide rates in the United States decreased by 14% between 1990 and 1999 *(8)*, in turn, dropping the manner of suicide from the 8th to the 11th overall associated causes of death. Rates decreased in every age bracket, in all races, and for both men and women during the last decade of the 20th century. In 2001, 24,672 men and 5950 women committed suicide in the United States, ranking the manner of suicide 8th and 19th for men and women, respectively, among leading associated causes of death *(9)*. The manner of suicide ranked as the third leading associated causes of death in 2001 between the ages of 10 and 34 years, after death by unintentional injury and homicide.

During the past century, certain features of suicidal trends have remained the same. The suicidal rates throughout the twentieth century have been the highest for white males and increase with advancing age. In 1999, the suicide rate in the United States was 10.7 per 100,000, whereas the rates for individuals over the age of 65 and 85 were 15.9 and 19.2, respectively *(8)*. Although the number of women aged 65 or older exceeds that of men in this age group in the population, the suicide rate of men is more than six times that of women *(15)*. Furthermore, non-Caucasian suicide rates peak by the fourth decade and subsequently decrease, unlike Caucasian rates, which continue to increase with age.

3.2. Methods of Suicide Trends

The earliest statistical reports of methods of suicide in the United States were documented in 1860 as a component of the Eighth Census *(16)*. The leading cause of suicidal death in 1860 was hanging, followed by poisoning and firearm. A total of 993 individuals, 789 men and 204 women, committed suicide. By 1900, poisoning emerged as the leading cause of suicidal death, followed by firearm and hanging *(17)*. Death by firearm was the primary method chosen by suicidal individuals every decade thereafter during the twentieth century in the United States *(9,17)*. Table 3 documents the leading causes of suicidal death, the suicide rate, the male to female ratio, and the race of suicide victims in the United States between 1860 and 2000 *(9,16,17)*.

Death by firearms has remained the leading cause of suicide in the United States since 1910 for both men and women *(17)*. Approximately 1 million firearm deaths were reported between 1933 and 1982 in the United States, of which suicide comprised 49% of these fatalities *(18)*. Throughout this time period, the firearm suicide rate exceeded that of homicide. The total number of deaths by firearm increased by 137% between 1962 and 1993, with suicide accounting for the largest percentage *(19)*.

An ongoing debate has focused on the relationship between household gun ownership and increased rate of suicide by firearm *(20–24)*. In a regional

Table 3
Suicide in United States With Respect to Methods, Gender, and Race: 1860–2000

Year[a]	Firearms	Poisoning	Hanging/ strangulation	Males/ females	White/ non-white
1860[b]	#3	#2	#1	789/204	N/A
1900 (10.2)	#2 (2.2)	#1 (3.1)	#3 (2.0)	15.7/4.7	N/A
1910 (15.3)	#1 (4.6)	#2 (4.1)	#3 (2.4)	23.0/7.2	15.4/11.8
1920 (10.2)	#1 (3.6)	#3 (1.6)	#2 (1.9)	14.5/5.7	10.8/3.6
1930 (15.6)	#1 (5.7)	#3 (2.5)	#2 (2.8)	24.1/6.9	16.8/5.0
1940 (14.4)[c]	N/A	4.3	N/A	21.9/6.8	15.5/4.6
1950 (11.4)	#1 (4.9)	#2 (2.6)	#3 (2.4)	17.3/4.9	11.6/4.7
1960 (10.6)	#1 (5.0)	#2 (2.4)	#3 (1.9)	16.6/5.0	11.1/5.4
1970 (11.6)[d]	#1	#2	#3	16.8/6.6	12.4/5.6
1981 (12.0)	#1 (7.0)	#2 (2.4)	#3 (1.6)	19.1/5.8	12.7/6.5
1990 (12.4)	#1 (7.6)	#2 (2.2)	#3 (1.8)	21.2/4.8	13.2/7.2
2000 (10.4)	#1 (5.9)	#3 (1.7)	#2 (2.0)	17.8/4.0	11.3/5.5

Note: [a]Suicide rate per 100,000 in parentheses. [b]In 1860, the rates of the cause of death were not available, and the male-to-female ratio represents the total number of victims in the United States. [c]In 1940, other causes of suicidal death besides poisoning are grouped with a rate of 10.1. [d]In 1970, the rates of the cause of death were not available.

# Indicates the prevailing cause of death.

and state-level analysis of the United States, a high correlation was obtained between the rates of household firearm ownership and suicide *(23)*. Individuals living in a high-gun state (Louisiana, Alabama, Mississippi, Wyoming, West Virginia, and Arkansas) were 3.8 times more likely to commit suicide using a firearm as compared to a low-gun state (Hawaii, Massachusetts, Rhode Island, and New Jersey). In a study of 803 suicides in both Shelby County, Tennessee and King County, Washington between 1987 and 1990, Kellermann et al. reported 326 (58%) suicides by firearm occurring in the victim's home *(24)*. Handguns were used in 72% of the cases, and the gun had been stored in the victim's home in 80% of the suicides by firearms. The majority of subjects kept guns in the home for months or years prior to their death whereas only a few victims had acquired the weapon within hours or days of their death. On this evidence they concluded that the ready availability of firearms increased the risk of suicide in the home and suggested that individuals should weigh the benefit of gun ownership when it may raise the possibility of a future suicide in a family member or self *(24)*.

3.3. International Trends

The comparison of suicidal trends between nations affords a unique outlook into the study of suicide and may provide an innovative method of confirming certain higher risk factors for suicide as a means of achieving the ultimate goal of treatment and prevention. Of the 62 countries reporting suicide data to the World Health Organization between 1960 and 1985, 42 (67.8%) nations experienced increased suicide rates during this period, reflecting an average percent change of 37% and a range from 66 to 437% *(25)*. This increase in rates was primarily observed in the 15- to 20-year age group worldwide. Diekstra pointed out that this age group constituted the children of the post-World War II baby boom whose large numbers spurred competition for jobs and participated in political uproar in Europe and the United States *(25)*. The year 1910 also marked a peak in youth suicide internationally *(25)*. Of interest, the first scientific conference on suicide was held in 1910 in Vienna, where Sigmund Freud was one of the participants. The rampant political, social, and cultural changes at the turn of the twentieth century, which culminated in World War I in 1914 and the Russian Revolution in 1917, may be associated with the increase in youth suicide in 1910. Between 1970 and 1984 the male suicide rate increased in 21 of 23 countries worldwide, while the female rate only increased in 14 nations *(26)*. During the 1980s and 1990s, two striking trends were noted internationally, namely a decrease in the female suicide rate and a substantial rise in the rate of adolescent suicide *(27)*.

In 1990, the highest suicide rates were recorded in Hungary and Sri Lanka, at 39.9 and 33.2, respectively; Kuwait and Egypt had the lowest rates, at 0.8 and 0.004, respectively *(27)*. The male suicide rate was higher than that of females in all countries in 1990 except China. Yip's study of suicide in Beijing between 1987 and 1996 found that the female suicide rate for ages 15 to 44 was higher than that of males *(28)*. Of special note, the female rate was 1.6 times greater than that of males in the age group 15 to 24. Factors attributed to the higher female rate were the patriarchal social structure in China, which may instill hopelessness and helplessness in women and promote suicide *(29)*. Furthermore, approximately half of Chinese women commit suicide by ingestion of poison, specifically, widely available lethal insecticides and herbicides.

Although an increase in adolescent suicide has been observed internationally, France has experienced a substantial rise in suicidal behavior among young adults. Approximately 40,000 adolescents are evaluated annually in a hospital after a suicide attempt, and the manner of suicide ranked as the second most common associated cause of death in the 15 to 24 year age group behind motor vehicle accidents and as the leading associated cause in the 25 to 35 year age group in 1993 *(30)*. Furthermore, a fivefold rise in suicide rate in

men aged 15 to 24 and a doubling of the rate in women in the same age group between 1955 and 1987 were recorded in New Zealand *(31)*.

Initially observed in 1910, death by firearm has remained the leading method of suicide in the United States throughout the twentieth century. In contrast to the United States, the three most common causes of death in 1862 suicides in northwest London between 1957 and 1977 were as follows: drug poisoning, in most cases barbiturates, in 48% of the victims, followed by carbon monoxide intoxication in 32%, and physical injury in 20% of the subjects *(32)*. The latter category included all methods of physical self-destruction, specifically shooting, stabbing, drowning, hanging, jumping from a height, and self-immolation. In all modalities, males constituted the majority of cases (77%). Internationally, there also appears to be a striking connection with gun ownership and increased suicide rates. Killias reported findings of an international survey of 11 European countries, Australia, Canada, and the United States focusing on the relationship between gun ownership and the suicide rate *(33)*. He observed a direct correlation between household gun ownership and use of a gun to commit suicide. The highest percentage of households with a gun (48.0%) and the highest rate of suicides using a gun (72.8 per million) were recorded in the United States. On the other hand, the Netherlands reported the lowest percentage of guns in the household (1.9%) and the lowest rate of self-inflicted gunshot wounds (2.8 per million).

3.4. Marital Status

Marriage tends to provide protection against suicide for both genders, more so for men than women *(34)*. Marriage resulting in children confers a protective factor against suicide, with a rate of 11 per 100,000 *(11)*. Although never-married people have a suicide rate twice that of married, divorced individuals have the greatest rate of suicide, where a rate of 69 per 100,000 for men and 18 per 100,000 for women applies. Never-married, divorced, widowed, or separated individuals with a mental illness have the highest risk of suicide *(34)*.

3.5. Employment

Unemployment has been closely associated with suicidal risk *(35,36)*. In particular, unemployed men and women ages 24 to 44 and men ages 45 to 64 are two to three times more likely to commit suicide than employed individuals of these ages *(35)*. Unemployment may increase the risk of suicide by either exacerbating a preexisting or provoking a mental illness, such as depression, anxiety disorders, and alcoholism. It may also intensify stressful life situations, including financial hardships and domestic strife.

Studies have documented an inverse relationship between socioeconomic status and suicide. In a study of 1210 suicides in Detroit, Michigan, the suicide rate of blue-collar workers was 44.2 compared with 17.8 for white-collar workers *(37)*. The suicide rate of laborers was 4.6 times that of professional-technical workers. Of the high-status societal positions, several studies have suggested that physicians and dentists have an increased risk of suicide *(11,38,39)*, most commonly resulting from substance overdoses rather than firearms, the primary modality among the general population *(11)*. Physicians who commit suicide have often suffered from a mental illness, including depression and substance abuse, in conjunction with a recent professional or domestic hardship. Individuals with a preemployment psychiatric illness often are enticed to pursue specific occupations. Persons with a depressive disorder who seek a high-status position in the medical field are drawn to psychiatry *(40)*. Artists also have a high rate of suicide, which may be attributed to a concomitant psychiatric disorder. Artists have a suicide risk 2.25 higher than that of the general population *(41)*. In a study of 30 creative writers, 80% suffered from an affective disorder, whereas 43% were afflicted with bipolar disorder *(42)*.

3.6. Seasons

Contrary to the popular view, suicide frequencies generally peak in the spring and summer months and are at their lowest in the autumn and winter months *(43)*. December frequencies fall 10% below other months, and the Christmas and New Years holidays are the least likely times to commit suicide of the entire year *(43,44)*. Social isolation and stressful situations during the holiday season can lead to suicidal ideation. However, the support system offered by family bonding and religious traditions in December may dispel suicidal thoughts and offer hope and a positive outlook for those contemplating suicide.

Studies have investigated the relationship between season and method of suicide *(43,45)*. Hanging peaked in the spring for both men and women, and drowning and jumping from heights were most common in the summer months *(45)*. Traffic suicides experienced a trough in the winter for both genders. The distribution of suicides throughout the week reveals that the number of suicides is highest on a Monday for both men and women and lowest on Saturday for men and Sunday for women *(46)*.

3.7. Physical Health

Physical illness has been associated with an increased risk of suicide (Table 4). Thirty-two percent of suicide victims have received medical atten-

Table 4
Suicidal Ideation Resulting From Disease States

Physical	Emotional
• Highest suicidal risk associated with diseases of central nervous system: epilepsy, multiple sclerosis, spinal cord and head injuries, Huntington's disease • Chronic and debilitating nature of physical disease	• Loss of control over one's life • Severing of ties with support systems • Feelings of impending mortality

tion within 6 months of death *(11)*. Patients with diseases of the central nervous system have the highest risk of suicide, with the most common being epilepsy, multiple sclerosis, spinal cord and head injury, and Huntington's disease *(11,47)*. Individuals suffering from a physical illness may experience a loss of control over their life, leading to helplessness and hopelessness, a severing of ties with one's support system, a feeling of mortality, and an inability to exercise logic and reason *(48)*. The pain often associated with chronic debilitating diseases forces sufferers to depend on others for all aspects of daily life, a dependency that may promote suicidal thoughts *(47)*. The timing of suicide after diagnosis of an illness varies. In certain cases cancer patients are more likely to commit suicide in the advanced stages of their illness when their prognosis is poor *(49)*, as opposed to others, who commit suicide within a short time of receiving their diagnosis, presumably because of the fear of the consequences that will ensue *(50)*.

3.8. Mental Health

Three psychiatric diagnoses have been closely linked to an increased risk of suicide: major affective disorder (including unipolar depression and bipolar disorder), schizophrenia, and chronic alcoholism (Table 5 *[34,51]*). A combination of a depressive-mood disorder and substance abuse significantly increases the risk of suicide, as 70 to 80% of suicide victims are diagnosed with comorbidity *(52)*. Furthermore, about 90% of adults who commit suicide have at least one of the *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition) psychiatric diagnoses. The suicidal risk is 3 to 12 times greater for psychiatric patients than the overall general population. The mean age of psychiatric suicide victims is generally younger than the typical older age of suicide victims in the same population *(11)*. The younger age of the

Table 5
Mental Illnesses Associated With Increased Risk of Suicide

Mental illness	Distinguishing characteristics in relation to suicide
Major affective disorder (unipolar depression, bipolar disorder)	• 25–30% lifetime probability of suicide • Depression: Initiation of end of depressive episode; Within 6 months of discharge from hospital • Bipolar disorder: First-degree family member suicide; History of suicide attempts
Schizophrenia	• 10–20% lifetime probability of suicide • Young male • Early stage of illness • Recent discharge from hospital • High intelligence and expectations • Illness well-controlled with antipsychotic medication
Chronic alcoholism	• 15–25% lifetime probability of suicide • Caucasian, middle-aged male • Unmarried • Within 6 weeks of interpersonal loss/ conflict • Increased aggression, psychological distress

psychiatric suicides is ascribed to the early-onset illness of major depressive disorder and schizophrenia. Suicide risk is increased in depressed patients at the initiation or end of a depressive episode and within 6 months of discharge from the hospital *(11,53)*. Only 5% of suicidal patients use their prescribed antidepressants as a mode to commit suicide *(54,55)*. These findings suggest that these medications may be underprescribed, ineffective in treating a suicidal patient, or may entice an individual with preexisting suicidal ideation to commit suicide. The volatility of bipolar disorder, which is characterized by inherent mania marked by irritability and excessive energy and coupled with the defeating depressive lows, embraces the escape mechanism that suicide

offers. Risk factors for an individual with bipolar disorder include both a first-degree family history of suicide and a history of suicide attempts *(56)*.

Approximately 10% of individuals with schizophrenia will commit suicide, whereas two to five times as many will attempt suicide *(57)*. Risk factors include being young, male, in the early stages of the illness, recently discharged from the hospital, and possessing high intelligence with high expectations. Similar to other suicide victims, schizophrenics who commit suicide often experience a recent loss or stress, feelings of hopelessness and isolation, and a lack of self-esteem. Paradoxically, schizophrenics are more likely to take their lives when their symptoms are under control, while compliantly taking their antipsychotic medications, without reference to an exacerbation of their illness *(58)*. Explanations for this pattern include postpsychotic depression that may lead to suicidal ideation or akathisia, an uncomfortable restlessness that often accompanies antipsychotic medication, which can induce suicidal behavior. A study by Chute et al. at the Office of the Chief Medical Examiner in Maryland evaluated 66 individuals with schizophrenia who died suddenly between 1994 and 1996. Thirteen (20%) schizophrenic victims committed suicide, compared with approx 6% of suicidal deaths in the general Maryland population *(59)*. The schizophrenics' most common causes of death were hanging and jumping from a height. They were less likely to commit suicide by firearms (<20%), as opposed to 53 to 58% of victims of a self-inflicted gunshot wound in the general population in the state.

Alcohol dependence affects 20.1% of men and 8.2% of women over their lifetimes; 200,000 individuals succumb to alcohol-related deaths annually *(60,61)*. The majority of alcohol-dependent suicide victims are Caucasian, middle-aged males who are unmarried and socially isolated *(11)*. As many as 50% had experienced an interpersonal loss or relationship disruption within 1 year and, in many cases, within 6 weeks of their suicide *(11,62,63)*. Alcohol consumption influences suicidal behavior through a variety of methods. Most common are its ability to increase aggressiveness and psychological distress, to crumble barriers during sobriety that protect against suicide in an individual with preexisting suicidal ideation, and to distort cognitive sensibilities that impair the development of coping strategies *(64,65)*. Alcohol dependence in association with comorbid psychopathology, such as major depressive disorder, increases the risk of suicidal ideation and attempts. The cause-and-effect relationship between alcohol consumption and suicidal ideation remains unclear in that alcohol use may aggravate or ameliorate psychopathology; conversely, one's psychopathology may lead an individual to consume alcohol *(65)*.

Table 6
Risk Factors for Suicide in Adolescents and Young Adults

- Psychiatric disorders: affective, conduct, and eating disorders, substance abuse, aggressive and antisocial behavior
- Interpersonal conflict with a lack of family support
- Family history of suicide
- Financial and legal difficulties
- Employment and school problems
- Physical and sexual abuse
- Risk-taking behavior: reckless driving, run away from home, theft, assault, truancy

4. Highlighting Three Populations: Young Adults, Older Adults, and Women

Special attention should be focused on high-risk groups, specifically adolescents and young unmarried adults and the elderly. Although women have a lower rate of suicide than men, women are significantly more likely to attempt suicide. Each of these groups of individuals possesses features that place them at risk for suicidal behavior.

4.1. Young Adults

The estimated ratio of attempted suicides to completed suicides for adolescents is 200:1 *(66)*, which is significantly higher than that of the general population with an estimated 10 to 25 attempts for every completed suicide *(8)*. In a study of 31 suicides in persons who were younger than 18 years of age, 42% had histories of risk-taking behaviors, including reckless motorcycle riding, running away from home, auto theft, assault, and truancy *(67)*. Numerous proposed psychosocial factors increase the risk of adolescent suicide and are tabulated in Table 6. The majority of adolescent suicide victims suffer from either single or combined psychiatric disorders, including affective disorders, substance abuse, anxiety or conduct disorders, eating disorders, and aggressive and antisocial tendencies *(68–73)*. Of special note is the adolescent "exposure to suicides" that poses a significant risk factor for adolescent suicide, namely either experiencing the suicidal death of a member of a young individual's peer group or indirectly gaining awareness of suicide through the media or discussions with friends *(72)*.

Studies have attempted to explain the impact of psychosocial dynamics on the developing and impressionable mind of an adolescent. It is postulated that the youth of our society strive to gain independence as they explore and

Table 7
Suicidal Characteristics of the Elderly

- Chronic and painful medical disease: cancer and cardiovascular disease
- Functional impairment
- Less inclined to share thoughts of suicidal ideation
- Social isolation
- Major depressive disorder
- Increased suicidal planning and intent
- Use methods that prove lethal caused by limited physical reserves

formulate a unique vision of their own identifying role in society. More than a century ago, Durkheim proposed that societies, in particular social institutions such as the family unit and religion, fail to adequately integrate the young person socially *(74)*. This inability to transform adolescents into productive and functioning members of society may lead to the hopelessness often linked to suicide. Society's quest for individualism and autonomy of its members may lead to detrimental consequences if not attained, resulting in a dichotomy between expectations and realities *(75)*. This sense of personal failure and lack of fulfillment may promote suicidal ideations within adolescents or young adults.

4.2. Older Adults

Compared with younger people, older adults face specific age-related suicidal risk factors. These characteristics are given in Table 7 *(76–78)*. Their psychosocial dynamics, consisting of an increased risk of comorbid depression, social isolation, a feeling of hopelessness and rare overt expression of suicidal intentions, play a role in suicidal behavior. Between 71 and 95% of suicide victims aged 65 or older have been diagnosed with a major psychiatric disorder prior to death *(79)*. The elderly are more likely to suffer from depression as opposed to psychotic illness (schizophrenia or delusional disorder), personality disorders, or anxiety disorders. Furthermore, alcoholism and substance disorders are infrequent in the elderly population compared to their younger counterparts *(80)*.

Older adults, as compared with younger people, are more likely to use more immediately lethal modes, which evince increased planning and intent *(77,79)*. They are less likely to survive their injury partly because of their limited physical reserves and of the decreased likelihood of being discovered before a fatality because of diminished social support. Attitudes toward death and dying expressed by elderly persons reveal that they rarely fear death and often experience greater anxiety facing stressful life situations *(78)*.

4.3. Women

Although women in the United States are twice as likely to experience major depression compared with men, the suicide rate among men in 2001 was four times that of women *(9,81)*. Estimates suggest that there are 10 to 25 nonfatal suicide attempts for every fatality, and women are three times as likely to attempt suicide vs men *(8)*. The fewer number of nonfatal suicidal acts by men as compared to women may be the result of underreporting, in part attributable to the social stigma of suicidal ideation—regarded inconsistent with masculinity—and the failure of researchers to detect suicidal clues in men *(82)*. However, most male suicides may be due to statistical underreporting of the female suicidal deaths, which have been categorized as accidents or undetermined deaths owing to drug-related toxicity.

A suicide attempt refers to intentional self-harm with an awareness that the act could result in a fatal outcome *(70)*. Individuals who attempt suicide, also known as parasuicides, often behave impulsively, alert others about their intentions, and use methods that are either ineffective or slowly acting *(83)*. At 35 years follow-up, less than 12% of all suicide attempters will succumb to suicide *(84)*. Both men and women are more inclined to select a drug overdose during a suicide attempt *(85)*.

Numerous hypotheses have been offered to explain the lower rate of suicide for women. Men have been socialized to lead independent lives and act decisively without approaching others in times of need *(85)*. Discussion of a man's suicidal ideation may be deemed as a sign of weakness. In turn, depressive and suicidal thoughts may become overpowering, culminating in a suicide. On the other hand, women value close emotional bonds with others and are more likely to disclose turmoil and hardships in their lives. In this respect, women are able to release frustrations and discuss negative thoughts prior to succumbing to the grip of suicide.

Historically, more women chose a drug overdose as their primary means of suicide *(86)*. Currently, the majority of women in the United States in 2001 selected a firearm to commit suicide *(9)*. This difference in method choice may reflect a woman's determination in ensuring a fatality, leaving no ability for method failure or escape. In addition, the increased incidence of self-inflicted gunshot wounds by women may be to the result of the greater availability of firearms in the homes of suicidal individuals and the increased social acceptance of women's use of firearms *(87)*.

5. Autopsy Findings

5.1. Firearms

A host of injuries may be detected at postmortem examination, which leads to the determination of a suicidal death. The primary mode of suicide for both males and females in the United States is by firearm. Kohlmeier et al. performed a 15-year retrospective review of 1704 firearm-related suicidal cases *(88)*. Both men and women between the ages of 20 and 29 were most likely to use a firearm. The entrance wound site was as follows: head (83.7%), chest (14.0%), abdomen (1.9%), and a combination of these sites (0.4%). Handguns were the most commonly chosen weapons for both sexes. Eisele et al.'s study of 266 suicides confirmed Kohlmeier et al.'s review with respect to entrance wound location; the head was the preferred entrance wound site (74%), followed by chest (18%), and neck and abdomen (both 4% *[89]*). In Cina et al.'s study of 86 fatalities caused by a self-inflicted gunshot wound to the head, 47% of the cases were temporal, 16% were intraoral, 16% of the entrances were located at the side of the head, 15% were aimed at the face, 3.5% were below the chin, and 2.5% were at the back of the head *(90)*. Handguns were used in 85% of the cases, and 97% of the victims had sustained a contact wound. Although individuals are more likely to shoot themselves on the same side of the head as their dominant hand, this act is not an absolute finding *(89)*.

The majority of suicidal gunshot wounds are single and directed against a specific area of the body; however, suicides resulting from multiple gunshot wounds of various bodily locations using one gun or two guns simultaneously have been reported *(91–95)*. Similarly, atypical features of entrance gunshot wounds are not reliable criteria to exclude a suicidal manner of death *(96)*. Multiple gunshot wounds of the body warrant utmost scrutiny to avoid the hasty judgment of labeling them homicidal. The necessity of multiple gunshot wounds of the head to ensure a fatality may result from the victim's lack of knowledge of anatomy, inability to damage vital centers, or defective weaponry or ammunition *(91,92,97)*.

A thorough scene analysis with particular attention to placement of the weapon is warranted in the investigation of a suicide by firearm. In a study of 574 suicidal gunshot wounds, the gun was discovered in the victim's hand in 24% of the cases, on or within 30 cm of the body in 69%, and more than 30 cm from the victim in 7% of the cases *(98)*. Attention should be addressed to the blood spatter patterns (Fig. 1), position of the firearm with reference to the victim (Fig. 2A–C), and tests of hand wipings for gunshot residues *(99,100)*.

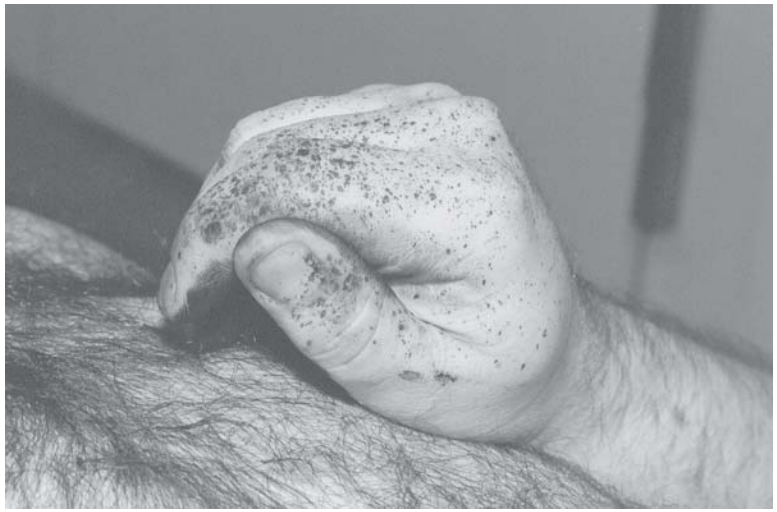

Fig. 1. Blowback on the shooting hand in a case of suicide. The weapon used in this case was an automatic pistol caliber 9 mm (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

5.2. Asphyxia

Asphyxial suicides include both ligature hanging and emplacement of a plastic bag placed over the head. In a series of 61 cases of asphyxial deaths by hanging, a ligature furrow denoting the demarcation of the overlying ligature was noted in each case and was located superior to the thyroid cartilage prominence in most victims *(101,102)*. The presence of conjunctival and facial petechiae was directly proportional to the extent of support below the ligature suspension. Of the victims, 26% had sustained hyoid bone and/or thyroid cartilage fractures and were primarily those individuals who had been discovered completely suspended. The percentage of fractures has been shown to be higher when the suspension time is less than 16 hours *(103)* and with increasing age of the victims *(101,103)*. In a study of 53 suicides using plastic bags, only 3% of the victims had evidence of petechiae, primarily of the conjunctivae *(104)*. Cutaneous markings about the neck were noted in 19% of victims, and a sole victim had sustained an internal neck injury, specifically thyrohyoid membrane hemorrhage.

Specific attention should be addressed to complex (combined) suicides involving hanging. The question whether capacity to execute a subsequent act was maintained after the infliction of primary injuries may become of major importance in such cases (Fig. 3).

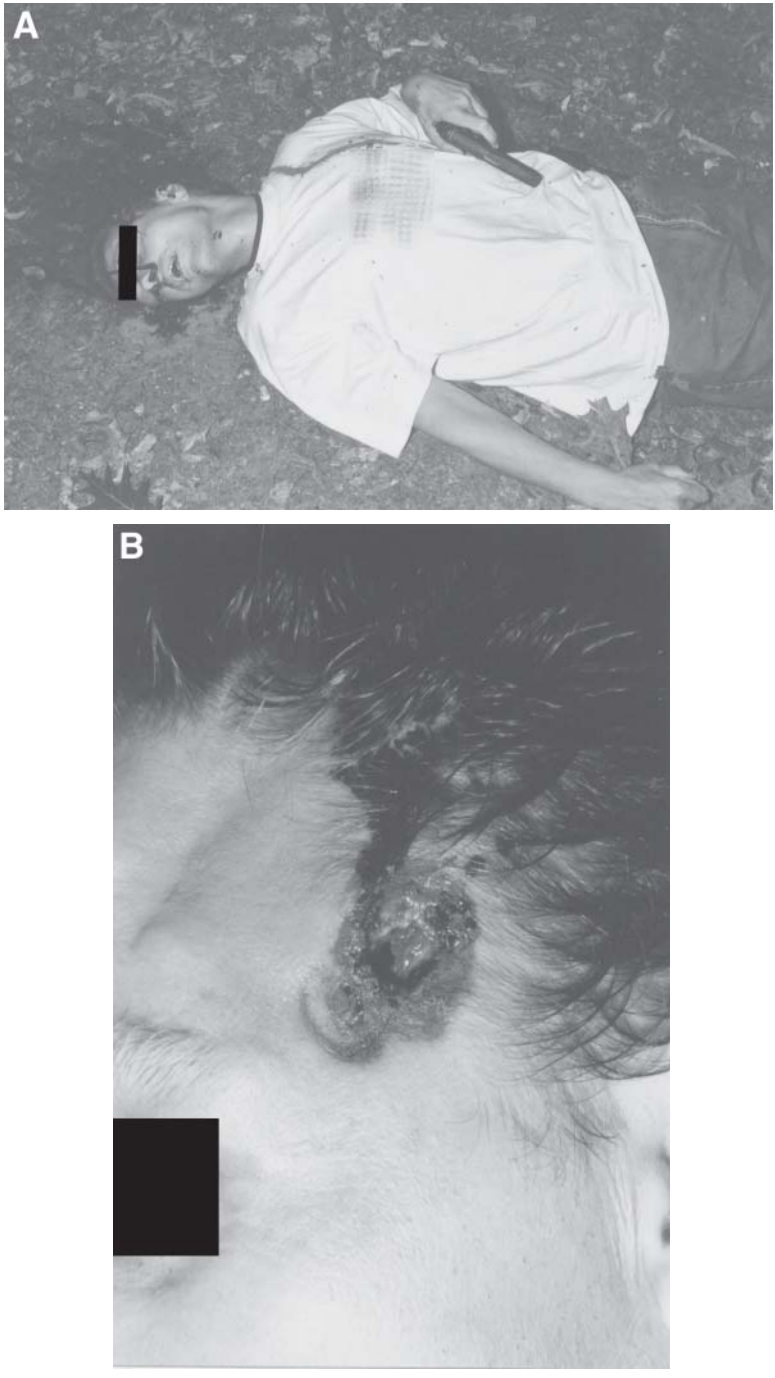

Fig. 2.

Fig. 2. Suicidal gun shot to the head. **(A)** Death scene. Original finding position of the body with the gun still in the hand of the victim. The bloodstain pattern on the left shoulder and chest of the victim´s tee-shirt extend from the blowback (drawback) of the shooting hand and pistol. **(B)** Contact wound on the left temple with muzzle imprint. **(C)** Muzzle of the automatic pistol used in this case. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

5.3. Sharp Force Injuries

Sharp force injuries, including stabbing and incised wounds, may be associated with a homicidal, suicidal, or accidental manner of death. Specific characteristics of these injuries may reflect a suicidal nature. Suicidal knife wounds are predominantly located in the throat, wrists, and chest *(105)*. Superficial sharp force injuries present at the wrists (Fig. 4A–C), inner forearms, face, or neck are often multiple and known as "tentative incisions" or "hesitation marks," likely reflecting the equivocal nature of the suicide attempt

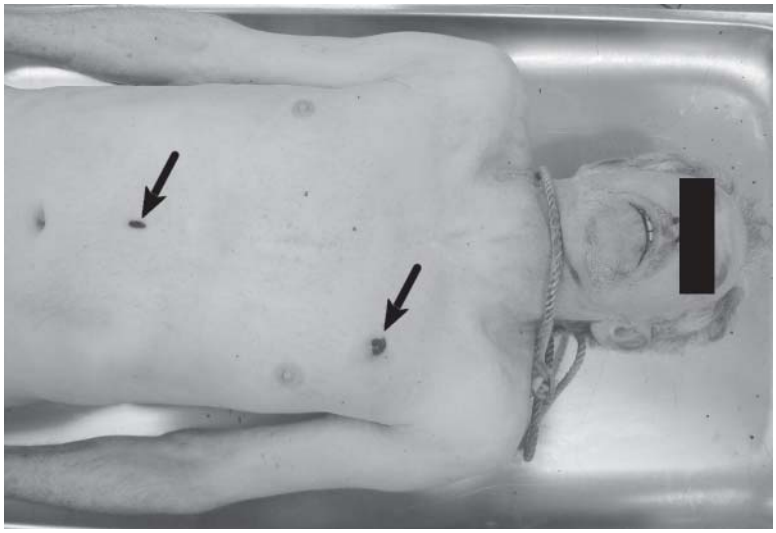

Fig. 3. Complex (combined) suicide of a 59-year-old male with two self-inflicted stab wounds on the midline abdomen and the precordial left chest (arrows). Both stab wounds were nonfatal, resulting in maintained capacity to execute a subsequent act. The victim hanged himself resulting in an asphyxial death by free suspension. A single-edged knife was found at the scene. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

Fig. 4.

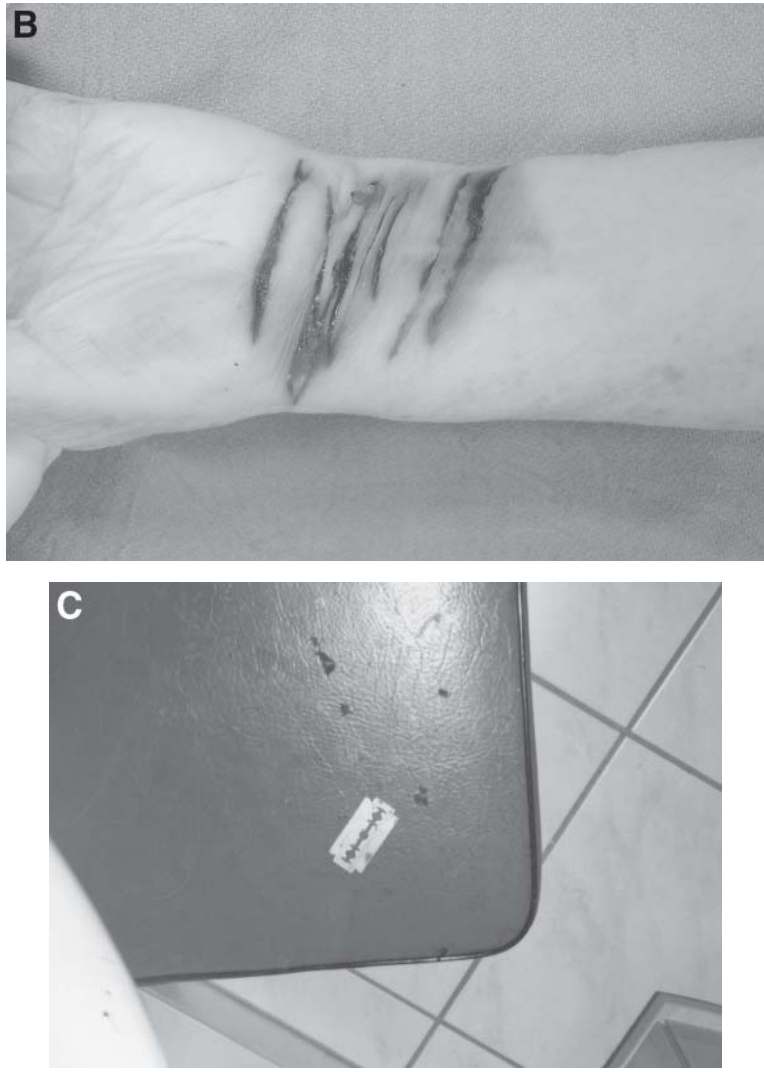

Fig. 4. Suicide by sharp force. **(A)** Death scene. Original body position of a 67-year-old woman who committed suicide by incising both wrists with a razor blade. Note the dark colored (bloody) water in the bathtub and drip blood pattern on the interior and exterior of the bathtub. **(B)** The fatal incisions on the victim's left inner wrist. **(C)** The razor blade found on a stool in the bathroom. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

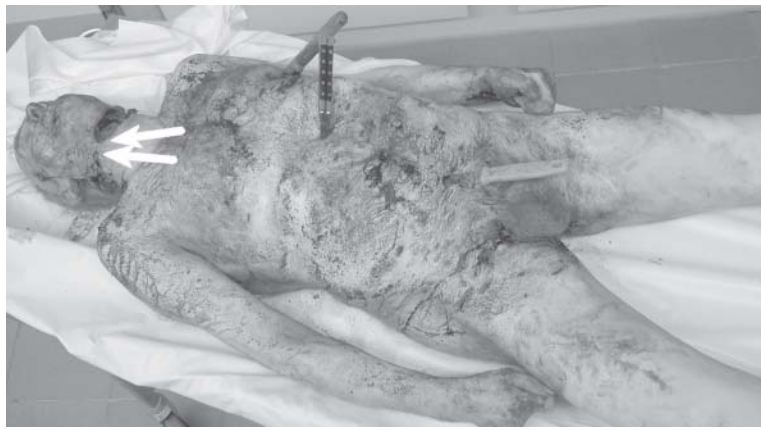

Fig. 5. Suicide by multiple sharp force injuries. Two deep incised wounds of the anterolateral neck (arrows) and multiple stab wounds to the chest, abdomen, and groin with three different knifes still in the body. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

prior to the lethal cut. Wrist cutting is rarely fatal, and scars may be discerned at autopsy of victims who used a different and more effective suicidal method. Although "tentative incisions" of the wrist or throat are more commonly associated with suicide, a thorough scene investigation is warranted to exclude other manners of death. In some cases, it may be very difficult to discern suicidal vs homicidal sharp force injuries, even when there are multiple wounds identified on the body (Fig. 5). The textbook description of a suicidal cut throat encompasses a single or several trial incisions followed by superimposed deeper cuts at the origin that gradually become shallower as they obliquely traverse the throat *(105)*. In reality, these cuts may be horizontal and do not vary in depth. On the other hand, homicidal incised wounds of the neck inflicted by a perpetrator facing the victim are often short and angled *(106)*. These slashes continue obliquely downward and medially across the neck. Wounds inflicted from the rear may extend deeply to the vertebral column.

Stab wounds constitute the majority of suicidal injuries to the chest and may be multiple and each life-threatening. Nonfatal linear incisions may scatter the precordium or extensive areas of the chest. Forensic investigators should be cognizant of self-inflicted injuries not deemed suicidal, which may be caused by an individual with a mental disorder as a form of self-mutilation (Fig. 6) or by one who deliberately harms oneself for motives of gain *(105)*. The wounds in self-mutilation are often superficial, multiple, and, although found anywhere on the body, not present in visible locations such as the lips, nose, and ears.

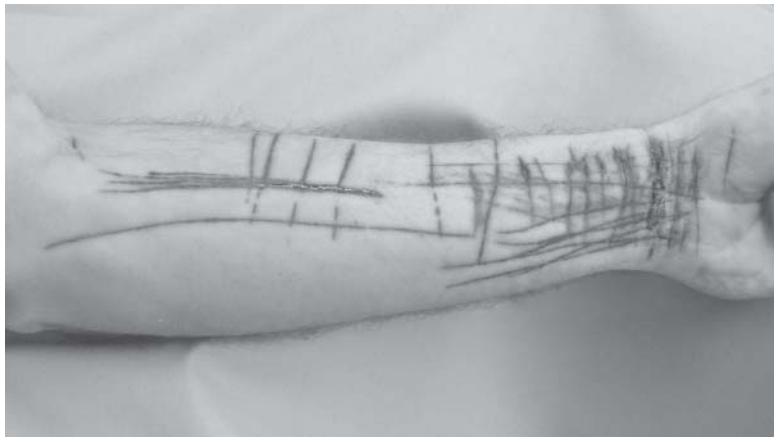

Fig. 6. Self-mutilation by sharp force in a 28-year-old schizophrenic male on the left inner forearm. These superficial self-inflicted incised wounds are arranged in a chessboard-like pattern. This man ultimately died as a result of jumping from a 16-meter height. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

5.4. Self-Immolation

Suicide caused by self-immolation, or self-incineration, is uncommon. However, certain autopsy findings may aid in confirming this manner of death. In a study of 32 self-immolation deaths, the majority of victims had evidence of soot in the airways combined with elevated blood carboxyhemoglobin (COHb) concentrations *(107)*. These findings confirmed that these individuals had been alive at the initiation of the fire and had succumbed to carbon monoxide poisoning caused by smoke inhalation. Of the victims, 88% used an accelerant, specifically gasoline in 86% of these cases. Although these cases are highly suggestive of suicide, the mere autopsy findings discussed in this report are not definitive.

5.5. Overdoses

The forensic investigation of overdose suicides may yield negative anatomical findings at autopsy and, therefore, centers primarily on the toxicological analysis. The accuracy of these results depends on numerous factors, including the timing and bodily site of sampling, the containers and preservatives used in the storage of the fluids or tissues, and the type of toxicological testing performed *(105)*. The diffusion effect resulting from the destruction of living cell components after death can profoundly affect the concentrations of physiological and foreign substances by drug redistribution. Postmortem col-

lection of peripheral vein blood, if available, is superior to cardiac blood for most toxicological analyses *(105)*.

5.6. Other Suicidal Deaths

Certain categories of suicidal death rely more heavily on the historical and scene investigation than the postmortem examination. Jumps from heights and impact motor vehicle collisions are prime examples. Suicide by self-inflicted blunt force injury is rare *(108)*. In these circumstances, investigatory confirmation of suicidal ideation in addition to a detailed scene reconstruction, complete autopsy, and toxicological evaluation may constitute the sole means of classifying a suicidal death.

The forensic pathologist plays an important role in the investigation of a suicide by performing the postmortem examination and confirming the self-inflicted nature of the death. A detailed scene analysis, an extensive analysis of the victim's background to uncover suicidal intent, and a complete autopsy with toxicology are warranted in these cases. Although specific injuries such as multiple gunshot wounds to the head or stabbing and incisional injuries may represent either a homicidal or suicidal act, the forensic pathologist should be cognizant of several distinctive findings during an autopsy that are associated with a suicidal death. A comprehensive analysis of suicidal trends in combination with a detailed review of significant postmortem evidence may shed light on the underlying motives behind a suicide and provide data for the formulation of strategies aimed at prevention.

Postscript

In the 20th century, international trends of suicide are similar to those experienced in the United States, with particular regard to the dramatic rise in youth suicide and prevalence of self-inflicted death by firearm in numerous countries worldwide over the twentieth century. International collaboration with mechanisms for coordination and sharing each country's unique struggle with suicide may offer a meaningful opportunity to combat it.

References

1. Rosenberg ML, Davidson LE, Smith JC, et al. (1988) Operational criteria for the determination of suicide. J Forensic Sci 33, 1445–1456.
2. Ho TP, Yip PSF, Chui CWF, Halliday P (1998) Suicide notes: what do they tell us? Acta Psychiatr Scand 98, 467–473.
3. Litman RE, Curphey TJ, Shneidman ES, Farberow NL, Tabachnick ND (1963) Investigations of equivocal suicides. JAMA 184, 924–929.

4. Denny KM (1995) Russian roulette: a case of questions not asked? J Am Acad Child Adolesc Psychiatry 34, 1682–1683.
5. Fishbain DA, Fletcher JR, Aldrich TE, Davis JH (1987) Relationship between Russian roulette deaths and risk-taking behavior: a controlled study. Am J Psychiatry 144, 563–567.
6. Tabachnick ND, Farberow NL (1961) The assessment of self-destructive potentiality. In Farberow NL, Shneidman ES, eds., The Cry for Help. McGraw-Hill Book Company Inc., New York, pp. 60–77.
7. World Health Organization. WHO Statistical Information System. Available at: URL: www.who.int/whosis; Internet; accessed September 22, 2004.
8. Maris RW (2002) Suicide. Lancet 360, 319–326.
9. Centers for Disease Control and Prevention. Web-based Injury Statistics Query and Reporting System (WISQARS) [online]. National Center for Injury Prevention and Control, Centers for Disease Control and Prevention (producer). Available at: URL: www.cdc.gov/ncipc/wisqars; Internet; accessed September 22, 2004.
10. Centers for Disease Control and Prevention (1997) Regional variations in suicide rates—United States, 1990–1994. MMWR 46, 789–793.
11. Kaplan HI, Sadock BJ (1998) Psychiatric emergencies. In Kaplan HI, Sadock BJ, eds., Kaplan and Sadock's Synopsis of Psychiatry, 8th ed. Williams & Wilkins, Baltimore, pp. 864–872.
12. Stafford MC, Weisheit RA (1988) Changing age patterns of U.S. male and female suicide rates, 1934–1983. Suicide Life Threat Behav 18, 149–163.
13. Centers for Disease Control and Prevention (1995) Suicide among children, adolescents, and young adults—United States, 1980–1992. MMWR 44, 289–291.
14. Centers for Disease Control and Prevention (1996) Suicide among older persons—United States, 1980–1992. MMWR 45, 3–6.
15. McIntosh JL (1992) Epidemiology of suicide in the elderly. Suicide Life Threat Behav 22, 15–35.
16. Statistics of the United States in 1860. 8th Census. Washington, DC, Government Printing Office Vol. 4, 1866.
17. Centers for Disease Control and Prevention. National Center for Health Statistics [online]. National Center for Injury Prevention and Control, Centers for Disease Control and Prevention (producer). Available at: URL: www.cdc.gov/nchs; Internet; accessed September 22, 2004.
18. Wintemute GJ (1987) Firearms as a cause of death in the United States, 1920–1982. J Trauma 27, 532–536.
19. Ikeda RM, Gorwitz R, James SP, Powell KE, Mercy JA (1997) Trends in fatal firearm-related injuries, United States, 1962–1993. Am J Prev Med 13, 396–400.
20. American Medical Association Council on Scientific Affairs (1989) Firearms injuries and deaths: A critical public health issue. Public Health Rep 104, 111–120.
21. Lester D, Murrell ME (1980) The influence of gun control laws on suicidal behavior. Am J Psychiatry 137, 121–122.
22. Markush RE, Bartolucci AA (1984) Firearms and suicide in the United States. Am J Public Health 74, 123–127.

23. Miller M, Azrael D, Hemenway D (2002) Household firearm ownership and suicide rates in the United States. Epidemiology 13, 517–524.
24. Kellermann AL, Rivara FP, Somes G, Reay DT, Francisco J, Banton JG (1992) Suicide in the home in relation to gun ownership. N Engl J Med 327, 467–472.
25. Diekstra RFW (1989) Suicide and the attempted suicide: An international perspective. Acta Psychiatr Scand 354, Suppl, 1–24.
26. Lester D (1990) Changes to suicide rates unique to Canada? Can J Public Health 81, 240–241.
27. Lester D (1997) Suicide in an international perspective. Suicide Life Threat Behav 27, 104–111.
28. Yip PSF (2001) An epidemiological profile of suicides in Beijing, China. Suicide Life Threat Behav 31, 62–70.
29. Zhang J (1996) Suicides in Beijing, China, 1992–1993. Suicide Life Threat Behav 26, 175–180.
30. Chastang F, Rioux P, Dupont I, Baranger E, Kovess V, Zarifian E (1998) Risk factors associated with suicide attempt in young French people. Acta Psychiatr Scand 98, 474–479.
31. Deavoll BJ, Mulder RT, Beautrais AL, Joyce PR (1993) One hundred years of suicide in New Zealand. Acta Psychiatr Scand 87, 81–85.
32. Gatter K, Bowen DA (1980) A study of suicide autopsies 1957–1977. Med Sci Law 20, 37–42.
33. Killias M (1993) International correlations between gun ownership and rates of homicide and suicide. Can Med Assoc J 148, 1721–1725.
34. Klerman GL (1987) Clinical epidemiology of suicide. J Clin Psychiatry 48:12, Suppl, 33–38.
35. Blakely TA, Collings SCD, Atkinson J (2003) Unemployment and suicide. Evidence for a causal association? J Epidemiol Community Health 57, 594–600.
36. Kposowa AJ (2001) Unemployment and suicide: a cohort analysis of social factors predicting suicide in the US National Longitudinal Mortality Study. Psychol Med 31, 127–138.
37. Stack S (1980) Occupational status and suicide: a relationship reexamined. Aggress Behav 6, 223–234.
38. Stack S (1998) Education and risk of suicide: an analysis of African Americans. Sociol Focus 31, 295–302.
39. Juel K, Mosbech J, Hansen ES (1997) Mortality and cause of death among Danish physicians, 1973–1992. Ugekr Laeger 159, 6512–6518.
40. Bedeian A (1982) Suicide and occupation: a review. J Vocational Behav 21, 205–223.
41. Stack S (1996) Gender and suicide risk among artists: a multivariate analysis. Suicide Life Threat Behav 26, 374–379.
42. Andreasen NC (1987) Creativity and mental illness: prevalence rates in writers and their first degree relatives. Am J Psychiatry 144, 1288–1292.
43. Ajdacic-Gross V, Wang J, Bopp M, Eich D, Rössler W, Gutzwiller F (2003) Are seasonalities in suicide dependent on suicide methods? A reappraisal. Soc Sci Med 57, 1173–1181.

44. Yip PSF, Chao A, Chiu CWF (2000) Seasonal variation in suicides: diminished or vanished. Experience from England and Wales, 1982–1996. Br J Psychiatry 177, 366–369.
45. Rasanen P, Hakko H, Jokelainen J, Tiihonen J (2002) Seasonal variation in specific methods of suicide: a national register study of 20,234 Finnish people. J Affect Disord 71, 51–59.
46. Massing W, Angermeyer MC (1985) The monthly and weekly distribution of suicide. Soc Sci Med 21, 433–441.
47. Maris RW, Berman AL, Silverman MM, Goldblatt MJ (2000) Physical illness and suicide. In Maris RW, Berman AL, Silverman MM, eds., Comprehensive Textbook of Suicidology. Guilford Press, New York, pp. 342–356.
48. Cassell EJ (1979) Reactions to physical illness and hospitalizations. In Usin G, Lewis JM, eds., Psychiatry in General Medical Practice. McGraw-Hill, New York, pp. 103–131.
49. Fox BH, Stanek EJ, Boyd SC, Flannerty JT (1982) Suicide rates among cancer patients in Connecticut. J Chronic Dis 35, 89–100.
50. Marzuk PM (1994) Suicide and terminal illness. Death Stud 18, 497–512.
51. Robins LN, Helzer JE, Croughan J (1981) National Institute of Mental Health Diagnostic Interview Schedule: Its history, characteristics and validity. Arch Gen Psychiatry 38, 381–392.
52. Moscicki E (2001) Epidemiology of suicide. In Goldsmith S, ed., Risk Factors for Suicide. National Academy Press, Washington, DC, pp. 1–4.
53. Angst J, Angst F, Stassen HH (1999) Suicide risk in patients with major depressive disorder. J Clin Psychiatry 60, Suppl 2, S57–S62.
54. Kelleher MJ, Daly M, Kelleher MJA (1992) The influence of antidepressants in overdose on the increased suicide rate in Ireland between 1971 and 1988. Br J Psychiatry 161, 625–628.
55. Müller-Oerlinghausen B, Berghofer A (1999) Antidepressants and suicidal risk. J Clin Psychiatry 60, Suppl 2, S94–S99.
56. Tsai SY, Kuo CJ, Chen CC, Lee HC (2002) Risk factors for completed suicide in bipolar disorder. J Clin Psychiatry 63, 469–476.
57. Siris SG (2001) Suicide and schizophrenia. J Psychopharmacol 15, 127–135.
58. Mann JJ (2002) A current perspective of suicide and attempted suicide. Ann Intern Med 136, 302–311.
59. Chute D, Grove C, Rajasekhara B, Smialek JE (1999) Schizophrenia and sudden death: a medical examiner case study. Am J Forensic Med Pathol 20, 131–135.
60. Kessler RC, McGonagle KA, Zhao S, et al. (1994) Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Arch Gen Psychiatry 56, 617–626.
61. Stoudemire A, Wallack L, Hedenark N (1987) Alcohol dependence and abuse. In Amler RW, Dull HB, eds., Enclosing the Gap: The Burden of Unnecessary Illness. Oxford University Press, New York, pp. 9–18.
62. Duberstein PR, Conwell Y, Caine ED (1993) Interpersonal stressors, substance abuse, and suicide. J Nerv Ment Dis 181, 80–85.

63. Murphy GE, Armstrong JW, Hermele SL, Fischer JR, Clendenin WW (1979) Suicide and alcoholism: interpersonal loss confirmed as a predictor. Arch Gen Psychiatry 45, 593–594.
64. Hufford MR (2001) Alcohol and suicidal behavior. Clin Psychol Rev 21, 797–811.
65. Gruenewald PJ, Ponicki WF, Mitchell PR (1995) Suicide rates and alcohol consumption in the United States, 1970–89. Addiction 90, 1063–1075.
66. Langley GE, Bayatti NN (1984) Suicides in Exe Vale Hospital, 1972–1981. Br J Psychiatry 145, 463–467.
67. Lee CJ, Collins KA, Burgess SE (1999) Suicide under the age of eighteen: a 10-year retrospective study. Am J Forensic Med Pathol 20, 27–30.
68. Rosewater KM, Burr BH (1998) Epidemiology, risk factors, intervention, and prevention of adolescent suicide. Curr Opin Pediatr 10, 338–343.
69. Chan KPM, Hung SF, Yip PSF (2001) Suicide in response to changing societies. Child Adolesc Psychiatr Clin N Am 10, 777–795.
70. Beautrais AL (2003) Suicide and serious suicide attempts in youth: a multiple-group comparison study. Am J Psychiatry 160, 1093–1099.
71. Koplin B, Agathen J (2002) Suicidality in children and adolescents: a review. Curr Opin Pediatr 14, 713–717.
72. Rosenberg ML, Smith JC, Davidson LE, Conn JM (1987) The emergence of youth suicide: an epidemiologic analysis and public health perspective. Annu Rev Public Health 8, 417–440.
73. Runeson BS (1993) History of suicidal behaviour in the families of young suicides. Acta Psychiatr Scand 98, 497–501.
74. Durkheim E (1970) Suicide: a study in sociology (Spaulding JA, Simson G, Trans.). Routledge and Kegan Paul, London (first published 1897).
75. Eckersley R, Dear K (2002) Cultural correlates of youth suicide. Soc Sci Med 55, 1891–1904.
76. Caine ED, Conwell Y (2001) Suicide in the elderly. Int Clin Psychopharmacol 16, Suppl 2, S25–S30.
77. Conwell Y (2001) Suicide in later life: a review and recommendations for prevention. Suicide Life Threat Behav 31, Suppl, 32–47.
78. Kastenbaum R (1992) Death, suicide and the older adult. Suicide Life Threat Behav 22, 1–14.
79. Conwell Y, Duberstein PR, Caine ED (2002) Risk factors for suicide in later life. Biol Psychiatry 52, 193–204.
80. Conwell Y, Duberstein PR, Cox C, Herrmann JH, Forbes NT, Caine ED (1996) Relationship of age and Axis I diagnoses in victims of completed suicide: a psychological autopsy study. Am J Psychiatry 153, 1001–1008.
81. Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, et al. (1994) Lifetime and twelve month prevalence of DSM-III-R psychiatric disorders in the United States: results from a national comorbidity survey. Arch Gen Psychiatry 51, 8–19.
82. Canetto SS, Sakinofsky I (1998) The gender paradox in suicide. Suicide Life Threat Behav 28, 1–23.

83. Edwards JE, Whitlock FG (1968) Suicide and attempted suicide in Brisbane, I and II. Med J Austr 1, I: 932–938, II: 989–995.
84. Dahlgren KG (1977) Attempted suicide—35 years afterward. Suicide Life Threat Behav 7, 75–79.
85. Murphy GE (1998) Why women are less likely than men to commit suicide. Compr Psychiatry 39, 165–175.
86. Doddakashi V, Wilcox RE (2003) Female suicides in major Texas cities, 1994 through 1998. Tex Med 99, 50–58.
87. Frierson RL (1989) Women who shoot themselves. Hosp Community Psychiatry 40, 841–842.
88. Kohlmeier RE, McMahan CA, DiMaio VJM (2001) Suicide by firearms: a 15-year experience. Am J Forensic Med Pathol 22, 337–340.
89. Eisele JW, Reay DT, Cook A (1981) Sites of suicidal gunshot wounds. J Forensic Sci 36, 480–485.
90. Cina SJ, Ward ME, Hopkins MA, Nichols CA (1999) Multifactorial analysis of firearm wounds to the head with attention to anatomic location. Am J Forensic Med Pathol 20, 109–115.
91. Jacob B, Barz J, Haarhoff K, Sprick C, Worz D, Bonte W (1989) Multiple suicidal gunshots to the head. Am J Forensic Med Pathol 10, 289–294.
92. Hudson P (1981) Multishot firearm suicide: examination of 58 cases. Am J Forensic Med Pathol 2, 239–242.
93. Introna F, Smialek JE (1989) Suicide from multiple gunshot wounds. Am J Forensic Med Pathol 10, 275–284.
94. Shields LBE, Hunsaker DM, Hunsaker JC 3rd, Rolf CM (2003) Multiple self-inflicted suicidal gunshot wounds of the head: a matter of timing and place[ment]—Simultaneous or sequential? ASCP Check Sample Forensic Pathology No. FP 03-2 (FP-283).
95. Parroni E, Caringi C, Ciallella C (2002) Suicide with two guns represents a special type of combined suicide. Am J Forensic Med Pathol 23, 329–333.
96. Skinker [Hunsaker] DM, Coyne CM, Lanham C, Hunsaker JC 3rd (1996) Chasing the casing: a 38 Special suicide. J Forensic Sci 41, 709–712.
97. DiMaio VJM (1999) Gunshot wounds: practical aspects of firearms, ballistics, and forensic techniques, 2nd ed. CRC Press, Boca Raton, FL.
98. Garavaglia JC, Talkington B (1999) Weapon location following suicidal gunshot wounds. Am J Forensic Med Pathol 20, 1–5.
99. Stone IC (1992) Characteristics of firearms and gunshot wounds as markers of suicide. Am J Forensic Med Pathol 13, 275–280.
100. Stone JC (1987) Observations and statistics relating to suicide weapons. J Forensic Sci 32, 711–716.
101. Shields LBE, Hunsaker DM, Hunsaker JC 3rd, Shouse B (2004) Autoerotic asphyxia: summary of a classic case. ASCP Check Sample Forensic Pathology No. FP 04-4 (FP-295).
102. Luke JL, Reay DT, Eisele JW, Bonnell HJ (1985) Correlation of circumstances with pathological findings in asphyxial deaths by hanging: a prospective study of 61 cases from Seattle, WA. J Forensic Sci 30, 1140–1147.

103. Morild I (1996) Fractures of neck structures in suicidal hanging. Med Sci Law 36, 80–84.
104. Haddix TL, Harruff RC, Reay DT, Haglund WD (1996) Asphyxial suicides using plastic bags. Am J Forensic Med Pathol 17, 308–311.
105. Knight B (1996) Forensic Pathology, 2nd ed. Arnold, London, Sydney, Auckland.
106. DiMaio VJ, DiMaio D (2001) Forensic Pathology, 2nd ed. CRC Press, Boca Raton, FL.
107. Shkrum M, Johnston KA (1992) Fire and suicide: a three-year study of self-immolation deaths. J Forensic Sci 37, 208–221.
108. Hunsaker DM, Thorne LB (2002) Suicide by blunt force trauma. Am J Forensic Med Pathol 23, 355–359.

11

Murder–Suicide

An Overview

Roger W. Byard, MD, MBBS

CONTENTS

SUMMARY

Murder–suicides form a distinct subset of homicides in which the perpetrator kills him or herself after dispatching his or her victims. More accurately called homicide–suicide, the most common scenario involves an estranged white male in his 40s killing his spouse and possibly children before committing suicide. Rates are relatively uniform among communities and are much less than simple homicide, or suicide, cases. A variety of categories have been identified that include spousal cases where *(a)* the action is precipitated by jealousy or concern over age or ill health, *(b)* familial cases where a parent usually kills all of the children and then themselves, and *(c)* a final mixed

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group consisting of disgruntled employees, cult members, and members of religious or political groups who target a large number of victims.

Key Words: Murder–suicide; homicide–suicide; dyadic death.

1. Introduction

Murder–suicide refers to a situation where the perpetrator of a homicide has taken his or her own life after the death of the victim(s) has occurred. It is well recognized, historically being described in the Ming dynasty in China and in Greek tragedies and has been known by a variety of names, including homicide–suicide and dyadic death *(1,2)*.

Although suicide may often immediately follow homicide, various authors have allowed up to 3 months between the events *(3)*. Most suicides, however, occur within a day or much less of the homicides *(4-7)*. It has been suggested that murder–suicides have distinctive epidemiological characteristics and overlap areas such as domestic homicide, mass murder, and suicide *(8)*. Generally, distinctive cultural conditions in which there may be multiple homicides followed by the suicide of the perpetrator, such as *amok* among Malays, are usually excluded from murder–suicide data, as are cases where a suicide attempt has failed, or where the victims were accidentally killed *(8)*.

2. Incidence

Murder–suicides are uncommon events and although suicides are a leading cause of death in many Western countries, murder-suicide rates are low, usually being much less than homicide rates *(6)*. Although rates of 0.2 to 0.3 per 100,000 persons per year have been cited *(5)*, determining exact numbers is often difficult because there is no standard classification and because cases tend to be recorded in official statistics as separate suicides and homicides. The percentage of homicides that are murder–suicides tends to be lower in countries and regions in which there is a high homicide rate and higher in regions in which homicides are rare *(9)*. For example, the percentage of homicides followed by suicide of the perpetrator in some series is 42% in Denmark, in which homicide rates are low, compared with 4% in the United States, in which homicide rates are much higher *(6)*. It was estimated in 1992 that there were about 1000 to 1500 such deaths per year in the United States, with relative stability in the numbers of such deaths in Western countries, although variations in definitions and reporting practices makes accurate assessment difficult *(8)*.

Table 1
International Rates of Murder–Suicide per 100,000 of the Population

Country	Years	Homicide-suicide rate	% of total homicides
New Zealand	1976–1989	0.05	3.4
Scotland	1986–1990	0.05	3
Iceland	1900–1979	0.06	8.5
England and Wales	1980–1990	0.07	7.2
Hong Kong	1961–1971	0.07	5
Sweden	1970–1981	0.09	15.6
Bermuda	1920–1979	0.13	5.5
Australia	1989–1991	0.16	8
Finland	1955–1970	0.18	8
Denmark	1946–1970	0.2	30
Canada	1961–1966	0.21	15.6

Data taken from ref *10*.

Looking at specific data *(10)*: the homicide rate in Atlanta from 1988 to 1991 was 38.8 per 100,000 of the population, compared with a much lower homicide rate of 1.11 per 100,000 in England and Wales from 1980 to 1990. The respective murder–suicide rates were 0.46 and 0.07, accounting for 1.4% of homicides in Atlanta and 7.2% in England and Wales. Murder suicides have accounted for between 1.4% of the total number of homicides in Atlanta and 67.8% in Israel. Homicide and murder–suicide rates have ranged from 2.35 and 0.13 for Bermuda, to 1.5 and 0.27 for Canada, and 0.72 and 0.06 for Iceland. The rates of murder–suicide and homicide for a variety of countries are summarized in Table 1 *(10)*.

3. *Investigation*

The investigation of murder–suicides is often difficult if family members are involved because the perpetrator is dead and those who are in the best situation to provide pertinent information to the investigators may also have been killed. Psychological autopsies with review of the medical records of the perpetrator and interviews with work colleagues and relatives may provide some insight into the events leading up to the fatal attack; however, this information is often not available on coroner's files *(11)*.

4. Characteristics

Although it has been suggested that the characteristic features of the perpetrators, victims, and the methods used tend to vary among communities and between countries, recent studies have shown similarities in rates among different ethnic, racial, and cultural groups *(6,10,12)*. For example, it was proposed that although the most common form of murder–suicide in the United States involved a married or estranged white male who shoots his female partner/spouse, maternal–child murder–suicides were more common in England *(8,11,13)*. This disparity has not been shown in more recent data, with the percentage of female perpetrators in many series ranging from only 3 to 8% and with shooting being the most common form of homicide in murder–suicides not only in the United States, but also in parts of England *(7,10)*. The percentage of female perpetrators does increase when murder–suicides involve child family members *(6,14)*. Perpetrators of murder–suicide also tend to be older than those who commit homicide alone and are usually about 40 to 49 years of age. There is usually a close personal relationship between the perpetrator and the victim; the killing of strangers is rare *(10,15,16)*. Characteristics of murder–suicide cases may, however, alter over time in the same community, with Hannah et al. *(17)* demonstrating a change from city-based, multiple killings by white perpetrators, to rural dyadic killings by black perpetrators in Central Virginia during a 10-year period.

Although homicides tend to occur more commonly within lower socioeconomic groups, murder–suicides have been found to be more a middle class phenomenon in several studies. Many deaths occur in the bedroom of family homes *(2,7,10)*.

Methods used may certainly vary depending on availability, with the high rate of murder–suicides involving firearms in the United States being attributed to the ready access to handguns in that country *(6)*. This may vary over time, with earlier studies from England showing a high rate of homicide using coal gas poisoning *(13)*, compared with later studies from the United Kingdom where domestic gas was no longer used once carbon monoxide had been eliminated *(18)*. Some studies have shown that murder–suicides may involve more violent methods than homicides alone, suggesting greater levels of frustration and aggression *(16)*.

5. Classification

A variety of classification systems have been proposed for murder–suicides. Marzuk et al. *(8)* proposed a classification system based on the type of victim–perpetrator relationship and possible motives or precipitating events,

Table 2
Classification of Murder–Suicide

Spousal or consortial

Perpetrator
a) Spouse
b) Consort

Type of homicide

i) Spouse-killing (uxoricidal)
ii) Murder of lover (consortial)

Familial

Perpetrator
a) Mother
b) Father
c) Child (<16 years)
d) Other adult family member (>16 years)

Type of homicide

i) Neonaticide (<24 hours)
ii) Infanticide (1 day to 1 year)
iii) Pedicide (1–16 years)
iv) Adult family member (>16 years)

Extrafamilial

Class:

a) Amorous jealousy
b) "Mercy killing"
c) "Altruistic or extended suicides"
d) Family, financial, or social stressors
e) Retaliation
f) Other
g) Unspecified

Data taken from ref. *8*.

dividing cases into those involving a spouse or partner, those involving other family members, and those that occurred outside families (Table 2).

5.1. Spousal

Spousal murder–suicides typically involve a male spouse or lover who suffers "morbid jealousy" or jealous rage precipitated by frustration *(5)*. The reported age range is 18 to 60 years and there often is a history of suspicions,

or knowledge, of spousal infidelity. Although some relationships are typified by abuse and actual infidelity, in others the suspicions may be delusional and involve psychotic breakdown. The "Othello syndrome" refers to a situation where delusions of infidelity lead to irritability, depression, and aggression. More than 90% of murder–suicides involving couples are perpetrated by males, who may also murder their spouse's lover. This has been termed triadic death *(8)*. Recent separation from a spouse has been noted in one Canadian study to increase the risk of murder–suicide, with 35.3% of estranged males who murdered their wives subsequently committing suicide, compared with only 21.6% of nonestranged spouses who committed suicide after spousal homicide *(8)*.

The other characteristic scenario of spousal murder–suicide involves an elderly couple who may have been married for many decades who are both suffering from significant illnesses, financial problems, and/or social isolation. Often the husband will either shoot or suffocate his wife and then commit suicide. This type of activity overlaps with so-called "mercy killings" and suicide pacts and also has been described in partners of acquired immunodeficiency syndrome victims who perform a "mercy killing" and then commit suicide *(8)*. Differentiating a suicide pact where a partner has been coerced to commit suicide, from murder–suicide may be difficult, and so-called "suicide pacts" between a parent and child may well be disguised murder–suicides *(6)*.

5.2. Familial

Familial murder–suicides often involve a parent who murders their children and then commits suicide. Suicide following infanticide is very uncommon in most countries, with only 10.5% of fathers and 2.3% of mothers who murder their infants committing suicide in some series. A higher incidence has been reported in Japan, where about 500 cases are documented annually *(2,6,8,14)*.

As noted, there are often higher numbers of females involved in murder–suicides with children, and it has been hypothesized that the action of killing the children is an extension of suicide and that the perpetrator is acting altruistically to "save" her children from the dangers of the world. Certainly, the methods used by mothers tend to be less violent than those used by fathers, consisting of poisoning, suffocation, and carbon monoxide exposure, compared to shooting, strangling and stabbing, methods favored by males *(13,14)*. Females may sedate their children prior to causing death and are also less likely to kill their spouse or other children who are not members of the family. This contrasts with males who often kill their own children, visiting children, their spouse, and family pets. The degree of violence exhibited by these individuals has led to the term "family annihilators" *(19)*.

Another form of familial murder–suicide may occur if a dependent adult child suffering significant physical or mental impairment is murdered by a parent who no longer feels capable of providing the necessary care because of age, infirmity, or financial problems. This has "altruistic" features in common with elderly spousal murder–suicides.

5.3. Extrafamilial

Murder–suicides involving individuals outside the family often take the form of a disgruntled employee or ex-employee seeking vengeance for real or perceived insults, harm, or ill-handling. These have been called "adversarial" murder–suicides *(6)*. Failure to achieve job promotion or attain monetary payment for services may be motivating factors that can reach the level of persecutory delusions. Cases also occur among peers when relationships have been characterized by antagonistic or competitive qualities, and several highly publicized cases of high school shootings have occurred in the United States involving disgruntled students who returned to school properties with firearms and sometimes with lists of potential victims.

In the "pseudo-commando" type of murder–suicide, a number of firearms may be used, including semiautomatic weapons, and a number of bystanders or so-called "secondary targets" may be killed. Perpetrators sometimes have stockpiled a small arsenal and intend to die in a "blaze of glory" *(6)*. These events often occur in public places where there is an opportunity to kill many victims. There is usually no escape plan for the perpetrator, who often forces the police to kill him. Pseudo-commando murder suicides have been divided into "indiscriminate" and "pseudo-community" subtypes. In the indiscriminate type, the perpetrator kills as many people as possible, their only common characteristic being proximity to the killer. In the pseudo-community subgroup a perpetrator targets a specific group. This was clearly demonstrated by Marc Lepine, who killed 14 female engineering students at Montreal's L'Ecole Polytechnique in December 1989. He had separated out the female from the male students and had said that he was fighting "feminism" *(6,11)*.

Murder–suicides occasionally occur in the setting of cults and may achieve considerable media coverage when a large number of deaths occur. Whether these types of deaths should be classified as murder–suicides, homicides, or suicides is often unclear, particularly given that most, if not all, witnesses are dead. The leaders of such cults have been described as charismatic and paranoid, and often may have persuaded their followers that death is a desirable outcome.

A final group consists of those involved in terrorist actions such as the Bali bombing that have been designated "kamikaze" murder–suicides *(5)*, where

the perpetrator dies as a result of the device or action that he or she has used to kill a large number of others. Suicide bombings in the Middle East would also fall into this special subcategory.

The logistics involved in cases where there may be hundreds of victims located in less-than-ideal circumstances may be highly complex, with issues such as preservation of the scene, preservation of bodies, preservation of evidence, and protection and provision of adequate working conditions for investigators being of paramount importance.

This classification system has been criticized on the grounds that it is difficult sometimes to determine what terms such as "consortial" mean. In addition, there is no provision for the inclusion of information such as race, gender, weapon type, and living arrangements. Hanzlick and Koponen *(4)* have therefore proposed an alternative typological system that enables the recording of much more circumstantial information on the demography and epidemiological features of individual cases, listed in the following sections.

5.3.1. Relationship of the Victim to the Perpetrator

Relationships are defined as spouse by marriage, common-law spouse, unmarried partner in relationship, extramarital consort (lover), real or perceived rival lover, parent, offspring, sibling, grandparent, grandchild, niece/nephew, aunt/uncle, cousin, family member other than those listed, acquaintance, stranger, same gender as perpetrator, opposite gender of perpetrator, same race as perpetrator, different race than perpetrator, lives in same household, lives in different household, no living witness(es), living witness(es), shot, stabbed/cut, beaten, other (asphyxia, drugged, etc.).

5.3.2. Cofactors

Cofactors are impending divorce, previously divorced, real or perceived loss of nonmarital partner in a relationship (boyfriend, lover, etc.), jealousy or retaliation for partner's real or perceived involvement with another person, retaliation against a real or perceived rival lover, mercy killing, altruism (to save from "evils of the world"), financial stressors, family stress or dysfunction, perpetrator intoxicated with alcohol, perpetrator intoxicated with drug(s) other than alcohol, perpetrator had known history of psychiatric illness, unspecified, and other or unknown factors.

5.3.3. Special Classifications

Special classifications are characterized as family annihilator, dyadic, triadic, followed a mass murder or serial murders committed by the perpetrator.

6. *Motivation*

As can be seen from the previous discussion, motivations for murder–suicide are quite complex and probably differ markedly from case to case. Determining the incidence of underlying psychological disturbance among perpetrators also is complicated by differences in psychiatric diagnostic approaches and mental illness classifications *(20)*. There are, however, some generalizations that can be made.

Psychological illness appears to play a major role in a number of the categories, although the literature does not contain abundant information on the psychological profiles of perpetrators prior to the fatal events. Psychological problems may take the form of paranoia, morbid jealousy, and psychosis in cases of spousal murder–suicides, or in familial cases where there has been recent estrangement. Alternatively, depression may play a significant role in cases where a parent murders his or her children; the incidence of depression has been reported as much higher generally in perpetrators of murder–suicide than of homicide alone. For this reason, it has been suggested that murder–suicide may merely be an extension of a suicidal act *(6)*. There may be a history of previous suicide attempts and consultation with mental health professionals *(11)*. There also may be delusional or psychotic elements with religious overtones, as in a case where a father murdered his children and then cut his hand off with an axe before fatally shooting himself (*Wherefore, if thy hand or thy foot offend thee, cut them off, and cast them from thee*, Matthew XVIII, 8). Perpetrators have been described as being impulsive with poor control of aggressive impulses and antisocial personalities; those who kill a large number of victims are more likely to have exhibited paranoid ideation. Loss of self-esteem, frustration, and low personal achievement have all been documented *(5,11)*.

Stressful life events also may be contributory factors, with cases occurring when there have been financial or work-related problems, including loss of employment. Marital discord with feelings of rejection may play a role in spousal and familial murder–suicides, as may significant physical ill health. Histories of domestic violence by the perpetrator toward the victim may be elicited *(21,22)*. Cases of elderly spousal murder–suicides may be consensual if there has been an agreement that this course of action is preferable to living with debilitating illness or unfavourable living conditions.

Alcohol and drug use may also be exacerbating factors in these cases, although drugs or alcohol are not always detected. Indeed some studies have shown lower levels of alcohol in victims and perpetrators compared to those involved in homicide alone *(6,14)*. Substance abuse has been reported in 17% of perpetrators *(11)*, with intoxication by psychoactive drugs being found in

10%, alcohol intoxication in 21% and both drugs and alcohol detected in 13%, in another series *(5)*.

Revenge may be a significant motive in cases of estranged spouses and also particularly in cases of disgruntled employees. Recently separated spouses may feel that if they cannot have their children or their spouse, then no one else will. Employees or individuals who have a particular issue with authorities may return to a work place or school and target a number of specific individuals. In these cases, it is not uncommon for bystanders to be injured or killed. Rather than killing themselves the perpetrators may place themselves in situations where it is inevitable that death will occur from police gunfire. This further extends the number of victims who may be psychologically traumatized by such an event.

Studies have attempted to determine whether murder–suicides represent either homicide with a suicidal element or suicide with a homicidal element. Cases are, however, often carefully planned ahead of time and so it is unlikely that the decision to commit suicide is only entertained once the significance of the act of killing has been considered. Analysis of cases also reveals that the demographic features of murder–suicide differ significantly from suicide. It is most likely, therefore, that murder–suicides form a separate entity to both suicide and homicide, although there are aspects that obviously overlap.

7. Conclusion

Murder–suicides are uncommon events that require careful investigation. Confusion of double or more homicides with murder–suicide is possible and therefore characteristic features of murder–suicide should be carefully looked for both in the history and at the death scene. Many perpetrators have histories of depression and contact with mental health services, and so cases may represent an extension of suicide. Cases that involve a large number of victims may lead to considerable logistical problems.

References

1. Copeland AR (1985) Dyadic death revisited. J Forensic Sci Soc 25, 181–188.
2. Berman AL (1979) Dyadic death: murder–suicide. Suicide Life Threat Behav 9, 15–23.
3. Allen NH (1983) Homicide followed by suicide: Los Angeles, 1970–1979. Suicide Life Threat Behav 13, 155–163.
4. Hanzlick R, Koponen M (1994) Murder–suicide in Fulton County, Georgia: comparison with a recent report and proposed typology. Am J Forensic Med Pathol 15, 68–73.

5. Palermo GB, Smith MB, Jentzen JM, et al. (1997) Murder–suicide of the jealous paranoia type. A multicenter statistical pilot study. Am J Forensic Med Pathol 18, 374–383.
6. Felthous AR, Hempel A (1995) Combined homicide–suicide: a review. J Forensic Sci 40, 846–857.
7. Milroy CM (1995) Reasons for homicide and suicide in episodes of dyadic death in Yorkshire and Humberside. Med Sci Law 35, 213–217.
8. Marzuk PM, Tardiff K, Hirsch CS (1992) The epidemiology of murder–suicide. JAMA 267, 3179–3183.
9. Coid J (1983) The epidemiology of abnormal homicide and murder followed by suicide. Psychol Med 13, 855–860.
10. Milroy CM (1995) The epidemiology of homicide–suicide (dyadic death). Forensic Sci Int 71, 117–122.
11. Buteau J, Lesage AD, Kiely MC (1993) Homicide followed by suicide: a Quebec case series, 1988–1990. Can J Psychiatry 38, 552–556.
12. Milroy CM, Dratsas M, Ranson DL (1997) Homicide–suicide in Victoria, Australia. Am J Forensic Med Pathol 18, 369–373.
13. West DJ (1965) Murder Followed by Suicide. Heinemann, London.
14. Byard RW, Knight D, James RA, Gilbert J (1999) Murder–suicides involving children—a 29 year study. Am J Forensic Med Pathol 20, 323–327.
15. Palmer S, Humphrey JA (1980) Offender-victim relationships in criminal homicide followed by offender's suicide, North Carolina, 1972–1977. Suicide Life Threat Behav 10, 106–118.
16. Wolfgang ME (1958) An analysis of homicide–suicide. J Clin Exp Psychopathol 19, 208–218.
17. Hannah S, Turf E, Fierro M (1998) Murder–suicide in central Virginia. Am J Forensic Med Pathol 19, 275–283.
18. Milroy CM (1993) Homicide followed by suicide (dyadic death) in Yorkshire and Humberside. Med Sci Law 33, 167–171.
19. Dietz PE (1986) Mass, serial and sensational homicide. Bull N Y Acad Med 62, 477–491.
20. Goldney RD (1977) Family murder followed by suicide. Forensic Sci 9, 219–228.
21. Currens S, Fritsch T, Jones D, Bush G, Vance J, Frederich K, et al. (1991) Homicide followed by suicide—Kentucky, 1985–1990. MMWR 40, 652–659.
22. Campanelli C, Gilson T (2002) Murder–suicide in New Hampshire, 1995–2000. Am J Forensic Med Pathol 23, 248–251.

Iatrogenic Injury

12

Iatrogenic Injury

A Forensic Perspective

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CONTENTS

SUMMARY

Iatrogenesis, the induction of illnesses by the activities of physicians and, by extension, all other health care clinicians, may best be regarded as injury attributable to the adverse effects of or mishaps associated with medical treatment, including various diagnostic and invasive procedures to which a patient has been subjected at any time that may result in serious morbidity or death. It spans the whole gamut of predominantly perioperative and nonperioperative

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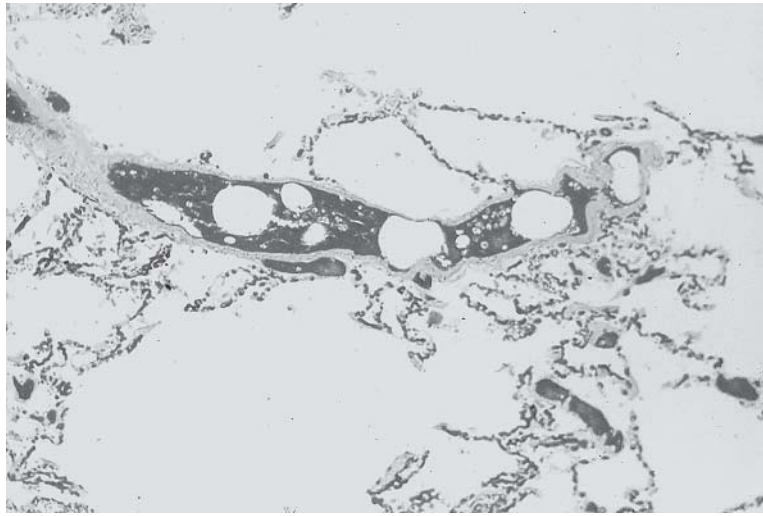

Fig. 9. Pulmonary fat embolism after total hip replacement surgery. This histological finding may sometimes be an artifact of vigorous resuscitation (hematoxylin and eosin stain, original magnification ×100).

Although it would not be feasible to conduct preoperative screening for generally uncommon or rare forms of hereditary thrombophilias such as deficiencies of antithrombin, proteins C and S, factor V Leiden, G20210A prothrombin mutation, and so on *(99,125–127)*, it appears that more could be done to prevent these deaths, for example, application of appropriate thromboprophylaxis (which, of course, is not without its hazards), to forestall the rather unpleasant medicolegal consequences, both criminal and civil, that may follow in their aftermath *(118)*.

2.5. Other Embolic Phenomena

Other forms of thromboembolism, including those of an iatrogenic etiology, are well documented in the forensic medical literature. Fat embolism (Fig. 9) tends to be associated with fractures, extensive soft tissue injury, septicemia, diabetes mellitus, acute pancreatitis, and burns. Fat embolism may also complicate surgical operations on fatty tissues (e.g., mastectomy), steroid injections *(128)*, spinal and orthopedic surgery (e.g., vertebroplasty for osteoporotic vertebral collapse *[129]*, total joint replacement operations *[130]*), and tumescent liposuction, which often is performed as an outpatient procedure and carries an estimated mortality rate of 1 in 5000 procedures *(131–*

Moreover, it has been reported that largely, preventable medical errors result in between 44,000 and 98,000 annual deaths in the United States alone *(5–8)*.

1.2. Role of the Forensic Pathologist

From the perspective of forensic pathology, iatrogenesis may be thought of as injury attributable, in part or in whole, to the adverse effects of or mishaps arising from medical treatment, including various diagnostic and invasive procedures to which a patient has been subjected at any time and which may result in serious morbidity or death. Accordingly, its reach may extend well beyond the proper confines of the medical profession as it were to embrace the iatrogenic potential of the ministrations of the expanding ranks of other health care professions allied to medicine. Fatal iatrogenesis has to be considered to be well within the ambit of forensic pathology *(9,10)* and that, accordingly, the detailed investigation of actual or suspected iatrogenic deaths constitutes a substantial forensic contribution to injury prevention, medical audit, continuing improvement in health care, as well as the elucidation and resolution of the attendant clinicopathological and medicolegal issues that almost inevitably arise in their wake *(11–13)*. This would be entirely in keeping with the contributions that forensic pathology has made (and continues to make) to public safety in general.

In the face of the mounting complexity of clinical practice afforded by increasing subspecialization and advances in medical technology (which renders it possible to treat greater numbers of older and gravely ill patients), it may be said that the medicolegal concerns now extend beyond the traditional confines of retained surgical instruments *(9)* or anecdotal reports of the wrong patients or the wrong parts of patients being subjected to unnecessary surgery (e.g., amputation of the wrong limb). Indeed, it might even be argued that cases of *res ipsa loquitur* are probably rare these days, apart from the still extant danger of prescribing or administering to a patient a drug to which he is known to be allergic.

1.3. Classification

Iatrogenic injury may be classified in a number of ways, depending on the premise and perspective adopted for its evaluation. For instance, iatrogenesis could be regarded—under the relevant circumstances—as being clinically anticipated or unanticipated, the direct or indirect cause of morbidity and death, an acceptable therapeutic risk or a cause for clinical, ethical, and medicolegal concern, or a systemic or individual error with the corresponding implications for medical audit, perhaps with particular emphasis on the robustness of

established clinical pathways, guidelines, and protocols that require periodic reviews.

However, for practical purposes that are in keeping with forensic practice, the following model of iatrogenesis, which is used throughout this chapter, is proposed:

- Predominantly procedural in nature (e.g., surgical or anesthetic complications including those attributable to invasive diagnostic and palliative procedures and minimal access surgery),
- Predominantly nonprocedural in nature (e.g., adverse drug reactions [ADRs]),
- Miscellaneous iatrogenic complications (e.g., adverse events related to diagnostic imaging, radiotherapy, or hemodialysis),
- Combined/complex iatrogenesis.

The following is a review of the wide spectrum of iatrogenic injuries documented by the Centre for Forensic Medicine, Health Sciences Authority, Singapore, as well as those instances described in the medical literature that one may well encounter in the course of conducting perioperative autopsies. Obviously, as therapeutic complications are legion, this does not, by any means, constitute an exhaustive treatise on the subject.

2. Complications Predominantly Related to Surgery and Invasive Procedures

Rapid and spectacular improvements in pre- and postoperative clinical investigations, particularly in the field of diagnostic imaging, have purportedly facilitated increasingly accurate diagnoses. Yet, although the apparent incidence of major discrepancies in perioperative deaths may have decreased progressively (from between approx 20 and 30% to possibly less than 15% during the past three decades or so *[14,15]*), one may frequently be surprised (pleasantly or unpleasantly, depending on one's perspective) by at least some of the autopsy findings in a perioperative death. Unfortunately, the decline in autopsy rates, which appears to be a global phenomenon and one which is repeatedly bemoaned *(16)*, has certainly not leant itself to maximizing the teaching potential of perioperative autopsies.

The collective experience of the Centre for Forensic Medicine, Health Sciences Authority, which undertakes all coronial casework in Singapore (a city-state with a population of approx 4 million) has been that medicolegal perioperative autopsies, albeit conducted selectively under the auspices of the state coroner, do shed considerable light on the nature and causes of such

Table 1
Coronial Perioperative Deaths (1989–1997) Examined by the Centre for Forensic Medicine, Health Sciences Authority, Singapore

Period	Perioperative autopsies (*n*)	% of Coroner´s autopsies	Iatrogenic rate (%)
1989–1991	132	2.0	15.2
1992–1994	170	2.6	28.8
1995–1997	270	4.4	24.4

deaths. Published data *(12,13)* have indicated a statistically significant increase in the necropsy incidence of coronial perioperative autopsies during the better part of the last decade, with a doubling of the iatrogenic death rate during the second triennium as compared to the first (Table 1). In the third triennial study, it was observed that the proportions of such deaths among patients subjected to multiple interventions or initial elective procedures were more than twice as high as amongst those undergoing single procedures and those initially classified as emergencies (35.6 vs 16.6% and 33.3 vs 13.2%, respectively; $p < 0.01$. *[11]*).

These studies have since evolved into an ongoing departmental review of all coronial cases of perioperative and iatrogenic mortality, with the view to supporting medical audit that the public (as well as private) hospitals and medical institutions in Singapore are required to undertake. It is estimated that since the year 2000 between 140 and 200 posttherapeutic (mostly perioperative) deaths have been reported to the coroner annually and that the corresponding coronial necropsy rates have been between 5 and 7% (of a total of approx 2000 autopsies per year) during that time. A detailed analysis of the nature of these iatrogenic injuries and their medicolegal implications is currently being undertaken.

2.1. Hemorrhage

Primary hemorrhage, presenting intraoperatively or postoperatively, may occur as a consequence of slipped hemostatic ligatures or direct intraoperative trauma to blood vessels or vascular organs. The following are examples of this adverse event:

- Hemopericardium may complicate coronary artery bypass graft surgery as the result of bleeding from vascular anastomoses or punctures, a condition that

necessitates urgent or immediate surgical reexploration. Notably, such a complication, which occurred at a frequency of 1 to 2% a decade or two ago *(17,18)*, is now considered rare in reputable cardiothoracic units, particularly with the current emphasis on pre- and postoperative pharmacological intervention to minimize blood loss in cardiac surgery *(19)*. Nevertheless, mediastinal hemorrhage still occurs, particularly in patients who have bleeding tendencies or are subjected to protracted periods of cardiopulmonary bypass. In this respect, it appears that off-pump coronary surgery might significantly reduce the need for blood transfusion *(20)*. Percutaneous transluminal coronary angioplasty (PTCA), which is commonly performed to relieve severe coronary stenosis and to treat acute coronary events, also may cause intrapericardial hemorrhage by way of significant coronary artery dissection and perforation.

In fact, an iatrogenic hemopericardium may, occasionally, result from relatively simple procedures, for example, as a consequence of a puncture of the anterior descending branch of the left coronary artery during fine needle aspiration of a breast lesion (the author having had the privilege of witnessing the autopsy on precisely such a case).

- Hemothorax may result from chest-tube insertion or following the removal of a fenestrated chest tube inserted during pneumonectomy.
- Hemoperitoneum may be the result of *(a)* partial gastrectomy, *(b)* colonic resection, *(c)* iliofemoral arterial dissection caused by intraaortic balloon pump insertion (Fig. 1) (usually applied in acute myocardial failure, decompensation during cardiac surgery, or cardiogenic shock *[21–25]*), *(d)* percutaneous liver and renal biopsies, *(e)* radiologically guided drainage of a liver abscess, *(f)* percutaneous transhepatic cholangiography and biliary drainage, *(g)* laparoscopic surgery, and *(h)* laser vaporization of intraperitoneal lesions such as endometriotic cysts. It might be worth noting that a percutaneous liver biopsy, which is a rather common invasive diagnostic procedure, is said to have a mortality rate of between 0.01 and 0.1% *(26,27)*. It has been suggested that there is no definite evidence that ultrasonographically guided biopsies are necessarily safer than those performed without ultrasonography *(28)*, although this is somewhat counterintuitive. Trocar-induced injuries associated with laparoscopic procedures are well known. On the matter of laparoscopic and other procedures (usually described as being minimally invasive in nature), it has been pointed out that these interventions are just as invasive as open surgery in their capacity to reach various organs and tissues and may not be minimally stressful to patients being treated by these means. A relevant example is a case report on an instance of massive retroperitoneal hemorrhage with a fatal outcome that arose as an unusual complication of percutaneous endoscopic gastrostomy (PEG), wherein it was deemed that the initial attempt at needle puncture of the stomach, under endoscopic guidance, had resulted in the iatrogenic perforation and laceration of the splenic and superior mesenteric veins *(29)*. Thus, the alternative appellation—minimal access surgery—appears to be more appropriate *(13,30,31)*.

Fig. 1. Extensive iliofemoral dissection resulting in severe retroperitoneal hemorrhage after the insertion of an intraaortic balloon pump. (Reprinted, with permission, from ref. *21.)*

- Hemorrhage may result from vascular injury (e.g., carotid artery "blow-out") attending radical neck dissection (often accompanied by partial mandibulectomy and/or glossectomy) for advanced oral/lingual, esophageal, pharyngeal, and laryngeal malignancies.

Primary hemorrhage also may occur as a consequence of perioperative coagulopathy, such as disseminated intravascular coagulation (DIC; Figs. 2 and 3 *[32]*) induced, for example, by *(a)* corrective surgery for complex congenital heart disease, *(b)* repair of an abdominal aortic aneurysm (which carries a mortality risk of 50% upon rupture and for which prior screening has been demonstrated to reduce overall deaths by as much as 42% *[33,34]* and postoperative mortality after an elective operation from 9 to 3% *[35,36]*), *(c)* massive abdominal surgery (such as a Whipple's operation for pancreatic carcinoma or extensive bowel resection for intestinal ischemia), and *(d)* necrotizing enterocolitis in premature neonates. The author also has observed that extensive operations on skeletal structures (e.g., reconstructive surgery for

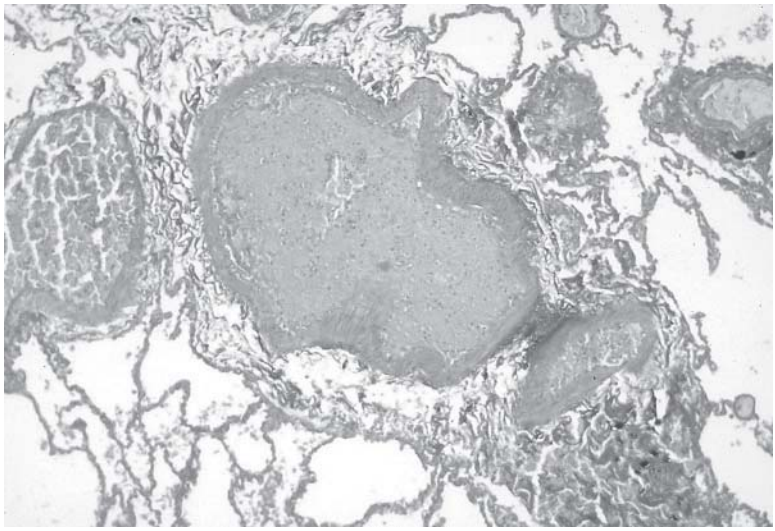

Fig. 2. DIC: platelet-fibrin thromboemboli within the pulmonary microvasculature (MSB, Martius, Scarlet, Blue; original magnification ×100).

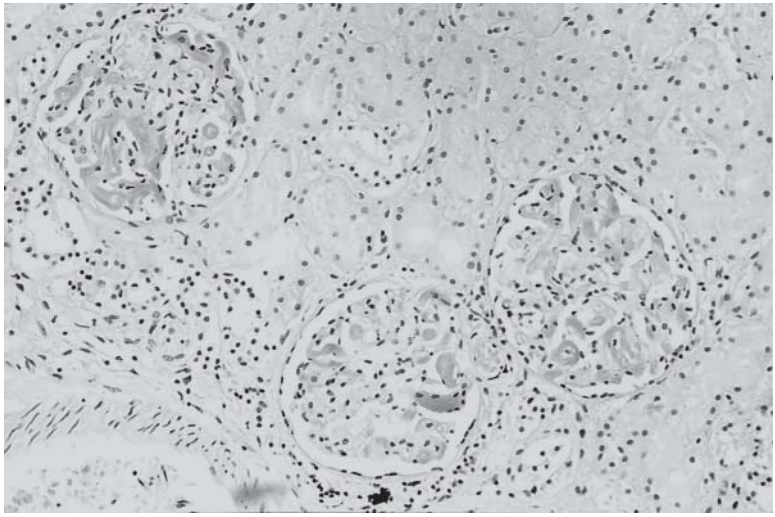

Fig. 3. DIC: Fibrin thromboemboli occluding the renal glomeruli (hematoxylin and eosin stain, original magnification ×100).

major congenital craniofacial anomalies) carry the risk of DIC that may, occasionally, manifest as fatal postoperative hemorrhage.

Not infrequently, surgical patients may also be predisposed to perioperative hemorrhage by various forms of comorbidity *(12,13)*. These include the following:

- Preoperative sepsis, malignancy, obstetric complications, impaired liver function, and bleeding diatheses;
- Preoperative anticoagulation (e.g., warfarin, heparin or enoxaparin [a low-molecular weight heparin] for thromboprophylaxis, anti-platelet agents such as aspirin, ticlopidine, and clopidogrel after a myocardial infarction (MI) or cerebrovascular accident, calcium antagonists that inhibit platelet aggregation *[37,38]* for anti-anginal and anti-hypertensive therapy); this author has encountered a case of severe acute intracerebral hemorrhage that could be attributed to the use of a standard dose of 5000 units of heparin during PTCA;
- Chronic or end-stage renal failure (often complicating diabetic nephropathy) or chronic glomerulonephritis.

Occasionally, self-removal of indwelling wide-bore intravascular catheters or the self-insertion of wide-bore butterfly needles by patients on regular hemodialysis also may induce fatal hemorrhage *(39)*.

2.2. Nosocomial Infections

Hospital and iatrogenically acquired infections not uncommonly supervene in surgical patients. Severely ill patients who require intensive care or who are diabetic and/or immunocompromisation seem to be particularly vulnerable to nosocomial infections (Figs. 4 and 5). It has been said that nosocomial infections in the United Kingdom alone affect almost 10% of patients and cause 5000 deaths annually *(40,41)*. According to one estimate, methicillin-resistant *Staphylococcus aureus* (MRSA), a surrogate marker for hospital acquired infections, may be responsible for between 47 and 68% of all cases of *S. aureus* bacteremia and surgical wound infection, respectively *(40,42)*. It appears that staff dressing wounds with MRSA may have an 80% risk of carrying the organism on their hands for up to 3 hours, whereas possibly 40% of patient–nurse interactions in intensive care may result in the transmission of *Klebsiella* species and *Clostridium difficile*, even after minimal contact *(40,43)*. Both MRSA and *Pseudomonas aeruginosa* often are implicated in postoperative respiratory infections. Indeed, multidrug resistant and newly emergent strains of pathogenic bacteria (e.g., vancomycin-resistant *Enterococci* species, *Burkholderia cepacia, Stenotrophomonas maltophilia*, extended spectrum

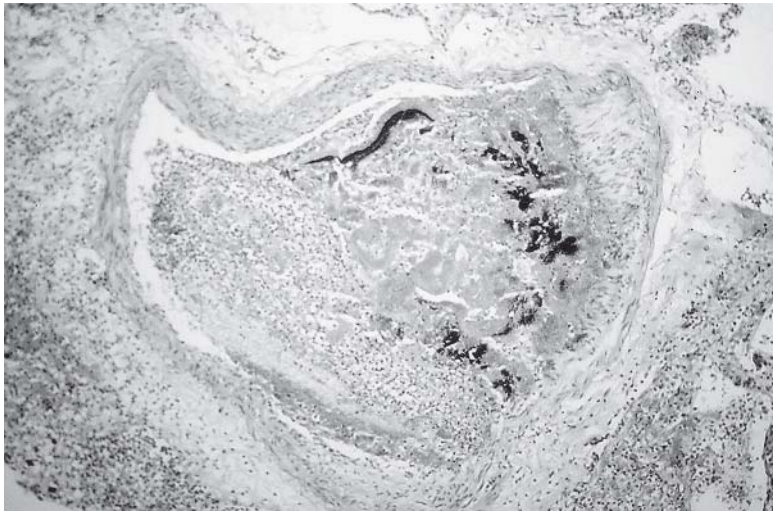

Fig. 4. Septic emboli within the pulmonary microvasculature in a case of severe postoperative MRSA septicemia (Gram stain, original magnification ×100).

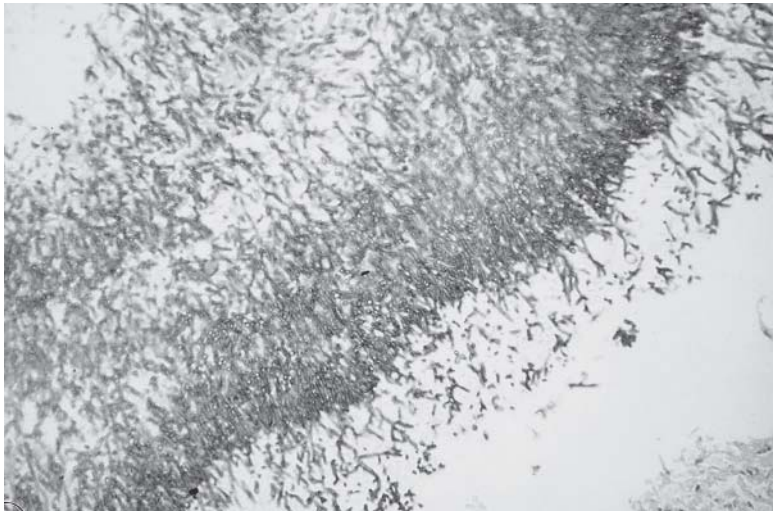

Fig. 5. Invasive pulmonary aspergillosis in an immunocompromised patient (GMS, original magnification ×100).

β-lactamase-producing coliforms such as *Escherichia coli* and *Klebsiella* species) and fungi (e.g., *Candida*, *Cryptococcus*) are commonplace in intensive care units, and organisms such as *Acinetobacter baumanii*, once considered to be merely opportunistic pathogens, may now be difficult to battle. Somewhat ironically, there has even been a report of an outbreak of sepsis from disinfectant contaminated with *Klebsiella oxytoca* in a neonatal and pediatric intensive care unit in Germany that resulted in the death of 28 infants *(44)*. Naturally, the ever-present hazard of sepsis associated with indwelling central venous catheters should be considered *(45,46)*. Whether this is the source of the lethal infection will highly depend on the clinical circumstances prevailing in any given case.

The following postsurgical infections are by no means uncommon *(12,13)*:

- Postoperative bronchopneumonia, urinary tract infection, and septicemia from prolonged immobilization;
- Acute peritonitis from an anastomotic leakage after partial gastrectomy, enteroenterostomy, or colorectal resection;
- Endoscopically induced perforations resulting in peritoneal soiling from rectosigmoid perforation during colonoscopy at an estimated risk of 0.1% *(47–49)*; perforation of the duodenum or a preexisting gastrojejunostomy from endoscopic retrograde cholangiopancreatography (ERCP); endoscopic sphincterotomy used in the treatment of biliary calculi may carry an overall mortality rate of 0.5 to 1%, which increases to 4% in elderly or high-risk patients *(40–52)*;
- Peritonitis and resultant septicemia resulting from biliary leaks induced by laparoscopic cholecystectomy *(53–57)*, which carries a risk of biliary tract injury that has variously been estimated to be between 0.2 and 1%, the upper limit being twice the incidence associated with conventional cholecystectomy *(58–60)*; a relatively recent study demonstrated that patients who suffered bile-duct injury during cholecystectomy have three times the risk of death in the ensuing 9 years compared to patients who do not incur the injury; the hazard of death, adjusted for age and comorbidity during that time, was 11% greater if the repairing surgeon was the same as the one who caused the biliary injury *(61,62)*;
- Spinal sepsis (leptomeningitis, epidural abscess) after laminectomy and decompression;
- Vertebral osteomyelitis after surgical stabilization of cervical dislocation (as observed by the author);
- Suppurative meningitis after neurosurgery, for example, transphenoidal resection of a pituitary adenoma and open resection of a meningioma;
- Chronic leakage around a PEG tube, resulting in localized cellulitis of the skin around the stoma and, occasionally, to lethal peritonitis and necrotizing fasciitis as a consequence of the intraabdominal migration of the intragastric bumper *(29)*.

In recent years there have been concerns about the danger of life-threatening pelvic retroperitoneal sepsis caused by *Bacteroides fragilis* and a *Clostridium* species that follows stapled hemorrhoidectomy and is possibly caused by the entry of these gas-producing organisms from the rectal lumen into the pararectal space, induced by the firing of the stapler *(63,64)*, not to mention the persistence of severe pain and fecal urgency in some patients *(65)*. There have also been reports of highly drug-resistant strains of tuberculosis being transmitted by contaminated bronchoscopes to a cancer patient and causing two outbreaks of the infection *(66,67)*.

Other unusual, but lethal nosocomial infections include the infection of the central nervous system by *Clostridium perfringens* and its subsequent, extensive intraabdominal and soft tissue dissemination attributed to intragluteal injections of diclofenac for back pain in an elderly diabetic patient *(68)*. Acute hepatic failure was observed in a group of 38 patients caused by the contamination of the water used for hemodialysis with the blue–green alga *Microcystis aeruginosa*, which produces a highly toxic cyclic heptapeptide known as microcystin-LR *(69)*. Fatal sepsis was reported following urea instillation abortion complicated by *Clostridium perfringens* and *Escherichia coli* infections *(70)*. There also has been a report of an outbreak of life-threatening sepsis caused by *Streptococcus pyogenes* with resultant multiorgan failure and systemic inflammatory response in three patients (of whom one died) following liposuction *(71)*.

Lately, concerns also have been expressed about the possibility of an iatrogenic variant of Creutzfeldt-Jakob disease being transmitted by contaminated surgical instruments (based on evidence that prion diseases can be spread via stainless steel appliances), although the actual risk involved is largely undetermined *(72–74)*.

2.3. Perioperative Trauma

Direct visceral trauma may complicate various invasive therapeutic and/or diagnostic interventions and result in lethal perioperative hemorrhage and sepsis or may be lethal in and of itself. Some of these complications have been described previously such as the consequences of biliary tract injury from laparoscopic cholecystectomy and endoscopically induced intestinal perforation. In respect of the latter, it has been observed that colonoscopic perforation could result in sudden and unexpected death, presumably from reflex cardiac inhibition *(13)*. An interesting experiment demonstrated that a possible sequence of such perforations might begin with the muscularis propria, followed by the serosa and, finally, the mucosa *(76)*. In any event serosal tears

without mucosal damage have been known to be a complication of colonoscopy *(77,78)*.

Iatrogenic tracheobronchial ruptures may, occasionally, be caused by bronchoscopy such as that performed with a stiff bronchoscope or during intubation. Although rare, such injuries carry a mortality rate as high as 42% *(79)*.

Acute hemorrhagic pancreatitis with its attendant sequel of DIC, shock, adult respiratory distress syndrome, systemic organ failure, sepsis, pancreatic abscess, and pseudocyst formation (amongst other complications) is not invariably the result of alcoholism, biliary disease, infection (e.g., Mumps, Coxsackievirus), hyperlipoproteinemia, or an ADR (e.g., thiazide diuretics, azathioprine). Iatrogenic causes such as perioperative injury and endoscopic procedures involving dye injection (e.g., ERCP) are well-documented etiological factors, too *(80,81)*.

Apart from the rare occurrence of fatal retroperitoneal hemorrhage and infection *(39,82–84)*, PEG also may be associated with gastric necrosis and hemorrhage, arising from excess tension between the intragastric bumper and the skin attachments of the tube, gastric wall dissection, acute gastric dilatation, tension pneumoperitoneum, or bowel obstruction *(82,85)*. There also have been rare reports of iatrogenic ruptures of the stomach associated with balloon tamponade (such as with a Sengstaken-Blackmore or Linton-Nachlas tube) for esophageal varices in the setting of hepatic cirrhosis *(86)*.

The iatrogenic causes of aortoesophageal fistula (Fig. 6), which usually result in massive gastrointestinal (GI) hemorrhage and present as hematemesis (typically after an initial episode of relatively mild "sentinel" hemorrhage) include thoracic irradiation, the use of a rigid esophagoscope (now rare), prolonged nasogastric tube placement, and anastomotic dehiscence following the prosthetic graft repair of a thoracic aortic aneurysm *(9,87–91)*. Another interesting, although rare, iatrogenic vascular complication is the splenic artery pseudoaneurysm whose principal etiological factors are pancreatitis, trauma, and intraabdominal iatrogenesis *(92)*. The author has encountered rare instances of atrial rupture during cardiac surgery, atrial or ventricular perforation attributable to cardiac catheterization as well as a hemothorax (or a pneumothorax) due to the subclavian vein being punctured during central venous access (for which imaging guidance has been urged *[93–95]*). An unusual variant of iatrogenic vascular injury attributable to central line insertion is the report of a case of fatal hydrothorax associated with subclavian vein cauterization for hemodialysis *(96)*.

Occasionally, the tips of chest drainage tubes might be embedded within the subpleural aspect of a lung, wedged deeply within a pulmonary fissure, or

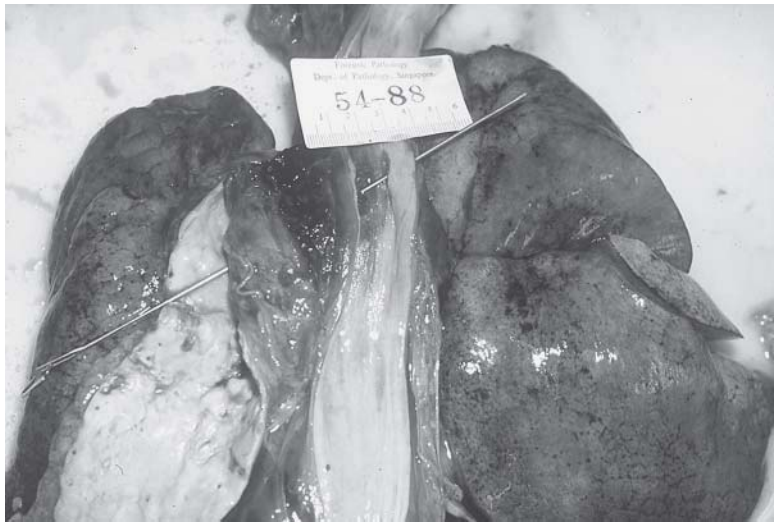

Fig. 6. An aorto-esophageal fistula caused by an ingested duck bone. Endoscopic removal of the foreign body resulted in torrential gastrointestinal hemorrhage.

apparently lacerating the posterior parietal pleura. Rare complications of tubes and drains include a reported case of sudden death in a patient with Arnold-Chiari malformation where the ventriculoperitoneal shunt (inserted to relieve hydrocephalus) had perforated the transverse colon resulting in ascending enterococcal meningitis *(97)* and another case with intracranial insertion of a nasogastric tube through the comminuted cribriform plate of the ethmoid in a victim of homicidal blunt force head trauma *(98)*.

Cervical, uterovaginal, or vaginal ruptures may sometimes attend forceps-assisted delivery, even in the absence of a history of previous cesarean section *(99)*, which is said to increase the risk of maternal morbidity and mortality considerably *(100–102)*. Although a trial of vaginal birth after a previous cesarean section has been recommended *(103)*, it also has been demonstrated that the risk of uterine rupture among women undergoing vaginal delivery after spontaneous labour was at least three times higher than in those who underwent a second cesarean delivery without spontaneous labor *(104,105)*. It should be noted that instrumental (vacuum- and forceps-assisted) delivery may, occasionally, result in fatal perinatal subgaleal hemorrhage *(106)*, while vacuum extraction itself may, even more rarely, cause neonatal intracranial (mainly subdural) hemorrhage *(107)*, resulting from tentorial laceration and rupture of the dural venous sinuses which in newborn infants tend to be

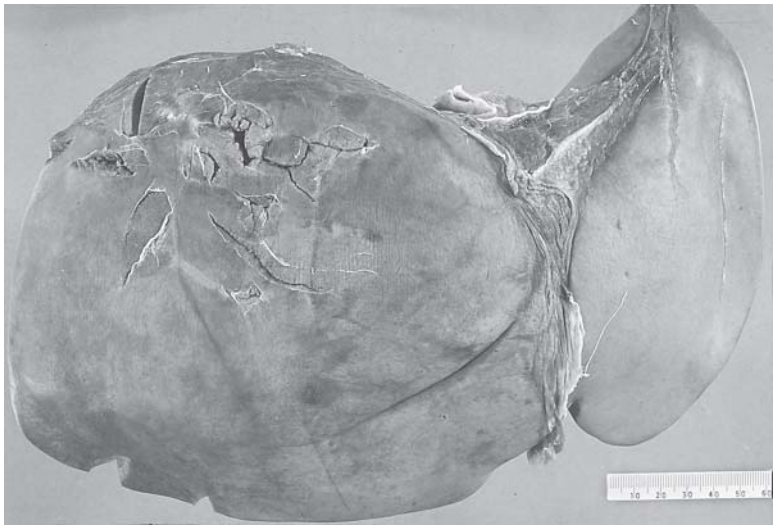

Fig. 7. Rupture of the liver as a sequel of cardiopulmonary resuscitation.

relatively large in relation to the brain and skull *(108–112)*. Although an early series reported a perinatal mortality rate of 15 per 1000 attributable to vacuum extraction *(107)*, it appears that the type of device employed may influence the occurrence of life-threatening iatrogenesis *(113)*.

Regarding the subject of iatrogenic trauma, it is worth noting that a wide range of resuscitative injuries may be observed at autopsy. Most of these are artifactual in nature and include bruising of the subcutaneous tissues and pectoral muscles, fractures of the sternum and rib cage, hemothorax, mediastinal bruising, cardiac rupture, pulmonary laceration, damage to the upper and lower airways, conjunctival petechial hemorrhages, retinal hemorrhages, rupture of the esophagus, stomach and intestines (including the mesentery, liver [Fig. 7], and spleen *[114]*), electric defibrillator pad markings, subarachnoid hemorrhage, and so on *(115)*. It would seem that the list is virtually endless and that almost any form of blunt force type injury could complicate vigorous and protracted cardiopulmonary resuscitation. Indeed, the author had witnessed a case of thoracic vertebral fracture dislocation in an elderly patient who had been subjected to precisely such ministrations. Occasionally, however, some of these injuries may have inadvertently been inflicted during the antemortem period and could, potentially, have contributed to morbidity or mortality *(116,117)*.

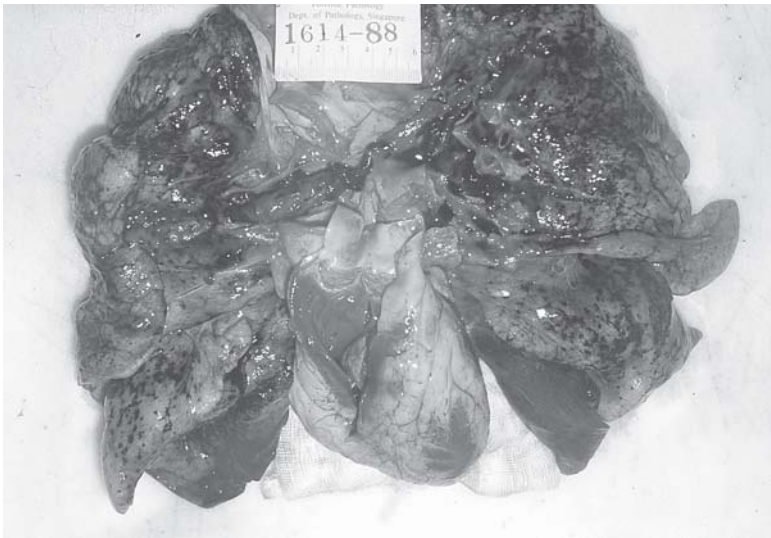

Fig. 8. Gross pathology of postoperative massive pulmonary thromboembolism that was clinically unsuspected.

2.4. Pulmonary Thromboembolism

In principle, pulmonary thromboembolism (Fig. 8), the often fatal sequel of deep vein thrombosis, essentially is a predictable and preventable perioperative complication. However, deep vein thrombosis may develop insidiously, and the resultant massive pulmonary thromboembolism may supervene with apparent and unexpected suddenness. Indeed, two consecutive necropsy series *(118,119)* suggested that fatal pulmonary thromboembolism is underdiagnosed in clinical practice. Both studies, which covered a time span of a decade, demonstrated that surgery was the most common risk factor detected (41.4% and 42.3%, respectively) after complete or relative immobility, followed by trauma, obesity, sepsis, malignancy, and pregnancy. They also consistently demonstrated a peak incidence of fatalities within the first week of immobilization or trauma rather than in the second or third weeks *(118,120)*, an observation that is supported by the results of another study *(121)*. Pulmonary thromboembolism was clinically unsuspected in nearly three-fourths of the deaths that occurred under medical care (77.1 and 74.6%, respectively). This, unfortunately, seems to be in accord with similar findings elsewhere, with between some 55 and 79% of such deaths being undiagnosed *(122–124)*.

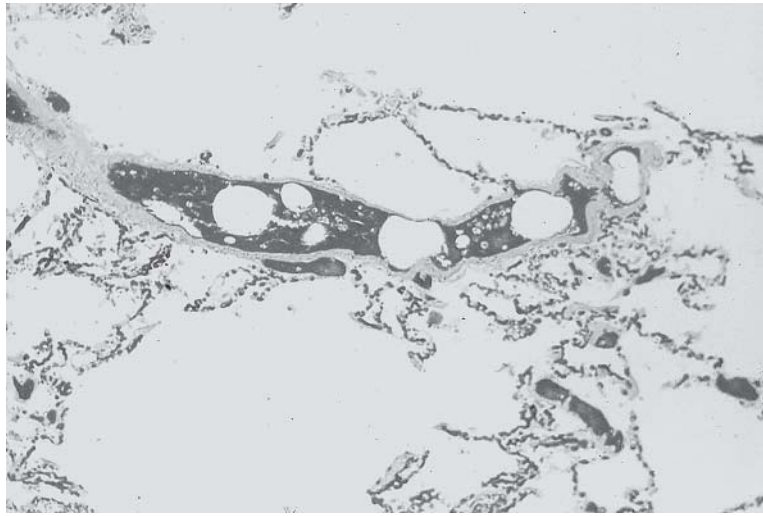

Fig. 9. Pulmonary fat embolism after total hip replacement surgery. This histological finding may sometimes be an artifact of vigorous resuscitation (hematoxylin and eosin stain, original magnification ×100).

Although it would not be feasible to conduct preoperative screening for generally uncommon or rare forms of hereditary thrombophilias such as deficiencies of antithrombin, proteins C and S, factor V Leiden, G20210A prothrombin mutation, and so on *(99,125–127)*, it appears that more could be done to prevent these deaths, for example, application of appropriate thromboprophylaxis (which, of course, is not without its hazards), to forestall the rather unpleasant medicolegal consequences, both criminal and civil, that may follow in their aftermath *(118)*.

2.5. Other Embolic Phenomena

Other forms of thromboembolism, including those of an iatrogenic etiology, are well documented in the forensic medical literature. Fat embolism (Fig. 9) tends to be associated with fractures, extensive soft tissue injury, septicemia, diabetes mellitus, acute pancreatitis, and burns. Fat embolism may also complicate surgical operations on fatty tissues (e.g., mastectomy), steroid injections *(128)*, spinal and orthopedic surgery (e.g., vertebroplasty for osteoporotic vertebral collapse *[129]*, total joint replacement operations *[130]*), and tumescent liposuction, which often is performed as an outpatient procedure and carries an estimated mortality rate of 1 in 5000 procedures *(131–*

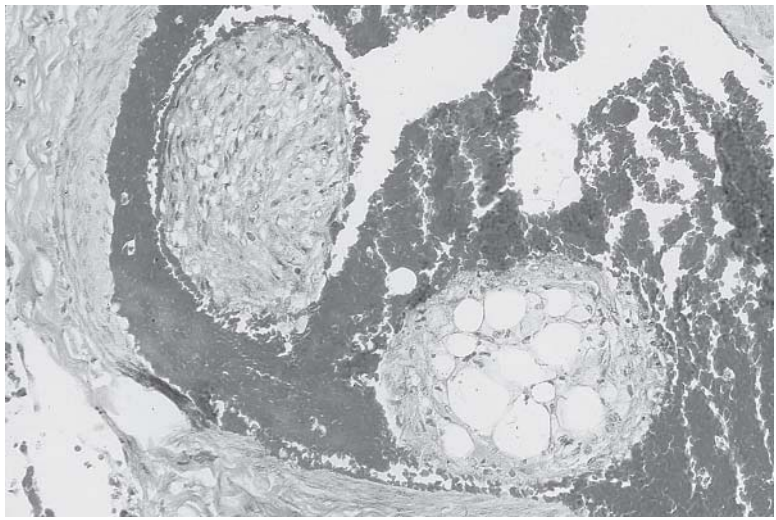

Fig. 10. Pulmonary bone marrow embolism with evidence of early organization (hematoxylin and eosin stain, original magnification ×100).

133). Although fat embolism may be pulmonary or systemic in distribution, bone marrow embolism (Fig. 10) tends to be restricted to the pulmonary circulation *(128)*. Not infrequently, both fat and bone marrow embolism may represent artifacts of resuscitation *(115,134)*.

Air embolism may present as a complication during operations on the head or neck (e.g., neurosurgical procedures and thyroid surgery *[135]*) and procedures involving central venous access such as the infusion of total parenteral nutrition or hemodialysis through the subclavian or internal jugular veins *(39)*.

Amniotic fluid embolism (Figs. 11 and 12), which are estimated to occur at a frequency in 1:8000 to 1:80,000 of pregnancies, usually is regarded as an unpredictable and unpreventable direct cause of maternal mortality but may be associated with various obstetric procedures (e.g., cesarean delivery, first- and second trimester abortions, hysterectomy, saline/prostaglandin-induced abortions, or amniocentesis). In this respect, the etiological role of oxytocic agents, if any, is uncertain *(99,136)*.

Rare instances of possibly iatrogenic pulmonary cartilage embolism (Fig. 13) have been reported (e.g., humeral fracture followed open reduction and internal fixation *[137]* or as a consequence or artifact of cardiopulmonary resuscitation *[138,139]*). These instances should be distinguished from the potentially lethal condition of fibrocartilaginous embolism resulting in spinal cord infarction or acute myelopathy, a condition whose etiology is unclear *(138)*.

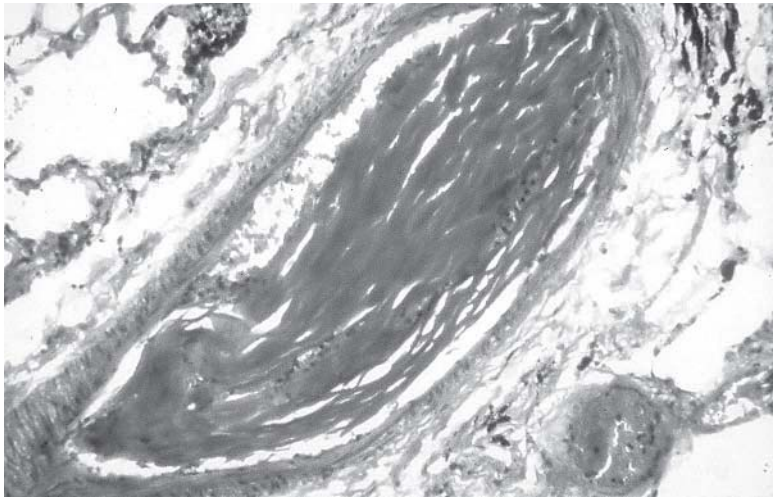

Fig. 11. Amniotic fluid embolism. Fetal squames in a pulmonary vessel (Phloxine-tartrazine stain, original magnification ×200). (Reprinted, with permission, from ref. *136*.).

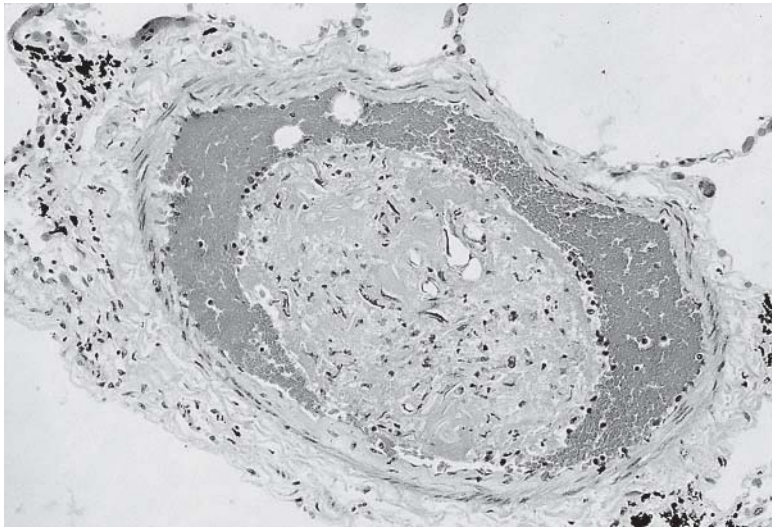

Fig. 12. Fetal squames admixed with fibrin in amniotic fluid embolism accompanied by severe coagulopathy (hematoxylin and eosin stain, original magnification ×100).

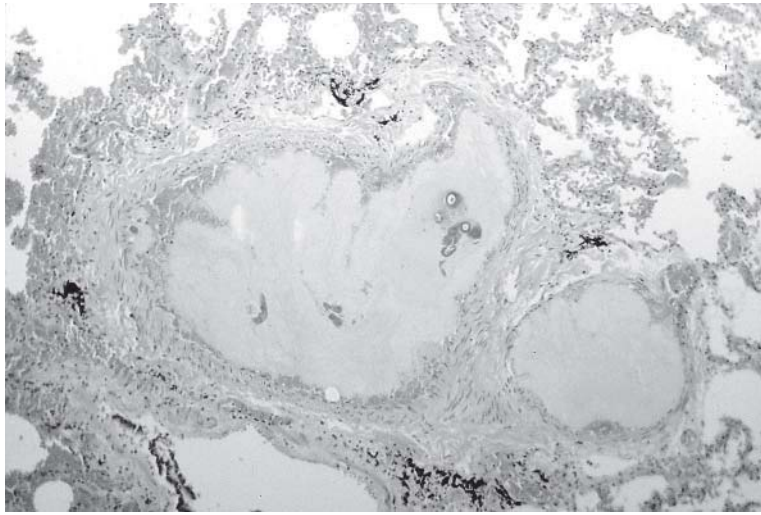

Fig. 13. Pulmonary cartilage embolism (hematoxylin and eosin stain, original magnification ×100).

Cholesterol embolism (with resultant acute renal failure and, possibly, multiorgan involvement accompanied by marked eosinophilia) may be observed in patients with generalized atherosclerosis, hypertension, diabetes, and aortic aneurysm. Predisposing factors include angiography, anticoagulation, thrombolysis, and any form of vascular surgery (e.g., coronary artery bypass grafting [CABG] or repair of an abdominal aortic aneurysm). The necropsy incidence is estimated to be 0.3 to 0.4% *(140,141)*.

Examples of iatrogenic foreign body embolism include the following:

- Suture embolism to the left anterior descending coronary artery or following mitral valve replacement surgery *(142)*;
- Occlusion of the left coronary artery by a fragment of a femoral artery during PTCA *(143)*;
- Systemic "meat and vegetable" embolization to the heart, kidneys, and brain arising from an esophageal-atrial fistula caused by a nasogastric tube *(144)*; and
- Intracardiac embolism of a distal fragment of an indwelling central venous catheter to the right ventricle and pulmonary trunk, presumably resulting in fatal cardiac arrhythmia *(145)*.

2.6. Perioperative Cardiac and Cerebrovascular Events

Acute or recurrent MI may occur intraoperatively or postoperatively *(13)*, possibly in the absence of any history or clinical suspicion of ischemic heart

disease. Usually, coronary risk factors such as systemic hypertension, diabetes mellitus, obesity, cigarette smoking, and hyperlipidemia are present in addition to the corresponding macroscopical and microscopical pathological features. Determining whether such an event was "natural" in origin or precipitated by the stress (both surgical and anesthetic) of the operation *(9)* is by no means an easy matter. It has been estimated that the risk of perioperative MI in healthy patients undergoing noncardiac surgery is less than 1%; the risk in patients with coronary risk factors or heart disease may be as high as 17% *(146)*.

Occasionally, perioperative myocarditis may supervene, usually presenting as an unanticipated viral infection or an ADR (*see* Subheading 3.1.). However, there have been rare instances of fatal giant cell myocarditis, believed to be an expression of a florid foreign body reaction to foreign material (such as cornstarch), that are introduced during mitral valve replacement surgery or CABG *(147–149)*.

Whether deaths after events such as stent embolism and restenosis after PTCA (with resultant worsening of the underlying myocardial ischemia or extension of the original MI) should be considered to be iatrogenic in nature, is, at least from a purely forensic perspective, a matter of debate. The author also has encountered several cases of sudden, unexpected postoperative deaths associated with myocardial tunneling of the anterior descending coronary artery and small coronary artery disease (Fig. 14) in the absence of any antecedent history of cardiovascular disease.

There appears to be evidence that patients with a history of stroke are at a 14-fold overall increased risk for a recurrence (with women having a relative risk more than twice that of men *[150]*). Women have higher morbidity and mortality rates after cardiac surgery than men *(151)*. It also has been said that patients who undergo coronary artery bypass graft shortly after experiencing unstable angina or non-Q-wave myocardial infarcts have a higher risk (perhaps twice) of stroke than those who forgo surgery or delay it *(152,153)*. In this respect it is interesting to note that morphological features such as reactive microgliosis, nuclear pyknosis, focal microscopic hemorrhage, global cerebral hypoxia, and necrosis have earlier been documented in patients who died after CABG and valve replacement surgery *(154)*.

2.7. Miscellaneous Postsurgical Complications

There appears to be a risk of malignancy supervening after various forms of surgery. For instance, there have been conflicting reports of remote partial gastrectomy for benign peptic ulcer disease being a risk factor for pancreatic

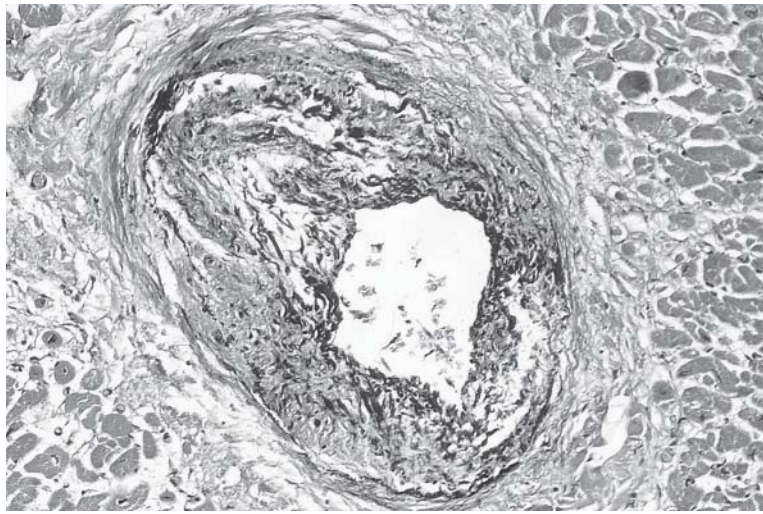

Fig. 14. Incidental small coronary artery disease with marked medial atrophy and a combination of florid myointimal and perivascular fibrosis. Note disruption of the internal elastic lamina (Verhoeff-van Gieson stain, original magnification ×200).

carcinoma. In one such study the overall increased risk of pancreatic carcinoma after gastrectomy was reported to be 1.8 on average (5–59 years postoperatively), increasing gradually to 3.6 after 35 years or longer after surgery *(155)*.

Malignant lymphoma may develop in the soft tissues at the sites of previous orthopedic operations, such as the internal fixation of a fracture or joint replacement surgery some 4 to 12 years later *(156)*. Of particular interest are the somewhat startling reports of the transmission of brain tumors through transplantation *(157)*. Noteworthy seems the report of a case of transmission of glioblastoma multiforme via liver transplantation *(158)*, the cadaveric liver having been harvested from a brain dead patient who had a frontal lobe glioma removed previously and underwent resection of a locally recurrent tumor shortly before her death. Indeed, a novel postsurgical complication of liver transplantation is massive hemoptysis in a patient with hereditary hemorrhagic telangiectasia who had irregularly thickened pulmonary arteries displaying concentric myointimal hyperplasia *(159)*.

2.8. Anesthetic Complications

Compared with iatrogenic injuries that are directly or indirectly attributable to surgical and other invasive procedures *per se*, significant anesthetic

Table 2
The American Society of Anesthesiologists' (ASA) Classification of Surgical Mortality Risk

Class	Description
ASA 1	A normal healthy patient
ASA 2	A patient with mild systemic disease
ASA 3	A patient with severe systemic disease that limits activity, but is not incapacitating
ASA 4	A patient with incapacitating systemic disease that is a constant threat to life
ASA 5	A moribund patient who is not expected to survive for more than 24 hours with or without an operation

mishaps seem to occur less frequently, but usually with devastating consequences. Thus, it has been estimated that, although anesthesia might contribute to death in 1:1,700 operations, it is probable that only 1:10,000 patients die exclusively from an anesthetic complication *(9)*. This appears to be in accord with this author's observation that there were only six deaths attributable to adverse anesthetic events of a total of 572 coronial perioperative autopsies performed between 1989 and 1997 at the Centre for Forensic Medicine in Singapore, with the necropsy incidence varying between 0.6 and 2.3%, whereas that for surgical iatrogenesis varied from 15.2 to 28.8% during this time *(12,13)*.

It is common practice to assess the risk of operative mortality in surgical patients by applying the relatively straightforward classification of the American Society of Anesthesiologists classification *(10,160)*, which stratifies patients into five levels of clinically appraised risk (Table 2). In this system, a patient who is assigned a score of 4 is very seriously ill (and hence at high operative risk), whereas a score of 5 quite literally means that death is imminent, whether an operation is conducted or not. In this respect, this author has observed that surgeons and anesthetists/intensive care clinicians not infrequently diverge rather sharply in their assessment of the operative risks of patients who are likely to be in the severe end of the spectrum, with the latter appearing to favor a more grave prognosis in general. This divergence is perhaps a reflection of the subjectivity that is inherent in such an ordinal method of clinical assessment.

Indeed, this author often marvels at the fact that major surgery—sometimes a series of extensive operations requiring general anesthesia—actually

Table 3
The Physiological, and Operative Severity Score for the Enumeration of Mortality and Morbidity (POSSUM)

Physiological factors
Age; Glasgow Coma Scale; respiratory function; cardiac status; electrocardiogram; blood pressure; pulse rate; hemoglobin level; white cell count; plasma urea, sodium, and potassium concentrations
Operative factors
Elective or emergency surgery; complexity of operation; multiple procedures; severity of hemorrhage; intraperitoneal contamination; extent of spread of malignancy

is conducted on some severely and chronically ill, elderly patients, each with a myriad of comorbidity involving almost every major organ system. In such instances it seems that the main value of the autopsy is to provide a morphological proof that any one of these conditions would have been sufficient in the ordinary course of nature to cause death, but that the perioperative complications that (inevitably?) supervened arguably constituted a *novus actus interveniens*, thereby abruptly or gradually converting what would otherwise have been death from natural causes to an iatrogenic one.

The development and implementation of a more comprehensive and sophisticated system of risk stratification, known as the Physiological and Operative Severity Score for the Enumeration of Operative Mortality and Morbidity (i.e., POSSUM *[10,161]*) might afford a more precise and objective assessment of surgical risk. This method involves the statistical analysis of 12 physiological and 6 operative factors (Table 3), each of which is given weightings of 1, 2, 4, or 8 in accordance with their variable substratification into two to four levels. The total physiological and operative scores (88 and 44, respectively) are then analyzed by logistic regression to produce composite risk estimates of morbidity and mortality. It appears that, despite it being somewhat cumbersome in nature, it has been widely accepted by the Scottish Audit of Surgical Mortality *(10)*.

Potentially lethal or devastating anesthetic complications include the following *(9,13)*:

- Oxygen deficiency resulting in hypoxic–ischemic encephalopathy and brain death (Fig. 15), for example, caused by failed intubation (inexperienced operator or

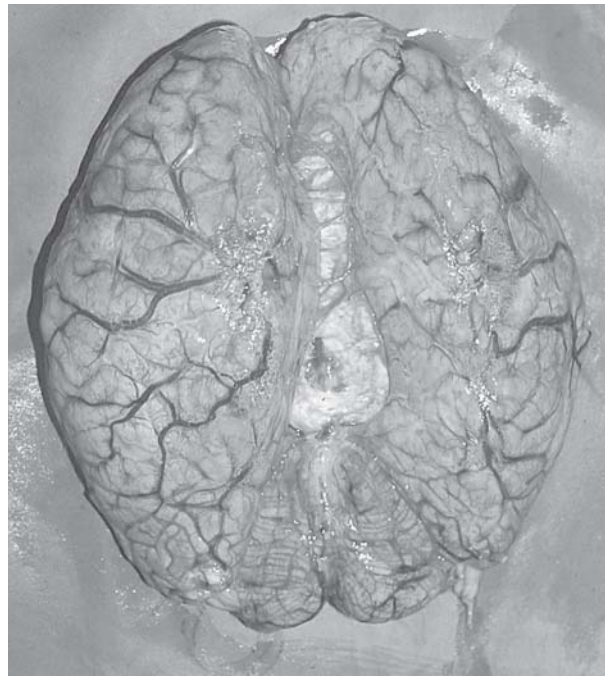

Fig. 15. Gross appearance of the brain in a patient who became brain dead as the result of oxygen deprivation in the course of an anesthetic mishap.

technical difficulties induced by anatomical variations, respectively), dislodgement of the endotracheal tube, faulty connections (minimized by the use of modern anesthetic apparatus with inlets and outlets of different designs and sizes), and prolonged reversal;

- Airway obstruction, for example, with blood, mucus, or foreign bodies (including pieces of gauze);
- Aspiration, for example, regurgitation of gastric contents, or aspiration of contrast medium *(9)*;
- Prolonged or excessive neuromuscular blockade; thiopentone, which is used in induction, may cause cardiorespiratory failure while trichloroethylene and atropine (used in premedication) may result in sudden circulatory failure *(9)*;
- Malignant hyperthermia, a rare autosomal dominant condition (with an estimated incidence of 1:5,000 to 1:70,000) that predisposes the patient to sudden uncoupling of oxidative phosphorylation with a resultant massive overheating, is induced by exposure to certain muscle relaxants (e.g., suxamethonium) and halothane *(9,162)*; it has been proposed that, in addition to the contracture test, which requires an open muscle biopsy sample, susceptibility to malignant hyperthermia could be predicted by the measurement of local carbon dioxide pressure in the

rectus femoris muscle after intramuscular injection of caffeine; this experiment demonstrated that susceptible individuals presented with a considerably higher intramuscular pCO_2 than controls *(163)*;

- Halothane hypersensitivity (with an estimated incidence of 1:6,000 to 1:600,000) may be associated with halothane-induced hepatitis or even massive hepatocellular necrosis, usually after repeated exposure to the agent *(9,164,166)*; there is evidence that halothane hypersensitivity is mediated by the immune sensitization of susceptible individuals (carrying CYP2E1) to trifluoacetylated liver protein neoantigens formation resulting from oxidative halothane metabolism; this particular pathogenetic pathway renders halothane-induced hepatitis amenable to prophylaxis with disulfiram which is converted to an active inhibitor of CYP2E1 in vivo *(165)*; isoflurane, another haloalkane which is minimally and nonreductively metabolized (<1% compared with 30% in the case of halothane) and does not undergo reductive alternative to halothane, has also been reported to cause fatal massive hepatocellular necrosis after a second exposure to this supposedly nonhepatotoxic alternative to halothane in a woman with a history of alcohol abuse and mild obesity who had also taken amitriptyline and acetaminophen (paracetamol *[166]*); instances of enflurane-induced hepatotoxicity have also been reported *(167)*;
- Propofol, a sedative-hypnotic agent used for the induction of anesthesia and for sedating mechanically ventilated patients in intensive care units, has been associated with fatal cardiac failure both in children *(168)* and in adult patients with head injuries *(169)*; in fact, the constellation of myocardial failure, metabolic acidosis, and rhabdomyolysis in children receiving propofol infusions for more than 48 hours has been termed the *propofol infusion syndrome (168)*; in addition, propofol is known to induce hypertriglyceridemia (apparently when used at a rate exceeding 100 μg $kg^{-1}min^{-1}$ for prolonged periods; ref. *170*) resulting in at least one case of nonfatal necrotizing pancreatitis *(171)*;
- Instances of iatrogenic cervical dislocation have been reported in children undergoing lymph node biopsy under general anesthesia; rotation of the head and neck during the procedure is believed to have caused atlantoaxial rotary dislocation, requiring neurosurgical intervention (open reduction *[172]*); from a forensic perspective, it is not difficult at all to appreciate that this is a potentially life-threatening complication;
- There have also been instances of toxic substances that were introduced during spinal or epidural anesthesia for elective cesarean delivery; in one case an unknown substance was introduced epidurally *(173)* whereas in another potassium chloride, instead of bupivacaine, was administered accidentally *(174)*; the former rendered the patient incontinent, while the latter, quite predictably, resulted in sudden death from potassium chloride toxicity; evidently, disastrous consequences may ensue after the application of neuraxial blockade despite evidence that it may reduce postoperative morbidity and mortality caused by deep vein thrombosis, pulmonary thromboembolism, pneumonia, respiratory depression, MI, and even renal failure *(175)*;

- Attention also has been drawn to the importance of maintaining perioperative normothermia (which could very well reduce the risk of hemorrhage, wound infection, and acute cardiac events), this being particularly emphasized for patients with cardiac risk factors *(176,177)*; it is conceivable that there might be occasions when such factors have to be taken into account when conducting a perioperative autopsy.

Interestingly, even common foods, such as potatoes, tomatoes, and aubergines, may contain naturally occurring solanaceous glycoalkaloids (which are naturally occurring insecticides that remain in the body for several days after ingestion). Even in small quantities, solanaceous glycoalkaloids may inhibit butyryl cholinesterase and acetylcholinesterase, thus resulting in the persistence of anesthetic agents and muscle relaxants in the body and a prolonged recovery time, which may last as long as 5 to 10 hours *(178)*. The potentially lethal consequence of this largely unanticipated complication is self-evident.

2.9. Hypoxia

The very mention of hypoxia in a health care setting tends immediately to evoke images of an anesthetic mishap, such as oxygen deprivation with consequent cardiorespiratory arrest, irreversible brain damage, and death (Fig. 16) caused by pulmonary aspiration *(179,180)*, faulty connection of the anesthetic circuit, disconnection from the apparatus, or failed esophageal intubation *(181,182)*. These, by and large, are medicolegally indefensible adverse events arising from preventable human errors. It has also been observed that anesthesia with mechanical ventilation may be attended by impairment of gaseous exchange, leading to decreased blood oxygenation. Indeed, atelectasis is said to occur in 85 to 95% of patients, often immediately after induction of general anesthesia, affecting some 20 to 25% of basal lung tissues *(183)*.

However, iatrogenically induced hypoxia may not always be related to anesthesia *per se*. Profound hypovolemic, septicemic, cardiogenic, or neurogenic shock as well as ventricular tachyarrhythmia may lead to protracted cardiorespiratory arrest and resultant hypoxic–ischemic encephalopathy. Although these predisposing factors could be the result of underlying disease conditions or trauma, they also may be the consequence of the various forms of surgical iatrogenesis described previously.

Indeed, oxygen desaturation (<90%) is known to occur during upper GI endoscopy (e.g., esophagogastroscopy, ERCP) performed under sedation. The incidence has variously been estimated to be between 55 and 91%, this being associated with a mortality risk as high as 1 in 2000 procedures, prompting

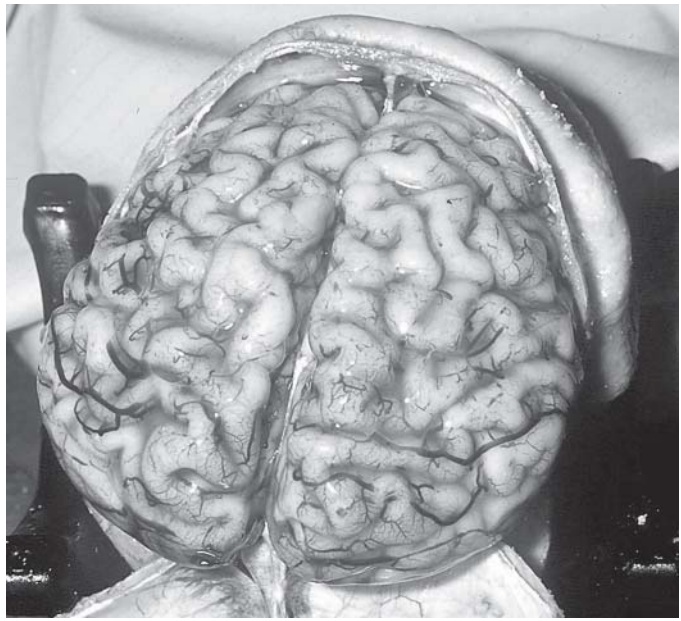

Fig. 16. A case of brain death: In situ formalin fixation of the brain before evisceration may help to solidify it and facilitate its easy removal. This technique should only be used as a last resort (e.g., if the brain is likely to disintegrate upon evisceration) because it may cause anatomical distortion of a brain that has liquefied as a result of autolysis and putrefacation.

calls for close monitoring of patients and the use of supplemental oxygen during these procedures *(184–187)*.

Occasionally, even common and usually mild therapeutic complications may have lethal consequences when the latter present with exceptional severity. Hence, there has been a report of an extensive postextraction hematoma (involving the floor of the mouth, larynx, and cervical muscles) presenting 8 hours after the extraction of an impacted mandibular right third molar that caused death from mechanical asphyxia *(188)*. This may be regarded as an adjunct to the better known Ludwig's angina, which is a life-threatening, diffuse infection (caused by a variety of Gram-negative organisms as well as *Streptococcus viridans*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*) of the submandibular and sublingual spaces that may spread inferiorly causing mediastinitis, empyema, pericarditis, and pericardial tamponade. Apart from the obvious danger of sepsis, Ludwig's angina can also cause critical airway obstruction requiring tracheostomy. Although dental abscesses, intra-

venous drug abuse, and pharyngotonsillitis are the more common etiological factors, Ludwig's angina may occasionally present as a postextraction complication *(189–193)*. The author has personally encountered a case of death in custody (involving a prisoner) attributed to postextraction Ludwig's angina.

3. Predominantly Nonprocedural Complications

3.1. Fatal Adverse Drug Reactions and Medical Errors

For practical purposes, it may be assumed that the therapeutic use of any and all pharmacologically active substances carries a variable risk of an adverse drug reaction (ADR), which may be defined as any undesirable effect of a drug exceeding its anticipated therapeutic properties. This has to be distinguished from more generic adverse drug events (ADEs) that comprise any untoward clinical occurrence after exposure to a specific drug that is not necessarily caused by its use *(194,195)*.

It has been said that ADRs account for 5% of all hospital admissions, occur in 10 to 20% of hospital inpatients, and are responsible for the deaths of some 0.1 and 0.01% of medical and surgical inpatients, respectively *(194)*. This seems to be in accordance with the observation that cases of fatal ADR constituted 0.3 to 0.5% of coronial autopsies during a 5-year period (1999–2003) at the Centre for Forensic Medicine in Singapore.

It is suggested that, for the purposes of the forensic evaluation of suspected cases of fatal ADR, the traditional classification of these reactions as being pharmacological (type A) or idiosyncratic (type B) in nature will mostly suffice (although more sophisticated and cumbersome methods have been proposed). The first are dose-dependent and represent reversible manifestations of the augmented pharmacological properties of a drug whereas the latter are described as being bizarre and largely unpredictable *(194,196)*. Type A reactions, which account for the vast majority (80%) of ADR, may be regarded as those that arise from the intrinsic toxicity of a therapeutic drug, in terms of its primary and secondary pharmacological effects *(194,197)* or from drug interactions where it appears that increasing age, comorbidity (e.g., renal failure), and polypharmacy may result in a considerable risk of ADR (as much as 50% if five drugs are used in combination *[194,198]*). The less common type B reaction, which tends to carry more serious consequences and accounts for a large number of ADR-related deaths, is associated with various complex immunologic (e.g., anaphylaxis), metabolic (e.g., CYP2D6 deficiency), cell receptor (e.g., malignant hyperthermia), and multifactorial pathogenic mechanisms (e.g., halothane-induced hepatitis *[194,199]*).

It is this author's experience that, from a thanatological perspective, the majority of fatal ADRs that come to forensic attention are usually idiosyncratic reactions resulting in anaphylaxis *(200)*, massive hepatocellular necrosis *(201)*, and severe skin or mucocutaneous drug reactions (e.g., Stevens-Johnson Syndrome, toxic epidermal necrolysis *[202]*) together with their attendant complications of sepsis, coagulopathy, and eventual multiorgan failure. These seem to be followed by comparatively lower frequencies of fatal cardiovascular *(203)*, nephrotoxic *(204)*, and neurotoxic *(205)* iatrogenesis.

The following review of ADRs, giving an outline of the main points, is confined to those instances that carry fatal or potentially life-threatening consequences. Given the immensity of this subject, which literally spans all organ systems, it is only possible to provide an overview of various conventional forms of potentially lethal ADRs and medication errors (including some unpublished cases observed or encountered by the author) supplemented by examples of reactions associated with the use of "novel" or relatively new drugs as well as agents employed in complementary therapy or as health supplements.

3.1.1. Hypersensitivity

Hypersensitivity reactions include the following:

- Anaphylaxis, for example, caused by penicillin allergy *(200)*;
- Myocarditis (caused by, e.g., penicillin, sulphonamides, tetracycline, streptomycin, sulphonylureas, or methyldopa *[203]*); and
- Stevens-Johnson syndrome/toxic epidermal necrolysis (Fig. 17) from penicillin, sulphonamides, hydantoins, barbiturates, salicylates, or antimalarials *(202)*.

3.1.2. Idiosyncratic Reactions

Idiosyncratic reactions include the following:

- Neuroleptic malignant syndrome caused by butyrophenones (haloperidol, droperidol), lithium, or phenothiazines *(206)*, and
- Malignant hyperthermia (*see* Subheading 2.8.).

3.1.3. Drug Toxicity

Examples of drug toxicity are *(a)* overdose from, e.g., paracetamol, salicylates, codeine (antitussives) caused by double or multiple prescriptions, ambiguous or inadequate instructions, and overzealous drug administration; and *(b)* intrinsic toxicity caused by massive hepatic necrosis (Figs. 18 and 19) from antituberculous therapy, e.g., rifampicin or isoniazid *(201)*, drug-induced (toxic or allergic) myocarditis (Figs. 20 and 21) induced by phenothiazines, barbiturates, theophylline, amphetamines, lithium, sulphonamides, tetracycline,

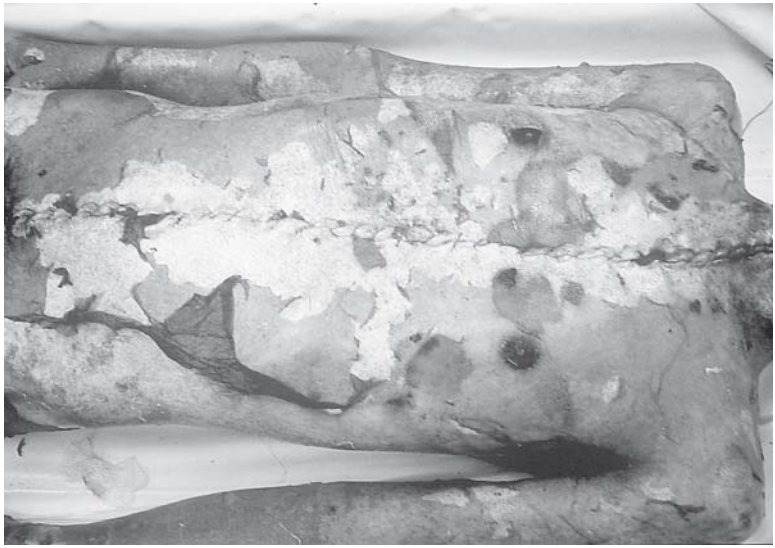

Fig. 17. Fatal toxic epidermal necrolysis involving approximately 80 to 90% of the body surface of this individual (photo taken after autopsy).

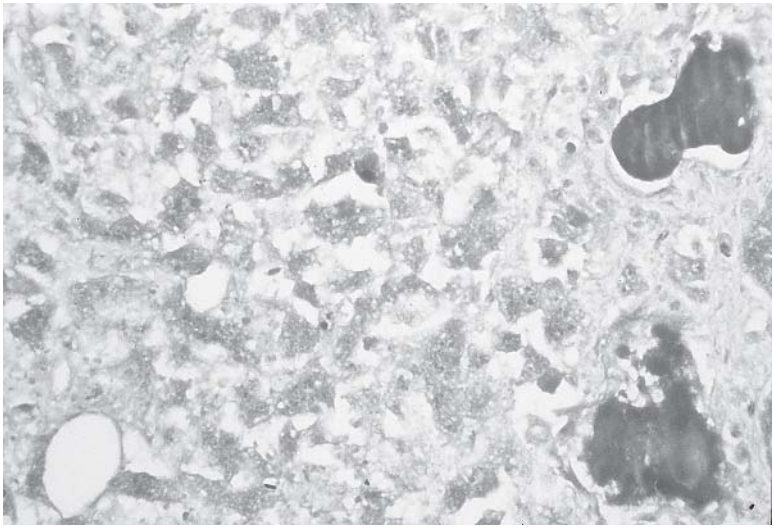

Fig. 18. Drug-induced massive hepatocellular necrosis with marked periportal cholestasis (Masson Trichrome stain, original magnification ×200). (Reprinted, with permission, from ref. *387*.)

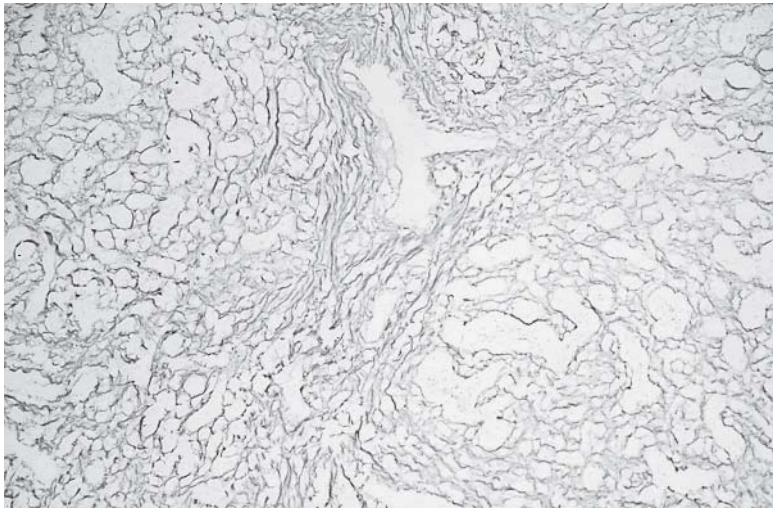

Fig. 19. Collapse of the reticulin framework in drug-induced massive hepatocellular necrosis (Gordon & Sweets stain, original magnification ×100). (Reprinted, with permission, from ref. *387*.)

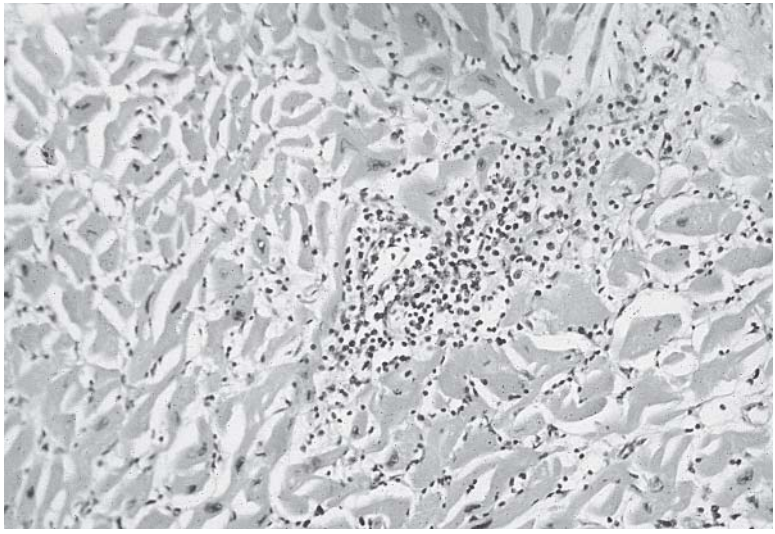

Fig. 20. Drug-induced myocarditis possibly induced by chlorpromazine (hematoxylin and eosin stain, original magnification ×40).

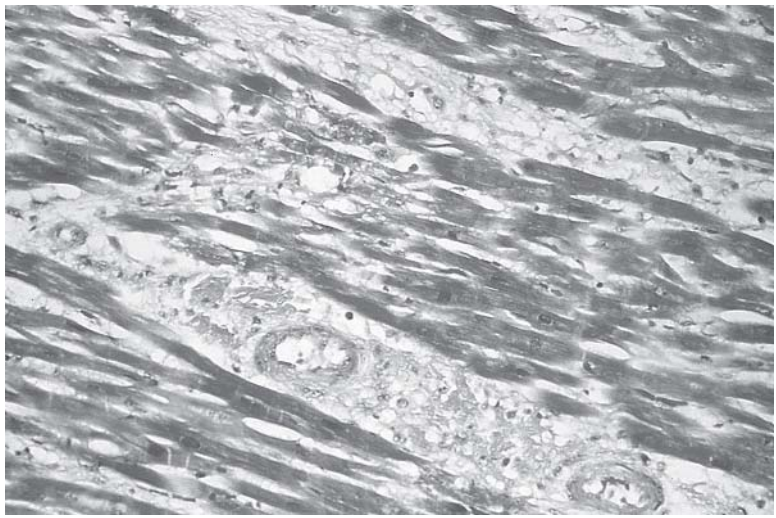

Fig. 21. Drug-induced myocarditis with focal coagulative necrosis of the myocardial fibers (Masson Trichrome stain, original magnification ×100). (Reprinted, with permission, from ref. *268*.)

streptomycin, penicillins, or phenylbutazone *(203)*, toxic cardiomyopathy from adriamycin, daunomycin, mitomycin, cyclosporin, arsenicals, chloroquine, or amphetamines (*203*), tubulointerstitial nephritis (Fig. 22) induced by sulphonamides, aminoglycosides (gentamycin), penicillins (methicillin, ampicillin), rifampicin, nonsteroidal antiinflammatory drugs (e.g., phenylbutazone), thiazide diuretics, or heavy metals (e.g., lead, mercury, arsenic, etc. *[204]*), and dapsone (diaminodiphenylsulphone) syndrome manifesting with jaundice, hepatitis, acute renal failure, peripheral motor neuropathy, toxic epidermal necrolysis, erythema nodosum, methemoglobinemia, and hemolytic anemia *(207)*.

3.1.4. Synergistic Drug Reactions

Synergistic drug reactions follow the combination of two or more drugs in therapeutic, nontoxic concentrations resulting in life-threatening or lethal potentiating effects in patients being treated for major depression, psychosis, or other psychiatric conditions *(208)*. This may, perhaps, be considered to be the interface between adverse pharmacological reactions and toxicology. Examples include, ethanol and benzodiazepines (diazepam, nitrazepam, temazepam); ethanol and antidepressants (imipramine, amitriptyline,

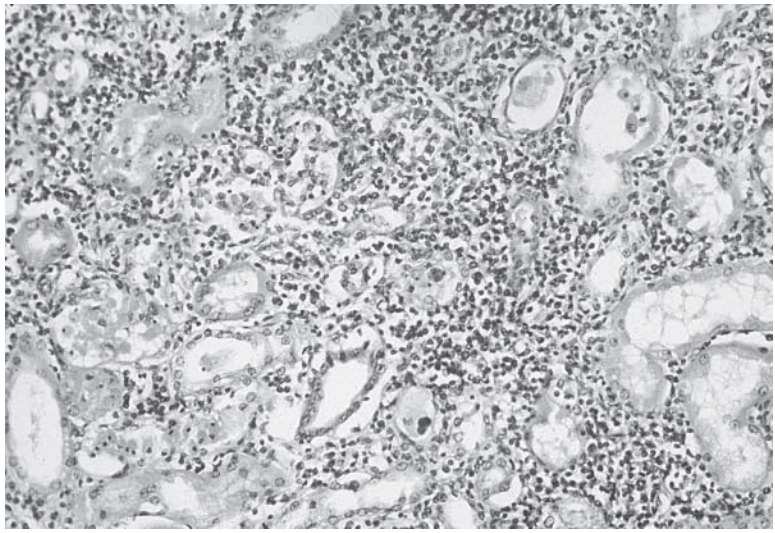

Fig. 22. Drug-induced tubulointerstitial nephritis caused by sulphsalazine (hematoxylin and eosin stain, original magnification ×100). Reprinted, with permission, from ref. *268*.)

dothiepin); and combinations of antidepressants, benzodiazepines, phenothiazines, and butyrophenones.

3.1.5. Wrong Mode of Administration

Examples of wrong modes of administration are intravenous potassium chloride (undiluted) in a single bolus resulting in cardiac arrest, intravenous adrenaline (undiluted), and intravenous theophylline (undiluted or administered too rapidly).

3.1.6. Wrong Route of Administration

An example of using the wrong route of administration is intrathecal or intraventricular vincristine neurotoxicity (Figs. 23 and 24; concurrent administration of intrathecal methotrexate and intravenous vincristine *[205]).*

3.1.7. Wrong Drug

In some circumstances, the wrong drug is used, for example, *(a)* erroneous prescription for oral propanolol, instead of prednisolone (20 mg tablets) issued to an asthmatic patient, thus resulting in acute bronchospasm and sudden death (*209*); *(b)* intramuscular digoxin instead of naloxone for a newborn after delivery (where the mother was given pethidine during labor); and *(c)*

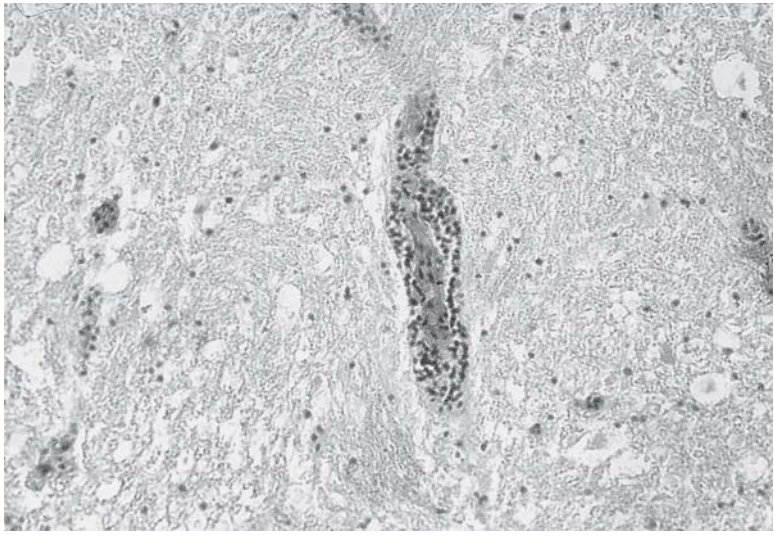

Fig. 23. Vincristine neurotoxicity: encephalitis after intraventricular administration of vincristine (hematoxylin and eosin stain, original magnification ×100).

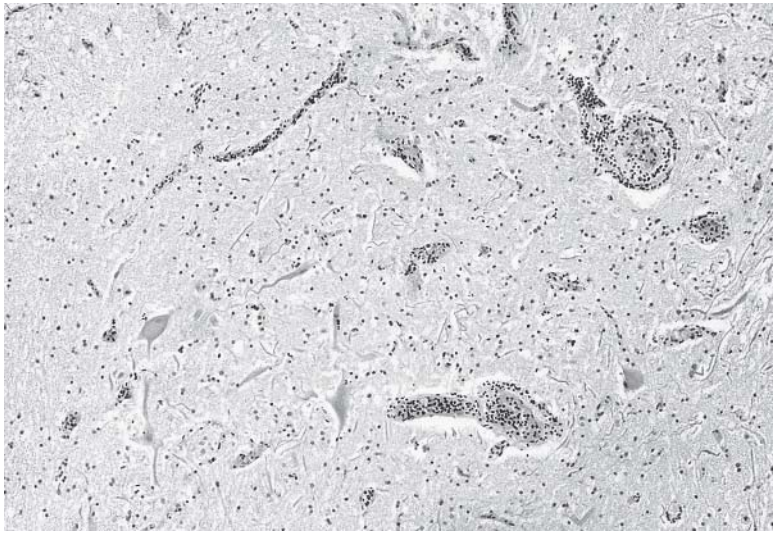

Fig. 24. Vincristine neurotoxicity: myelitis with extensive damage to the anterior horn cells (hematoxylin and eosin stain, original magnification ×100).

intravenous promethazine instead of phenobarbitone for control of convulsions in a premature neonate.

3.1.8. Treatment of Malignancies

Complications during treatment of malignancies include *(a)* immunosuppression predisposing to recurrent nosocomial infections, *(b)* general complications of long-term steroid therapy, and *(c)* secondary acute myeloid leukemia following autologous bone marrow transplantation for Hodgkin's disease, non-Hodgkin's malignant lymphoma, acute leukemia, and breast cancer *(210)*.

3.2. Adverse Reactions Associated With Specific Therapeutic Agents

In recent years, there have been numerous calls for a heightened awareness of ADRs (particularly in relation to newly approved drugs) and strategies to reduce medication errors *(211–216)*. It may be argued that forensic pathology and toxicology, by elucidating or confirming the precise nature of suspected fatal ADRs, may substantially contribute toward medical audit and, thereby, the achievement of these objectives. A survey of some adverse reactions of potential medicolegal significance is provided here. It is clear that these ADRs span the full gamut of pharmacological toxicity and idiosyncrasy related to the use of contemporary therapeutic drugs.

3.2.1. Antimicrobial Agents

Antibiotic-associated diarrhea occurs in 5 to 30% of patients in the early phase of antibiotic therapy or may occur up to 2 months after the end of therapy. Antibiotic-associated diarrhea is caused by *Clostridium difficile, Clostridium perfringens, Staphylococcus aureus, Klebsiella oxytoca,* and *Salmonella* and *Candida* species. Antibiotic-associated diarrhea may result in fulminant pseudomembranous colitis with resultant toxic megacolon, perforation, and shock *(217–219)*.

Macrolide-induced intracranial hypertension can be induced by the use of tetracycline, doxycycline (e.g., antimalarial prophylaxis), and minocycline *(220,221)*. Possible interference with the energy-dependent absorption mechanism at the arachnoid granulations exists *(222)*.

Clarithromycin has been reported as the cause of pulmonary infiltration and eosinophilia in a patient with bronchial asthma, allergic rhinitis, and sinusitis; symptoms vanished after antibiotic withdrawal *(223)*.

The use of ofloxacin for the treatment of lobar pneumonia has been associated with reversible diabetes insipidus (which may also occur with diverse

other agents, such as lithium, demeclocycline, vitamin D, or methoxyflurane *[224]*). Both ofloxacin and ciprofloxacin have been implicated in drug-induced hepatitis *(225)*. Moxifloxacin may cause prolongation of the QT interval with consequent tachycardia *(226)*.

The use of thiacetazone (as an inexpensive companion drug in antituberculous therapy used for the treatment of relapses and initially isoniazid-resistant strains) has been complicated by toxic epidermal necrolysis *(227)*. Topical ketoprofen as antifungal medication has been implicated in acute renal failure despite its marginal cutaneous absorption of only 5 to 8% *(228)*.

3.2.2. Antiviral Agents and Vaccines

Anti-retroviral drugs such as zidovudine and nevirapine, which are used in the treatment of HIV–1 infection, have been complicated by Stevens-Johnson syndrome and toxic epidermal necrolysis *(229,230)*. Nevirapine has also been reported as cause of severe drug-induced hepatitis *(231)* and liver damage severe enough to warrant liver transplantation *(232)*.

Zanamivir may cause bronchospasm and serious respiratory deterioration when it is used for the treatment of influenza *(233)*. The use of the 17DD and 17D–204 vaccines (yellow fever vaccines) has been associated with hepatitis, renal failure, hemorrhagic diatheses, multisystem failure, and death *(234–237)*.

3.2.3. Cardiovascular Drugs

There may be an increased risk of GI hemorrhage among elderly hypertensive patients being treated with calcium antagonists such as nifedipine, verapamil, and diltiazem compared with those receiving β-blockers or angiotensin-converting enzyme (ACE) inhibitors. Risk of GI hemorrhage seems to be particularly increased when there is concurrent use of aspirin, nonsteroidal antiinflammatory drugs (NSAIDs), or warfarin or when there is a history of peptic ulcer disease, reflux esophagitis, or GI malignancy *(238,239)*. Mibefradil has been reported to interfere with the cytochrome P450 system in the liver, with potentially lethal drug interactions between it and other calcium antagonists, β-blockers, and digoxin, culminating in cardiogenic shock *(240)*.

The use of ACE inhibitors may be associated with potentially life-threatening acute angioedema, probably mediated by sharp rises in plasma bradykinin concentrations, in 0.1 to 0.5% of patients *(240,241)*. Omapatrilat, another vasopeptidase inhibitor that interrupts ACE as well as neutral endopeptidase with a resultant increase in the concentration of natriuretic peptides, also carries a similar risk of 0.7% *(241)*; cholestatic hepatotoxicity has also been reported *(243)*. Adverse effects of angiotensin II receptor antagonists include

cholestasis (irbesartan), pancreatitis, angioedema, acute nephritic syndrome (candesartan), and Henoch-Schonlein purpura (losartan *[244–247]*).

Ticlopidine, an inhibitor of adenosine diphosphate-induced platelet aggregation that is commonly used in the primary or secondary prevention of acute coronary or cerebrovascular events, has been associated with neutropenia, thrombotic thrombocytopenic purpura, bone marrow aplasia *(248)*, as well as acute polyarthritis that could be a manifestation of drug-induced hypersensitivity (leukocytoclastic) vasculitis *(249)*. Clopidogrel, which is hydrolyzed to its carboxylic derivative (i.e., ticlopidine), shares similar adverse effects.

The use of amiodarone, an antiarrhythmic and antianginal agent with vasodilatory properties that is used in the treatment of severe cardiomyopathy and coronary artery disease complicated by supraventricular and ventricular tachyarrhythmias, may cause hepatic and thyroid impairment. In approx 5 to 10% of patients, amiodarone also may induce pulmonary toxicity ("amiodarone lung") through a direct cytotoxic effect on the alveolar-capillary membrane in the lung thus resulting in obstructive-restrictive impairment of lung function that may, occasionally, have a fatal outcome *(250)*.

There has been a report of laryngeal edema (possibly caused by laryngeal irritation) after the repeated use of isosorbide dinitrate spray in combination with sublingual nifedipine in a patient who had collapsed and presumably died from intracerebral hemorrhage *(251)*.

3.2.4. Antiinflammatory and Related Drugs

3.2.4.1. Cyclooxygenase 2 Inhibitors

Although selective inhibitors of cyclooxygenase 2 (i.e., COX-2 inhibitors) such as rofecoxib and celecoxib appear to be significantly less ulcerogenic than conventional NSAIDs such as naproxen and ibuprofen, they may be associated with a higher risk of acute cardiovascular and cerebrovascular events, such as ischemic stroke, unstable angina, and MI *(252–256)*. Celecoxib has been associated with cholestatic hepatitis *(257)*, not unlike some conventional NSAIDs, such as diclofenac sodium and nimesulide, which have a predilection to serious hepatotoxicity *(257–259)*. The use of both celecoxib and rofecoxib has also been complicated by acute temporary visual impairment *(260)*. High-dose meloxicam has been reported to cause ischemic colitis *(261)*.

3.2.4.2. Salicylates and Related Drugs

Aspirin (acetylsalicylate) is an ulcerogenic drug that may lead to potentially fatal GI hemorrhage, even when it is used in low doses for its antithrombotic properties in the primary and secondary prevention of acute coronary and cerebrovascular events as well as the prophylaxis of systemic

embolism in patients with atrial fibrillation *(262)*. Reye's syndrome, comprising an acute viral prodrome followed by acute encephalopathy, selective hepatic derangement (raised liver transaminases and hyperammonemia), and metabolic decompensation (acidosis, hypoglycemia, electrolyte imbalances) attributed to mitochondrial failure, still occasionally occurs when acetylsalicylate is administered to children *(263)*. It seems that the ability of salicylates to enhance the expression of inducible nitric oxide synthase generated by interferon-γ, may exacerbate potentially infectious diseases including falciparum malaria as well as viral infections, which trigger the development of Reye's syndrome *(264)*.

Mesalazine, a slow-release formulation of 5-aminosalicylic acid (5-ASA) used in the treatment of inflammatory bowel disease, has been reported to cause acute and chronic hepatitis *(265)* as well as interstitial nephritis of late onset, after 1 to 5 years of continuous treatment *(266)*.

Balsalazide (5-ASA linked by a diazo bond to 4-aminobenzoyl-β-alanine, a biologically inert carrier molecule) is used in the treatment of ulcerative colitis. It has been associated with a hypersensitivity reaction, comprising a constellation of pericarditis, pericardial effusion, vasculitis, cholestasis, and a lupus-like syndrome *(267)*.

Sulphasalazine (principally comprising 5-ASA and sulphapyridine) possesses a combination of antibacterial, antiinflammatory, and immunosuppressive properties that are variously harnessed in the treatment of inflammatory bowel disease and active rheumatoid arthritis. It has been associated with hypersensitivity myocarditis and tubulointerstitial nephritis (possibly in response to the sulphonamide component) as well as with an immuno-allergic reaction known as the *3-week sulphasalazine syndrome*, consisting of fever, dermatitis, lymphadenopathy, and hepatitis, a pathological condition that may culminate in massive hepatocellular necrosis *(268)*.

3.2.5. Alosetron

Alosetron, a type 3 serotonin (i.e., 5-HT_3) receptor antagonist that is used in the treatment of irritable bowel disease, has been reported to cause severe constipation and ischemic colitis with potential fatal outcome *(269)*.

3.2.6. Psychotropic Drugs

The neuroleptic malignant syndrome (NMS) is a clinical tetrad of fever (<38.5°C), lead pipe rigidity, altered sensorium (confusion, agitation, lethargy, stupor, coma), and autonomic dysfunction (tachycardia, tachypnea, diaphoresis, labile blood pressure, respiratory stridor, urinary incontinence) usually accompanied by leukocytosis. Intense muscle contraction often leads to muscle

damage, as indicated by elevated serum creatine kinase concentration (up to 100,000 IU/L) and possible rhabdomyolysis with resultant myoglobinuria and acute renal failure. NMS occurs in 0.5 to 1% of patients receiving a wide range of neuroleptic drugs (phenothiazines, butyrophenones, thioxanthenes, and dopamine antagonists) and other psychotropic agents (such as tricyclic antidepressants, selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, anticonvulsants, and lithium). NMS also may be associated with the use of recreational drugs such as 3,4-methylene-2,5-dioxymethamphetamine (i.e., MDMA or ecstasy) and lysergic acid diethylamide (LSD). The onset is usually within the first week of treatment and the syndrome may last 5 to 10 days after withdrawal from the triggering agent. Currently, the mortality rate is about 10%. A wide spectrum of skeletal muscle changes have been noted, including conspicuous edema, variable involvement of the muscle fascicles, replacement of the sarcoplasm with tiny to large vacuoles, necrosis, contraction bands separating segments of edematous myofibrils, lack of regeneration, severe endomysial edema, and scanty mononuclear infiltrates. Extensive clinicopathological correlation is required for the postmortem diagnosis or confirmation of this idiosyncratic response to antipsychotic drugs and to exclude other conditions such as heat stroke, sepsis, viral encephalitis, cerebrovascular accidents, autoimmune disease, and endocrinopathies responsible for the aforementioned histopathological changes *(206,270)*.

3.2.6.1. Cardiotoxicity

Antipsychotic drugs such as those used in the treatment of schizophrenia (e.g., thioridazine [also prescribed for learning disabilities], clozapine haloperidol, droperidol, risperidone) are known to prolong the QT interval, thereby predisposing to torsade de pointes and sudden death *(271,272)*. Clozapine may cause lethal drug-induced myocarditis and cardiomyopathy within 3 weeks to 3 years after commencement of therapy *(273,274)*. Clozapine has also been implicated in the pseudopheochromocytoma syndrome (hypertension, tachycardia, diaphoresis, and raised urinary catecholamine concentrations in the absence of an adrenal lesion), a pathological condition resolving upon withdrawal of the drug *(275)*.

3.2.6.2. Hepatotoxicity

Hypnotic drugs of the imidazopyridine group (e.g., zolpidem, alpidem) have been associated with drug-induced acute hepatitis mimicking biliary lithiasis *(276)*. Treatment of bipolar affective disorders with lithium may be complicated by diabetes insipidus with consequential life-threatening water depletion and hypernatremia attributed to lithium's ability to interfere with

the action of adenylate cyclase and the generation of cAMP in the cell membranes of distal tubular cells *(277)*. Some of the newer antipsychotic agents may interfere with glucose metabolism and hyperlipidemia, for example, olanzapine may be associated with a sixfold increase in the risk of developing diabetes mellitus in users as compared to nonusers of antipsychotics *(278)*. There exists evidence that the use of certain selective serotonin reuptake inhibitors, such as sertraline, citalopram, escitalopram, and paroxetine, as well as selective norepinephrine reuptake inhibitors such as venlafaxine, in the treatment of major depressive disorders in children and adolescents may increase the risk of suicidal thoughts and self-harm *(279,280)*. Tramadol hydrochloride, a weak opioid, was reported to cause auditory hallucinations in a patient with lung cancer who was receiving palliative care *(281)*.

3.2.7. Neurological Drugs

3.2.7.1. Anticonvulsants

Lamotrigine has been reported to induce agranulocytosis *(282)* and fulminant hepatic failure *(283)*. Levetiracetam has been associated with marked weight loss of 2.3 to 7 kg per month *(284)*.

3.2.7.2. Migraine Drugs

Sumatriptan (a $5HT_{1b}$ and $5HT_{1c}$ receptor agonist) has been associated with atrial fibrillation and chest pain, probably because of myocardial ischemia resulting from coronary vasospasm *(285)*. Chronic ergotamine use may be complicated by life-threatening small bowel ischemia induced by mesenteric arterial vasospasm. Ergotamine has similar effects on the carotid, coronary, renal and retinal arteries *(286)* with ischemic and hemorrhagic strokes having been reported *(287)*.

3.2.7.3. Baclofen

Baclofen, a muscle relaxant used to relieve chronic severe spasticity from multiple sclerosis or spinal trauma has been associated with convulsions (including status epilepticus) after withdrawal, both in adults and in a neonate exposed to the drug *in utero (288)*.

3.2.8. Antiasthmatic Drugs

3.2.8.1. Inhaled Corticosteroids

When used in high doses, beclomethasone, budesonide, and fluticasone propionate may result in adrenal suppression and secondary hypoglycemia *(289)*. Fluticasone propionate may also predispose to intracranial hyperten-

sion, as may other steroids when given systemically or topically or after their withdrawal *(290)*.

3.2.8.2. Leukotriene Receptor Antagonists

These drugs counteract leukotriene-induced inflammation, bronchospasm, and airway edema. Zafirlukast therapy has been associated with Churg-Strauss syndrome (allergic angiitis and granulomatosis), of which cardiac involvement is a potentially fatal complication (coronary arteritis, myocarditis, and even dilated cardiomyopathy), but this may be confounded by concurrent steroid withdrawal *(291)*.

3.2.9. Antineoplastic Agents

3.2.9.1. Vincristine

Vincristine is a highly neurotoxic vinca alkaloid that causes microtubular damage and neuronal destruction. The agent is used intravenously in the treatment of acute lymphoblastic leukemia, lymphoma, and a range of other malignancies. Vincristine does not penetrate the blood–brain barrier well, but there have been a number of reports of accidental intrathecal or intraventricular vincristine administration with resultant fatal myeloencephalopathy *(292,293)*. In some instances, the attending physicians were convicted of manslaughter for having caused this avoidable iatrogenic injury *(294)*.

3.2.9.2. Methotrexate

Methotrexate is an inhibitor of dihydrofolate reductase (and consequently of thymidine and purine synthesis) commonly used in the treatment of acute lymphoblastic leukemia. Neurotoxic complications associated with intrathecal administration include hemiparesis, aphasia, convulsions, arachnoiditis, and leukoencephalopathy (chronic neurotoxicity owing to demyelination *[292,295,296]*). Deaths also have occurred as a consequence of bone marrow suppression (when used in low doses) and from prescription and dosing errors with oral methotrexate used in the treatment of severe rheumatoid arthritis and psoriasis *(297,298)*.

3.2.9.3. Treatment of Breast Cancer

Taxane-based chemotherapy, involving the use of docetaxel for metastatic breast cancer, has been associated with ischemic colitis, leading to fatal bowel infarction and neutropenia *(299)*. Life-threatening constrictive pericarditis was reported as a complication during high-dose adjuvant chemotherapy (involving fluorouracil, epirubicin, cyclophosphamide, carboplatin, and thiotepa) during a period of 8 weeks *(300)*. There also was a report of tamoxifen-

induced severe fatty liver (attributed to its ability to interfere with lipid metabolism) that occurred a year after commencement of oral therapy *(301)*. Trastuzumab, a monoclonal antibody against the HER–2 receptor, may precipitate cardiac failure in patients with underlying heart disease, hypertension, and hypercholesterolemia *(302)*.

3.2.9.4. Pentostatin

Pentostatin, an inhibitor of adenosine deaminase derived from *Streptomyces antibioticus* that is used in the treatment of hairy cell leukemia and other low-grade lymphoid tumors, has been associated with fatal erythroderma *(303)*.

3.2.9.5. Temozolomide

Fatal dosing errors of temozolomide, a substance used in the treatment of refractory anaplastic astrocytoma have resulted in leukopenia, thrombocytopenia, and pancytopenia *(304)*.

3.2.9.6. Intravesical Bacille Calmette-Guerin Instillation

Intravesical Bacille Calmette-Guerin instillation, used in the treatment of in situ and recurrent low grade bladder cancers, may cause systemic granulomatous disease *(305)*.

3.2.9.7. Antiemetics

Certain 5-HT_3 receptor antagonists (e.g., tropisetron, ondansetron) used during chemotherapy may lead to hypersensitivity reactions, resulting in anaphylactic shock and acute asthma attacks *(306)*.

3.2.10. Immunotherapy

Intravenous immunoglobulin (Ig) therapy may increase the risk of thrombotic events, resulting in congestive cardiac failure and MI. The underlying pathophysiological mechanism is possibly the increase of serum viscosity (particularly in patients with hypercholesterolemia) and/or induction of cryoglobulinemia and hypergammaglobulinemia *(307)*. Intravenous IgG infusion has been associated with transverse sinus thrombosis in a patient with a history of recurrent bacterial chest infection from IgG1 deficiency *(308)*. High-dose Ig in the setting of idiopathic thrombocytopenic purpura may cause complications, including aseptic meningitis *(309)*.

3.2.11. Antidiabetic Therapy

Troglitazone, a thiazolidinedione that increases insulin sensitivity, has been associated with fatal hepatotoxicity *(310)*. Gabapentin (an anticonvul-

sant used in the treatment of diabetic peripheral neuropathy) may induce cholestasis *(311)*. Precipitation of lispro insulin results in sharp and erratic blood glucose fluctuations *(312)*.

3.2.12. Hormone Therapy

3.2.12.1. Hormone Replacement Therapy

It has been shown that hormone replacement therapy, although effective in mitigating menopausal symptoms and protective against osteoporotic fractures, carries increased risks of carcinomas of breast, endometrium, and ovaries as well as venous thromboembolism, heart disease, and stroke in 20 to 50% of patients *(313–320)*. However, it is most difficult to apply these epidemiologically derived conclusions (robust as they may be) to the forensic evaluation of such deaths, which tend to be regarded as being due to natural causes and are certified accordingly.

3.2.12.2. Contraceptives

It appears that third-generation contraceptives (e.g., gestodene, desogestrel, drospirenone plus ethinylestradiol) are associated with thromboembolic risks as high or higher (1.4 to 4 times) than that of the second-generation pills *(321,322)*. Again, invoking this as the antecedent cause of massive pulmonary thromboembolism would be difficult to prove in the usual forensic pathological setting.

3.2.12.3. Growth Hormone

Growth hormone, which is commonly used in the treatment of growth hormone deficiency or insufficiency and in individuals with poor growth because of renal failure (and often is misused to promote athletic power), may predispose the user to diabetes mellitus, fluid retention, and hypertension *(323)*.

3.2.13. Noncardiac Drugs With Life-Threatening and Fatal Adverse Cardiovascular Effects

The nonsedating antihistamines terfenadine and astemizole have been associated with QT prolongation, torsades de pointes, and sudden death *(324,325)*. Antimicrobial drugs, such as macrolides, quinolones, antimalarials, and imidazole antifungal agents, may cause QT prolongation and torsades de pointes *(326–329)*. Sildenafil has been reported to cause ventricular tachyarrhythmia, MI, sudden death, hypertension as well as tonic–clonic convulsions and cerebrovascular hemorrhage *(330,331)*. Cisapride, which is used in the treatment of gastroesophageal reflux, may cause QT prolongation, ventricular fibrillation, complete heart block, and sudden death *(332,333)*.

Latanoprost, a prostaglandin $F_{2\alpha}$ analog used in the treatment of open angle glaucoma, may exacerbate angina pectoris in the presence of ischemic heart disease *(334)*. Bromocriptine, a synthetic brominated ergopeptide derivative with dopaminergic agonist properties that may be used to suppress pituitary prolactin secretion and, hence, lactation in postpartum women, may occasionally cause acute coronary thrombosis *(335)*.

3.2.14. Antiobesity Drugs

Fenfluramine and dexfenfluramine, used as appetite suppressants, are associated with cardiac valvulopathy (namely mitral and aortic regurgitation, although right-sided valves have been reported to be also involved) with histopathological features similar to carcinoid-induced valvular disease *(336–338)*. The use of fenfluramine and phentermine in combination also has been known to cause complications, particularly valvular disease and pulmonary hypertension (fibroproliferative lesions in the pulmonary arteries and arterioles *[339–341]*). Orlistat is a tetrahydrolipostatin lipase inhibitor that exerts a topical action within the stomach and small bowel with minimal systemic absorption. There have been reports of reversible orlistat-induced hypertension and hepatitis as well as a case of massive hepatocellular necrosis associated with its use *(342–344)*.

3.3. Some Examples of Drug-Induced Electrolyte Imbalance

3.3.1. Hyponatremia

Anticonvulsants (e.g., lamotrigine, carbamazepine), which act on voltage-sensitive sodium channels and decrease calcium conductance, may induce inappropriate secretion of antidiuretic hormone, with consequent hyponatremia *(345)*. Severe hyponatremia (serum sodium <120 mmol/L), a pathological condition associated with serious neurological complications and mortality, may supervene in elderly patients taking diuretics or antidepressants, particularly if they are suffering from preexisting cardiac diseases, hepatic failure, or pulmonary infection *(346,347)*. Iatrogenic postoperative hyponatremia may result from the injudicious use of isotonic dextrose, thus resulting in cerebral edema and death. Rapid correction of severe hyponatremia may induce cerebral myelinolysis (*347*).

3.3.2. Hyperkalemia

Spironolactone, used in the treatment of severe congestive cardiac failure, is known to cause renal dysfunction and even severe hyperkalemia (serum potassium <6.0 mmo/L *[348]*).

3.3.3. Hypocalcemia

Biphosphonates (e.g., alendronate, risedronate, clodronate, pamidronate, zoledronic acid) are used in the treatment of metabolic bone disease and as prophylaxis against metastatic bone cancer. Intravenous administration of zoledronic acid and disodium pamidronate has been reported to cause severe hypocalcemia (serum calcium 1.11–1.66 mmol/L) in patients undergoing chemotherapy *(349)*.

3.3.4. Hyperphosphatemia

The use of phosphorus-containing laxatives and enemas, for example, in the setting of bowel preparation for colonoscopy, may be complicated by severe hyperphosphatemia (serum phosphate <6 mmol/L), hypocalcemia, hypomagnesemia, hypokalemia, metabolic acidosis and QT prolongation, particularly in patients with chronic renal and hepatic failure *(350,351)*.

3.3.5. Water Intoxication

Water intoxication may result in severe hyponatremia, convulsions, coma as well as cerebral and pulmonary edema. This pathological condition has been associated with the compulsive consumption of water by psychiatric patients and may be iatrogenically induced by gastric lavage (e.g., treatment of poisoning), enema, fluid infusion, and certain drugs (e.g., intravenous cyclophosphamide, oxytocin *[352,353]*). Overconsumption of fluids by athletes has also resulted in fatal hyponatremic encephalopathy *(354)*.

3.4. Some Fatal or Potentially Lethal Drug Interactions

Some possible, occasionally fatal, drug interactions are listed below:

- The combination of warfarin and any of the following:
  - NSAIDs: increased risk of GI hemorrhage from anticoagulation and mucosal damage *(355)*.
  - Paracetamol: anti-coagulant instability with unexpectedly high international normalized ratio (INR) values greater than 6 compared to target range of 2.0 to 3.0 *(356)*.
  - Levonorgestrel: enhancement of the anticoagulant effect of warfarin by displacing the latter from the binding site of human α_1-acid glycoprotein, the main plasma drug transport protein *(357)*.
  - Interferon-α (used in the treatment of chronic hepatitis C): it inhibits hepatic microsomal enzymes (CYP–450) that metabolize drugs, thereby potentiating warfarin activity *(358)*.

  - Topical miconazole cream: miconazole also inhibits hepatic CYP–450 enzymes; even minimal systemic absorption after topical application may result in a significant increase in the INR value *(359)*.

With respect to thromboprophylaxis, it has been found that the minimum risk of death was attained at INR values of 2.2 to 2.3 and that an increase of 1 unit of INR greater than 2.5 doubles the risks of death from cerebral bleeding *(360)*.

- The combination of cyclosporin (used for transplant conditioning) and
  - High-dose cyclophosphamide (which inhibits CYP–450 enzymes), prednisolone, vincristine, and methotrexate: neurotoxicity, manifesting as convulsions, auditory and visual hallucinations, hypertension, Parkinsonian tremor, and rigidity, were reported in a 9-year-old boy undergoing chemotherapy for monoclonal posttransplantation lymphoproliferative disease after cardiac transplantation for congenital heart disease *(361)*.
  - Fluoxetine (a potent inhibitor of CYP2D6): increase of blood cyclosporin levels *(362)*.
  - Ticlopidine: reduction of cyclosporin concentration–dose ratio *(363)*.
  - Statins: rhabdomyolysis is a potential complication; a combination of high-dose cerivastatin and gemfibrozil has been implicated in a number of fatalities amongst elderly patients *(364)*.
- Combinations of spironolactone and ACE inhibitors and angiotensin II receptor blockers (e.g., enalapril, captopril, ramipril, closartan, candesartan), respectively: severe hyperkalemia may occur in elderly patients with diabetes mellitus and renal impairment *(365)*.
- Combinations of cisapride and macrolides (e.g., erythromycin, clarithromycin) and quinolones (e.g., fluconazole) may cause induction of torsade de pointe, cardiac arrest, and sudden death (*355*).
- The combination of hydroxyurea and didanosine used in the treatment of HIV infection has been associated with acute pancreatitis *(366)*.
- Combinations of adrenaline and
  - Pseudoephedrine and diphenhydramine: there has been a report of ventricular tachycardia and myocardial ischemia after the use of adrenaline for the treatment of non-life-threatening angioedema in a patient taking pseudoephedrine and diphenhydramine for sinusitis *(367)*.
  - Cocaine: reuptake of noradrenaline at sympathetic nerve terminals induced by cocaine (causing local vasoconstriction) is intensified by adrenaline; however, unusually rapid and sometimes delayed systemic absorption could still occur, resulting in catecholamine-induced ventricular fibrillation and myocardial ischemia *(368)*.
- Anaphylaxis from crossreactivity: An apparent crossreaction between oral mesalazine and budesonide, taken 2 years apart, was reported in a patient with

Crohn's disease *(369)*. Interestingly, steroids (despite their antiallergic properties) may occasionally induce anaphylaxis, with sensitivity to aspirin having been postulated as a risk factor *(370,371)*.

4. Complications Associated With Complimentary Therapy and Health Supplements

There is emerging evidence that various forms of complimentary or alternative therapy as well as what are widely regarded as health supplements may not be free from adverse effects and could interact with conventional therapeutic drugs, thus having potentially serious consequences. Accordingly, the somewhat simplistic notion that because medicinal herbs and herbal products are obtained or derived from natural sources they are thereby absolutely safe for consumption should be discarded. That herbal medicines may alter human physiology is evidenced by abnormal test results (e.g., raised liver enzymes, increased INR value) that may be associated with their use. Some of these herbs such as *chan su* and comfrey are intrinsically toxic and may cause death *(372)*.

Given the increasing popularity of such products, it is entirely conceivable that serious iatrogenesis associated with their use could well have an impact on the practice of forensic pathology. Some of the better-known complications are as follow:

- St. John's wort (*Hypericum perforatum*) is used in the treatment of depression, neuralgia, and burns. Its extracts comprise a mixture of bioflavonoids, xanthones, napthodiantrons, and other components. It induces CYP3A4, which breaks down a variety of drugs and toxins, including more than half of the antineoplastic agents currently in use. It has been reported to diminish the chemotherapeutic effects of irinotecan (used against colonic carcinoma) and possibly etoposide, teniposide, anthracycline, paclitaxel, docetaxel, and tamoxifen. Its metabolic interaction with cyclosporin also has been implicated in acute heart transplant rejection, apparently because of the latter's decreased oral bioavailability *(373,374)*. Likewise, low concentrations of theophylline, digoxin, and indinavir could also be induced by St. John's wort.
- A number of herbal medications and supplements are known to increase the perioperative risks of hemorrhage (garlic, *gingko*, *ginseng*, ginger, feverfew), hypoglycemia (*ginseng*), MI and stroke (ephedra, also known as *ma-huang*), allergic reactions and immune suppression (echinacea), and prolonged anesthesia (kava, valerian; ref. *375,376*).
- Kava (*Piper methysticum*), which contains pharmacologically active lactones and is used as an effective herbal anxiolytic, has been suspected of being hepatotoxic

and associated with fatal outcome in some cases *(377–379)*. Other hepatotoxic herbs include echinacea, comfrey, mistletoe, chaparral, and germander *(372)*.

- *Ginkgo biloba* extract, which is often used as a supplement to improve mental alertness, is a potent inhibitor of platelet-activating factor, and its long-term use has been associated with spontaneous subarachnoid hemorrhage *(380,381)*.
- Drug–herb interactions have been reported, too. These include combinations of coumarin anticoagulants (namely warfarin) with St. John's wort, *dong quai*, *ginseng*, garlic, *gingko*, and kava because of their additive anticoagulant properties *(382)*. Ephedra may interact with antidepressants and antihypertensives, resulting in raised blood pressure and tachycardia *(383)*.
- Contamination of herbal medication by heavy metals (e.g., mercury, arsenic, lead), steroids, and even conventional therapeutic agents are by no means uncommon *(372,384)*. There has been a series of reports on weight-reducing herbal extracts adulterated with nitrosofenfluramine with resultant hepatotoxicity which, in some instances, progressed to massive hepatocellular necrosis, culminating in either liver transplantation or death *(385–387)*. It should be noted that nitrosamines, as a whole, are variably hepatotoxic (and carcinogenic) because of the binding of their active metabolites to cytosol molecules by way of alkylation *(201)*. Accordingly, the hepatic catabolism of nitrosofenfluramine may generate ethylcarbonium ions with the capacity to mediate DNA damage, leading to hepatocellular necrosis *(387)*.

It should be added that unorthodox methods of alternative therapy may, occasionally, have fatal consequences *(388)*. For instance, a patient who had declined surgery, radiotherapy, and chemotherapy for maxillary sinus carcinoma died from severe GI hemorrhage because of fulminant hepatorenal failure and profound coagulopathy, attributed to hydralazine-induced hepatotoxicity *(389)*.

5. Forensic Pathological Investigation of Suspected Cases of Fatal ADRs

Given the vast array of pharmacologically active agents and their seemingly unlimited potential for causing life-threatening toxic and idiosyncratic reactions (both directly and synergistically), the forensic pathological investigation of fatal adverse reactions to conventional and complementary/alternative therapeutic agents is by no means easy.

Consequently, the forensic process often involves a tedious, clinicopathological exclusionary process of eliminating various natural and nontoxic causes of multiorgan injury. However comprehensive this may be, it can never be truly exhaustive and must take into account the confounding effect of

polypharmacy (e.g., concurrent use of multiple drugs, of which several could be responsible for the adverse reaction[s] observed) that tends to attend fatal cases of ADRs. Moreover, the comorbidity that underlies the use of these therapeutic agents often constitutes substantial contributory, if not concomitant, causes of death. Where anaphylaxis is concerned, the postmortem findings may be nonspecific (e.g., laryngeal, pharyngeal, and pulmonary edema; mucous plugging; hyperinflated lungs) and even ancillary investigations, such as serum mast cell tryptase and IgE concentrations must be interpreted with great caution and strictly in context, as they may be significantly elevated in the presence of cardiovascular disease, trauma, and a prolonged postmortem interval *(390,391)*.

Certainly, a number of tools have been devised to facilitate the assessment of ADRs and their diverse implications *(392–396)*. These include the expansion of the original classification of adverse reactions into types A (pharmacological; dose-dependent and predictable reactions) and B (idiosyncratic; dose-independent and unpredictable) to embrace types C (chronic, dose- and time-dependent reactions), D (delayed reactions), E (withdrawal reactions), and F (treatment failure). Indeed, a sophisticated model of probabilistic analysis, derived from a three-dimensional classification system based on the parameters of dose-relatedness, timing, and patient susceptibility (conferred by genetic, pathological, and other biological factors) and displayed as a series of three-dimensional graphs or equivalent normograms, has been proposed *(392)*.

However, these elegant methods of analysis tend to have a marked clinical orientation and it may be rather difficult to apply these methods of assessment to routine forensic casework where there is really no substitute for the exercise of sound professional judgment and the judicious application of the principles of evidence-based medicine to establish a clinicopathological correlation which forms the very basis of a considered opinion on the culpability of any putative agent.

6. *Miscellaneous Iatrogenic Complications*

Some iatrogenic complications are not readily resolved into either of the two main categories described previously, or may straddle them. Examples of these are given below.

6.1. Radiation Overdose

There has been some concern that neonates, infants, and children undergoing computed tomography (CT) scans may have been exposed to doses of

radiation that are fivefold greater than necessary and, consequently, might be at an increased risk of cancer mortality in the ensuing decades *(397–399)*. The forensic evaluation of such a possibility would obviously be fraught with great difficulty, given the various other predisposing factors that would almost inevitably confound the issue.

6.2. Magnetic Resonance Imaging

A series of fatal and potentially lethal incidents involving ferromagnetic objects being magnetically drawn as projectiles into the magnetic resonance imaging suites have been reported. The objects include nitrous oxide and oxygen tanks, a defibrillator, a wheelchair, a respirator, ankle weights, a toolbox, a vacuum cleaner, and even mop buckets *(400,401)*. Intracranial aneurysm clips may be torqued or dislodged, resulting in fatal intracerebral hemorrhage *(402)*, whereas other deaths have been attributed to the inadvertent scanning of patients with cardiac pacemakers *(403)*.

6.3. Adjuvant Radiotherapy for Breast Cancer and Cardiotoxicity

It has been shown that the risk of fatal MI may be threefold higher among patients irradiated for left-sided tumors compared with controls *(404)*. Scintigraphic studies have demonstrated regional hypoperfusion in these patients, possibly arising from radiation-induced damage to the microcirculation *(405)*. Irradiation of the internal mammary lymph nodes may present an additional risk factor for cardiac mortality *(406)*.

6.4. Brachytherapy

Intraluminal endobronchial irradiation, used as palliation for advanced or recurrent bronchogenic carcinoma, may be complicated by acute hemorrhage, radiation bronchitis, and stenosis *(407–411)*.

6.5. Complications Associated With Contrast Media

There has been an instance of fatal barium poisoning after a barium swallow (with serum and cerebrospinal fluid levels as high as 370 µg/mL and 440 µg/mL, respectively), possibly because of progressive absorption of barium sulphate contrast medium occasioned by intestinal obstruction after a CT scan of the digestive tract *(412)*. Fatal anaphylaxis also has occurred during fluorescein angiography in an elderly patient without any history of allergy *(413)*. The more common contrast-induced nephropathy, presenting as acute renal failure, may occasionally culminate in end-stage renal failure and death. Risk

factors include diabetic nephropathy, myeloma, and intravascular volume depletion. Its overall incidence is said to be as high as 14.5%, but may increase to 30 to 50% in the presence of preexisting renal impairment *(414–416)*.

The former use of thorotrast (containing thorium–232, a highly radioactive substance with a half-life of nearly 14 billion years that is barely excreted from the body) has been associated with late onset fibrosis at the site of injection as well as with malignancy (e.g., hepatic angiosarcoma and cholangiosarcoma after a latency of 12 to 45 years; osteosarcoma, chondrosarcoma, and other soft tissue sarcoma local at the site of previous puncture). It may also induce granuloma formation, there being an instance of a thorotrast granuloma mimicking retropharyngeal tuberculosis in an elderly patient who underwent carotid angiography more than four decades previously *(417,418)*.

6.6. Some Complications Related to Renal Dialysis

Apart from the complications of hemorrhage *(39)*, infection *(69)*, and air embolism *(39)* hemodialysis may, occasionally, be complicated by fatal microcystin toxicity *(419)* and aluminium poisoning *(420)* arising from the use of contaminated sources of water in the dialysate. Deaths associated with a particular type of membrane dialyzer also have been reported *(421)*. It appears that the use of icodextrin (a polymer of glucose) as the osmotic agent in peritoneal dialysis may cause spurious hyperglycemia in diabetic patients with potentially fatal consequences (owing to the ability of maltase [a metabolite of systemically absorbed icodextrin] to interfere with certain types of glucose assays *[422]*).

6.7. Intravenous Colloids

There has been some concern about the safety of using colloids for fluid management in critically ill patients with life-threatening hypovolemic shock, burns, and hypoproteinemia. There appears to be evidence that the use of human albumin in these situations may actually lead to excess mortality (6 additional deaths for every 100 patients treated). The reasons postulated for this include the following *(423–425)*:

- Cardiac decompensation arising from rapid volume replacement and volume retention caused by an increase in colloid-osmotic pressure, thus resulting in pulmonary edema;
- Leakage of albumin and water through the capillary membrane in patients with increased capillary permeability worsening pulmonary edema and thereby compromising tissue oxygenation with eventual multiorgan failure;

- Increased blood loss in postsurgical or trauma patients owing to the antihemostatic and platelet-lowering properties of albumin; and
- Impairment of sodium and water excretion thus aggravating renal failure.

However, it appears that albumin might have a better safety profile than certain artificial colloids such as hydroxyethyl starch, dextran, and gelatin, in terms of their potential to cause anaphylactoid reactions and coagulopathy *(426)*.

6.8. Hematological Interventions

Some lethal and/or unusual complications resulting from the transfusion of blood and blood products include the following:

- Acute noncardiogenic pulmonary edema from acute lung injury after blood or platelet transfusion, said to occur at a frequency of approx 1:5000 transfusions *(427)*;
- An increased risk of death from hepatocellular carcinoma and chronic liver disease among hemophiliac men and boys given hepatitis C-contaminated blood products *(428)*;
- A possibly increased (doubled) risk of non-Hodgkin's lymphoma associated with blood transfusions *(429)*;
- Spontaneous rupture of the spleen during peripheral blood stem cell (PBSC) transplantation in a patient with acute myeloid leukemia *(430)*; and
- PBSC mobilization with recombinant human granulocyte colony-stimulating factor (rhG-CSF) in a healthy donor convalescing after Epstein-Barr infection *(431)*.

6.9. Nutritional Interventions

The complications of parenteral nutrition are well known and are not discussed here except to mention the fact that long-term parenteral nutrition in children may be associated with manganese neurotoxicity (Parkinsonism caused by accumulation of magnesium in the basal ganglia) and hepatotoxicity (cholestatic liver disease) *(432)*.

An interesting, but potentially fatal condition known as the *refeeding syndrome* may supervene when patients recommence eating after a period of prolonged hunger (this was first observed in malnourished prisoners of war following World War II). Its features comprise rhabdomyolysis, respiratory failure, cardiac failure, hypotension, cardiac arrhythmias, convulsions, coma, and sudden death, all pathological conditions thought to be precipitated by hypophosphatemia. It is believed that in a malnourished state, the secretion of insulin is decreased as a result of reduced carbohydrate intake (accompanied

by catabolism of the body's fat and protein stores for energy production) resulting in an intracellular loss of electrolytes, particularly phosphate. Resumption of feeding (both enteral as well as parenteral) induces a sudden shift back to carbohydrate metabolism, leading to increased secretion of insulin, which stimulates cellular uptake of phosphate with consequent hypophosphatemia. Presently, the refeeding syndrome may arise in the setting of cancer, alcoholism, anorexia nervosa, as well as in patients with neurological dysphagia being fed through nasogastric or PEG tubes *(433–436)*.

6.10. Postsurgical Metabolic Complications

Apart from the ever-present danger of perioperative fluid and electrolyte imbalance alluded to previously metabolic complications may supervene as a direct consequence of surgery itself. For instance, urinary diversion with an ileal conduit (which exposes the small bowel to urine) may cause hypopotassemia and hyperchloremia as well as drug toxicity from reabsorption (e.g., phenytoin, methotrexate, lithium *[437]*). Likewise, a jejunoileal bypass (such as for morbid obesity) may result in (secondary) enteric hyperoxaluria with resultant nephrolithiasis and oxalate tubulointerstitial nephritis *(438)*.

7. Complex/Combined Iatrogenesis

In the author's experience, cases of complex and combined forms of fatal iatrogenesis, encompassing the perioperative and nonperioperative complications discussed above, are by no means uncommon. The postmortem examination and forensic evaluation of such cases is often extremely difficult, time-consuming, and must be undertaken with great care. A series of vignettes is appended to the end of this chapter to illustrate the varying levels of complexity with which these cases inevitably present. It has been suggested that the retrospective medicolegal (coronial) adjudication of these cases is by no means an easy matter and that, at least in some jurisdictions, anything short of blatant or criminal negligence might not attract judicial censure, although the errant physician may still be subjected to statutory professional disciplinary proceedings. In addition, some of these cases illustrate the principle that, although human agency is the immediate cause of iatrogenesis, systemic failure may well account for almost 80% of harmful errors by providing conditions under which these errors tend to thrive *(3,13)*.

8. *The Postmortem Examination*

A proper forensic evaluation of any death that is possibly iatrogenic in nature rests upon the establishment of a comprehensive clinicopathological correlation and an awareness of the corresponding medicolegal implications. As such, the autopsy, which constitutes the mainstay of such an exercise, is an essential component of the process but is inadequate in itself unless the autopsy findings are interpreted in the appropriate clinical context. For this purpose, the attendance of the principal clinicians, particularly at perioperative autopsies, is to be encouraged and might even be thought of as being crucial to a satisfactory conduct of the autopsy, given the fact that the altered anatomy, resulting from various (often multiple) surgical and other therapeutic or even invasive diagnostic procedures, could present enormous difficulties to the pathologist conducting the autopsy.

Pre- and postautopsy consultations with clinical colleagues (unconnected to the case at hand) practicing in the relevant specialties or subspecialities may be necessary to enable the pathologist to obtain a reasonable grasp of the critical management issues, namely the indications and complications of various diagnostic, therapeutic, and palliative procedures as well as supportive measures and their associated risks of morbidity and mortality.

Certainly, a pathologist should refrain from the formal expression or provision of opinions on clinical management issues, just for the simple reason that he or she (usually) is not a clinician and because clinical practice has evolved into a highly sophisticated and subspecialized state so that it is no longer possible for any medical practitioner to be truly knowledgeable about and both adequately skilled and sufficiently experienced in all fields of medicine. Nevertheless, being a medical practitioner tasked with the responsibility of ascertaining the cause of a perioperative death and determining whether it was likely to have been iatrogenic in nature, the attending pathologist must have a sound understanding of these issues.

Moreover, it may be argued that although it is not given to a forensic pathologist to opine on matters pertaining to clinical propriety, it is entirely valid for him or her to raise questions (which may have to be answered by other relevant specialists) should the postmortem investigation, conducted at the behest of the proper legal authorities, reveal causes for concern. Indeed, it is in this area that forensic pathology may be seen to contribute substantially to medical audit and injury prevention.

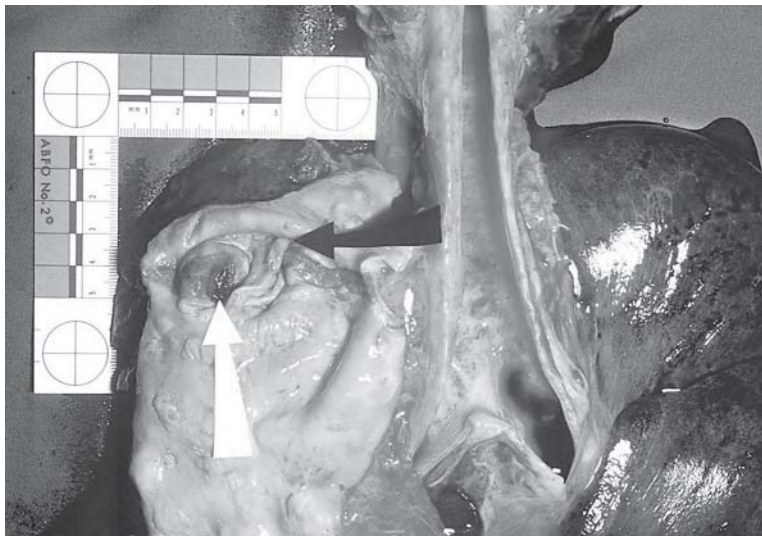

Fig. 25. Perforated thoracic aneurysm developing at the site of previous surgical correction of aortic coarctation nearly two decades ago; death was caused by pulmonary hemorrhage. Prior knowledge of the medical history was crucial to the evaluation of this (possibly iatrogenic) fatality. (Reprinted, with permission, from ref. *440*.)

8.1. Prelude to the Autopsy

From a thanatological perspective, the primary purpose of the forensic perioperative autopsy is to determine whether death was caused by *(a)* the underlying disease or abnormality for which the procedures were performed, *(b)* comorbidity or traumatic injury other than that for which the procedures were conducted, *(c)* an operative mishap, *(d)* an anesthetic mishap, or *(e)* patient-specific factors not directly related to disease or perioperative complications (e.g., a fatal head injury caused by falling from a bed postoperatively or a patient who deliberately pulled out intravenous lines *[9,13]*).

Therefore, it is of the utmost importance that all relevant clinical records are made available to the attending pathologist before the autopsy proper. This will enable the pathologist to review the deceased patient's medical history in its entirety and identify the key issues of clinicopathological significance and medicolegal concern that will serve to guide the very conduct of the postmortem examination. In this respect, it is imperative that the pathologist does not perform the autopsy "blind" or solely based upon incomplete information (Fig. 25), as this would inevitably lead to preventable technical difficulties encoun-

tered in the course of the autopsy as well as irretrievably deleterious consequences for the unwary pathologist.

If complex issues are identified such as unusual drug interactions, significant fluid and electrolyte derangement (a bewildering subject even in the best of times, and certainly not a popular one with most forensic pathologists), multiple surgical interventions resulting in extensive or gross anatomical alterations (as in some instances of colorectal, upper GI, and hepatobiliary surgery), a rare infection (caused by an erstwhile unheard of pathogen), or an anesthetic mishap, then a preautopsy consultation is clearly warranted.

8.2. The Autopsy Proper

An autopsy on an iatrogenic death begins with an external examination. The location, characteristics, and dimensions of various marks of both recent and previous therapeutic procedures (e.g., fresh surgical incisions or scars, injection and puncture marks) as well as the proper placement of tubes, drains, vascular catheters, and other medical devices have to be documented. Thus, it is imperative to note whether an endotracheal tube has been placed within the tracheal lumen, as opposed to esophageal intubation, or had slipped out of a tracheostomy. It also is important to establish whether a central venous line has been inserted correctly through an internal jugular or subclavian vein and terminates at the confluence of the superior vena cava and right atrium or whether a chest tube had punctured the lung. All these features should be carefully documented and reflected on the ensuing autopsy report. Accordingly, there should be a standing arrangement with the relevant healthcare and medicolegal authorities that all medical devices present at the time of death should not be removed before the autopsy, but retained in situ.

When a fatal ADR is suspected, attention should be paid to the presence of cutaneous or mucocutaneous eruptions (e.g., Stevens-Johnson syndrome/toxic epidermal necrolysis) and the extent of body surface involvement has to be determined accordingly.

During evisceration, it is advisable to avoid incising through or across existing surgical incisions, so that they can be examined in their original state. However, it is acknowledged that it would be difficult to bypass midline sternotomy and laparotomy incisions, as alternative postmortem incisions would be rather cumbersome. In any event, as long as every reasonable effort is made to conserve the external and internal features of prior therapeutic intervention, it is really quite permissible to vary the standard evisceration and dissection techniques to facilitate a thorough postmortem examination. For instance, it may be necessary to inspect the GI tract and dissect parts of it *in situ* to iden-

tify the sites of anastomotic dehiscence before evisceration, so as to avoid causing artifactual dehiscence of these delicate structures, rendered all the more fragile by variable postmortem autolysis. The same principle might apply to deaths after corrective surgery for complex congenital heart disease.

As a rule, it is important to note the presence of all collections of effusions, hemorrhage, exudates, and abscesses and to measure their quantities before the organs are eviscerated. After evisceration, the rest of the autopsy could proceed in much the usual manner, although particular vigilance will have to be exercised when examining and dissecting the surgical sites. Attention should also be paid to the presence of concurrent pathology of major organs (especially the cardiovascular, respiratory, and central nervous systems) other than those subjected to therapeutic intervention, as comorbidity is often a significant contributory or even causative factor in perioperative deaths.

8.3. Ancillary Investigations

It is recommended that autopsies conducted in cases of perioperative and iatrogenic deaths are complemented by comprehensive postmortem histopathology. This may be essential to the ascertainment of the cause of death (e.g., drug or toxin-induced massive hepatocellular necrosis, myocarditis, or tubulointerstitial nephritis), but may also provide much enlightening information that enables a thorough clinicopathological correlation to be made.

Postmortem toxicology (predominantly performed on specimens of blood, urine, bile, gastric contents, liver, kidney, and, occasionally, hair and nails) may help to confirm or exclude suspected drug toxicity, interaction, or overdose resulting from medication errors. However, as the patient may be expected to have died after an interval of some days or even weeks after admission to hospital, the analysis of autopsy blood samples will often yield negative or inconclusive results. In such an instance, it might be necessary to obtain (through the proper medicolegal or law enforcement authorities, as the case may be) postadmission samples of blood for retrospective analysis.

Selective recourse to postmortem biochemistry may provide supporting or corroborating evidence of an ADR (e.g., serum tryptase and IgE concentrations in a case of drug-induced anaphylaxis), although these results must always be interpreted with due caution and strictly in the appropriate context. Similarly, vitreous urea and electrolyte levels must be interpreted with equal care and correlated with the antemortem clinical blood biochemistry wherever possible. Although the presence of a high vitreous glucose level may indicate antemortem hyperglycemia, the opposite finding may simply be a reflection of the postmortem interval *(439)*.

Postmortem microbiological cultures may reveal the presence of a particularly fulminant pathogen but would have to be obtained with the prescribed aseptic techniques. However, it is the author's experience that, even if strict asepsis was obtained (as much as this is possible in a normal postmortem room), it would not prevent the inevitable postmortem growth of commensal microbes in the bowel and elsewhere, nor their subsequent spread throughout the body during the postmortem interval. This often results in polymicrobial flora being obtained (usually a combination of various enteric bacteria and some fungal species) that are extremely difficult to interpret. Viral cultures may be required to confirm or exclude a viral infection in cases of suspected drug-induced cardiotoxicity or in those rare cases of fatal perioperative myocarditis. Serology for viruses known to cause hepatitis may assist in the evaluation of suspected drug-induced hepatotoxicity. In any event, the assistance of clinical microbiologists and virologists in interpreting these results should be assured.

In keeping with developments in pharmacogenomics, the elucidation of patient susceptibility to certain ADRs, such as those resulting in cardiotoxicity (e.g., QT prolongation and tachyarrhythmias) and hepatotoxicity, the latter often attributable to genetically determined metabolic factors (e.g., various CYP–450 enzyme polymorphisms), may be in order.

Occasionally, it may be necessary to conduct postmortem angiography, for example, after coronary artery bypass grafts, to assess the integrity and patency of these vessels (Fig. 26) as well as their proximal and distal anastomoses if there is reason to suspect anastomotic leakage as a cause of fatal postoperative hemorrhage. Other examples include the identification of iatrogenic vascular trauma after percutaneous transluminal angioplasty or sites of iatrogenic perforation associated with the insertion of central lines. However, this would require the ready availability or access to fluoroscopic radiology facilities.

There also may be situations where recourse to external expertise is necessary such as the examination of medical devices (e.g., anesthetic machines, laparoscopes, surgical lasers) by biomechanical engineers for possible defects suspected to have caused or contributed to death.

8.4. The Autopsy Report

Ideally, the report should set forth, in full, the findings of the postmortem examination *per se* as well as provide a concise and, as far as possible, neutrally worded interpretation of the autopsy findings in a way that is objective and nonpartisan. Admittedly, the process can be extremely taxing and the

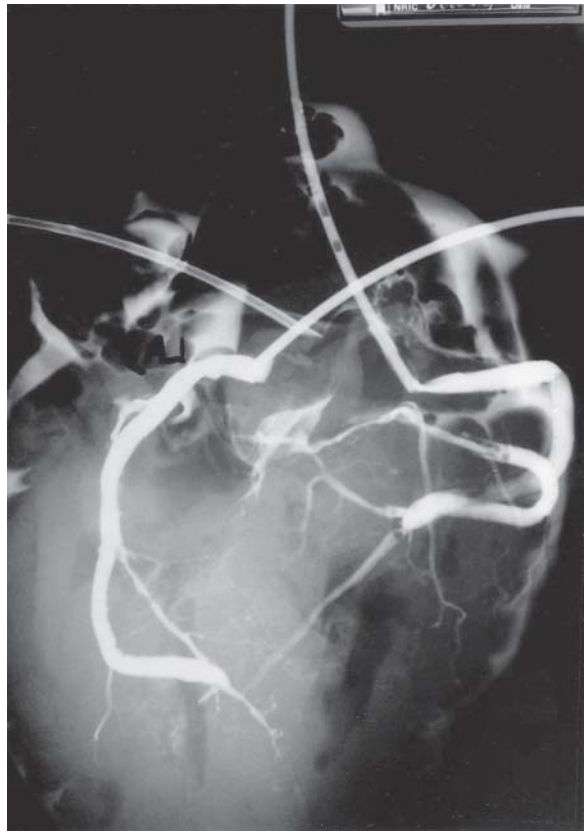

Fig. 26. Postmortem angiogram showing the patency and integrity of the saphenous venous grafts in a case of death following coronary artery bypass grafting.

pathologist must avoid entrenching himself in a position based on a rigid and narrow interpretation of the facts or without due recourse to relevant clinical consultation.

9. *Conclusion*

Iatrogenesis is a vast and fascinating subject that touches on practically all spheres of medical practice. The forensic postmortem evaluation of fatal iatrogenesis is an extremely challenging, sometimes overwhelming, process with an enormous potential for almost limitless clinicopathological and medicolegal exploration, undertaken in the public interest. The forensic postmortem evaluation of fatal iatrogenesis can also serve as a conduit for forensic

pathology to become firmly entrenched in medical practice, as the rapid medical advances currently taking place will, in all probability, be attended by ever novel and unpredictable forms of iatrogenesis. It is truly amazing how adverse events arising from the well intended ministrations of clinicians could contribute substantially to the intellectual life and the professional development of forensic pathologists, for which the latter should truly be grateful.

Appendix: Vignettes of Complex Perioperative Deaths

Case 1

A 36-year-old female with Down syndrome underwent elective total hysterectomy (with left salpingo-oophorectomy) for the management of poor menstrual hygiene. The operation was uneventful. Postoperatively, she was heavily sedated with intravenous midazolam and continuous morphine infusion. On the first postoperative day, she collapsed on the ward and was diagnosed with hypoxic encephalopathy. Subsequently, she acquired various nosocomial respiratory infections (including MRSA) and died 3.5 months later.

Autopsy confirmed that the cause of death was a combination of bronchopneumonia and hypoxic encephalopathy. At the coroner's inquest, the consultant anesthetist in charge was named as the potential defendant, and two independent clinical experts were engaged. The following lapses of care were identified:

1. Failure to appreciate the clinical significance of a low sedation score, at least on the part of the nursing staff assigned to monitor the patient during the immediate postoperative period;
2. Failure of communication among the medical and nursing staff involved in the clinical management of the patient;
3. Failure to recognize the likelihood of a patient with Down syndrome being particularly prone to morphine and sedative drugs leading to respiratory depression from a combination of oversedation and upper airway obstruction (due to the presence of a large tongue); and
4. Failure to manage such a patient with a commensurate degree of care (despite the use of sedation and pain scores and other hourly parameters) and to titrate the dose of morphine infusion accordingly, as evidenced by (a) the fact that the patient received continuous morphine infusion, consistently at a rate of 1.5 mg per hour during a period of some 8 hours postoperatively and (b) the lack of SaO_2 monitoring for more than 5 hours during this time).

The coroner concluded that, although the patient was likely to have suffered desaturation postoperatively, it was not possible to particularize the time at

which the deceased sustained irreversible hypoxic brain damage. Accordingly, an open verdict was returned.

Case 2

A 40-year-old female underwent cystectomy for a right ovarian endometriotic cyst. The operation was performed satisfactorily, but on the second postoperative day she complained of abdominal pain that she attributed to "severe winds." The following day, abdominal distension was noted, and a fleet enema was administered without relief. The patient subsequently became distressed and hypotensive. She collapsed and died later that day, despite prolonged and aggressive resuscitation.

Autopsy showed evidence of acute suppurative peritonitis (comprising 1L of turbid peritoneal fluid). There was no evidence of any significant intraabdominal hemorrhage or visceral perforation.

The independent clinical expert (a senior obstetrician and gynecologist) postulated that the patient might have succumbed to toxic shock syndrome caused by group A streptococci infection, a condition often not associated with a definite portal of entry. He stated that the operation was indicated and that there was no negligence on the part of the attending gynecologist. An open verdict was recorded.

Case 3

A 57-year-old female was admitted for the management of acute epiglottis with laryngeal stridor. She had a medical history of systemic hypertension, diabetes mellitus, bronchial asthma, a recent cerebrovascular accident, and epilepsy (on treatment with phenytoin). The patient was severely obese (body mass index at autopsy was 38.3). Emergency tracheostomy was performed under general anesthesia with some difficulty. During the procedure, it was observed that the patient had a short neck and that the trachea was small; also, the epiglottis and pharynx were severely edematous. She was then transferred to the surgical intensive care unit (SICU) for postoperative ventilation. Some 4 hours after the operation, the patient developed surgical emphysema, compounded by respiratory distress and cyanosis when the attending ear, nose, and throat (ENT) surgical registrar decided to place the patient on spontaneous respiration with a tracheostomy mask, during which the ventilator was disconnected. A subsequent attempt at passing a soft-tip-suction catheter through the tracheostomy tube failed. The surgeon then proceeded to remove the existing tube and attempted to insert a new one in its place, but without success. During this time, the patient suffered cardiac arrest and died 5 hours after the operation.

A senior consultant ENT surgeon, who was engaged on behalf of the state coroner as an independent clinical expert, opined that the most likely cause of the postoperative surgical emphysema was slippage of the tracheostomy tube, but did not consider the failed attempt at changing the tracheostomy tube in the SICU to be in any way negligent. A verdict of misadventure was recorded.

Case 4

A 67-year-old Malay man with a medical history of systemic hypertension underwent craniectomy and evacuation of a spontaneous hematoma in the left basal ganglia (mainly the left putamen) with intraventricular extension. On the seventh postoperative day, tracheostomy for prolonged intubation and bronchopneumonia was performed by a team of two neurosurgical registrars. However, the next morning the patient developed respiratory distress, and slippage of the tracheostomy tube was suspected. The existing tube was removed and attempts at reinsertion failed. Autopsy showed that death was primarily the result of acute surgical emphysema with bilateral pneumothoraces.

One of the neurosurgical registrars who performed the tracheostomy stated in a written report that postoperative chest radiograms indicated proper tube placement and the absence of pneumothorax and subcutaneous emphysema. However, a senior ENT surgeon, acting as an independent expert for the ensuing coroner's inquiry, pointed out that these radiological observations were not documented in the case records. He also took issue with the fact that between the tracheostomy and the time when the patient was found to be breathless (an interval of some 13 hours), there was very poor clinical documentation of the patient's progress. As such, it would not be possible to determine the time point at which the tracheostomy tube was out of position. He further gave the opinion that the "vertical slit" for the creation of the tracheostomy, as was used by the neurosurgical registrars, was prone to closure and would pose considerable difficulty to attempts at reinsertion, whereas a "window" or a circular opening in the anterior tracheal wall would facilitate reinsertion, should slippage of the tube occur. A verdict of misadventure was returned.

Case 5

A 54-year-old Chinese male was diagnosed with advanced adenocarcinoma of the gallbladder accompanied by biliary obstruction. He underwent cholecystectomy and extensive liver resection (right hepatic lobe and segment

tify the sites of anastomotic dehiscence before evisceration, so as to avoid causing artifactual dehiscence of these delicate structures, rendered all the more fragile by variable postmortem autolysis. The same principle might apply to deaths after corrective surgery for complex congenital heart disease.

As a rule, it is important to note the presence of all collections of effusions, hemorrhage, exudates, and abscesses and to measure their quantities before the organs are eviscerated. After evisceration, the rest of the autopsy could proceed in much the usual manner, although particular vigilance will have to be exercised when examining and dissecting the surgical sites. Attention should also be paid to the presence of concurrent pathology of major organs (especially the cardiovascular, respiratory, and central nervous systems) other than those subjected to therapeutic intervention, as comorbidity is often a significant contributory or even causative factor in perioperative deaths.

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periventricular and brainstem necroses, together with midbrain and pontine petechial hemorrhages. There also was a central intracerebellar hematoma occupying the vermis. Diffuse hypoxemic neuronal degeneration and necrosis were present. There was no evidence of stent failure or of perforation or rupture of the left anterior descending artery. Death was attributed to acute cerebral and intracerebellar hemorrhage, consistent with perioperative heparin administration. A verdict of misadventure was returned.

Case 7

A 61-year-old female developed DIC. Despite heroic efforts at hemostasis and resuscitation supplemented by massive transfusions of blood, fresh-frozen plasma, colloids, and crystalloid solutions, she died during subtotal hysterectomy for a suspected uterine malignancy.

Autopsy showed the presence of a large necrotic and hemorrhagic tumor occupying much of the uterus (which was received as a separate surgical specimen) and measuring 14 cm in diameter. On cross sections, the tumor had a whorled appearance. There also were severe subpleural, whitish, metastatic tumor nodules found in both lungs. A diagnosis of uterine leiomyosarcoma was made on postmortem histological examination and immunohistochemistry. Extensive tumor embolization within the pulmonary arterial microvasculature was detected histologically, too. It is possible that the latter could have occurred, at least to some extent, during surgical manipulation at the uterus and the uterine blood vessels and might, in part, have engendered DIC with fatal consequences.

Subsequent inquiries revealed that the patient had consulted a number of other gynecologists prior to surgery, apparently without the knowledge of the gynecologist who performed the ill-fated operation. It appears that investigations conducted in the course of the previous consultations had documented ultrasonographic and radiological evidence of a highly vascular uterine tumor and possible pulmonary metastases, respectively. A verdict of misadventure was recorded.

Case 8

A 53-year-old Chinese male was diagnosed and investigated for obstructive jaundice. Two attempts at ERCP failed, whereas a second attempt at percutaneous transhepatic cholangiography (PTC) and drainage was successful. However, several hours later the patient developed pain and tenderness over the operated site and deteriorated rapidly. He subsequently collapsed and could not be resuscitated.

Autopsy showed 600 mL of liquid blood and 900 g of clotted blood in the peritoneal cavity with preference of the right subphrenic space. The PTC catheter had been inserted through the right eighth intercostal space, laterally, and then through the right diaphragmatic dome. It then proceeded through the anterolateral surface of the right hepatic lobe and continued downwards to terminate within the right hepatic duct, there being no evidence of pulmonary or hepatic laceration or parenchymal hemorrhage. Apart from hemobilia, the intra- and extrahepatic biliary tract was free of any mechanical obstruction by tumor or biliary calculi; there was also no evidence of ampullary stenosis. In addition, there was evidence of severe coronary atheroma and hypertensive heart disease.

Postmortem histology showed features consistent with intrahepatic cholestasis (for which a drug-induced etiology could not be excluded). There was also accompanying mild hepatitis and focal cholangitis, consistent with mild iatrogenic injury engendered by ERCP and PTC as well as occasional megamitochondria within some hepatocytes. Subsequent police inquiries revealed that the patient had neither ingested any herbal preparation nor did he have a history of alcoholism.

Two independent clinical experts were engaged for the coroner's inquiry. The radiological opinion was that the second PTC was indicated as the patient was becoming increasingly jaundiced. In view of the slightly prolonged PTT of 47.3 s (normal range: 29.0–40.5 s) in the face of a normal blood platelet count, infusion of one or two units of fresh-frozen plasma prior to and during the procedure might have been in order. However, this expert declined to comment directly on the issue of negligence. The surgical opinion was largely similar and emphasized the difficulty in distinguishing between intra- and extrahepatic causes of jaundice. A verdict of misadventure was recorded.

Case 9

A 56-year-old male, recently diagnosed with inferior MI, underwent cardiac cauterization, which demonstrated triple vessel disease with total occlusion of the right coronary and left circumflex arteries. He apparently tolerated coronary angiography well but became hypotensive immediately after the left ventriculogram was completed, this being associated with a rash and wheezing.

An anaphylactic reaction to the radiocontrast medium was immediately diagnosed and the patient was resuscitated accordingly. However, as he was deemed to have suffered irreversible cardiogenic shock he was placed on percutaneous cardiopulmonary support and emergency coronary bypass surgery was performed as a salvage procedure. Unfortunately, perioperative DIC supervened and he died toward the end of 5 hours of operation. There was

apparently no past history of any drug allergy. The coroner's verdict was that of misadventure.

Case 10

A 71-year-old Chinese female underwent anterior resection for moderately differentiated carcinoma of the rectum. She developed a postoperative intestinal obstruction, which necessitated adhesiolysis and small bowel resection on postoperative day 16. Subsequently, she became septicemic from a combination of MRSA urinary tract infection, MRSA contamination of an indwelling central venous catheter inserted for total parenteral nutrition, bronchopneumonia (with sputum cultures yielding *Pseudomonas* and *Klebsiella* species), and surgical wound infection. She died 3 months later.

Autopsy and postmortem histological examination demonstrated infective endocarditis with perforation of the posterior cusp of the mitral valve accompanied by acute suppurative pancarditis and focal myocardial necrosis. There also was evidence of bronchopneumonia, features consistent with adult respiratory distress syndrome, focal renal cortical infarcts, and acute splenitis. The left cerebral parietal cortex was infarcted with evidence of acute vasculitis, septic embolization, and focal abscess formation. Postmortem cultures of the vegetations arising from the mitral valve yielded MRSA, *Klebsiella* species and *Escherichia coli*.

In this instance, infective endocarditis could have arisen as a consequence of septicemia caused by MRSA contamination of the central venous catheter or a combination of this and MRSA bronchopneumonia. The coroner's verdict was that of misadventure.

References

1. Sharpe VA, Faden AI (1998) Medical Harm. Historical, Conceptual and Ethical Dimensions of Iatrogenic Illness. Cambridge University Press, Cambridge, p. 1.
2. Walton J, Barondes JA, Lock S (1994) The Oxford Medical Companion. Oxford University Press, Oxford, p. 397.
3. Sharpe VA, Faden AI (1998) Medical Harm. Historical, Conceptual and Ethical Dimensions of Iatrogenic Illness. Cambridge University Press, Cambridge, pp. 137–140.
4. Brennan TA, Leape LL, Laird NM, et al. (1991) Incidence of adverse events and negligence in hospitalised patients. N Engl J Med 324, 370–376.
5. Kohn LT, Corrigan MJ, Donaldson MS (2000) To Err is Human: Building a Safer Health System. Institute of Medicine, National Academy Press, Washington, DC, pp. 1–2.
6. Chartran F (1999) Medical errors kill almost 100,000 Americans a year. BMJ 319, 1519.

7. Chartan F (2000) Senators introduce bill to improve patient safety. BMJ 320, 465.
8. Woods D (2000) Estimate of 98,000 deaths from medical errors is too low, says specialist. BMJ 320, 1362.
9. Saukko P, Knight B (2004) Knight's Forensic Pathology, 3rd ed. Arnold, London, pp. 480–491.
10. Grocott MPW, Ingram S (2003) Perioperative deaths. In Payne-James J, Busuttil A, Smock A, eds., Forensic Medicine: Clinical and Pathological Aspects, Greenwich Medical Media, London, pp. 201–212.
11. Juvin P, Teissiere F, Brion F, Desmonts JM, Durigon M (2000) Postoperative death and malpractice suits: is autopsy useful? Anesth Analg 91, 344–346.
12. Lau G (1995) Perioperative deaths: a comparative study of coroner´s autopsies between the periods of 1989–1991 and 1992–1994. Ann Acad Med Singapore 25, 509–515.
13. Lau G (2000) Perioperative deaths: a further comparative review of coroner's autopsies with particular reference to the occurrence of fatal iatrogenic injury. Ann Acad Med Singapore 29, 486–497.
14. Shanks JH, McClugage G, Anderson NH, Toner PG (1990) Value of the necropsy in perioperative deaths. J Clin Pathol 43, 193–195.
15. Sonderegger-Iseli K, Burger S, Muntwyler J, Salomon F (2000) Diagnostic errors in three medical eras: a necropsy study. Lancet 355, 2027–2031.
16. O'Grady G (2003) Death of the teaching autopsy. BMJ 327, 802–804.
17. Wong JWW, Tong MC, Tan NC, Lim YC, Ong KK (1990) Left main coronary artery obstruction: surgical experience with 93 patients. Ann Acad Med Singapore 19, 37–40.
18. Saw H (1990) Coronary artery bypass surgery in the elderly. Ann Acad Med Singapore 19, 45–50.
19. Levi M, Cromheecke ME, de Jonge E, et al. (1999) Pharmacological strategies to decrease excessive blood loss in cardiac surgery: a meta-analysis of clinically relevant endpoints. Lancet 354, 1940–1947.
20. Angelini GD, Taylor FC, Reeves BC, Ascione R (2002) Early and mid-term outcome after off-pump and on-pump surgery in Beating Heart Against Cardioplegic Arrest Studies (BHACAS 1 and 2): a pooled analysis of two randomised controlled trials. Lancet 359, 1194–1199.
21. Lau G (1994) Fatal hemorrhage following intra-aortic balloon counterpulsation: a case report and a brief review of its clinico-pathological and medico-legal aspects. Med Sci Law 34, 111–116.
22. Goldberger M, Tabak SW, Shah PK (1986) Clinical experience with intra-aortic balloon counterpulsation in 112 consecutive patients. Am Heart J 111, 497–502.
23. Gottlieb SO, Brinker JA, Borkon AM, et al. (1984) Identification of patients at high risk for complications of intra-aortic balloon counterpulsation: a multivariate risk factor analysis. Am J Cardiol 53, 1135–1139.
24. Iverson LIG, Herfindahl G, Ecker RR, et al. (1987) Vascular complications of intra-aortic balloon counterpulsation. Am J Surg 154, 99–103.
25. Kantrowitz A, Wasfie T, Freed PS, Rubenfire M, Wajszczuk W, Schork MA (1986) Intra-aortic balloon pumping, 1967 through 1982: analysis of complications in 733 patients. Am J Cardiol 57, 976–983.

26. Piccinino F, Sagnelli E, Pasquale G, Giusti G (1986) Complications following percutaneous liver biopsy. A multicentre retrospective study on 68,276 biopsies. J Hepatol 2, 165–173.
27. McGill DB, Rakela J, Zinmeister AR, Ott BJ (1990) A 21-year experience with major hemorrhage after percutaneous liver biopsy. Gastroenterology 99, 1396–1400.
28. Vautier G, Scott B, Jenkins D (1994) Liver biopsy—blind or guided? BMJ 309, 1455–1456.
29. Lau G, Lai SH (2001) Fatal retroperitoneal hemorrhage: an unusual complication of percutaneous endoscopic gastrostomy. Forensic Sci Int 116, 69–75.
30. Cuschieri A (1999) Technology for minimal access surgery. BMJ 319, 1304.
31. Ferriman (1998) Surgeons carry out too many operations on dying patients. BMJ 318, 728–730.
32. Aster JC (2005) Red blood cell and bleeding disorders. In Kumar V, Abbas AK, Fausto N, eds., Robbins and Cotrain Pathologic Basis of Disease, 7th ed, Elsvier Saunders, Philadelphia, PA, pp. 656–659.
33. Earnshaw JJ, Shaw E, Whyman MR, Poskitt KR, Heather BP (2004) Screening for abdominal aortic aneurysms in men. BMJ 328, 1122–1124.
34. Ashton HA, Buxton MJ, Day NE, et al. (2002) The Multicentre Aneursym Screening Study Group (MASS) into the effect of abdominal aortic aneurysm screening in mortality in men: a randomised controlled trial. Lancet 360, 1531–1539.
35. Greenhalgh RM (2004) National screening programme for aortic aneurysm. BMJ 328, 1087–1088.
36. Irvine CD, Shaw E, Poskitt KR, Whyman MR, Earnshaw JJ, Heather BP (2000) A comparison of the mortality rate after elective repair of aortic aneurysms detected either by screening or incidentally. Eur J Vasc Endosvasc Surg 20, 374–378.
37. Wagenknecht LE, Furberg CD, Hammon JW, Legault C, Troost BT (1995) Surgical bleeding: unexpected effect of a calcium antagonist. BMJ 310, 776–777.
38. Zuccala G, Pahor M, Landi F, et al. (1997) Use of calcium antagonists and need for perioperative transfusion in older patients with hip fracture: observational study. VMJ 314, 643–644.
39. Lau G (1995) Iatrogenically-related, fatal hemorrhage occurring in end-stage renal failure: a series of three cases. Forensic Sci Int 73, 117–124.
40. Teare L, Cookson B, Stone S (2001) Hand hygiene. BMJ 323, 411–412.
41. National Audit Office (2000) The management and control of hospital acquired infection in acute NHS trusts in England. Stationery Office, London (www.nao.gov.uk).
42. Public Health Laboratory Service (2000) Nosocomial infection national surveillance scheme (1997–9). PHLS, London.
43. Pratt RJ, Pellowe C, Loveday HP, Robinson N, Smith GW, Barrett S, et al. (2001) The epic project: developing national evidence-based guidelines for preventing healthcare associated infections. Phase I: Guidelines for preventing hospital-acquired infections. J Hosp Infect 47, Suppl, S3–S82.
44. Reiss I, Borkhardt A, Fussle R, Sziegoleit A, Gortner L (2000) Disinfectant contaminated with Klebsiella oxytoca as a source of sepsis in babies. Lancet 356, 310–311.

45. O'Leary M, Bihari D (1998) Central venous catheters—time for a change? BMJ 316, 1918.
46. Dobbins BM, Kite P, Wilcox MH (1999) Diagnosis of central venous catheter related sepsis—a critical look inside. J Clin Pathol 52, 165–172.
47. Hall C, Dorricott NJ, Donovan IA, Neoptolemos JP (1991) Colon perforation during colonoscopy: surgical versus conservative management. Br J Surg 78, 542–544.
48. Christie JP, Marrazzo J (1991) "Mini-perforation" of the colon—not all postpolypectomy perforations require laparotomy. Dis Colon Rectum 34, 132–135.
49. Farley DR, Bannon MP, Zietlow SP, Pemberton JH, Illstrup DM, Larson DR (1997) Management of colonoscopic perforations. Mayo Clin Proc 72, 729–733.
50. Tham TCK, Carr-Locke DL (1999) Endoscopic treatment of bile duct stones in elderly people. BMJ 318, 617–618.
51. Freeman ML, Nelson DB, Sherman S, Haber GB, Herman ME, Dorsher PJ, et al. (1996) Complications of endoscopic biliary sphincterotomy. N Engl J Med 335, 909–918.
52. Targarona EM, Ayuso RMP, Bordas JM, et al. (1996) Randomised trial of endoscopic sphincterotomy with gallbladder left in situ versus open surgery for common bile duct calculi in high-risk patients. Lancet 347, 926–929.
53. Woods MS, Shellito JL, Santoscoy GS, et al. (1994) Cystic duct leaks in laparoscopic cholecystecomy. Am J Surg 168, 560–563.
54. Wise US, Glick GL, Landeros M (1996) Cystic duct leak after laparoscopic cholecystectomy. A multi-institutional study. Surg Endosc 10, 1189–1193.
55. Barkun AN, Rezieg M, Mehta SN, et al. (1997) Postcholecystectomy biliary leaks in the laparoscopic era: risk factors, presentation and management. Gastrointest Endosc 45, 277–282.
56. Adamsen S, Hansen OH, Funch-Jensen P, Schulze S, Stage JG, Wara P (1997) Bile duct injury during laparoscopic cholecystecomy: a prospective nationwide series. J Am Coll Surg 184, 571–578.
57. Paul A, Troidl H, Peters S (1994) Fatal intestinal ischemia following laparoscopic cholecystectomy. Br J Surg 81, 1207.
58. Terpstra OT (1996) Laparoscopic cholecystectomy: the other side of the coin. BMJ 312, 1375–1376.
59. Macintyre IMC, Wilson RG (1993) Laparoscopic cholecystectomy. Br J Surg 80, 552–559.
60. Gouma DJ, Go PM (1994) Bile duct injury during laparoscopic and conventional cholecystectomy. J Am Coll Surg 178, 229–233.
61. Gottlieb S (2003) Injury to bile duct during cholecystetomy nearly triples risk of death. BMJ 327, 946.
62. Flum DR, Cheadle A, Prela C, Dellinger P, Chan L (2003) Bile duct injury during cholecystectomy and survival in Medicare beneficiaries. JAMA 290, 2168–2173.
63. Molloy RG, Kingsmere D (2000) Life threatening pelvic sepsis after stapled hemorrhoidectomy. Lancet 355, 810.

64. Fazio VW (2000) Early promise of stapling technique for hemorrhoidectomy. Lancet 355, 768–769.
65. Cheetham MJ, Mortensen NJM, Nystrom P, Kamm MA, Phillips RKS (2000) Persistent pain and fecal urgency after stapled hemorrhoidectomy. Lancet 356, 730–733.
66. Larkin M (1997) TB transmitted by contaminated bronchoscopes. BMJ 350, 1009.
67. Agerton T, Valway S, Gore B, et al. (1997) Transmission of a highly drug-resistant strain (strain W1) of *Myocbacterium tuberculosis*. Community outbreak and noscomial transmission via a contaminated bronchoscope. JAMA 278, 1073.
68. Gliemroth J, Heise S, Missler U (1996) A 64-year-old man with diabetes and ascending paraplegia. Lancet 347, 516.
69. Dunn J (1996) Algae kills dialysis patients in Brazil. BMJ 312, 1183–1184.
70. Katherine MJ, Shakir M, Perper J (1993) Fatal *Clostridium perfringens* and *Escherichia coli* sepsis following urea-instillation abortion. Am J Forensic Med Pathol 14, 151–154.
71. Simini B (1999) Liposuction surgery in Italy leads to *Streptococcus pyogenes* sepsis. Lancet 353, 1164.
72. Froth A, Joyce R, Johnson A (2001) Iatrogenic vCJD from surgical instruments. BMJ 322, 1558–1559.
73. Hill F, Butterworth RJ, Joiner S, et al. (1999) Investigation of variant Creuzfeldt-Jakob disease and other human prion diseases with tonsil biopsy samples. Lancet 353, 183–189.
74. Bernoulli C, Siegfried J, Baumgartner G, et al. (1977) Danger of accidental person-to-person transmission of Creutzfeldt-Jakob disease by surgery. Lancet 1, 478.
75. Zobeley E, Flechsig E, Corrizio A, Enari M, Weissman C (1999) Infectivity of scrapie prions bound to a stainless steel surface. Mol Med 5, 240–243.
76. Uno Y, Morita T (1997) Colonic perforation and serosal tears associated with colonoscopy. Lancet 349, 1888.
77. Kozarek RA, Earnest DL, Silverstein ME, Smith RG (1980) Air-pressure-induced colon injury during diagnostic colonscopy. Gastroenterology 78, 7–14.
78. Ehrlich CP, Hall FM, Joff N (1984) Postendoscopic perforation of normal colon in an area remote from instrumentation. Gastrointest Endosc 30, 190–191.
79. Hofmann HS, Rettig G, Radke J, Neef H, Silber RE (2002) Iatrogenic ruptures of the tracheobronchial tree. Eur J Cardiothorac Surg 21, 649–652.
80. Hruban RH, Wilentz RE (2005) The Pancreas. In Kumar V, Abbas AK, Fausto N, eds., Robbins and Cotran Pathologic Basis of Disease, 7th ed, Elsevier Saundus, Philidelphia, PA, pp. 942–948.
81. Cuschieri A, Fayers P, Fielding J, et al. (1996) Postoperative morbidity and mortality after D_1 and D_2 resections for gastric cancer: preliminary results of the MRC randomised controlled surgical trial. Lancet 347, 995–999.
82. Botterill I, Miller G, Dexter S, Martin I (1998) Deaths after delayed recognition of percutaneous endoscopic gastrostomy tube migration. BMJ 317, 524–525.

83. Chowdhury MA, Batey R (1996) Complications and outcome of percutaneous endoscopic gastrostomy in different patient groups. J Gastroenterol Hepatol 11, 835–839.
84. Calton WC, Martindale RG, Gooden SM (1992) Complications of percutaneous endoscopic gastrostomy. Mil Med 157, 358–360.
85. Feussner H, Hannig CH, Weiser HF (1989) Trans-gastric perforation of a percutaneous endoscopic feeding tube with a fatal outcome. Endoscopy 21, 45–46.
86. Walter R, Ennemoser O, Tributsch W, Ambach E (1995) Iatrogenic ruptures of the stomach after balloon tamponade. Am J Forensic Med Pathol 16, 135–139.
87. Lewandrowski KB, Southern JF, Medeiros J, Jacobs M (1989) Aortoesophageal fistula arising as a complication of prolonged nasogastric tube placement. Human Pathol 20, 709–711.
88. Sosnowik D, Greenberg R, Bank S, Graver M (1988) Aortoesophageal fistula: early and late endoscopic features. Am J Gastroenterol 83, 1401–1404.
89. Wasmuth H, Verhage CC (1989) Hematemesis due to fistula between aorta and eosphagus and later to fistula between left common carotid artery and eosphagus. Acta Chir Scand 155, 67–69.
90. Borrero E, Aylward CA, Logan WD (1989) Aortoesophageal fistula: early postoperative complication at the distal anastomosis of an aortic graft. South Med J 82, 927–930.
91. Grey TC, Mittleman RE, Wetli CV, Horowitz S (1988) Aortoesophageal fistula and sudden death. Am J Forensic Med Pathol 9, 19–22.
92. Tessier DJ, Stone WM, Fowl RJ, et al. (2003) Clinical features and management of splenic artery pseudoaneurysm: case series and cumulative review of literature. J Vasc Surg 38, 969–974.
93. Adam A (1995) Insertion of long term central venous catheters: time for a new look. BMJ 311, 341–342.
94. Muhm M (2002) Ultrasound guided central venous access (2002) BMJ 325, 1373–1374.
95. Hind D, Calvert N, McWilliams R, et al. (2003) Ultrasonic locating devices for central venous cannulation: meta-analysis. BMJ 327, 361–367.
96. Zeien LB, Noguchi TT (1992) Fatal hydrothorax associated with subclavian vein catheterization for hemodialysis. Am J Forensic Med Pathol 13, 326–328.
97. Byard RW, Koszyca B, Qiao M (2001) Unexpected childhood death due to a rare complication of ventriculoperitoneal shunting. Am J Forensic Med Pathol 22, 207–210.
98. Castiglione AG, Bruzzone E, Burrello C, Pisani R, Ventur F, Canale M (1998) Intracranial insertion of a nasogasric tube in a case of homicidal head injury. Am J Forensic Med Pathol 19, 329–334.
99. Lau G (2002) Are maternal deaths on the ascent in Singapore? A review of maternal mortality as reflected by coronial casework from 1990 to 1999. Ann Acad Med Singapore 31, 261–275.
100. Shennan A, Bewley S (2001) How to manage term breech deliveries. BMJ 323, 244–245.

101. Hall MH, Bewley S (2001) Maternal mortality and mode of delivery. Lancet 354, 776.
102. Waterstone M, Bewley S, Wolfe C (2001) Incidence and predictors of severe obstetric morbidity: case control study. BMJ 322, 1089–1094.
103. Wagner M (2000) Choosing cesarean section. Lancet 356, 1677–1680.
104. Josefson D (2001) Vaginal delivery after cesarean section triples risk of uterine rupture. BMJ 323, 68.
105. Lydon-Rochelle M, Holt VL, Easterling TR, Martin DP (2001) Risk of uterine rupture during labour among women with a prior cesarean delivery. N Engl J Med 345, 3–8.
106. Plauche WC (1980) Subgaleal hematoma. A complication of instrumental delivery. JAMA 24, 1597–1598.
107. Plauche WC (1979) Fetal cranial injuries related to delivery with the Malmstrom vacuum extractor. Obstet Gynecol 53, 750–757.
108. Mohammed G, Ahmed M (2003) Serious fetal intracranial hemorrhage associated with the vacuum extractor. Br J Obstet Gynecol 110, 436–438.
109. Choudhari K, Choudhari Y (2003) Posterior fossa hemorrhage in a preterm infant following vacuum assisted delivery. Br J Obstet Gynecol 110, 787.
110. Kent A, Lemyre B, Losley-Millman M, Pes B (2001) Posterior fossa hemorrhage in a preterm infant following vacuum assisted delivery. Br J Obstet Gynecol 108, 1008–1010.
111. Hanigan WC, Morgan AM, Stahlbert LK, Hiller JL (1990) Tentorial hemorrhage associated with vacuum extraction. Pediatrics 85, 534–539.
112. Huang LT, Lui CC (1995) Tentorial hemorrhage associated with vacuum extraction in a newborn. Pediatr Radiol 25, Suppl 1, S230–S231.
113. Vacca A (2001) Operative vaginal delivery: clinical appraisal of a new vacuum extraction device. Aust N Z J Obstet Gynecol 41, 156–160.
114. Wolf DA (2001) Heimlich Trauma. Am J Forensic Med Pathol 22, 65–67.
115. Pekka S, Knight B (2004). Knight's Forensic Pathology, 3rd ed. Arnold, London, pp. 40–41.
116. Lau G (1994) A case of sudden maternal death associated with resuscitative liver injury. Forensic Sci Int 67, 127–132.
117. Fitchet A, Neal R, Bannister P (2001) Splenic trauma complicating cardiopulmonary resuscitation. BMJ 322, 480–481.
118. Lau G (1995) Pulmonary thromboembolism is not uncommon—results and implications of a five-year study of 116 necropsies. Ann Acad Med Singapore 24, 356–365.
119. Lau G, Thamboo TP, Lai SH (2003) Fatal pulmonary thromboembolism in Singapore: has anything changed? Med Sci Law 43, 307–314.
120. Owing JT, Kraut E, Battistella F, Cornelius JT, O'Malley R (1997) Timing of the occurrence of pulmonary embolism in trauma patients. Arch Surg 132, 862–867.
121. Knight B, Zaini MRS (1980) Pulmonary embolism and venous thrombosis: a pattern of incidence and predisposing factors over 70 years. Am J Forensic Med Pathol 1, 227–232.

122. Mandelli V, Schmid C, Zogno C, Morpurgo M (1997) "False-negatives" and "false-positives" in acute pulmonary embolism: a clinical post-mortem comparison. Cardiologia 42, 205–210.
123. Morgenthaler TI, Ryu JH (1995) Clinical characteristics of fatal pulmonary embolism in a referral hospital. Mayo Clin Proc 70, 417–424.
124. Pineda LA, Hathwar VS, Grant BJ (2001) Clinical suspicion of fatal pulmonary embolism. Chest 120, 791–795.
125. Baglin T (2000) Thrombophilia testing: what do we think the tests mean and what should we do with the results? J Clin Pathol 53, 167–170.
126. Svensson PJ, Dahlback B (1994) Resistance to activated protein C as a basis for venous thrombosis. N Engl J Med 330, 517–522.
127. Andrew TA, Fairweather R (2003) Prothrombin G20210A mutation and sudden death. Am J Forensic Med Pathol 24, 377–380.
128. Pekka S, Knight B (2004). Knight's Forensic Pathology, 3rd ed. Arnold, London, pp. 343–345.
129. Heini PF, Orler R (2004) Vertebroplasty in severe osteoporosis. Technique and experience with multi-segment injection [in German]. Orthopäde 33, 22–30.
130. Nazon D, Abergel G, Hatem CM (2003) Critical care in orthopedic and spine surgery. Crit Care Clin 19, 33–53.
131. Ross RM, Johnson GW (1988) Fat embolism after liposuction. Chest 93, 1294–1295.
132. Graze FM, de Jong RH (2000) Fatal outcomes from liposuction: census survey of cosmetic surgeons. Plast Reconstr Surg 105, 436–446.
133. Platt MS, Kohler LJ, Ruiz R, Chole SD, Ravichandran P (2002) Deaths associated with liposuction: case reports and review of the literature. J Forensic Sci 47, 205–207.
134. Mason JK (1993) Resuscitation artefacts, including bone marrow. In Mason JK, ed., Forensic Medicine: An Illustrated Reference, Oxford University Press, Oxford, pp. 39–42.
135. Pekka S, Knight B (2004). Knight's Forensic Pathology, 3rd ed. Arnold, London, pp. 346–348.
136. Lau G (1994) Amniotic fluid embolism as a cause of sudden maternal death. Med Sci Law 34, 213–220.
137. Veinot JP, Edwards WD (1994) Trauma-related embolization of cartilage to the lungs. Case report of a 41-year-old man. Am J Forensic Med Pathol 15, 138–141.
138. Lau G (1995) Pulmonary cartilage embolism: fact or artefact? Am J Forensic Med Pathol 16, 51–53.
139. Bots GTAM, Watendorff AR, Burma OJS, Roos RAC, Endtz LJ (1981) Acute mylopathy caused by fibrocartilaginous emboli. Neurology 31, 1250–1256.
140. Dupont PJ, Lightstone L, Clutterbuck EJ, Gaskin G, Pusey CD, Cook T, Warrens N (2000) Cholesterol emboli syndrome. BMJ 321, 1065–1067.
141. Moolenaar W, Lamers CB (1996) Cholesterol crystal embolization in the Netherlands. Arch Intern Med 156, 653–657.
142. Cina SJ, Raso DS, Crymes LW, Upshur JK (1994) Fatal suture embolism to the left anterior descending coronary artery. Am J Forensic Med Pathol 15, 142–145.

143. Veijola M, Ikaheimo M, Valkam J, Hirvonen J (1997) Fatal occlusion of the left coronary main stem by a fragment from the femoral artery: a previously unreported complication of cardiac catheterisation. Forensic Sci Int 1997, 111–116.
144. Bell MD, Tate LG, Hensley GT (1992) Esophageal-atrial fistula resulting in systemic "meat and vegetable" emboli. Am J Forensic Med Pathol 13, 137–141.
145. Suarez-Penaranda JM, Guitian-Bareiro MD, Concheiro-Carro L (1995) Longstanding intracardiac catheter embolism. Am J Forensic Med Pathol 16, 124–126.
146. Ashton CM, Petersen NJ, Wray NP, Kiefe CI, Dunn JK, Wu L, et al. (1993) The incidence of perioperative myocardial infarction in men undergoing noncardiac surgery. Ann Intern Med 118, 504–510.
147. Rashid AMH, Williams RM (1994) Sudden death caused by giant cell myocarditis following coronary artery bypass. Am J Forensic Med and Pathol 15, 82–86.
148. Rabson AB, Choen FJ, Warhol MJ, Mudge GH, Collins JJ Jr (1984) Giant cell myocarditis after mitral valve replacement: case report and studies of the nature of giant cells. Hum Pathol 15, 585–587.
149. Brynjolfsson G, Eschaghy B, Talano JV, Gunnar R (1977) Granulomatous myocarditis secondary to cornstarch. Am Heart J 94, 353–358.
150. McCarthy M (1999) Study identifies heart surgery patients at risk for stroke. Lancet 354, 575.
151. Ramsay S (2001) Stroke contributes to women's higher mortality after cardiac surgery. Lancet 357, 1419.
152. Josefson D (2001) Early bypass surgery increases risk of stroke. BMJ 323, 185.
153. Cronin L, Mehta SR, Zhao F, et al. (2001) Stroke in relation to cardiac procedures in patients with non-ST-elevation acute coronary syndrome. Circulation 104, 269–274.
154. Herczeg L, Gorombey S, Vaszily M (1995) Morphological damage to the central nervous system (CNS) following open heart surgery. Forensic Sci Int 79, 103–111.
155. Tascilar M, van Rees BP, Sturm PDJ, et al. (2002) Pancreatic cancer after remote peptic ulcer surgery. J Clin Pathol 340–345.
156. Radhi JM, Ibrahiem K, Al-Tweigeri T (1998) Soft tissue malignant lymphoma at sites of previous surgery. J Clin Pathol 51, 629–632.
157. Healey P, Davis CL (1998) Transmission of tumours by transplantation. Lancet 352, 2–3.
158. Frank S, Muller J, Bonk C, Haroske G, Schackert HK, Schackert G (1998) Transmission of glioblastoma multiforme through liver transplantation. Lancet 352, 31.
159. Aseni P, Vertemati M, Minola E, Banacina E (2003) Massive hemoptysis after living donor liver transplantation. J Clin Pathol 56, 876–878.
160. Dripps RL, Lamont A, Eckenhoff JE (1961) The role of anesthesia in surgical mortality. JAMA 178, 261–266.
161. Copeland GP, Jones D, Walters M (1991) POSSUM: a scoring system for surgical audit. Br J Surg 78, 355–360.
162. Ranklev E, Fletcher R, Krantz P (1985) Malignant hyperpyrexia and sudden death. Am J Forensic Med Pathol 6, 149–150.
163. Anetseder M, Hager M, Muller CR, Roewer N (2002) Diagnosis of susceptibility to malignant hyperthermia by use of a metabolic test. Lancet 359, 1579–1580.

164. Warden JC, Horan BF, Holland R (1997) Morbidity and mortality associated with anesthesia. Acta Anesthesiol Scand 41, 949.
165. Kharasch ED, Hankins D, Mautz D, Thummel KE (1996) Identification of the enzyme responsible for oxidative halothane metabolism: implications for prevention of halothane hepatitis. Lancet 347, 1367–1371.
166. Gelven PL, Cina SJ, Lee JD, Nichols CA (1996) Massive hepatic necrosis and death following repeated isoflurane exposure. Case report and review of the literature. Am J Forensic Med Pathol 17, 61–64.
167. Lewis JH, Zimmerman JH, Ishak KG, Mullick FG (1983) Enflurane hepatotoxicity: a clinicopathologic study of 24 cases. Ann Intern Med 98, 984–992.
168. Bray RJ (1998) Propofol infusion syndrome in children. Pediatr Anesth 8, 491–499.
169. Cremer OL, Moons KGM, Bouman EAC, et al. (2001) Long-term propofol infusion and cardiac failure in adult head-injured patients. Lancet 357, 111–115.
170. Metkus AP, Trabulsy PP, Scholobohm RS, Hickey MS (1996) A firefighter with pancreatitis. Lancet 348, 1702.
171. Boyle WA, Shear JM, White PF (1990) Tolerance and hyperlipidemia during long-term sedation with propofol. Anesthesiology 73, Suppl, A245.
172. Casey ATH, O'Brien MO, Kumar V, Hayward RD, Crockard HA (1995) Don't twist my child's head off: iatrogenic cervical dislocation. BMJ 311, 1212–1213.
173. Brahams D (1994) Epidural injection of toxic substance. Lancet 344, 1218.
174. Meel B (1998) Inadvertent intrathecal administration of postassium chloride during routine spinal anesthesia. Am J Forensic Med Pathol 19, 255–257.
175. Rodgers A, Walker N, Schug S, McKee A, Kehlet H, van Zundert A, et al. (2000) Reduction of postoperative mortality and morbidity with epidural or spinal anesthesia: results from overview of randomised trials. BMJ 321, 1493–1496.
176. Harper CM (2003) Maintaining perioperative normothermia. BMJ 326, 721–722.
177. Fricker J (1997) Warm operating theatres may prevent problems. Lancet 349, 1075.
178. Tanne JH (1998) Food and drugs alter response to anesthesia. BMJ 317, 1102.
179. Warner MA, Warner ME (1993) Clinical significance of pulmonary aspiration during the perioperative period. Anesthesiology 78, 56–62.
180. Kallar SK, Everett LL (1993) Potential risks and preventive measures for pulmonary aspiration: new concepts in preoperative fasting guidelines. Anesth Analg 77, 171–182.
181. Taylor TH (1992) Avoiding iatrogenic injuries in theatre. BMJ 305, 595–596.
182. Dyer C (1994) Anesthetist loses final appeal. BMJ 309, 78.
183. Rothen HU, Sporre Bengt, Engberg G, Wegenius G, Reber A, Hedenstierna G (1995) Prevention of atelectasis during general anesthesia. Lancet 345, 1387–1391.
184. Charlton JE (1995) Monitoring and supplemental oxygen during endoscopy. BMJ 310, 886–887.
185. Quine MA, Bell GC, McCloy RF, Charlton JE, Devlin HB, Hopkins A (1995) Prospective audit of upper gastrointestinal endoscopy in two regions of England: safety, staffing, and sedation methods. Gut 36, 462–467.

186. Bowling TE, Hadjiminas CL, Polson RJ, Baron JH, Foale RA (1993) Effects of supplemental oxygen on cardiac rhythm during upper gastrointestinal endoscopy: a randomised controlled double blind trial. Gut 34, 1492–1497.
187. Moller JT, Witrup M, Johansen JH (1990) Hypoxemia in the post anesthesia care unit: an observer study. Anesthesiology 73, 890–895.
188. Funayama M, Kumagai T, Saito K, Watanabe T (1994) Asphyxial death caused by postextraction hematoma. Am J Forensic Med Pathol 15, 87–90.
189. Doldo G, Albanese I, Macheda S, Caminiti G (2001) Ludwig angina: a disease of the past century. Case report [in Italian]. Minerva Anestesiol 67, 811–814.
190. Neff SP, Merry AF, Anderson B (1999) Airway management in Ludwig´s angina. Anesth Intensive Care 27, 659–661.
191. Parhiscar A, Har-El G (2001) Deep neck abscess: a retrospective review of 210 cases. Ann Otol Rhinol Laryngol 110, 1051–1054.
192. Furst IM, Ersil P, Caminiti M (2001) A rare complication of tooth abscess—Ludwig's angina and mediastinitis. J Can Dent Assoc 67, 324–327.
193. Simon E, Matee M (2001) Post-extraction complications seen at a referral dental clinic in Dar Es Salaam, Tanzania. Int Dent J 51, 273–276.
194. Pirmohamed M, Breckenridge AM, Kitteringham NR, Park BK (1998) Adverse drug reactions. BMJ 316, 1295–1298.
195. Asscher AW, Parr GD, Whitmarsh VB (1995) Towards the safer use of medicines. BMJ 311, 1003–1005.
196. Rawlins MD, Thomas SHL (1998). Mechanisms of adverse drug reactions. In Davies DM, ed., Textbook of Adverse Drug Reactions, 5th ed. Chapman and Hall Medical, London, pp. 40–64.
197. Einarson TR (1993) Drug-related hospital admissions. Ann Pharmacother 27, 832–840.
198. Atkin PA, Chenfield GM (1995) Medication-related adverse reactions and the elderly: a literature review. Adverse Drug React Toxicol Rev 14, 175–191.
199. Park BK, Pirmohamed M, Kitteringham NR (1992) Idiosyncratic drug reactions: a mechanistic evaluation of risk factors. Br J Clin Pharmacol 34, 377–395.
200. Pumphrey RSH, Roberts ISD (2000) Postmortem findings after fatal anaphylactic reactions. J Clin Pathol 53, 273–276.
201. Zimmerman HJ, Ishak KG (2002) Hepatic injury due to drugs and toxins. In MacSween RNM, Burt AD, Portmann BC, Ishak KG, Scheuer PJ, Anthony PP, eds., Pathology of the Liver, 4th ed. Churchill Livingstone, London, pp. 621–709.
202. Weedon D (2002) Skin pathology, 2nd ed. Churchill Livingstone, London.
203. Lewis W, Silver MD (2001) Effects of drugs on the cardiovascular system. In Silver MD, Gotlieb AI, Schoen FJ, eds., Cardiovascular Pathology, 3rd ed. Churchill Livingstone, New York, pp. 541–561.
204. Ordonez NG, Rosai J (2004) Urinary Tract. In Rosai J, Ackerman LV, eds., Rosai and Ackerman´s Surgical Pathology, 9th ed. C.V. Mosby, New York, pp. 1224–1229.
205. Eillison D, Love S, Chimelli L, Harding BN, Lowe J, Vinters HV (2004) Neuropathology. A Reference Text of CNS Pathology, 2nd ed. Mosby, London.

206. Ellenhorn MJ, Schonwald S, Ordog G, Wasserberger J (1996) Neuroleptic drugs. In Ellenhorn MJ, Schonwald S, Ordog G, Wasserberger J, eds., Ellenhorn's Medical Toxicology: Diagnosis and Treatment of Human Poisoning, 2nd ed. Williams and Wilkins, Baltimore, pp. 662–683.
207. Lau G (1995) A fatal case of drug-induced multi-organ damage in a patient with Hansen's disease: dapsone syndrome or rifampicin toxicity? Forensic Sci Int 73, 109–115.
208. Ellenhorn MJ, Schonwald S, Ordog G, Wasserberger J (1996) Alcohols and glycols. In Ellenhorn MJ, Schonwald S, Ordog G, Wasserberger J, eds., Ellenhorn's Medical Toxicology: Diagnosis and Treatment of Human Poisoning, 2nd ed. Williams and Wilkins, Baltimore, pp. 1127–1165.
209. Spitz DJ (2003) An unusual death in an asthmatic patient. Am J Forensic Med Pathol 24, 271–272.
210. Kumar L (1995) Secondary leukemia after autologous bone marrow transplantation. Lancet 345, 810.
211. Phillips DP, Christenfield N, Glynn LM (1998) Increase in US medication-error deaths between 1983 and 1993. Lancet 351, 643–644.
212. Jofeson D (2002) Doctors warned to be wary of new drugs. BMJ 324, 1113.
213. Taxis K, Barber N (2003) Ethnographic study of incidence and severity of intravenous drug errors. BMJ 326, 684–687.
214. Cuervo LG, Clarke M (2003) Balancing benefits and harms in health care. BMJ 327, 65–66.
215. Mayor S (2004) Report calls for strategies to reduce medication errors. BMJ 328, 248.
216. Smith J (2004) Building a safer NHS for patients. Improving medication safety. Department of Health. Available at: http://www.doh.gov.uk/buildsafenhs/medicationsafety.
217. Barbut F (2002) Managing antibiotic associated diarrhoea. BMJ 324, 345–346.
218. Wistrom J, Norrby Sr, Mybre EB, et al. (2001) Frequency of antibiotic-associated diarrhoea in 2462 antibiotic-treated hospitalised patients: a prospective study. J Antimicrob Chemother 47, 43–50.
219. McFarland LV (1998) Epidemiology, risk factors and treatments for antibiotic-associated diarrhoea. Dig Dis 16, 292–307.
220. Lochhead J, Elston JS (2003) Doxycycline induced intracranial hypertension. BMJ 326, 641–642.
221. Digre KB (2003) Not so benign intracranial hypertension. BMJ 326, 613–614.
222. Chiu AM, Chuenkongkew WL, Cornblath WT, et al. (1998) Minocycline treatment and pseudotumour cerebri syndrome. Am J Opththalmol 126, 116–121.
223. Terzano C, Petroianni A (2003) Clarithromycin and pulmonary infiltration with eosinophilia. BMJ 326, 1377–1378.
224. Bharani A, Kumar H (2001) Diabetes insipidus induced by ofloxacin. BMJ 323, 547.
225. Jones SE, Smith RH (1997) Quinolones may induce hepatitis. BMJ 314, 869.

226. Siepmann M, Kirch W (2001) Tachycardia associated with moxifloxacin. BMJ 322, 23.
227. Ipuge YAI, Rieder HL, Enarson DA (1995) Adverse cutaneous reactions to thiacetazone for tuberculosis treatment in Tanzania. BMJ 346, 657–660.
228. Krummel T, Dimitrov Y, Moulin B, Hannedouche T (2000) Acute renal failure induced by topical ketoprofen. BMJ 320, 93.
229. Warrren KJ, Boxwell DB, Kim NY, Drolet BA (1998) Nevirapine-associated Stevens-Johnson syndrome. Lancet, 351, 567.
230. Murri R, Antinori A, Camilli G, Zannoni G, Patriarca G (1995) Fatal toxic epidermolysis induced by zidovudine. Clin Infect Dis 23, 640–641.
231. Wooltorton E (2004) HIV drug nevirapine (Viramune): risk of severe hepatotoxicity. CMAJ 170, 1091.
232. Gotlieb S (2001) Nevirapine should not be prescribed for needlestick injuries. BMJ 322, 126.
233. Yamey G (2000) Drug company issues warning about flu drug. BMJ 320, 334.
234. Mortimer PP (2002) Yellow fever vaccine. BMJ 324, 439.
235. Vasconcelos Pedro FC, Luna EJ, et al. (2001) Serious adverse events associated with yellow fever 17DD vaccine in Brazil: a report of two cases. Lancet 358, 91–97.
236. Martin M, Tsai TF, Cropp B, et al. (2001) Fever and multisystem organ failure associated with 17D–204 yellow fever vaccination: a report of four cases. Lancet 358, 98–104.
237. Chan RC, Penney DJ, Little D, et al. (2001) Hepatitis and death following vaccination with 17D–204 yellow fever vaccine. Lancet 358, 121–122.
238. Gordon RD (1996) Calcium antagonists and gastrointestinal hemorrhage: the balancing act. Lancet 347, 1056.
239. Pahor M, Guarainki JM, Furber CD, Carbonin P, Havlik RJ (1996) Risk of gastrointestinal hemorrhage with calcium antagonists in hypertensive persons over 67 years old. Lancet 347, 1061–1065.
240. Macready N (1998) Drug causes cardiogenic shock. BMJ 316, 1927.
241. Messerli FH, Nussberger J (2000) Vasopeptidase inhibition and angio-oedema. Lancet 356, 608–609.
242. Nussberger J, Cugno M, Amstutz C, Cicardi M, Pellancani A, Agostoni A (1998) Plasma bradykinin in angio-oedema. Lancet 351, 1693–1697.
243. Hagley MT, Hulisz DT, Burns CM (1993) Hepatotoxicity associated with angiotensin converting enzyme inhibitors. Ann Pharmacother 27, 228–231.
244. Hariraj R, Stoner E, Jader S, Preston DM (2000) Prolonged choestasis associated with irbesartan. BMJ 321, 547.
245. Morton A, Muir J, Lim D (2004) Rash and acute nephritic syndrome due to candesartan. BMJ 328, 25.
246. Andersson OK (1998) Tolerability of a modern antihypertensive agent: candesartan cilexetil. Basic Res Cardiol 939, Suppl 2, S54–S58.
247. Bosch X (1998) Henoch-Schonlein purpura induced by losartan therapy. Arch Intern Med 158, 191–192.

248. Quinn MJ, Fitzgerald DJ (1999) Ticlopidine and clopidogrel. Circulation 100, 1667–1672.
249. Dakik HA, Salti I, Haidar R, Uthman IW (2002) Ticlopidine associated with acute nephritis. BMJ 324, 27.
250. Jessurun GA, Crijns HJGM (1997) Amiodarone pulmonary toxicity. BMJ 314, 619–620.
251. Silfvast T, Kinnunen A, Varpula T (1995) Laryngeal oedema after isosorbide dinitrate spray and sublingual nifedipine. BMJ 311, 232.
252. Gottlieb S (2001) FDA refuses companies' request to drop ulcer warning. BMJ 322, 385.
253. Gottlieb S (2001) COX 2 inhibitors may increase risk of heart attack. BMJ 323, 471.
254. Josefson D (2001) FDA warns Merck over its promotion of rofecoxib. BMJ 323, 767.
255. Bombardier C, Laine L, Reicin A, et al. (2000) Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. N Engl J Med 343, 1520–1528.
256. Mukherjee D, Nissen SE, Topol EJ (2001) Risk of cardiovascular events associated with selective COX–2 inhibitors. JAMA 286, 954–959.
257. O'Beirne JP, Cairns SR (2001) Cholestatic hepatitis in association with celecoxib. BMJ 323, 23.
258. Traversa G, Bianchi C, Da Cas R, Abraha I, Menniti-Ippolito F, Venegoni M (2003) Cohort study of hepatotoxicity associated with nimesulide and other non-sternoidal anti-inflammatory drugs. BMJ 327, 18–22.
259. Iveson TJ, Ryley NG, Kelly PM, Trowell JM, McGee JO, Chapman RW (1990) Diclofenac associated hepatitis. J Hepatol 10, 85–89.
260. Coulter DM, Clark DW, Savage RL (2003) Celecoxib, rofecoxib, and acute temporary visual impairment. BMJ 327, 1214–1215.
261. Garcia B, Ramaholimihaso F, Diebold MD, Cadiot G, Thiefin G (2001) Ischemic colitis in a patient taking meloxicam. Lancet 357, 690.
262. Tramer MR (2000) Aspirin, like all other drugs, is a poison. BMJ 321, 1170–1171.
263. McGovern MC, Glasgow JFT, Stewart MC (2001) Lesson of the week: Reye's syndrome and aspirin: lest we forget. BMJ 322, 1591–1592.
264. Clark I, Whitten R, Molyneux M, Taylor T (2001) Salicylates, nitric oxide, malaria, and Reye's syndrome. Lancet 357, 625–627.
265. Deltenre P, Berson A, Marcellin P, Degott C, Biour M, Pessayre D (1999) Mesalazine (5-aminosalicylic acid) induced chronic hepatitis. Gut 44, 886–888.
266. Popoola J, Muller AF, Pollock L, O'Donnell P, Carmichel P, Stevens P (1998) Late onset interstitial nephritis associated with mesalazine treatment. BMJ 317, 795–797.
267. Adhiyaman V, Vaishnavi A, Froese S (2001) Drug points: hypersensitivity reaction to balsalazide. BMJ 323, 489.
268. Lau G, Kwan C, Chong SM (2001) The 3-week sulphasalazine syndrome strikes again. Forensic Sci Int 122, 79–84.
269. Lievre M (2002) Alosetron for irritable bowel syndrome. BMJ 325, 555–556.
270. Behan WMH, Madigan M, Clark BJ, Goldberg J, McLellan DR (2000) Muscle changes in the neuroleptic malignant syndrome. J Clin Pathol 53, 223–227.

271. Hennessy S, Bilker WB, Knauss JS, et al. (2002) Cardiac arrest and ventricular arrhythmia in patients taking antipsychotic drugs: cohort study using administrative data. BMJ 325, 1070–1074.
272. Davies SJC, Cooke LB, Moore AG, Potokar J (2002) Discontinuation of thioridazine in patients with learning disabilities: balancing cardiovascular toxicity with adverse consequences of changing drugs. BMJ 324, 1519–1521.
273. Coulter DM, Bate A, Meyboom RHB, Lindquist M, Edwards IR (2001) Antipsychotic drugs and heart muscle disorder in international pharmacovigilance: data mining study. BMJ 322, 1207–1209.
274. Kilian JG, Kerr K, Lawrence C, Celermajer DS (1999) Myocarditis and cardiomyopathy associated with clozapine. Lancet 354, 1841–1845.
275. Krentz AJ (2001) Pseudopheochromocytoma syndrome associated with clozapine. BMJ 322, 1213.
276. Karsenti D, Blanc P, Bacq Y, Metman EH (1999) Hepatotoxicity associated with zolpidem treatment. BMJ 318, 1179.
277. Waise A, Fisken RA (2001) Unsuspected nephrogenic diabetes insipidus. BMJ 323, 96–97.
278. Koro CE, Fedder DO, L'Italien GJ, Weiss SS, Magder LS, Kreyenbuhl J, et al. (2002) Assessment of independent effect of olanzapine and risperidone on risk of diabetes among patients with schizophrenia: population based nested case-control study. BMJ 325, 243–247.
279. Ramchandani P (2004) Treatment of major depressive disorder in children and adolescents. BMJ 328, 3–4.
280. Jureidini JN, Doecke CJ, Mansfield PR, Haby MM, Menkes DB, Tonkin AL (2004) Efficacy and safety of antidepressants for children and adolescents. BMJ 328, 879–883.
281. Keeley PW, Foster G, Whitelaw L (2000) Hear my song: auditory hallucinations with tramadol hydrochloride. BMJ 321, 1608.
282. de Camargo OAK, Bode H (1999) Agranulocytosis associated with lamotrigine. BMJ 318, 1179.
283. Makin AJ, Fitt S, Williams R, Duncan JS (1995) Fulminant hepatic failure induced by lamotrigine. BMJ 311, 292.
284. Hadjikoutis S, Picersgill TP, Smith PEM (2003) Weight loss associated with levetiracetam. BMJ 327, 905.
285. Morgan DR, Trimble M, McVeigh GE (2000) Atrial fibrillation associated with sumatriptan. BMJ 321, 275.
286. Christopoulos S, Szilagyi A, Kahn SR (2001) Saint-Anthony's Fire. Lancet 358, 1694.
287. Paez de la Torre E, Lasic-Toccalino G, Mercado-Diez F, Torres-Calloni CM, Balcarce-Bautista PE (2003) Multifocal brain hemorrhage associated with migraine and medication abuse [in Spanish]. Rev Neurol 37, 840–842.
288. Ratnayaka BDM, Dhaliwal H, Watkin S (2001) Drug points: Neonatal convulsions after withdrawal of baclofen. BMJ 323, 85.
289. Drake AJ, Howells RJ, Shield JPH, Prendiville A, Crowne EC (2002) Symptomatic adrenal insufficiency presenting with hypogylcemia in children with asthma receiving high dose inhaled fluticasone propionate. BMJ 324, 1081–1083.

290. Bond DW, Charlton CPJ (2001) Benign intracranial hypertension secondary to nasal fluticasone propionate. BMJ 322, 897.
291. Churg A, Churg J (1998) Steroids and Churg-Strauss syndrome. Lancet 352, 32–33.
292. Lau G (1996) Accidental intraventricular vincristine administration: an avoidable iatrogenic death. Med Sci Law 36, 263–265.
293. Dettmeyer R, Driever F, Becker A, Wiestler OD, Madea B (2001) Fatal myeloencephalopathy due to accidental intrathecal vincristine administration: a report of two cases. Forensic Sci Int 122, 60–64.
294. Dyer C (1993) Manslaughter verdict quashed on junior doctors. BMJ 306, 1432–1433.
295. Yim YS, Mahoney DH, Oshman DG (1991) Hemiparesis and ischemic changes of the white matter after intrathecal therapy for children with acute lymphocytic leukemia. Cancer 67, 2058–2061.
296. Bernini JC, Fort DW, Gierner JC, Kane BJ, Chappell WB, Kamen BA (1995) Aminopylline for methotrexate-induced neurotoxicity. Lancet 345, 544–547.
297. Sosin M, Handa S (2003) Low dose methotrexate and bone marrow suppression. BMJ 326, 266–267.
298. Mayor S (2003) UK introduces measures to reduce errors with methotrexate. BMJ 327, 70.
299. Ibrahim NK, Sahin AA, Dubrow RA, Lynch PM, Boehnke-Michaud L, Valero V (2000) Colitis associated with docetaxel-based chemotherapy in patients with metastatic breast cancer. Lancet 355, 281–283.
300. Tulleken JE, Kooiman CGHM, van der Werf TS, Zijlstra JG, de Vries EGE (1997) Constrictive pericarditis after high-dose chemotherapy. Lancet 350, 1601.
301. Ogawa Y, Murata Y, Nishioka A, Inomata T, Yoshida S (1998) Tamoxifen-induced fatty liver in patients with breast cancer. Lancet 351, 725.
302. Feldman AM, Lorell BH, Reis SE (2000) Trastuzumab in the treatment of metastatic breast cancer. Circulation 102, 272–274.
303. Ghura HS, Carmichel AJ, Bairstow D, Finney R (1999) Fatal erythroderma associated with pentostatin. BMJ 319, 549.
304. Holquist C, Phillips J (2003) Fatal medication errors associated with Temodar. Drug Topics 7, 42–43.
305. Mooren FC, Lerch MM, Ullerich H, Burger H, Domschke W (2000) Systemic granulomatous disease after intravesical BCG instillation. BMJ 320, 219.
306. Dataja V, de Bruijn K (1996) Hypersensitivity reactions associated with 5-hydroxytryptamine$_3$-receptor antagonists. Lancet 347, 584–585.
307. Gottlieb S (2002) Intravenous immunoglobuin increases risk of thrombotic events. BMJ 324, 1056.
308. Evangelou N, Littlewood T, Anslow P, Chapel H (2003) Transverse sinus thrombosis and IVIg treatment: a case report and discussion of risk-benefit assessment for immunoglobulin treatment. J Clin Pathol 56, 308–309.
309. Picton P, Chisholm M (1997) Aseptic meningitis associated with high dose immunoglobulin: case report. BMJ 315, 1203–1204.
310. Mitchell P (1997) Shock as troglitazone withdrawn in UK. BMJ 350, 1685.

311. Richardson CE, Williams DW, Kingham JGC (2002) Gabapentin induced cholestasis. BMJ 325, 635.
312. Wolpert HA, Faradji RN, Bonner-Weir S, Lipes MA (2002) Metabolic decompensation in pump user due to lispro insulin precipitation. BMJ 324, 1253.
313. Josefson D (2000) Women taking combination HRT are at greater risk of breast cancer. BMJ 320, 333.
314. McPherson K (2004) Where are we now with hormone replacement therapy? BMJ 328, 357–358.
315. Minelli C, Abrams KR, Sutton AJ, Cooper NJ (2004) Benefits and harms associated with hormone replacement therapy: clinical decision analysis. BMJ 328, 371–376.
316. Burgermeister J (2003) Head of German medicines body likens HRT to thalidomide. BMJ 327, 767.
317. Mayor S (2003) Hormone treatment increases breast cancer risk, study shows. BMJ 327, 359.
318. Spurgeon D (2003) Long term use of combined HRT doubles cancer risk. BMJ 327, 9.
319. Josefson D (2001) Heart association advises against HRT for heart protection. BMJ 323, 252.
320. Gottlieb S (2001) Oestrogen replacement increases cancer risk, study shows. BMJ 322, 756.
321. Drife JO (2001) The third generation pill controversy ("continued"). BMJ 323, 119–120.
322. van Grootheest K, Vrieling T (2003) Thromboembolism associated with the new contraceptive Yasmin. BMJ 326, 257.
323. Hintz RL (2004) Growth hormone: uses and abuses. BMJ 328, 907–908.
324. Rankin AC (1997) Non-sedating anti-histamines and cardiac arrhythmic. BMJ 350, 1115–1116.
325. Yap YG, Camm J (2000) Risk of torsades de pointes with non-cardiac drugs. BMJ 320, 1158–1159.
326. Gitler B, Berger LS, Buffa SD (1994) Torsades de pointes induced by erythromycin. Chest 105, 368–372.
327. White NJ, Looareesuwan S, Warrell DA (1983) Quinine and quinidine: a comparison of EKG effects during the treatment of malaria. J Cardiovasc Pharmcol 5, 173–175.
328. Nosten F, ter Kuile FO, Luxemburger C, et al. (1993) Cardiac effects of antimalarial treatment with halfantrine. Lancet 341, 1054–1056.
329. Honig PK, Wortham DC, Zamani K, Conner DP, Mulin JC, Cantilena LR (1993) Terfenadine-ketoconazole interaction. Pharmacokinetic and electrocardiographic consequences. JAMA 269, 1513–1518.
330. Gilad R, Lampl Y, Eshel Y, Sadeh M (2002) Tonic-clonic seizures in patients taking sildenafil. BMJ 325, 869.
331. Moreira SG, Brannigan RE Jr, Spitz A, Orejuela FJ, Lipshultz LI, Kim ED (2000) Side-effect profile of sildenafil citrate (Viagra) in clinical practice. Urology 56, 474–476.
332. Josefson D (1998) FDA warns about heartburn drug. BMJ 317, 101.

333. Gottlieb S (2000) FDA tells doctors to use heartburn drug as last resort. BMJ 320, 336.
334. Mitra M, Chang B, James T (2001) Exacerbation of angina associated with latanoprost. BMJ 323, 783.
335. Loewe C, Dragovic LJ (1998) Acute coronary artery thrombosis in a postpartum woman receiving bromocriptine. Am J Forensic Med Pathol 19, 258–260.
336. Ault A (1997) Anti-obesity drugs recalled from global market. BMJ 350, 867.
337. Centres for Disease Control (1997) Cardiac valvulopathy associated with exposure to fenfluramine or dexfenfluramine: US Department of Health and Human Services Interim Public Health Recommendations, November 1997. MMWR 46, 1061–1066.
338. Shively BK, Roldan CA, Gill EA, Najarian T, Loar SB (1999) Prevalence and determinants of valvulopath in patients treated with dexfenfluramine. Circulation 23, 2161–2167.
339. Rich S, Rubin L, Walker AM, Schneeweiss S, Abenhaim L (2000) Anorexigens and pulmonary hypertension in the United States: results from the surveillance of North American pulmonary hypertension. Chest 117, 870–874.
340. Gross SB, Lepor NE (2000) Anorexigen-related cardiopulmonary toxicity. Rev Cardiovasc Med 1, 80–89.
341. Tomita T, Zhao Q (2002) Autopsy findings of heart and lungs in a patient with primary pulmonary hypertension associated with use of fenfluramine and phentermine. Chest 121, 649–652.
342. Lau G, Chan CL (2002) Massive hepatocellular necrosis: was it caused by orlistat? Med Sci Law 42, 309–312.
343. Christidis C, Mal F, Gayet B, Guettier C (2000) Hepatotoxicity caused by orlistat [English translation of the original French article]. Gastroenterol Clin Biol 24, 374.
344. Persson M, Vitols S, Yue QY (2000) Orlistat associated with hypertension. BMJ 321, 87.
345. Mewasingh L, Aylett S, Kirkham F, Stanhope R (2000) Hyponatremia associated with lamotrigine in cranial diabetes insipidus. Lancet 356, 656.
346. Seed BO, Beaumont D, Handley GH, Weaver JU (2002) Severe hyponatremia: investigation and management in a district general hospital. J Clin Pathol 55, 893–896.
347. Crook M (2002) The investigation and management of severe hyponatremia. J Clin Pathol 55, 883.
348. Svensson M, Gustafsson F, Galatius Soren, Hildebrandt PR, Atar D (2003) Hyperpotassemia and impaired renal function in patients taking spironolactone for congestive heart failure: retrospective study. BMJ 327, 1141–1142.
349. Peter R, Mishra V, Fraser WD (2004) Severe hypocalcemia after being given intravenous biphosphonate. BMJ 328, 335–336.
350. Escalante CP, Weiser MA, Finkel K (1997) Hyperphosphatemia associated with phosphorus-containing laxatives in a patient with chronic renal insufficiency. South Med J 90, 240–242.
351. Nir-Paz R, Cohen R, Haviv YS (1999) Acute hyperphosphatemia caused by sodium phosphate enema in a patient with liver dysfunction and chronic renal failure. Ren Fail 21, 541–544.

352. Chen X, Huang G (1995) Autopsy case report of a rare case of acute iatrogenic water intoxication with a review of the literature. Forensic Sci Int 76, 27–34.
353. McCarron MO, Wright GD, Roberts SD (1995) Water intoxication after low dose cyclophosphamide. BMJ 311, 292.
354. Noakes TD (2003) Overconsumption of fluids by atheletes. BMJ 327, 113–114.
355. Merlo J, Liedholm H, Lindblad U, Bjorck-Linne A, Falt J, Lindberg G, et al. (2001) Prescription with potential drug interactions dispensed at Swedish pharmacies in January 1999: cross sectional study. BMJ 323, 427–428.
356. Larkin M (1998) Warfarin and paracetamol do not always mix. BMJ 351, 729.
357. Ellison J, Thomson AJ, Greer IA (2000) Apparent interaction between warfarin and levonorgestrel used for emergency contraception. BMJ 321, 1382.
358. Adachi Y, Yokoyama Y, Nanno T, Yamamoto T (1995) Potentiation of warfarin by interferon. Lancet 311, 292.
359. Devaraj A, O'Beirne JP, Veasey R, Dunk AA (2002) Interaction between warfarin and topical miconazole cream. BMJ 325, 77.
360. Oden A, Fahlen M (2002) Oral anticoagulation and risk of death: a medical record linkage study. BMJ 325, 1073–1075.
361. Tweddle DA, Windebank KP, Hewson QC, Yule SM (1999) Cyclosporin neurotoxicity after chemotherapy. BMJ 318, 1113.
362. Horton RC, Bosner RS (1995) Interaction between cyclosporin and fluoxetine. BMJ 311, 422.
363. Verdejo A, de Cos MA, Zubimendi JA (2000) Probable interaction between cyclosporin A and low dose ticlopidine. BMJ 320, 1037.
364. Chartan F (2001) Bayer decides to withdraw cholesterol lowering drug. BMJ 323, 359.
365. Lane R, Phillips M (2003) Rhabdomyolysis. BMJ 327, 115.
366. Longhurst HJ, Pinching AJ (2001) Pancreatitis associated with hydroxyurea in combination with didanosine. BMJ 322, 81.
367. Johnstone SL, Unsworth J, Gompels MM (2003) Adrenaline given outside the context of life-threatening allergic reactions. BMJ 326, 589–590.
368. Nicholson KEA, Rogers JEG (1995) Cocaine and adrenaline paste: a fatal combination? BMJ 311, 250–251.
369. Heeringa M, Zweers P, de Man RA, de Groot H (2000) Anaphylactic-like reaction associated with oral budesonide. BMJ 321, 927.
370. Corominas N, Mane JM, Codina C, Paz MA, Ribas J (1992) Hydrocortisone anaphylaxis: a new case report. Pharm Weekbl Sci 14, 93–94.
371. Dajani BM, Sliman NA, Shubair KS, Hamzeh YS (1981) Bronchospasm caused by intravenous hydrocortisone sodium succinate (Solu-Cortef) in aspirin-sensitive asthmatics. J Allergy Clin Immunol 68, 201–204.
372. Dasgupta A (2003) Review of abnormal laboratory test results and toxic effects due to use of herbal medicines. Am J Clin Pathol 120, 127–137.
373. Josefson D (2002) St. John's wort interferes with chemotherapy, study shows. BMJ 325, 460.
374. Ruschitzka F, Meier PJ, Turina M, Luscher TF, Noll G (2000) Acute heart transplant rejection due to Saint John's wort. Lancet 355, 548–549.

375. Larkin M (2002) Safe use of herbal products before surgery proposed. Lancet 358, 128.
376. Ang-Lee MK, Moss J, Yuan CS (2001) Herbal medicines and perioperative care. JAMA 286, 208–216.
377. Ernst E (2003) Herbal medicines put into context. BMJ 327, 881–882.
378. Schulze J, Raasch W, Siegers CP (2003) Toxicity of kava pyrones, drug safety and precautions—a case study. Phytomedicine 10, Suppl 4, S68–S73.
379. Escher M, Desmeules J (2001) Hepatitis associated with kava, a herbal remedy for anxiety. BMJ 322, 139.
380. Vale S (1998) Subarachnoid hemorrhage associated with Ginkgo biloba. Lancet 352, 36.
381. Chung KF, Dent G, McCusker M, Page CP, Barnes PJ (1987) Effect of a ginkolide mixture (BN 52063) in antagonising skin and platelet responses to platelet activating factor in man. Lancet 1 (8527), 248–251.
382. Fugh-Berman A, Ernst E (2001) Herb-drug interactions: review and assessment of report reliability. Br J Clin Pharmcol 52, 587–595.
383. Larkin M (1999) Surgery patients at risk for herb-anesthesia interactions. Lancet 354, 1362.
384. Gould M (2001) Patients warned of dangers of Chinese medicines. BMJ 323, 770.
385. Adachi M, Saito H, Kobayashi H, et al. (2003) Hepatic injury in 12 patients taking the herbal weight loss aids "Chaso" or "Onshido." Ann Intern Med 139, 488–492.
386. Anonymous (2003) Results of the investigation into China-made slimming products classified as health foods (non-approved medicine) [English translation]. Pharmaceutical and Food Safety Bureau, Ministry of Health, Welfare and Labour, Japan. Available at: http://www.mhlw.go.jp/houdou/2003/02/h0212–1.html; Internet; accessed September 23, 2004.
387. Lau G, Lo DST, Yao YJ, Leong HT, Chan CL, Chu SS (2004) A fatal case of hepatic failure possibly induced by nitrosofenfluramine: a case report. Med Sci Law 44, 252–263.
388. Eisenberg DM, Davis RB, Ettner SL, et al. (1998) Trends in alternative medicine use in the United States, 1990–1997: results of a follow-up national survey. JAMA 280,1569–1575.
389. Hainer MI, Tsai N, Komura ST, Chiu CL (2000) Fatal hepatorenal failure associated with hydrazine sulfate. Ann Intern Med 133, 877–880.
390. Pumphrey RSH, Roberts ISD (2000) Postmortem findings after fatal anaphylactic reactions. J Clin Pathol 53, 273–276.
391. Horn KD, Halsey JF, Zumwalt RE (2004) Utilization of serum typtase and immunoglobulin E assay in the postmortem diagnosis of anaphylaxis. Am J Forensic Med Pathol 25, 37–43.
392. Aronson JK, Robin RE (2003) Joining the DoTS: new approach to classifying adverse drug reactions. BMJ 327, 1222–1225.
393. Riedl MA, Casillas AM (2003) Adverse drug reactions: types and treatment options. Am Fam Phys 68, 1781–1790.

394. Kanjanarat P, Wionterstein AG, Johns TE, Hatton RC, Gonzalez-Rothi R, Segal R (2003) Nature of preventable adverse drug events in hospitals: a literature review. Am J Health Syst Pharm 60, 1750–1759.
395. Mittmann N, Knowles SR, Gomez M, Fish JS, Cartotoo R, Shear NH (2004) Evaluation of the extent of under-reporting of serious adverse drug reactions: the case of toxic epidermal necrolysis. Drug Saf 27, 477–487.
396. Runciman WB, Roughead EE, Semple SJ, Adams RJ (2003) Adverse drug events and medication errors in Australia. Int J Qual Health Care 15, Suppl 1, S49–S59.
397. Larkin M (2001) Radiation overdose common in CT scans of children. Lancet 357, 289.
398. Brenner DJ, Elliston CD, Hall EJ, Berdon WE (2001) Estimated risks of radiation-induced fatal cancer from pediatric CT. AJR 176, 289–296.
399. Paterson A, Frush DP, Donnelly LF (2001) Helical CT of the body. Are settings adjusted for pediatric patients? AJR 176, 297–301.
400. Spurgeon D (2001) "Flying" vacuum cleaners are among hazards in MRI suites. BMJ 323, 357.
401. Chaljub G, Kramer LA, Johnson RF III, Johnson RF Jr, Singh H, Crow WN (2001) Projectile cylinder accidents resulting from the presence of ferromagnetic nitrous oxide or oxygen tanks in the MR suite. AJR 177, 27–30.
402. Klucznik RP, Carier DA, Pyka R, Haid RW (1993) Placement of a feromagnetic intracerebral aneurysm clip in a magnetic field with a fatal outcome. Radiology 187, 855–856.
403. Schenck JF (2000) Safety of strong, static magnetic fields. J Magn Reson Imaging 12, 2–19.
404. Rutqvist LE, Lax I, Fornander T, Johansson H (1992) Cardiovascular mortality in a randomised trial of adjuvant radiation therapy versus surgery alone in primary breast cancer. Int J Radiat Oncol Biol Phys 22, 887–896.
405. Gyenes G, Fornander T, Carlens P, Glas U, Rutqvist L (1996) Myocardial damage in breast cancer patients treated with adjuvant radiotherapy: a prospective study. Int J Radiat Oncol Biol Phys 36, 899–905.
406. Kunkler I (2000) Adjuvant irradiation for breast cancer. BMJ 320, 1485–1486.
407. Santos RS, Raftopoulos Y, Keenan RJ, Halal A, Maley RH, Landreneau RJ (2004) Bronchoscopic palliation of primary lung cancer: single or multimodality therapy? Surg Endosc 18, 931–936.
408. Lo TC, Beamis JF Jr, Villanueva AG, Gray AW Jr, Wu TR (2001) Intraluminal brachytherapy for malignant endobronchial tumours: an update on low-dose rate versus high-dose rate radiation therapy. Clin Lung Cancer 3, 65–68.
409. Bedwinek J, Petty A, Bruton C, Sofield J, Lee L (1991) The use of high dose rate endobronchial brachytherapy to palliate symptomatic endobronchial recurrence of previously irradiated bronchogenic carcinoma. Int J Radiat Oncol Biol Phys 22, 23–30.
410. Spieser BL, Spratling L (1993) Remote afterloading brachytherapy for the local control of endobronchial carcinoma. Int J Radiat Oncol Biol Phys 25, 379–387.

411. Speiser BL, Spratling L (1993) Radiation bronchitis and stenosis secondary to high dose rate endobronchial irradiation. Int J Radiat Oncol Biol Phys 25, 389–397.
412. Pelissier-Alicot AL, Leonetti G, Champsaur P, Allain P, Mauras Y, Botta A (1999) Fatal poisoning due to intravasation after oral adminstration of barium sulfate for contrast radiography. Forensic Sci Int 106, 109–113.
413. Fineschi V, Monasterolo G, Rosi R, Turillazzi E (1999) Fatal anaphylactic shock during a fluorescein angiography. Forensic Sci Int 100, 137–142.
414. Oliveira DBG (1999) Prophylaxis against contrast-induced nephropathy. Lancet 353, 1638–1639.
415. McCullough PA, Wolyn R, Rocher LL, Vevin RN, O'Neill WW (1997) Acute renal failure after coronary intervention: incidence, risk factors, and relationship to mortality. Am J Med 103, 368–375.
416. Weisberg LS, Kurnik BR (1994) Risks of radiocontrast nephropathy in patients with and without diabetes mellitus. Kidney Int 45, 259–265.
417. Polacarz SV, Laing RW, Loomes R (1992) Thorotrast granuloma: an unexpected diagnosis. J Clin Pathol 45, 259–261.
418. Plent S, Shah S, Westmore GA (1990) Thorotrast granuloma: a renascence. J Laryngol Otol 104, 355–357.
419. Pournia S, de Andrade A, Barbosa J, et al. (1998) Fatal microcystin intoxication in hemodialysis unit in Caruaru, Brazil. Lancet 352, 21–26.
420. de Wolff FA, Berend K, van der Voet GB (2002) Subacute fatal aluminum poisoning in dialyzed patients: postmortem toxicological findings. Forensic Sci Int 128, 41–43.
421. Bosch X (2001) Baxter withdraws dialyser after 12 patients die. BMJ 323, 529.
422. Riley SG, Chess J, Donovan KL, Williams JD (2003) Spurious hyperglycemia and icodextrin in peritoneal dialysis fluid. BMJ 327, 608–609.
423. Offringa M (1998) Excess mortality after human albumin adminsitration in critically ill patients. BMJ 317, 223–224.
424. Schierhout G, Roberts I (1998) Fluid resuscitation with colloid or crystalloid solutions in critically ill patients: a systematic review of randomised trials. BMJ 316, 961–964.
425. Cochrane Injuries Group (1998) Cochrane Injuries Group albumin reviewers. BMJ 317, 235–240.
426. Barron ME, Wilkes MM, Navickis RJ (2004) A systematic review of the comparative safety of colloids. Arch Surg 139, 552–563.
427. Virchis E, Patel RK, Contrerras M, Navarrete C, Kaczmarski RS, Jan-Mohamed R (1997) Acute non-cardiogenic lung oedema after platelet transfusion. BMJ 314, 880–882.
428. Darby SC, Ewart DW, Giangrande PLF, et al. (1997) Mortality from liver cancer and liver disease in hemophilic men and boys in UK given blood products contaminated with hepatitis C. Lancet 350, 1425–1431.
429. Alexander FE (1997) Blood transfusion and risk of non-Hodgkin lymphoma. Lancet 250, 1414–1415.

430. Kasper C, Jones L, Fujita Y, Morgenstern GR, Scarffe JH, Chang J (1999) Splenic rupture in a patient with acute myeloid leukemia undergoing peripheral blood stem cell transplantation. Ann Hematol 78, 91–92.
431. Falzetti F, Aversa F, Minelli O, Tabilio A (1999) Spontaneous rupture of spleen during peripheral blood stem-cell moblisation in a healthy donor. Lancet 353, 555.
432. Fell JME, Reynolds AP, Meadows N, et al. (1996) Manganese toxicity in children receiving long-term parenteral nutrition. Lancet 347, 1218–1221.
433. Hearing SD (2004) Refeeding syndrome. BMJ 328, 909–910.
434. Crook MA, Hally V, Panteli JV (2001) The importance of the refeeding syndrome. Nutrition 17, 632–637.
435. Weinster RI, Krumdieck CL (1980) Death resulting from overzealous total parenteral nutrition: the refeeding syndrome revisted. Am J Clin Nutr 34, 393–399.
436. Cumming AD, Farquhar JR, Bouchier IAD (1987) Refeeding hypophosphatemia in anorexia nervosa and alcoholism. BMJ 295, 490–491.
437. Alhasso A, Bryden AA, Neilson D (2000) Lithium toxicity after urinary diversion with ileal conduit. BMJ 320, 1037.
438. Hicks K, Evans GB, Rogerson ME, Bass P (1998) Jejuno-ileal bypass, enteric hyperoxaluria, and oxalate nephrosis: a role for polarised light in the renal biopsy. J Clin Pathol 51, 700–702.
439. Saukko P, Knight B (2004) Knight's Forensic Pathology, 3rd ed., Arnold, London, pp 88–90.
440. Lau G (2002) Pulmonary hemorrhage caused by a perforated and possibly iatrogenic thoracic aortic aneurysm. Forensic Sci Int 126, 167–170.

Imaging Techniques in Forensic Pathology

13

Forensic Radiology

Tzipi Kahana, PhD and Jehuda Hiss, MD

Contents

Summary

Imaging techniques are powerful tools in forensic sciences. Medical examiners, forensic pathologists, and anthropologists are required to interpret findings from imaging studies to further medicolegal investigations. Often, the forensic investigator calls on the radiologist, whose expertise might prove invaluable in forensic consultations. Radiological studies are instrumental in medicolegal investigations involving the location of foreign bodies within the body (i.e., bullets, gas emboli), documentation of fractures, and other mechanical injuries. Virtual autopsy (virtopsy), which involves a full-body computed tomography and magnetic resonance imaging examination to obtain two-dimensional and three-dimensional documentation has been proposed as an alternative to conventional autopsy in cases when the next of kin oppose

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the necroscopy and as a complementary tool for better visualization of postmortem findings. Antemortem and postmortem radiographic comparison is a common procedure in the identification of unknown human remains in most forensic facilities throughout the world. Computerized record keeping, available in most hospitals, expedites the retrieval of individual x-ray films, making radiographic comparison one of the most common techniques used by forensic pathologists and anthropologists to establish positive identification of unknown remains. The use of radiographs in routine and mass disaster identification has long been in effect, and its application in necroidentification is efficient, swift, and relatively easy. Age estimation of the living as well as of cadavers relies heavily on data regarding growth and developmental stages of the individual as obtained from dental and skeletal radiographs. Medical practitioners should be aware of the importance of storing radiographs for prolonged periods of time and of efficient record keeping methods because of various legal problems that might arise requiring the films for later interpretation or for their presentation in court.

Key Words: Forensic radiology; forensic anthropology; imaging techniques; identification; age estimation; mass disasters; nonaccidental injury; physical child abuse.

1. INTRODUCTION

The importance of imaging techniques in forensic medicine is widely recognized. Forensic anthropologists and odontologists routinely rely on the comparison of antemortem and postmortem radiographic plates to establish identity. The location of foreign bodies and gas emboli, and the documentation of fractures and other types of injuries make x-rays an integral element of most medicolegal procedures. Furthermore, radiographic studies of the body are crucial in early recognition of hazardous objects because exploding bullets that penetrated the body unactivated, undetonated explosive material, and retained sharp souvenirs (foreign bodies) can cause severe injuries to the forensic investigators if undetected before the autopsy. Necroscopic as well as clinical radiological examination plays a significant role in the diagnosis of nonaccidental injury in children and adults, in establishing medical negligence, and estimating biological age in disputed cases. Finally, there is an increasing demand for noninvasive techniques to replace the conventional autopsy in cases in which cultural and religious demands ban invasive postmortem procedures. The aim of this review is to describe the various uses of radiology within the medicolegal realm.

2. *Necroscopic Examinations*

Postmortem radiological examination is fairly common in most modern forensic facilities. The permanent nature of x-ray plates makes them available for reevaluation and reinterpretation as additional evidence accumulates regarding the case investigated. The objective and noninflammatory character of radiographic records makes them a valuable tool for presenting evidence in court. The stage at which radiology is implemented during autopsy will vary according to the circumstances surrounding a particular case. Generally, radiographs will be taken after the external examination and before the dissection, except in medicolegal investigation of bombings and charred bodies.

In natural death cases as well as in investigations of assumed medical malpractice, the implementation of various imaging techniques is the most adequate method for detecting pathological features such as pneumothorax, pneumoperitoneum, barotrauma injuries, and air embolisms. Moreover, when traumatic subarachnoid hemorrhage is suspected, vertebral angiography, if available, is recommended *(1)*. When the body to be examined is badly decomposed, for instance, in exhumed cadavers, it is highly recommended to conduct a full body radiographic study that might help visualize otherwise hidden injuries and pathological findings.

As a rule, in all gunshot wound cases it is strongly recommended that x-rays are taken. This includes instances where the bullet is known to be in the body as well as those in which it has allegedly exited (Fig. 1*[2]*). This relatively easy task can sometimes fail—a bullet can be overlooked even by experienced radiologists because of "professional blinkers phenomenon" *(3)*. In localizing bullets, it is important to remember that they might migrate; thus, the radiographic examination should include the whole body. The path of the bullet can be observed as a cloud of minute metallic particles detached from the projectile, the so-called "lead snowstorm" *(4)*, or by using more sophisticated imaging techniques such as computed tomography (CT *[5]*).

The type of bullet (high or low velocity) might be deduced by the pathologist through the different injuries detected on radiographs, whereas the determination of the type of weapon and the distance from the target should be left to the ballistics experts. Establishing the bullet's caliber from a radiograph should be avoided because angulation and distance from the beam can distort the image.

Radiography is useful not only to locate a projectile but also in determining whether metallic fragments are present within the body because even very small fragments of a bullet's jacket may suffice for a ballistic identification.

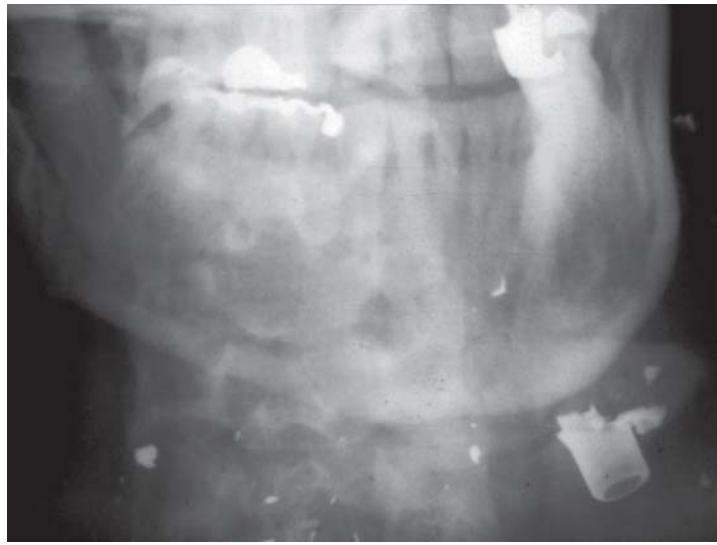


Fig. 1. Fragmented bullet within the head and neck areas of a gunshot wound victim. The attending physician at the hospital was puzzled by the presence of one entrance and one exit wound while the projectile appeared retained on radiographic examination, which during autopsy was discovered to be a metallic dental jacket on the second left maxillary molar.

The presence of "souvenirs" within the body can be revealed through radiographic examination of the body before autopsy. The precise location of a foreign body is better visualized in multiple radiographic planes, including antero-posterior and lateral ones.

In victims of bombings, the examination of radiographic plates before handling the body is imperative; one of the threats posed by the centrifugally expanding wave of the blast is the presence of undetonated components of the device, which can remain embedded within the body cavities of the victims (Fig. 2), placing the unsuspecting forensic pathologist in danger of detonating these parts by manipulating them during body handling and examination. Location and documentation of the shrapnel within the body is a key step in this type of criminal investigation because the identification of the terrorist cell involved in the act can be expedited since the kind of shrapnel added by the perpetrators acts as a signature to specific terrorist groups (Fig. 3A,B *[6]*). Occasionally, in victims of nautical sporting accidents, the injuring agent, for example, a propeller blade fragment, can be evinced on radiography, and thus retrieved for physical matching with a suspected boat.

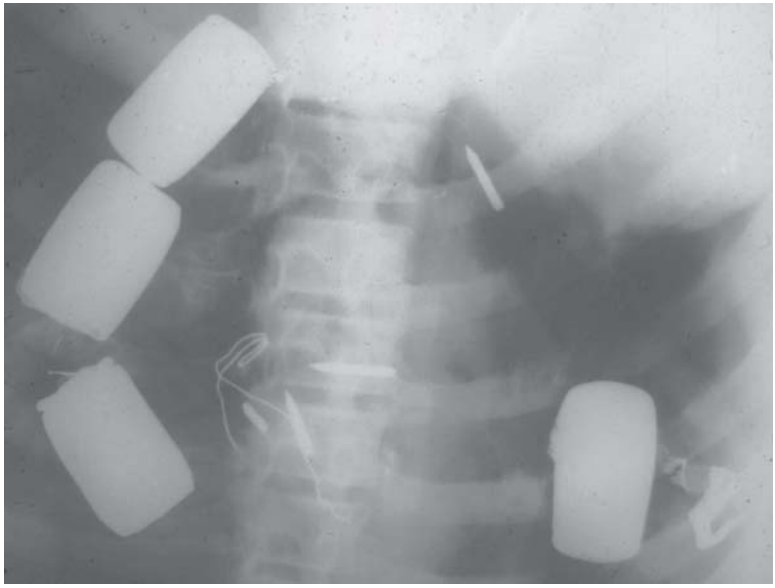
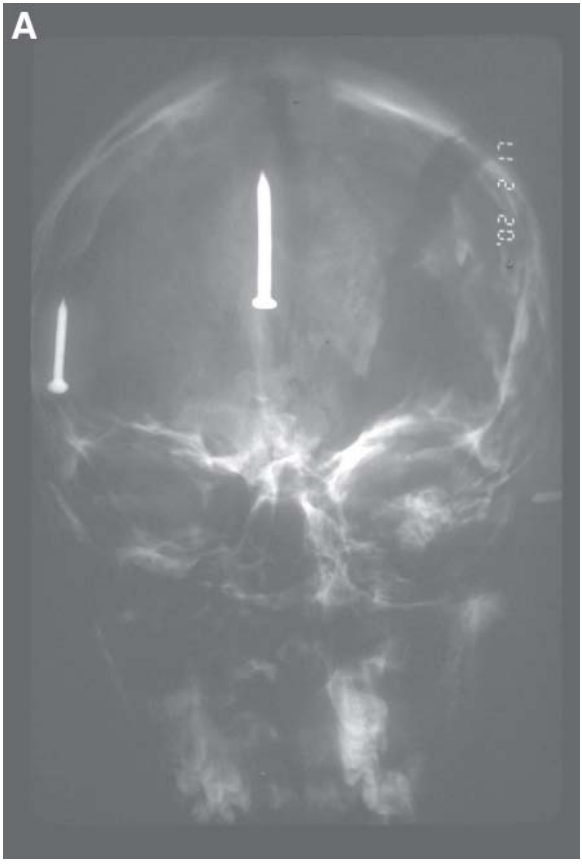


Fig. 2. Unexploded detonators embedded within the thoracic cavity of a victim of a suicidal bombing.

The estimation of age at death of unidentified victims also can be achieved through radiographic evaluation of epiphyseal closure when, for different reasons, an autopsy is not feasible. The poor visualization on x-ray of certain articular phases like the pubic symphysis makes radiographic age determination of the adult nonadvisable.

For certain cultures and religions conventional autopsy is stigmatized or even forbidden; various imaging techniques, like ultrasound, CT, and magnetic resonance imaging (MRI) allow conducting medicolegal investigations, sparing the feelings of the victim's next of kin.

During the 1990s, a variety of alternative noninvasive procedures were proposed as substitute for conventional autopsy. The concept of "virtopsy" (virtual autopsy) was born of the desire to overcome the obstacles posed by relatives of the deceased and to provide objective and indestructible documentation of postmortem evidence. The technique combines whole-body examination by CT and MRI to obtain two-dimensional and three-dimensional documentation. The advantages of virtopsy lie in the ability to "freeze" the findings at the moment of the investigation without causing damage and allowing the investigator to recapitulate the results later on, either for presentation in court, teaching, consultation through telemedicine, and/or quality con-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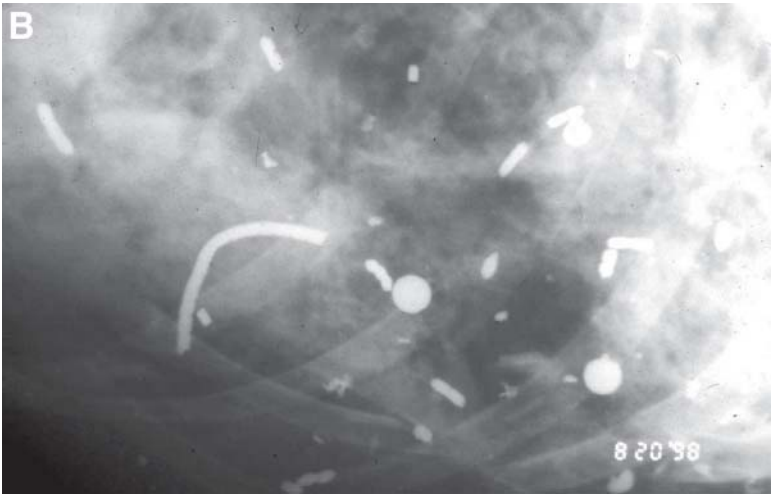
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trol *(7,8)*. Additional advantages of radiologic necropsy include safe and easy examination of infected cadavers minimizing the hazards to the practitioner. The importance of a full radiological examination of victims of suspected physical abuse is discussed later in Section 5.

3. Identification of Human Remains

The identification of human remains is one of the most essential aspects of forensic pathology. Unidentified human remains constitute approx 10% of the caseload of most forensic practitioners. This fraction includes skeletonized, decomposed, and burnt victims along with cases of extensive mechanical trauma to the face. During the 1990s, the socioeconomic changes in the European eastern block and the opening of frontiers in most European and American countries brought large waves of illegal foreign immigrants, some of whom became homeless and, when they died, often left unidentified bodies. Currently, there are no comprehensive statistics dealing with the number of unidentified cadavers and human remains within the European community and Middle East, but the numbers seem to be increasing.

There may be variations in the state of preservation of human remains caused by factors such as normal thanatological processes affecting the cadaver, the mechanism of death, or animal scavenging. The skeleton, or at least some of its components, usually offers resistance towards environmental, mechanical, and physical processes and hence nearly always can be examined radiographically. Furthermore, because radiography is a common diagnostic tool for a great variety of dental and medical conditions, it frequently is possible to obtain antemortem radiographs for positive identification *(9)*.

Personal identification of human remains is achieved when specific features detected on the cadaver match data recorded during the life of the individual. Radiographic identification can be accomplished when there is a lead as to the possible identity of the victim; usually, this is the result of comparing an anthropological profile (gender, age, stature, and ethnic affinity) with police or military “missing persons” reports. The correct radiographic identification of the deceased greatly depends on the similarity of the conditions in which the antemortem and postmortem radiographs were taken, that is, position and intensity *(10)*.

Fig. 3. (A) Nails used as additional shrapnel by homicidal bombers to increase the lethality of the explosive device. **(B)** Metallic spheres in the thoracic cavity encountered in explosive devices prepared by a different terrorist group.

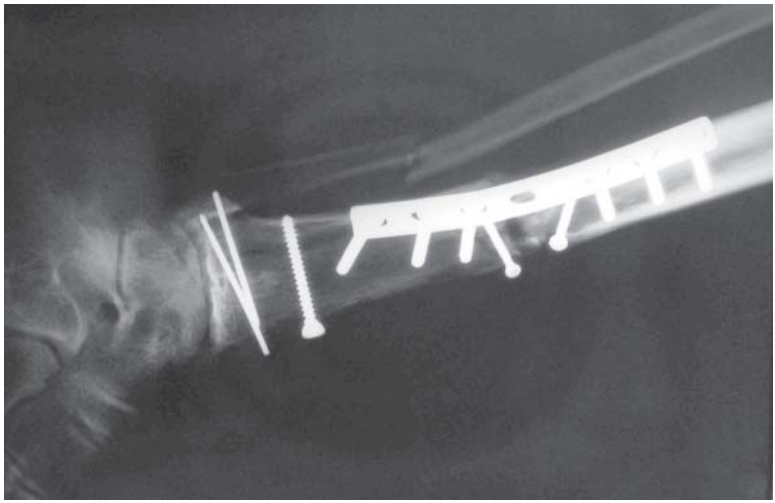

Fig. 4. Radiography of the right leg of an unidentified victim of a light plane crash. The presence of surgical scars and the consecutive radiographic study showing extensive orthopedic surgery were instrumental in the positive identification of the body.

Positive radiographic identification is accomplished by meticulous comparison of the details present on the film; however, unlike fingerprints there is no established minimum number of points of comparison that must be concordant to determine identity. The features depicted on radiographs must comply with two requirements in order to be of forensic identification value; on the one hand the feature has to be unique to each individual, and on the other hand it has to remain stable over time despite ongoing life processes and aging. Usually, one to four unique analogous features and no discrepancies are considered enough for a positive identification *(11)*.

Radiographic positive identification of unknown human remains is often attained by comparison of some markers present on the antemortem and postmortem plates, for example, signs of previous medical intervention such as old surgical or orthopedic procedures (Fig. 4) as well as prosthetic devices, evidence of healed trauma, normal anatomical variation like the variation and configuration of the frontal and paranasal sinuses *(12)*, osseous and vascular degenerative changes, congenital malformations, and certain slow growing neoplasms that might be evident within the remains *(13)*.

Bearing in mind that teeth are composed of the most resilient structures of human tissues, for example, enamel, and that the materials used in dental restorations are extremely resistant to destruction by chemical and physical

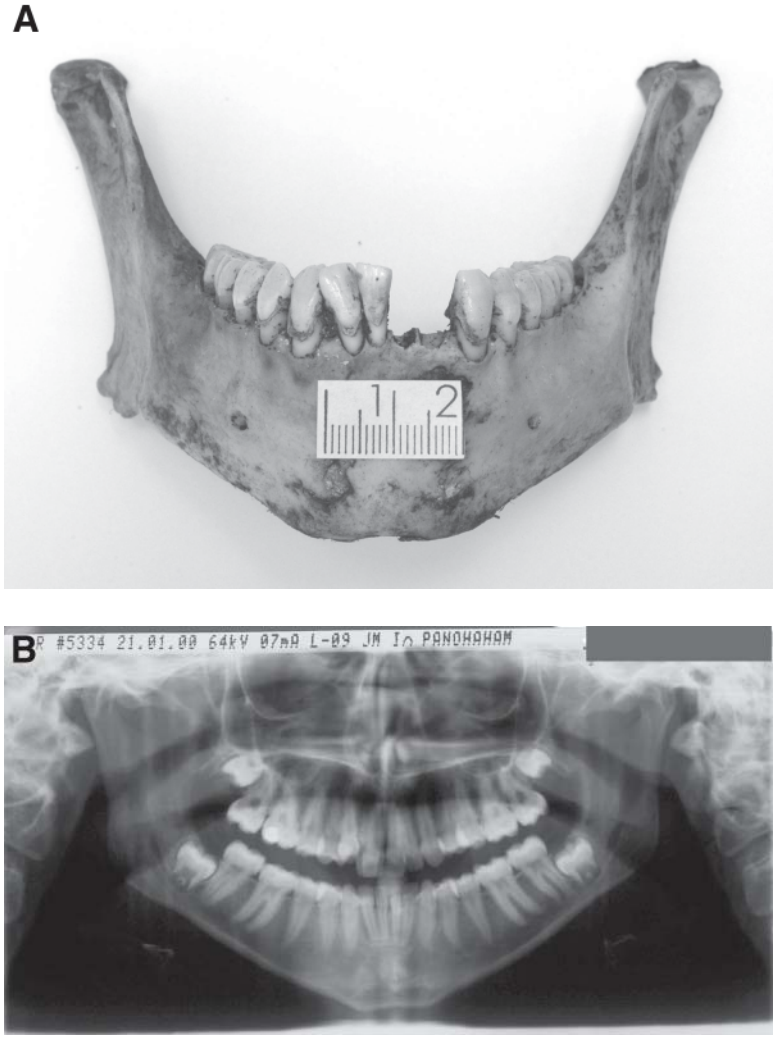

Fig. 5. (A) Human mandible encountered in the woods near the area where a 19-year-old woman had disappeared 2 years before. **(B)** Orthopantogram of the missing woman taken when she was 16 years old. Note the unerupted mandibular third molars. **(C)** Periapical radiograph of the left mandibular third molar. Note the position of the impacted tooth, which rules out the possibility of this being the mandible of the missing woman because during the time elapsed between the antemortem radiograph and her being missing, the tooth could have not rotated to the present position underneath the bulge of the second molar.

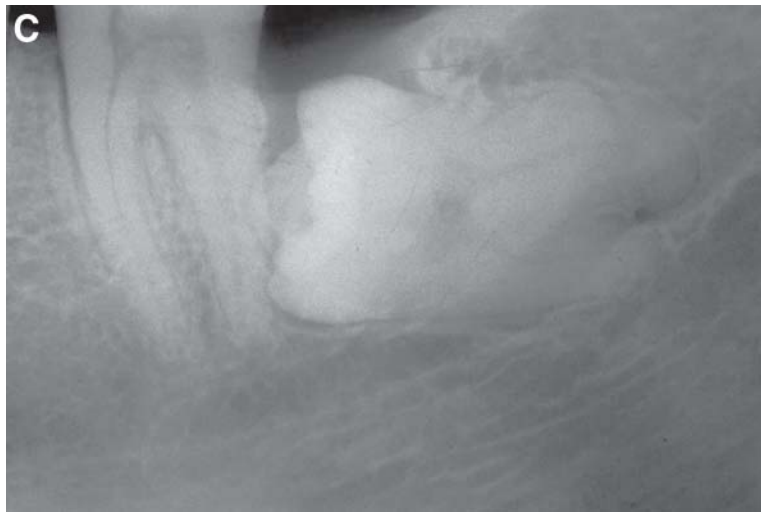

Fig. 5. *(Continued)*

agents, the innumerable combinations of missing teeth, carious lesions, restorations, and prostheses involving the surfaces of deciduous and permanent dentition, together with the normal morphological variation of crowns and roots, render dental identification the most useful and powerful tool. Most often, radiographic identification techniques are implemented in daily casework (Fig. 5A–C), as well as in mass disaster situations *(14)*. Panoramic radiographs, which enable the visualization of most structures of the jaws and related areas on a single film, have been advocated for mass screening, such as of military personnel *(15)*.

Manipulation of the radiographs is very common in modern roentgenography. With digitalization of all kinds of radiographic equipment and image-processing software becoming more easily available, it is not an unusual practice to enhance and correct the image, especially for clinical purposes. In the forensic setting, digital image processing is very useful. Contrast enhancement, brightness correction, and segmentation of images are all acceptable procedures to facilitate radiographic identification. However, any manipulations that distort radiologically visible structures by changing their angular relationship are inadmissible; the use of drawing tools which can retouch, accentuate, or fade out contours should be avoided *(16,17)*.

The potential value of comparison between antemortem and postmortem radiographs in forensic pathology is nowadays fully appreciated. Similar com-

parisons between antemortem and postmortem CT images can yield successful personal identification *(18)*. This type of comparison is becoming more feasible as CT equipment is growing to be more available to forensic facilities worldwide.

4. Age Estimation of the Living

Age determination of living individuals is crucial in many legal issues. In developing countries, where many a birth takes place in rural venues with deficient record keeping, when the suspicion of fraudulent registration arises—especially in light of the recent increasing illegal emigration from third-world countries and the lack of proper official documentation of these individuals—the forensic practitioner's expert opinion plays a key role in legal rulings.

In general, every country has an official age limit underneath which an individual will be considered a minor and will be granted certain leniency, for example, will be judged in juvenile court in cases of criminal charges, will not be enrolled into military service, or will be excluded from certain labors on the one hand, however, the individual who has not reached this official age limit will be denied certain financial and legal rights, such as the right to legal binding contracts, marry, the right to have sexual intercourse with an adult, and so on.

When the chronological age of an individual is questioned, the investigator must resort to the evaluation of the individual's biological age, that is the growth and developmental stage the individual has reached. The estimation of the biological age is obtained from combining clinical and radiological data on dental and osseous development.

The development of the deciduous and permanent dentition spans the infant and juvenile years. Dental age is estimated from the combined observation of the degree of mineralization of dental buds, the presence of individual teeth erupted, and the extent of root formation.

Skeletal age can be evaluated by the sequence of development and fusion of epiphyses of long bones and development of centers of ossification in small bones. The point in time of each epiphysis' fusion varies greatly according to their anatomy and physiology. The pace of growth and development differs between the sexes and between various ethnical groups, and the onset of the diverse age indicators is affected by genetic and environmental factors; thus, they should be taken into consideration. Atlases of the various stages have been developed for hands and knees *(19)*. Other radiological developmental techniques include the careful estimation of bone-by-bone development of the hand *(20)*.

The most accurate source of information for age estimation during the juvenile phase is the sequence of fusion of epiphyses and the unification of the three bones of the os coxa *(21)*. The standard deviation of the estimate in this stage is greater than the assessment based on the appearance of the centers of ossification during childhood and oscillates between 2 and 4 years, depending on the gender and ancestry of the individual. The correlation between chronological and dental age is stronger than that of chronological and skeletal age because dental development is less affected by adverse environmental conditions. For individuals in good health, skeletal age can be more than 1 year older or younger than chronological age.

The stage of fusion of the basilar synchondrosis (spheno-occipital fissure) has been regarded as a trustworthy indicator of biological age. A number of authors proffer that the synchondrosis remains open throughout childhood and adolescence and coalesces as the individual reaches adulthood, whereas others propose that fusion commences during the adolescent stage, concomitant with the eruption of the second permanent molars *(22)*. The stage of fusion of the basilar synchondrosis (spheno-occipital fissure) has been regarded as a trustworthy indicator of biological age. Albeit the assertions regarding the basilar synchondrosis as a good age indicator for the adolescence period, forensic investigators should be aware of the great variability in the time of closure of this trait in male individuals. Various ages of fusion of the spheno-occipital fissure have been reported by different researchers, from as early as 10 years old to as late as 25 years *(23,24)*. Age estimation of the living adult is extremely inaccurate; the correlation between degenerative processes of the various osseous elements, such as those evinced in radiographs of the vertebral column is low and can be affected by extraneous factors such as physical activity, nutrition, and pathological conditions. Furthermore, reliable osseous markers, such as pubic symphysis or sternal rib articulation, cannot be visualized in conventional radiographs. A CT scan of the clavicle has been suggested as a reliable age indicator for individuals younger than 21 to 25 years of age *(25)*.

5. *Nonaccidental Injury*

Radiological technology plays an important role in diagnosing nonaccidental injury (physical abuse) in children. In fact, more than 80% of all identified child abuse-related injuries in the United States are detected through medical imaging.

Although radiographs for the detection of fractures are normally not a requisite during autopsy because the bones themselves can be inspected and

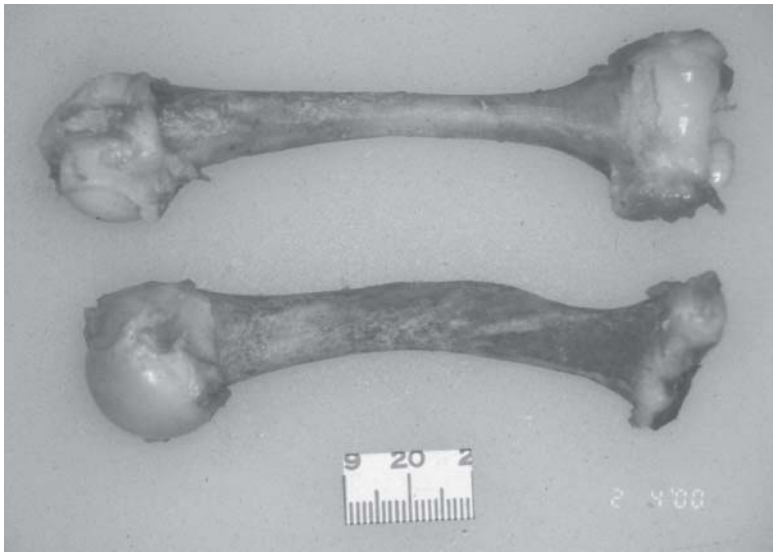

Fig. 6. Humeri of a 3-month-old baby who died of severe head injuries. Note the callus on the right humerus resulting from a spiral fracture sustained while he was 4 weeks old.

the fractures—new and healed ones—be well documented (Fig. 6), in cases of suspected nonaccidental injuries, a complete radiographic study is mandatory. This radiographic skeletal survey should include the entire axial and appendicular skeleton and not a single radiograph of the entire child (i.e., "babygram"), which is considered diagnostically inadequate *(26)*.

Some techniques such as skeletal scintigraphy are highly sensitive in the detection of rib, spinal, and diaphyseal fractures and have a low sensitivity for cranial fractures. This procedure should be considered as a supplemental examination in suspected cases of nonaccidental injuries *(27)*.

The mechanisms of trauma associated with the various types of fractures have been discussed in the relevant literature *(11,28,29)*; in this section, the authors emphasize only the most prominent radiological findings.

The most common fractures associated with inflicted injuries of children are diaphyseal, spiral-oblique, or transverse fractures (Fig. 7). Metaphyseal–epiphyseal fractures are less common. All these are considered diagnostic of nonaccidental injury because the forces necessary to produce such fractures cannot be generated from simple falls or other accidents. Caffey (1946) coined the term "bucket-handle" fracture to describe metaphyseal fractures of long bones, which are typical of abused children *(30)*.

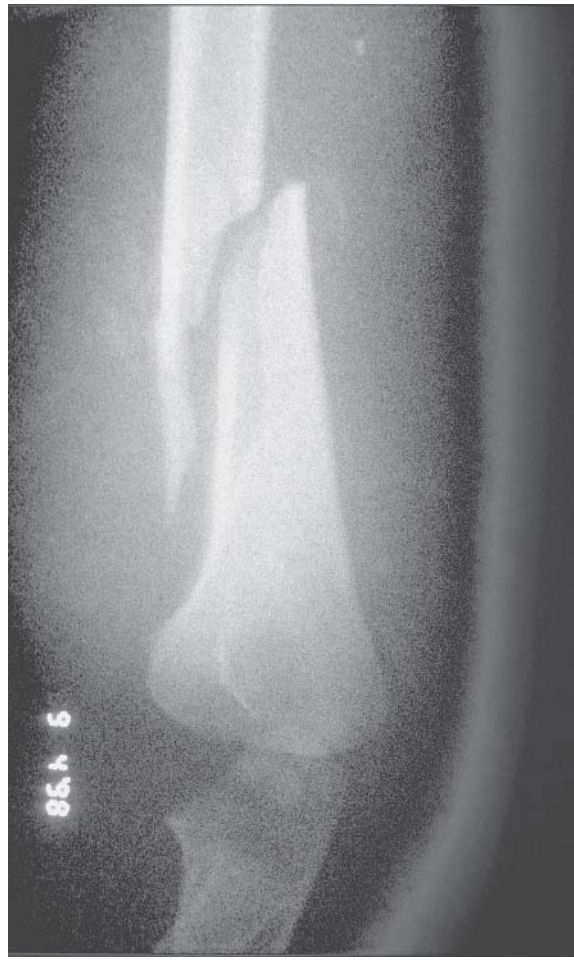

Fig. 7. Lateral view of an oblique fracture of the left humerus of a 20-month-old baby. This type of fracture is mostly consistent with twisting of the limb.

Another pathognomonic sign of abuse is the presence of multiple rib fractures (seen in 5–27% of physically abused children). These fractures are rarely seen in motor-vehicle accidents or after resuscitation attempts. Multiple rib fractures may be difficult to diagnose on radiographs in the acute setting and might be best detected with bone scanning *(31)*.

Accidental cranial fractures in infants usually are simple, linear, and unilateral, affecting the parietal bone, and do not branch or cross sutures. In general, falls from beds, sofas, diaper-changing tables, or stairs (commonly referred

to as "short falls") produce relatively minor trauma *(32)*. Abusive fractures are often complex, wide at presentation, multiple or depressed, and bilateral *(33)*. There are some descriptions in the literature of fatal "short falls," but the majority of experts concur that these are unlikely *(34)*.

Subdural hemorrhages are a common sequel to violent shaking of an infant. The relatively large, heavy, and poorly supported head predisposes an infant to violent acceleration and deceleration forces in the "whiplash shaken syndrome," causing disruption and bleeding of the bridging veins into the subdural space. The contemporary literature on the subject contends that these forces often are insufficient to cause permanent damage and that the mechanism includes some type of blunt injury to the head *(35)*. Diagnosis of the syndrome is made by CT scan *(26,30)* and MRI *(26,34,36)*. There is no universal consensus as to the best imaging procedure for detection of nonaccidental cranial injuries. Forensic radiologists suggest different techniques for specific head injuries. CT is recommended for detection of subarachnoid hemorrhages, whereas MRI is superior in revealing subdural hematomas, concussive injuries, and shear injuries. CT and MRI are equally efficient for demonstrating epidural hematomata, and for detection of fractures, CT is advocated *(32)*.

Age assessment of cranial injuries is rather imprecise. As a rule, cranial CT is considered both sensitive and specific in defining acute (recent up to several days old) extracerebral blood collections because fresh subdural blood collections are of high density on CT. The density gradually diminishes over the first week after the injury, and at that time, MRI is superior to CT in depicting subacute (a few weeks old) and chronic (more than 3 months old) extracerebral bleedings and deep cerebral injuries. Subacute, early chronic subdural blood produces a distinct, high signal intensity with T_1-weighted images (short echo time [TE] and short relaxation time [TR]). As subdural blood evolves, it manifests increasing signal intensity with T_2-weighted images (long TE and long TR *[37,38]*). It has been postulated that posttraumatic brain swelling can be detected on head CT as early as 1 hour and 17 minutes after the injury *(39)*.

6. *Conclusions*

Imaging techniques are a powerful tool in forensic sciences. Medical examiners and forensic anthropologists are less versed in the finer points of roentgenology than are radiologists; nevertheless, they are required to interpret findings from imaging studies to further medicolegal investigations. Often, the forensic investigator calls on the radiologist whose expertise might prove invaluable in forensic consultations.

Radiological investigation during autopsy is priceless not only in gunshot wound cases, but in all instances when the practitioner might be required to locate foreign bodies within the cadaver, like in suicidal bombing victims.

The implementation of other imaging techniques, such as MRI and CT, are strongly advocated for cases of suspected air embolisms and for abuse victims. In the last decade of the 20th century, investigators have suggested the use of "virtopsy" (virtual autopsy) as the best tool to be used in conjunction with conventional postmortem examination to reveal the greatest amount of details pertinent to the case at hand *(7,8)*.

The importance of complete radiological examination of postmortem as well as clinical cases of suspected abuse cannot be overestimated. Radiological evidence of skeletal trauma commonly is found in abused children aged 18 months and younger; the location, nature, and multifocal aspect of these injuries are considered specific for nonaccidental injuries. Radiological imaging plays a crucial role in evaluating craniospinal injury, and the implementation of CT and MRI is advised in all cases of suspected nonaccidental cranial injuries *(40,41)*.

In forensic anthropology and odontology, radiographic examination plays a key role in positive identification of unknown human remains. This often is attained by comparison of antemortem and postmortem radiographs. Some of the markers frequently collated in the plates are signs of medical intervention, normal anatomical variation, and evidence of healed trauma. There are numerous accounts of cranial, dental, and postcranial radiographic features useful for identification. The correct radiographic identification of the deceased depends greatly on the similarity between the antemortem and postmortem films. Positioning of the questioned anatomical specimens prior to radiograph is of paramount importance for comparison since the investigator strives to duplicate as closely as possible the antemortem object-film angulation.

Biological age estimation of living individuals, an undertaking that is becoming more common worldwide in most forensic anthropology practices, is mostly supported by radiographic evaluation of dental and skeletal maturation.

The importance of careful record keeping in medical facilities and private practices for as long as feasible cannot be overemphasized. In most countries, radiographs pertaining to inactive patient's files are stored at least for 5 years *(42)*. The radiographic information can be stored on a magnetic media when facing space constrains, thus allowing one to save data for 20 years *(43)*.

References

1. Knight B (1996) Forensic Pathology, 2nd ed. Arnold, London.
2. Hiss J, Kahana T (2002) Confusing exit gunshot wound. Two for the price of one. Int J Legal Med 116, 47–49.
3. Bajanowski T, Karger B, Brinkmann B (2001) Scratched pustule or gunshot wound? A medical odyssey. Int J Legal Med 114, 267–268.
4. DiMaio VJM (1999) Gunshot Wounds. Practical Aspects of Firearms, Ballistics, and Forensic Techniques, 2nd ed. CRC Press, Boca Raton.
5. Thali MJ, Schweitzer W, Yen K, et al. (2003) New horizons in forensic radiology. The 60-second "digital autopsy": Full body examination of a gunshot victim by multi-slice computed tomography. Am J Forensic Med Pathol 24, 22–27.
6. Hiss J, Kahana T (2000) Trauma and identification of victims of suicidal terrorism in Israel. Mil Med 165, 889–893.
7. Thali MJ, Vock P (2003) Role and techniques in forensic imaging. In Payne-James J, Busuttil A, Smock W, eds., Forensic Medicine: Clinical and Pathological Aspects. Greenwich Medical Media, London, pp. 731–746.
8. Thali MJ, Yen K, Vock P, et al. (2003) Image-guided virtual autopsy findings of gunshot victims performed with multi-slice computed tomography and magnetic resonance imaging and subsequent correlation between radiology and autopsy findings. Forensic Sci Int, 138, 8–16.
9. Kahana T, Hiss J (1999) Forensic radiology. Br J Radiol 72, 129–133.
10. Kahana T, Hiss J (1997) Identification of human remains: Forensic radiology. J Clin Forensic Med 4, 7–15.
11. Di Maio DJ, Di Maio V J (1989) Forensic Pathology. Elsevier, New York.
12. Ribeiro F de A (2000) Standardized measurements of radiographic films of the frontal sinuses: an aid to identifying unknown persons. Ear Nose Throat J 26, 32–33.
13. Kahana T, Goldin L, Hiss J (2002) Personal identification based on radiographic vertebral features. Am J Forensic Med Pathol 23, 36–41.
14. Hiss J, Freund M, Motro U, Kahana T (2002) The medicolegal investigation of the El Aqsah Intifada. Isr Med Assoc J 4, 549–553.
15. Kahana T, Hiss J (2002) Forensic odontology in Israel. Alpha Omegan 95, 47–48.
16. Du Chesne A, Benthaus S, Brinkmann B (1999) Manipulated radiographic material—capability and risk for the forensic consultant? Int J Legal Med 112, 329–332.
17. Richardson ML, Frank MS, Stern EJ (1999) Digital image manipulation: what constitutes acceptable alteration of a radiologic image? Am J Roentgenol 164, 228–229.
18. Kahana T, Goldstein S, Kugel C, Hiss J (2002) Identification of human remains through comparison of computerized tomography and radiographic plates. J Forensic Ident 52, 151–158.
19. Greulich WE, Pyle SI (1966) Radiographic atlas of skeletal development of the hand and wrist. Stanford University Press, Stanford.
20. Tanner JM, Whitehouse RH, Cameron N, Marshal WA, Healy NJR, Goldshetein H (1991) Assessment of skeletal maturity and prediction of adult height (TW2 Method), 2nd edition, Academic Press, London.
21. Stewart TD (1979) Essentials in Forensic Anthropology. Charles C Thomas, Springfield, IL.

22. Kahana T, Birkby WH, Goldin L, Hiss J (2003) Estimation of age in adolescents—the basilar synchondrosis. J Forensic Sci 48, 504–508.
23. Okamoto K, Ito J, Tokiguchi S, Furusawa T (1996) High-resolution CT findings in the development of the sphenooccipital synchondrosis. Am J Neuroradiol 17, 117–120.
24. Schmeling A, Olze A, Reisinger W, Rosing FW, Geserick G (2003) Forensic age diagnostics of living individuals in criminal proceedings. Homo 54, 162–169.
25. Brown T (1995) Radiography's role in detecting child abuse. Radiol Technol 66, 389–390.
26. Merten DF, Carpenter BLM (1990) Radiologic imaging of inflicted injury in the child abuse syndrome. Orthop Clin North Am 37, 815–837.
27. Mandelstam SA, Cook D, Fitzgerald M, Ditchfield MR (2003) Complementary use of radiological skeletal survey and bone scintigraphy in detection of bony injuries in suspected child abuse. Arch Dis Child 88, 387–390.
28. Lonergan GB, Baker AM, Morey MK, Boos SC (2003) From the archives of the AFIP. Child abuse: radiologic-pathologic correlation. Radiographics 23, 811–845.
29. Carty H (1997) Non-accidental injury: a review of the radiology. Eur Radiol 7, 1365–1376.
30. Hobbs CJ, Hanks HGI, Wynne JM (1993) Child Abuse and Neglect. A Clinical Handbook. Churchill Livingston, Edinburgh.
31. Cramer EC (1996) Orthopedic aspects of child abuse. Orthop Clin North Am 43, 1035–1051.
32. Brogdon BG (1998) Forensic Radiology. CRC Press, Boca Raton, FL.
33. Hiss J, Kahana T (1995) The medicolegal implications of bilateral cranial fractures in infants. J Trauma 38, 1–5.
34. Reiber GD (1993) Fatal falls in childhood. How far must children fall to sustain fatal head injury? Report of cases and review of the literature. Am J Forensic Med Pathol 14, 201–207.
35. Blumenthal I (2002) Shaken baby syndrome. Postgrad Med J 78, 732–735.
36. Rubin DM, Christian CW, Bilaniuk LT, Zazyczny KA, Durbin DR (2003) Occult head injury in high-risk abused children. Pediatrics 211, 1382–1386.
37. Sato Y, Yuh WT, Smith WL, Alexander RC, Kao SC, Ellerbroek CJ (1989) Head injury in child abuse: Evaluation with MR imaging. Radiology 73, 653–657.
38. Mimkin K, Kleinman PK (1997) Imaging of child abuse. Pediatr Clin North Am 44, 615–635.
39. Willman KY, Bank DE, Senac M, Chadwick DL (1997) Restricting the time of injury in fatal inflicted head injuries. Child Abuse Neg 21, 929–940.
40. Jaspan T, Griffiths PD, McConachie NS, Punt JA (2003) Neuroimaging for non-accidental head injury in childhood: a proposed protocol. Clin Radiol 58, 44–53.
41. Demaerel P, Casteels I, Wilms G (2002) Cranial imaging in child abuse. Eur Radiol 12, 849–857.
42. Berlin L (1997) Malpractice issues in radiology. Storage and release of radiographs. Am J Radiol 168, 895–897.
43. Mason J K (1983) Forensic Medicine for Lawyers, 2nd ed. Butterworths, London.

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