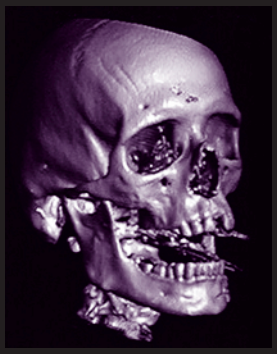

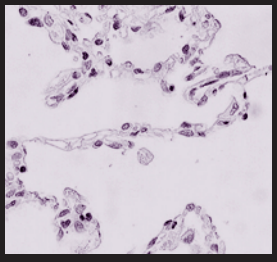

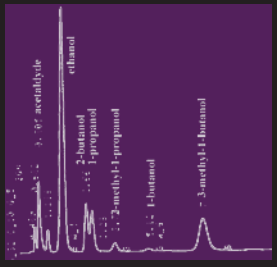

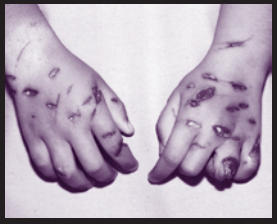

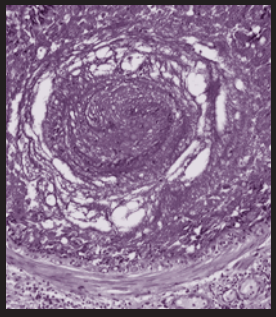

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Volume 4

Edited by

Michael Tsokos, MD

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Dedication

To my son Titus, once again

—Michael Tsokos, MD

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

Never before has so much information on current criminal cases and the associated forensic pathological matters been so readily available to so many people. The media seem to carry an increasing number of crime stories that touch detail nearly every day. The Internet provides widespread and virtually unlimited access to information (and misinformation) on all types of forensic pathological and medicolegal scenarios.

More than ever, there is a need for fixed points of reference by which both common forensic pathological entities and specific pathological conditions that are only rarely encountered in the autopsy room can be judged based on expert review, evaluation, and recommendation. The *Forensic Pathology Reviews* series has the goal of increasing a point of reference.

Of course, solving forensic pathological problems effectively requires a strong base of knowledge of pathology combined with personal experience and critical appraisal skills. Successful forensic pathologists and medical examiners weigh the importance and validity of the data they collect from the victims of homicide, suicide, and accidents as well as from those who expire from natural causes using sound reasoning based on probabilities, and a broad knowledge of the pathological and clinical features presented by these fatalities, in order to generate a thoughtful, convincing, and appropriate approach to establishing the cause and manner of death and the reconstruction of events. Encountering and solving problems in forensic pathology—particularly challenging cases—continues to be a source of great professional satisfaction.

It is a pleasure to express my gratitude to the many colleagues from all around the world who have so generously contributed to the *Forensic Pathology Reviews* series by making their scientific and practical knowledge accessible to the broad international readership of this series.

***Michael Tsokos,* MD**

Contents

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Death From Environmental Conditions

1

Pathological Features of Death From Lightning Strike

Stephan Seidl, MD

Contents

Summary

Lightning strikes cause more deaths in the United States than other natural disasters, such as hurricanes, tornadoes, volcanoes, and floods. Lightning is a transfer of an electrical charge and results from the sudden environmental discharge of static electricity. The power of lightning is estimated to be between 10,000 and 200,000 A of current, with estimated voltage ranging from 20 million to 1 billion V. The effects of lightning on the human body depend on a number of features, such as the intensity of the current, the time it spends passing through the body, the pathway involved, the activity and position of the person at the time of the event in relation to the ground, and the kind of strike (direct strike, contact voltage, side splash, ground strike, or wire-mediated lightning). Lightning strikes result in multisystem dysfunction, and survivors may experience prolonged disability following recovery from the initial insult.

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Electrical energy causes muscular spasm and necrosis, thrombosis, blood vessel tears, unconsciousness, and motor and sensory function abnormalities. Most deaths after lightning strikes occur either because of primary cardiac arrest or hypoxia-induced secondary cardiac arrest. If multiple persons are struck, attention at the scene should be directed first to those who appear dead, because they may be in respiratory arrest and in urgent need of immediate cardiopulmonary resuscitation that can be successful in lightning strike victims for far longer than would seem reasonable in other types of injury.

Key Words: Lightning strike; lightning injuries; direct strike; ground strike; contact voltage; side splash; side flash; wire-mediated lightning; flashover; Lichtenberg figures; burns.

1. Introduction

Lightning is a transfer of electrical charge resulting from the sudden environmental discharge of static electricity sandwiched between an upper negative-charged region, such as thunderheads, and a lower positive area *(1)*. When voltage between two oppositely charged fields exceeds atmospheric resistance, discharge occurs *(2)*. The most common types of lightning strikes are intracloud (the majority of discharges) and cloud-to-ground (some 20%), with ground-to-cloud and cloud-to-cloud lightning occurring only rarely *(2–4)*. However, a lightning strike may occur under fair weather conditions from a clear sky, far from any thunderstorm clouds *(5-8)*. Cherington and colleagues *(9)* discussed a case where the discharge originated in a cumulonimbus cloud that was approx 10 miles away and that was obscured by mountains. They called this phenomenon a "bolt from the blue."

The power of lightning is estimated to be between 10,000 and 200,000 A of current, with estimated voltage ranging from 20 million to 1 billion V *(10-12)*. A strike produces an intense burst of thermal radiation of up to 30,000 K within milliseconds and is accompanied by a shock wave of up to 20 atm that can contuse or perforate human organs *(10,11,13,14)*. Electrical energy follows the path of least resistance. Because tissues that have a low water and electrolyte content have a higher resistance, tissue resistance decreases in the following order: bone, fat, tendon, skin, muscle, blood vessel, and nerve *(4,11,15,6)*. The most important resistor to the flow of current is skin. Skin resistance varies from 1000 ohms on a sweaty palm to 1 million ohms on a dry, calloused hand *(11)*. The phenomenon of current traveling on the surface of wet skin without much penetration to deeper tissues is called "flashover" *(1-3,14)*. If it is raining or the person is perspiring, the water can vaporize with such force that the clothes are shredded and the shoes are blown off *(2,14)*.

Estimates reveal that there are approx 50,000 thunderstorms and 8 million lightning flashes per year worldwide *(17)*. Surprisingly, despite the vast amounts of energy involved, the overall mortality rate following lightning injury is only 30% *(8,18)*. The morbidity rate in survivors, however, is close to 70% *(2,19)*. Lightning strikes, although uncommon, still reportedly cause more deaths in the United States than other natural disasters, such as hurricanes, tornadoes, volcanoes, and floods *(16,18,20,21)*. In the United States, several thousand people are struck by lightning but survive, and approx 100 to 600 people die from lightning injuries *(2,4,8,10,14,17–19,22)* each year. These deaths occur primarily from May to September, between 3 and 6 PM, and mostly affect a young, active group less than 40 years of age *(8,18,19)*. The effects of lightning on the human body depend on a number of features, including the intensity of the current, the time it spends passing through the body, the pathway actually involved, and the activity and position of the person at the time of the event in relation to the ground *(1,10,13)*. In addition to these, a factor of major importance is the kind of strike, as a person may be hit by lightning in several ways *(4)*.

2. The Different Forms of Lightning Strikes

2.1. Direct Strike

Lightning may strike a person directly, usually entering through the head or an outstretched arm *(1,12,18,23,24)*. The current is then transferred to the body, and, in most of these cases, the exit pathway is through the soles of the feet because the lightning victim is normally standing, earthing him or herself through the ground *(12,14)* (Fig. 1). A direct lightning strike is the most damaging because the current discharges directly through the body and causes extensive thermal injuries and barotrauma *(2,12,19,23,25)*.

2.2. Contact Voltage

The difference between a direct strike and a side splash is the contact voltage. It occurs if people are wearing or carrying something metal, such as an umbrella, golf club, or a weapon *(2,23,26)*. If lightning strikes this metal object, the current will flow to the body of the victim, if this pathway is the way of least resistance *(23)*.

2.3. Side Splash

A side splash or side flash strike occurs when lightning discharges—after a primary strike to an adjacent object such as a tree—to a victim who has no

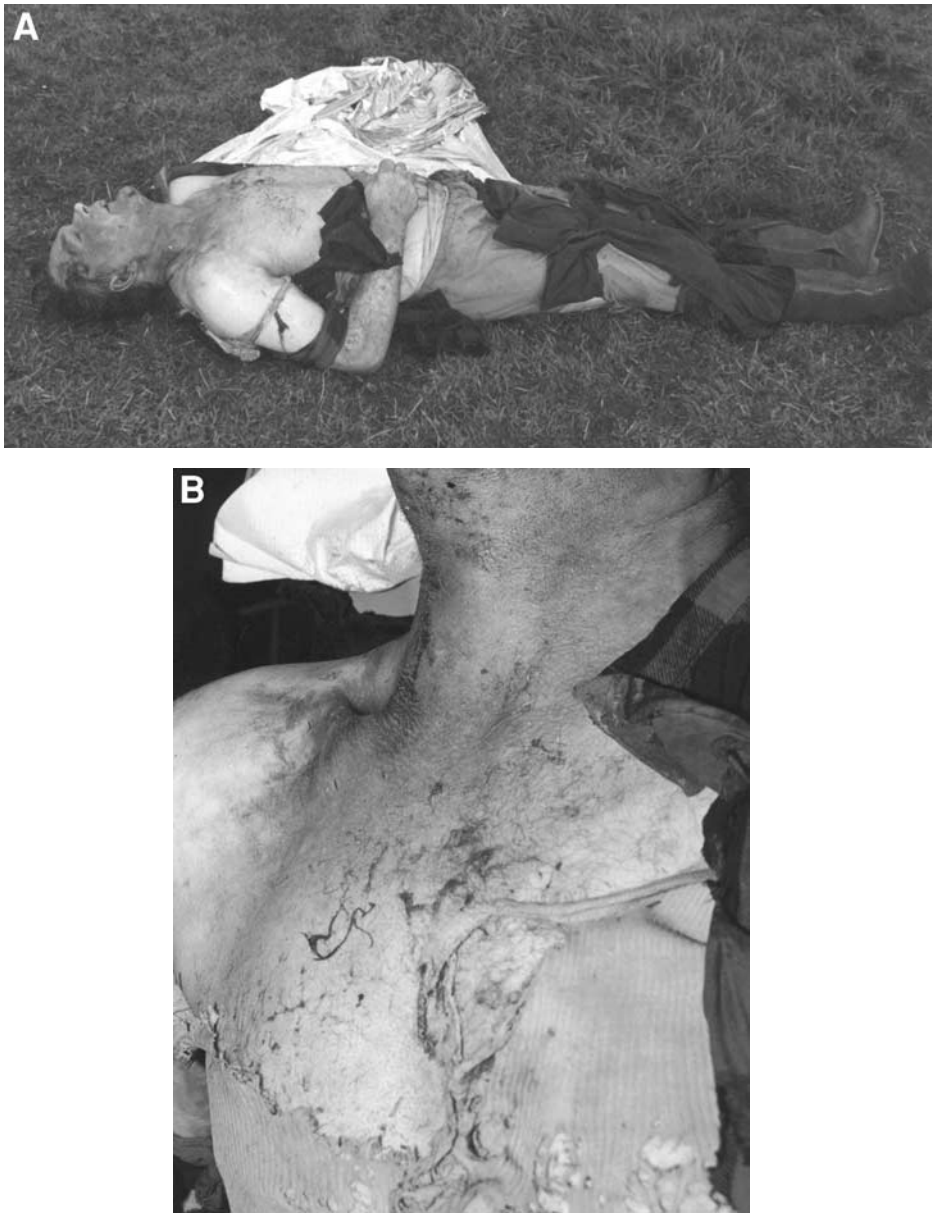
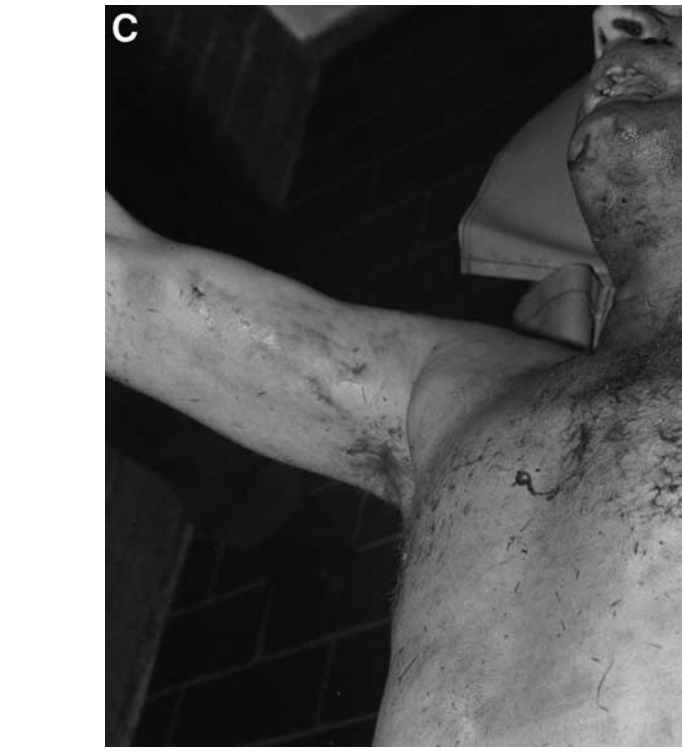


Fig. 1. Fatal direct lightning strike of a 63-year-old farmer. **(A)** Scene of death was an open field on a rainy day. The clothes are partially shredded, but the rubber boots are not blown off. **(B,C)** The skin of chest and right arm shows a fern-like pattern below the entrance point (neck) of the lightning strike. **(D)** The exit point was characterized by a melted sole of the right rubber boot, **(E)** a defect of the stocking, and **(F)** a plantar second- to third-degree burn with melted fabric from the sock. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.) *(Figure continues on two following pages.)*



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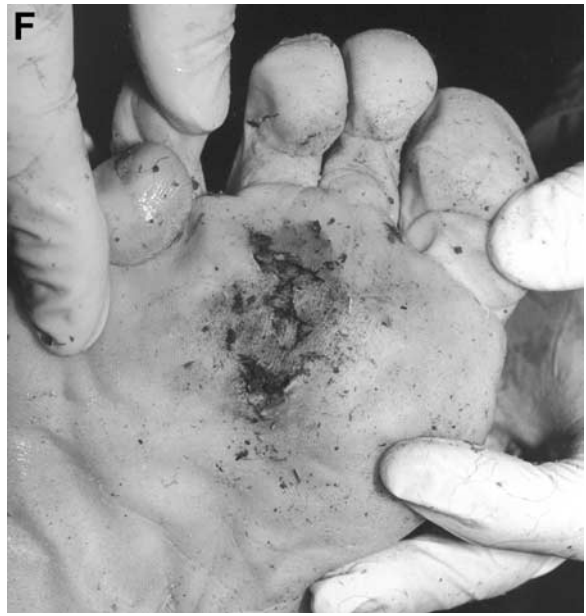

Fig. 1. *(continued)*

direct contact with the object *(2,19,23,24,27,28)*. The lightning may be conducted down the tree or it may jump to a path of less resistance, such as the person or persons standing under or near the tree *(19)*. The side splash seems to be the most frequent form of lightning strike *(22)*. As with ground strikes, the side splash often leads to injuries of several people *(23,26)*.

2.4. Ground Strike

Ground strike (also known as "stride potential" and "step voltage") occurs when lightning strikes the ground and a person is standing or walking nearby. An electrode voltage is encountered between the victim's two legs, and lightning can travel up one leg of a victim and down the other, causing a current to flow through the lower extremities and trunk *(2,12,20,23)*. Ground strikes are less severe because the force of the lightning dissipates rapidly when it travels through soil *(25)*. The heart and brain are usually spared by this type of injury, but the victims are frequently rendered transiently paraplegic and show severe burns on both legs *(12,23)*.

2.5. Wire-Mediated Lightning

People may rarely be struck by lightning indoors while using a telephone or an electrical appliance *(18,29,30)*. In these cases, the lightning surge may energize the structure of a house, causing current to flow through the victim into the grounded telephone or appliance. Likewise, lightning may strike a cable, causing connected telephones to become electrically charged *(18,31)*. Morgan and colleagues *(32)* reported the case of a 52-year-old man who was struck by lightning via the telephone line. They assumed that lightning passed through the man from head to toe and grounded via arcs from the right big toe and left scapular region. Qureshi *(33)* described the case of a 27-year-old woman who was indirectly struck by lightning while using the telephone during a thunderstorm. She heard a loud bang mediated through the telephone into her left ear and was thrown across the room. She was briefly unable to speak and hear. The left half of her face and body felt numb and she suffered from temporary left-sided hemiparalysis.

3. Effects of Lightning Strikes on the Human Body

Lightning injuries result from electrical energy, thermal energy, or the enormous blast force of a thunderous lightning strike *(23)*. Although lightning is essentially a high-voltage direct current, the effects of lightning are much different than those caused by other types of high-voltage electricity *(18)*. Whereas high-voltage electricity usually produces charring of tissue, a lightning strike is characterized by an extremely short duration of electrical contact. Although it is just a small proportion of the current that passes through the victim's body, the magnetic field that is formed is high enough to affect the neuromuscular, cardiovascular, and central nervous systems *(9,15,34)*. Lightning strikes result in multisystem dysfunction, and survivors may experience prolonged disability

following their recovery from the initial strike *(14)*. Electrical energy affects the permeability of cell membranes, can denature active intracellular proteins, and cause muscular spasm and necrosis, thrombosis, blood vessel tears, unconsciousness, motor and sensory function abnormalities, such as transient paraplegia and aphasia, amnesia, burns, contusions, edema, cataracts, decreased renal clearance with myoglobinuria, necrosis, and other injuries, including cerebral bleeding, skull fractures, or heart contusion *(3,4,8,14,15,27,35,36)*. However, lightning is anything but invariably fatal: cases have been reported of one person who was struck by lightning and merely stunned, whereas his companion, who was struck with the discharge after it emerged from the elbow of the original target, was killed. Other authors reported electrocutions of entire crowds, with some killed, others burned, and others merely showing various minor or major neurological sequelae *(37,38)*. The degree of injury is dependent on many factors, including current strength, resistance of the conducting tissue, current pathway, and duration of current flow.

3.1. Skin Injury

The vast majority of lightning injuries are first- and second-degree burns to the skin, covering between 1 and 20% of the body *(13,14,39,40)*. However, the points at which the current enters and leaves the body by arcing usually show third-degree burns *(32,37)*. Most of the current flows along the outside of the body, thus decreasing the extent of internal burning and tissue damage *(13,38,41)*. Immediately after the strike, lightning victims may be too hot to touch *(14)*. Burns associated with lightning appear in several forms: feathering, linear, punctuate, thermal (from the ignition of clothing), contact (from metal objects, such as jewellery or zippers [Fig. 2]), and flash *(1,4)*. Celiköz and colleagues reported the case of a 21-year-old man who had taken shelter under a thick nylon cover in the center of a group of five soldiers. While the soldiers sheltered themselves from heavy rain, lightning struck the nylon cover and a flash discharge with full-thickness scalp burn 12 cm in diameter occurred in the victim *(1)*. Most lightning burns, however, are superficial and require only local care, such as dry gauze dressings *(2,14)*; sometimes they reach from the entry point nearly to the exit point, where full-thickness burns are often found *(14,40,42,43)*. In contrast to electrical skin injuries from alternating current, significant myonecrosis is only rarely seen *(42)*. The feathering, arborescent, or fern-like pattern of skin injury is a transient, hyperpigmented mark that appears within an hour of the strike, radiates outward from a central spot, and disappears after several hours *(8,27,33,38)*. This so-called “Lichtenberg figure” (Figs. 3 and 4) is thought to be a fractal pattern of positive electrical discharge,

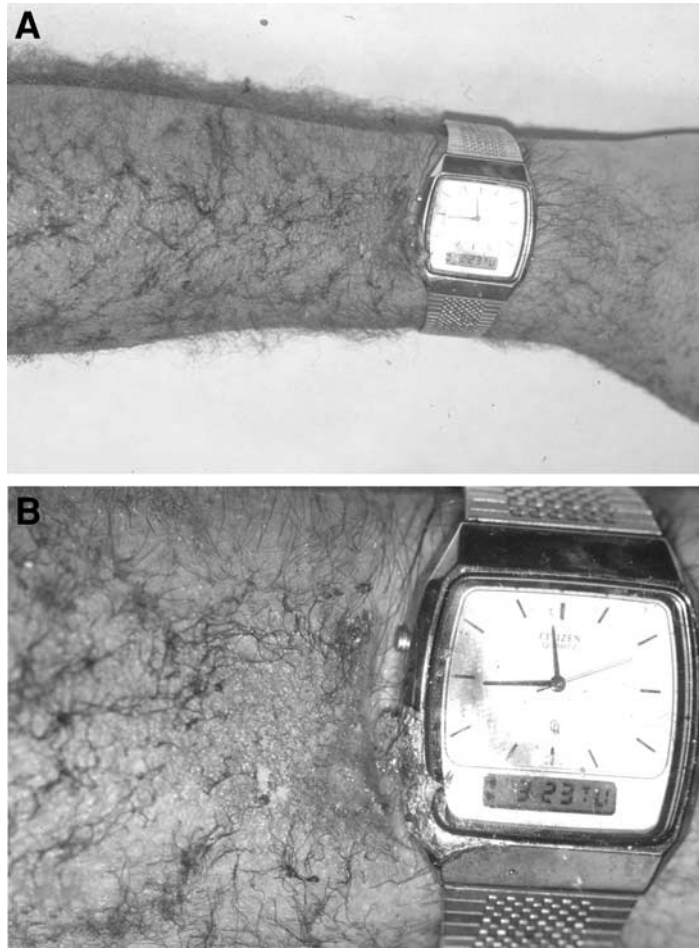

Fig. 2. (A) Contact burn at the left forearm caused by a partially melted watch following a lightning strike. **(B)** Detail. (Courtesy of Dr. Roland Hausmann, Erlangen, Germany.)

and has been reported in approx 30% of lightning strike cases *(4,8,14,38,44)*. Histological examination of the arborizing pattern revealed normal epidermis and dermis. An extravasation of red blood cells in the subcutaneous fatty tissue, as proposed by Resnik and Wetli *(38)*, is not mandatory. Other authors described only a dilatation of small blood vessels in the corium, sometimes combined with an elongation and nuclear streaming (palisading) of the cells of the basal epidermal layer *(45)*.

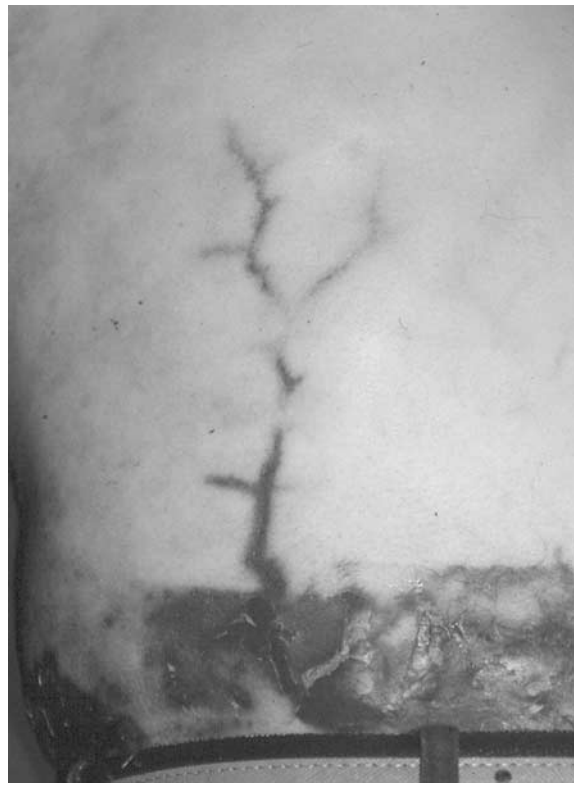

Fig. 3. Fatal direct lightning strike of a 52-year-old man. An arborescent pattern of skin injury is clearly visible at the left loin region. (Courtesy of Dr. Roland Hausmann, Erlangen, Germany.)

Jonas et al. *(46)* reported the case of a couple who was hit by a lightning strike. Both survived the thunderbolt without permanent injury. However, whereas the man was unconscious for only a few minutes, his wife fell into a coma for 24 hours. The lightning, which entered her body behind the left ear and went out at her left shoe, caused a partial evaporation of a gold ornamental chain on her neck. After recovery, the woman got a tattoo on her neck. Two skin biopsies taken 6 months later showed particles of different shapes and sizes up to 2 mm deep in the skin that accumulated in histiocytes, multinucleated giant cells, and, to a small extent, in fibroblasts. Element analysis by electron energy loss spectroscopy, energy-dispersive X-ray analysis, and atomic absorption spectroscopy showed that the detected particles were gold (70%), silver (21%), and copper (9%).

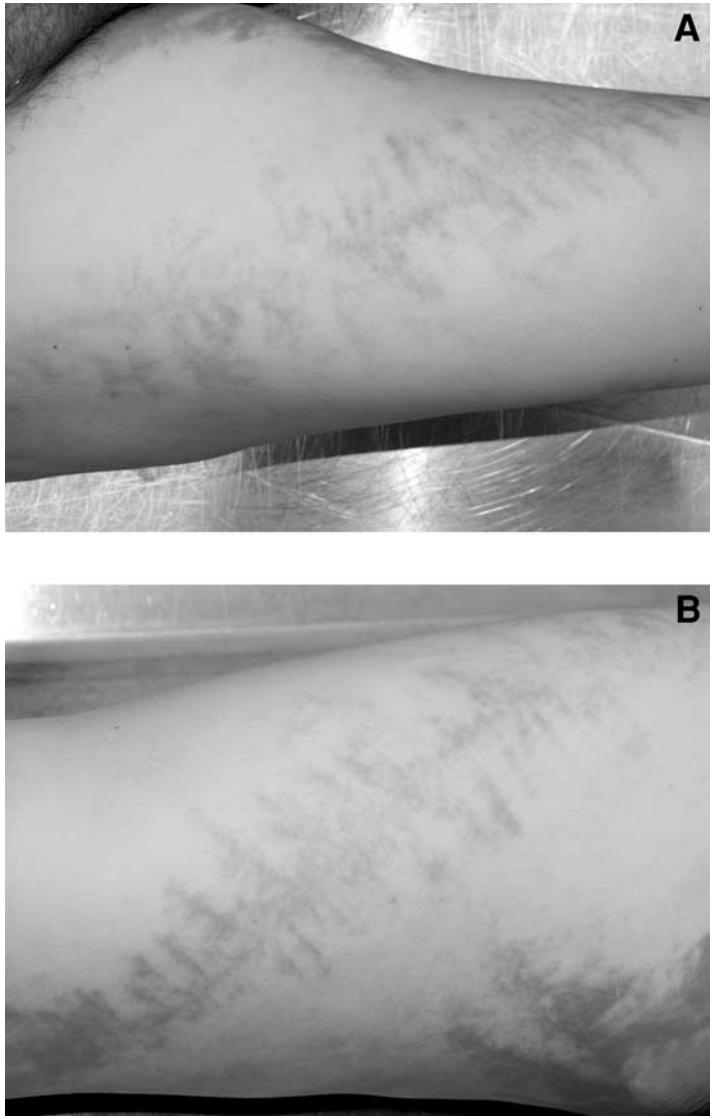

Fig. 4. Fatal direct lightning strike of a 42-year-old woman. **(A,B)** Fern-like patterns ("Lichtenberg figures") are visible on the thigh. (Courtesy of Dr. Wolfgang Huckenbeck, Düsseldorf, Germany.)

3.2. Heart Injury

Most deaths following lightning strikes occur because of primary cardiac arrest or are the result of hypoxia-induced secondary cardiac arrest *(2,14)*. Because lightning is direct current, it has the effect of cardioversion and induces ventricular standstill, as opposed to atrial and ventricular fibrillation *(2,4,8,14,32,41,47,48)*. Despite the fact that 50 to 75% of those who have cardiac arrest will die, cardiopulmonary resuscitation (CPR) can be successful in lightning victims far longer after the initial strike than in other types of injury *(2,4,18,37,49)*.

Other cardiovascular effects of lightning are transient hypertension and tachycardia, both thought to be secondary to adrenal stimulation with excess catecholamine release or autonomic stimulation *(19,49)*. Chia *(39)* reported the case of a 24-year-old woman who was struck by lightning and was found to be in congestive cardiac failure at the time of hospital admission. Kleiner and Wilkin portrayed the case of an 18-year-old man with T-wave inversion in electrocardiograph (ECG) showing a rapidly developing severe bilateral pulmonary edema 3 hours after admission *(49)*. Even in cases of primary cardiac arrest, ECG abnormalities are not necessarily seen *(14)*. If ECG changes occur, they consist of deep T-wave inversions in multiple leeds and patterns of ischemic changes, myocardial infarction, or subepicardial injury that normally resolve within several months *(19,39,41,48–50)*. These changes are explained by epicardial hemorrhages, muscle fiber necrosis, and a peculiar spiral malformation of the myocardial fibers *(41,51)*. Animal experiments by Karobath et al. *(47)* confirmed the existence of myocardial necroses owing to lightning strikes. Zack and colleagues *(35)* portrayed a case of side splash where a 27-year-old man showed severe acute myocardial infarction, affecting virtually all parts of the myocardium. Lichtenberg and colleagues *(19)*, however, showed that the potential for, and mechanisms of, injury to the cardiovascular system obviously differ in each type of lightning strike. They found in 19 cases that a direct lightning strike results in a high incidence of severe cardiac injury that can be manifested early as life-threatening pericardial effusion or severe global cardiac dysfunction. ST elevation and prolonged QTc intervals in the ECG were limited to direct strikes, whereas myocardial injuries manifesting as creatine kinase-MB release can be seen in any type of strike; this occurred in 75% of victims of a direct hit, 66% of victims of a side splash, and 12% of victims of a ground strike. ECG changes after splash and ground strikes were limited to nonspecific ST-T-wave changes *(19)*.

3.3. Central Nervous System

The most serious injuries from lightning strikes involve the cardiovascular and central nervous systems *(2,9,16)*. Because of their clinical course, the effects of direct strikes to the head can be divided into the following four groups:

1. Fatal coma from hypoxic encephalopathy.
2. Global encephalopathy with partial recovery.
3. Slow resolution of focal neurological dysfunction.
4. Transient neurological symptoms with rapid complete recovery *(9, 18)*.

Mechanisms of neuronal damage in lightning strikes include prolonged depolarization, direct neuronal damage (heat and blunt trauma) as well as secondary tissue damage from edema, ischemia, and reperfusion injury *(10,52)*. It has been well-described that lightning strikes cause intense vasospasms and ischemia *(10,53)*. Whereas skull fractures are mostly linked to falls, brain lesions are common in the absence of falls. In fact, most neurological lesions in lightning-strike patients are not linked to trauma from falls, but occur as a result of hypoxia caused by paralysis of the respiratory musculature or failure of the medullary respiratory center *(14,36)*. Comparable to other tissues, brain damage of lightning victims is triggered thermally or electrically *(54)*. Subarachnoid and intraventricular hemorrhages often follow a particular pattern with regard to the location in the brain *(12,36,55)*. The most vulnerable sites are the basal ganglia and brainstem. Although small petechial hemorrhages are seen in the brainstem, larger hemorrhages are found in the basal ganglia *(12,36,56,57)*. The pathophysiology of basal ganglia hemorrhages related to lightning is not likely a product of either mechanical trauma or intense peripheral vasoconstriction resulting in acute hypertension, but may reflect the path of current flow in the brain *(36)*. Ozgun and Costillo *(56)* pointed out that electrical conductivity is highest in the cerebrospinal fluid (CSF), and heating of the CSF to 145°F has been recorded after legal electrocution. Additionally, blood vessels and neural tissue have been found to carry more current per area than other tissues, and to become damaged before surrounding tissues in an animal model *(56,58)*. Ozgun and Costillo concluded that preferential conduction along Virchow-Robin spaces in the anterior perforated substance plays a major role in the production of basal ganglia injury following lightning strikes *(56)*.

Severe head trauma can occur as a consequence of falling, being thrown to the ground by the enormous blast force of a thunderous lightning strike, or

as a direct effect of the lightning strike *(8,25,32,36)*. Blunt head trauma patients often present with subarachnoid and intraventricular hemorrhages, as well as mass lesions of epidural and subdural hematomas *(32,36)*.

Ongoing neurological deficit following a lightning strike is an important issue. The majority of sequelae following a lightning strike are neurological, as seen in 70% of survivors *(14,43)*. Injuries arise as a result of the primary strike (hemorrhages, edema, or neuronal injury) or are owing to a hypoxic insult from the cardiac arrest *(14)*. Nearly 80% of lightning-strike survivors suffer from amnesia, loss of consciousness, or confusion, and 50% are afflicted with paraplegia, which is normally transient and resolves within hours to days *(14,40,43)*. Persisting neurological deficits, however, indicate spinal cord damage, which is a rare but well-documented phenomenon following lightning strikes *(14,59)*. Freeman and colleagues *(59)* reported the case of a 58-year-old man who was struck by lightning while sleeping in a tent:

> After short-term loss of consciousness, he was unable to move his arms and legs. Over the next few hours, he gradually noticed some return of movement and feeling in the fingers, arms, and legs. … Two days after admission, the patient was discharged with no persistent sensory loss or paresthesias. The only remaining deficit was some difficulty in walking.

Approximately 6 weeks after the lightning strike hit the man, he began to notice numbness, tingling, and dysesthesia in both hands.

> Over the next week, the paresthesia gradually migrated into his forearms to the elbows and became noticeable in his feet. He also complained of weakness in his hands and legs. Finally, the patient described severe shock going through his arms, trunk, and feet elicited by minor neck flexion.

CSF analysis revealed a moderate elevation in protein (0.89 g/L) with normal cell count. Nerve conduction studies were normal. Magnetic resonance imaging (MRI) revealed hyperintense signals within the posterolateral region of the spinal cord from C1 to C3 bilaterally. Clinically, the symptoms were consistent with a diagnosis of delayed myelopathy secondary to electric injury with predominant dorsal column involvement. The abnormal spinal cord signal had resolved at a follow-up MRI performed 6 weeks later. At this time, the patient described some improvement with less stiffness in his hands, improved sensation, and improved grip *(59)*.

Marleschki *(60)* portrayed the case of a lightning strike during the fourth month of pregnancy. Aside from skin burns and transitory symptoms of left ventricular ischemia, the 27-year-old woman suffered from temporary hypoesthesia, muscular hypotonia, and palsy of the right arm and both legs. As move-

ments of the fetus were not palpable for 12 hours, Marleschki assumes that the lightning stroke caused a reversible palsy of the child *(60)*.

Van Zomeren and colleagues *(61)* tried to objectify neuropsychological impairments in six survivors of lightning stroke between 1 and 4 years after injury. Patients reported fatigue and lack of energy as their main complaints. In addition, poor concentration, irritability, and emotional lability were mentioned often. Neuropsychological tests disclosed mild impairments in memory, attention, and visual reaction times. The authors speculated that most complaints of these survivors are caused by a vegetative dysregulation, a disorder that has often been noted in the literature as an aftermath of electrical injury to the nervous system *(61)*.

3.4. Other Injuries

Victims of lightning strikes may also suffer ophthalmic, otological, psychiatric, pulmonary, renal, gastrointestinal, musculoskeletal, and peripheral neurovascular complications *(18)*. Dilated, unreactive pupils are usually a short-term effect of autonomic nervous system involvement *(14)*.

Ear lesions may vary from mild burns of the auricle to complete destruction of the hearing organ. Absence of the organ of Corti, rupture and collapse of Reissner´s membrane, strial degeneration, and decreased spiral ganglion cell population were reported *(17,62,63)*. Sound pressure levels of 150 to 160 dB have been recorded thus, ruptured tympanic membranes are common in lightning-strike patients, but no case of auditory ossicle injury following lightning strike has been reported so far *(3,14,17,25,33,64–66)*. Symptoms related to the inner ear following lightning strike may be cochlear, such as hearing loss, or vestibular, such as temporary vertigo, positional vertigo, and endolymphatical hydrops *(15)*. A case of hearing loss caused by a lightning strike was reported by Cankaya and colleagues *(16)*. Aside from a first-degree burn of the auricle, the 33-year-old man showed two perforations with hemorrhagic edges on the right tympanic membrane that were attributed to the blast effect of lightning. The middle-ear mucosa was hyperemic and edema was present. At pure-tone audiometry, a 40-dB conduction-type hearing loss was detected, which resolved within 6 months. Sensorineural hearing loss caused by lightning strike, however, does not have a high rate of resolution *(15)*. In the case of a lightning strike via a telephone wire mentioned earlier, the 27-year-old woman complained of tinnitus and deafness in her left ear *(33)*. Although the tinnitus improved, deafness persisted at a re-examination 6 months later.

Todd and Meyers *(17)*, as well as Silbergleit and Trenkner *(67)*, reported cases of lightning strike-induced dysphagia that persisted several months and was attributed to an isolated central nervous system defect.

As the lens of the eye seems to be very sensitive to electrical current or the heat set free by the current, the most common permanent ocular sequela of lightning is cataract *(10,29)*. The cataract can occur soon after a lightning strike or it may develop over many months *(2,68)*. Problems with vision may be a sign of retinal detachment. Burns of the periorbita and the eyelids, keratitis, iritis, papillary abnormalities, cornea lesions, lens dislocations, retina damages, and thermal optic nerve papillitis were reported in patients injured by lightning *(10)*.

The muscular effects of electricity consist of muscular contraction in response to electric stimulation *(32)*. Because lightning is a modified form of alternating current, consisting of a brief, highly damped discharge, no injury is usually associated with this contraction. Indeed, a rupture of muscles may occur occasionally as sometimes observed in cases of electrocution *(69)*, or the victim may fall, receiving secondary injuries *(32)*. In contrast with other electrical injuries, muscle necrosis with resulting renal impairment is rarely seen after lightning strikes *(14)*. The myoglobinuria from lightning injury results from tissue damage caused by heat generation and coagulation necrosis. Myoglobinuria has clearly been shown to precipitate acute tubular necrosis, which progresses in some cases to renal failure *(70)*.

4. First Aid After Lightning Strike

Anyone struck by lightning should have a complete physical examination, including ophthalmological and otological check-up. Care for these patients with multisystem involvement requires a team approach and attention to the whole patient *(19)*. Multiple lightning victims have to be treated in the opposite way from those involved in other disaster situations *(2)*: the recommendation of the American Heart Association is that after a lightning strike, all attention should be given to the most sick, particularly to those who appear moribund or even dead, because these persons may be in respiratory arrest and in need of immediate CPR *(2–4,18,37,49,70)*.

5. Conclusions for the Forensic Pathologist

In regions with high lightning-strike densities, lightning strikes should always be considered as a potential differential diagnosis when investigating unexpected, unwitnessed deaths, especially when they occurred outdoors *(66)*. The diagnosis of a lightning fatality should be considered even when witnesses report that the sky was cloudless and they heard no thunder *(66)*. Because knowledge of the weather conditions, even in the surrounding area, is of obvious value, further information is available from meteorological services like the National Lightning Detection Network of the United States *(8)*.

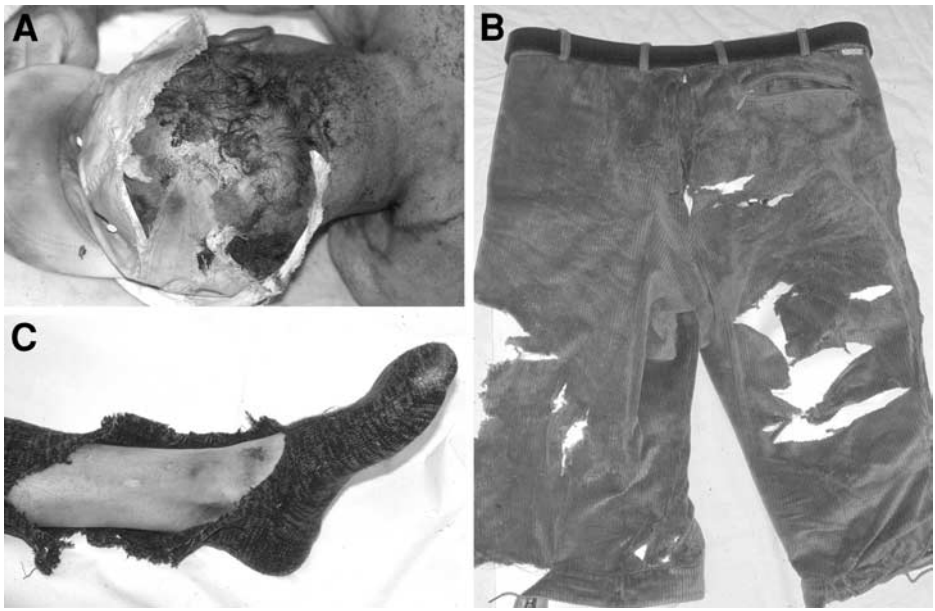

Fig. 5. Fatal direct lightning strike of a 52-year-old man. Because of an effect called "flashover," **(A)** the cap, **(B)** the trousers, and **(C)** a sock exploded into shreds. (Courtesy of Dr. Roland Hausmann, Erlangen, Germany.)

If there are no or no reliable witnesses, a diligent search for evidence at the scene of death is needed *(18)*. Externally visible forensic clues that suggest lightning strike at the scene include damage to nearby trees, such as splitting or removal of bark, or charred arc marks on the walls of nearby structures *(18)*. The ground may display a keraunographic fern-like pattern in the grass resembling the cutaneous changes (Lichtenberg figures) observed in some victims *(8,18)*. If the scene of death is inside a building, nearby electrical and telephone equipment should be examined for signs of lightning damage *(18)*. The clothing of lightning-strike victims may be burned, the trouser legs, for example, may be exploded into shreds, and the shoes may be melted or "exploded" *(8,14)* (Fig. 5). Clothing of lightning-strike victims, particularly shoes, may have been torn by the lightning energy. Finding a victim in a remote location with the clothing in disarray may even suggest a sexual attack *(18)*. Skin observations of burns and fern-like cutaneous patterns, as well as singed hair, may then point to the true cause of death *(66)*.

In any case of death attributed to lightning, a thorough autopsy is mandatory. Besides the lightning injuries mentioned, such as skin burns or fern-like

injuries, the forensic pathologist has to look for fractures that may have been caused by the lightning strike's explosive effects or by electrically induced muscular contractions *(18)*. Further points of interest are the tympanic membranes, aortic injury (mostly consisting of endothelial elevations and tearing of a portion of the media *[8]*), cardiac injury (such as heart contusions or myocardial ischemia), and head injury, including cerebral hemorrhages.

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2

Elder Abuse

Challenges for Clinical Forensic Specialists and Forensic Pathologists in the 21st Century

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CONTENTS

SUMMARY

The ever-expanding growth of the geriatric population increases the likelihood of abuse and neglect both in the home and in caregiving institutions. In the United States alone, hundreds of thousands of elders are maltreated each year. Only recently has there been a clear public and governmental awareness of elder abuse in all its forms. Elder maltreatment, including abuse and neglect, comprises an act or omission resulting in morbidity and/or mortality of older persons. Six recognized categories of elder maltreatment include physical, sexual, and psychological abuse; financial exploitation; neglect, and a miscellaneous classification that often includes the violation of the elder's rights. A

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strong familial relationship between the abused and the abuser exists. In more than two-thirds of cases, an adult child or spouse is the perpetrator. Domestic violence in the family is also a common underlying factor. Recognition of this phenomenon is the initial step in reaching a correct diagnosis. Despite efforts to educate health care providers of elder maltreatment, physicians in the United States have reported only 2% of all abuse cases in recent years. Achieving full recognition of elder maltreatment requires a multidisciplinary effort from many fields, including clinicians, social workers, medicolegal death investigators, and law enforcement to establish comprehensive research and governmental funding. The ultimate goal of such an agenda is to define evidence-based markers for accurate diagnostic methods, which differentiate causes of injury and death by abuse and neglect from those related to normal aging and senescence. In addition to the painstaking scrutiny of the putative victim's medical and psychological background, circumstantial and scene findings that may suggest elder maltreatment, in any form, must be meticulously investigated.

Key Words: Elder abuse; elder neglect; chronic debilitating diseases; maltreatment; domestic violence; forensic pathology; clinical forensic medicine; sexual abuse; decubitus ulcers; institutionalization; health care providers; caregiver.

1. INTRODUCTION

> "Honor your father and mother"—which is the first commandment with a promise—"that it may go well with you and that you may enjoy long life on the earth." (Ephesians 6:2–3 *[1]*)

With the rapidly growing cohort of elders in the general population, both society and individuals are bound to address with greater emphasis the health evaluation, maintenance, and care of the aged, both logistically and financially. In the United States alone, it is anticipated that more than 70 million "baby boomers" aged 65 years or older will retire over the coming decade. Families or other caregiving institutions increasingly face the inevitable task of establishing long-term care arrangements for the elderly as increasing longevity continues unabated. The care of the chronically diseased and disabled elders portends significant strains on the family. Failure to establish appropriate long-term planning earlier in the elder's life, before the onset of significant disability, increases the likelihood of both familial and societal disequilibrium. This prospect represents an unprecedented, emerging public health crisis with global impact. In the United States, the "traditional" family unit (composed of a father, mother, and children) has undergone progressive deterioration and, to a great extent, no longer exists. As a consequence, reliance on caregivers from the

broader, extended family unit, as well as from private or state-supported institutions, may further complicate strained familial relationships. In these circumstances the care of an elder becomes extremely suboptimal.

Between 1 and 2 million older Americans are likely to experience some form of elder mistreatment each year *(2,3)*. Elder mistreatment denotes all types of elder abuse and elder neglect. Elder abuse and neglect were defined by the American Medical Association's Council on Scientific Affairs of 1987 as the omission of actions or the actions resulting in harm or threatened harm to the welfare or the health of the elderly *(4,5)*. In essence, such mistreatment represents a failure by the lawfully designated caregiver to reasonably exercise the duty to accord fundamental legal and human rights.

The United Nations in the Universal Declaration of Human Rights, coupled with many subsequent statements of principles, covenants, and commitments in support of the rights of older persons, lays out the fundamental entitlements of all humans and provides a basis for legislation. For example, the Older Americans Act enacted by the US Congress in 1992 specifically defines elder abuse in terms similar to those of the American Medical Association (42 USC §3002[13]). At the state level, as a further example, Kentucky law defines elder abuse as "the infliction of physical pain, mental injury, or injury of an adult" (KRS 209.020[7]).

Maltreatment of the elderly can be divided into the following categories: physical, psychological, financial/material neglect; financial/material abuse; or violation of civil rights; or any combination of these *(6)*. Elder neglect, although currently less well-defined, often is the most commonly investigated form of elder abuse.

Although elder abuse and neglect have achieved ever-increasing recognition in the medical and law enforcement arenas, reporting of these instances is still woefully inadequate. Only one in five cases is reported to the appropriate authority. Such a failure in official recognition of the problem still exists, although most US jurisdictions have legislated mandatory reporting laws *(3,7,8)*. Kentucky is a mandatory reporting state requiring that any person (physicians, law enforcement officers, nurses, social workers, cabinet personnel, coroners, medical examiners, alternate care facility employees, caretakers, and attorneys outside the attorney–client relationship) report when the observer has reason to believe that a vulnerable adult has been abused or neglected (KRS 209.030[2]). All 50 states and the District of Columbia have legislation touching on elder abuse in either domestic or institutional environments, setting up systems for both identifying and reporting suspected cases, and establishing adult protective services (APS) for investigation of allegations and assistance to victims. Hamp provides a current and comprehensive compilation of state legislation in

the United States *(9)*. Yet, uniformity is lacking owing to the jurisdiction-dependent variety of legislative approaches. This is exemplified by the following various points of interjurisdictional divergence:

- Age covering the victim.
- Definition of elder abuse.
- Classification as to criminal or civil.
- Kinds of abuse included.
- Mandatory versus voluntary reporting requirements.
- Procedures specifically covering investigation.
- Remedies for abuse and neglect.

Such official enactments have prompted more executive and judicial scrutiny; however, because of the lack of systematic training and knowledge about elder mistreatment in the legal sectors, among health care providers, and by medicolegal investigators, cases of elder abuse are still being identified only at critical stages of illness or at autopsy. Many are never investigated thoroughly. In contrast to the policy of vigorously investigating unexplained or suspicious deaths of younger humans, the standard for analyzing deaths among geriatric groups is considerably lower; risk factors for abuse are not well-recognized and well-established medical conditions, which overlap with features of abuse, are often regarded as sufficient to explain death. As the magnitude of elder abuse is not fully clear, many consider it to be a hidden crime impacting a significant number of the elderly. Complicating this deficit in official oversight is the common phenomenon whereby the elder may be uncooperative because of fear of changing his or her environment, for example, removal from the familiar home setting to a nursing home.

Elder mistreatment is, in many ways, similar to child abuse, and subtle findings on physical examination may be the only indication of abuse or neglect. Clinicians and forensic investigators have developed considerable skill and expertise in identifying and documenting features of nonfatal and fatal child abuse. With recent emphasis on clinical geriatric medicine, more specialists are now competent to address the health concerns of the aged, including suspected abuse. Thoughtful, perceptive interviewing by clinical specialists tends to assist the elder in realizing that he or she may indeed be a victim of abuse. A thorough, goal-directed physical examination—with complete review of the medical history, evaluation of the patient's quality of mentation, and laboratory results—constitutes the optimal approach for the forensic investigator in uncovering abuse and neglect. The same exhaustive investigatory approach applies in cases of death, although the same kind and degree of specialized training for postmortem death investigators have not kept pace with that of their clinical

counterparts *(10)*. Nonetheless, the autopsy remains the final opportunity for determining whether abuse or neglect caused or contributed to death. This chapter covers findings or markers of elder abuse in the clinical context, as well as at autopsy, including commonly encountered decubitus ulcers. Once recognized, all such indicators require explanation prior to exclusion of abuse or neglect by the caregiver.

2. The Definition and Scope of Elder Maltreatment

2.1. Epidemiology of the Graying Phenomenon

The worldwide population older than 65 years is projected to be 550 to 973 million from 2000 to 2030. This reflects a drastic rise in the global population from 6.9 to 12%. Such projections forecast that the number of persons in developing countries will nearly triple from 249 million in the year 2000 to an estimated 690 million in the year 2030. These numbers represent both a decline in fertility and an increase in life expectancy. Even in third-world countries with relatively less effective health care provision, the life expectancy has increased to 76 to 80 years. In the wake of the growing geriatric population, increased demands on medical and public health services have accrued. Concomitant with the growth of this clearly emerging, distinctive group are projected increased long-term health care costs attributable to a greater prevalence of chronic diseases, various disabilities, and illnesses related to injuries and mental dysfunction *(11)*. The 60- to 65-year-old age bracket represents the fastest growing age group in the United States. By 2030, elder groups will have grown to 19.6% of the US population. By 2030, there will be 71 million elderly Americans *(2)*. By the year 2050, the average life span in the United States will increase by 10 years. The estimated number of elders 80 years of age or older will grow from approx 9.3 million in 2000 to 19.5 million in 2030 *(11)*.

2.2. Elder Abuse Definition: Forms of Maltreatment

In 1975, G. R. Burston wrote a letter to the editor published in the *British Medical Journal* raising the specter of "granny-battering," which documented various cases of elder maltreatment perpetrated by family members *(12)*. During the last 30 years, the prevalence of elder maltreatment has been further defined with greater public awareness. The American Medical Association (AMA) provided the following standard definition of elder abuse in a 1987 position paper:

> "Abuse" shall mean an act or omission which results in harm or threatened harm to the health or welfare of an elderly person. Abuse includes inten-

> tional infliction of physical or mental injury; sexual abuse; or withholding of necessary food, clothing, and medical care to meet the physical and mental needs of an elderly person by one having care, custody or responsibility of an elderly person. *(4,5,13)*

The National Aging Resource Center on Elder Abuse (NARCEA) divides elder maltreatment into five classes of elder abuse, including physical, psychological (emotional), financial, sexual, and neglect. NARCEA includes two additional categories: self-abuse (neglect) and a miscellaneous group *(2)*. Several of these classes may, and often do, occur simultaneously *(14)*.

Elder abuse is a generic or umbrella term that encompasses both elder abuse and neglect. The term "maltreatment" is synonymous with elder abuse. As recently as the 1990s, the prevalence of maltreatment in the elderly population ranged from 1 to 5%, affecting hundreds of thousands of elderly people in all classes of society *(8,13)*. As recognition and reporting of elder abuse currently in the United States have improved, the incidence is only slightly less than that of child abuse *(8)*. Ten percent of elders 65 years of age and older experience some form of maltreatment annually. Four percent of these elders will experience some type of moderate-to-severe abuse *(4,14,15)*.

In some forms of domestic violence, there is a general ebb and flow of elder abuse activity in the home or maltreatment in a long-term care facility. Such actions are typified by alternating periods of quiescence and exacerbation. Ultimately, the situation-specific frequency of abuse escalates over ever-shorter intervals and reaches the point at which it does not spontaneously resolve. Even seemingly trivial incidents may be debilitating or potentially lethal for the susceptible elder with fewer psychological or physical reserves. Stressors that are commonly overcome by younger, healthier persons may well set in motion rapid decline and death in the seniors. Intervention is necessary at that critical juncture if the elder is to fully recover *(2)*.

Dyer et al. *(16)* offer a comprehensive review of evidence-based markers suggestive of elder abuse and neglect, while acknowledging that no gold standard yet exists and emphasizing that extensive research is mandatory in order to optimize diagnosis (Table 1). Their discussion incorporates a description of both physical and, frequently coexistent, nonphysical markers, the manner in which changes owing to aging relate to and are distinguishable from these markers, and the current state-of-the-art armamentarium that facilitates the diagnosis of abuse and neglect derived from findings both clinically and at autopsy.

2.2.1. Physical Abuse

Physical abuse of elders consists of violent acts intended to cause pain or injury. Egregious cases of physical abuse, such as firearm injury, extensive

Table 1
Potential Forensic Markers of Abuse and Neglect in the Elderly: Applications to Clinical and Postmortem Investigation

Bruises	Dehydration/malnutrition	Financial exploitation
Abrasions/lacerations	Improper medication use	Hygiene
Fractures	Decubitus ulcers	Restraints
Burns	Sexual abuse	Cognitive and mental health problems

Adapted from ref. 16.

blunt force trauma, sharp force injuries, cutaneous markings resulting from inappropriate or excessive employment of various kinds of restraints, and human bite marks, present little challenge in diagnosis even to the uninitiated medicolegal investigator. Slapping and striking the victim with various objects constitute subtler, typical acts of violence. Shoving and/or pushing are other types of physical abuse. The resulting physical findings include cutaneous blunt force injuries (bruises, abrasions, and lacerations) sharp-force injuries (incised wounds and stab wounds), skeletal fractures, skin burns, and ligamentous injuries *(2,3,13)*. Evaluation of physical injuries necessitates consideration of the type, amount and extent, pattern, location, and potential pathophysiological consequences *(17)*.

2.2.1.1. Blunt Force Injuries

The physical appearance of blunt traumatic wounds, including the magnitude and severity, rests on the following well-established factors:

- Degree of applied force.
- Time interval of applied force.
- Character of body surface impacted (flat, curvilinear, bone-supported).
- Area of body surface to which force is applied.
- Nature of the blunt instrument.

Contusions (bruises) of skin and other external body surfaces (e.g., sclerae) result from impact of variable force by or against a blunt object either perpendicular to or at an acute angle to the surface. The consequence is tearing of the subcutaneous (subconjunctival) vasculature with extravasation of variable degrees of whole blood or blood constituents to the surrounding tissue and dissection along and into tissue planes. The timing of the appearance and the location of contusions are variable. Relative superficial injury may be apparent on the surface soon after impact, and may exhibit a pattern implicating the offend-

ing blunt instrument. Imprints from knuckles or fingers, including those in subaxillary regions of the arms and around the ankles, are occasionally discernible and indicative of forceful slapping, fist pounding, or movement of the victim through grasping the arms below the shoulders or pulling at the feet. Parallel, "railroad-track" bruise patterns are characteristic of impact by a firm, smooth-surfaced, cylindrical object, such as a pipe, switch, or rod *(17–19)*. Localization of contusions is valuable in distinguishing those resulting from a fall (which is not uncommon among the elderly and is often the explanation for the bruises provided by the caregiver), as opposed to nonaccidental wounding. The most common sites of inflicted wounds are the face and neck, thoracoabdominal areas, hips, and the palms and soles of the feet *(18,19)*. The dating of bruises by appearance (size and color) is inexact and forensically unreliable because of great individual variation in response to injury. Although more recent bruises are said to be varying shades of red, blue, or purple and those in "healing" stages appear as either blue-green, yellow-green, or brown, great caution is mandatory in interpretation of the approximate age of the injury. Evaluation requires close correlation with the offered history of occurrence. Histological examination of bruises to determine their age is also of quite limited value. In a comprehensive review designed to age bruises in 89 subjects ranging from 10 to 100 years, Langlois and Gresham stated that "it was only possible to conclude that a bruise with a yellow color was more than 18 hours old" *(20)*. Multiple bruises that are indisputably in different stages of healing suggest physical abuse. In fatal cases without internal injury where extensive external soft tissue contusions are documented and the extravasated blood volume is semiquantitated, consideration of hypovolemic shock as the mechanism of death is appropriate *(21)*. In the same setting where the loss of blood is insufficient to invoke fatal exsanguinating shock, the forensic pathologist may examine the myocardium to possibly determine whether histopathological features of "stress cardiomyopathy" caused by multiple, repeated blunt impacts are present *(22)*.

Abrasions and lacerations of skin occur from impacts by or against blunt objects of various shapes, and result from motion over a rough surface or forceful separation of the skin, respectively, causing a surface split and disruption from the subjacent connective tissue. Either kind of wound is common among the elderly because of age-related loss of skin thickness, elasticity, and tensile strength *(23,24)*. Such injuries in the geriatric group in the institutional setting have been observed quite frequently on the arms, of which known causes (approx 50%) were reported as falls, stumbling against objects in the environment, or mishaps with wheelchairs. A multiplicity of such injuries, especially those not present on the extremities, is suspicious for abuse or neglect. Abrasions in particular frequently form a pattern that reflects more accu-

Table 2
Phases of Skin-Wound Healing

Singer and Clark (Wounds)	Robertson and Hodge (Abrasions)
Inflammation	Scab formation
Tissue formation	Epithelial regeneration
Epithelization	
Formation of granulation tissue	
Neovascularization	
Tissue remodeling	Subepithelial granulation and epithelial hyperplasia
Wound contraction and extracellular matrix reorganization	
	Regression of epithelium and granulation tissue

Adapted from refs. 25 and 28.

rately than bruises the configuration of the causative instrument. The microscopical dating of abrasions and lacerations, which is scientifically based on an understanding of the dynamics of cutaneous wound healing, has significant limitations *(25)*. All stages are subject to multifactorial conditions that overlap in time and are dependent on constitutional factors. For example, diabetes mellitus, which is common in the geriatric population, impairs healing *(26)*, whereas fibroproliferative disorders predispose the elder to formation of keloids and hypertrophic scars *(27)*. Recognizing these, the forensic expert is adequately prepared to make some general conclusions by considering the timing of the stages of healing *(28)*.

With regard to survival after injury, inflammation, predominantly the infiltration of polymorphonuclear leukocytes (PMN), is typically observable microscopically within 4 to 6 hours after the nearly immediate investment of serum, erythrocytes (red blood cells [RBCs]), and fibrin in the abrasion. The scab progresses after 12 hours to form a three-layered zone of fibrin and RBCs, neutrophils, and collagen, and PMN continue to aggregate over the next 12 to 18 hours. Epithelial overgrowth becomes visible between 30 and 72 hours in most cases. By 5 to 8 days after injury, chronic inflammatory infiltrates and neovascularization form granulation tissue; by 9 to 12 days postinjury, the appearance of keratin superjacent to the thickened epithelial cells is evident. Regression with remodeling of the epithelium is usually underway by day 12. Cautious application of these brackets of time allows the expert to offer reasonable generalizations about the time elapsed since injury (Table 2) *(25,28)*.

Table 3
Phases of Fracture Healing

Phase	Time after injury	Features
Initial repair	Up to 24 hours	Tearing of periosteum and endosteum with enlarging fibrin-laden hematoma.
Inflammation	24–48 hours	Edema, fibrin, population of neutrophils followed by macrophages.
Granulation tissue	48 hours–days	Proliferation of fibroblasts and mesenchymal cells → granulation tissue; demarcation of osteocytic necrosis from live bone; exuberant periosteal and endosteal cell proliferation.
Incipient callus	Days–1 week	Periosteal collar and capillary ingrowth in hematoma; osteoclasts → bone trabeculae;chrondroblasts → cartilage.
Callus	2–3 weeks	Osteoblasts → bone matrix and calcification → immature "woven" bone.
Hard callus	3–4 weeks	Periosteal/endochondral ossification.
Healed new bone	> 4 weeks	Remodeled immature bone → mature bone.

Adapted from ref. *17*.
PMN, polymorphonuclear leukocyte.

Fractures are lacerations of bone leading to structural splitting, separation, or compression. A host of aging changes, including cancer and prolonged immobility, make the bones of the elder more fragile and fracture-prone. Pathological fractures from cancer may occur at any skeletal site. Common age-related fractures occur in the hips and wrists from falls. Osteoporotic women commonly experience vertebral and hip fractures. Sites of fractures that raise suspicion of abuse fall into two main categories: mid- and lower facial fractures (fractured or avulsed teeth, and fractures of the mandible and zygoma) *(29)*, and spinal and truncal fractures *(30)*. Spiral fractures and those caused by rotation are highly suspicious for physical abuse *(16,31)*. Ante- and postmortem radiographs facilitate the detection, type, and age of fractures, and assess bone density. In regard to timing, the same general principles and cautions that are applicable to evaluation of skin wounds also apply to the histological examination of fractures as healing progresses. In adults, the principal dependent variable is the state of nutrition of the victim. DiMaio and DiMaio *(17)* present the consensus view of the phases of fracture healing as given in Table 3.

Cutaneous bite marks are a special form of blunt force injury. They consist of abrasions, contusions, lacerations, impressions (or any combination thereof), represent a patterned injury (prototypically circular or oval separate, curved arches in apposition that approximate the size and shape of the biting surface of the front teeth), and are commonly inflicted by humans, cats, or dogs *(32)*. The forensic significance of human bites is that they are often a component of violence and abuse, even though they infrequently cause significant morbidity or death. It is beyond the scope of this article to cover the specialty of forensic dentistry. Properly evaluated and documented bite marks on the elder's skin permits the forensic odontologist to assess the concordance between the patterned injury and the suspect's dentition for purposes of exclusion or inculpation. Reference to this kind of specialist underscores the necessity for a multidisciplinary collaboration in the investigation of suspected elder abuse.

Another potential marker of elder abuse involving the head is "traumatic alopecia," which exhibits an atypical pattern of hair loss associated with abnormalities of the scalp *(33)*.

Resorting to mechanical restraints, such as Posey vests or various kinds of ankle and wrist binding, prompts suspicion when there is no reasonable evidence to believe that such an effort to control the elder's behavior is indicated to avoid significant harm. Further, restraint without prior medical evaluation only heightens suspicions of abuse or neglect. Physical restraint in the absence of an emergency or life-threatening situation is significantly associated with injury and death. The frail elder is susceptible to getting entrapped in thorax/chair restraints and succumbing to positional asphyxia, hanging, or thorax compression (Fig. 1) *(34,35)*. Patterned blunt skin injuries of the thorax and neck, including a cervical ligature furrow created by the strap qua noose, correlate well with such a predicament. Physical markers of restraints include patterned blunt force injuries—primarily abrasions or contusions—encircling the wrists or ankles that form corresponding scars upon healing. Immobility from restraint predisposes to the formation of decubitus ulcers (*see* Section 4.5.).

2.2.1.2. Burns

Although it is clear that elderly persons older than 65 years of age die from complications of burns at a rate well above the national average, the relationship of this kind of injury to elder abuse is less clear in comparison to historical evidence-based patterns of child abuse. Burns ascribed to abuse are frequently present on the palms, soles of the feet, and hips *(36)*. At least two retrospective studies suggest that burns represent a marker of neglect or abuse of the aged, ranging from 36 to 70% of cases *(37,38)*. White recommends that

Fig. 1. Accidental positional asphyxia secondary to chest compression from partial body trapping under bed railing of nursing home's bed.

nurses regard burns in the elderly (Fig. 2) as suspicious for abuse *(39)*. In rare cases, thermal body burns or scalds may mimic decubitus ulcers (Fig. 3) and may need to be distinguished in assessing whether abuse has occurred *(40)*.

2.2.1.3. Sexual Abuse

Sexual abuse is reported in less than 1% of all elder abuse cases *(4,5,13)*. Any contacts with the genitalia, mouth, or anus are considered sexual molestation *(14)*. The acts of kissing, fondling, or improper handling during grooming are included in this definition. Elderly who are incapable of rendering consent because of weakness or various cognitive deficiencies more commonly fall victim to sexual abuse *(2)*. The diagnosis of a sexually transmitted disease, primarily among elder women, is suspicious for abuse. Mucosal contusions of the

Fig. 2. Elder with **(A)** second-degree immersion burns to the left proximal second through fifth digits and **(B)** bullous second-degree burn on the left inner foot. This elder had bilateral mirror-image burns of both feet and ankles.

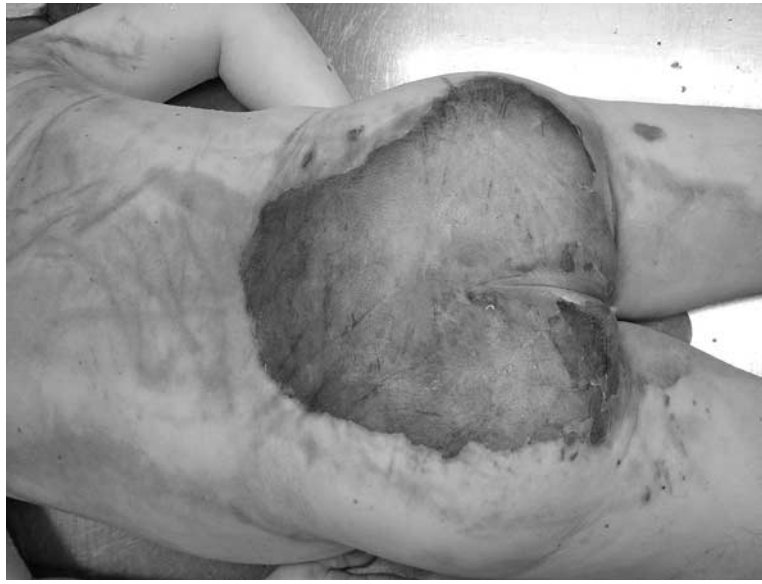

Fig. 3. Autopsy appearance of a scald over the sacral and gluteal region of an 89-year-old man. The scald was caused by application of hot water by a male nurse to the demented patient who was institutionalized in a senior citizen home. The patient died 2 days later from septic multiple organ failure. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

palate and uvula are consistent with nonconsensual fellatio *(29,41)*. Cutaneous and mucocutaneous lacerations, abrasions, and contusions of the anogenital regions likewise are findings highly suspicious for abuse *(42)*. The prevalence of urinary tract infections among older women unrelated to geriatric physiology may indicate abnormal sexual contact *(43)*.

2.2.2. Elder Neglect

Neglect of the elderly may be intentional (active) or unintentional (passive). Intentional neglect is the deliberate or willful failure to fulfill legal or contractual responsibilities in order to punish or harm the elderly. These may include withholding items of necessity, such as bathroom privileges, food, or medication. Unintentional neglect is commonly attributed to the physical or psychological impairment of the caregiver, who is in fact incapable of providing proper care to the elderly. Passive neglect may also result from the caretaker's ignorance or lack of skills. Overall, neglect of any kind usually results in the inability of the elderly to properly thrive in a community. Without objec-

tive documentation and evidence-based interpretation of findings, it may be difficult to meet the legal or administrative standards of proof for establishing the seeming paradox of "intentional neglect" *(2,3,15)*.

Abandonment, a type of neglect, is the desertion of the elder by the person who is responsible for providing care. Desertion of the elder may occur in various settings, such as in the person's own home, in a care facility, or at a public location *(7,44,45)*.

Limitations in activities of daily living (ADLs) often necessitate long-term elder care and dependency by the disabled elder on other individuals *(46)*. Elders who are not able to perform ADLs are more often neglected, especially in cases of an inherent problem with eating. Those elderly who cannot perform ADLs are more likely to sustain physical abuse *(2)*.

2.2.3. Nonphysical Abuse

Nonphysical abuse includes psychological humiliation, financial fraud, and violation of the elder's rights. These less-recognized forms of elder maltreatment practiced by the perpetrator(s) are frequently difficult to establish and prosecute.

2.2.3.1.Psychological Humiliation

The psychological abuser intends to cause emotional pain to the elder, and this form of abuse often accompanies physical abuse. Verbal humiliation and threatening to abandon or institutionalize the elder are important forms of psychological abuse *(3)*. Humiliation, nonverbal threats or insults, and verbal insults are frequent components of psychological or emotional abuse *(2)*. Any type of mental anguish in the elder caused by inappropriate caregiver conduct is also classified as psychological abuse *(13)*.

2.2.3.2. Financial Fraud

Misappropriation of money or property by the caregiver or outside "con artist" is the prime example of material or financial exploitation in the elderly. Changing of wills or other legal documents and financial coercion are other examples of material exploitation *(3)*. Financial fraud also consists in lottery, sweepstakes, and home-improvement scams. Many elders will basically "buy friendship" by providing financial gain to the person who willingly takes the elder's money in return for companionship *(47,48)*.

2.2.4. Violations of Rights

The miscellaneous category proposed by NARCEA comprises medical abuse, abandonment, and various violations of rights *(2,14)*. Examples of vio-

lations of rights are denial of an individual's participation in any decision-making processes pertinent to health, personal issues, or marriage, and may also include denial of the inviolable right to privacy.

The ratio of male to female victims of abuse is 1:1. However, the frequency of reporting injuries derived from the abuse is more common in females *(2)*. In a 10-year retrospective review of both living and deceased victims of abuse, the investigators concluded that more males were victims of neglect, as contrasted to the higher number of female victims who experienced physical and sexual abuse *(49)*.

Another broad classification of elder maltreatment distinguishes among domestic elder abuse, institutional elder abuse, and self-neglect or self-abuse *(2,14)*. Domestic situations of abuse refer to cases where the elder is mistreated—either in the elder's home or in the home of the caregiver—by someone who has a special, close relationship with the elder, such as a spouse, child, other relative, or friend acting as caregiver. Institutional abuse occurs in various residential facilities: extended care facilities, nursing homes, or board and care facilities. The perpetrators are institutional and have a fiduciary or contractual obligation to care for the elder in that facility *(47)*.

2.3. Differential Presentations: Mimics of Elder Abuse

2.3.1. Self-Neglect

Self-neglect refers to the elder who lives alone in the community and is not able to provide for his or her mental health *(3)*. Self-neglect or self-abuse occurs when the presumptively competent elder refuses assistance with his or her ADLs, despite knowing the consequences. Self-neglect applies whenever the inaction threatens the patient's safety or health *(2)*. Nonspecific but typical signs and symptoms of self-neglect include poor personal hygiene, dehydration and malnutrition; hazardous, unsanitary, or unclean living conditions, lack of specific medical aids that help with ADLs, inadequate housing, or homelessness *(47)*.

Syllogomania, or hoarding behavior, is an obsessive-compulsive disorder that is subsumed within the self-neglect typology. Specifically, dysfunctional hoarding relates to the apparent incapacity to make a decision to discard "trash." Typically, the home is filled with trash to the point where the environment is unsafe to inhabit *(50,51)*. In a National Elder Abuse Incidence Study (NEAIS) from 1996, surveying American elders 60 years of age or older representing 20 US counties, found that 101,000 experienced some form of self-neglect. This study also estimated 450,000 of the sampled elders to be victims of elder neglect or abuse *(7)*.

2.3.2. Chronic Diseases: Age-Related Morbidities and Mortality

Neglect and subtle signs of abuse among the aged are often difficult to diagnose because of the common presence of chronic or debilitating diseases *(14,52)*. Ten percent of elders suffer from various types of degenerative or debilitating diseases. Approximately 80% of all elderly persons 65 years of age or older in the United States have at least one chronic condition, and about 50% have at least two. Diabetes mellitus affects one in five (18.7%) of these individuals. The prevalence of Alzheimer's disease and other forms of dementia doubles every 5 years after 65 years of age *(48)*. The inevitable and possibly untreatable aspects of chronic illness in the aged may be confused with neglect. Common manifestations include urinary or fecal incontinence, delirium—which may be either organic or caused by insufficient or excess drug-administering patterns—and dementia-related behavioral disorders *(15)*.

3. Participant Characteristics and Risk Factors for Elder Abuse and Neglect

Familial ties to elder maltreatment occur in 90% of cases. In two-thirds of these cases, the perpetrator is typically an adult child or the spouse of the elder *(53)*.

3.1. Characteristics of Elders at Risk

Both progressive inability of the elder to perform ADLs and cognitive impairment are the two critical factors predisposing to elder maltreatment *(13)*. In addition, elder victims of maltreatment may have one or more of the following characteristics: bowel and/or bladder incontinence, minimal social interaction, and residence in close proximity or shared living arrangement with the abuser *(2,3,54,55)*. Other risk factors for elder maltreatment include low level of education, history of prior domestic violence, and limited access to local resources, such as governmental aid. Another factor predisposing the elder to mistreatment is that many elderly persons prefer to live in the situation of abuse or neglect rather than be sent to a nursing home *(13)*. Conversely, epidemiological surveys do not implicate the elder's economic, educational, or religious background or the elder's use of alcohol as risk factors for maltreatment or abuse *(2)*.

3.2. Characteristics of the Abusive Caregiver

Risk factors specific to the caregiver include substance abuse and alcoholism, psychiatric and personality disorders, and financial, legal, and emo-

tional dependence on the elder *(3,46)*. A history of violence in the family, particularly the spouse, is a higher predictor of abuse toward the elder. Opportunities for conflict occur in shared living environments *(3)*. The "caregiver-stress hypothesis" postulates that the caregiver develops stress and resentment from the day-to-day chronic care of the disabled elder. The financial and emotional dependence of the caregiver on the patient is significantly correlated with abuse. The relative proportion, namely the relationship of the caregiver/perpetrator and abuse to the elder, has been reported as follows: children in 16 to 55%; spouses in 14 to 58% of cases; other relatives in 6 to 15%; and nonrelatives less than 5%. *(2,46)*. Perpetrators of sexual abuse operate in various settings. Some abusers are affiliated with a long-term care institutions (commonly, other patients who have underlying psychiatric disorders or dysfunction, or institutional personnel), whereas others in the domestic situation are friends or family members *(2,49)*.

Elderly residing in nursing homes averaged 1.5 million in 1997. These patients suffered predominantly from various chronic illnesses and functional disabilities. Death in these individuals may, in certain cases, be the result of some form of elder maltreatment or therapeutic misadventure. Nursing home elder deaths usually are not autopsied medical examiner cases because the decedent has a long and complicated medical history that can be linked to the death *(56)*.

4. Reporting Issues and the Medical Examination

In the United States, recognition and reporting of elder abuse has been minimum, at best. With increased attention and education to elder maltreatment by law enforcement, health care providers, prosecutors, and society, "at-risk" elders may have a better chance of receiving the necessary medical or psychological care and financial aid.

4.1. Health Care Provider Reporting

Elder mistreatment is under-recognized by physicians. Less than 10% of elder abuse cases are reported by clinicians. A few states have voluntary reporting laws; however, most have mandatory reporting laws *(2)*. Clinicians are less inclined to report elder abuse for a variety of reasons: denial of the maltreatment by the adult patient, the physician's lack of knowledge about the jurisdictional statues mandating a report of suspected abuse, the evaluator's fear that a report will result in the irretrievable breakdown of the relationship between the family and patient, the desire to avoid court appearances, and the frequent diagnostic confusion in differentiating between neglect and/or abuse and natural

disease states. Tied to this mindset among clinicians is the current absence of easy-to-use protocols or screens to aid in the diagnosis of elder maltreatment *(15)*. A detailed patient history and a separate account of the illness provided by the caregiver are crucial for the diagnosis of elder maltreatment. Physicians may be the only outside connection to the elder or the caretaker. As the initial and sole evaluator of the elder's status, the physician is best situated to make the diagnosis *(13)*. As part of the evaluation, physicians are required to have sensitive interviewing skills in asking the elder patient about abuse even if there is no evidence of injuries or psychological changes to suggest it *(3)*.

Elder mistreatment is most often identified during routine office visits. However, subtle or obscure presentations of elder maltreatment may prevent health care professionals from interpreting or reporting their physical or psychological findings. These subtle findings include poor hygiene, disorderly clothing, dehydration and malnutrition, or decubitus ulcers *(13)*. Common elder complaints include failure of self-care, dehydration, and falls. Such complaints are etiologically nonspecific, but should raise suspicion in physicians administering to the aged population. As any of these complaints can suggest either self-neglect or general neglect and abuse, prudence requires that mistreatment be high on the initial differential diagnosis *(2)*. Physicians must be in tune with the wide scope of elder abuse and neglect spanning ethnic and socioeconomic groups *(15)*.

The elder population utilizes emergency medical services twice as much as any other age group. In one urban study, the elderly comprised 22% of all emergency department (ED) patients *(2,57)*. Precisely at this critical time, the ED physicians have the opportunity to evaluate "accidental injuries" in older adults and determine whether the trauma may have resulted from neglect, physical abuse, or self-neglect *(2)*.

4.2. Legal Aspects

In the United States, all physicians and health care providers must report suspected elder maltreatment mandated by law. APS must be alerted when elder maltreatment is suspected. If such a service does not exist, the state's Office of Child and Family Services can be contacted. Such notification sets in motion the process by which a social worker investigates the case *(13)*. In the United States, APS enlists the assistance of volunteer reporting of suspected abuse or neglect to state authorities *(3)*. The law and definition of elder abuse vary in either minor or significant ways among the respective jurisdictions *(9)*. Multidisciplinary teams effectively communicate issues of elder maltreatment among the geriatric physician, forensic pathologist, social workers, case managers, nurses, legal experts, members of APS, senior advocacy volunteer groups,

Table 4
Elder Mistreatment Screening Questions

GENERAL SITUATIONAL
- Do you feel safe where you live?
- Are you having difficulties with the living situation?
- How are you getting along with your _____?
- Are you getting out with your friends?
- Are you afraid of _____?

SPECIFICS ABOUT INJURY
- Has anyone hurt you?
- Does anyone hit you?
- Has anyone touched you (sexually) without your consent?
- How and when did it happen? Is it still going on?
- How often has this type injury occurred?
- How much time has passed since injury and treatment?

GENERALIZED HEALTH AND WELL-BEING
- What kinds of food are you eating and when?
- Do you see a doctor regularly?
- Are you taking your medicines as prescribed?

FINANCIAL
- What are the specific living arrangements at your home?
- Who handles your finances? i.e., check book?
- Has anyone taken something that belongs to you when you did not want them to take it?

SPECIFICS ABOUT ACTIVITIES OF DAILY LIVING
- Are you able to get around your environment without any assistance?
- If not, do you have help? If so, who helps you around the house?
- Do you pay them to help you?
- What are you missing at home that would make it easier to get around? Why don't you have this?

EMOTIONAL AND PSYCHOLOGICAL STATUS
- Do you have frequent disagreements with your _____? If so, what happens?
- Are you often alone?
- Have you ever been with anyone who did not provide care for you when you needed it?
- Have you been threatened or scolded?

For further details, *see* refs. *2,3,7,13,15*.

including the Ombudsman, and other local government interest groups *(13)*. The Older Americans Act of 1976 established the Ombudsman Program in nursing homes and provides for standards and methods to address facility maltreatment. This program affords anonymous avenues for the elder resident to file complaints in nursing home *(2)*.

4.3. Clinical Forensic Medicine: Evaluation in Living Patients

4.3.1. History

In the living patient, a thoroughly painstaking history is an indispensable tool in elucidating mistreatment. Interviews of the elder and the caregiver should be done separately. As part of a complete history, posing simple, direct, nonjudgmental questions in a neutral, private, and relaxed setting is often more conducive to eliciting specific complaints. Many elder victims do not want to volunteer any information about their maltreatment owing to shame, fear, and love for the abuser. Open-ended questions allowing adequate time for the elder to respond is the most effective way to glean information (Table 4). Direct questions should be asked during the office visit. Specific inquiries about the elder's daily activity level, medications, social encounters, and the list of daily meals are helpful in accessing the overall functioning. Questions about care rendered by the nursing facility and staff are also acceptable *(3,13,15)*.

In 1992, the American Medical Association published diagnostic guidelines with questions directed to all elders pertaining to abuse and neglect, irrespective of whether physical findings were present *(2,6)*.

Legal documentation for possible criminal neglect or abuse must be complete and accurate. Photographs together with indicated laboratory and radiographic examinations are all components of a thorough documentation. Laboratory tests should include a complete blood count, coagulation profile, and blood chemistries, including blood urea nitrogen, creatinine, calcium, total protein, and albumin. If old, healing, or new fractures are discovered, images obtained either by X-ray, computed tomography, or magnetic resonance imaging, alone or in combination, are necessary features of the diagnostic process. These require clinicians or radiologists skilled at interpretation that differentiates between natural disease processes and trauma consistent with neglect or abuse *(13,41)*. Toxicological examination of blood and urine, coupled with hair and fingernail analysis, may be utilized for possible drugs or toxins. Appropriate therapeutic drug monitoring may uncover undermedication, which can prompt investigators to determine whether the caregiver withheld or misappropriated the patient's drugs. Evidence of overmedication may point to efforts by the caregiver to calm down or even sedate the victim with tranquilizers *(41)*.

In some cases in which the physician identifies maltreatment, the patient refuses to allow the doctor to report the abuse by virtue of the competent patient's right within the doctor–patient relationship *(13)*. The abused elder may not seek medical care or other types of governmental assistance from fear that any type of assistance may show that he or she exhibits signs of abuse. Further, pursuant to state laws mandating reporting, their injuries will be subject to further investigation, which may terminate the typical home situation *(8)*. The competency or capacity of the patient must be established in these situations (*see* Section 4.3.2.). If the elder is competent to make decisions, he or she may refuse to proceed with further intervention by law enforcement. If the elder is deemed incompetent to make decisions, all jurisdictions authorize the judiciary to declare the elder a guardian of the state so that appropriate actions for care and oversight of the elder can be implemented. Part of this is effectuated through notification of and intervention by local and state social services agencies *(13)*.

4.3.2. Psychological, Neurological, and Cognitive Evaluation

Victims of abuse may have diminished capacity—the lack of the ability to make and communicate informed and rational decisions reflecting an understanding of the attendant risks and benefits. Capacity may be both situational and transient. Elders with dementia or mental illness may retain some degree of capacity, the assessment of which is time-consuming and requires skill by the evaluator. Approximately 50% of elder nursing home patients exhibit some form of dementia *(56)*. The Minnesota Multiphasic Personality Inventory and the Wechsler Adult Intelligence Scale are helpful in further evaluation of possible elder abuse victims. The Zung Self-Rating Depression Scale, the Hamilton Rating Scale, Yesavage Geriatric Depression Scale, the Cambridge Mental Disorders of the Elderly Examination, the Beck Depression Inventory, and the Popoff Index of Depression all may be used in the neuropsychological evaluation of the victims. The Michigan Alcoholism Screening Test (geriatric version) may also be helpful in identifying alcoholic victims or alcoholic caregivers *(41)*.

4.3.3. Physical Examination and Laboratory Studies

Paramount to the differential diagnosis of abuse or neglect is the examiner's high index of suspicion. At the outset the physician must be aware that abuse occurs in all races and spans all socioeconomic groups *(14)*. Inconsistency between the physical appearance and pattern of trauma and explanations offered for creation of the injuries is a strong indicator of the fact and, more specifically, type of elder abuse. Hallmark presentations highly suspicious for

neglect or abuse include delays between injury and medical intervention, exemplified by healing fractures of the extremities and ribs and cutaneous lacerations healing by secondary intention at the time of examination *(3,14,25)*. Although physical signs do not always yield conclusive diagnosis of abuse, the following examples heighten the index of suspicion: bilateral or parallel injuries of the extremities, which suggest control marks or forceful restraining; burns or scalds on the soles, palms, or buttocks sustained during hot water baths; and poorly treated, extensive, deep, or purulent decubitus ulcers *(2,3,14)*.

In summary, the clinician having the first opportunity to examine the elderly patient has a host of historical, physical, psychological, and laboratory factors to weigh in assessing whether abuse is present: frequent visits to the ED for chronic debilitating disease without proper care; signs and symptoms attributable to lack or misadministration of medicines; injuries or illnesses that are not explained adequately by the patient or the abuser; and levels of drugs—whether subtherapeutic or toxic—that are inconsistent with the reported care provided *(3,14)*.

4.4. Forensic Autopsy

Autopsy is the final opportunity to make the diagnosis of elder abuse *(14)*. Physical findings at autopsy, coupled with appropriate investigation and laboratory studies, frequently overlap with those observed in the clinical setting (Table 5). At autopsy or in the clinical setting, the physical findings cataloged in this table should be explained and ruled out from possible sources of abuse or neglect *(3,41)*. Many postmortem studies that make up the forensic specialist's armamentarium in the diagnosis of elder maltreatment are available and should be used, depending on the nature of the case: appropriate imaging studies, histopathological examination, and laboratory analysis (vitreous humor chemistry, toxicology, which may include organs in addition to fluids, and microbiology) *(58,59)*.

4.5. Medicolegal Interpretation of Decubitus Ulcers

If suspected by the domestic or institutional caregiver, decubitus ulcers (pressure ulcers/pressure sores)—especially those that are large, foul smelling, and necrotic—are not difficult to observe and diagnose (Fig. 4). Because they constitute a usually treatable, yet potentially lethal, condition in the elder, the medical forensic specialist must have expertise in understanding the pathology and pathophysiology of decubitus ulcers in order to proffer evidence-based opinions on the potential causal connection to morbidity and mortality *(60)*. Such opinions play an indispensable role in evaluating quality of care with significant impact in both civil and criminal forums.

Table 5
Physical Findings in the Clinical Forensic Setting or at Autopsy

GENERAL
- Overall hygiene
- Weight and height
- If necessary, possible anthropometric measurements for body mass index

GENITOURINARY
- Ecchymosis of vulva, anus, or scrotum may indicate forced sex
- Vaginal fistula
- Sexually transmitted diseases

HEAD AND NECK
- Ecchymosis of uvula, hard and/or soft palates may indicate forced oral sex
- Poor dentition including tooth fractures or ill-fitting dentures may suggest neglect
- Retinal detachment, orbital fractures, or traumatic cataracts may suggest chronic abuse
- Subconjunctival hemorrhage may suggest acute trauma including possible strangulation
- Neck abrasions or contusions may suggest attempts of strangulation
- Traumatic alopecia
- Closed head injuries

SKELETAL
- Acute or occult rib fractures
- Fractures of long bones may occur secondary to decreased bone tensile strength in immobilization, Sudeck's atrophy (reflex sympathetic dystrophy), osteoporosis

TORSO
- Acute or occult bruises of the chest, abdomen, or back
- Pneumothorax
- Rupture of the spleen, lacerations of the liver, and/or free intraabdominal blood may suggest abdominal trauma
- Bronchopneumonia
- Unhealed decubitus ulcers with infection or infestation with maggots

EXTREMITIES
- Restraint associated ligature marks that may include abrasions, lacerations, or bruises to the wrists or ankles
- Burns, lacerations, abrasions, bruises, especially if patterned, may suggest blunt force trauma injuries
- Immersion burns may be clearly delineated (must differentiate between trauma and natural disease state, such as Raynaud's phenomenon, digital ischemia, or vitamin C deficiency

Modified from refs. *3* and *41*.

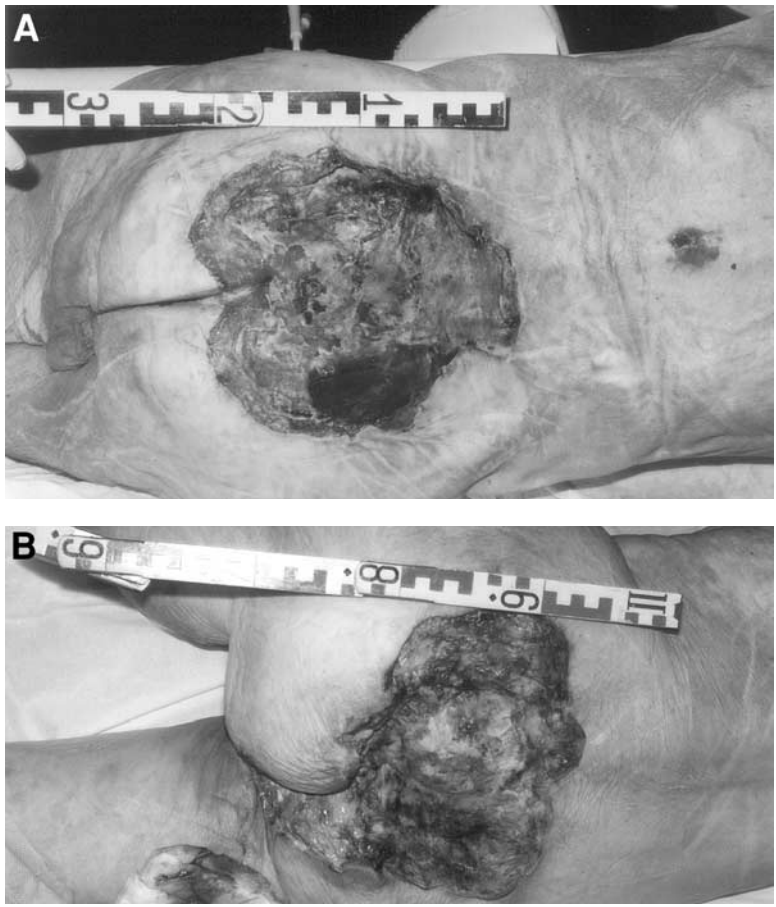

Fig. 4. Gross appearance of decubitus ulcers at external examination. **(A,B)** Extensive grade IV decubitus ulcers on the sacrum with inflamed wound margins and large zones of necrotic debris indicating neglect/maltreatment prior to death. **(C)** Grade III decubitus on the sacrum showing inconspicious, neatly excised wound margins according to surgical intervention shortly before death. **(D)** Grade IV decubitus on the trochanter. **(E)** Grade IV decubitus on the heel. **(F)** Grade IV decubitus on the thoracic vertebral column. **(G)** Grade III decubitus on the back of the head. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.) *(Figure continues on following pages.)*

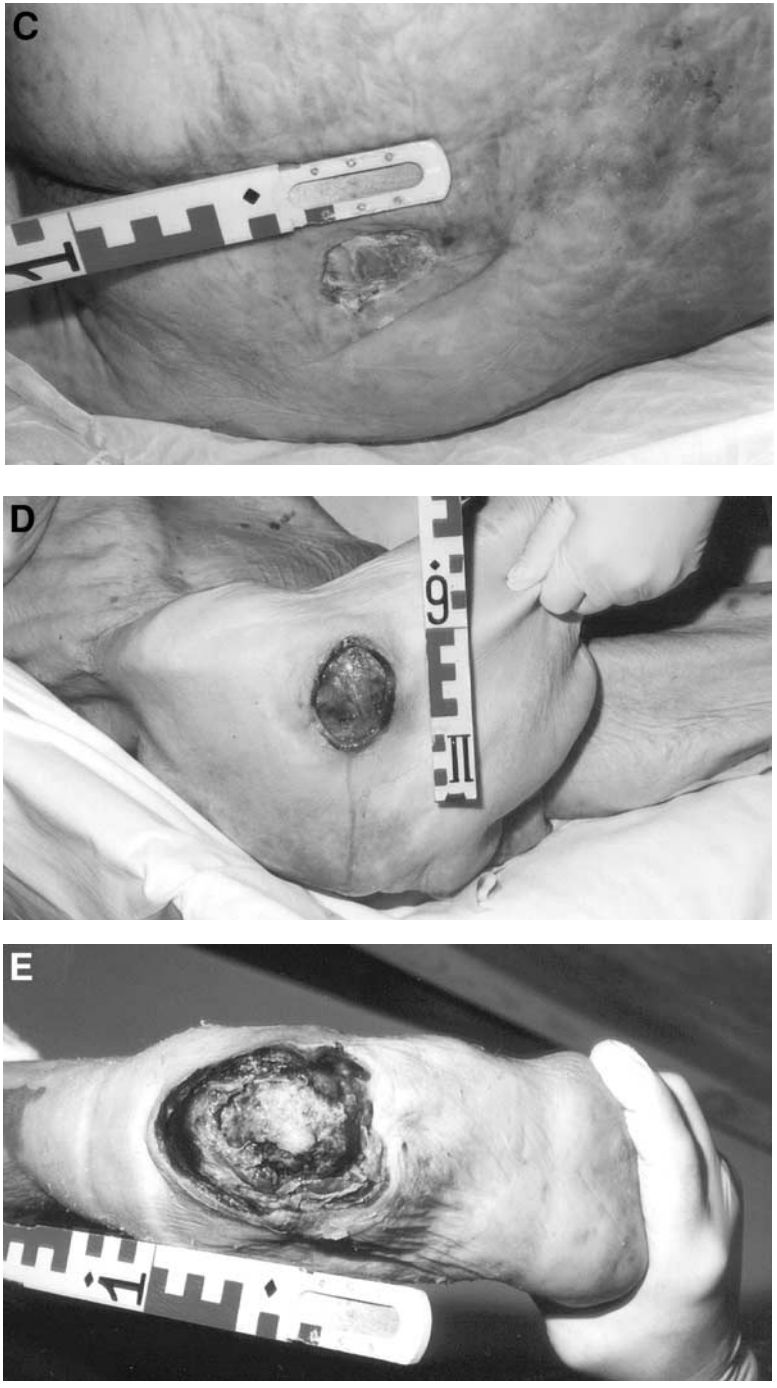

Fig. 4. *(continued)*

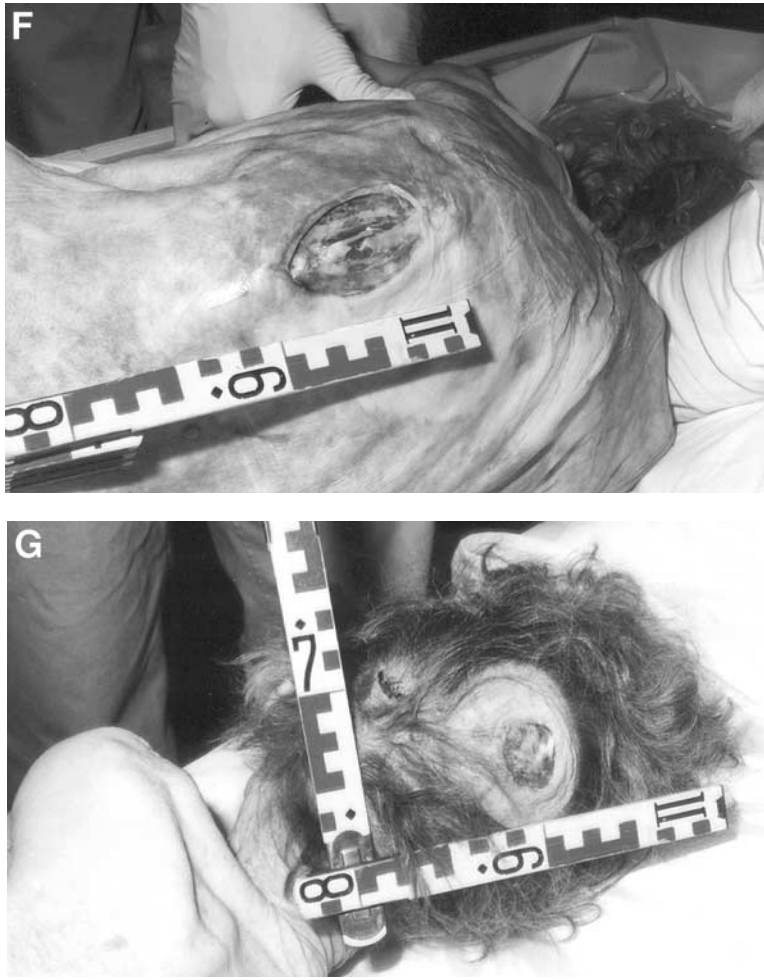

Fig. 4. *(continued)*

Various disease states, in which individuals are bedridden, ranging from quadriplegics to demented elderly, augment skin breakdown. Even round-the-clock medical care meeting current standards may be insufficient to prevent lethal infectious complications of decubitus ulcers, as exemplified by the highly publicized death in 2004 of the quadriplegic actor, Christopher Reeve *(61)*. The resultant decubitus ulcers in both appearance and extent inevitably raise questions about the quality of health care, neglect, and mistreatment. Conventional

wisdom among many physicians and other health care providers has held for many years that the development of decubitus ulcers, especially in the elderly patient in a long term care setting, is inevitable—a consequence of "normal" disease processes. In practice, this has come to mean that, for example, an infectious complication of decubitus ulcers is regarded as a feature of natural disease processes. Accordingly, the manner of death on the death certificate is recorded as natural. With increasing recognition of, and ever-improving treatment for, this type of potentially lethal chronic skin ulcer, however, decubitus ulcers and their complications are frequently, but not inevitably, preventable. As a result, it behooves clinicians and medicolegal death investigators, especially forensic specialists, to diagnose accurately the type of chronic ulcer, to understand the pathophysiology of their formation, and to determine whether the quality of care met civil or criminal standards. This applies particularly to the judgment of forensic pathologists, who must opine whether the fatal outcome was not the consequence of natural causes, but rather caused by neglect or malfeasance by someone other than the deceased (e.g., the caregiver failing in the legal duty to prevent the contributory cause of death, the decubitus ulcer). In some instances, the manner of death may be appropriately regarded as unnatural. If classified as an accident, this may raise questions of medical malpractice. In other circumstances, the manner may be correctly assigned as homicide and therefore lead to criminal prosecution *(62)*. As the geriatric cohort continues to increase, such issues will assume an even greater role in the practice of forensic pathology.

The geriatric population is particularly susceptible to the development of chronic cutaneous ulcers, which may occur in any setting of care. Health care providers need to understand the pathophysiology of decubitus ulcers, not only for proper diagnosis and treatment, but also as a method for ruling out those resulting from abuse and neglect. Takahashi et al. *(63)* provide a useful scheme in differentiating the major types of geriatric-associated chronic ulcers (Table 6).

Considerably less common forms of chronic ulcers are those caused by vasculitic small-vessel disease and various dermatological disorders *(63)*. Reference to the correct differential diagnosis is fundamental to the examiner in determining whether the chronic ulcer is related to some form of elder abuse.

It is equally significant to recognize in the differential diagnostic process that some skin wounds are "mimickers," appearing to be decubitus ulcers when in fact the wound resulted from another pathophysiological insult. Tsokos reported the case of an 85-year-old patient with severe peripheral artery disease dying shortly after hospitalization. Clinically, death was ascribed to sepsis from decubitus ulcers. Thorough medicolegal investigation, which included review

Table 6
Differentiation of Chronic Ulcers

Type	Cause
Decubitus ulcers (pressure ulcers)	Prolonged compression of soft tissue between bony prominence and external surface
Ischemic ulcers	Diminished or no circulation to area, commonly owing to peripheral arterial disease
Venous ulcers	Lower extremity edema owing to venous incompetency, various systemic conditions, or drugs
Neuropathic ulcers	Various neuropathies; lack of sensation over pressure area resulting in ischemia

Adapted from ref. 63.
According to refs. 66–68.

of medical history and events prior to admission, autopsy, and extensive histopathological evaluation of the wounds, prompted a significant change in the cause-of-death statement on the certificate of death: complications of third-degree body burns owing to the application of an electric heating pad to his back at home shortly before hospitalization *(40)*.

Physical findings, such as poorly treated decubitus ulcers and other cutaneous conditions—including burns or nonhealing skin rashes—in the anogenital and perineal regions, may represent neglect and/or abuse *(64)*. These cutaneous lesions pose a significant source of morbidity and mortality in the elderly (as well as nonelderly, immobile, dependent adults). A sixfold increase in mortality is related to nonhealed decubitus ulcers *(65)*.

The presence of one or more decubitus ulcers on the elder requires special scrutiny. Under most circumstances, decubitus ulcers are preventible or treatable. Such findings raise a red flag requiring that neglect or abuse must be ruled out. In a study by Shields et al., approx 95.4% of postmortem neglect cases exhibited decubitus ulcers *(49)*.

There is a high prevalence of decubitus ulcers in females in the age group 80 years of age or older *(66)*. Underlying factors, such as trauma, and neurological diseases, such as senile dementia, and malnutrition, are associated with the presence of decubitus ulcers in elderly *(66)*. Decubitus ulcers in this cohort account for serious infectious complications and/or sepsis. Failure to recognize the causal link to the infectious etiology may lead to underdiagnosis and underreporting *(66)*. The grading of decubitus ulcers, which not only describes the

Fig. 5. Multiple decubitus ulcers over the sacrum and thoracic vertebral column in a case of fatal neglect of an 87-year-old female who died in a senior citizen home. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

severity, but also predicts significant morbidity or mortality, is highlighted in the classification according to Shea (*67*) (Table 7). The location of chronic ulcers is important in differentiating decubitus ulcers from other types: 69.6% of these chronic ulcers were located on the sacrum, whereas 10.9% of multiple decubitus ulcers were present on the sacrum. A combination of multiple decubitus ulcers (Fig. 5) appeared both on the trochanter and sacrum (4%), the hip and sacrum (3.2%), and the thoracic vertebral column and sacrum (2.4%) *(66)*. Ulcers in other locations, such as on the front of the legs from prolonged crossed position or on the chest from extended arm pressure, may be a marker of negligent failure by the caregiver to reposition the elder at appropriate intervals. Tsokos and co-workers (*66*) have formulated a panel of questions in reference to decubitus ulcer formation as a means of sorting out whether they resulted from willful negligence, including alleged medical malpractice (Table 8).

Medicolegally, it is important to directly link causality of fatal infectious sequelae including sepsis to the untreated or maltreated decubitus ulcer. Advanced decubitus ulcer grades include exposure of the subcutaneous tissue penetrating to muscle and/or bony tissue *(66)*. Clinically and at autopsy, the proper evaluation of the consequences of the decubitus ulcer includes macroscopical description and photographic documentation, adequate histopatholog-

Table 7
Decubitus Ulcer Grades—Macroscopical and Histopathological Features

Grade	I	II	III	IV	
Gross findings	Erythema of infected skin	Superficial dermal ulceration; Possible bullous formation; no underlying subcutaneous involvement	Bone or joint involvement with no anatomical limits; possible osteomyelitis and/or joint dislocation	Ulceration, necrosis, and associated inflammation into subcutaneous and skeletal muscle tissue	
Histopathological findings	Vascular congestion	Mixed inflammation; epidermolysis	Ulceration of epidermis with mixed inflammation and necrosis into the dermis and subcutaneous tissues; fat and skeletal muscle necrosis	Type IVA	Type IVB
				chronic osteomyelitis alone	acute and chronic osteomyelitis
Incidence	4% in group ≤70 years; 10% ≤90 years		4% in group ≤90 years combined with IV	4% in group ≤90 years combined with III	

Table 8
Tsokos Decubitus Care Questions

- Where, when, and under what circumstances did the decubitus ulcer develop?
- Was a decubitus ulcer risk calculation used on admission of the patient?
- What intrinsic and extrinsic patient risk factors were identified?
- Was prevention management adequately adjusted to the identified risk factors?
- Was the patient consistently monitored, and were skin changes in the areas at risk registered?
- If the formation of a decubitus ulcer was noted, was treatment subsequently undertaken?
- Was the institutional documentation sufficient and are there enough data for a conclusive argument?

Taken from ref. 66.

ical sampling and review of the soft and, where indicated, boney tissue, and selective microbiology of blood and other affected tissues in circumstances where artifactual contaminants (e.g., prolonged postmortem interval with decomposition) do not render the results uninterpretable. Colonization of persistent, open, and nontreated decubitus ulcers infected by various microorganisms has been causally linked to bacteremia, sepsis, and osteomyelitis. Such infectious complications have a mortality rate of up to 50% *(66)*. On gross examination it may be difficult to distinguish between subcutaneous infection in decubitus ulcers, which do not accompany bone infection, and osteomyelitis. Moreover, pressure-induced noninflammatory changes of the involved bone subjacent to the ulcer irrespective of the presence of osteomyelitis may be found with the cortical bone layer intact. Decubitus ulcer-associated osteomyelitis is reported to occur in a frequency between 17 and 58%. This may be an overestimation based on clinical diagnosis alone without the presence of a bone biopsy or osteotomy at autopsy. Severe osteomyelitis may not be found in many grade IV decubitus ulcers if sepsis is not present. Türk et al. recommend that examination of bone specimens adjacent to deep decubitus ulcers without sepsis is probably not indicated *(68)*. It is important to recognize, however, that even though sepsis and other systemic inflammatory complications may stem solely from the soft tissue in grade IV decubitus ulcers, bacteriological blood cultures and tissue cultures may not be sufficient to identify osteomyelitis *(69)*. Additional studies may be needed to diagnose and treat osteomyelitis in the clinical setting and to document it as the etiopathogenetic factor causing lethal sepsis.

The recent study of the medicolegal aspects of decubitus ulcers by Heinemann et al. showed a risk of 0.5% for developing pressure ulcers within

the general German population of 400,000 geriatric patients. This translates into the prospect that approx 50% of the patients are at risk for developing decubitus ulcers *(70)*. Approximately 50% of patients who have severe decubitus ulcers may experience periods of sepsis with associated mortality rates of 48 to 55% *(70)*.

4.6. Starvation, Malnutrition, and Dehydration as Markers of Neglect

Malnutrition and its extreme, starvation, both result from inadequate consumption of essential, nutritious foodstuffs, and are potentially fatal. Among the elderly, the most common type is protein-–caloric malnutrition. Many age-related conditions ranging from edentia and poor-fitting dentures through loss of appetite to dementia can contribute to being underweight and malnourishment. Commonly prescribed psychotropic drugs repress the desire to eat. Neurological disease causing inefficient mastication and deglutition may prompt the caregiver to either force-feed or ignore the elder who is eating. In either scenario, a lethal choking episode (a variation of the fatal "café coronary") or aspiration pneumonia may ensue *(71)*. Malnutrition *per se* can also result from caregiver neglect. Quinn and Tomita define significant weight loss as follows: 5% (below baseline) in 1 month, 7% in 3 months, and 10% in 10 months *(36)*. Body weight loss of more than 40% may be fatal. Clinically, anemia resulting from malnutrition may be a mark of neglect. Measurement of serum albumin is more specific for indicating malnutrition than weight loss alone *(72)*. Malnutrition commonly initiates a vicious cycle, predisposing to infection and skin ulcers, which in turn require more calories and protein for healing. At autopsy, in addition to assessment of body and visceral mass, the forensic specialist should document the contents of the entire gastrointestinal tract and examine the adrenal glands microscopically for stress-related changes of the cortex. In the study by Shields et al., 36.4% of the deceased patients that were considered victims of neglect were classified in the anorexic category according to determination of the body mass index (BMI) (BMI <17.5 kg/m^2) (Fig. 6). Nearly 40.9% were underweight (BMI = 17.5–20 kg/m^2) *(49)*.

Dehydration, an insufficient level of total body water, frequently accompanies malnutrition. The aged are prone to dehydration as a result of age-related alterations in control of fluid homeostasis. Illness (infection or diarrhea) and medications, such as diuretics, may explain dehydration. Nevertheless, dehydration may also be a result of caregiver neglect when monitoring fluid intake and supplying required fluid are absent. Clinical signs of dehydration may be difficult to sort out because of overlap with other conditions in the elder. Mark-

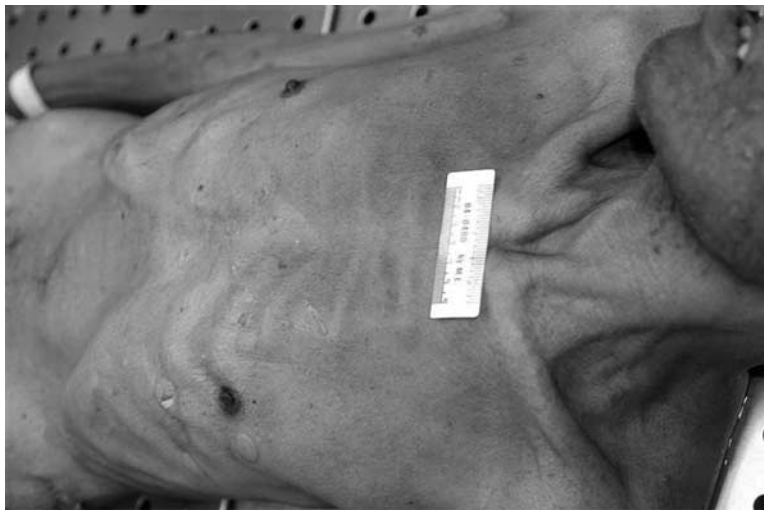

Fig. 6. Cutaneous chest retraction and scaphoid abdomen secondary to malnutrition of an elder (same case as Fig. 2). His body mass index was 14.8 kg/ m^2. The elder also exhibited hypernatremic and hyperosmolar dehydration at the time of his arrival in the emergency department.

ers at autopsy for dehydration include sunken eyes, dry mucosal, mesothelial, and serosal membranes, lack of skin turgor, and fecal impaction. Laboratory studies helpful in diagnosing dehydration include vitreous humor chemistry evidencing the classic "dehydration pattern," and toxicology for drugs that cause fluid loss or impair appetite *(17,58)*.

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Homicide

3

Homicides by Sharp Force

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CONTENTS

SUMMARY

Injuries caused by sharp or pointed objects are common. They rarely cause fatal injuries, however, and the fatality rate is estimated to be 3% at most. Most fatalities caused by sharp force are homicides. The ratio of homicide to suicide is estimated at 6:1 to 5:2. When investigating deaths owing to sharp force, the forensic pathologist is expected to give an opinion on the following points: the type of injuries; the number and anatomical distribution of injuries; the shape, size, length, and depth of injuries; the object (weapon) used; the amount of force needed to inflict the injuries; the extent of internal injuries; the cause of death; and the victim's capability to act. These points are of decisive importance for the reconstruction of the sequence of events and, thus, are essential for distinguishing between self-infliction and involvement of another party. Most homicides by sharp force are committed by males, often under the influence of alcohol. The most common tool used is a knife, but other pointed

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objects, such as scissors, ice picks, forks, or broken glass, may also be used. The victims are usually family members or acquaintances. Homicides committed by females are comparatively rare. In such cases, the victims are mostly the life partners. The scene of death is most frequently the victim's home. Fatal stabs are usually located in the precordial or cervical region. The number of stabs does not allow the drawing of conclusions as to the mode of death, the motive, or sex of the perpetrator. When the number of stabs is higher than necessary to kill the victim, this is referred to as "overkill," and may point to a strong emotional conflict between the perpetrator and the victim.

Key Words: Homicide; sharp force injury; stab wounds; overkill; clinical examination of offenders.

1. Introduction

The term "sharp force" refers to a form of mechanical impact causing injuries by means of pointed and/or sharp-edged instruments. Such instruments are ubiquitously available as objects of daily use (e.g., knives, scissors, forks, bottles or glasses, screw-drivers, ball-point pens, axes, or saws) and may cause serious or fatal injuries when used inappropriately.

Injuries caused by sharp or pointed objects are common. Their number cannot be estimated, as most of them heal without any medical treatment. Most of these injuries are domestic accidents *(1)*; fatal injuries are rare. According to Walton et al., the mortality rate is 3% without taking the anatomical location of the injuries into account *(2)*. A higher percentage (17%) was found by Webb et al. *(3)*, but in a selected study material: the authors reported on 120 victims of knife attacks who had to undergo medical treatment in Edinburgh hospitals between 1992 and 1996. Despite rapid medical intervention, 20 of them died.

Deaths caused by sharp force account for about 2% of the forensic autopsy material *(4)*, most of them homicides. Statements as to the ratio between homicides and suicides with sharp objects vary in the literature. Gill and Catanese, who systematically investigated 120 deaths occurring in New York City in 1999, found a ratio of 6:1 *(5)*. The figures quoted in the European literature range between 4:1 *(4,6)* and 5:2 *(7)*. Accidental deaths resulting from sharp force are generally very rare and are hardly mentioned in the literature *(7–10)*.

This chapter focuses on homicides resulting from the use of sharp force. General aspects of injuries owing to sharp force are discussed only on the fringe. For details, refer to the forensic pathological literature and textbooks in particular *(11–16)*.

2. EPIDEMIOLOGY

2.1. General

Although in the United States homicides are predominantly committed with guns, as these are comparatively easy to obtain *(17–22)*, the use of sharp force (usually stabbing with knives) is the most frequent method of homicide in European countries *(10,23–28)*. The share of homicides by sharp force in the total number of killings is similar in many regions. For example, Oslo, Norway, reports 27% *(25)*, Copenhagen, Denmark, 33% *(26)* and 36% *(25)*, respectively, and Geneva, Switzerland, 37% *(27)*. Outside Europe, 35% were reported for New Foundland *(29)* and 34% for Auckland, New Zealand *(30)*. A high percentage of 55% was found by Kua et al. in a study from Singapore *(31)*.

The victims of homicidal deaths caused by sharp force form a heterogeneous group without any specific constellation of characteristic features. A retrospective analysis of our own material including 82 homicidal deaths owing to sharp force injuries from 1990 to 2000 in the region south of Baden (southwestern Germany) *(32)* showed a relatively broad frequency peak for the victims in the age group between 20 and 50 years of age. The average age was 37.5 years. These figures are in line with those of other studies *(7,10,23,33)*. The sex ratio was nearly equal (52% male, 48% female). Similar sex ratios in the victims were also found by Bajanowski et al. (60% male, 40% female) *(7)*, Hunt and Cowling *(33)*, as well as Padosch et al. (50% male and female each) *(34)*. In contrast to this, only 21% of the victims were female in the study by Ormstad et al. from Stockholm, Sweden *(10)*. In other Scandinavian studies, women also accounted for a considerably lower share among the victims than men: 36% in Copenhagen, Denmark, 31% in Oslo, Norway, and 24% in Stockholm, Sweden *(23,25,26)*.

Concerning the perpetrators' ages, our study showed a peak in the age distribution between the 20th and 30th year of life with an average age of 33.9 years *(32)*. In the study performed by Hunt and Cowling *(33)*, in which 91 homicidal deaths owing to sharp force from all parts of Great Britain were investigated, the peak age of the perpetrators was observed between the 20th and 40th year of life, just as in the study by Karlsson from Stockholm, Sweden *(23)*. Among the perpetrators in our study material, males were represented with a share of 87%. Only 13% of the perpetrators were women. Similar sex distributions were also reported in other studies: Rogde et al. reported 85% male and 15% female perpetrators *(26)*, Ormstad et al. 88% males and 12% females *(10)*, Padosch et al. reported 86% males and 14% females *(34)*, and Hunt and Cowling reported 80% male and 20% female offenders *(33)*. The constellation of a female perpetrator and a female adult victim is a rarity. In some

studies, this constellation did not occur at all *(10,23)*. Hunt and Cowling reported on an attempted murder–suicide carried out by a depressive woman who stabbed her mother to death before trying to kill herself *(33)*.

2.2. Motives and the Relationship Between Perpetrator and Victim

In most publications dealing with homicides by sharp force it is emphasized that usually the perpetrator and the victim knew each other before the attack, and that, in many cases, there was an even closer relationship (life partnership) *(7,25,26,32,33)*. Homicides without a previous personal relationship between the perpetrator and the victim range between 11% *(7)* and 23% *(25)*. Consequently, the dominant motives are interpersonal conflicts. Rogde et al. *(26)* found domestic conflicts in female victims and arguments in male victims to be the most frequent causes leading to attack. Hunt and Cowling *(33)*, as well as Ormstad et al. *(10)*, expressed the same opinion. Only 5 to 8% of the cases are sexually motivated *(10,32,33)*, although the use of sharp force in sexually motivated homicides is not uncommon *(35–39)*.

A high percentage of the perpetrators are intoxicated by alcohol or drugs when committing the offense. In the study by Karlsson, only 9% of the perpetrators were sober *(23)*. In 20% of the cases the offenders had taken drugs of abuse in addition to alcohol, but the victims were also usually under the influence of alcohol and in most cases, their blood alcohol concentration was more than 150 mg/dL. Moreover, drug components were found in the blood of 22% of the victims *(23)*. Similar results were observed in the investigations conducted by Ormstad *(10)* and Rogde et al. *(26)*. In our own study material, only 15% of the perpetrators and 12% of the victims had blood alcohol concentrations above 200 mg/dL *(32)*.

3. Scene Findings

In fatalities caused by sharp force injuries, most frequently the death scene is the victim's home *(25,26,32,33)*. According to our own investigations, 56% of the female victims and 42% of the male victims were killed in their own residence. The second most frequent death scene was out in the open, where 28% of the male victims and 15% of the female victims had been killed. The high number of crimes committed in the victim's home can be explained by the fact that homicides owing to sharp force are often offenses of a domestic nature between life partners. This applies especially to the constellation of a female victim and a male perpetrator (Fig. 1). In contrast to this, offenses involving a male victim and a male perpetrator are often committed in the open or in pub-

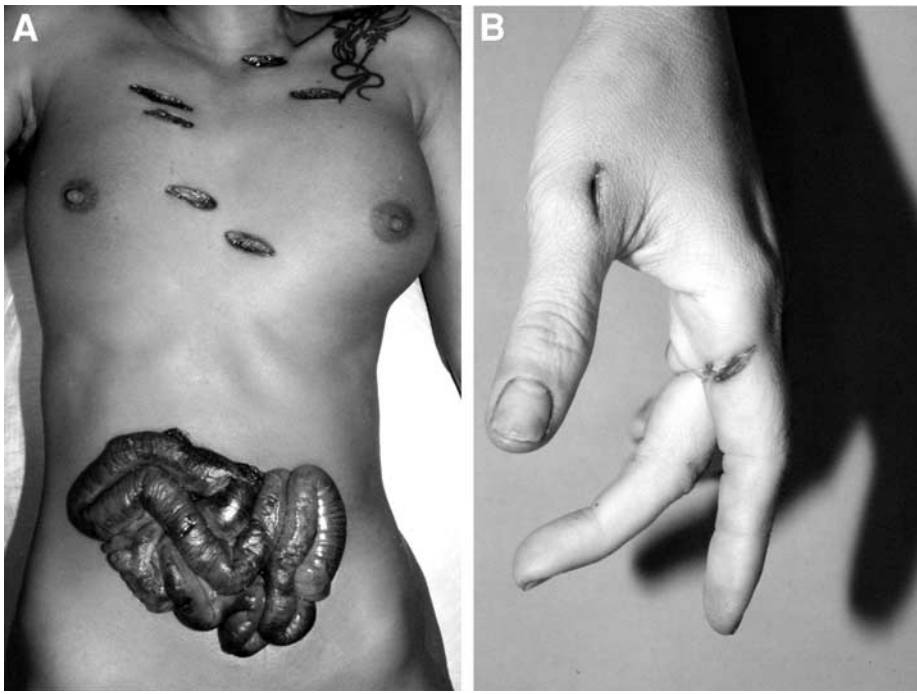
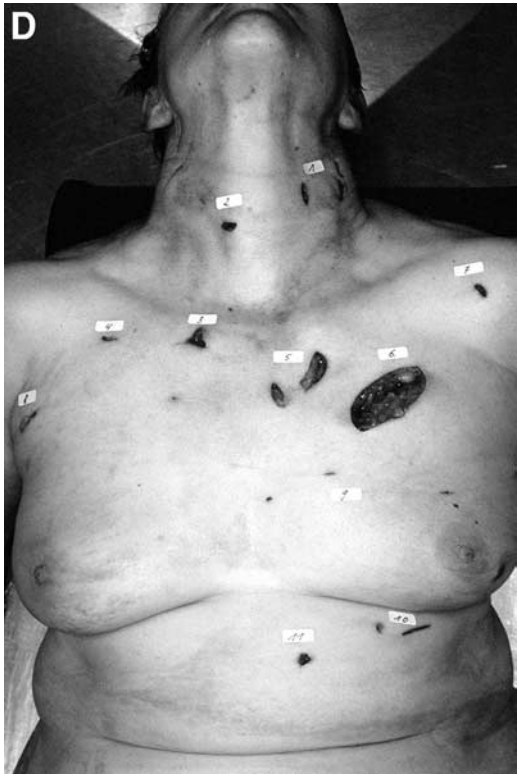


Fig. 1. Homicide of a 32-year-old woman who was killed by her jealous lover by **(A)** knife stabs to the chest, the neck, and the abdomen. **(B)** "Active" defense wounds on the left hand.

lic places. Here, personal disputes between (often intoxicated) parties are the dominant factor *(32)*.

On principle, the findings at the scene are important to determine whether death was caused by homicide, suicide, or accident (Fig. 2). The importance of a joint visit to the scene by the police and the forensic pathologist cannot be emphasized enough. The purpose of this visit to the scene is to reconstruct the sequence of events in the given case. It has turned out that, for this purpose, it may often be helpful to inspect the scene again after having performed the autopsy, as only then will the extent of the injuries and the cause of death be known. For example, for the evaluation of the bloodstain pattern, it is important to know whether and, if so, which arteries of the victim were injured and how long the capability to act may have been maintained. The analysis of bloodstain patterns is a special discipline in forensic science on which comprehensive literature is available *(40–48)*.

Fig. 2. Homicide of a 73-year-old woman killed in the bathroom by her psychotic son by multiple stab wounds inflicted by a knife. **(A)** Position of the victim at the crime scene; the woman was attacked while standing near the bathtub and fell backward. **(B,C)** Details, with remarkable bloodstain patterns, indicating that the killing of the woman was committed at the scene where the victim was found, and that she was not placed into the bathtub but fell into it. **(D)** Stab wounds on the victim's body surface. **(E)** Numerous stab wounds at the left side of the thorax and on the inner aspect of the left upper arm indicating that the left arm was elevated for defensive actions while the stab wounds were inflicted.



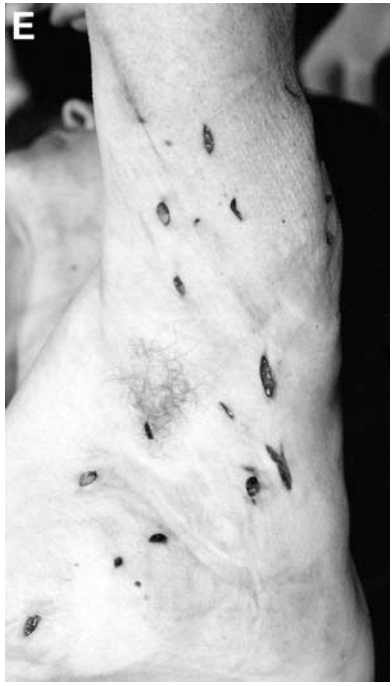


Fig. 2. *(Continued)*

4. Forensic Medical Evaluation

4.1. General

When investigating deaths caused by sharp force, the forensic pathologist is expected to give an opinion on the following points:

1. The type of injuries.
2. The number and anatomical distribution of injuries.
3. The shape, size, length, and depth of injuries.
4. The object (weapon) used.
5. The amount of force needed to inflict the injuries.
6. The extent of internal injuries.
7. The cause of death.
8. The victim's capability to act *(1)*.

These points are of general importance for the reconstruction of the sequence of events and thus are also essential for the central distinction between self-infliction and involvement of another party.

4.2. Wound Morphology

Injuries are classified according to their shape as stabs, cuts, and slashes or chops. The distinctive characteristic of a stab is that the depth of the wound is greater than its length. The wound has sharp edges, which can be brought into apposition. The instrument is pointed and hits the surface of the victim's skin at an approximately perpendicular angle. Cuts are also sharp-edged incisions on the skin that can be approximated, but here the instrument hits the skin more at a tangential angle. Cuts are longer than they are deep. They show shallow extensions at the ends of the wound and their depth varies along the course. The causative instrument is sharp-edged. Slashes are caused by "half-sharp" force. Slashing tools (axes, cleavers, etc.) are sharp-edged and often rather heavy so that the wounds produced by them contain elements of both blunt and sharp force in terms of morphology, e.g., a sharp-edged incision with an abraded wound margin. The morphology of sharp-force injuries is thoroughly described in current textbooks *(1,11,14,49,50)*. There is nothing new to be added to the existing information, therefore this chapter will not discuss morphological findings in detail.

4.3. Identification of the Object (Weapon) Used

The shape of a wound does not necessarily provide information as to the weapon used. The most frequently used object is a knife *(7,23)*. Common types

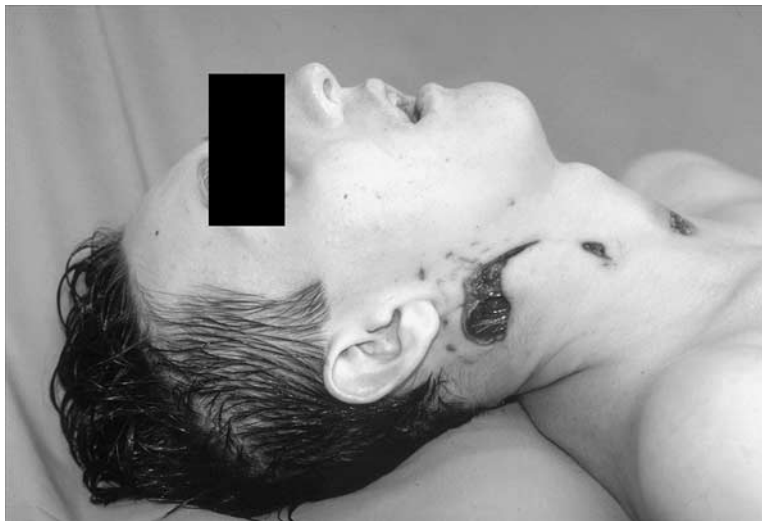


Fig. 3. An 18-year-old man who was killed by stabbing with a broken glass.

are single-edged kitchen or pocket knives with a blade length between 10 and 13 cm *(4,11,51)*. Less often, injuries are caused by pieces of glass (Fig. 3), especially broken-off bottle necks or glasses *(11,14,50,52–55)*, scissors *(11,14,50,56,57)*, screwdrivers *(11,14,50,58–60)*, ice picks *(11)*, or forks *(11,14,50)*.

Although a stab wound will usually provide information as to whether a single- or double-edged knife was used, it is rather rare that conclusions can be drawn with regard to its general shape. Differences in wound morphology are mainly the result of the dynamics of the fight (movements of the victim and the perpetrator during the stabbing) and not so much a consequence of the shape of the knife. Accordingly, caution must be employed when drawing conclusions as to the width of the blade from the length of an incision. First, the blade needs not to have penetrated into the body with its full length. Second, many stab wounds are the result of both a stabbing and cutting movement so that the length of the wound slit suggests a wider blade than the knife actually used. Moreover, the length of the wound track is not necessarily indicative of the length of the blade. First, the blade needs not to have entered the body up to the handguard or hilt. However, it is possible that the soft tissue was compressed as the blade was thrust in. After withdrawing the stabbing instrument, the pressure and the compression produced by it ceases, thus resulting in a wound track that is longer than the penetrating blade. The question of whether a blade was fully

plunged into the body can be definitely answered only if a punch mark of the knife´s handguard is discernible on the skin around a stab wound. In certain tools used for stabbing, this mark may have a characteristic shape and, thus, give a clue to the weapon used.

Injuries caused by the same knife may be shaped differently, suggesting the use of different knives. However, wounds produced by different weapons may resemble each other. Exceptions are knives with unusual shapes. An example is given by Pollak *(61)*, who reported on wound findings after the use of a survival knife (so-called "Rambo" knife). Such a knife has a serrated back which causes characteristic parallel, superficial abrasions on scraping over the skin *(61)*. If very long knives are used or if the victim is a small person, e.g., a child (Fig. 4), they may fully perforate not only the neck or the extremities, but also the trunk *(62)*. The exit wounds of such stabs must not be mistaken for the entrance wounds. If the blades of a pair of scissors were closed, injuries caused by scissors are often "Z"-shaped *(56)*. If the blades of a pair of scissors were open, the injuries may at first sight be similar to those produced by a knife. An overview of various forms of the wound morphology produced by scissors is given in *Knight's Forensic Pathology (14)*.

Generally, injuries can be assigned to an object best if the DNA (from blood or tissue) of the victim can be found on the weapon used. In those rare cases in which the tip of the knife has broken off in the victim's body after striking a bone, this part recovered from the body at autopsy can be morphologically assigned to the presumed weapon *(11)*.

4.4. Dynamics of Stab Wounds

In the forensic pathological literature there are numerous publications discussing the physical aspects of stabbing *(63–73)*; the authors tried to quantify aspects of the amount of force used (stabbing intensity) and the dynamics of the stab.

The amount of force required to inflict a stab wound depends on the type and shape of the object (weapon) used, the speed of approach, the kind of clothing and its thickness, as well as whether bony structures have to be penetrated or not. If a pointed, sharp object is used, the amount of force is comparatively smaller than with a blunt object having a rounded blade tip (such as a bread knife).

Essential resistance to a pointed, sharp object is offered by clothing, skin, and bony structures. The opinion occasionally expressed in the literature that the deeper soft tissue layers (fatty tissue, muscles) do not offer any major resistance to the stabbing object has not gone unchallenged *(67,72)*. Weber et al. *(72)*, as well as Kaatsch et al. *(67)*, were able to demonstrate in their stabbing

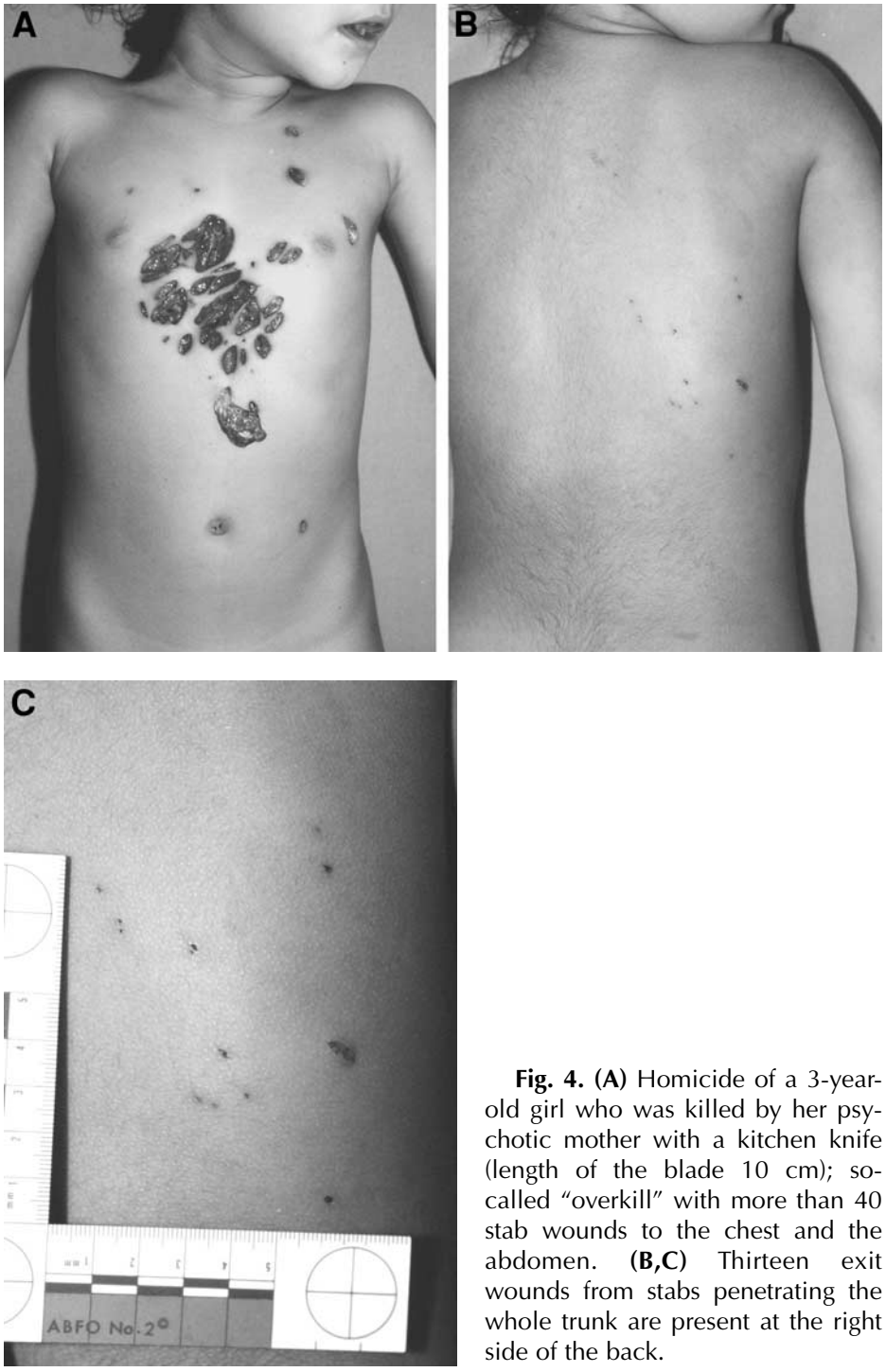


Fig. 4. (A) Homicide of a 3-year-old girl who was killed by her psychotic mother with a kitchen knife (length of the blade 10 cm); so-called "overkill" with more than 40 stab wounds to the chest and the abdomen. **(B,C)** Thirteen exit wounds from stabs penetrating the whole trunk are present at the right side of the back.

experiments that with a higher amount of force, a blade will penetrate deeper into a body or a body model. This does not mean, however, that the resistance of the internal organs or soft tissues is equal to that of clothing, skin, or bone. If no bony structures are affected and the clothing consists only of a T-shirt, a relatively small amount of force is required to inflict the injury, provided that a pointed, sharp object is used, the knife is held firmly in the hand, and the stab is effected at a high velocity. If the knife-holding hand is moved slowly towards the victim, a considerably higher amount of force will be required.

O'Callaghan et al. *(73)* investigated the amount of force required to penetrate soft tissue with a knife. For a 10-cm-deep stab through human skin, subcutaneous fatty tissue, and muscle, an average amount of 49.5 N was required. In contrast to this, investigations by Kaatsch et al. *(67)* using pig cadavers showed that for a stab through soft tissue (though with penetration of a thin textile layer), a force of 150 to 240 N was necessary. In studies performed on corpses, Fazekas et al. *(65)* found that a single-edged, normal, moderately sharp stiletto knife penetrates skin covered by a single layer of clothing at a force of 15.33 to 17.86 kp (corresponding to 150–175 N) on average. With loose clothing, the stabbing force values were higher than with tight clothing.

Weber and Milz conducted investigations on the speed of stabs *(70)*. They were able to demonstrate that adult males reached average speeds of 6.14 m per second with their dominant hand and 5.27 m per second with their nondominant hand. Female subjects reached a mean value of 4.16 m per second for the dominant hand and 3.68 m/second for their nondominant hand.

Weber and Schweitzer conducted investigations on the momentum of stabs *(71)*. Their results showed that with a single-edged bread knife (blade length 155 mm, width up to 22 mm) a momentum of 1.3 kg m per second produced a stab with an average depth of 59 mm; a momentum of 2.6 kg m per second produced a stab with an average depth of 126 mm; and a momentum of 3.6 kg m per second produced a stab with a depth of 150 mm in an intercostal space of a body.

4.5. Number and Distribution of Wounds

Homicides by sharp force can be committed by a single stab or a multitude of stabs. The share of homicides by single stab wounds varies in the different studies: Bajanowski et al. *(7)* reported an isolated stab wound only in 22% of their cases, Karlsson in 34% *(23)*, Ormstad et al. in 44% *(10)*, Hunt and Cowling in 54.5% *(33)*, and Blomquist et al. in 67% (*74*).

The number of stabs shows a certain correlation with the gender of the perpetrator *(23,32,33)*. In homicides caused by sharp force that were committed by female perpetrators, the victims had fewer stab wounds on average than

in homicides that were committed by male perpetrators. It seems that female perpetrators often inflict only one singular stab to their victim, especially if the victim is a man *(10,32,33)*. On the contrary, Karlsson found in his study material from Stockholm, Sweden, that singular stabs occurred mostly in the course of arguments between intoxicated men, whereas women who killed men usually inflicted two to nine stabs *(23)*.

Concerning their anatomical distribution, stabs are most often located on the thorax and the neck. According to the results of the study by Bajanowski et al. *(7)*, the injuries were located on the anterior left side of the trunk in 50% of the cases. In singular fatal stabs, the precordial region is strongly overrepresented. Hunt and Cowling *(33)* found the injuries to be located in the precordial region in 69% of their cases; in our own autopsy series, they were found in that location in 60% of the cases. The anatomical distribution of the stabs has a certain relation to the total number of stabs. Whereas in homicides with a single stab the chest was hit in 60% of all cases, the share of stabs to the chest decreased in inverse proportion to the total number of stabs. In the presence of more than 50 stabs only 15% were located in the precordial region. With a rising number of stabs they were distributed over all body regions. The probability of stabs in the dorsal region increases as well. Single fatal stabs in the back were found neither in our study *(32)* nor in those by Ormstad et al. *(10)* or Hunt and Cowling *(33)*, whereas Metter and Benz *(79)* reported them in 9% of the cases.

The location of the stabs does not allow for the drawing of conclusions as to the gender of the perpetrator *(32)*; in both sexes, the most common stabbing sites are the chest and the neck. No relation is found between the location of the stabs and the motive of the offense or the type of relationship between the perpetrator and the victim.

4.6. Overkill

The term "overkill" refers to the infliction of massive injuries by a perpetrator by far exceeding the extent necessary to kill the victim *(39)*. Overkill indicates that the offender was in a state of strong excitement; very often this is because of deep personal conflicts between the perpetrator and the victim that may have a long history *(10,23)*. It is also possible, however, that the victim assumes the role of a substitute and becomes the projection surface for the killer. This applies especially to sexually motivated homicides *(37–39)*. An overkill can also be the consequence of an escalating fight, for example, in the course of a robbery. Finally, it has to be considered that psychotic or psychopathic offenders sometimes commit very bizarre homicides (Fig. 4) in which there may be a large number of stab wounds inflicted on the victim *(23,62,75,76)*. An overkill, there-

fore, does not necessarily indicate that the perpetrator and the victim knew each other personally, although experience shows that killings with a multitude of stab wounds tend to have a personal background *(23)*, exceptions are not uncommon. For example, the authors had homicidal deaths in their own study material that had occurred in connection with a robbery and showed more than 30 stab wounds *(32)*. On the basis of our own investigations, no clear correlation between the motive (assignment according to the Crime Classification Manual *[39]*) and number of stab wounds could be confirmed.

A large number of injuries alone does not necessarily suggest infliction by another party *(4,77)*. Lieske et al. *(4)*, for example, reported on a suicide with 120 self-inflicted knife stabs. Although a large number of injuries are considered to be indicative of homicide, one would expect defense injuries on the victim´s body, especially in the presence of a multitude of fatal stab wounds. According to our investigations, these were always present in homicides in which the victim had suffered more than 30 stab wounds *(32)*.

4.7. Defense Injuries

Defense injuries resulting from sharp force provide specific evidence of infliction by another party and prove that the victim was, at least initially, conscious and able to ward off the assault to a certain degree. Defense injuries may occur when the victim raises the hands or arms for protection ("passive" defense injuries) or tries to seize the weapon or the attacker's weapon-holding hand ("active" defense injuries, defense injuries sustained on grasping the weapon) *(11,78)*. The distinction between "active" and "passive" defense injuries is the result of tradition and does not sufficiently take into account the dynamics of a fight and the numerous possibilities of interaction between the victim and the perpetrator *(7,78–80)*.

"Active" defense injuries are located on the palms, the flexor sides of the fingers and the interdigital spaces. "Active" defense injuries are seen particularly often in the region of the thumb, the index finger (Fig. 1) and the pertinent metacarpal regions I and II, especially near the intermetacarpal space I (Fig. 5) (*11,49,78,80*). "Passive" defense injuries are located on the extensor sides of the forearms and the back of the hands (Fig. 6). They occur when victims raise their hands for protection or move their arm upward to ward off a stab effected from the top downwards (*49*).

Under morphological aspects, defense injuries caused by sharp force are cuts, stabs, or wounds with both cutting and stabbing elements. Relative movements of the blade in the wound may—as in other parts of the body—cause stab wounds with different angles *(79,80)*. Perforations of forearms or hands are not uncommon, especially with tangential stabs and thin soft tissue, such as on the

Fig. 5. "Active" defense injuries at the intermetacarpal space I of the left hand.

hand *(78,80)*. Perforating stabs through the extremity held in front of the body for protection with secondary reentry in another part of the body are possible. Cuts of the hands are considered the most frequent form of defense injuries resulting from sharp force *(28,78)*.

In several autopsy studies, the incidence of defense injuries ranged between 37.1 and 49.5% *(5,26,28,33,78,81,82)*. Significantly different figures (5.8–31.8%) were reported in studies conducted on a comparatively smaller number of cases or preselected material *(7,83–85)*. With an increasing number of injuries on the remaining body, the number of additional defense injuries rose as well, although this relation is not linear *(28,32,33,78,81)*. In victims suffering a single fatal stab or cut wound, defense injuries are relatively rare: Katkici et al. found them in 3.3% *(81)*; Hüttemann in 5.6% *(32)*, Metter and Benz in 8.3% *(78)*, and Hunt and Cowling in 15.4% *(33)* of these cases. Some investigators *(26,33,81)* observed defense injuries in female victims more often (54.5–79.0%) than in male victims (27.3–36.0%). Rouse et al. *(28)*, on the other hand, did not see any significant difference in this respect.

Preferential sites for defense injuries are the left forearm and left hand, with approximately two-thirds of all defense injuries being located on the left-hand side (Katkici et al: 61.1% *[81]* and Metter and Benz 69% *[78]*). Bajanowski et al. *(7)* found almost the same distribution of defense injuries on the right and left arm, although these results are based on a very small number of examined victims.

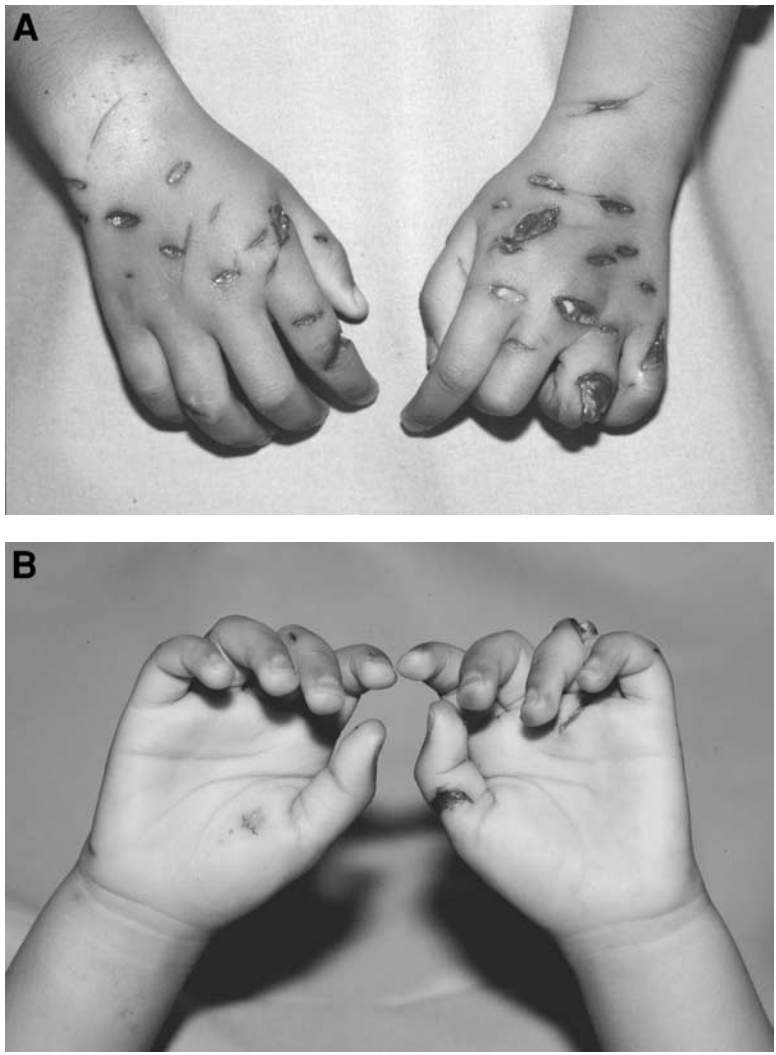


Fig. 6. (A,B) Numerous "passive" defense wounds restricted to the back of both hands (same case as Fig. 4).

4.8. Causes of Death

The most frequent cause of death in homicides caused by sharp force is exsanguination because of injuries of major vessels. How fast death will occur depends on the number of vascular hits, the type of vessels affected (arteries, veins), and their size (diameter). In this context, not only the amount of blood

lost, but also the speed of blood loss, is a deciding factor. Arterial hemorrhages from major vessels may lead to death relatively fast. A loss of more than 1 L of blood from a major vessel may already be fatal. However, several liters of blood may be lost from smaller venous vessels before death occurs. Thus, there is a close link between the question of the cause of death and the ability to act (*see* Subheading 4.9).

An external sign of acute anemia is sparse postmortem lividity. Apart from accumulations of blood in the tissue near the injured vessels or in the body cavities, pale organs are a characteristic finding at autopsy. In stab injuries of the trunk, it may happen that there is only minor external bleeding with most of the blood accumulating in the thoracic and abdominal cavity. By a shift in the soft tissue layers (so-called "Kulissenphänomen" in German) the wound tracks may close, in most cases, only partially. Under these circumstances, only minor traces of blood may be found on the clothing and at the scene, despite massive injuries.

When the stab or cut wounds are located on the neck, two other potential causes of death have to be considered: aspiration of blood and air embolism. For aspiration of blood, exposure of the respiratory tract is a precondition. A lesion of the great cervical vessels is not mandatory. Loss of blood from small vessels (e.g., the thyroid gland) may be sufficient enough to cause fatal aspiration of blood. In gaping wounds of the jugular veins, air may be sucked in when the body is in an upright position. If a sufficient amount of air is transported to the right ventricle via the bloodstream, air embolism may occur, which can lead to death alone or in combination with loss of blood, or occasionally with aspiration of blood.

Less frequent causes of death owing to the impact of sharp force are cardiac tamponade and failure of central regulatory processes. Cardiac tamponade subsequent to a stab wound will occur if the blood flowing from the heart or the adjacent great vessels cannot escape through the opening in the pericardium. This is possible if the opening in the pericardium is comparatively small, for example, when a narrow, pointed object was used *(49)*. In this context, Karger et al. reported a suicide in which the left ventricle was punctured with a cannula *(86)*. Central regulatory failure is possible after stabs to the skull injuring the medulla oblongata *(11,87,88)*.

4.9. Ability (Capability) to Act

Assessment of the ability to act (capability to act) after suffering stab and cut wounds is essential for the reconstruction of the sequence of events. Generally, immediate incapacitation is to be expected only if central regulatory centers are injured or if the victim becomes unconscious, e.g., after blunt traumatization of the skull. On the other hand, lesions of the heart, the lungs, or

Table 1
Graduation of Ability to Act According to Petersohn (89) and Staak and König (90)

Grade 1: Complex, carefully directed actions that require full consciousness.
Grade 2: Instinctive actions that are appropriate to the situation.
Grade 3: Reflexes and automatisms also appearing in the unconscious.
Grade 4: Incoherent, rapidly exhaustible sequences of movements, such as extension spasms.

vessels in the vicinity of the heart by no means always result in immediate incapacitation, even if the injuries are ultimately fatal. According to Petersohn *(89)*, as well as Staak and König *(90)*, the ability to act is classified into four grades (Table 1).

In forensic practice, it is very common after stab injuries to the heart that the ability to act is maintained at least for a short period of time *(49)*. In a retrospective analysis of 37 deaths caused by sharp force, Zimmer at al. *(91)* found some deaths resulting from exsanguination after penetration of the heart in which the victim maintained the ability to act (grade 1 to 2) for up to 15 minutes. Other authors also reported that occasionally the ability to act was maintained over prolonged periods of time after injuries of the heart or the brain were sustained *(8,86,90,92–95)*. There are even reports in the literature of stabs to the heart that were survived for several hours, especially in cases in which the left ventricle was penetrated only a few millimeters *(93–95)*. The duration and extent of the ability to act after stabs to the heart seems to depend essentially on the size of the lesion and thus, the amount and speed of blood loss *(86,90,91,96)*, and not so much on the anatomical location in the heart *(90)*.

In lesions of the abdominal aorta, the ability to act may be maintained over prolonged periods of time, whereas in injuries of the thoracic aorta, incapacitation generally occurs within seconds *(49)*. Injuries of the lungs or abdominal organs do not lead to immediate incapacitation.

4.10. The Differentiation Between Suicide and Homicide

The most important issue in evaluating deaths caused by sharp force is the differentiation between suicides and homicides. In the literature, the signs indicative of suicide are the following *(4,9,11,51,82,97,98)*:

1. Location of the injuries in the precordial region.
2. Grouped injuries with a similar direction.
3. Concomitant, shallow, tentative stabs.

4. Combination with trial cuts (mostly on the arms).
5. The absence of defense injuries.
6. Exposure/undressing of stab region.
7. Stabs often descending.
8. Avoidance of body regions with high sensitivity to pain.

Multivariate analyses of deaths owing to sharp force have shown that the simultaneous presence of several of these features is highly indicative of suicide *(97)*. One must emphasize, however, that an accumulation of these characteristic features is only a clue pointing toward suicide, but not definitive proof. In particular, it is not possible to draw the reverse conclusion that the absence of the above findings means homicide. This aspect has been pointed out in many case reports in which the above criteria were partially or totally absent.

A rarely mentioned but also significant criterion is the extent of blood on the hands of the victim. Although suicides often show extensive traces of blood on the hands, this is usually not the case in homicide victims, unless they have suffered defense injuries, which points also to infliction by another party *(49,99)*.

4.11. Injuries on the Knife-Holding Hand of the Perpetrator

The clinical forensic examination of living individuals becomes more and more important. This does not only refer to victims of actual or fictitious violent crimes, but also to suspected offenders. These are presented to the forensic pathologist by the police not only to preserve DNA traces as proof of contact between the suspect and the victim, but also to determine potential consequences of fights with the victim. In this respect, the reconstruction of the offense is of primary importance *(80,100)*.

In the course of bodily injuries or homicides by sharp force, the perpetrator may involuntarily injure him- or herself *(11)*. Cut wounds on the knife-holding hand of the offender may occur when the hand slips onto the blade. This is possible if the knife used does not have an adequate hand guard and the tip of the blade hits a solid resistance, e.g., a bony structure of the victim, thus being abruptly decelerated. Depending on how the knife is held by the hand, characteristic lesions may be created. When the stab is effected with the blade protruding on the ulnar side of the fist, the little finger will be predominantly affected *(101)*. When the knife is held with the blade protruding on the radial side, injuries may occur on the thumb or index finger, or even on both of them, especially if a double-edged blade is used *(100)*. When the cuts are deep enough, the flexor tendons of the fingers may also be severed; in this

connection, strongly retracted tendon stumps suggest that the fist was firmly closed at the moment of traumatization or the tendons injured were at least under tension *(101)*. Thus, specific injuries on the perpetrator's hand allow for reconstructive conclusions. For correct evaluation it is, of course, indispensable to consider the pattern of injuries as a whole, as victims of sharp force and suicides may show similar lesions on the hands *(9,98,101)*.

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Death From Natural Causes

4

Sudden and Unexpected Death in Marfan Syndrome

Roger W. Byard, MBBS, MD

CONTENTS

SUMMARY

Marfan syndrome is one of the most common inherited connective tissue disorders, and it is caused by abnormalities in the *fibrillin-1* gene. This results in a series of cardiovascular and musculoskeletal abnormalities that may be responsible for sudden and/or unexpected death. Review of cases and the literature shows that, although sudden death is most commonly the result of hemorrhage from acute aortic dissection, there may be a variety of other lethal mechanisms. These include intra- and extracranial arterial dissection, intra- and extracranial arterial aneurysm rupture, mitral valve prolapse, aortic valvular

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incompetence with acute cardiac failure, ventricular arrhythmia, endocarditis, and brainstem compression from atlanto-occipital instability. Pregnancy, exercise, and trauma may exacerbate underlying tissue weaknesses in individuals with the syndrome. The phenotypic manifestations of Marfan syndrome vary considerably among individuals, making postmortem diagnosis difficult. Marfan syndrome should be suspected in tall individuals with vascular complications, with appropriate tissues and blood taken for DNA extraction (or storage) for genetic analysis if confirmation of the diagnosis is required.

Key Words: Marfan; aortic dissection; aneurysm; sudden death; arterial aneurysm rupture; mitral valve prolapse.

1. Introduction

Marfan syndrome was first described by Antoine-Bernard Marfan in 1896 in Paris in a 5-year-old girl with long fingers (*pattes d'araignée* or "spider fingers") and toes and tall stature. Aortic involvement was first described in 1943 *(1)*. Marfan syndrome is a heritable disorder of connective tissue caused by mutations in the gene encoding for *fibrillin-1*, which is a large major glycoprotein lying within 10- to 12-nm extracellular matrix microfibrils responsible for elastogenesis and the elasticity and maintenance of elastin fibers *(2)*. Alterations to the gene may affect connective tissues throughout the body, resulting in characteristic phenotypic features that can be used clinically to identify potential cases. Although there has been a significant increase in the life-span of individuals with Marfan syndrome over the past 30 years owing to elective aortic root replacement and closer medical monitoring and management, sudden and/or unexpected death remains a well-recognized phenomenon *(3–5)*. The observed increase in life expectancy in people with Marfan syndrome may also have been influenced by the overall improvement in life expectancy in the population, and also by the increase in the number of cases being diagnosed with less severe manifestations of the syndrome *(4)*. Although the most common cause of death involves internal hemorrhage from aortic dissection, a wide variety of other lethal events may occur involving other organ systems, not all of which will be necessarily obvious at autopsy (Table 1).

2. Illustrative Cases

A review of the files of the Forensic Science SA in Adelaide, Australia, revealed a number of cases where the possibility of Marfan syndrome had been raised, usually based on tall stature, arachnodactyly, and aortic dissection. Confirmation of the diagnosis by obtaining a positive family history or by genetic testing was usually not possible and so the diagnosis in many cases remained conjectural. The following cases were chosen to demonstrate certain features

Table 1
Findings in Sudden and/or Unexpected Death in Marfan Syndrome

A. Causes of death related directly to Marfan syndrome
  1. Aortic dissection
    i. Hemopericardium/cardiac tamponade.
    ii. Hemothorax.
    iii. Vascular compromise.
      a. Coronary artery occlusion.
      b. Other artery occlusion.
    iv. Arteriovenous shunting.
  2. Other vessel dissection
    i. Coronary artery dissection with myocardial infarct.
    ii. Aortic branch dissections.
  3. Aneurysm rupture with hemorrhage
    i. Intracranial.
    ii. Ductus arteriosus.
    iii. Other.
  4. Mitral valve prolapse
  5. Aortic valve dilatation with left ventricular failure
  6. Ventricular arrhythmias
  7. Endocarditis
    i. Native valves.
    ii. Prosthetic valves.
  8. Postvascular/valvular surgical complications
  9. Atlanto-occipital instability with cord/brainstem compression
B. Conditions/Activities exacerbating underlying pathological features of Marfan syndrome
  1. Pregnancy
  2. Exercise
  3. Trauma
  4. Drug abuse
C. Lethal events not necessarily related to Marfan syndrome
  1. Accidents
  2. Homicides
  3. Coincidental natural diseases

and problems that may arise in clinical and forensic practice in determining the sequence of terminal events and their relationship to possible Marfan syndrome.

2.2. Case 1

A 28-year-old woman presented at the hospital with sudden onset of abdominal pain with diarrhea and vomiting. Initial diagnoses were of possible

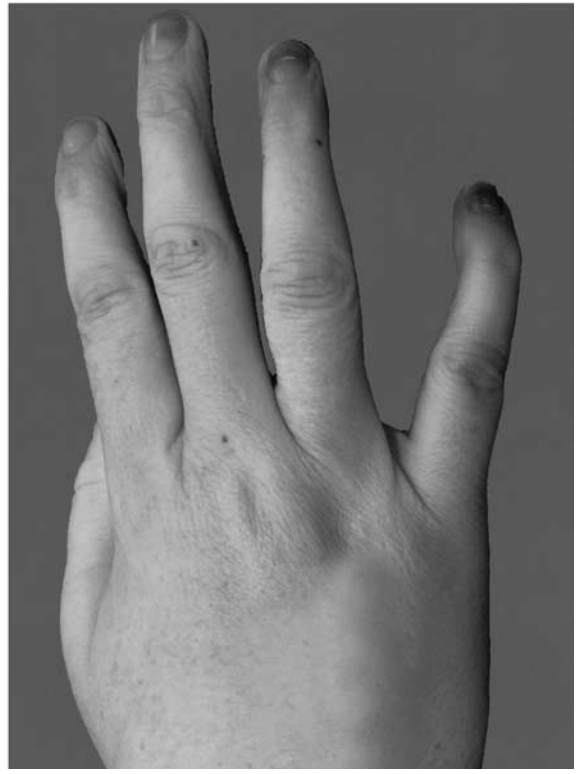

Fig. 1. Typical arachnodactyly of the fingers in a case of Marfan syndrome.

renal colic or gastroenteritis. She had one child and gave a past history of asthma and hypothyroidism. Precipitate clinical deterioration occurred with the development of peritoneal irritation and collapse. A laparotomy was performed that demonstrated an inoperable small and large intestinal infarction. Her abdomen was closed and death occurred soon after. A subsequent history was obtained from a family member of Marfan syndrome that had been diagnosed in infancy at an interstate hospital.

At autopsy, the deceased was well built without a marfinoid habitus. She did have, however, long fingers and toes (Fig. 1), pectus carinatum, and dorsal kyphoscoliosis. The most critical finding was of thoracic aortic dissection extending from an intimal tear at the origin of the left subclavian artery distal to the bifurcation of the common iliac arteries (Fig. 2). This was associated with a small amount of posterior mediastinal hemorrhage (Fig. 3) and small bilateral accumulations of serosanguinous pleural fluid. The aorta was not dilated and there were no abnormalities of other vessels or of the aortic or mitral valves.

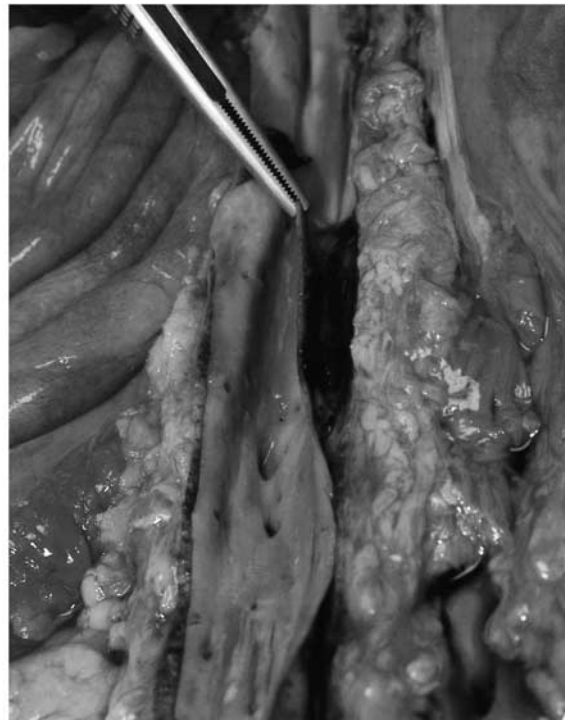

Fig. 2. Aortic dissection in a case of Marfan syndrome showing peeling of the outer from the inner aortic wall layers around the origin of the renal and gut arteries.

The most significant effect of the dissection had been compromise of the origins of the celiac axis and superior and inferior mesenteric arteries supplying the gut with ischemic damage/infarction of the stomach and small and large intestines (Fig. 4). Microscopically, cystic medial necrosis of the aorta was confirmed (Fig. 5), and infarction of the intestine with focal areas of acute inflammatory response and bacterial overgrowth was demonstrated. Death was caused by multiple organ failure following intestinal infarction resulting from dissection of a nonaneurysmally dilated aorta in an individual with previously confirmed Marfan syndrome.

2.2. Case 2

A 38-year-old woman suffered cardiac arrest in bed after an episode of coughing. She had no significant past medical history. At autopsy, the body had a marfinoid habitus, being tall and thin with long fingers. There was no pectus exca-

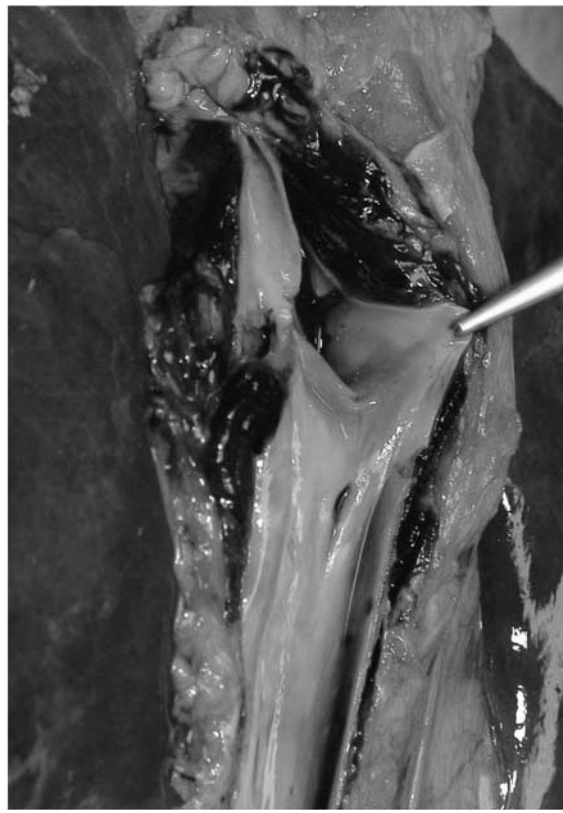

Fig. 3. Posterior mediastinal hemorrhage adjacent to a dissection of the thoracic aorta in a case of Marfan syndrome.

vatum or high-arched palate. The only significant finding was that of mitral valve prolapse with no other abnormalities of the heart, aortic valve, or aorta. Death was, therefore, attributed to mitral valve prolapse possibly related to underlying Marfan syndrome. Genetic confirmation was not sought by her family.

2.2. Case 3

A 57-year-old man with known Marfan syndrome collapsed with chest pains after digging in the garden. His past history included ischemic heart disease with previous myocardial infarction and three coronary artery stents. At autopsy, the body was tall (190 cm) but not thin, with long fingers and a high-arched palate. The mitral valve showed mild prolapse. There was no aortic dilatation, aortic dissection, or aortic valve abnormality. There was significant

Fig. 4. Early intestinal infarction due to aortic dissection that caused compromise of the celiac axis and the superior and inferior mesenteric arteries.

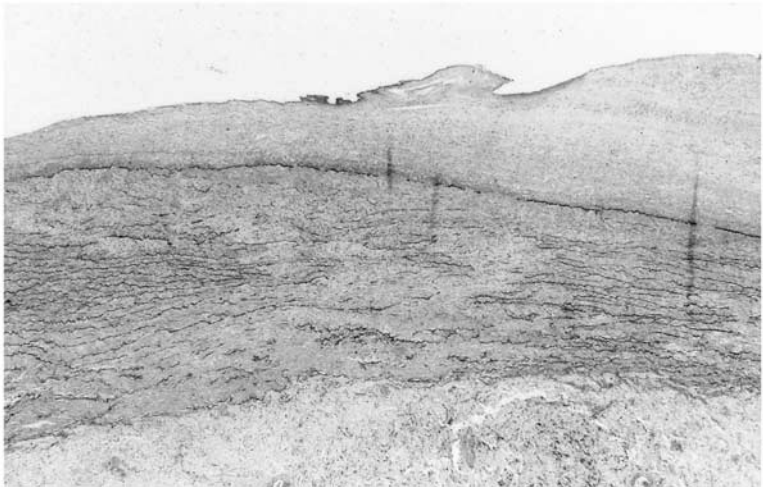

Fig. 5. Cystic medial necrosis of the wall of the aorta showing characteristic disruption of elastin fibers with increased amounts of glycosaminoglycans.

cardiomegaly with extensive scarring of the left ventricular free wall and the interventricular septum associated with marked atherosclerotic narrowing of the right and left anterior descending coronary arteries despite previous stenting. Death was caused by coincidental ischemic heart disease in an individual with known Marfan syndrome.

3. Incidence and Inheritance

Marfan syndrome is one of the most common inherited disorders of connective tissue, occurring in approx 2 to 3 people in every 10,000 in all ethnic groups *(1)*. It may be either an autosomal dominant condition associated with over 200 mutations of the *fibrillin-1* gene located on chromosome 15q21.1, or a sporadic occurrence in 25 to 30% of cases.

4. Characteristics

Defects in the *fibrillin-1* gene may result in a very characteristic phenotype with variable and seemingly unrelated abnormalities in the eyes and cardiovascular and skeletal systems, with arachnodactyly (long fingers and toes), dolichostenomyelia (long arms and legs), high-arched palate, kyphoscoliosis, pectus excavatum or carinatum, and cutaneous stria *(1)*. Affected individuals tend to be tall and thin with an arm span-to-height ratio greater than 1.05. There may be a history of recurrent joint dislocations. Additional findings that may be demonstrated clinically or at autopsy include dolichocephaly (long, narrow skull), hypoplastic or retrognathic mandible, flattened cheek bones, dural ectasia, mitral valve prolapse, and dilatation of the ascending aorta. In addition, there are a number of ocular abnormalities that may have been detected clinically including ectopia lentis (lens subluxation), microspherophakia, hypoplasia of dilator pupillae muscles, blue sclera, glaucoma, and retinal detachment *(1,5)*. Specific features must be present before the clinical diagnosis of Marfan syndrome can be made and have been defined in the "Ghent" diagnostic criteria *(2)*.

The differential diagnosis includes a wide variety of conditions, such as homocystinuria and Stickler syndrome. Homocystinuria, an inherited disorder of amino acid metabolism, has an autosomal dominant mode of inheritance and is characterized by thrombotic events and mental retardation, which are not features of Marfan syndrome. Although individuals with Stickler syndrome, also known as hereditary arthro-opthalmyopathy, may have tall stature, midface hypoplasia, joint problems, retinal detachment, and an autosomal dominant inheritance, they also have vitreoretinal degeneration and a family history of deafness and cleft palate *(1)*.

5. Lethal Mechanisms

Lethal mechanisms usually involve the cardiovascular system, with the most common terminal event being aortic dissection related to weakness in the aortic media and progressive dilatation. The most common site of dissection is the ascending aorta when tearing of the intima allows blood to track into the aortic wall; this area is subject to high sheer and tensile stresses. The risk of dissection increases with the aortic diameter and may occur either in infancy or not until late adult life. Microscopically, in addition to evidence of fresh hemorrhage, there is a background of disrupted elastin fibers with an accumulation of excessive amounts of glycosaminoglycans, so-called "cystic medial necrosis." Severe aortic incompetence may be caused by dilatation of the aortic root, and there may be life-threatening mitral valve prolapse.

Aortic dissection may extend proximally and rupture into the pericardial sac producing hemopericardium, with compromise of cardiac function owing to cardiac tamponade. Alternatively, there may be a rupture of the aorta into the pleural cavities, with significant hemothoraces and death occurring as a result of hypovolemic shock exacerbated by pulmonary atelectasis. Rarely, dissection may occur into the adjacent superior vena cava and result in lethal acute cardiac failure owing to marked arteriovenous shunting *(6)*. Dissection may also compromise smaller aortic branches, such as the coronary and carotid arteries, causing critical reduction in blood flow and end-organ ischemia. Lethal arrhythmias have been documented in Marfan syndrome without coronary artery involvement in association with left ventricular dilatation and mitral valve prolapse *(7)*.

As the changes of infarction may take a number of hours to develop before they can be seen microscopically, there may be no specific histological changes detectable in end organs if death has occurred rapidly. Alternatively, there may be early changes in the heart, with loss of cellular details with myocyte eosinophilia and contraction band necrosis. Early cerebral parenchymal changes may include patchy neuronal eosinophilia.

Aortic dissection may also extend into branch arteries or may compromise their blood supply by causing proximal occlusion. Such was the situation in the first case where intestinal infarction was the immediate cause of death, prompted by critical reduction of gut blood supply owing to a thoracic dissection extending into the abdominal aorta.

Other vascular abnormalities in Marfan syndrome include aneurysms of a wide range of extra- and intracranial arteries *(8,9)*. These may rupture, resulting in significant hemorrhage that may be life-threatening, particularly when it is located intracranially *(10)*. Isolated dissections not associated with aortic dis-

section may also occur within blood vessels supplying the brain at extra- and intracranial sites and the heart with subsequent ischemic complications *(11–15)*. Affected vessels may appear elongated and tortuous, and on microscopic examination, may show intimal proliferation, medial degeneration, and fragmentation of the internal elastic lamina *(10,16)*. Aneurysms and dissection of the ductus arteriosus, sometimes with lethal outcomes at very early ages, have been reported in fetuses and young infants *(17,18)*.

Endocarditis may be found involving prolapsed mitral valves or prosthetic valve replacements *(19,20)*. This may cause local complications or lead to sepsis. Myocarditis has also been reported as an associated feature in one family *(21)*. Standard surgical complications may arise from the implantation of prosthetic vascular grafts or valves. Rarely, a cardiomyopathy may be present *(1)*.

Hypermobility of the atlanto-occipital joint with an increase in height of the odontoid peg is another feature of Marfan syndrome that may cause sudden death in affected individuals resulting from compression of the cervicomedullary junction *(22)*. This may also present problems during intubation for general anesthesia *(1)*. Postmortem radiographs of the cervico-occipital junction with careful dissection will, therefore, be required to demonstrate the abnormally long odontoid and laxity of the joint fibroconnective tissue. Spontaneous pneumothoraces are a feature of Marfan syndrome but are usually not lethal. There may be a predisposition to them under certain circumstances, such as flying in unpressurized aircraft or scuba diving.

6. Exacerbating Conditions and/or Activities

6.1. Pregnancy

On occasion, the underlying tissue weaknesses present in individuals with apparently stable disease may be exposed when they are subjected to extra physiological or traumatic stresses. For example, hemodynamic changes associated with pregnancy are known to precipitate dissection in women with Marfan syndrome, and it has been recommended that pregnancy should be avoided in those with an aortic root diameter of more than 40 mm. Studies have shown, however, an increased risk of aortic dissection during pregnancy even in those with no detectable preconceptional cardiovascular abnormalities *(23)*. Aortic rupture most commonly occurs in the third trimester. Pregnancy in women with Marfan syndrome has a 1% risk of fatal complications and may be associated with aortic root dilatation, worsening aortic incompetence, and postpartum hemorrhage. There is also an increased risk of spontaneous abortion and preterm labor caused by cervical incompetence, and a risk of inheritance of the

syndrome to offspring. Postpartum hemorrhage may be due to uterine inversion or lack of adequate connective tissue support, and it has also been proposed that connective tissue weakness may predispose the individual to injury from instrumentation during delivery *(23,24)*.

6.2. Exercise

Exercise may also precipitate aortic dissection, and sudden death has been reported in a number of high-profile athletes in whom the diagnosis of Marfan syndrome was not made until after death. Sports involving competition with intense physical activity and/or emotional stress causing increased heart rate and blood pressure or sports with contact or collision may precipitate dissection *(25)*. Dissection may not, however, occur for many years, presumably not until aortic dilatation reaches a critical point or a particular activity causes more than the usual amount of hemodynamic stress *(26)*. The possibility of an effect from stimulant drugs, such as amphetamines, should prompt toxicological studies if suspected.

7. Autopsy Problems

Problems at autopsy may arise in cases where there are pathological findings, such as aortic dissection at a young age that suggest Marfan syndrome but where there is a paucity of well-defined external phenotypic features to support the diagnosis, or where there are phenotypic features that are atypical of Marfan syndrome *(27)*. This was the situation in the Case 2 where prolapse of the mitral valve was associated with a marfanoid habitus and long fingers, but no other anatomical findings were present to support a possible diagnosis. Quite often, as in Case 2, there is no detailed history available, and Marfan syndrome has not been suggested by attending physicians to police, leaving the examining forensic pathologist in a difficult situation. Discussing such a case with a medical geneticist may be useful as there may be information on the case or on the decedent's family in the local Department of Medical Genetics. The department may also be willing to process skin for fibroblast cultures and blood and tissues for DNA analyses. Even if families are not interested in definitively establishing a diagnosis around the time of death, taking material for DNA extraction and tissues for storage enables this option to be pursued at a later date if they subsequently change their minds.

As the potential implications of a diagnosis of Marfan syndrome may be of considerable significance for current and future family members, a high index of suspicion must be maintained in the forensic setting. Initially, when genetic mutations were being found for a variety of heritable conditions, there

was a hope that genotype–phenotype correlations would help predict prognoses and manifestations in other family members, and that an autopsy with subsequent molecular investigations/diagnoses would, therefore, have far-reaching consequences in terms of directing genetic counseling. Unfortunately, a problem that has arisen in Marfan syndrome, and in other conditions that are characterized by large genes with multiple genetic mutations, is that individual families may have unique mutations of completely uncertain significance. Thus, although referral of a family for formal medical genetic counselling is an option to consider, it may be of limited clinical usefulness.

8. Alternative Causes of Death

Merely because an individual is suffering from a potentially life-threatening condition, such as Marfan syndrome, does not, of course, preclude other fatal conditions. For example, individuals with Marfan syndrome may die of a host of natural diseases that are completely unrelated to the underlying disorder of fibrillin. This was illustrated in Case 3 by the 57-year-old man who had a proven diagnosis of Marfan syndrome, but who succumbed to coincidental underlying coronary artery atherosclerosis with ischemic heart disease.

Those with Marfan syndrome may also die from homicidal or accidental trauma. Whereas trauma may be unrelated to the syndrome, there may be a predisposition to injury because of the underlying connective tissue defect. For example, aortic valve tearing in Marfan syndrome following a motor vehicle accident *(28)* may be sustained with less force than would be required for a normal individual to suffer a similar injury. Conversely, aortic dissection and rupture or some other catastrophic event in a vehicle driver who has Marfan syndrome *(29)* may precipitate, rather than result from, an impact.

9. Conclusions

Marfan syndrome is associated with an array of apparently unrelated abnormalities involving the cardiovascular, ocular, and musculoskeletal systems. A full autopsy examination requires documentation of the range of features present and determination as to whether the cause of death was related to the underlying disorder of fibrillin or was merely a coincidental event. Accuracy of diagnosis is of considerable importance at autopsy given the likelihood of an inherited basis for many cases of Marfan syndrome. Uncommon mechanisms of death include sepsis and structural instability of critical joints with brainstem compression. Further testing may involve microbiological screening,

neck radiographs, toxicological testing, and the submission of blood and tissues for fibroblast cultures and DNA analyses.

Acknowledgment

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5

Asthma Deaths

Phenomenology, Pathology, and Medicolegal Aspects

Michael Tsokos, MD

Contents

> No man is an island, entire of itself; …any man's death diminishes me
> —John Donne, 1624

Summary

Asthma bronchiale is an inflammatory disease of the airways characterized, in part, by reversible airflow obstruction, airway narrowing, and airway hyperresponsiveness. The prevalence of asthma is continuously rising in Western countries. Fatal asthma attacks may produce dubious death scenes: doors and/or windows might be wide open at the scene where the deceased is found. When involvement of a third party can be excluded, this finding can be explained by the circumstance that the affected individual opened the respective

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doors and/or windows prior to death for better oxygen supply during the acute attack. The external examination of the body gives no special hints towards the disease. At autopsy, the lungs are hyperinflated and overexpanded, appearing ballooned and occupying the whole thoracic cavity. Petechial hemorrhages beneath the visceral pleura are sometimes present. Greyish-whitish mucus plugs that occasionally completely occlude the airways and involve airways of all sizes are a gross pathological hallmark of asthma. The mucous material appears relatively dry, is adherent to the mucosal surface of the airways, and has a tenacious consistency. The histological features of asthma include mucous plugs in the airway lumen, edema and inflammation of the airway walls, thickening of the subepithelial basement membrane, epithelial desquamation, smooth muscle cell hyperplasia, goblet cell hyperplasia, ectasia of bronchial gland ducts, and vascular congestion. Curschmann spirals seen microscopically on transversal sections through bronchi are the most characteristic feature of mucus appearance in asthma. Some authors have described histological differences regarding the time interval from the onset of the asthma attack to fatal outcome by investigating the airways of patients who died from slow-onset asthma compared with cases of fatal sudden-onset asthma, the latter characterized by a relative paucity of eosinophils in the face of an excess of neutrophils in the airway submucosa. Viral upper respiratory infections have been associated with 80% of asthma exacerbations in children, and some investigators assume that even as much as 50% of all asthma episodes in adults are triggered by viral infections. Whether atypical bacterial infections are an epiphenomenon or a pathogenic event in asthma has not yet been clarified. Respiratory symptoms in patients having an anaphylactic reaction may be misdiagnosed as an acute asthma attack. Deaths of asthmatics may be to the result of the disease alone, to other unrelated causes, or both in combination. Only a thorough autopsy is the final word in suspected asthma deaths. Clinical and autopsy studies have suggested an association between the relatively nonselective β-agonist, fenoterol, one of the most commonly used asthma medications in many Western countries, and asthma deaths. A number of case–control studies have confirmed that among patients prescribed fenoterol, the risk of death is significantly elevated. Preventable asthma deaths still occur, particularly in relation to inadequate treatment. It is crucial for the forensic pathologist to recognize the different phenomenological and morphological features under which asthma deaths may present, as well as the associated pathogenetic mechanisms in order to determine the correct manner of death. In addition, a profound knowlegde of medicolegal issues raised by asthma deaths is essential for everyone involved in death investigation.

Key Words: Asthma bronchiale; sudden death; sudden-onset asthma attack; slow-onset asthma attack; status asthmaticus; mucous plugging; lactoferrin; mucins; smooth muscle cell hyperplasia; Curschmann spirals; accuracy of death certification; forensic histopathology; autopsy.

1. INTRODUCTION

Asthma bronchiale is an inflammatory disease of the airways characterized, in part, by reversible airflow obstruction, airway narrowing, and airway hyperresponsiveness. Narrowing of the airways occurs because of airway remodeling and mucous hypersecretion, and is exacerbated as the airways become hyperresponsive to various stimuli. Remodeling of the bronchial wall is characterized by the thickening of the reticular layer underneath the subepithelial basement membrane, accumulation of inflammatory cells, increased vascularity, hypertrophy of smooth musculature, and goblet cell hyperplasia. As a sequel of airway remodeling, reduced airflow rate and air trapping within the lungs leads to permanent airflow limitation with decreased blood oxygenation and increased carbon dioxide, and pulmonary hypertension with subsequent right ventricular systolic overload.

The prevalence of asthma (approximately 3–5% in the population) is continuously rising. To a certain degree, this rise can be attributed to improvements in diagnosis, but environmental factors so far not sufficiently elucidated are most likely responsible for the rising prevalence as well *(1)*. Despite recent advances in the understanding of the pathogenesis and pathophysiology of asthma, the availability of new modes for the diagnosis of the disease, and the development of various therapeutic options, a gradually increasing death rate from asthma has been reported worldwide *(2)*.

Classically, asthma was divided into two major categories: Extrinsic (allergic, atopic) asthma, referring to those cases where bronchoconstriction was believed to be induced by inhaled allergens; and intrinsic (idiopathic, nonatopic) asthma, which was considered unrelated to immune mechanisms. These distinctions imply that the pathogenetic mechanisms that govern the disease process are quite disparate. However, new findings suggest possible common pathogenetic effector pathways leading to the development of a unique inflammatory response causing the characteristic pathophysiological and clinical manifestations of asthma.

Asthma is not a single disease. The term *asthma* refers to a heterogenous collection of clinical phenotypes as opposed to a single condition. The key feature of persistent asthma is the development of airway inflammation and airway hyperresponsiveness. How this hyperresponsive state is acquired is not clearly

understood yet but it is likely to result from several cellular and molecular effector mechanisms, some of which may be operative in one individual suffering from asthma but not in another. In addition, airway remodeling may by itself contribute to hyperresponsiveness through purely mechanical means.

In the popular imagination, asthma is, for the most part, the result of air pollution. Undoubtedly, air pollution exacerbates preexisting asthma but despite a decrease of air pollution, the prevalence of asthma increases. In many cases of fatal asthma there is an identifiable trigger, such as viral upper respiratory tract infections, drug abuse, cigarette smoke, house dust, smog, or other irritants including a wide a range of nonspecific stimuli. The most common trigger of asthma exacerbation in children is viral infection, whereas in adolescence, infections are thought to account for only a small proportion of asthma exacerbations *(3,4)*. The "hygiene hypothesis" proposes that the relatively sterile environment present in industrialized Western countries has contributed to the recent epidemic of asthma and atopy *(5)*.

In 1960, Messer et al. conducted a large autopsy study of asthma bronchiale, retrospectively reviewing 304 cases *(6)*. The authors stated, "*There is a widespread impression that bronchial asthma seldom causes death. This study shows clearly that the disease terminates fatally more often than is generally thought.*" This statement, as any forensic pathologist with daily autopsy practice will answer in the affirmative, is still effective more than 45 years later. Asthma deaths affect all age groups and, despite substantial advances in treatment over the last decade, asthma still represents a significant cause of sudden, unexpected death.

Asthma deaths often occur rapidly and in the absence of any witnesses. Fatal asthma attacks often concern younger individuals, and most often occur outside the hospital. Asthma deaths may occasionally produce dubious death scenes that look at first sight as if a third party was involved (e.g., doors or windows have been opened by the affected individual prior to death for better oxygen supply during the acute asphyxic attack). The diagnosis of asthma may not have been made prior to death, and the disease is sometimes first diagnosed at autopsy.

2. Acute Severe Asthma Attacks: Sudden- and Slow-Onset Asthma

In 1965, Roe was the first to draw attention to the distinction between death following a prolonged asthma attack and sudden, unexpected death as a sequel of sudden onset of an asthma attack *(7)*. Since then, a number of studies have documented that acute severe asthma may come with a sudden- or slow-

onset *(8–11)*. Sudden-onset asthma attacks are characterized by a sudden development of airway obstruction, sometimes with fatal consequences. This rapid development contrasts with that of patients with slow-onset asthma attacks. Slow-onset asthma attacks are associated with a progressive but slow clinical and functional deterioration. The prolonged duration of the attack and the time frame during which symptoms develop can give an indication of the development of acute airflow inflammation, whereas a sudden deterioration suggests predominant airway smooth muscle contraction *(12)*. The inhalation of large doses of allergen by sensitized patients with asthma and high levels of specific immunoglobulin (Ig)E to the allergen usually cause sudden-onset asthma attacks *(13,14)*.

Data from Rodrigo and Rodrigo support the earlier notion that sudden-onset asthma attacks constitute a distinct but uncommon acute asthma presentation, with a predominance in males *(12)*. Their study suggests that upper respiratory tract infection is not a significant trigger factor in the affected individuals.

Arnold and co-workers prospectively studied the speed-of-onset of acute severe asthma attacks in a total of 261 consecutive episodes *(8)*. The speed-of-onset of an attack was rapid (defined as <24 hours) in 46% of episodes and was less than 1 hour in 13%. Sudden-onset attacks occurred more frequently in younger patients and were no more or less severe than attacks of slower evolution. This study also reported an association between age and duration of the attack: sudden-onset attacks occurred more frequently in younger patients. In contrast, Rodrigo and Rodrigo did not find a similar association with age in their clinical trial using a prospective cohort study enrolling 403 patients *(12)*.

Clinically, sudden-onset asthma attacks are defined by a sudden development of airway obstruction with less than 6 hours' duration in contrast to slow-onset asthma attacks, which are associated with a progressive clinical and functional deterioration *(12)*. For medicolegal purposes, it is most useful to apply stricter chronological dividing lines to asthma fatalities: fatal sudden-onset asthma cases defined as the occurrence of death in a specific case within 1 hour of the onset of the asphyxic asthma attack and fatal slow-onset asthma defined as a fatality in which the time interval between onset of asthma attack and death was longer than 1 hour *(15)*. This distinction is recommended in medicolegal inquiries because hints toward a prolonged duration of the attack and the time frame over which symptoms developed (e.g., as reconstructed from witness reports) in an asthma death undergoing forensic investigation can indicate if inadequate management might have contributed to the fatal outcome, despite there being enough time for effective treatment.

3. Pathology of Asthma Bronchiale

3.1. Death Scene

Asthma deaths occur mostly outside the hospital, often in the absence of any witnesses. Especially in the younger age group, asthma deaths occur more often during nighttime or in the early morning than during daytime *(16–21)*, possibly owing to a pronounced diurnal variation in airflow limitation *(22–25)*.

Fatal asthma attacks may produce dubious death scenes. Doors and/or windows might be wide open at the scene where the deceased is found. When involvement of a third party can be excluded, this finding can be explained by the circumstance that the affected individual opened the respective doors and/or windows prior to death for better oxygen supply during the acute asphyxic attack *(24)*. Occasionally, the place where the fatality took place may present in a state of disorder because the deceased rummaged his personal belongings in search of his antiasthmatic drugs during the attack. An inhaler may be found in the hand, close to the deceased, or somewhere else at the death scene *(26)*.

3.2. External Examination

The external examination gives no special hints toward the disease. Petechial hemorrhages of the conjunctivae or facial skin may be seen occasionally in asthma deaths, depending on the severity of right ventricular overload. However, one has to bear in mind that these petechiae—resulting from engorgement of venules owing to obstruction of venous return to the right ventricle—are highly unspecific because they are frequently seen in a variety of forensic autopsy cases, e.g., in fatalities resulting from ligature or manual strangulation, in individuals who underwent forceful resuscitative chest compression prior to death, or those who died from acute decompensation of a chronic right ventricular cardiac disease.

3.3. Gross Pathology

In fatalities that occurred during an acute asthma attack or status asthmaticus, the lungs—after opening the intercostal spaces of the rib cage—do not collapse, thus giving a first hint towards expiratory airflow obstruction before full opening of the thoracic cavities. The lungs are hyperinflated and overexpanded, they frequently appearing ballooned, and occupy the whole thoracic cavity.

Giant emphysematous lung bullae (Fig. 1) are not an uncommon finding in patients with a history of severe persistent asthma. Pneumothorax and pneu-

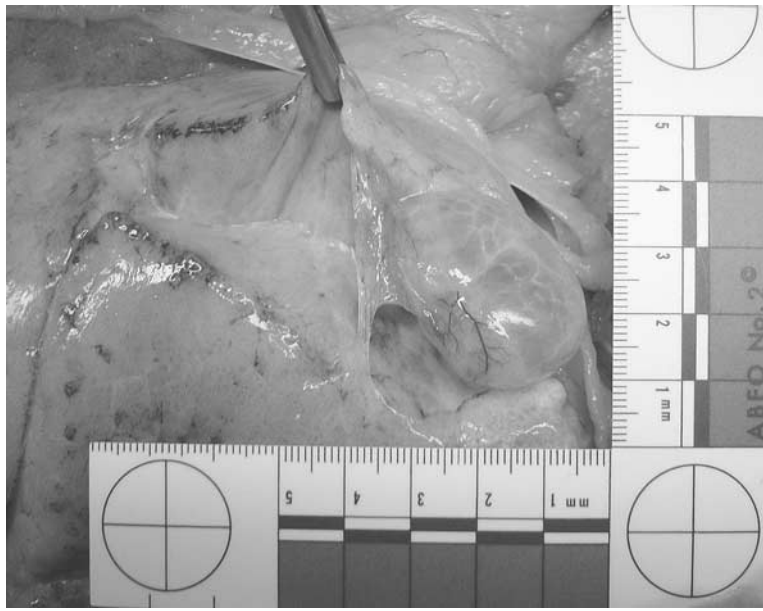

Fig. 1. Giant emphysematous lung bulla seen after opening of the thoracic cavity in a deceased patient with a history of severe persistent asthma.

momediastinum may be the result of rupture of such bullae and should be carefully looked for at autopsy.

After evisceration of the chest organs, the outlines of the ribs may be apparent as indentations on the pleural surfaces of the ballooned lungs. Petechial hemorrhages beneath the visceral pleura (Tardieu's spots) are occasionally present. These visceral petechiae in conjunction with conjunctival and facial petechiae are highly unspecific toward the underlying cause of death; nevertheless, their presence indicates some type of asphyxia preceding death.

In an acute asthma attack, the margins of the lungs are outlined sharply (Fig. 2) and the hyperinflated lung parenchyma has lost most of its capacity to retract, which can be demonstrated by compressing the outer surface of the non-incised lungs with the thumb or a blunt object: this artifactual indentation will remain for a considerable period of time (Fig. 3). The ballooning of the lungs and the proof of loss of capacity of retraction is only demonstrable in fresh lungs and may have vanished in cases where extensive cardiopulmonary resuscitation attempts, including forceful chest compressions with overcoming of expiratory airflow resistance, were carried out before death.

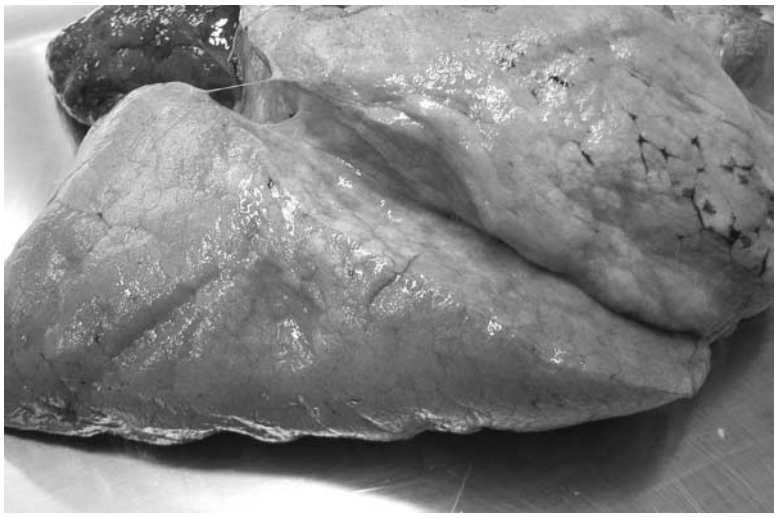

Fig. 2. Sharply outlined margins of the lobes of the lungs in a fatality caused by an acute asthma attack.

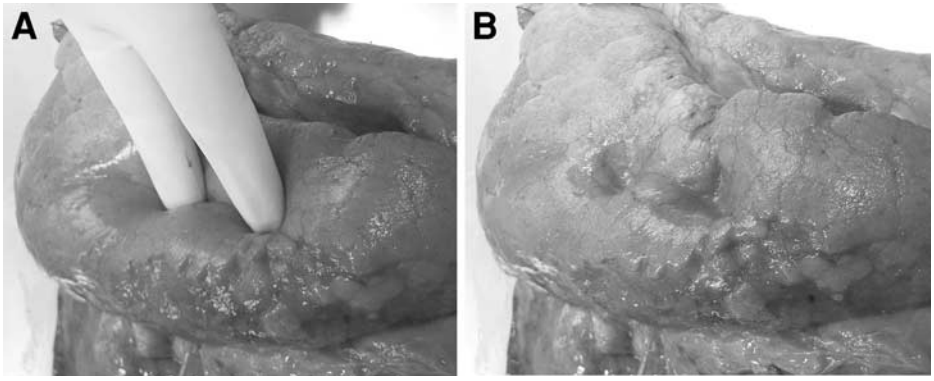

Fig. 3. Demonstration of the loss of the lung parenchyma's capacity to retract owing to hyperinflation in fatal asthma. **(A)** Compressing the outer surface of the nonincised lung with the fingers leads to **(B)** artifactual indentation that remains for a considerable period of time.

Greyish-whitish mucous plugs, which occasionally completely occlude the airways and involve airways of all sizes from the lobar bronchus down to the bronchioli, are a pathological hallmark of asthma. The mucous material appears relatively dry, is adherent to the mucosal surface of the airways, and has

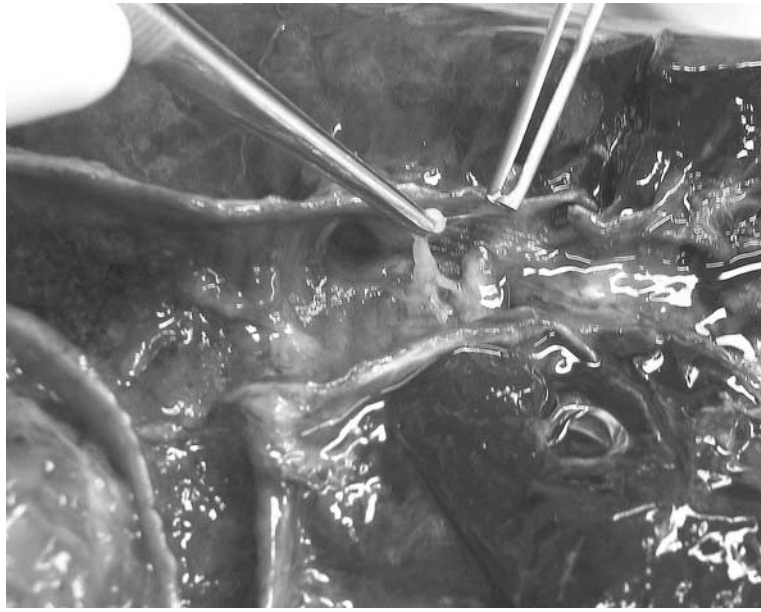

Fig. 4. Gross appearance of mucous plugging of the airway lumen in asthma. The mucous material adhering to the mucosal surface has a tenacious consistency demonstrated here by pulling out a mucous plug with a pair of tweezers.

a tenacious consistency that can be demonstrated by pulling out a plug with a pair of tweezers, with the mucus appearing like a cobweb or spinning yarn (Fig. 4). After wiping the mucous plugs off the airways, the mucosa of the airways appears edematous, injected, and reddened; however, this redenning of the airway mucosa is a highly subjective observation. Whether fatal sudden-onset and slow-onset asthma cases can be distinguished by the amount of mucous plugging seen at gross examination of the airways is debated controversially *(16,17,27–29)*.

Bronchial wall thickening is another frequent finding on cut surfaces of the lungs, indicating a previous history of long-term asthma.

Occasionally, laryngeal edema with total or near-total obstruction of the larynx may be seen in acute asthma deaths, but in such cases an acute anaphylactic reaction has to be considered above all as a potential differential diagnosis of acute asphyxic asthma; the possibility of a coexistence of allergen exposure inducing anaphylaxis and asthma exacerbation has to be considered as well.

Atelectasis and bronchiectasis indicate chronic airway occlusion as a sequel of chronic persistent asthma.

A distinct but by no means pathognomonic extrapulmonary finding in acute fatal asthma attacks is an acute dilatation of the right ventricle and atrium of the heart sometimes accompanied by the right ventricular myocardium showing a pale appearance when compared with the color intensity of the left ventricular myocardium. However, right ventricular dilatation may be missed in cases where strong rigor mortis is present in the cardiac muscle, as usually seen in the early phase of the postmortem interval.

3.4. Histopathology

The histological features of asthma include mucous plugs in the airway lumen, edema and inflammation of the airway walls, thickening of the subepithelial basement membrane, epithelial desquamation, smooth muscle cell hyperplasia, goblet cell hyperplasia, ectasia of bronchial gland ducts, and vascular congestion.

It is well established that infiltration by eosinophils, activated T-lymphocytes, and mast cells plays the central role in asthmatic airway inflammation. The mucosa, and especially the submucosa, appear edematous, although this is difficult to quantify, and contain a mixed inflammatory cell infiltrate with eosinophils and lymphocytes usually constituting the predominant cell types (Fig. 5). Mediators released by these cells cause exaggerated bronchoconstriction and induce airway smooth muscle cell proliferation *(30–36)*. However, more recent studies suggest that neutrophils, basophils, and macrophages, via the expression of cytokines, leukotrienes, and cellular adhesion molecules, also play a crucial role in modulating submucosal airway inflammation *(37–41)*.

Some authors have described histological differences regarding the time interval from the onset of the asthma attack to fatal outcome by investigating the airways of patients who died from slow-onset asthma compared with cases of fatal sudden-onset asthma, the latter characterized by a relative paucity of eosinophils in the face of an excess of neutrophils in the airway submucosa *(28,41)*. Three possible pathophysiological mechanisms are put forward in the literature for the role of neutrophils in acute severe asthma *(42–44)*: (a) neutrophil proteases, especially neutrophil elastase, stimulate goblet cell and submucosal gland secretions; (b) mediator release from neutrophils may affect the airway microvessels, thus resulting in increased vascular permeability and plasma exudation into the airway lumen; and (c) neutrophil elastase causes degranulation of eosinophils *(46)*.

In 1996, Carroll et al. examined inflammatory cell profiles and airway structures in two study groups of fatal asthma related to the duration of the fatal attack (short duration group: cases where death occurred within 2 hours of the fatal attack; long duration group: cases where death occurred >5 hours after the

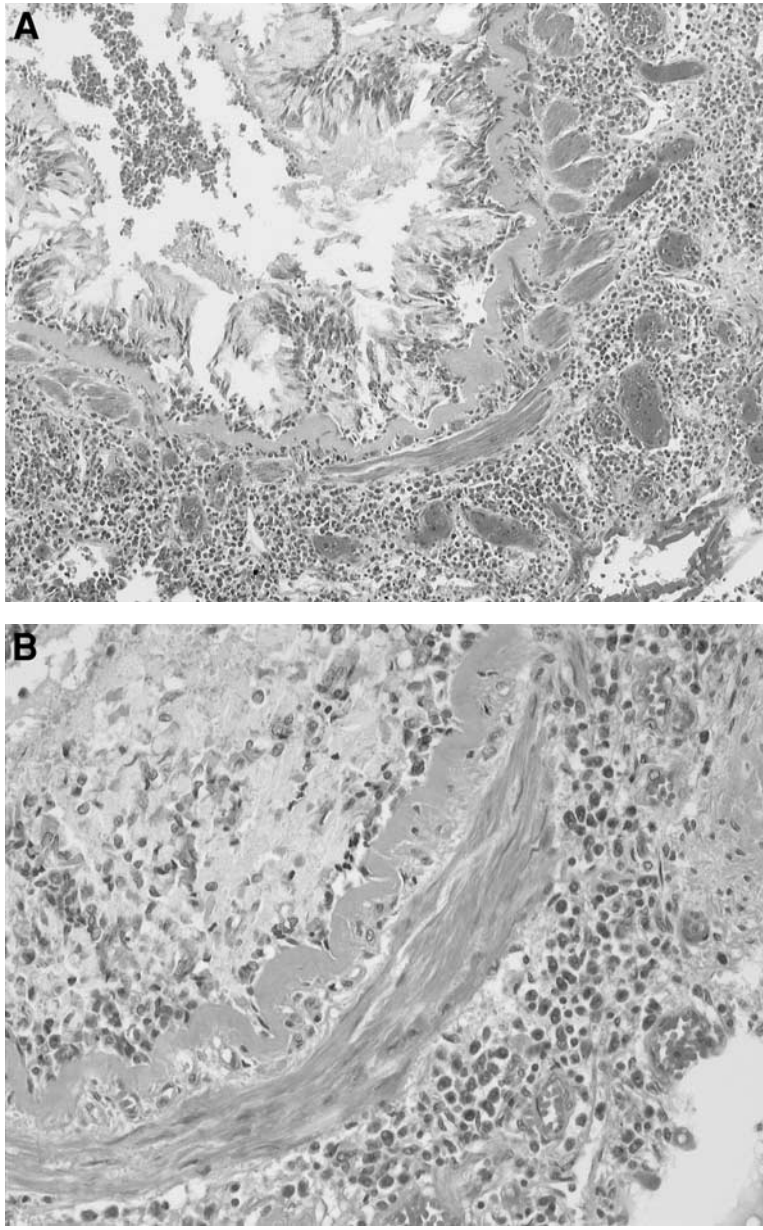

Fig. 5. Histopathology of asthma bronchiale. **(A)** Panoramic view of a small bronchus with goblet cell hyperplasia, thickening of the subepithelial basement membrane, and smooth muscle cell hyperplasia. The submucosa shows an inflammatory cell infiltrate and vascular congestion (hematoxylin and eosin [H&E]). **(B)** Closer view of a bronchus wall in asthma with marked thickening of the subepithelial basement membrane and smooth muscle cell hyperplasia. The submucosa is edematous and shows a mixed inflammatory cell infiltrate consisting of eosinophils, lymphocytes, and mast cells (H&E).

onset of the fatal attack). In cases with fatal attacks of short duration, the numbers of neutrophils and the mucous gland area were increased and the numbers of eosinophils were reduced compared with fatal attacks of long duration *(45)*. However, the routine microscopic examination of pulmonary tissue samples from asphyxic asthma deaths alone will never enable the forensic pathologist to make a clear distinction as to whether he is dealing with a sudden-onset or slow-onset asthma attack. At most, the predominance of eosinophils or neutrophils may support the chronological sequence of events preceding death as implied, for example, by witness reports.

The question of whether neutrophils, as suspected by the clinical investigation of sputum from subjects with asthma exacerbation, constitute the dominant leukocyte subtype in acute severe asthma attacks was recently addressed in 2000 by Ordoñez et al. *(46)*. These authors analyzed tracheal aspirates from patients intubated emergently for acute severe asthma and from patients intubated electively for nonpulmonary reasons. The numbers of neutrophils in tracheal aspirates collected within 12 hours of intubation from the severely ill asthmatic patients was eightfold higher than the numbers of eosinophils in the same samples as determined by cell count and cell differential *(46)*. Still, some doubt the pathoanatomical significance of this clinical observation because the authors did not comment on whether (a) any microbiological investigations where performed in the asthmatic patients, (b) if attention was paid to the probable trigger of the asthma attack, and (c) whether any attempts were undertaken toward the exclusion of concomitant (e.g., inflammatory) lung diseases in the patients studied.

Mucous plugging of the airway lumen (Fig. 6) occurs in airways of all sizes *(47)*. Curschmann spirals, seen on transversal sections through bronchi and best demonstrated histologically by periodic acid-schiff stain, are the most characteristic histological feature of mucus appearance in asthma (Fig. 7). Although mucus hypersecretion is to the result of hyperplasia of goblet cells (Fig. 8) and hypertrophy and hyperplasia of submucosal glands (Fig. 9), the mucous plugs are not only composed of mucin glycoproteins (mucins) from goblet cell and submucosal gland secretions, but also of plasma exudate, desquamated epithelial cells, and inflammatory cells (mostly eosinophils) *(25,43)*. Goblet cell hyperplasia is established as a histopathological characteristic of mild, moderate, and severe asthma. Abnormalities in goblet cell numbers are accompanied by changes in stored and secreted mucins. The mechanism by which goblet cells increase in number in asthma is poorly understood, but it has been put forward that goblet cell metaplasia occurs by conversion of nongranulated secretory cells to goblet cells *(48,49)*. This may occur in part because of activation of mucin gene expression *(50)*.

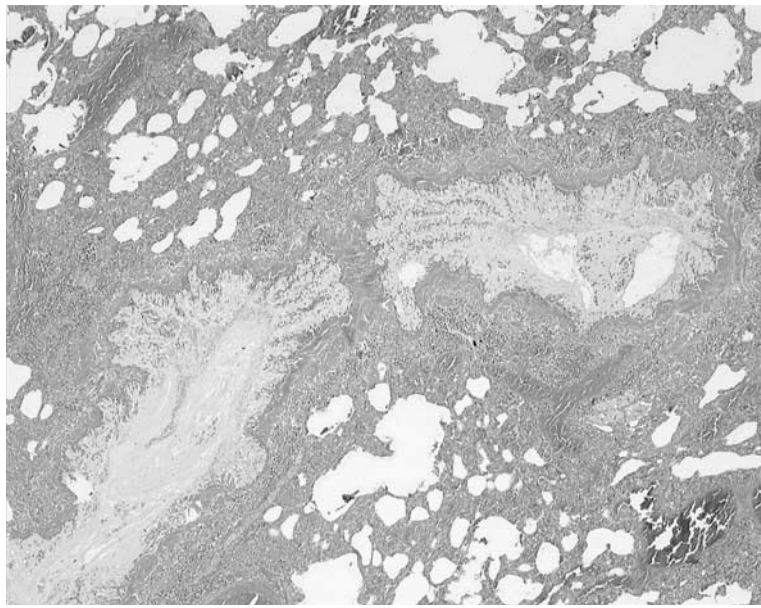

Fig. 6. Panoramic view of two bronchi occluded by mucous plugs in asthma bronchiale (hematoxylin and eosin).

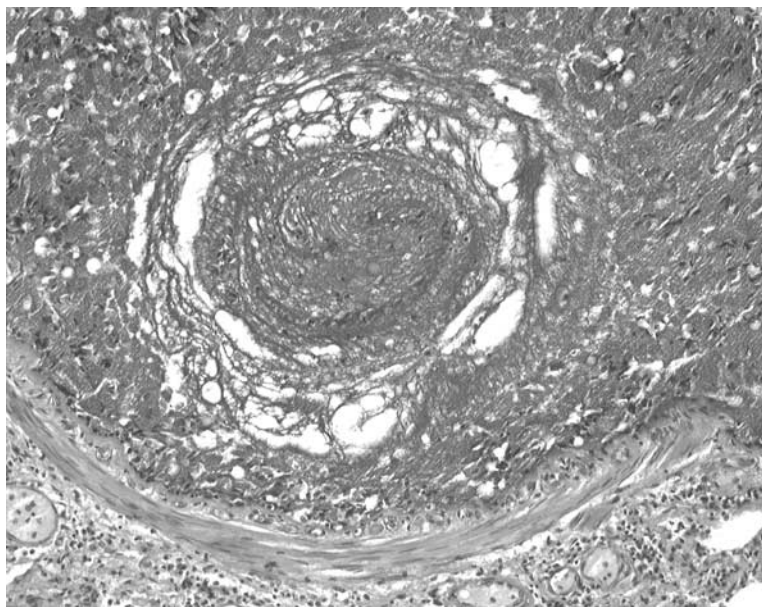

Fig. 7. Closer view of a Curschmann spiral occluding a small bronchus (periodic acid-Schiff stain).

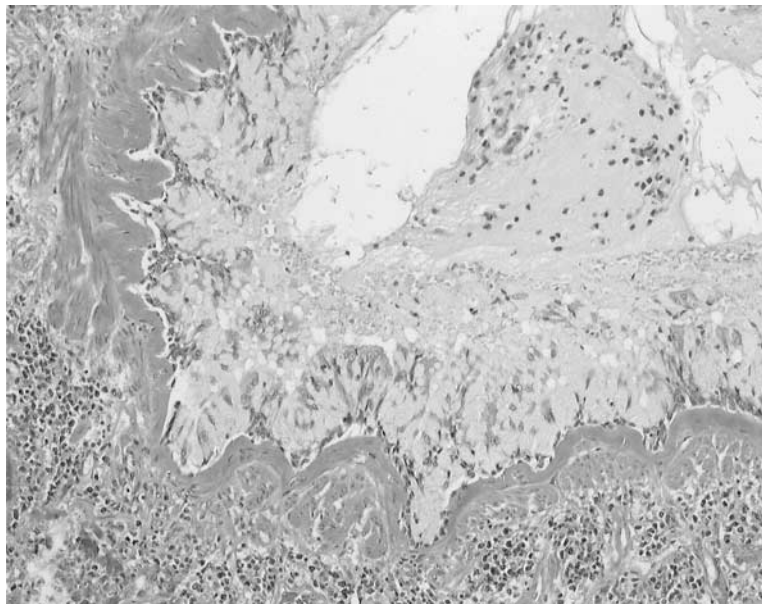

Fig. 8. Mucus hypersecretion with hyperplasia of goblet cells. Note the inflammatory cells, mostly eosinophils, within the mucus in the lumen of the bronchus (hematoxylin and eosin).

The presence of mucus contributes to bronchial collapse and reduces bronchial reversibility by modifying surface tension properties. Mucus may extend from the bronchial gland ducts to fuse with airway luminal masses (Fig. 10). Bronchial gland duct ectasia (Fig. 11) is another common histological feature seen in more than two-thirds of fatal cases *(51)*; a periductal inflammatory cell infiltrate mainly composed of eosinophils is characteristic.

In earlier textbooks of pathology, Charcot-Leyden crystals (also known as Charcot-Robin's crystals or asthma crystals) have been considered a highly specific micromorphological feature of asthma bronchiale when found in mucous plugs within the airway lumina from autopsy cases or in sputum obtained in vivo. These acidophilic, hexagonal, bipyramidal crystals, described by Jean-Martin Charcot and Charles-Philippe Robin in 1853 and by Ernst Victor von Leyden in 1872 *(52,53)* are indeed associated with chronic respiratory diseases, particularly asthma; however, these small particles, most probably deriving from eosinophilic granules of leukocytes, are not exclusively limited to airway secretions because they are also found in a broad variety of extrapulmonary secretions and human tissues. Their presence is usually associated with an increased number of peripheral blood or tissue eosinophils. Apart from

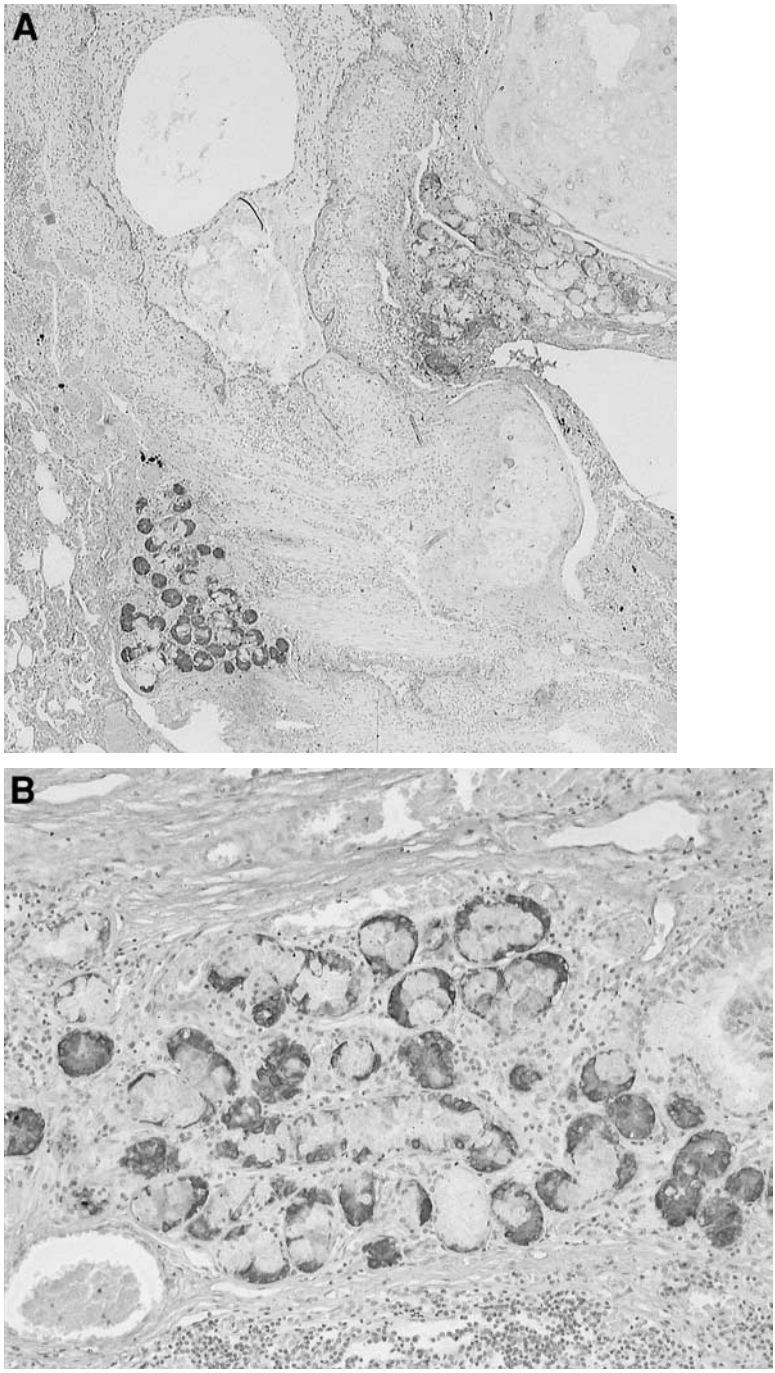

Fig. 9. Hypertrophy and hyperplasia of submucosal glands (lactoferrin immunohistochemistry). **(A)** Panoramic view. **(B)** Close-up view of strong positive immunohistochemical staining reaction pattern in serous parts of subepithelial seromucous glands of the bronchi in a case of fatal sudden-onset asthma (lactoferrin immunohistochemistry).

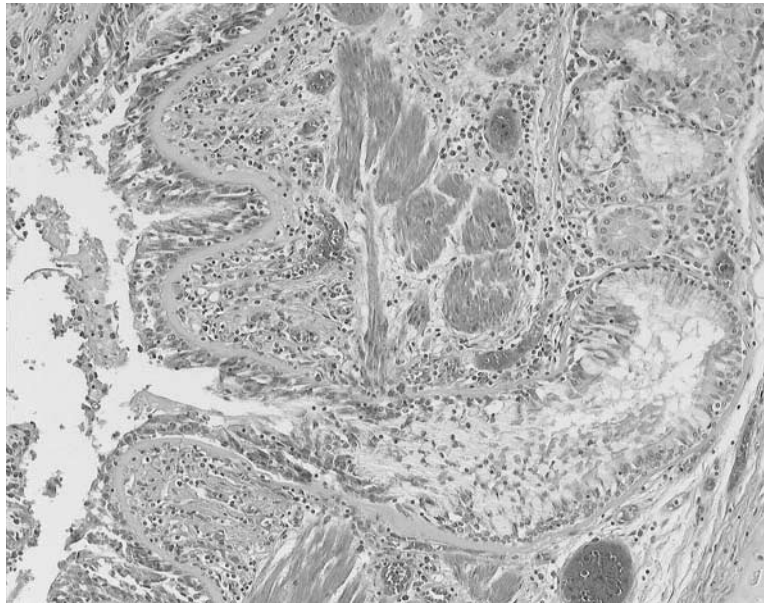

Fig. 10. Mucous masses extending from a bronchial gland duct fusing with airway luminal mucus (hematoxylin and eosin).

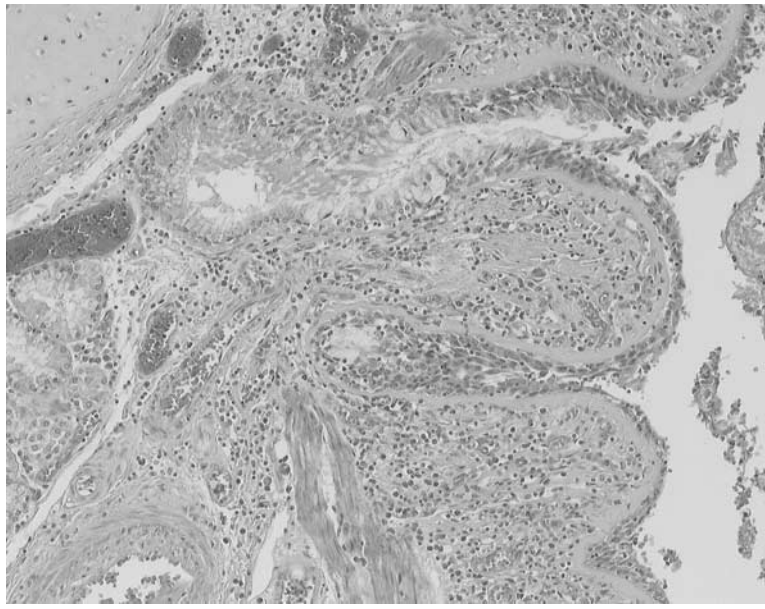

Fig. 11. Bronchial gland duct ectasia in asthma bronchiale. Note the periductal inflammatory cell infiltrate (hematoxylin and eosin).

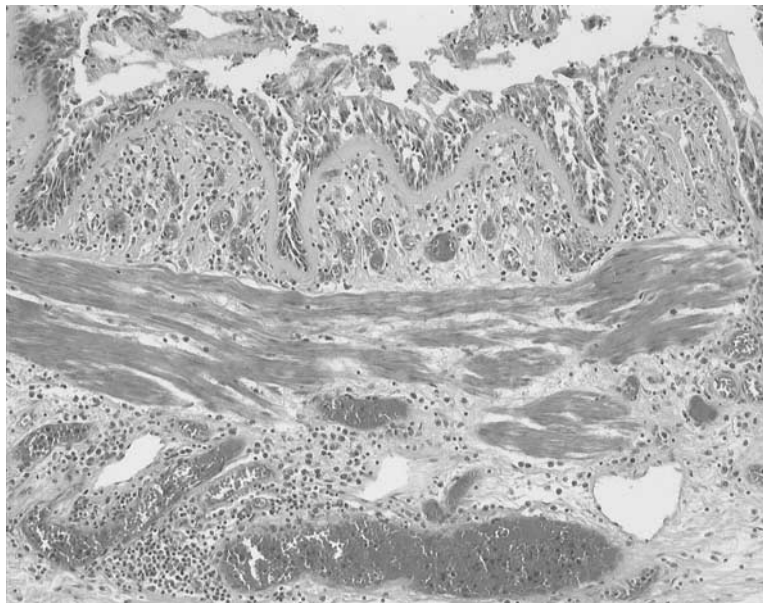

Fig. 12. Airway remodeling in chronic asthma bronchiale: marked thickening of the subepithelial basement membrane and smooth airway muscle hyperplasia (hematoxylin and eosin).

chronic respiratory diseases, such as chronic obstructive lung disease (COPD) or aspergillosis, the finding of Charcot-Leyden crystals has been described in different parasitic and allergic processes, e.g., in amoebic and ulcerative colitis.

Thickening of the subepithelial basement membrane, as well as smooth airway muscle hyperplasia, are further characteristic histological features of airway remodeling in asthma contributing to airway narrowing (Fig. 12). Airway remodeling may by itself contribute to airway hyperresponsiveness in asthmatics through purely mechanical means. By using electron microscopy, Roche and coworkers showed that subepithelial basement membrane thickening is in fact subepithelial fibrosis as a result of collagen deposition owing to fibroblast activation in the reticular basement membrane, which is located directly underneath the light microscopically visible subepithelial basement membrane *(54)*.

Dilatation of the airways' vessels is another factor contributing to excessive airway narrowing in status asthmaticus. A perivascular inflammatory infiltrate (Fig. 13) mainly composed of eosinophils and monocytes, as well as inflammatory infiltrates within the adventitia of smaller and medium-sized vessels, is frequently seen in recurrent and prolonged asthma attacks.

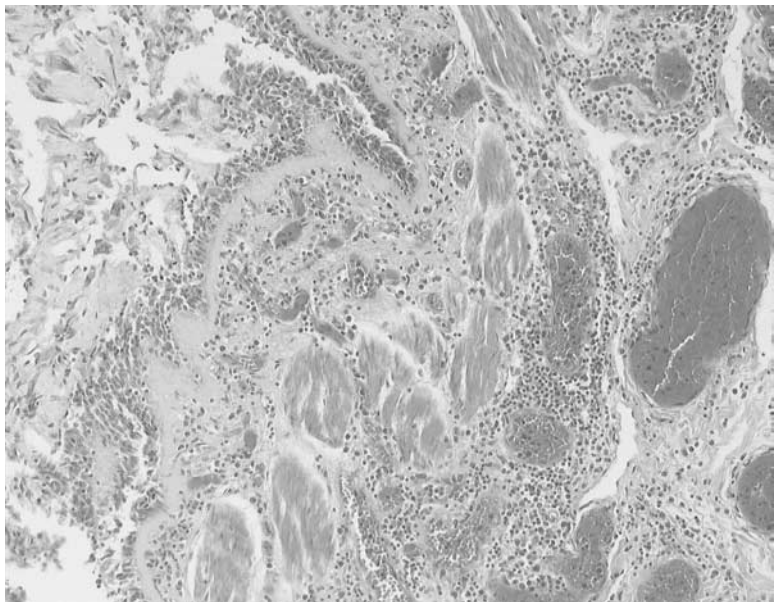

Fig. 13. Dilated airways vessels with perivascular inflammatory infiltrates in a deceased patient who suffered from chronic asthma bronchiale (hematoxylin and eosin).

Mediators released from these cells, such as cytokines and adhesion molecules, directly affect the vessels' walls, making them much more permeable to macromolecules with subsequent exudation of plasma through the airway wall, thus resulting in airway wall edema and increased mucosal thickening *(43,55)*.

Epithelial desquamation has been regarded as one of the classical pathological features of asthma for decades. In pulmonary autopsy specimens, the term *epithelial desquamation* refers to the finding of epithel cell loss ("denudation") with strips of endothelium seen within the airway lumen (Fig. 14). Epithelial desquamation has been observed in postmortem specimens *(47,56)* and in endobronchial biopsies from patients with asthma *(57,58)*. The clinical observations of clumps of epithelial cells in sputum from patients with asthma (so-called Creola bodies) and increased numbers of epithelial cells in bronchoalveolar lavage fluid from subjects with asthma has supported the concept of epithelial desquamation being a pathological characteristic of asthma *(59,60)*. It has been hypothesized that airway hyperreactivity in asthma may be related to epithelial desquamation through mechanisms involving loss of epithelium-derived relaxing factor *(62,63)*.

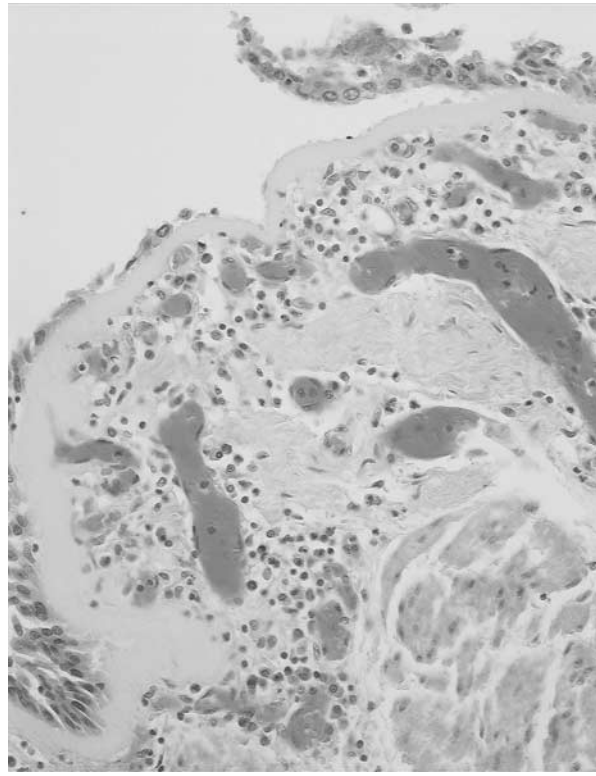

Fig. 14. Epithelial desquamation in asthma bronchiale (hematoxylin and eosin).

However, the pathological and pathophysiological significance of epithelial desquamation has recently been questioned. It was claimed that desquamation of the epithelium in asthma may not represent true pathology but may instead be an artifact of inadequate tissue sampling and handling *(50,63)*, a statemant that has been vehemently contradicted by another study group *(64)*. Because of the ongoing uncertainty about whether epithelial desquamation represents a pathological feature of asthma or an artifactual finding, one should be cautious in regarding epithelial cell loss as pathognomonic for asthma in doubtful cases.

Peripheral airway inflammation, as indicated by elevated numbers of lymphocytes and eosinophils within the interstitium of the alveolar compartment (Fig. 15), is another characteristic feature of asthma cases where the disease is the primary or major contributing cause of death *(65,66)*.

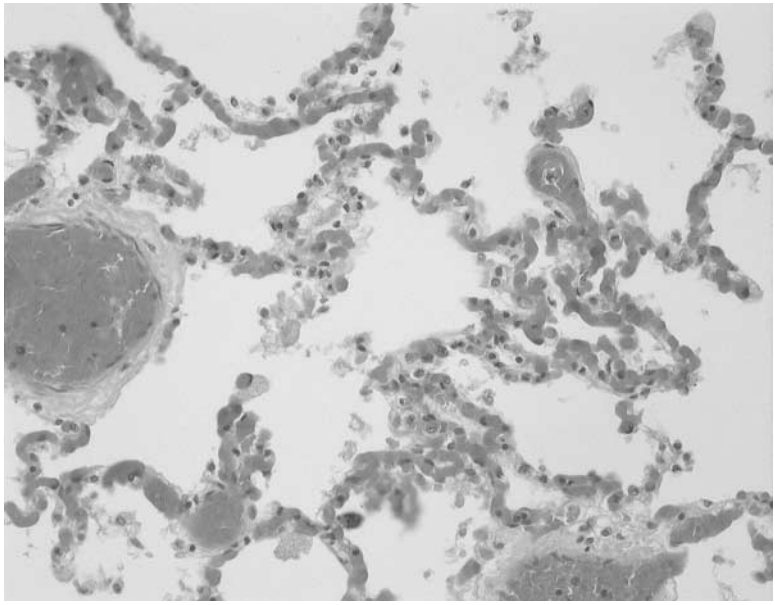

Fig. 15. Peripheral airway inflammation with lymphocytes and eosinophils within the interstitium of the alveolar compartment (hematoxylin and eosin).

3.5. Immunohistochemistry

For practical purposes, immunohistochemical investigations are usually not required to establish or support the diagnosis of asthma in forensic pathological casework because eosinophils and leukocytes can be easily identified by conventional staining techniques, such as hematoxylin and eosin. However, for the sake of completeness, some remarks on immunohistochemical studies in asthma deaths are made here.

To begin, one has to be aware that the use of archival autopsy and surgical specimens for immunohistochemical studies impose a number of limitations for drawing clear-cut conclusions on the pathological condition being investigated. To objectify tissue fixation in the archival samples being investigated, even when applying comparison of staining intensity to an internal positive control, is generally not possible, and differences in cell fixation may lead to both intraindividual and case-to-case variation in immunostaining sensitivity.

Faul and co-workers examined the relative proportion of lymphocyte and macrophage subsets and eosinophils in proximal and distal tissues from cases of sudden-onset asthma deaths compared with stable asthma cases using

immunohistochemical and immunofluorescence techniques *(67)*. These authors found large numbers of CD8+ T-cells in fatal sudden-onset asthma, suggesting again distinct qualitative, as well as quantitative, characteristics in the immunopathology of sudden asthma deaths.

Kepley and co-workers used a basophil-specific monoclonal antibody (2D7) to quantify postmortem lung basophil numbers in individuals who died from asthma and compared the results to subjects who had died from nonasthmatic causes (both patients who had a history of asthma but died from nonasthmatic causes, as well as patients with no previous history of asthma) *(39)*. Basophils express high levels of the high-affinity IgE receptor and are effector cells in systemic allergic inflammation because of their ability to release histamine and other preformed mediators from granules upon stimulation. Basophil numbers are reportedly increased in both the upper and lower airways during the late-phase response of allergic patients. Kepley et al. *(39)* found basophils scattered throughout the large and small airways, airway epithelium, submucosa, and alveolar walls in both asthma deaths and controls. However, basophil infiltration was significantly increased in lungs from fatal asthma cases when compared with the numbers of basophils in lungs from individuals who had a previous history of asthma but died from nonasthmatic causes and in those with no previous history of asthma. From these authors' points of view, these findings support the hypothesis that basophils are involved in the pathogenesis of severe asthma with sometimes fatal consequences. Kepley et al. *(39)* put forward the idea that increased numbers of basophils in the lungs could contribute to the pathogenesis of fatal asthma in a variety of ways because, aside from mast cells, basophils are the only cell type that stores and releases large amounts of histamine, which induces both bronchoconstriction and secretion of mucus in asthmatics. In addition, basophils store and release large amounts of leukotrienes, which are powerful bronchoconstrictor agents as well.

Popper and colleagues took a look at the immunohistochemical expression pattern of different adhesion molecules in bronchitis associated with asthma *(42)*. They found a strong expression of very late-activation antigen-4 and lymphocyte function-associated antigen-1 on lymphocytes in all asthma cases compared to a weak, sporadic expression of these adhesion molecules on lymphocytes in controls. Vascular cell adhesion molecule (VCAM)-1 was strongly expressed on endothelial cells of pulmonary venules and capillaries only in biopsies from patients with clinically active disease, which was also positively correlated with a greater density of infiltrating lymphocytes and eosinophils; VCAM-1 was not detectable in control tissue. The authors concluded that expression of VCAM-1 on endothelial cells and its ligand, very late-activation antigen-4, on lymphocytes and eosinophils is a somehow specific

finding in asthma, and that the activation of cellular adhesion molecules in asthma obviously follows selective pathways.

Tsokos and Paulsen *(15)* investigated the immunohistochemical expression pattern of lactoferrin (LF) in pulmonary tissue sections deriving from fatal slow-onset asthma (time interval between onset of asthma attack and death >2.5 hours) and fatal sudden-onset asthma (cases in which death occurred within 1 hour of the onset of the asphyxic asthma attack) compared with controls (sudden death owing to diseases other than respiratory disorders). LF is a nonspecific modulator of airway inflammation located in subepithelial seromucous glands of the bronchi and in specific granules of leukocytes. In airway inflammation, LF is substantially enriched on mucosal surfaces. LF plays a central role in the modulation of airway inflammation, referring to its ability of binding free iron, thus preventing iron-mediated catalysis of hydroxyl radical formation and promoting leucocyte adherence to endothelial cells, hence amplifying the cellular response at inflammatory sites. LF was applied to paraffin sections using a standard peroxidase-labelled streptavidin-biotin technique. LF immunoreactivity was graded semiquantitatively in relation to different histoanatomic distribution sites of LF on a five-category ordinal scale. There was a statistically significant difference between an enhanced expression of LF in both asthma groups compared with the controls ($p < 0.004$ and $p < 0.001$, respectively). When both asthma groups were compared, there was a statistically significant difference in LF immunoreactivity between the slow-onset and sudden-onset groups ($p < 0.001$). The different expression pattern of LF observed in fatal sudden-onset and slow-onset asthma can be of medicolegal relevance because conclusions may be drawn toward the preceding period of time between the asphyxic asthma attack and death.

3.6. Infections: Triggers and Complications

Clinical and experimental evidence suggests an important role for respiratory tract infections in the development of asthma attacks. Viral upper respiratory infections have been associated with 80% of asthma exacerbations in children, and some investigators assume that even as much as 50% of all asthma episodes in adults are triggered by viral infections *(68)*. In children, a common cold, usually caused by human rhinovirus, has been implicated as the principal virus associated with asthma episodes. In a recent study, Watson et al. *(69)* tried to detect rhinovirus in archival, wax-embedded peripheral airway tissue obtained postmortem from patients who died from asthma using reverse transcription-polymerase chain reaction. All tissues samples examined were negative for the presence of rhinovirus mRNA. The authors concluded that rhinovirus infection of the lower airways may be an uncommon cause of fatal

asthma or alternatively, rhinovirus may not extend to the peripheral airways. Whether investigation of more proximal tissue samples would have detected rhinovirus, and thereby determined a possible association between rhinovirus respiratory tract infection and asthma, remains a matter of speculation, but in this author's opinion, the design of this study was too insufficient to draw any conclusions.

Studies indicate that atypical bacteria, such as *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*, may precipitate asthma symptoms and induce an acute attack *(70)*. Whether atypical bacterial infections are an epiphenomenon or a pathogenic event in asthma has not yet been clarified *(68)*; the pathology of *M. pneumoniae* and its forensic pathological relevance has been recently reviewed elsewhere *(71)*.

Upper aerodigestive tract candidiasis is a well-known but uncommon sequel of steroid inhalation therapy, seen especially in immunocompromised subjects *(72–76)*.

3.7. Differential Diagnoses

Rainbow and Browne *(77)* reported two cases of fatal anaphylaxis involving a 4-year-old boy and a 9-year-old girl who were clinically misdiagnosed to have severe asthma. Both children did not respond to bronchodilator treatment and died in hospital shortly after the onset of symptoms. This observation highlights the fact that a predominance of respiratory symptoms in patients having an anaphylactic reaction may be misdiagnosed as an acute asthma attack.

Mucous plugs within the bronchi can be occasionally seen on cut surfaces of the lungs in fatalities caused by pulmonary thromboembolism and should not be mistaken for plugging of mucus as seen in asthma deaths. Pathophysiologically, mucous plugs seen in pulmonary thromboembolism are a result of acute pulmonary hypertension: the increase of intraluminal pulmonary artery pressure is transmitted to the anatomically neighboring bronchi, thus forcing mucus out of the bronchial mucosa into the bronchial lumen. However, mucus as a result of pulmonary thromboembolism does not have the tenacious appearance as is the case in asthma. Microscopical examinations will, in most instances, not be necessary to differentiate between mucous plugs as a result of pulmonary thromboembolism or asthma because incision and inspection of the pulmonary artery tree during the routine removal of the lung *(78)* will easily resolve the differential diagnosis. However, mucus as a result of pulmonary thromboembolism is easily distinguished histologically from true asthmatic mucous plugs by the lack of eosinophils within the mucus and the absence of the airway wall characteristics seen in asthma.

3.8. Causes of Death

Deaths of asthmatics may be caused by the disease alone or its sequelae, by other unrelated causes, or a combination of both. Fatal asthma attacks usually occur against a background of a long medical history of the disease, chronic persistent symptoms, required prolonged medication, and repeated hospitalization.

Death may occur during status asthmaticus or may be quite sudden and unexpected without any respiratory symptoms preceeding death. Whereas in the first instance death is attributable to acute asphyxia as a sequel of massive narrowing of the airways, the exact pathophysiological mechanisms of death in the second instance are not yet completely understood *(79)*. In such cases where no or only minor respiratory symptoms preceeded death, it has been proposed that sudden cardiac death may occur as a manifestation of neurally mediated hypotension-bradycardia syndrome, occasionally influenced by adverse adrenergic effects of some anti-asthmatic drugs. However, it is difficult to obtain adequate clinical data because of the rapidity of the time course from apparent wellness to death and the occurrence outside the hospital *(80)*. Some asthmatics may indeed have additional, undiagnosed cardiac abnormalities contributing to sudden death rather than true asthma deaths.

The immediate cause of death in acute severe asthma attacks is usually attributable to asphyxia as a sequel of excessive airway narrowing primary owing to a combination of muscle spasm and increased production of mucus with subsequent mucous plugging. However, it may be difficult in some asthma deaths to clearly distinguish between asphyxia and primary right heart failure as the immediate cause of death at autopsy as stressed by Cordes and Püschel *(24)*, who retrospectively reviewed the files of 18,391 autopsies performed at the Institute of Legal Medicine, Hamburg, Germany, between 1983 and 1999. They found 21 cases where asthma was the immediate cause of death (corresponding to a prevalence of asthma of 0.11% in the autopsy material) with more than 70% of the asthma deaths showing a hypertrophy of the mycardium of the right ventricle at autopsy.

In their autopsy study of 304 decedents with asthma bronchiale at the time of death, Messer et al. found that 11.5% of the cases were the result of status asthmaticus, 10.2% occurred from complications of the disease, and 78.3% were unrelated to the disease *(6)*.

Jones and co-workers conducted a prospective study including a review of hospital records and autopsy reports and inquiring of relatives and respective general practitioners of subjects whose deaths were attributed to asthma, whether wholly or partly *(81)*. In their study, asthma alone appeared to be the critical factor in causing death in only 16 of the 79 certified cases investigated.

In another 17 cases, a wide variety of additional, comorbid disorders appeared to have contributed to fatal outcome. These 17 cases included, during the 24 hours preceding death, gastric juice aspiration, sepsis, a single dose of a β-blocker, the abuse of organic solvents or illicit drugs, and, possibly, an inadvertent exposure to horse allergen. More chronic causes of comorbidity were ischemic heart disease, COPD, thoracic cage deformity, and alcohol abuse *(81)*.

Kravis and Kolski *(82)* and Champ and Byard *(83)* investigated sudden deaths from asthma in childhood. In their clinical study, Kravis and Kolski revealed that the cause of death may be medication-related, as exemplified by patient abuse of inhaled adrenergic drugs with concomitant erratic use of theophylline and corticosteroid drugs or by physician failure to appreciate the need for corticosteroids *(82)*. In the clinicopathological study by Champ and Byard, sudden and unexpected death from asthma occurred only in children with significant underlying chronic diseases *(83)*.

Fatal sepsis resulting from upper aerodigestive tract candidiasis as a complication of steroid inhalation therapy is extremely rare.

4. Medicolegal Aspects of Fatal Asthma

4.1. Accuracy of Death Certification

The accuracy of death certification related to asthma deaths has been questioned repeatedly over the last decades. Wright and co-workers reviewed asthma deaths in Northern Ireland that had been registered between 1981 and 1984. With clinical evidence, death certificates, and coded cause of death, a 31% false-negative rate for death certification was reported in the 0- to 64-years-of-age group in this study (*84*). Bucknall and co-workers reviewed the general practice and hospital records of 235 patients dying in mainland Scotland between 1994 and 1996 with a principal diagnosis of asthma as the cause of death that was recorded by the Registrar General's Office. Only 95 deaths (40%) were confirmed as being the result of asthma *(85)*.

In a Danish population, Sidenius and coworkers *(86)* more recently evaluated the accuracy of 218 death certificates where asthma was coded as cause of death. Medical information on all subjects with asthma coded as the underlying cause of death was obtained by reviewing hospital records and performing interviews with general practitioners and relatives. A panel of four pulmonologists each examined the obtained information and independently assessed the cause of death. In 16 (9%) of the subjects, death from asthma was judged to be the definite cause of death and in another 12 (7%), death from asthma was possible. Of 151 nonasthma deaths coded as asthma deaths,

109 were judged to have suffered or died from COPD and 14 from heart disease. These authors concluded that the accuracy of death certification in asthma deaths is poor and that the true asthma mortality is substantially lower than officially recorded *(86)*.

The aforementioned substantial discrepancies between the true cause of death and the cause of death given on death certificates are not surprising to the forensic pathologist because when comparing the clinical and postmortem diagnoses of the causes of death, diagnoses are only in total agreement in approx 50 to 60% of cases *(87,88)*.

4.2. Exogenic Trigger Factors Exacerbating Asthma

The consensus hypothesis attributes bronchial hyperresponsiveness in asthma to an inflammatory reaction to diverse stimuli. Allergens seem to be the most common triggers for asthma exacerbation. The incidence of asthma triggered by allergens is increasing. There are many theories explaining this rise including improvement of diagnostics, urban living, higher exposure to dust mites, atmospheric pollution, nutrition, lifestyle changes, maternal smoking, diesel fumes, geography, the "hygiene hypothesis," etc. Of specific medicolegal and forensic pathological interest are several extrinsic noxae ("exogenic trigger factors"), such as substances of abuse that may trigger acute life-threatening asthma exacerbation, potentially resulting in fatal outcome. Heroin insufflation is a common trigger for asthma symptoms among individuals with preexisting asthma, and several reports provide data that (uncontaminated) morphine and heroin produce bronchospasm, possibly through an allergic mechanism (*89–93*). In 2003, Krantz et al. described asthma exacerbations associated with heroin insufflation in 13 of 23 patients (56%) who required intensive care unit admission for a life-threatening asthma attack *(94)*. These cases were characterized by childhood onset of asthma in 45%, heroin insufflation as the sole method of use, except for one patient, and sudden onset of symptoms after insufflation.

Different underlying pathophysiological mechanisms for heroin-induced bronchospasm have been proposed. Pulmonary mast cell degranulation may be one possible mechanism because histamine release occurs in one-fifth of patients receiving postoperative analgesia with morphine *(95)*. Heroin powder and its contaminants and/or cutting agents may serve as nonspecific airway irritants *(94)*.

Alcoholic beverages are also capable of triggering asthma. In a survey of asthmatics, more than 40% reported the triggering of allergic or allergic-like symptoms following alcohol consumption and 30 to 35% reported a worsening of their asthma following alcoholic beverage consumption *(96)*. Among alco-

holic beverages, wine is clearly the most commonly reported trigger, with the histamine in wine held responsible for such adverse reactions *(96)*.

Hormonal variations during the female menstrual cycle are considered to play an important role in the exacerbation of asthma. About 30 to 40% of female asthmatics report worsening of asthma symptoms during their premenstrual and/or menstrual period. Exogenous sex hormones and/or contraceptive pills are also believed to precipitate an asthma attack in individual cases *(97)*.

More or less strenuous exercise can precipitate an acute asthma attack. Whether exercise-induced bronchospasm is the result of vascular congestion of the bronchi, water loss of bronchial epithelium, or mediator release is still a matter of debate. The complex interplay of a variety of factors responsible for exercise-induced asthma exerbation and sudden death in childhood has been accentuated by Byard and co-workers *(98)*.

4.3. Adverse Effects of Asthma Medications

Inhaled β(2)-adrenoceptor agonists (β[2]-agonists) are the most commonly used asthma medications in many Western countries. Earlier studies have suggested an association between the relatively nonselective β-agonist fenoterol and asthma deaths. Recently, a number of case–control studies have confirmed that among patients prescribed fenoterol, the risk of death is significantly elevated. It is probable that nebulized and oral β(2)-agonists are also associated with an increased risk of cardiovascular death, ischemic heart disease, and cardiac failure *(99)*.

Beasley et al. *(100)* reviewed numerous studies that examined the effects of β-agonist drugs on morbidity and mortality. With regard to morbidity, there was considerable evidence that the regular use of β-agonist drugs could potentially lead to a worsening of asthma because of the effects on bronchial hyperresponsiveness, the development of tolerance and reduced protection against provoking stimuli, an increased allergen load, and masking of the symptoms of deteriorating asthma. With regard to deaths, Beasley et al. found evidence indicating that high-dose preparations of fenoterol and isoproterenol were associated with increased mortality and were the major causes of the epidemics of asthma mortality observed in some countries *(100)*.

Reports from relatives give indications that asthmatics tend to use higher doses of inhalation medications than prescribed. Because of the effectiveness of these drugs in providing symptomatic relief, this may cause patients to fail to recognize the severity of their disease and delay seeking medical help.

Development of oropharyngeal and esophageal candidiasis is a frequently reported adverse effect of inhaled corticosteroid use *(101,102)*.

4.4. The Medical Expert Witness and Asthma Deaths: Issues of Medical Malpractice

The forensic pathologist is occasionally confronted with asthma deaths in the following constellations: (a) the death of an asthmatic who is known to have consulted a physician prior to death but the correct diagnosis was not established; (b) the death of an asthmatic who had consulted a physician prior to death (it does not matter whether this was during an acute state of the disease or not) and the correct diagnosis was established but treatment was inadequate or even worsened the attack; or (c) the sudden, unexpected death of an asthmatic occurring outside the hospital or out of a physician's surveillance, witnessed or unwitnessed, with or without a previous history of asthma.

Burr and co-workers conducted a confidential inquiry of 52 fatalities where asthma was the underlying cause of death *(103)*; 15 cases could be attributed to deficiencies in medical care. The authors established the following demographic characteristics of asthma deaths: (a) nearly all asthma deaths in persons younger than 65 years of age occur outside hospital; (b) in half the cases, the disease is chronic and severe; an acute catastrophic attack occurs in one-third of patients whose disease is moderate or mild; (c) other factors contributing to fatal outcome other than the severity of the disease can be identified in 70% of asthma deaths; (d) in 60% of cases, a "patient factor," particularly relating to psychosocial problems and poor compliance with treatment, can be identified; and (e) in 30% of cases, some aspect of the medical care may have contributed to the death, particularly lack of inhaled steroids, inadequate follow up, or contraindicated medication.

To prove that a physician has not taken sufficient care of his patient and that fatal outcome of asthma has undoubtedly arisen from insufficient diagnostics and/or treatment requires a detailed evaluation of the case with knowledge of the deceased's medication and activities preceding death (*see* Subheadings 4.2. and 4.3.), information on the background medical history, and detailed knowledge of autopsy and histological findings. Whether the required standards of medical care have been applied properly in the individual case has to be scrutinized against the background of all circumstantial data available.

Rainbow and Browne *(77)* highlighted the fact that a predominance of respiratory symptoms in patients having an anaphylactic reaction may be misdiagnosed as an acute asthma attack. The question of whether the diagnosis of an acute asphyxic asthma attack was correctly established and if treatment was adequate antemortem is of major importance. In most asthma deaths where the diagnosis was established correctly antemortem, inadequate medical treatment

in view of the known severity of the disease can be held responsible for fatal outcome (e.g., failure to prescribe adequate inhalation therapy, prescription of drugs that may have an adverse effect on asthma, such as β-blockers, aspirin, or nonsteroidal antiinflammatory preparations, and inadequate arrangements for the patient's follow-up). Nonadmission to the hospital despite a life-threatening condition owing to inadequate assessment of the patient's condition may also be a relevant factor contributing to a patient's death.

Whether a physician can be held liable for the fatal outcome from the medicolegal point of view highly depends on the individual patient's characteristics mentioned in Subheadings 3.6., 4.2., and 4.3. Although often very difficult to verify, poor patient compliance or inadequate agreement between the patient and the physician in charge of treatment are further common issues. Delay in seeking medical attention, failure to keep appointments or comply with treatment, symptom denial, and alcohol or drug abuse are frequently contributors to a fatal course of asthma, especially in persons with a low socioeconomic status. On the other hand, irrespective of their socioeconomic status, serious self-management errors occur in a high proportion of patients with acute severe asthma.

The results of the study by Robertson and coworkers *(104)* seems to be representative of the issues discussed before. These authors examined the circumstances surrounding the deaths of 163 patients who died from asthma in the state of Victoria, Australia, over a 12-month period. Thirteen percent of the affected individuals had a history of trivial or mild asthma, 22% had a history of moderate asthma, and 65% had a history of severe asthma. Death occurred outside the hospital in 150 subjects (92%). In the fatal attack, 58% had a sudden onset and collapsed within minutes, 20% were found dead, and 27% had an acute progression of an established attack. Twenty-nine percent of the deaths were retrospectively assessed as preventable. Preventable factors included inadequate assessment or therapy of prior asthma (35%), poor compliance with therapy (33%), and delay in seeking help (43%). A significant number of subjects in this survey could not be classified as belonging to the "high-risk" patient group.

It can not be accentuated enough that the final exacerbation phase usually occurs very suddenly and that this terminal attack is usually short, with death often occurring within 1 hour or less. Therefore, especially in asthma attacks that occur outside the hospital, respiratory failure and right ventricular overload may progress so rapidly that there is insufficient time to obtain adequate therapy. Another point is that patients may, for miscellaneous reasons, not respond to treatment that appears to be adequate.

5. Conclusions

Asthma bronchiale represents a major health issue and an important cause of sudden, unexpected death, and will likely remain so for decades. All major studies evaluating the accuracy of death certificates coding asthma as cause of death agree that death certificate coding is an unreliable guide to asthma death, and that a thorough autopsy is the final word in suspected asthma deaths. Preventable asthma deaths still occur, particularly in relation to inadequate treatment. It is crucial for the forensic pathologist and medical examiner, respectively, to recognize the different phenomenological and morphological features under which asthma deaths may present, as well as the associated pathogenetic mechanisms that may have exacerbated the acute, fatal asthma attack in order to determine the correct manner of death. In addition, a profound knowledge of medicolegal issues raised by asthma deaths is essential for everyone involved in death investigation.

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6

Peliosis of the Liver and Spleen

Pathological Features and Forensic Pathological Relevance of Two Rare Diseases With Potentially Fatal Outcome

Michael Tsokos, MD and Andreas Erbersdobler, MD

CONTENTS

SUMMARY

Peliosis is a comparetively rare pathological entity characterized by the gross appearance of multiple cyst-like, blood-filled cavities within parenchymatous organs. Peliosis has been related to several underlying debilitating illnesses, such as tuberculosis, hematological malignancies, AIDS, and posttransplant immunodeficiency, as well as intravenous drug abuse, chronic alcoholism, and in conjunction with intake of oral contraceptives or the abuse of anabolic–androgenic steroids. The classical pathoanatomical concept is based on the opinion that peliosis exclusively develops in organs belonging to

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the mononuclear phagocytic system (liver, spleen, bone marrow, and lymph nodes). In the liver, at gross inspection, the peliotic lesions give the cut sections a “swiss cheese” appearance. Microscopically, two different types of peliosis can be distinguished in the liver: (1) “parenchymal peliosis” consisting of irregular cavities that are neither lined by sinusoidal cells nor by fibrous tissue; and (2) “phlebectatic peliosis” characterized by regular, spherical cavities lined by endothelium and/or fibrosis. One of the differential diagnoses that most closely resembles peliosis hepatis is secondary hepatic congestion owing to veno-occlusive disease or the Budd-Chiari syndrome. In addition, electrocoagulation during surgery may cause vacuolated lesions within the liver parenchyma histologically resembling peliosis hepatis. In the spleen, the peliotic lesions may be arranged sporadically, disseminated, or in clusters in an uneven distribution pattern. Histologically, the cavities show frequently well-demarcated margins that may be focally lined by sinusoidal endothelium or totally lacking a clear cell lining. Differential diagnoses of peliosis of the spleen are hemangiomas and involvement of the spleen in hairy-cell leukaemia. Because the disease may culminate in spontaneous rupture of the affected organ and thus may mimick a violent death at autopsy, awareness of peliosis at autopsy, as well as an appreciation for the histopathological changes, is an important issue for the forensic pathologist.

Key Words: Peliosis; liver; spleen; sudden death; intraperitoneal hemorrhage; autopsy; forensic histopathology.

1. Introduction

Peliosis is a comparetively rare pathological entity characterized by the presence of multiple blood-filled cavities within parenchymatous organs *(1)*. The Greek word “pelios” (blackish-bluish with sugillation) was first used by Wagner in 1861 to describe the gross appearance of the disease on cut surfaces of the liver *(2)*. The diagnosis of peliosis may be occasionally established at autopsy by the unique gross appearance of multiple cyst-like, blood-filled cavities on cut surfaces of the affected organ, but usually, the disease is histologically diagnosed *(3–8)*. Peliosis has been related to several underlying debilitating illnesses or medications, and different theories have been proposed concerning the pathogenesis of this unique disorder over the years. Although some investigators favor congenital malformation of vessels or microcirculatory disturbances manifesting under altered local intravascular pressure conditions as responsible for the development of the disease *(5,9–11)*, others believe that an acquired vascular disorder triggered by toxic noxae may play a key role in the pathogenesis *(12,13)*. However, none of the chronic or toxic conditions

described in association with peliosis, or the pathogenetic mechanisms they may induce, can satisfactorily explain the pathological findings in all cases and organs involved, respectively. The morphological data obtained by different investigators suggest that there is more than one path of formal pathogenesis to the onset and maintenance of peliosis.

The classical pathoanatomical concept is based on the opinion that peliosis exclusively develops in organs belonging to the mononuclear phagocytic system (formerly known as the "reticuloendothelial system") and therefore, only affects the liver, spleen, bone marrow, and lymph nodes *(13,14)*.

This review adresses our present day knowledge of the pathological features of peliosis pointing out toward the forensic pathological relevance of the disease.

2. Peliosis of the Liver

The first well-described cases of peliosis were reported for the liver (*2,15*). This may be because of the observation that the liver seems to be the organ most frequently involved in the disease. Another reason could be the very conspicuous macroscopical appearance of peliotic lesions, particularly in the liver. Instead of the homogeneous or slightly spotted, tan, or brown aspects of the normal liver parenchyma, peliosis of the liver displays randomly distributed, well-defined, reddish-blackish spots. On cut sections through the organ, these spots turn out to be blood-filled, cystic spaces, measuring between several millimeters and a few centimeters, giving the section a "swiss cheese" appearance (Fig. 1).

The histological characteristics of hepatic peliosis have been described in numerous case reports and some small series of cases *(12,13,16)*. Two different types of lesions have taken shape by these microscopical analyses *(17)*: the first type is called "parenchymal peliosis" and consists of irregular cavities that are neither lined by sinusoidal or endothelial cells nor by fibrous tissue; the adjacent hepatic cells are sometimes necrotic (Fig. 2). The second type is known as "phlebectatic peliosis" (Fig. 3A,B); here, regular, spherical, blood-filled cavities are lined with endothelium and sometimes surrounded by fibrosis. Even ultrastructural examinations with semithin and ultrathin sections could not clarify whether the dilatation affects the sinusoids or the spaces of Disse within the liver. Rather, it seems as if both possibilities may coexist and that there is an unusually wide communication between these two compartments in the peliotic liver *(18)*. Immunohistochemical investigations showed that collagen types III and IV, as well as laminin, are deposited along the dilated cavities. These observations were interpreted as evidence for the trans-

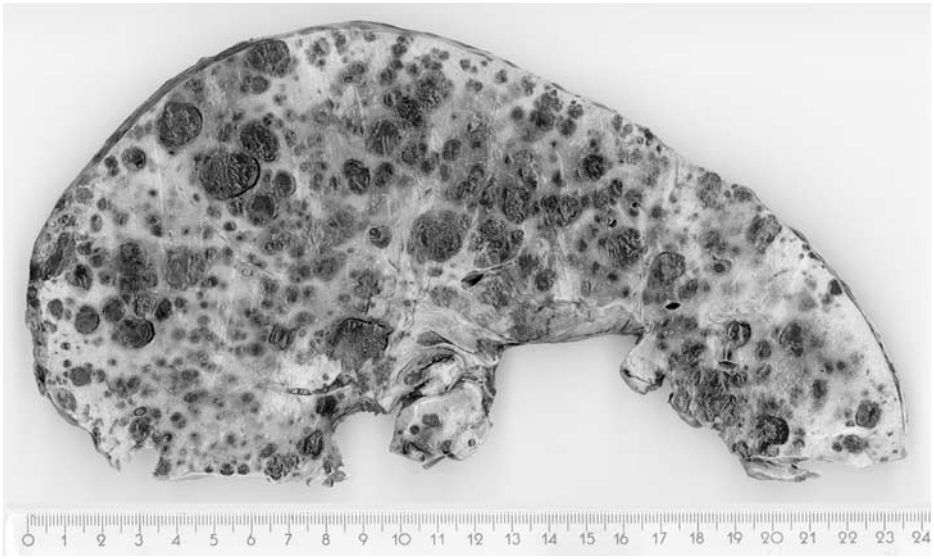

Fig. 1. Peliosis hepatis. Cut surface of liver parenchyma displaying multiple blood-filled cystic spaces.

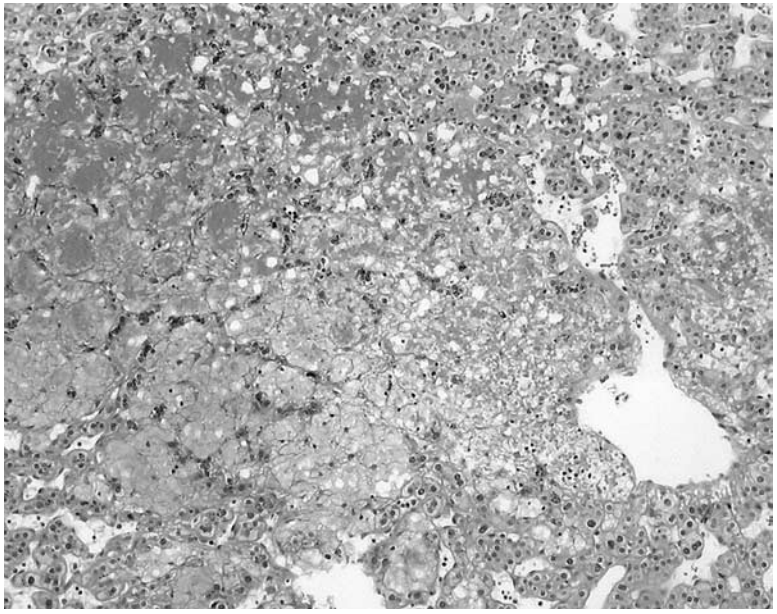

Fig. 2. Liver resection specimen removed because of a cyst (probably echinococcus); peliosis as an incidental finding in the adjacent parenchyma. Irregular, blood-filled, dilated sinusoidal spaces are visible. Hematoxylin and eosin, original magnification ×250.

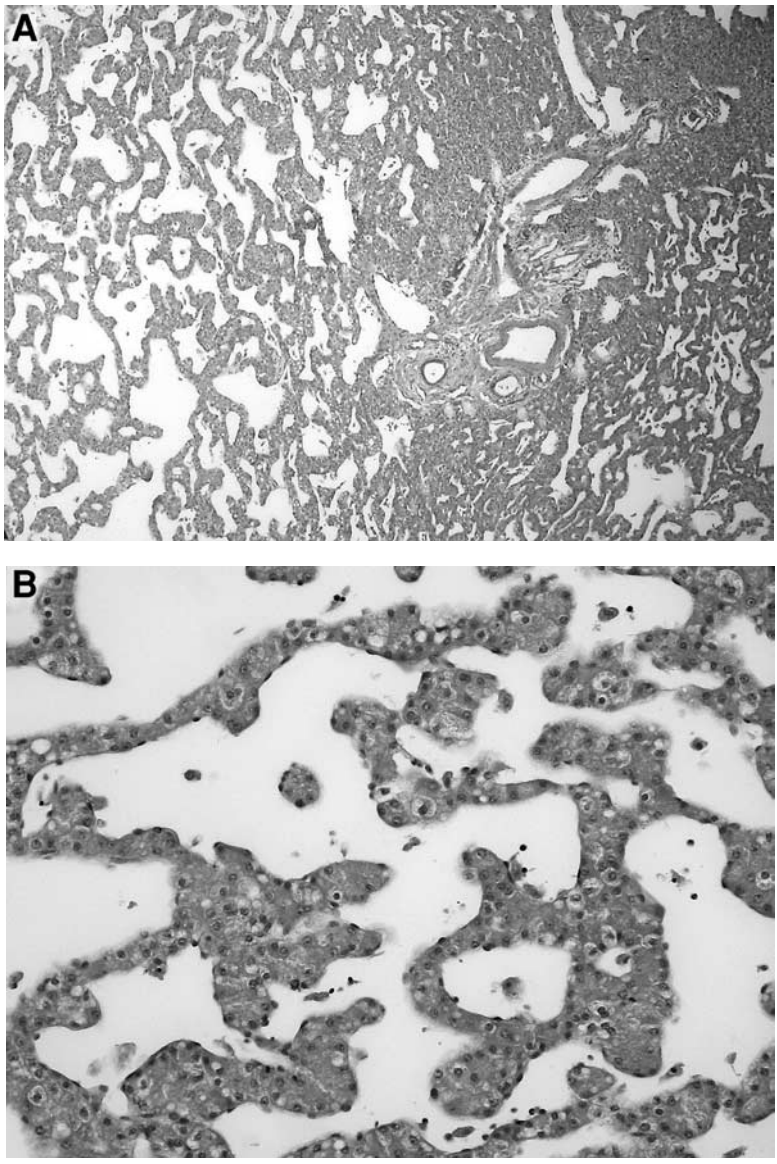

Fig. 3. (A,B) Liver from a cadaveric transplant donor showing previously unsuspected peliosis. The liver was not used for transplantation. Note dilated sinusoidal spaces lined with littoral cells. Spaces are empty because of pretransplant perfusion with preversation solution. Hematoxylin and eosin, **(A)** original magnification ×100. **(B)** original magnification ×400.

formation of hepatic stellate cells into myofibroblast in response to endothelial cell injury *(19)*.

Since the first descriptions of peliosis, much has been speculated about its causes. A wide variety of underlying disorders, most often chronically wasting diseases, such as tuberculosis and malignant tumors, were reported in earlier publications *(12,16)*. In fact, tuberculosis has been replaced as the disease most often accompanying peliosis by AIDS or posttransplant immunodeficiency *(6)*. Furthermore, a connection between the chronic intake of oral contraceptives or anabolic–androgenic steroids and the occurence of hepatic peliosis has become evident *(3,20,21)*. The formal pathogenesis of peliosis hepatis is still obscure. It is not quite clear whether the dilated cavities are the result of a locally elevated vascular pressure or if hepatocellular necrosis or apoptosis provides the space for the cystic lesions *(16)*. Some authors even hypothesize a neoplastic process, representing the utmost benign end on the spectrum of vascular tumors in the liver.

Secondary congestion owing to veno-occlusive disease or the Budd-Chiari syndrome is among the differential diagnoses that, histologically, most closely resemble peliosis of the liver. Taken alone, the sinusoidal dilatation ocurring in these ralated diseases may be indistinguishable from peliosis. Some of the earlier reports in which severe hepatic congestion or fibrotic alterations of the central veins were described *(12,16)* may in fact have been a chronic form of veno-occlusive disease rather than peliosis of the liver. Only the simultaneous dilatation of the portal vein in the neighboring portal field may provide a clue to an underlying, severe congestion (Fig. 4A). Of course, organizing thrombi within small central venules or larger hepatic veins (Fig. 4B) confirms the diagnosis of veno-occlusive disease or the Budd-Chiari syndrome *(22)*. Furthermore, these two disorders differ from peliosis with respect to their clinical presentation. Instead of the severe portal hypertension and the rapid deterioration of hepatic function, which is observed following hepatic outflow obstruction *(22)*, peliosis of the liver was an incidental finding in surgical specimens or at autopsy in most cases reported.

Another histological differential diagnosis of peliosis hepatis is the occurrence of thermally induced alterations of the liver parenchyma following electrocoagulation during surgery. Schulz et al. observed vacuolated, peliosis-like lesions (Fig. 5) directly underneath the gallbladder's liver bed following laparoscopic cholecystectomy *(23)*.

Only a few publications exist describing liver failure directly attributable to peliosis of the liver. However, a peculiar complication of hepatic peliosis may be the rupture of the lesions resulting in severe abdominal hemorrhage with potentially fatal outcome. Because of this, peliosis of the liver may mimic a

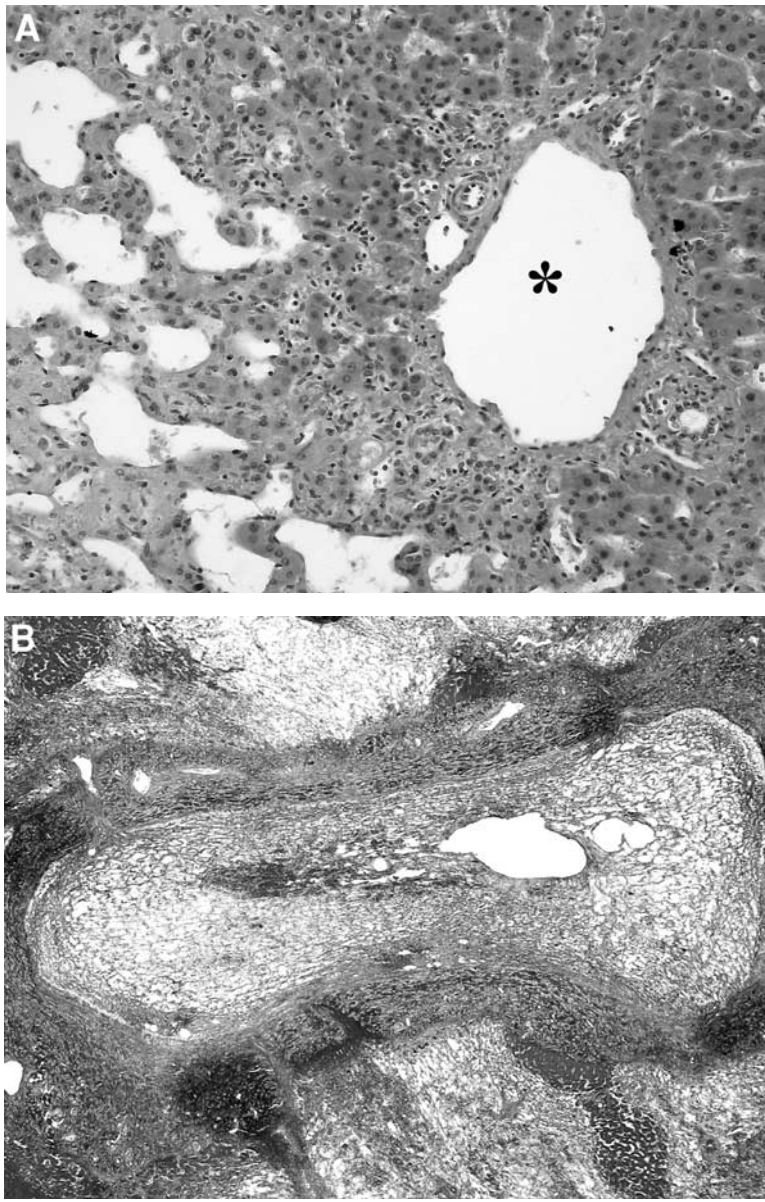

Fig. 4. Explanted liver removed because of liver failure owing to Budd-Chiari syndrome. **(A)** Peliosis-like dilated sinusoidal spaces due to severe congestion. Note also dilated portal vein (asterisk). Hematoxylin and eosin, original magnification ×250. **(B)** Large, central vein with luminal narrowing due to an organized thromus. Masson's trichrome stain, original magnification ×25.

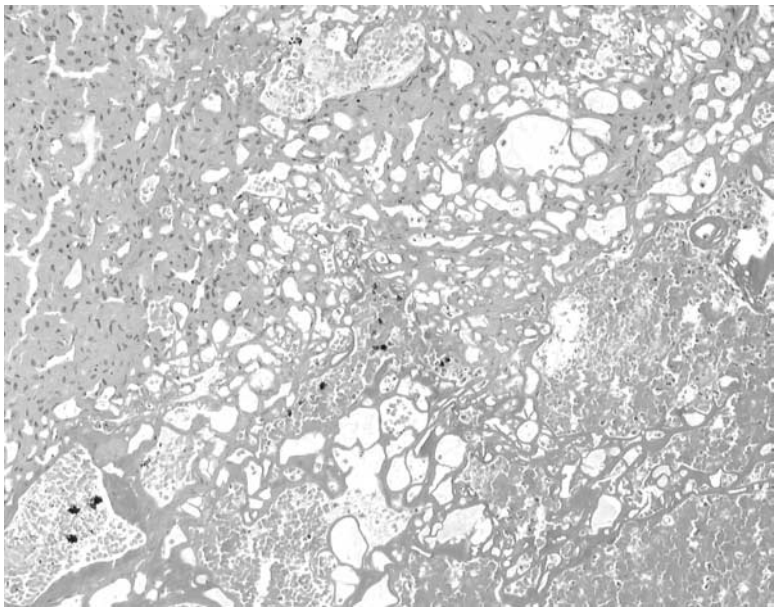

Fig. 5. Vacuolated, peliosis-like lesions of the liver parenchyma, thermally induced by electrocoagulation during laparoscopic cholecystectomy. Hematoxylin and eosin, original magnification ×50.

traumatic event preceding death and accordingly the knowledge of this rare disorder is important for both the forensic and clinical pathologist.

3. Peliosis of the Spleen

The first well-documented autopsy case of peliosis of the spleen dates from 1866 when Cohnheim described the case of a 27-year-old man who died suddenly as an inpatient of a psychiatric ward *(24)*. Autopsy revealed approx 1 L of fluid and clotted blood within the abdominal cavity. The source of the bleeding was found to be a rupture on the surface of the spleen. Cross-sections of the spleen revealed multiple cystic spaces reaching up to several centimeters in diameter that were filled with clotted blood. On cut surfaces of the right lobe of the liver, smaller blood-filled spaces were apparent as well. Although Cohnheim did not observe any endothelial cells lining the cavities at microscopical examination, he interpreted the cystic blood-filled spaces in both the liver and the spleen as some type of underlying phlebectasis of unknown etiology.

Since the publication by Cohnheim, numerous cases of peliosis of the spleen—mostly incidental findings at autopsy that were in association with

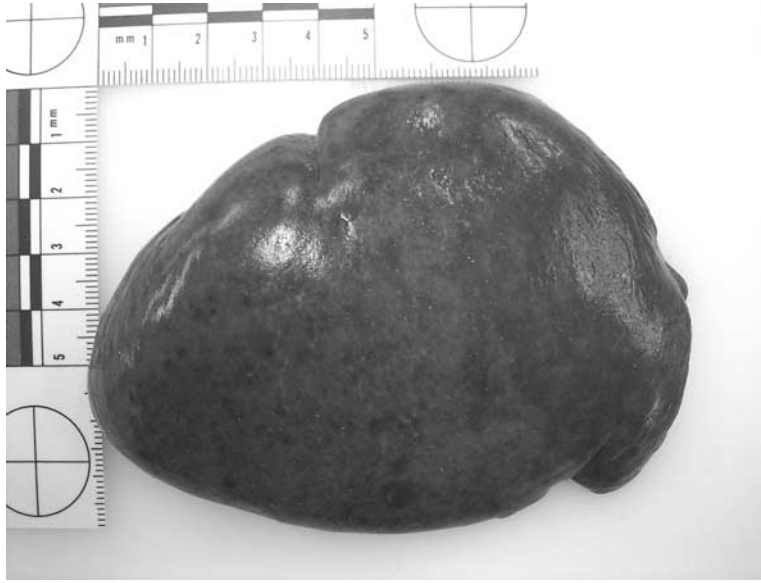

Fig. 6. Gross appearance of peliosis of the spleen. Multiple blood-filled, unevenly distributed cavities are seen beneath the capsule.

peliosis of the liver—have been published. So far, only 35 cases of isolated peliosis of the spleen have been reported in the Anglo-American literature *(8)*, making the isolated occurrence of peliosis of the spleen a rare pathological entity.

At autopsy, multiple blood-filled cavities, round to oval in shape and occasionally measuring up to several centimeters in diameter, can be seen beneath the capsule (Fig. 6) or within cut sections of the spleen (Fig. 7A,B). The blood-filled cavities may be arranged sporadically, disseminated, or in clusters in an uneven distribution pattern within the parenchyma of the spleen. At gross examination, the blood occupying the cavities may appear liquid, clotted, or with thrombi formation in a state of organization. Despite the obvious space-occupying effect of the blood-filled peliotic lesions, there is inconsistency in the reporting of splenomegaly accompanying the finding of peliosis of the spleen by different investigators.

Histologically, the peliotic lesions in the spleen are frequently well demarcated from the surrounding parenchyma and most often strictly limited to the splenic red pulp. The well-defined margins may appear focally lined by sinusoidal endothelium *(4,9,10)* (Fig. 8) but may also totally lack a clear cell lining (Fig. 9) *(8,25,26)*. The remainder of the red pulp, as well as the white pulp,

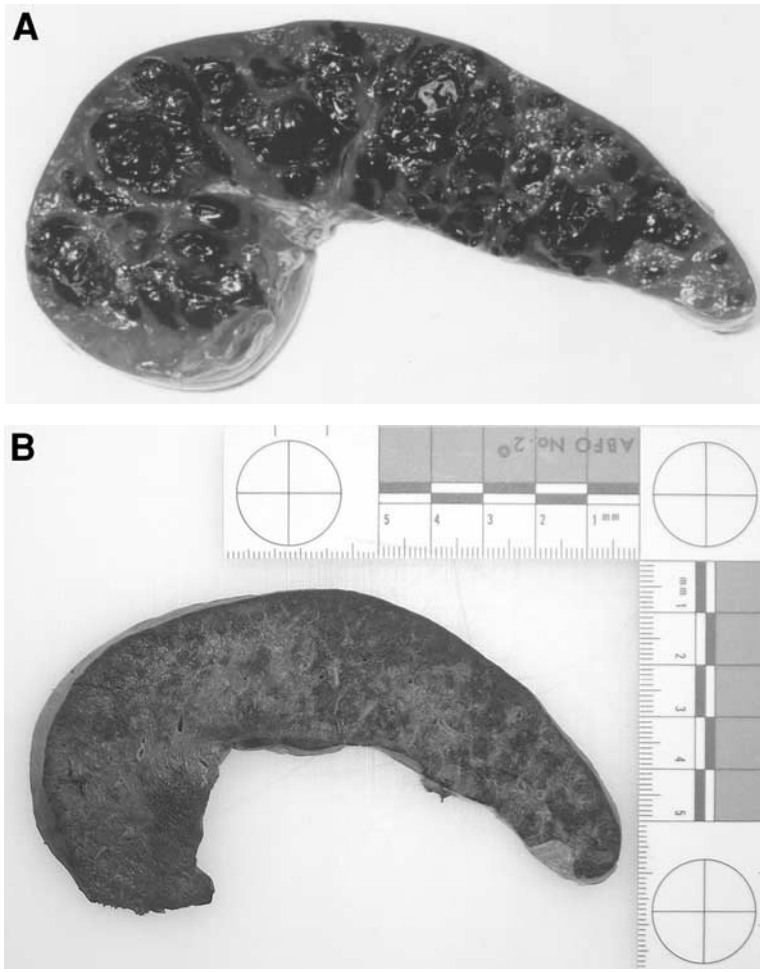

Fig. 7. Peliosis of the spleen. **(A,B)** Cyst-like cavities on cut surfaces of the spleen.

appear mostly unremarkable. On the ultrastructural level, reticular fiber dissolution has been observed in the margins of peliotic lesions in the spleen *(7)*.

Potential differential diagnoses are hemangiomas and involvement of the spleen in hairy-cell leukemia *(10,27)*. Hemangiomas of the spleen, usually occurring focally, are characterized histologically by vascular channels that show endothelial cell lining and separation by fibrous tissue septa. In hairy-cell leukemia, endothelial lining cells of the splenic sinusoids are replaced by hairy-

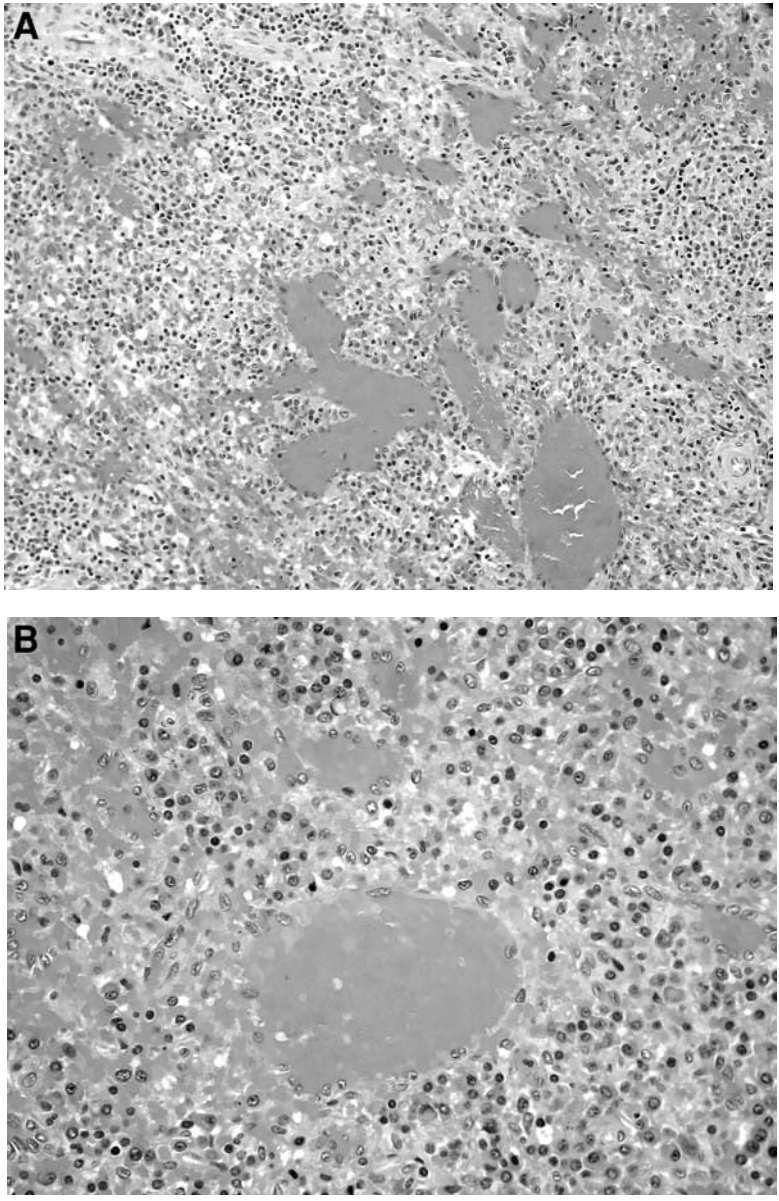

Fig. 8. Peliosis of the spleen. **(A)** Multiple blood-filled cavities varying in size and shape (Note the endothelial cell lining). Hematoxylin and eosin (H&E), original magnification ×200. **(B)** High power view of peliotic lesion. The marginal zone is focally lined by sinusoidal endothelium. H&E, original magnification ×400.

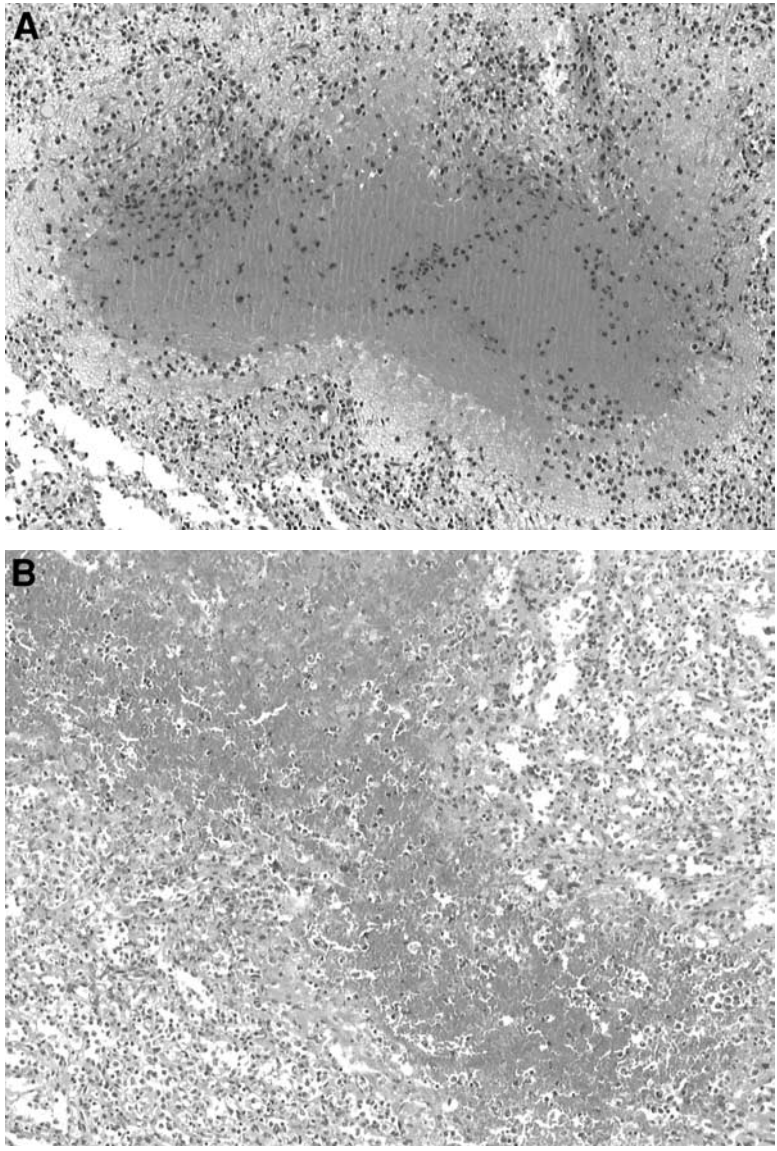

Fig. 9. Peliosis of the spleen. **(A,B,C)** The margins of the peliotic lesions lack a clear cell lining. Hematoxylin and eosin, **(A)** original magnification ×100. **(B)** original magnification ×200. **(C)** original magnification ×100. *(Figure continues)*

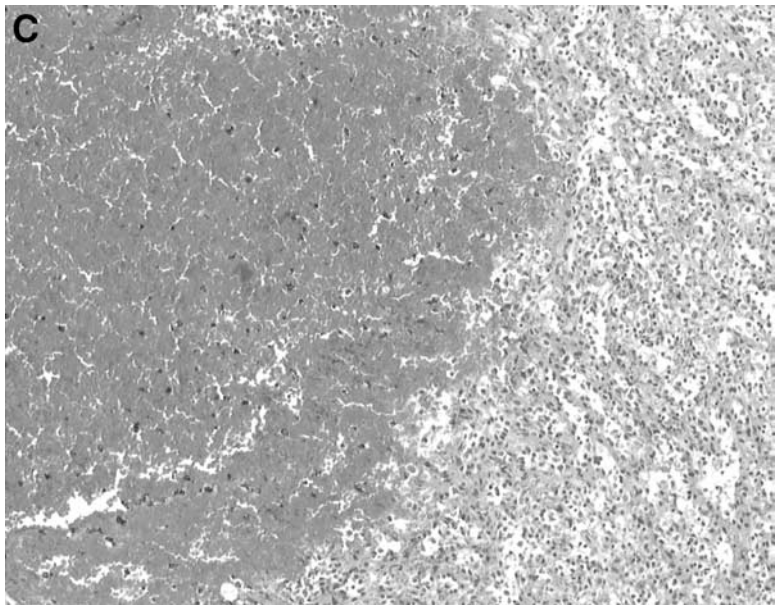

Fig. 9. *(Continued)*

cells leading to degeneration of ring fibers and a pseudo-angiomatous sinusoid pattern of the spleen.

Peliosis of the spleen has been described in association with tuberculosis, liver cirrhosis, hematological malignancies, intravenous drug abuse, chronic alcoholism, chemotherapy, and treatment with steroids *(5,7,8,10,25,28–30)*, but in some cases, no associated disease or predisposing factors could be established *(5,9,30)*.

Regarding the formal pathogenesis of peliosis of the spleen, Tsokos and Püschel *(8)* recently supposed the concept that local microcirculatory disturbances manifesting under altered local intravascular pressure conditions in the spleen may, at least to a certain degree, be responsible for the peliotic lesions. In this case, the peliotic lesions would represent abnormal venous drainage patterns (equivalent to portal-systemic collaterals) dilated by portal hypertension and increased blood flow through the splanchnic system caused by increased hepatic resistance. Whether these venous drainage systems represent physiologically preexisting vascular channels, are a consequence of maldevelopment in embryogenesis, or a hitherto undefined acquired vascular disorder remains unclear at present; it is also uncertain which factors induce or influence the

development of the vascular lesions in the presence of increased portal hypertension in one person but not in the other.

Complications of peliosis of the spleen arise when the peliotic lesions rupture, either from intrinsic pressure or extrinsic trauma, with resultant intraperitoneal hemorrhage *(7,8,25)*.

4. Forensic Pathological Relevance

Both clinical presentation and outcome of hepatic peliosis vary according to the underlying disease processes. In most of the cases reported, peliosis of the liver was a pure incidental finding in surgical specimens or at autopsy *(3,12,13,19,32)*. However, in a small number of studies, liver failure as the primary cause of death was attributed directly to peliosis hepatis *(3,20)*. In addition, rupture of peliotic liver lesions may occur, thus leading to intraperitoneal hemorrhage with potential fatal outcome *(33–36)*. In a recent publication, Karger at al. reported the case of a sudden death of a 2.5-year-old boy where autopsy revealed a ruptured subcapsular hematoma of the liver with 600 mL of blood within the peritoneal cavity causing death by hemorrhagic shock. At gross examination, both lobes of the liver showed numerous circular blood foci measuring up to 2 cm in diameter. Histologically, the blood cysts turned out to be peliotic lesions, thus ruling out the initial suspicion of a fatal trauma *(37)*.

Of forensic pathological relevance may also be the frequent occurrence of peliosis associated with the chronic intake of anabolic-androgenic steroids abused as performance enhancing drugs *(38)*.

5. Conclusions

When detecting the aforementioned characteristic features at gross examination or histological examination in the liver and/or spleen, respectively, the pathologist should keep peliosis as a possible diagnosis in mind. Of course, peliosis of parenchymatous organs may be a pure incidental finding at autopsy or under the microscope, respectively, that has neither any relation between cause and effect of the underlying disease nor any causal connection with, or pathological relevance to, the cause of death. Nevertheless, forensic pathological significance may arise in distinct cases because peliosis may culminate in spontaneous rupture of the affected organ with resultant intraperitoneal or intrathoracic hemorrhage, thus mimicking a violent death at autopsy.

Peliosis is far more than just another morphological curiosity. Awareness of peliosis at autopsy as well as an appreciation for the histopathological changes may occasionally become a highly important issue for the forensic pathologist.

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Infectious Diseases

7

Pathology of Human Endothelium in Septic Organ Failure

Annette M. Müller, MD and Michael Tsokos, MD

Contents

Summary

Human sepsis is a spectrum of pathophysiological changes in the host system resulting from a generalized activation and systemic expression of the

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host's inflammatory pathways in response to infection. The endothelium plays a key role in the pathogenesis of sepsis, and studies of endothelial derangement and its underlying pathophysiological mechanisms in sepsis have become of considerable interest in both the clinical and pathological fields. Recognition, as well as contact formation, between leukocytes and the endothelium is dependent on the presence of both cytokines and adhesion molecules that mediate leukocyte–endothelial cell adhesive interactions. The adherence of leukocytes on the vascular endothelial cell surface and the transmigration through the endothelial layer is regulated by at least three adhesion molecule families: the selectins (E-Selectin, L-Selectin, P-Selectin), the integrins (e.g., lymphocyte function-associated antigen-1, Mac-1, very late activation antigen-4), and the immunoglobulin superfamily (e.g., intercellular adhesion molecule-1, vascular cell adhesion molecule-1). The constitutively expressed platelet endothelial cell adhesion molecule-1, already strongly expressed by endothelial cells lacking inflammatory stimuli, is localized at the cell-to-cell borders of endothelial cells and mediates a common final step in the extravasation of monocytes and neutrophils during the inflammatory response from the vascular lumen through the endothelium. In addition, platelet-activating factor, released in response to endotoxin, is another mediator of considerable relevance for endotoxin-induced leukocyte recruitment into tissue in sepsis, leading to loss of fluid from the intravascular into the extravascular space, thus contributing to the progressive loss of circulating blood and, thereby, to a depression of cardiac output. Studies have demonstrated that this loss of fluid is not the result of changes in hydrostatic and/or osmotic pressures within the vascular compartment, but rather the breakdown of endothelial barrier function, thus allowing emigration of fluid and macromolecules—including proteins—into the extravascular space. Separation of tight junctions between endothelial cells, influenced by inflammatory mediators and white blood cells, and dysfunction rather than destructive changes of endothelial cells leading to defects in endothelial cell volume regulation are discussed as the main underlying pathophysiological mechanisms. Two major pathways are involved in initiation of apoptosis in sepsis: a receptor-initiated caspase-8-mediated pathway and a mitochondrial-initiated caspase-9-mediated pathway. In vitro studies have revealed apoptotic cell death of endothelial cells in response to LPS and tumor necrosis factor-α, as well as to certain microorganisms. A number of studies suggest that endotoxin induces expression of antiapoptotic molecules in microvascular endothelial cells and neutrophils. The latter could play a role in the accumulation of neutrophils during sepsis, as well as in prolongation and/or augmentation of the inflammatory response.

Key Words: Sepsis; systemic inflammatory response syndrome (SIRS); human endothelium; acute respiratory distress syndrome (ARDS); adhesion molecules; cytokines; interleukins.

1. INTRODUCTION

Sepsis is a generalized systemic inflammatory response of the host to the presence of microorganisms. It is associated with mortality rates as high as 30 to 90% *(1,2)*.

As the endothelium plays a key role in the pathogenesis of sepsis, current therapeutic approaches for septic conditions including antimicrobial chemotherapy, oxygen therapy, mechanical ventilation, and hemodialysis do not have any substantial impact on the mortality associated with septic shock *(3)*; studies of endothelial derangement and its underlying pathophysiological mechanisms in sepsis have become of considerable interest in both the clinical and pathological fields.

Under physiological conditions, the endothelium—today considered both an endocrine and paracrine organ—maintains a barrier function, controls the nutrient and cellular transvascular trafficking, regulates the vasomotor tone, as well as angiogenesis, and influences coagulation. In sepsis, the endothelium is involved in, as well as influenced by, the excessive release of a broad variety of mediators. The imbalance between proinflammatory and antiinflammatory processes causes impaired tissue oxygenation and leukocyte accumulation, vasodilatation, and increased microvascular permeability.

In general, local inflammatory responses to infection are mainly regulated through the production of cytokines. Macrophages, triggered and activated by bacterial products, such as lipopolysaccharids (LPS), secrete cytokines including interleukin (IL)-6, IL-12, interferon (INF), and platelet-activating factor (PAF) into their surrounding microenvironment *(4–6)*. In response to this, the endothelium modulates the inflammatory cell function through the production and release of adhesion molecules and proinflammatory cytokines.

This chapter adresses our present-day knowledge of the role of the human endothelium in sepsis. In addition, several factors and pathophysiological mechanisms which are, in accordance with current opinions, pivotal for the pathogenesis of sepsis and the development of its clinical sequelae, such as organ dysfunction and multiple organ failure (MOF), are emphasized. Finally, aspects of sepsis-related deaths are briefly discussed from the forensic pathologist's viewpoint.

2. Definition of Sepsis

Human sepsis is a spectrum of pathophysiological changes in the host system resulting from a generalized activation and systemic expression of the host's inflammatory pathways in response to infection. Sepsis complicates other disease states and involves a characteristic spectrum of pathological changes in the host system *(7–11)*. Sepsis, sepsis syndrome, and septic shock represent the increasingly severe stages of the same disease *(9,12,13)*. The pathophysiology of sepsis is, as yet, not completely understood. However, sepsis is known to result from dysregulated immune and metabolic responses of the host to infection or injury.

The term *sepsis*, in popular usage, implies a clinical response arising from infection. However, it is apparent that a similar, or even identical, deleterious generalized systemic inflammatory reaction can arise in the absence of infection in response to a variety of life-threatening clinical conditions, e.g., major trauma, burns, extensive surgical procedures, protracted hemorrhagic or cardiac shock, or pancreatitis *(14,15)*. A consensus conference of the American College of Chest Physicians/Society of Critical Care Medicine stressed this concept for practical usage and recommended the term systemic inflammatory response syndrome (SIRS) to describe this generalized inflammatory process independent of its cause *(7)*. SIRS is clinically defined by two or more of the following clinical criteria: body temperature higher than 38°C or less than 36°C, heart rate higher than 90 beats per minute, tachypnea with more than 20 breaths per minute or PCO_2 less than 4.3 kPa, white blood cell count higher than 12,000 cells/mm^3 or less than 4000 cells/mm^3, or the presence of more than 10% immature neutrophils *(7)*.

2.1. Sepsis, Severe Sepsis, and Septic Shock

SIRS can result from either noninfectious or infectious conditions. The presence of at least two of the aforementioned SIRS components when triggered by infection is termed sepsis *(7)*. Infection leading to sepsis may be bacterial, fungal, parasitic, protozoan, or viral.

Sepsis and other critical illnesses produce a biphasic inflammatory (immunological, hormonal, and metabolic) response. The acute phase is marked by an abrupt rise in the secretion of so-called stress hormones with an associated increase in mitochondrial and metabolic activity. The combination of severe inflammation and secondary changes in endocrine profile diminish energy production, metabolic rate, and normal cellular processes, with potential MOF *(16)*.

Severe sepsis is defined by a deterioration in the presence of hypotension, organ dyfunction and hypoperfusion; the term *septic shock* is reserved for

severe sepsis with hypotension despite fluid resuscitation and resultant perfusion abnormalities *(7)*. The progression from sepsis to severe sepsis and to septic shock is a continuum reflecting the host's inflammatory response to infection. During this process, an increasing proportion of patients develop acute respiratory distress syndrome (ARDS), organ dysfunction syndrome, and MOF.

The clinical criteria such as fever or hypothermia, tachycardia, tachypnea, and leukocytosis or leukopenia are common clinical signs of systemic inflammation. However, these manifestations are neither specific nor sensitive for sepsis. Owing to the wide range and variability of potentially sepsis-associated symptoms, physicians must be aware of the multiple differential diagnoses and the many ways in which an underlying septic condition may present.

3. Pathophysiology of Sepsis

Human sepsis, usually a complication of other disease states, is associated with a generalized activation and expression of the host's inflammatory pathways, but the sequence and detailed mechanisms of the cellular and molecular events involved in the pathogenesis of sepsis are not completely understood yet *(10)*. Although the clinical picture of sepsis has proposed a common process of inflammation and tissue injury induced by the combined attack of exogenous noxae and the host's proinflammatory response, this image is incomplete. The critically ill patient reflects the complex interaction of both pro- and antiinflammatory processes. Because pro- and antiinflammatory processes are often expressed simultaneously, their combination usually creates the clinical picture *(10)*.

Most of the initial mediators of SIRS are cytokines, such as tumor necrosis factor (TNF)-α, IL-1, IL-6, and IL-8. These proteins that, with few exceptions, have to be synthesized *de novo* are released in response to infection or injury and/or ischemia to eliminate pathogens and to promote wound healing. Under most conditions, cytokine signaling appears to function as a local process, but once these cytokines gain access to the systemic circulation in levels capable to overcome the pure local response, they induce a systemic response of the host organism. In the healthy (nonseptic) individual, this proinflammatory response is then downregulated by the release of antiinflammatory cytokines and other mediators, resulting in the restoration of homeostasis. In the septic individual, however, local defense mechanisms are insufficient to eliminate the infectious agent and overstimulation of the host's immune effector cells occurs. In sepsis, the overwhelming systemic proinflammatory reaction is frequently followed by an overactive, compensatory antiinflammatory mediator release. As a consequence, however, the balance between the pro- and

antiinflammatory response is frequently lost. This state of immunological imbalance ranges from persistent massive inflammation to ongoing immune suppression *(17,18)*. The severity of sepsis is proportional to the intensity of the host's immune and metabolic response to infection.

The migration of leukocytes from the vascular system to sites of pathogenic exposure is one of the key events in the process of inflammation. The inflammatory reaction, in the healthy individual enabling the host to defend himself against infectious agents, has also the potential to cause deleterious effects if the regulatory mechanisms of the inflammatory response are altered or immune responses to residual infectious products trigger a persistent inflammatory reaction. In such instances, the defense mechanisms of the leukocytes can turn against the host and lead to harmful tissue destruction *(19)*.

4. Interaction Between Endothelial Cells and Leukocytes

The entry of leukocytes into sites of injury or infection requires molecular mechanisms that enable the leukocytes to recognize such sites from within the vasculature and to get in contact with the vessel-lining endothelium in order to perform diapedesis through the vessel wall. Recognition, as well as contact formation, with the endothelium is dependent on the presence of both cytokines and adhesion molecules that mediate neutrophil-endothelial cell adhesive interactions. During sepsis, the lung is especially susceptible to injury, and a progressively impaired lung function is the major complication in sepsis *(20–22)*. The extravasation and sequestration of leukocytes during acute lung injury is dependent on a complex intercellular communication based on (a) activation of mononuclear cells and release of proinflammatory cytokines *(23–27)* with subsequent (b) surface expression of endothelial adhesion molecules and neutrophil-derived adhesion molecules *(28–30)*, resulting in (c) enhanced rolling, adhesion and transendothelial migration of leukocytes *(31–33)*.

Leukocyte recruitment in inflammation is a multistep process in which a broad variety of soluble factors and membrane-bound adhesion molecules on activated endothelial cells and leukocytes are involved. Endothelial activation as found in systemic inflammation is not only caused by cytokines, such as IL-1, IL-6, IL-8, INF, or TNF-α, but also by proteases (e.g., thrombin, factor Xa), various growth factors, vasoactive compounds (e.g., bradykinin, histamine, endothelin-1), and complement factors *(11)*.

The adherence of leukocytes on the vascular endothelial cell surface and the transmigration through the endothelial layer is mainly regulated by three adhesion molecule families: the selectins (E-Selectin, L-Selectin, P-Selectin), the integrins (e.g., lymphocyte function-associated antigen [LFA]-1, very late

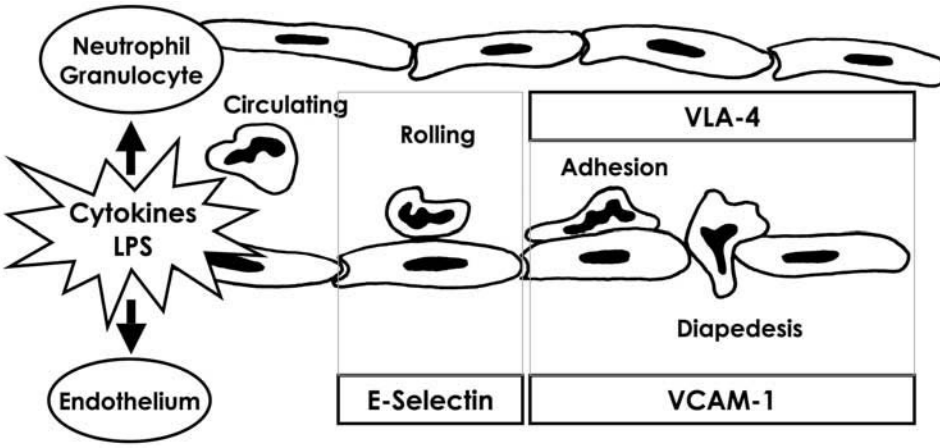


Fig. 1. Schematic presentation of the complex intercellular interactions between leukocytes (examplary demonstrated by a neutrophil granulocyte) and endothelial cells. The extravasation and sequestration of leukocytes during acute lung injury is dependent on a multistep cascade of different molecular interactions. Inflammatory stimuli such as cytokines and bacterial lipopolysaccharids induce upregulation of cellular adhesion molecules. The selectins mediate the initial cell-to-cell contact between the leukocyte and the endothelium; this results in rolling of leukocytes along the endothelial surface. Then, leukocytic integrins (e.g., very late antigen activator-4) bind to members of the immunoglobulin superfamily (e.g., vascular cell adhesion molecule-1) expressed on the endothelial cell surface, leading to firm adhesion of the leukocyte on the endothelial surface which is a prerequisite for direct diapedesis of the leukocyte through the vascular endothelium into the interstitium.

activation antigen [VLA]-4, Mac-1), and the immunoglobulin superfamily (e.g., intercellular adhesion molecule [ICAM]-1, vascular cell adhesion molecule [VCAM]-1) *(32,34,35)*. The selectins mediate the initiation of the cell-to-cell contact between leukocytes and endothelium. Selectin-mediated docking of leukocytes to the blood vessel wall in combination with the flowing bloodstream leads to the rolling of leukocytes on the endothelial cell surface. In contrast to the rapidly flowing blood cells in the bloodstream, the rolling leukocytes are able to sense signals from the endothelium, which stimulates them to adhere firmly to its surface. Then, stimulated by cytokines, leukocytic integrins bind to members of the immunoglobulin superfamily expressed on the endothelial cell surface (Fig. 1).

Firm adhesion via activated integrins is a prerequisite for leukocyte diapedesis through the layer of the endothelial cells. Very little is known about the

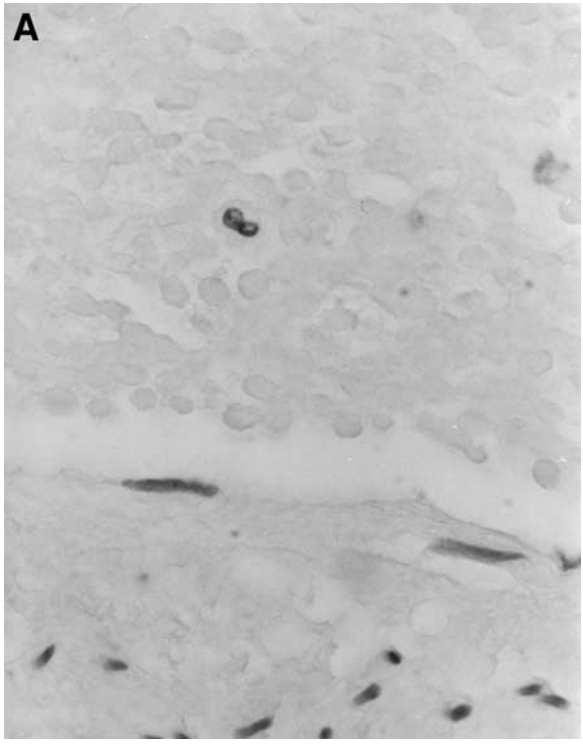
exact mechanisms of diapedesis, namely if the leukocytes transmigrate through the junctions between adjacent endothelial cells or directly through a single endothelial cell *(19)*.

In vitro, IL-1, TNF-α, and LPS induce the expression of E-Selectin and VCAM-1 and an upregulation of ICAM-l *(36–40)*. The transferability of these results to in vivo conditions has been demonstrated by Redl et al. in an animal model of baboons with septic shock *(41)*. In addition, the pivotal role of TNF-α in sepsis has been proven for animals by producing symptoms of septic shock by TNF-α infusion, as well as protecting animals against endotoxin-induced septic shock by administration of anti-TNF-α antibodies, thus leading to an increased endothelial cell synthesis of proinflammatory cytokines, including IL-1, IL-3, IL-6, and IL-8, as well as the upregulation of endothelial cell adhesion molecules *(11,42–47)*.

Van der Poll et al. *(48)* demonstrated that endothelial expression of E-Selectin (Fig. 2) followed by the release of soluble E-Selectin into the blood stream in vivo is induced by LPS.

E-Selectin mediates the initial adhesion of leukocytes to and rolling on cytokine-activated endothelial cells *(49,50)*. ICAM-1 and VCAM-1 together mediate the firm adhesion between leukocytes and endothelium and initiate subsequent diapedesis. Whereas integrin ligands for ICAM-1 are present on granulocytes and lymphocytes, VCAM-1 interacts with VLA-4 on lymphocytes and monocytes (Fig. 3), but not neutrophils. Normally, the intact endothelium expresses undetectable or low levels of E-Selectin and VCAM-1 *(51)*. In vitro, endothelial upregulation of E-Selectin after stimulation by endotoxin or cytokines is time-dependent (Fig. 4). Cultured endothelial cells express E-Selectin within 1 to 2 hours after stimulation with interleukins, with maximum expression at 6 hours and a return to baseline after 24 hours *(39,52,53)*. In contrast, the level of ICAM-1 expression reaches a maximum between 12 and 24 hours and remains elevated for at least 48 hours *(54)*. Because soluble E-Selectin levels in vivo are endotoxin dose-dependent *(54)*, today this molecule is regarded as a quantitative marker of inflammation-induced endothelial activation *(55)*.

Although E-Selectin has been regarded as an early mediator of endothelial leukocyte adhesion, being present in early and active states of inflammation, VCAM-1 expression, on the other hand, has been thought to characterize later phases of inflammation. Because of studies on animal and human tissue, these assumptions have to be revised. According to Fries et al. *(39)*, the cytokin-induced expression of E-Selectin and VCAM-1 in animals can persist for more than 3 days. In addition, the studies by Huang et al. *(56)* and Tsokos et al. *(57)* indicate that endothelial activation and the expression of E-Selectin correlates with a continuous presence of cytokines.



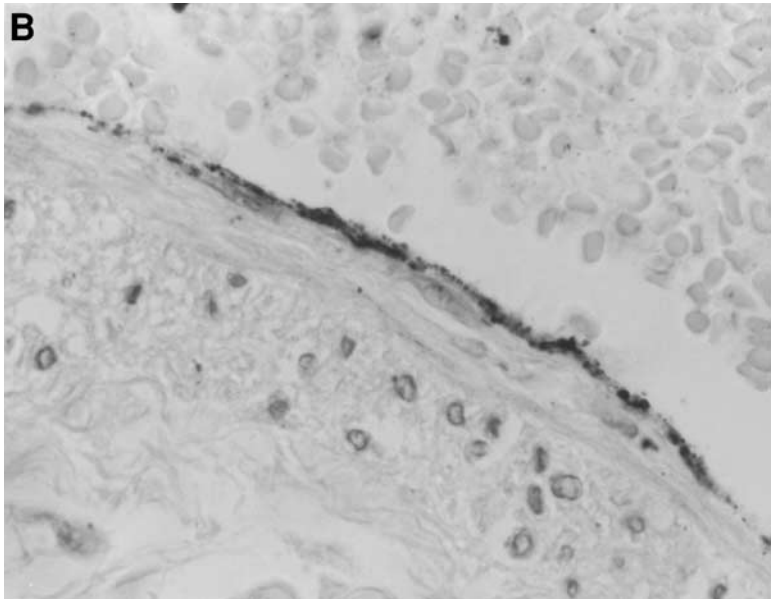


Fig. 2. (A) Lacking immunohistochemical E-Selectin expression along the endothelial lining of a nonseptic small pulmonary vessel (CD 62E immunostaining). **(B)** Strong E-Selectin expression on endothelial cells of a small pulmonary vessel in sepsis (CD62E immunostaining).

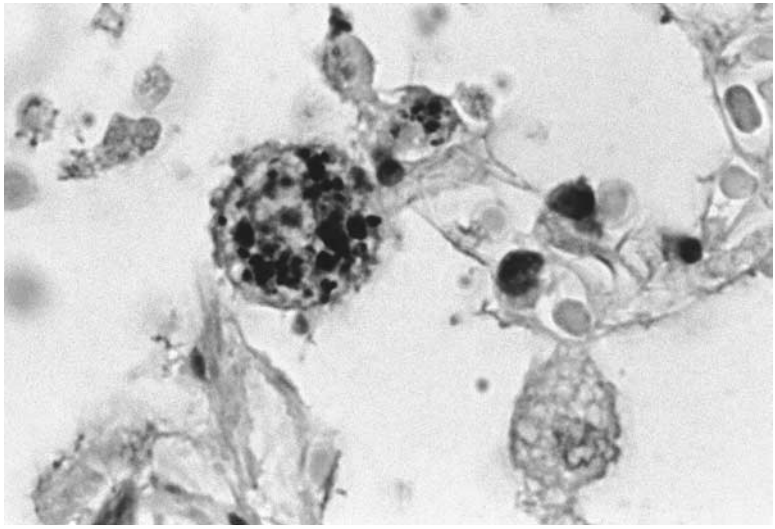


Fig. 3. Intraalveolar very late antigen activation-4 immunopositive monocyte in sepsis-induced lung injury (CD49d/CD29 immunostaining).

Although time correlations of cellular adhesion molecules have been studied extensively in vitro and in animal models *(36,41,58,59)*, and more recently by studying plasma and serum levels in humans *(48)*, pathomorphological studies of human tissue (e.g., from the lung) deriving from patients with Gram-negative shock are rare. This is probably caused by the difficulties in obtaining parenchymal specimens from patients with Gram-negative sepsis and septic shock, respectively. Consequently, hypotheses on the endothelial expression patterns of cellular adhesion molecules in human tissue—although central for the development of sepsis-induced MOF—are mainly based on results of in vitro experiments and animal models *(60–62)*.

The complexity of the in vivo situation has been demonstrated by our studies on endothelial cell adhesion molecule expression in heart valve endothelial cells in degenerative and active endocarditic valve disease *(63)*. Although we found a nearly identical intensity of ICAM-1-expression on fibrotic, as well as on inflamed, valves, cultured endothelial cells and endothelial cells in an organ culture showed an increase of a low basic expression of ICAM-1 after treatment with cytokines, such as IL-1, TNF-α, as well as ionizing radiation *(40,64)*. Combe et al. *(65)* provided evidence that monocytes stimulate and amplify their adhesion to endothelial cells by induction of ICAM-1, thus constituting a self-perpetuating positive feedback system. The E-Selectin

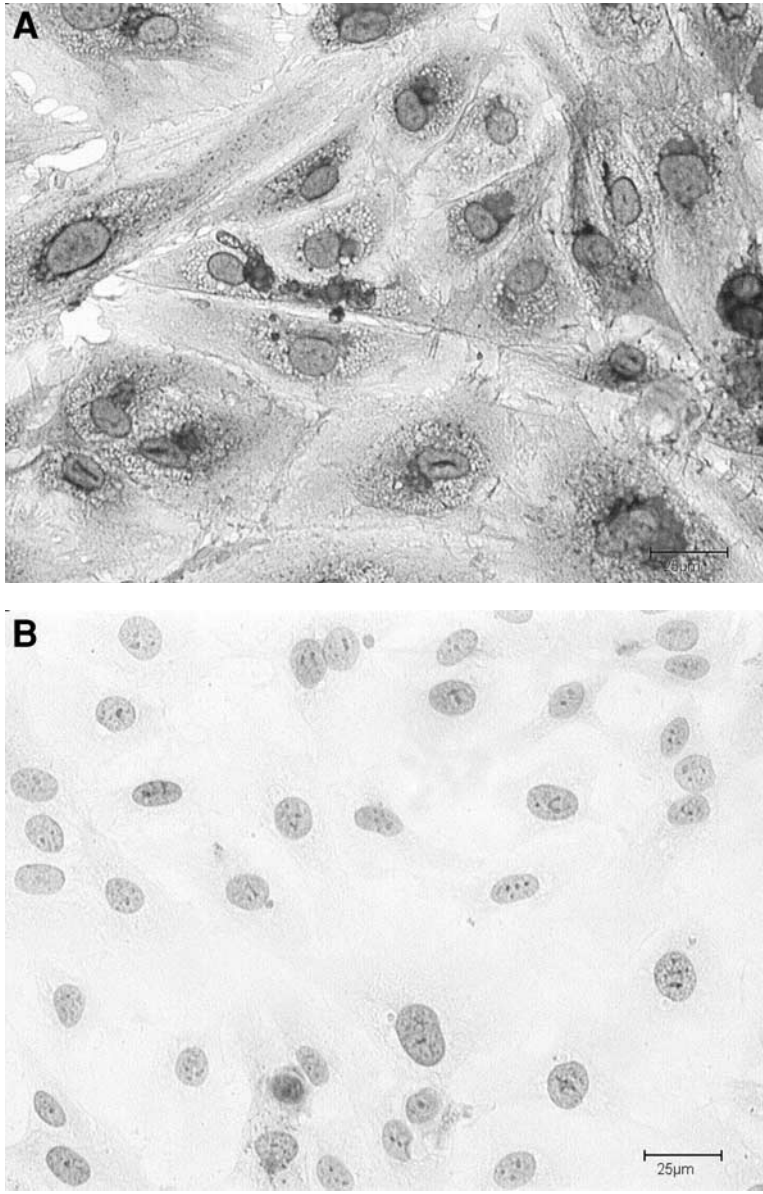


Fig. 4. E-Selectin expression by cultured endothelial cells after stimulation with lipopolysaccharid. **(A)** Strong expression of E-Selectin by human pulmonary microvascular endothelial cells after 6 hours and **(B)** no expression by human umbilical vein endothelial cells 28 hours after stimulation.

expression in some cases of degenerative valve disease without acute inflammatory signs *(63)* has been one of the first reports of the complexity of the expression patterns of inducible adhesion molecules in the human organism.

Another proof for the different expression kinetics of endothelial cells in vivo compared with endothelial cells in vitro or in animal models with a fixed time of endotoxin application and sacrifice *(66)* is the strong expression of inducible adhesion molecules in lungs from patients with ARDS that we observed more than 48 hours after the onset of shock *(51)*. Our studies on human tissue indicate that repeated stimulation of endothelial cells by proinflammatory cytokines and bacterial toxins in patients with ARDS and sepsis generally represents the principal difference between the status quo in vivo and that in experimental studies; in the latter, the endothelial cell stimulation, owing to a single dose of toxin or cytokines, has a completely different time scale *(51,57)*. On the basis of our experience, and in keeping with that of Kayal et al. *(67)*, it seems fair to conclude that plasma levels of E-Selectin shed from the endothelial cell plasma membrane increase rapidly after the onset of sepsis and continue to increase for 3 to 4 days, especially in patients who do not survive.

5. Platelet Endothelial Cell Adhesion Molecule-1

Besides the so-called inducible adhesion molecules discussed above, we would like to focus attention towards the constantly expressed platelet endothelial cell adhesion molecule (PECAM)-1, especially as its role in sepsis is seldom appreciated. This might be because of the fact that the overall expression of PECAM-1 appears unaltered in sepsis *(68)*. The constitutively and strongly expressed PECAM-1, localized at the cell-to-cell borders of the endothelial cells (Fig. 5), mediates a common final step in the extravasation of monocytes and neutrophils during the inflammatory response from the vascular lumen through the endothelium and the perivascular basement membrane into the interstitium *(69)*. According to DeLisser et al. *(70)*, the activity of PECAM-1 is regulated by variations in its cytoplasmatic domain. Nevertheless, PECAM-1 is already strongly expressed by endothelial cells lacking inflammatory stimuli *(64,71–74)*. Therefore, this homogeneous strong expression by all endothelial cells in all vessels of nonseptic and septic lungs underlines the importance of PECAM-1 as a panendothelial marker for morphological endothelial integrity, e.g., in tissue specimens from septic individuals. According to Romer et al. *(75)*, TNF-α and INF-γ elicit a change in PECAM-1 cytoskeletal association with a change in localization at the cell surface. Stewart et al. *(76)* found that treatment of endothelial cells with TNF-α and/or IFN-γ led to dramatic decreases in steady state levels of PECAM-1 mRNA transcripts. Moreover, engagement of

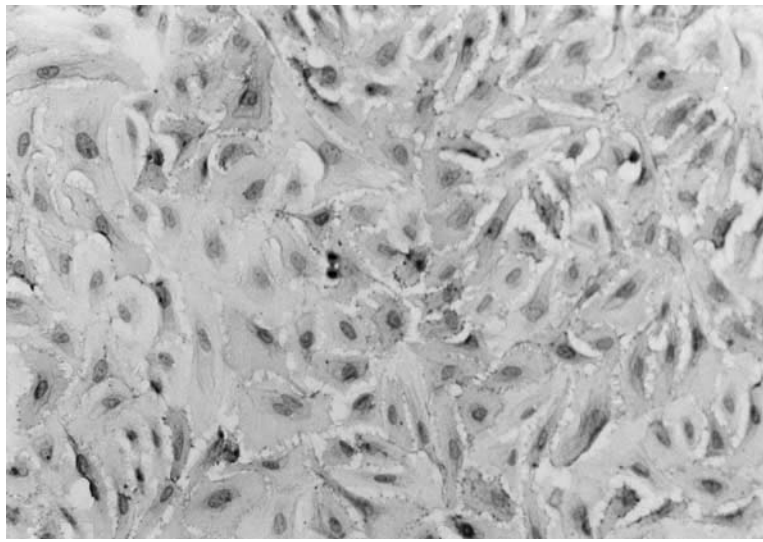


Fig. 5. Localization of the constitutively expressed platelet endothelial cell adhesion molecule-1 at the cell-to-cell borders of human umbilical vein endothelial cells (HUVEC).

PECAM-1 may alter a number of endothelial cell functions, including the secretion of vasoactive mediators *(77)*.

6. Effects of Endotoxin on Endothelium

Endotoxin, consisting of LPS, is the main toxic component of Gram-negative bacteria and is regarded as the leading stimulus of the inflammatory cascade in sepsis and septic shock, respectively.

Clinical observations in septic patients and in animal models have proven that the presence of endotoxin/LPS results in the activation and adhesion of leukocytes to endothelial cells *(78)*. In addition, the production of vasoactive products (such as bradykinin) and activation of complement factors is promoted, both of which can enhance endothelial permeability.

LPS binds with a high affinity to a 60-kDA glycoprotein, the LPS-binding protein (LBP), an acute-phase protein primarily synthesized by hepatocytes *(79)*. CD14 on monocytes, macrophages, and granulocytes interacts with the LPS–LBP complex, whereas the leukocyte integrin complexes CD11/CD18 binds with LPS *(80)*.

Sepsis owing to Gram-positive bacteria is based on the interaction of CD14/LPB with components of the Gram-positive bacterial cell wall. Similar

effects as LPS show soluble peptidoglycan and lipoteichoic acids (LTA) *(61,81)*. The signaling induced by these bacterial components occurs primarily through toll-like receptors (TLRs), 10 of which are identified so far. Endothelial cells express TLR-4 and, at low levels, TLR-2 *(82)*, the expression of both being regulated by inflammation-related factors, such as LPS, TNF-α, and INF-γ *(83)*. For endothelial activation by endotoxin, an initial binding of endotoxin to LPB and subsequent complex binding to the CD14–TLR-4 membrane complex on the cell surface is required *(84)*. Further details will soon become obvious as different types of TLRs seem to respond to specific bacterial DNA sequences *(85–89)*.

Although the intracellular signaling cascades activated by endotoxin are complex, several important kinases and nuclear factors are crucial for the systemic inflammatory process in sepsis. Several studies have demonstrated the importance of the mitogen-activated protein kinase (MAPK) family, composed of p38, ERK1 and 2, and Janus kinase (JNK), for inflammation-mediated gene transcription through, and their effect on, modulation of key proinflammatory nuclear factors, such as AP-1 and nuclear factor (NF)-κB *(78,85,86,90)*, as well as nitric oxide (NO) *(91)*. This inflammation-induced intracellular signaling leads to the transcription of numerous inflammatory genes, hence the generation of inflammatory products, such as ICAM-1, IL-8, and cyclooxygenase (COX)-2. The observations by Carlos and Harlan *(32)*, namely that the injection of endotoxin upregulates the expression of adhesion molecules on leukocytes (e.g., CD11b/CD18) and on endothelial cells has, among others, been supported by Tamura et al. (*92*). Tamura et al. reported that p38 MAPKs mediate upregulation of ICAM-1 expression *(92)*. Liu et al. *(93)* showed a reversion of the upregulation of adhesion molecules/leukocyte recruitment in rats after inhibition of NF-κB with pyrrolidone dithiocarbamate. Hence, NF-κB regulates not only the degree of cytokine expression but is also responsible for the regulation of transcription of adhesion molecules, immunoreceptors, procoagulatory factors (e.g., tissue factor), and acute phase proteins *(56,94,95)*. Activation and regulation of NF-κB is tightly controlled by another transcription factor family with inhibitory functions, the IκBs *(96)*.

Data support the hypothesis that NF-κB-independent pathways exist to induce the expression of cell adhesion molecules under proinflammatory stimuli, such as endotoxin, TNF-α, or IL-1β. Using inhibitors of protein kinases, Kirkpatrick and co-workers showed that cytokine-induced upregulation of E-Selectin and ICAM-1 is markedly reduced, both at the gene product and at the mRNA level, without reducing the translocation of NF-κB to the nucleus *(94,97)*.

Another factor for endotoxin-induced leukocyte recruitment into diverse tissues is platelet activating factor (PAF), released in response to endotoxin. Blockade of the PAF-receptor effectively prevents leukocyte adhesion and subsequent tissue injury *(98)*. Schmidt et al. *(99)* showed that administration of a C1 esterase inhibitor in rats attenuated endotoxin-induced leukocyte adhesion. On the other hand, endotoxin-induced leukocyte recruitment and adhesion in the pulmonary microcirculation and red blood cell velocity can be enhanced by administration of arachidonic acid, the precursor of a number of lipid mediators, such as leukotrienes or thromboxane *(100)*.

7. Edema and Breakdown of Endothelial Barrier Function

In sepsis, the loss of fluid from the intravascular into the extravascular space contributes to the progressive loss of circulating blood and thereby, to a depression of cardiac output. Studies have demonstrated that this loss of fluid is not caused by changes in hydrostatic and/or osmotic pressures within the vascular compartment *(101,102)*, but is rather owing to the breakdown of endothelial barrier function, thus allowing emigration of fluid and macromolecules—including proteins—into the extravascular space. In addition, the increase of endothelial permeability in vitro is induced by a number of sepsis-related factors (e.g., TNF-α and LPS) *(103,104)*.

Separation of tight junctions between endothelial cells *(103)* influenced by inflammatory mediators and white blood cells *(105)* and dysfunction rather than destructive changes of endothelial cells *(106)* leading to defects in endothelial cell volume regulation are widely discussed as the main underlying pathophysiological mechanisms *(107)*. Recently, there has been evidence that an LPS-induced increase of endothelial permeability is achieved by enzymatic cleavage of adherens junction proteins *(108)*.

8. Heterogenity of Endothelial Cells

The principle functions of the endothelium are spatially as well as timewise differentially, regulated between different sites of the vasculature, correlating with a vessel type-specific molecular profile of endothelial cells *(109–114)*.

In septic shock, generally characterized by refractory hypotension and profound vasodilation, blood flow is diminished in some vascular beds, such as the splanchnic circulation, indicating that some vasoconstrictor mechanisms are activated in reponse to endotoxin stimuli *(115–118)*. Kubli et al. *(119)* demonstrated that the capacity of the endothelium to produce signals for vasorelaxation remains intact in the cutaneous microcirculation of patients with septic shock.

The inhomogenity of pulmonary endothelial cells as described for nonseptic lungs by Feuerhake et al. *(120)* is supported by the findings of an inhomogeneous von Willebrand factor (vWf) expression in capillaries (Fig. 6) *(113)*. Interestingly, in sepsis, the exchange between the lumina and the extravascular space takes place in the capillaries and not in the venules. However, although LPS-mediated induction of E-Selectin is greater in the lung compared with other organs *(121)*, studies on the expression of the inducible adhesion molecules E-Selectin and VCAM-1 demonstrate that they are upregulated in all vessels but capillaries *(23,51,57)*. This almost total absence of E-Selectin and VCAM-1 in capillary endothelial cells in lungs of ARDS patients can be explained by the existence of a definitive functional heterogeneity of endothelial cells, at least within the lung, with respect to cell adhesion molecule expression. Doerschuk et al. *(122)* emphasized that in the pulmonary microvessels there is no requirement for adhesion molecule expression because activated leukocytes are trapped in capillaries owing to their relatively larger size compared with pulmonary capillary segments.

9. Changes in Hemostasis in Sepsis

Normally, the endothelium possesses anticoagulant/antithrombotic properties in expressing, e.g., tissue factor pathway inhibitors, thrombomodulin, nitric oxide (NO), and prostacyclin *(123)*. As with the adhesion molecules, stimulation of the intact endothelium results in vascular bed-specific changes in hemostatic properties *(124)*. Besides, in sepsis the coagulation and the fibrinolytic systems are activated by endotoxin *(125)*. Hence, during the pathogenesis of sepsis, changes in the expression of coagulation-involved factors occur *(126)*: tissue factor (a procoagulant glycoprotein) is released by endothelial and subendothelial cells *(127)*; a dysregulated balance of tissue-type plasminogen activator and plasminogen activator inhibitor-1 leads to increased coagulation and suppressed fibrinolytic activity *(128)*. The systemic administration of LPS to mice results in a down-regulation of the procoagulant vWf mRNA expression in the lung, compared with an upregulation in heart and kidney *(129)*. With the occlusion of microvessels by microthrombi, a lack of nutrients and hypoxic conditions develops in the tissue, contributing decisively to organ failure.

10. Nitric Oxide

The vasodilatative NO is produced by constitutively expressed endothelial NO synthase (eNOS). NO is believed to play a key role in the pathogenesis of septic shock by contributing to hypotension *(130)* and vascular unresponsiveness (vasoplegia) *(131,132)*. In several experimental models, endotoxin has

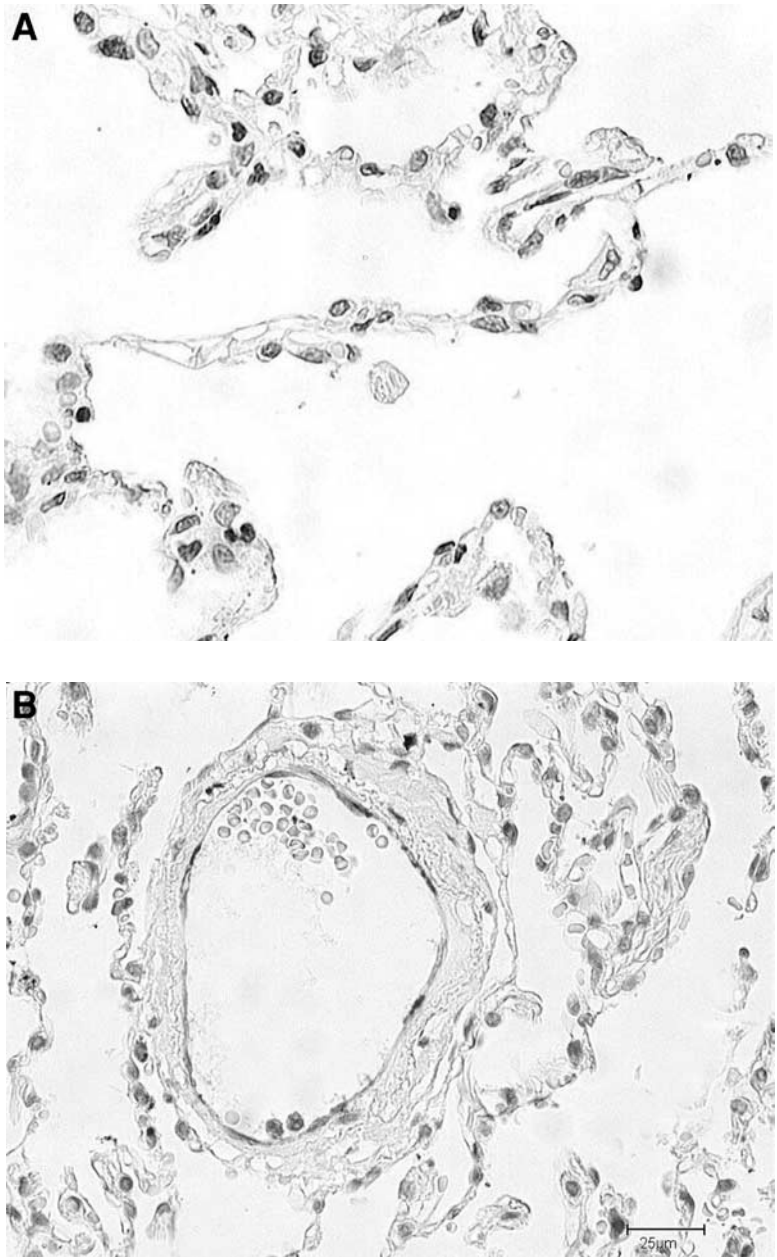


Fig. 6. Inhomogenous expression of von Willebrand factor (vWF) and CD34 by pulmonary endothelial cells in lung tissue without signs of inflammation. **(A)** Very weak-to-absent vWF expression on endothelium of pulmonary capillaries and **(B)** distinct vWF expression by endothelial cells of a venule. **(C)** In contrast, a strong CD34 expression by endothelial cells of pulmonary capillaries, but nearly no immunopositivity on venous endothelium. *(Figure continues)*

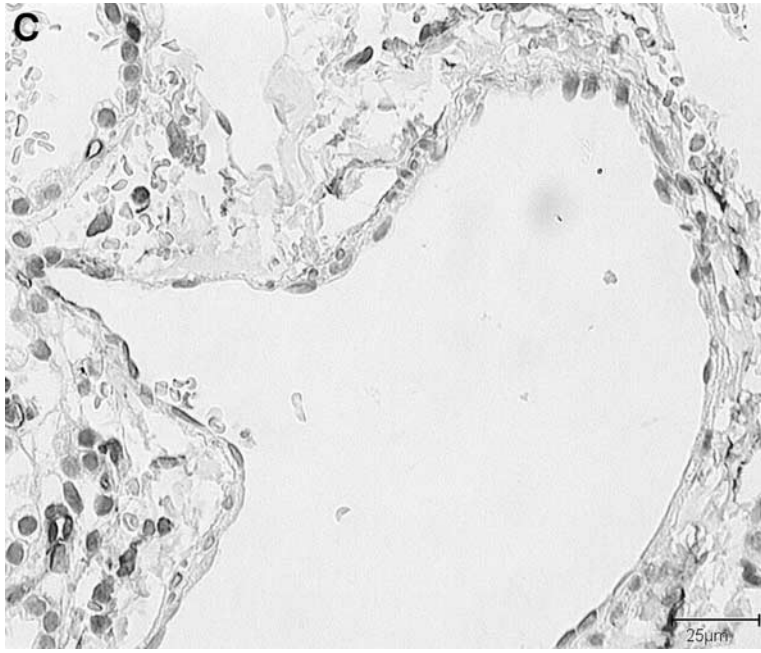


Fig. 6. *(Continued)*

been shown to increase the constitutive release of NO by the endothelium, as well as the activity of inducible NOS (iNOS) *(133)*. Recently, Song et al. demonstrated that NO release from iNOS regulates aspects of sepsis-induced immune dysfunction by the activation of p38 MAPK *(91)*.

11. Apoptosis

Apoptosis (programmed cell death) describes a set of regulated physiological and morphological changes leading to cellular death, hence eliminating senescent or dysfunctional cells. Although apoptotic cell death in general plays a critical role in sepsis *(134)*, studies on endothelial apoptosis in sepsis are inconclusive so far.

Two major pathways are involved in initiation of apoptosis: a receptor-initiated caspase-8-mediated pathway and a mitochondrial-initiated caspase-9-mediated pathway *(135)*. Either caspase-8 or caspase-9 can activate caspase-3, which is involved in the final common pathway of the cell death program. Caspase-8 can be activated by a number of ligands, including TNF-α, whereas the mitochondrial caspase-9-mediated pathway can be activated by a diverse num-

ber of stimuli, including reactive oxygen species, radiation, and chemotherapeutic agents *(135)*.

A number of in vitro studies revealed apoptotic cell death of endothelial cells in response to LPS and TNF-α *(136,137)*, as well as to certain microorganisms. Sylte et al. *(138)* reported that viable bacterias, as well as filtrates of *Haemophilus somnus*, a common cause of pneumonia and vasculitis in cattle, causes apoptosis in bovine endothelial cells in vitro. Likewise, uptake of *Staphylococcus aureus* by cultured endothelial cells induced apoptosis in infected cells *(139)*. On the other hand, studies suggest that endotoxin induces expression of antiapoptotic molecules in microvascular endothelial cells *(134)* and neutrophils *(140)*. The latter could play a role in the accumulation of neutrophils during sepsis, as well as prolongation and/or augmentation of the inflammatory response. Hotchkiss et al. *(141)* found no electron microsopical evidence of endothelial cell apoptosis in aortas from septic rats. Furthermore, in pulmonary vessels from mice with *Pseudomonas aeruginosa* pneumonia, alveolar capillary endothelial cell apoptosis was observed extremely rarely, whereas neutrophil and lymphocyte apoptosis was frequently noted *(141)*.

12. Death Owing to Sepsis: A Postmortem Diagnosis Occasionally Placing Heavy Demands on the Forensic Pathologist

Although diagnosing a septic condition may even present a problem in the living patient, the primary diagnosis of sepsis after death is far more difficult because the major limitation to a precise postmortem diagnosis of sepsis is the often nonspecifity of autopsy findings encountered in such fatalities.

Postmortem microbiological investigations are of little or no value in sepsis-associated fatalities *(142)*. The reason for this is the possibility of gut translocation of bacteria which is defined as the passage of gastrointestinal microflora across the lamina propria to local mesenteric lymph nodes and from there into the systemic circulation. Three primary mechanisms that promote bacterial translocation in sepsis have been identified: intestinal bacterial overgrowth, increased permeability of the intestinal mucosal barrier, and deficiencies in host immune defenses. Migration of organisms across the bowel wall may occur by pinocytosis in epithelial cells. This mechanism has been proposed as the principle factor for translocation in the presence of an intact mucosal barrier. However, many studies have identified alterations in intestinal permeability in critically ill patients. Under normal circumstances, bacteria reaching the mesenteric lymph nodes are phagocytosed by macrophages, but in the immunocompromised septic individual, the normal defense mechanisms fail, permitting

bacteria access to distant extraintestinal sites *(143,144)*. Therefore, heart and spleen blood obtained at autopsy from septic individuals often show polymicrobial culture growth of bowel flora.

In the clinicopathological field—regarding fatalities occuring in hospital—there often is, at least in a relevant proportion of cases, acceptable evidence of an underlying infectious condition in a deceased prior to death, based on the knowledge of the medical history and results of diagnostic procedures preceding death. Furthermore, there is usually a good interdisciplinary communication between the physicians that were in charge of the deceased and the clinical pathologist who is performing the autopsy. The latter can make use of this in obtaining additional information on the clinical course before starting with the postmortem examination. In sepsis-related fatalities of inpatients, the primary task of the clinical autopsy and the subsequent investigations is not to establish the primary diagnosis of sepsis at autopsy, but rather (a) to provide feedback on the accuracy of the clinical diagnosis, (b) to search for underlying disease processes that could have been overlooked but nonetheless contributed to the onset of sepsis and fatal outcome, (c) to verify a suspected, or uncover an uncontrolled focus of sepsis, and (d) to demonstrate pyemic abscess formations or superinfection. In contrast, the postmortem diagnosis of sepsis is by far more difficult to establish in forensic pathology. In the majority of forensic autopsy cases, clear-cut information about the circumstances of death is often not available. Data on the medical history and clinical course of a deceased or an individual's symptoms prior to death are often not reported or not available at the time of autopsy, especially in outpatient fatalities. Similar difficulties may turn up for the forensic pathologist in cases where the fatality took place abroad or where the patient was not in a condition to give a history at admission and/or the duration of the hospital stay before death was too short to achieve any relevant diagnostic findings. Therefore, in a great number of fatalities there is hardly any valuable clinical information available for the forensic pathologist at the time of autopsy. Even in later phases of the postmortem investigation of death of individuals hospitalized for a longer period antemortem, problems can arise when the hospital/institutional documentation is containing incomplete data on the deceased´s clinical course *(145–147)*.

Initiated by a variety of causes and triggered by endogenous mediators, shock events can lead to hypoperfusion with subsequent hypoxia and accumulation of various metabolites resulting in the development of so-called "shock lesions" that are not specific for sepsis or septic shock as they are found also in ischemic episodes of other causes with or without underlying SIRS.

Many sepsis-induced tissue alterations arise symptomless and, therefore, may be late in giving rise to clinical symptoms, thus the pathological age may be greater than the assumed clinical age estimated by the time elapsed from the onset of the first symptoms.

Apart from septicopyemic abscess formations in internal organs, distinct pathomorphological alterations that can be considered pathognomonic for an underlying septic condition in a deceased do not exist. The overwhelming majority of autopsy and microscopic findings in sepsis-related cell and tissue injury, induced by germs or their products and mediated by the broad cascade of endogenous inflammatory mediators are neither specific nor sensitive for sepsis and, as a result, lack evidence when considered as isolated findings. Nevertheless, the detection of diverse, potentially sepsis-induced pathological alterations by routine histological examination and immunohistochemistry can be considered characteristic to a certain degree within the framework of the entire case history and may, therefore, add relevant information to the postmortem elucidation of potentially sepsis-related fatalities.

In the course of the medicolegal investigation, clinical expertises may be necessary to interpret the clinical data against the background of autopsy findings, histopathology, immunohistochemistry, postmortem microbiological results, and further analytical work-ups. All findings brought to light by the postmortem investigation may become evidence in later trial proceedings, and the forensic pathologist may testify as an expert witness against the deceased's physicians. Consequently, any personnel or nonauthorized communication between the forensic pathologist and the deceased's physicians has to be strictly avoided.

From the clinical point of view, a number of sudden, unexpected deaths occuring outside hospital as the sequel of a rapidly progressive course of infection will have to be regarded as unavoidable. However, under medicolegal aspects, the principal question to deal with is whether a causality between an exogenous noxa (e.g., occupational or traffic accident, sharp or blunt external force, decubitus ulcer, indwelling catheter, injection, surgical procedure, etc.) and infection and death can be proven.

13. Some Aspects of Sepsis-Related Fatalties Encountered in Forensic Pathological Autopsy Practice

On various occasions, the forensic pathologist may be confronted with the question if a deceased suffered from a septic condition prior to death and thus, if sepsis caused or at least contributed to fatal outcome in this case.

Typical scenarios of infection-related deaths routinely encountered in forensic autopsy practice are, e.g., infection associated with vascular catheters, delayed diagnosis of Waterhouse-Friderichsen syndrome (WFS) in infancy and childhood, pseudomembranous colititis following the uncritical use of broad spectrum antibiotics for the treatment of minor infections, gas gangrene following surgical procedures, pyomyositis or necrotizing fasciitis resulting from introduction of pathogens into injured tissue (e.g., as a result of an assault), posttraumatic meningitis, infection following intravenous drug abuse, or infected decubitus ulcers. The postmortem investigation and following medical expertise in such fatalities will often concentrate on a specific mode or portal of entry of microorganisms, respectively. Most of these cases are investigated against the background of an allegation of medical malpractice, nursing injury or neglect, most often raised by close relatives of the deceased. In other, rarer instances, the accusation may focus on (grievous) bodily harm.

For example, when advanced grade decubitus ulcers are not mentioned on the death certificate but show up in a deceased, e.g., within the scope of the external examination, or these decubitus ulcers are not sufficiently taken into consideration on the death certificate regarding a potential causal relationship with the cause of death by the physian carrying out the external examination of the corpse, questions concerning the cause and manner of death (unnatural death caused by sepsis as a consequence of the decubitus ulcer?) may arise. Apart from fluid and protein loss, the colonization of a persistent open decubitus with microorganisms may lead to infectious complications, such as osteomyelitis and sepsis. If the deceased was in need of a nursing service or medical care prior to death, the forensic investigator may be also confronted with questions concerning neglect or the existence of an actual nursing injury. In particular cases of decubitus ulcer-associated fatalities, an iatrogenic origin or even medical malpractice has to be considered as well. The development of sepsis as a consequence of decubitus ulcer formation has to be regarded as an unnatural cause of death when the responsibility for the development of the decubitus can be attributed to neglectful care, incorrect nursing, or medical malpractice.

Another example illustrating the relevance of a watertight medicolegal argument that even meets the requirements of the legal authorities' reasonings concerning the causal relationship between death and an underlying sepsis is the manifestation of sepsis following invasive iatrogenic procedures, such as injections, operations, and intravenous or urinary catheters. Sepsis is the most severe and life-threatening complication of iatrogenic injection techniques in use for therapeutic or diagnostic purposes. Although the clinical diagnosis of an iatrogenic injection-induced infection can be difficult in the absence of

signs of inflammation around the insertion site of an intravenous catheter or the puncture site of an intramuscular injection, this diagnosis is even more difficult to establish in forensic autopsy practice when information on a preceding iatrogenic injection therapy is lacking. To enable etiopathogenetic conclusions concerning the causal relationship between, e.g., catheter-related infection and fatal outcome, the proof that a given tissue injury, such as the insertion site of a peripheral venous catheter or a gluteal abscess following intramuscular injection, represents the only and exclusive portal of entry has to be established in the first place. Next, the question whether inoculation of microorganisms through the established portal of entry could have been avoided by all probabilities if the responsible medical staff had acted *lege artis* (e.g., in view of hygiene regulations) and therefore, death, as a result of infection, can be ascribed to medical or nursing malpractice, will be the main focus of medicolegal interest.

Another example of medicolegal relevance is any fatality resulting from WFS in infancy and childhood. In such cases, almost inevitably the question arises if the child could have been saved if there was an earlier diagnosis *(148)*. Especially if a physician has been consulted in the beginning of the disease, medical malpractice seems to be obvious for the parents. Because of the rapid clinical course of the disease and the rather unspecific findings in its beginning, it can be impossible even for the clinical professional to distinguish WFS from a common cold or an enteritis. Even if medical help is sought in an early stage of the disease, it is impossible to predict the outcome in individual cases *(149)*.

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8

Special Aspects of Crime Scene Interpretation and Behavioral Analysis

The Phenomenon of "Undoing"

Judith Schröer, MD and Klaus Püschel, MD

CONTENTS

SUMMARY

A careful reconstruction of the sequence of events and the assessment of offender behavior are playing a more important role in unsolved homicide cases. The method of behavioral analysis was developed in the United States. It is defined as a complex information-processing system for the purpose of enabling an overall view of a given criminal case and of supporting the casework in ongoing investigations. The analytical process is of central importance for further deductions (e.g., offender aims, organized/disorganized components of offender behavior, escalation). *Undoing* is a special form of offender behavior representing a symbolic reversal of the crime. This special form of *personation* occurs at a crime scene when there is a close association between the

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offender and the victim or when the victim represents someone of importance to the offender.

Key Words: Behavioral analysis; homicide investigation; undoing; personation; modus operandi; signature; staging; posing; forensic pathology

1. Introduction

Crime scene analysis may lead to different ways of interpretation and classification of a crime investigated. Additional information can be obtained by methods of behavioral analysis where an overall view of the criminal case is provided and conclusions regarding the underlying motive(s) can be drawn *(1–10)*. Behavioral analysis in unsolved homicide cases is playing a more important role in the field of police work *(11–13)*. The method of behavioral analysis was developed in the United States and further developed in different European countries in the late 1980s. Today, it is routine practice for police agencies to perform analytical procedures in unsolved homicide cases in close cooperation with experts from different fields, such as psychiatry, psychology, and forensic pathology. This analytical process should not be confused with *offender profiling*.

The forensic pathologist investigating the death scene and examining the victim's body will most often contribute valuable information to a careful reconstruction of the sequence of events at the scene of a crime *(14)*. Without establishing the pathological features of a given case in detail, it will be extremely difficult to assess the offender's behavior.

In behavioral analysis, one differentiates between *modus operandi* and *personation (15)*. The exact discrimination between these terms is of significance regarding the assessment of offender behavior. *Modus operandi* refers to behavioral patterns of an offender for the purpose of successfully completing the offense. The term "personation," on the other hand, refers to behavior that indicates the offender's emotional needs. If the same behavior is repeated in a series of offences, this is called *signature*.

This chapter focuses on the so-called phenomenon of undoing ("undoing-behavior"), which can be regarded as a special form of personation. Undoing represents the symbolic reversal of a crime by the offender.

2. Case Reports

Four cases from the Institute of Legal Medicine at the University of Hamburg, Germany, investigated between 2000 and 2003, that illustrate the phenomenon of undoing are presented here.

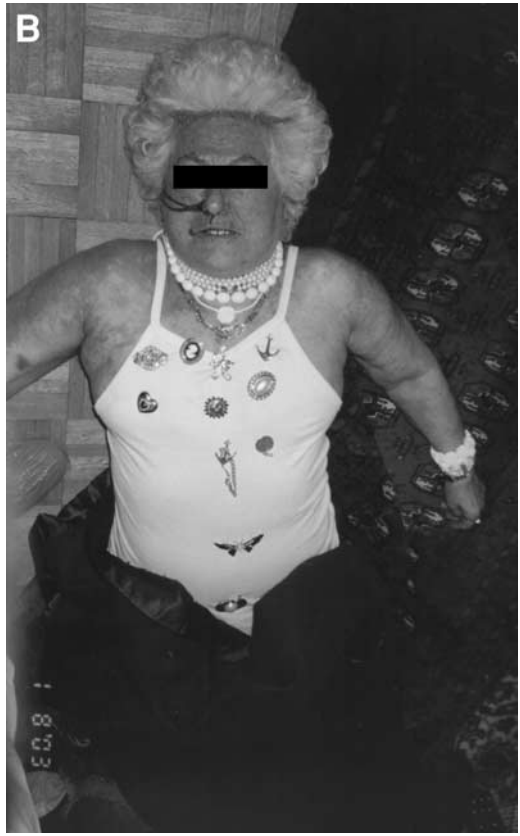
Fig. 1. Case 1: a 67-year-old female; death by ligature strangulation. **(A)** Covering of lower part of the body by offender with placement of jewelry on chest, neck, forehead, and wrists. *(Figure continues)*

Case 1

After a prostate gland operation, a 68-year-old man developed a psychotic disorder that culminated in committing murder–suicide. The man killed his 67-year-old wife by ligature strangulation. She was found in the living room, the lower part of her body covered with a blanket. Most of her jewelry was placed on her chest, as well as around her neck, forehead, and wrists (Fig. 1). The necklaces covered and concealed the strangulation mark at the crime scene. After the man had failed to kill himself by cutting his wrists, he hung himself from an iron bar in a door frame.

Case 2

A 32-year-old man killed his 30-year-old ex-girlfriend because he could not cope with the fact that she had left him. He struck the sleeping woman's head with a big bottle of red wine and then manually strangled her. After the homicide, the blankets of the bed where the victim had died were found pulled very accurately and straight. Additionally, a towel was placed on the lower part of her face to cover the tongue that was protruding owing to the strangulation (Fig. 2).



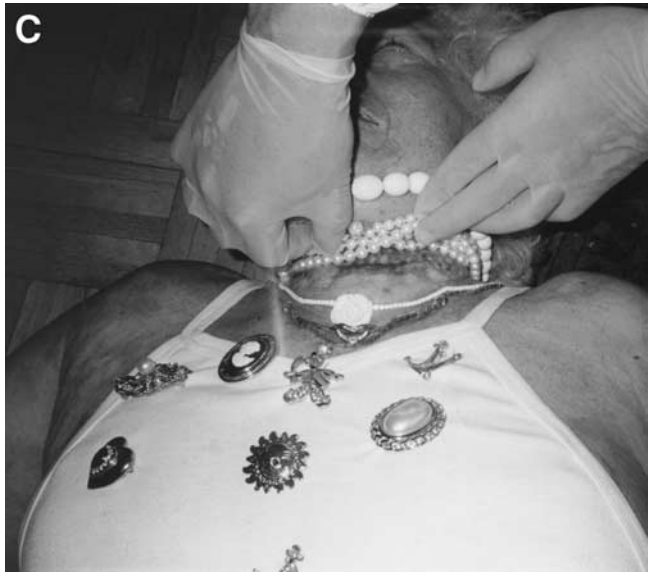


Fig. 1 *(Continued)* **(B)** The ligature mark on the neck covered and concealed by the necklaces. **(C)** Ligature mark seen after partly removal of the several necklaces.

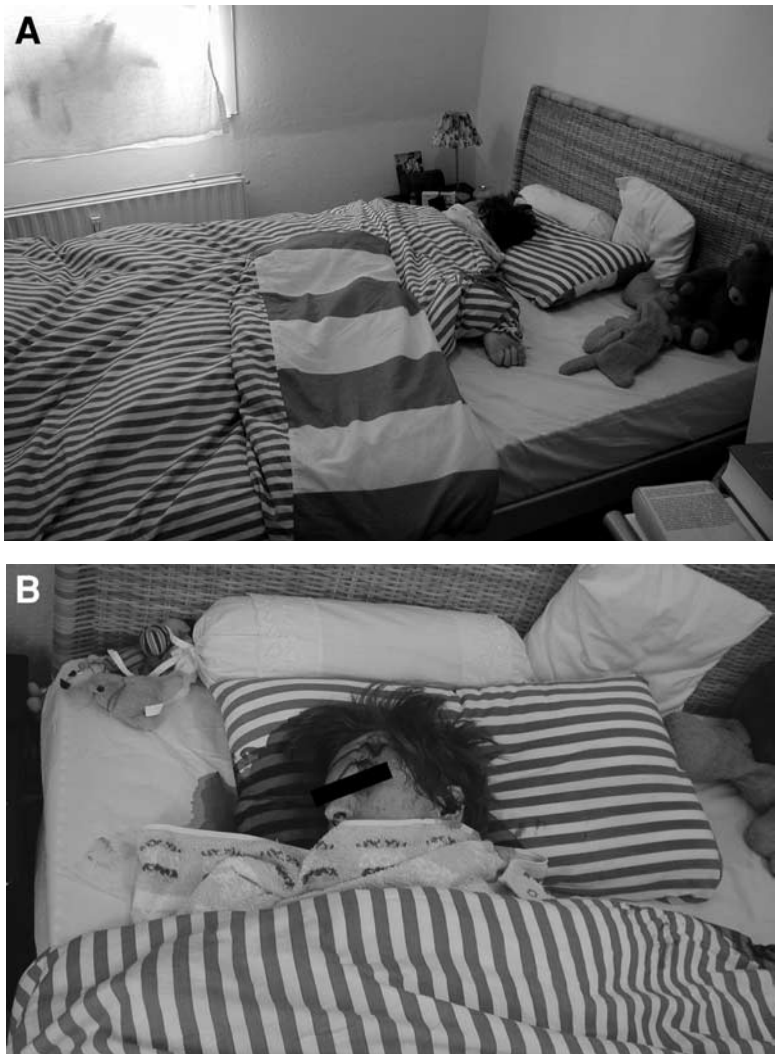


Fig. 2. Case 2: a 32-year-old female; death by manual strangulation. **(A)** Blankets pulled straight over the victim. **(B)** Towel placed on the lower part of the victim's face.

Case 3

A 26-year-old woman was found dead in her apartment. Undoing behavior in the form of covering of the victim with a blanket, a feather boa draped around the head of the decedent, and an artificial rose placed in front of the bed was apparent at the crime scene (Fig 3). Because of the advanced decomposi-

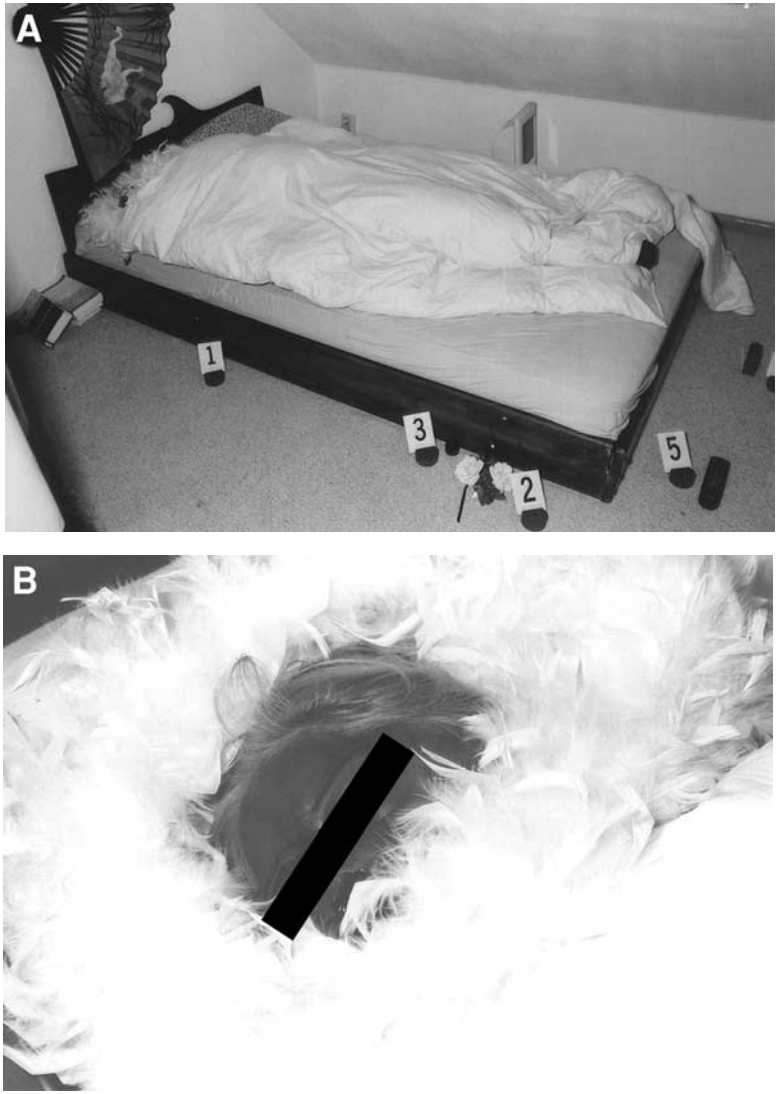


Fig. 3. Case 3:a 26-year-old female; death by manual strangulation. **(A)** The victim is covered with a blanket, a feather boa is draped around the head, and an artificial rose is placed in front of the bed. **(B)** A closer look at the feather boa draped around the face. Note that the body is in a state of advanced decomposition.

tion of the body, autopsy produced poor evidence. Manual strangulation was assumed as the most probable cause of death but could not be proved by pathoanatomical means. The 33-year-old boyfriend of the victim was under strong suspicion, and when he was confronted with the facts from the crime scene in the main hearing, he surprisingly made a confession. He had killed his girlfriend after putting her under pressure to marry him. She had refused and wanted to bring the relationship to an end because she had lived in constant fear of his previous repeated violent attacks. According to the man, he had sexually assaulted and then killed her by manual strangulation. In the verdict, the undoing behavior was mentioned as follows:

> After he had committed the crime, the accused realized that he had done wrong, and he felt remorse. He decorated the deceased with a feather boa that he draped around her head, covered the body with a blanket and put an artificial rose, that he had found in the apartment, in front of her bed.

Because of a paranoid psychotic disorder, the man was found not responsible for his actions. As his personality was assessed as dangerous to the general public, the court sentenced him to life-long detainment in a psychiatric ward.

Case 4

A 46-year-old woman was found dead lying on the bed in her apartment (Fig. 4). Her naked body was covered with a blanket and a sweater. Many items, such as plants, flowers, dolls, artificial butterflies, grapes, etc., were placed around the decedent's head on the bed linen. Someone had obviously colored the victim's eyelids and lips. The offender was the 47-year-old partner who had met the woman during therapy in a psychiatric hospital. He had killed the woman by ligature strangulation. Years ago, he had been sentenced for having murdered his aunt. Although the woman had already reported to the police because of physical violence by her partner, strict separation had not been established. Aside from alcohol addiction, a borderline personality disorder of the offender was diagnosed by the psychiatric expert; he was sentenced to 10 years imprisonment and consecutive life-long safekeeping in a psychiatric hospital.

3. Discussion

Any forensic pathologist dealing with unsolved homicide cases should have at least some basic knowledge of the principles and the methods of behavioral analysis *(16)*. In any given case of suspected homicide, it is self-evident that the forensic pathologist who performs the autopsy should visit the death scene because all injuries must be examined within the context of the event. There are

Fig. 4. Case 4: 46-year-old female; death by manual strangulation. **(A)** The body of the victim is covered with a blanket and a sweater and different utensils are placed around the dead woman's face. **(B)** A closer look at different utensils placed around the face. Note coloration of eyelids and lips of the victim.

still far too many cases where this does not occur, thus making it impossible to carry out an exact reconstruction of the sequence of events in later stages of the criminal investigation. In numerous cases, however, the initial situation is inadvertently changed by police forces or rescue teams that first arrive at the crime scene. As a result, the initial scene is often not sufficiently documented, and changes may lead to misinterpretation in the future analysing process.

The term "undoing" first appeared in the literature when the FBI started to study and classify offender behavior systematically in the 1970s. In these systematic studies, it turned out that if forms of undoing behavior were present at a crime scene, the two most likely constellations were (a) a close association between the offender and the victim or (b) that the victim represented a person of importance to the offender. That is why the definition of undoing is summarized in the FBI's Crime Classification Manual in the section of "domestic homicide." Undoing behavior should be differentiated from acts of staging or posing. *Staging* is defined as the purposeful alteration of the crime scene to make it appear to be something it is not. *Posing* means the positioning of a victim's body—usually in a degrading position—for the offender's own pleasure or to shock the finder of the body *(17)*.

We were not able to find any relevant quantitative analyses or statistical figures about the phenomenon of undoing in the available literature. Within the case material of the city-state of Hamburg, Germany, we investigated the police files and autopsy protocols with respect to this special aspect of personation after sexually motivated killings *(18)*. Undoing behavior was registered in 11% of the solved homicides. Among these cases, a long-lasting relationship between victim and offender was shown in all cases except for one.

At first sight, these aspects might be of little significance for the forensic pathologist's work at a crime scene, but they may be helpful for first considerations regarding the person who committed the crime in question and, therefore, provide valuable investigative leads *(19)*.

The four cases demonstrated here confirm the hypothesis of preceding deep emotional relations between victim and offender leading to undoing behavior: exclusively the female partner or ex-partner was killed in the cases presented here.

However, the differential diagnosis of deception factors and false tracks should not be disregarded. One single behavior should never be interpreted outside its context, because the same behavior may represent different offender needs. For example, cleaning of the victim may be part of an offender's *modus operandi* for the purpose of destroying trace evidence, whereas in another case, it can be a form of undoing behavior. Therefore, a behavior's meaning to the offender can only be interpreted when regarded in its context *(20)*.

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Toxicology

9

Neogenesis of Ethanol and Fusel Oils in Putrefying Blood

Wolfgang Huckenbeck, MD

Contents

Summary

The main problems in determining alcohol concentrations after death are changes in blood alcohol concentration (BAC) that can occur both within the blood of corpses as well as in blood samples that are improperly stored or contaminated with bacteria or fungi. The possibility that neogenesis of ethanol after death might give rise to an incorrect estimation of BAC is the most relevant factor. Therefore, the interpretation of postmortem alcohol levels is still problematic in legal medicine. Detection of fusel oils does not give proof of postmortem ethanol neogenesis; however, congener analysis can help to identify putrefactive alcohols. A discrimination of fusel oils from higher alcohols originating

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from alcoholic beverages consumed premortem may be problematic, but the postmortem identification of methanol is a definite indicator of alcohol consumption before death. Proper postmortem collection of blood samples is critical for a precise and reliable alcohol determination in blood from corpses. In this respect, difficulties may arise depending on the degree of putrefaction or in cases of mutilated or burnt corpses. A number of ubiquitous microorganisms are capable of forming or degrading alcohol, and neogenesis of ethanol is only a byproduct of bacterial decomposition processes. The reactions that take place are mainly influenced by temperature, pH value, concentrations of available carbohydrates, and the presence of other utilizable nutrients. The main problems in the assessment of ethanol concentrations in blood from corpses include the potential of water loss, autolysis, putrefaction, and postmortem glycogenolysis. Whereas advanced putrefaction of a blood sample can be recognized macroscopically and by its odor, the transitional phase into putrefaction presents difficulties. Certain bacteria, which can be recognized by their synthesis of putrefactive amino acids (e.g., production of α-aminobutyric acid, γ-aminobutyric acid and/or δ-aminovaleric acid) play an important role in the postmortem neogenesis of alcohol. In case of uncertainty, an amino acid analysis can confirm suspected putrefaction.

Key Words: Alcohol; ethanol; putrefactive alcohols; putrefactive flora; putrefactive amino acids; ethanol neogenesis; congeners of alcoholic beverages; alcohol diffusion.

1. INTRODUCTION

The term “alcohol” is derived from the Arabic “al-kuhul,” a black powder prepared from antimony that was used as eye shadow in ancient times. In medieval times, alchemists initially used the word to describe fine powders, and later for purified substances yielded by distillation. The term “alcohol” is often used as a synonym for ethyl alcohol, i.e., drinking alcohol or the active ingredient of alcoholic beverages. This chapter deals with the determination of postmortem ethyl alcohol concentration, which is an important topic in forensic and legal medicine. The main problems in determining alcohol concentrations after death are changes in concentration that can occur both within the blood of corpses and in stored blood samples that are contaminated with microorganisms. The possibility that neogenesis of ethanol after death might give rise to an incorrect estimation of blood alcohol concentration (BAC) is the most relevant factor in legal proceedings *(1)*. In this chapter, such ethanol will be referred to as “putrefactive alcohol” to distinguish it from saprogenic alcohols. The term “saprogenic alcohols” is used to describe all other (higher) alcohols originating

Table 1
Proof of Fusel Oils and Their Derivatives in Postmortem or Contaminated Blood Samples According to Different Investigators

Authors (ref. no.)	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
Substance											
1-propanol	•	•	•	•	•	•	•	•	•	(•)	(•)
2-propanol	•	•	•	•		•	•	•	•	(•)	(•)
1-butanol	•	•	•	•		•	•	•	•		
1-butanol	•	•	•	•		•	•		•		
Isobutanol	•	•	•	•			•	•	•		
Isopentanol	•										
Acetaldehyde	•	•		•			•	(•)		•	•
Acetone	•	•		•		•	•	(•)	•	•	•
Ethyl methyl ketone	•							(•)			
Diethyl ether	•										
Formaldehyde	•										
Phenyl ethanol	•										
Hydroxyphenyl ethanol	•										
3-methyl-1-butanol		•	•		•	•	•	•			
2-methyl-1-butanol		•					(•)				
1-pentanol		•									
2-methyl-1-propanol				•		•					
2-pentanol									•		

from putrefactive processes, either in blood samples stored in vitro or in the corpse itself. In a broader sense, acetaldehyde, acetone, and methylethyl ketone should be included in this class of substance, whereas methanol is not included.

Interestingly, and unfortunately, the composition of this class of substance is very similar to that of the so-called congeners of alcoholic beverages—a circumstance that occasionally gives rise to problems in forensic practice. Therefore, detection of fusel oils does not give proof of ethanol neogenesis. Our own studies *(2)* have, however, shown that bacterial decomposition in human tissue does not lead to neogenesis of methanol. In cases where methanol is found it can be concluded that alcoholic beverages were consumed before death.

Table 1 provides a summary of putrefactive alcohols detected in putrefying blood from corpses and in inadequately stored and contaminated blood samples *(1–11)*. In principle, the processes of in vitro putrefaction in such blood samples are comparable to those that take place in putrefying blood within a corpse.

It was speculated in the 19th century that alcohol is formed during putrefaction of corpses *(12,13)*, but substantiating evidence could not be provided until the 20th century *(14)*.

The development of new analytical methods led to continual improvements in the identification of alcohols in the blood of corpses. The extensive literature available is very heterogeneous with respect to the analytical methods used, which makes comparison of results difficult and therefore, requires that such results must be interpreted with caution.

A more precise differentiation of putrefactive alcohols has only been made possible by the introduction and development of gas chromatography. Osterhaus and Johannsmeier *(6)* provided instructions on how to determine ether, acetone, and alcohols using a gas-chromatographic method. Using the same method, Weinig et al. *(3)* identified methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, isobutanol, 2-butanol, and 2-methyl-1-butanol as compounds found in putrefactive alcohols.

Besides the variety of analytical methods used, the different experimental conditions applied also make the evaluation and interpretation of the available literature difficult *(2–7,10,11,15,16)*.

However, a comparison of the various studies demonstrates that alcohol concentration often changes in putrefying blood. These changes might be caused by either a change in the level of ethanol or to the formation of higher alcohols, aldehydes, and ketones.

Even though "fresh" samples of blood from corpses can contain a variety of higher alcohols (that can at least partially be attributed to congener alcohols imbibed while the subject was alive), the expected concentrations in putrefying blood are exceeded by several orders of magnitude. Therefore, the presence of such alcohols must be a result of neogenesis within the corpse or the stored blood sample. Using the appropriate analytical technology, the concentration changes should normally be detectable, especially when one considers the high concentrations found. However, the situation is more complex in cases of congener analyses, and when it is suspected that putrefaction has commenced. Figure 1 shows a typical chromatograph obtained from the analysis of a putrefied blood sample from a corpse *(2)*.

2. Importance of Ethanol Determination in Medicolegal Autopsy Samples

It is beyond question that the determination of alcohol concentration is of great importance in medicolegal autopsy blood samples, regardless of whether civil, criminal, or insurance law is involved. Medicolegal problems

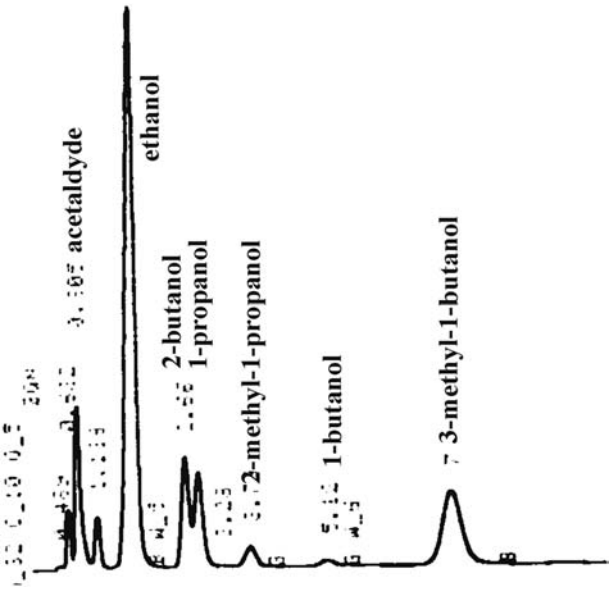

Fig. 1. Typical chromatogram of a blood sample from a corpse.

are not confined to determining alcohol as either a sole or joint cause of death, such as in accidents, but also include the problem of distinguishing true alcoholic poisoning.

Marcinkowski and Przybylski *(17)* reported BACs of up to 0.636% in autopsy samples. Concentrations ranging from 0.225 to 0.603% were found by Stead and Moffat *(18)* when analyzing cases of lethal alcoholic poisoning in Poland. In the absence of evidence suggesting any other cause of death (e.g., asphyxia), Johnson *(19)* estimated an alcohol level of 0.25% to be a possible fatal dose. Heatley and Crane *(20)* calculated an average value of 0.355% for 175 deaths in Ireland owing to acute ethanol poisoning. In a South African study on 948 autopsies, Loftus and Dada *(21)* found alcohol was present in 52.5% of cases with an average of 0.18% (0.008–0.48%).

In a study of 18,000 young, ex-US soldiers, Pollock et al. *(22)* found 133 alcohol-related causes of death within the 426 deaths analyzed. Hain et al. *(23)* examined 874 casualties from 12 to 25 years of age. In the 16–21-year age group, 53% of those examined were found to have been under the influence of alcohol at the time of accident. In the 21–25-year age group, the level rose to 68%. Average BAC values were 0.14 or 0.18%, respectively. In 1984, Plueckhahn *(24)* determined blood alcohol values of 135 people who accidentally

drowned between 1959 and 1983. Thirty-seven percent of the males had BAC values higher than 0.08%. Forty-two percent of the males older than 30 years showed BAC values greater than 0.15%. In Denmark, Kringsholm et al. *(25)* carried out a study on deaths by drowning which occurred over a time period of 3 years. Of 74 cases, 53% showed levels of alcohol above 0.01%, whereas levels of 0.03% and above were found in 10% of cases. Analyzing autopsy samples ($N = 6431$) in Berlin from 1978 to 1988, Wiese et al. *(26)* reported evidence of acute alcoholic influence or indications of chronic alcohol abuse in 22% of all samples studied. In cases where an accident was stated as the manner of death, this percentage rose to 35%. Abel and Zeidenberg *(27)* analyzed the involvement of alcohol in 3358 deaths by acts of violence during a period of 10 years. Alcohol was found to be involved in more than 48% of road casualties, more than 45% of homicides, and more than 35% of suicides. The Federal Statistical Office of Germany reported 64,400 recorded traffic accidents in which the driver was under the influence of alcohol in 2001 *(28)*. For fatal accidents in which two-wheeled vehicles were involved, Lutz and Kreidel *(29)* estimated an involvement of alcohol in 22% of cases.

Christopoulos et al. *(30)* reported the presence of alcohol in more than 50% of postmortem examinations that included toxicological analyses. According to Gariott *(31)*, significant ethanol levels are found in 50% of drivers involved in fatal road accidents, and in 60% of pedestrians killed in the United States. Within a 3-month period, Lindblad and Olsson *(32)* reported 24 patients having a BAC of more than 0.5%—one patient even surviving a BAC of 0.78%.

A retrospective study performed by this author based on postmortem examinations carried out from 1991 to 1997 at the Institute of Legal Medicine of the Heinrich Heine University in Düsseldorf, Germany, showed the presence of alcohol (>0.01%) in 23.6% of the total of 8169 cases evaluated *(2)*. The highest values found were 0.617 and 0.635%. The corresponding distribution of BAC values is given in Fig. 2. A follow-up study over subsequent years revealed no significant change in this BAC distribution (Huckenbeck W, 2004, unpublished data).

3. Blood Collection From Corpses

Proper postmortem collection of blood samples is critical for a precise and reliable alcohol determination in blood from corpses. In this respect, difficulties may arise depending on the degree of putrefaction or in cases of mutilated or burnt corpses. Contrary to expectations, general questions, such as from where blood should ideally be collected, are topics of controversial (when viewed internationally) discussions *(33–35)*.

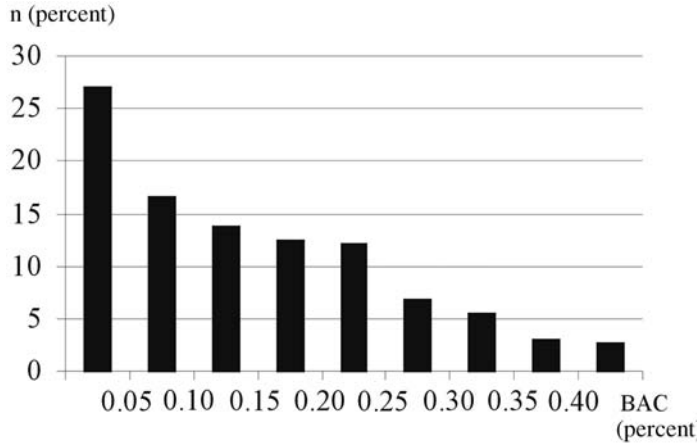

Fig. 2. Distribution of blood alcohol concentration greater than 0.1 mg ethanol per gram in medicolegal autopsies in Düsseldorf, Germany, from 1991 to 1997 (*n* = 8169).

Circumstances valid for living patients cannot be transferred to corpses *(36,37)* because at the time of death, changes in the permeability of vascular walls commence. In addition to water draining from the whole blood, clotting, separation, and sedimentation processes can be observed, as already described by Weinig in 1936 *(38)*. Thus, when drawing samples from blood of the living, the liquid within the blood vessels constitutes a well-mixed medium. By contrast, in the case of corpses, the zones into which a hypodermic needle is introduced may contain material of different viscosity, ranging from thin liquid to viscous or corpuscle-rich material *(39)*.

However, Falconer and Falconer *(40)* examined nine corpses without signs of putrefaction and found identical (within the accuracy of measurement) BACs in all blood vessels analyzed. Iffland and Palm *(39)* analyzed samples from various blood vessels, urine, bile, and cerebrospinal fluid from 75 corpses without signs of putrefaction, but they could not confirm the results of Falconer and Falconer *(40)*. In contrast, Iffland and Palm *(39)* found considerable concentration differences in 18 cases, even in corresponding vessels. Postmortem diffusion, bacterial activities at low blood vessel filling level, death in resorption phase, and contamination during preparation of samples were discussed as possible reasons for these discrepancies.

The different states of the liquid within the blood vessels can be taken into account by determining the water content of the postmortem blood sample *(41–45)*, a factor which is dealt with later.

Although the scientific basis was precisely analyzed and described much later, the collection of cardiac blood has been unanimously advised against in German-speaking countries since the 1930s *(46)*, a development which is not necessarily true for English-speaking countries, especially not for the United States.

Sjövall and Widmark *(47)* compared alcohol concentrations in blood samples from corpses and found equal concentrations in both the longitudinal sinus and pulmonary vein, whereas values for blood from the iliac vein differed significantly. The studies carried out by Sjövall and Widmark *(47)* were resumed by Guldberg *(48)*, who recommended collecting blood samples from corpses as far away from the stomach as possible. Laboratory experiments and studies on corpses with regard to alcohol diffusion from the gastrointestinal tract were also carried out by Huber *(49)*. This author strongly advised against the collection of cardiac blood for the purpose of determination of alcohol concentration.

Studying the diffusional permeability of stomachs from corpses, Schleyer *(50)* found that the wall of the stomach behaves like a membrane in the first 40 hours postmortem resulting in a regular alcohol efflux corresponding to the concentration gradient. Subsequently, alcohol is discharged in waves, which, according to the author, is a result of autolysis. After measuring alcohol in femoral vein blood, cardiac blood, and pericardial fluid from 29 corpses, Schweitzer *(51)* introduced a 15% alcoholic solution into the abdominal cavity and redetermined the concentration after 12 to 31 hours storage at 4°C. In one-third of the cases, an increase in cardiac blood ethanol concentrations of up to 0.03% was measured and could be attributed to diffusion. Two-thirds of the cases showed an increase of up to 0.3% in ethanol levels within the pericardial fluid. Interestingly, high values found in pericardial fluid did not necessarily correspond to increases in ethanol concentrations in cardiac blood. An explanation for the various findings could not be provided by Schweitzer. However, his studies give evidence for ethanol diffusion through the diaphragm into the heart.

Hebold *(52)* introduced an alcoholic solution into the stomachs of 12 corpses and determined the alcohol concentration in pericardial fluid and cardiac blood up to 24 hours later. In one case, an increase in ethanol level of 0.08% was observed after just 2 hours. In a case report, Abele and Scholz *(53)* found an increase in BAC (in cardiac blood) of 0.06% within 60 hours. Because alcohol consumption shortly before death could be proven, the authors discussed diffusion originating from the stomach.

In the Anglo-American literature, Gifford and Turkel *(54)* reported postmortem diffusion following instillation of whiskey into the digestive tract of 11 corpses. A significant diffusion of ethanol into cardiac blood, pleural, and peri-

cardial fluid was described. Based on these findings, the authors recommended collection of blood samples from the femoral vein. Turkel and Gifford *(55)* compared blood drawn from the femoral vein with blood collected from the pericardium during autopsy. In 35 of 51 cases, the authors found an increase in alcohol concentration in "pericardial blood." This comparison was criticized by Muehlberger *(56)*, who pointed out differences in the composition of blood that is collected in the pericardium. In addition, the study of Turkel and Gifford (*55*) was criticized by Harger *(57)*, who attributed the differences in alcohol levels between femoral and cardiac blood to normal absorption in the extremities.

Based on 61 autopsies, Briglia et al. *(58)* analyzed blood samples from the right and left ventricles, femoral vein, pericardial and cerebrospinal fluid samples, vitreous humor, urine samples, stomach contents, and brain tissue. The measured differences in alcohol concentration in the samples from each corpse ranged from 1.8 to 428%. Larger differences between different points of blood collection were found in cases of higher alcohol concentration in the stomach.

Using Widmark's and gas chromatographic methods, Schwarz et al. *(59)* compared ethanol levels in blood samples collected from the right atria and the femoral veins of 250 corpses, the postmortem collection intervals of which were up to several days. The authors found changes in the water content of the femoral vein blood to be more pronounced when compared to the cardiac blood in the first days. On average, alcohol concentrations were lower in cardiac blood than in the femoral vein. In individual cases, however, differences of up to 50% were found.

Following introduction of alcohol (ethanol and isopropanol) into the abdominal cavities of 13 corpses, Günther *(60)* determined alcohol concentrations in urine and blood, the latter partially collected from the heart. The BAC in femoral veins increased up to a level of 0.74%, whereas isopropanol could not be detected in cardiac blood. The author concluded that blood collected from the prepared heart of corpses is suitable for forensic investigations. Although these conclusions cannot be wholly accepted, his experimental study raised awareness of the problem of blood collection from the femoral vein in cases of abdominal injury.

Based on experiments on alcohol diffusion from aspirated vomit, Pounder and Yonemitsu *(61)* strongly recommend collecting blood from the femoral vein. However, depending on the position of the corpse, vomit can diffuse into other body fluids, such as vitreous humor *(62)*.

Pounder and Smith *(63)* introduced a methanol–ethanol solution into the stomachs of corpses. Alcohol concentrations arising from diffusion were the highest in pericardial fluid, followed by left pulmonary vein, aorta, left ventricle, pulmonary artery, superior vena cava, inferior vena cava, right ventricle,

right pulmonary vein, and femoral vein. The rate of diffusion correlated with the alcohol concentration applied. This time-dependent process was found to be significantly reduced when the corpse was stored at 4°C. On the basis of their experimental results, the authors considered puncture of the femoral vein to be the best solution *(63)*.

It has been reported by Winek and Esposito *(64)* that, in the United States, blood from corpses is often collected by thoracic puncture. Among a total of 6000 cases, they found 19 cases of BACs above 0.5%, of which 8 corpses were also found to have gastrointestinal injuries.

Analyzing cardiac blood from eight corpses in an advanced state of putrefaction, for which premortem alcohol consumption could be excluded, Plueckhahn et al. *(33)* found alcohol concentrations of up to 0.127%. The ratio of alcohol concentration in cardiac to femoral vein blood was determined by Prouty and Anderson *(65)* in 100 corpses. In most instances, they found alcohol levels in cardiac blood to be increased by more than 0.02%. In contrast to other authors, they nevertheless regarded determination of alcohol concentrations in cardiac blood as a suitable method.

Using stomachs isolated from corpses, Erkens et al. *(66)* analyzed the influence of temperature and time on ethanol diffusion. The diffusion rate was found to vary considerably; the time until ethanol could first be detected in a surrounding NaCl solution varied between 7 and 54 hours. Independent of the starting concentration of ethanol introduced, in the initial phase, a steep decrease in alcohol concentration was observed within the stomachs, without an equivalent increase in the surrounding solution.

For blood collection from femoral veins, it was recommended by Druid and Holmgren *(67)* that blood be drawn from bottom to top in order to prevent influx from blood in the inferior vena cava.

Winek et al. compared BACs from thoracic punctures with alcohol concentrations determined in cardiac blood. Seven cases of gastrointestinal injury were observed *(68)*. Based on these findings, these authors recommended that if such injuries exist, only blood from the intact ventricle or other body fluids should be used for BAC determination.

As described by Winek et al. *(68,69)* and Günther *(60)*, when collecting blood from the femoral vein, misinterpretations are possible, if, because of gastrointestinal injuries, alcohol-containing matter leaks from the stomach into the abdominal cavity and gives rise to subsequent diffusion. Experiments on corpses carried out by Jäckel *(70)* showed that depots of alcohol in the minor pelvis might lead to an increase in ethanol concentration in the femoral vein. The relevance of abdominal cavity injuries was pointed out by Jäckel.

Therefore, an alternative sampling site, remote from the stomach, was required. Franz *(71)* tested 100 corpses for evidence of postmortem diffusion of alcohol from the stomach into the subclavian veins. Despite the topographical relation between the left subclavian vein and the stomach, this study clearly showed that alcohol levels in the left subclavian vein were not significantly higher than those in the right subclavian vein. Only a few instances of an increased BAC compared with that found in the femoral vein were observed. Stomach and diaphragm ruptures showed no detectable influence. The shortest diffusion time measured was 15 hours. The author recommended the subclavian vein for blood collection from corpses *(71)*.

Two studies, reported by Brettel *(72)* and Brettel and Franz *(73)*, respectively, also studied the potential of collecting blood from the subclavian vein for postmortem determination of alcohol levels. Experiments were carried out on 100 corpses, into the stomach of which an alcoholic solution was injected. Although in nine cases alcohol diffusion from the stomach could clearly be detected (with femoral vein blood being negative), the authors recommended the method for routine use.

To sum up, as a method of choice, blood from corpses should be collected from the femoral vein. If gastrointestinal injuries exist or are suspected, a puncture of the subclavian vein should also be carried out. Alcohol determination in cardiac blood should be restricted to special circumstances, e.g., if peripheral blood cannot be sampled, in which case an additional alcohol determination of the stomach contents is strongly recommended. At this point, the casuistry of Logan and Lindholm *(74)*, who reported on contamination with stomach contents when blind-puncturing the heart, is referred to, for which histological evidence was given. The extreme BAC value of 1.7% (!) in corpse blood reported by Christopoulos et al. *(30)* is certainly attributable to the collection method (cardiac blood). The same is true for the casuistry reported by Chikasue et al. *(75)*.

In principle, the recommendation of Brettel *(46)* to collect blood from the femoral vein according to the method described by Grüner *(76)*, and alternatively from the subclavian vein, can be agreed on. But even when applying this method of vasopuncture, proper procedure has to be followed. The case reported by Zollinger et al. *(77)*, who detected a contamination of stomach contents (owing to use of the same ladle) when drawing off blood from the dissected femoral vein, which resulted in a BAC of more than 0.9%, shows the advantage of using disposable syringes. In order not only to avoid unintentional collection of blood from the inferior vena cava, but also to ensure sufficient material is collected, injection should be carried out distally and blood be drawn off in a proximal direction (lifting the leg may help in this regard).

The following is a short description of a proven method for collecting blood from the femoral vein *(78)*:

> The tip of the thumb is placed on the anteriosuperior iliac spine and the end phalanx of the middle finger on the pubic symphysis. The spread of these fingers limits the movement of the forefinger, which parallels the direction in which the femoral vein runs. Approximately a hand's width below the inguinal ligament, the sartorius muscle can be felt. The vascular bundle of femoral artery and vein runs directly within its interior.
>
> A cardiac canula is introduced into this area in an angle of 30–40 degrees. In order to access the vein, the artery has to be penetrated. This penetration can be recognized by the tangible resistance when the canula is introduced into this blood vessel. After pushing through the artery, the tip of the canula is located in the vein and blood can be aspirated with a syringe.
>
> This procedure can also be applied to putrefying corpses, in which case the syringe will initially be filled with putrefactive gas, which can be exhausted by leaving the canula in the vein. Peripheral gas pressure then forces the blood into the syringe. If, in individual cases, this method of blood collection should fail, dissection has to be carried out. Having located the vessel, a 10 cm incision is made along the inner rim of the sartorius muscle. Following exposure of the upper tissue layers and subcutaneous fatty tissue, the muscles lie exposed. Sartorius and pectineus muscles can then be identified. The vascular bundle of the thigh runs in between the two muscular cords. The muscular sheath separating both muscles can be detached so that artery and vein are clearly visible. The vein lies below the artery and can be recognized by its strong bluish-reddish color. Puncture can now be easily carried out, at an angle of 30 to 40 degrees, in order to avoid transpenetration of the vein or aspiration of the venous valve. After blood collection, the skin incision is stitched using sectional needle and suture.

The procedure is illustrated in Fig. 3.

4. Factors Influencing the Alcohol Level

4.1. Water Content of Sample Material

Whole blood consists of corpuscular elements and plasma. Water makes up approx 90% of the serum (blood plasma minus fibrinogen) and 78 to 80% of whole blood. Because it is difficult to obtain a homogeneous blood sample prior to analysis, routine alcohol determination is carried out using serum and the concentration is calculated using a correction factor. However, this procedure cannot usually be applied to blood from corpses, for reasons such as

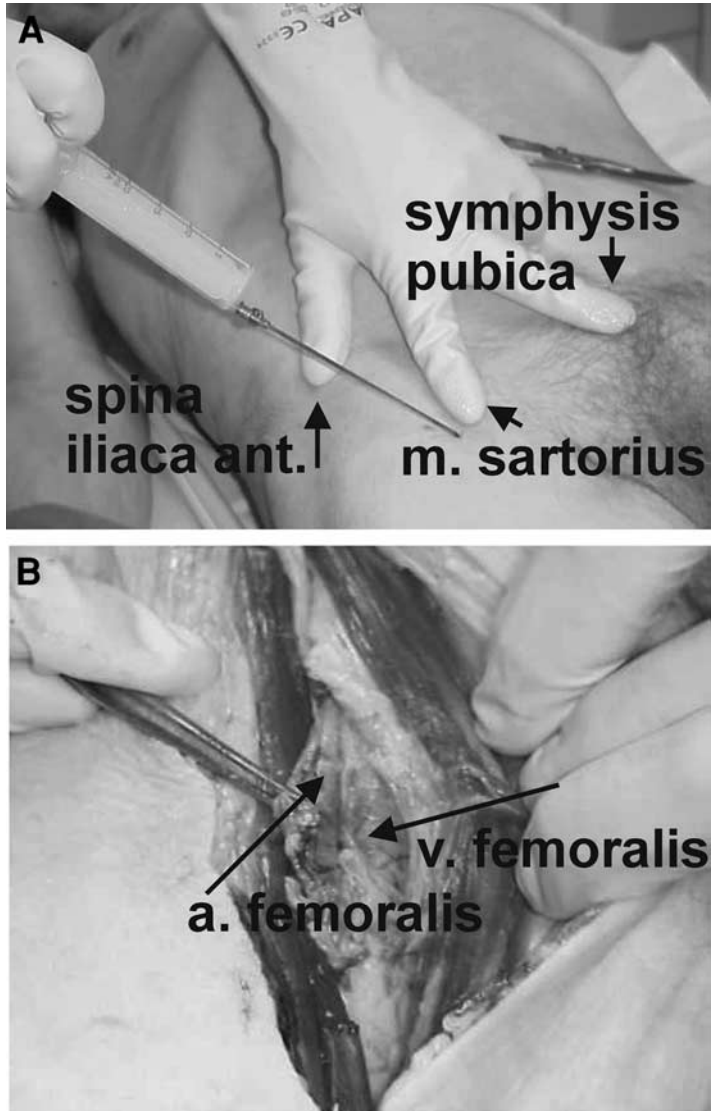

Fig. 3. (A,B) Postmortem blood sampling from the vena femoralis (modified according to ref. *79*).

permeability disorders, liquid transfer into tissues, etc., as discussed in refs. *79–81*.

Grüner *(82)* analyzed the distribution of alcohol in drinking experiments and found that practically the only solvent in question is water in the body. In

order to compensate for the considerably higher variability in corpses as opposed to living subjects, an individual determination of the water content in blood from corpses must be carried out. According to Berghaus and Dotzauer *(83)*, the water content in blood samples from corpses varies between 60 and 90%. These values have been backed up by several studies. Brettel *(43)* analyzed 960 samples of blood from corpses and observed considerable variations in water content (58.8–89.3%), even within corresponding blood vessels from the same corpse. In 1970, the same author analyzed blood samples from 66 corpses *(44)*. Blood was taken from each femoral vein after various postmortem time intervals. He found a correlation in the decrease in BAC and water content, which was particularly pronounced in the first days postmortem.

Variations in water content between 61 and 90% were reported by Audrlicky *(41)*, who analyzed 194 blood samples collected from different body regions of 20 corpses. He pointed out the considerably high probability of error for the alcohol determination if these variations (+33% to –10%) are not taken into account. Further investigations by Audrlicky and Pribilla *(42)* were related to vitreous humor and synovial fluid. These studies showed that water content is much more constant in these body fluids than in blood. Schwarz et al. *(59)* found changes in water content of femoral vein blood to be more pronounced than those in cardiac blood over the first days after death. On average, alcohol concentrations were lower in cardiac blood than in the femoral vein. In individual cases, however, differences of up to 50% were found.

Felby and Nielsen *(84,85)* analyzed BAC and water content in samples from 71 corpses. The first sample was collected within the initial 10 hours postmortem, the second after a time interval of 8 to 230 hours postmortem. For fresh corpses the influence of water content on BAC values was found to be significant, whereas in case of putrefying corpses other factors predominated. The authors pointed out that after hemorrhagic shock and if blood is collected within a few hours of death, the BAC value should be corrected if the water content is higher than 82%.

With regard to the problem of the water content in drowned bodies, the investigations of Grüner et al. *(86)* showed that water can pass through the skin of the corpse and accumulate in muscular and subcutaneous fatty tissue. In blood samples collected from the femoral vein, however, the water content was not increased. Thus, according to these authors, in case of death by drowning, a substantial dilution cannot generally be assumed. According to Paltauf *(87)*, Wachholz *(88)*, and Mueller *(89)*, water accumulation in drowned bodies primarily occurs during the process of drowning. Studies published by Völpel *(90)*, Mijnlieff *(91)*, and Mueller and Gorgs *(92)* clearly showed that during classic cases of drowning large amounts of liquid enter the body leading to

decreasing BAC values. This was also demonstrated by Schweitzer *(93)* in animal experiments.

According to investigations by Baierl *(94)*, in addition to liquid transfer through the skin, an increase in fluid in the subcutaneous fatty tissue and in the muscles of the corpse occurs after drowning. Brettel and Emig *(95)*, however, ruled out a significant diffusion of alcohol through the intact skin of the corpse. A decrease in alcohol blood levels in corpses proportional to the postmortem interval in water was discussed by Simonin *(96)*. In animal drowning experiments, Kubisch *(97)* found a decrease in BAC in femoral vein blood, and a significant decrease in cardiac blood (on average by 25%) in all cases.

In another animal study, Ameno and Nanikawa *(98)* found ethanol levels to be decreased following bleeding to death. The characteristics of the BAC curve in case of blood loss was also investigated in the studies of Forster *(99)*, Ditt and Schulze *(100,101)*, and Gumbel *(102)*. Brettel and Maske *(103)* analyzed the BAC curve characteristics on 20 surgical patients undergoing blood volume replacement while being in intensive care. These authors observed that the water content in whole blood is considerably more increased than that in serum. Thus, application of regular conversion factors favors probands because the divisor actually needed for converting serum into whole-blood values is often lower than 1.1. These findings have to be taken into account in cases of corpses that underwent surgery prior to death.

Using the table published by Brettel *(45)* for correction via determination of water content has proved successful in laboratory practice.

4.2. Storage of Samples

Another factor influencing results of alcohol determination is the storage of samples. This applies to both samples of blood from corpses and from living subjects *(69,104–107)*.

The most important factors are storage time and temperature *(108,109)*. Earlier studies on the evaporation effects in incompletely filled, sealed sample tubes *(110–113)* can be disregarded according to the results of various authors *(114–118)*. The latter studies are at least partially in contrast to findings reported by Unterdorfer and Umach *(119)* who stored 100 collection vessels filled to different levels for two days. Reanalysis of samples from vessels containing small amounts of blood showed an average loss of ethanol of 4.1% (compared with the first determination).

Obviously, changes in stored blood samples cannot always be avoided. Dick and Stone *(120)* detected decreasing ethanol levels in several blood samples stored for alcohol determination. Even without opening collection vessels,

a change in alcohol levels can occur within the first few days, ranging from a decrease by 0.008% to an increase by 0.07%, as demonstrated by Schwerd and Hillermeier *(121)*, who analyzed 287 stored blood samples. According to these authors, this is caused by catalase activity.

However, Somogyi et al. *(122)* did not observe significant changes in alcohol levels during a 12-week period in sealed tubes with high concentrations of ethanol (>0.19%) without sodium fluoride added or in open sample tubes with natrium fluoride (NaF) added.

Analyzing 194 blood samples to which fluoride had been added, Kisser *(123)* did not detect an increase in ethanol levels even after a storage period of 12 months. Sixteen percent of the control samples without fluoride (n = 50) showed alcohol neogenesis, amounting up to 0.032% (12% of initial concentration). In some of the samples, however, a loss of ethanol was observed.

Occasionally, a sample tube might not be sealed correctly, in which case significant evaporation effects occur, as was described by Weiler and Klöppel *(118)*. These authors identified temperature, sample surface evaporation area, and air circulation as the main influencing factors of alcohol loss in vials that had been stored unsealed. Within approx 1 week, the loss amounted to 50% at room temperature, whereas at 5°C, a loss of 20% was measured. In the first few days, the occurring loss was proportional to the evaporation area.

Sachs *(116)* explained the alcohol loss in stored blood samples by the fact that opening intervals of tubes were too long. Quite a number of authors attributed the decrease in alcohol in stored samples of blood from corpses to microbial activities and postmortem oxidation *(124–127)*.

Thus, the question relating to the extent of possible changes in stored blood samples and to what extent correction of the BAC values is possible has not been convincingly answered up to now. According to Somogyi et al. *(122)*, the measurable alcohol decrease in samples with an alcohol level in the range of 0.1% can be characterized by a linear function with defined increment. The formula given by the authors is: $BAC_{initial} = BAC_{neo}$ + (increment * number of weeks).

A deduction of 0.01% per 2 days—with a maximum of 0.1%—was recommended by Osterhaus and Johannsmeier *(128)* for blood from corpses. If exclusively ethanol is analyzed, the maximum amount to be deducted should be 0.05%. The forensic usability of blood samples stored unsealed was studied by Hausmann et al. *(129,130)*. In serial experiments at 4°C, a stringently linear decrease in ethanol concentration was found, which was dependent on the initial concentration. According to these studies, the initial ethanol concentration can be estimated using the formula: Y(0) = Y(t)*exp(t*).

Because no sufficiently precise data for supplementary correction of measured values is available, the only remaining option is to optimize the storage conditions of blood samples. Freezing or storing in the cold after addition of fluoride was recommended by Christopoulos et al. *(30)*. Blume and Lakatua *(131)* observed that addition of 1% sodium fluoride after autopsy inhibits most microbial activity (one exception being *Candida albicans*). At deep-freeze temperatures, alcohol level changes were also ruled out by Bonte and Hey *(7)*.

4.3. Glucose Levels in Blood

Glucose in blood from corpses must be regarded as a potential ignition spark for bacterial alcohol neogenesis. According to Merkel and Ausbüttel *(132)*, who determined glucose levels in blood from 158 recently deceased individuals, the highest postmortem blood glucose values are found in the portal vein, followed by right atrium, inferior caval vein, femoral vein, and in cerebrospinal fluid. Levels found in the last two samples mentioned are often below intravital values. The authors observed a slow decrease in glucose levels in corpses. These findings confirmed the results of Hill *(133)*, which were obtained from animal experiments. Further results were published by Tonge and Wannan *(134)* and Fekete and Kerenyi *(135)*.

Naumann *(136)* analyzed cerebrospinal fluid and blood from the right and left ventricle for glucose concentration in 170 corpses. He found both glycolysis and glycogenolysis to have taken place, the latter giving rise to an increase in glucose levels in the right ventricle. Altogether, Naumann considered the glucose level more stable in cerebrospinal fluid than in blood.

Routine use of quick tests for postmortem determination of glucose (e.g., Glucotest strips) helps avoid possible misinterpretations of alcohol analysis caused by increased blood glucose levels *(137)*.

The particular factors regarding urinary alcohol in diabetics, owing to glucose concentration, have been reported by Alexander et al. *(138)*, Kronert et al. *(139)*, and Saady et al. *(140)*. In addition, "endogenous" urinary alcohol may be induced by bacteria to a considerable extent in such cases *(123)*.

4.4. Postmortem Milieu

Postmortem decay that can be observed in human bodies can be divided into four groups of processes, which partly occur consecutively and partly run in parallel. These are (a) putrefaction, (b) decomposition, (c) autolysis, and (d) mummification. Detailed descriptions of early postmortem decomposition of the corpse are given by Laves *(142)*, Schmidt *(143)*, and Schwarzfischer *(144)*.

4.4.1. Putrefaction

Putrefaction is caused by microbial degradation and transformation reactions. The onset of putrefactive processes depends on the distribution and rate of bacterial growth *(145)*. Degradation of nitrogen-containing substances by bacterial enzymes results in the release of ammonia, biogenic amines, and ptomaines. After just a few hours postmortem, bacteria in the human body enter the internal organs through the intestinal wall (which becomes permeable). In addition, microorganisms from the external environment may invade the human body *(146)*. Altogether, this gives rise to the development of the so-called "putrefactive flora." In contrast to autolysis (in which decay processes are mainly catalyzed by endogenous enzymes), protein, carbohydrate, and fat degradation is brought about by bacterial enzymes.

Saccharolysis—mentioned here as an example—is of relevance in the degradation of connective tissues *(147)*. Other important enzymes in degradation and transformation processes in the corpse are structure-disintegrating enzymes, e.g., gelatinase, lecithinase, hyaluronidase *(148–157)*, elastase *(158)*, and collagenolytic bacterial activities *(159–164)*.

Application of antibiotics or other noxious agents that affect the bacterial flora suppresses or inhibits putrefaction *(165)*. Likewise, environmental conditions, such as temperature, humidity, and motion of air, exert considerable influence on the extent and rate of putrefaction *(166)*.

5. Alcohol Neogenesis by Bacteria and Fungi

For decades, it has been unanimously agreed that bacteria cause the formation of putrefactive alcohols. Weinig *(38)* reported an increase in alcohol levels in people who died of sepsis. Domenici *(167)* described the formation of reducing substances being dependent on temperature, time, and integrity of the skin. The origin of the bacteria involved need not be discussed in detail here—the intestinal tract of the corpse with its innumerable bacteria and the microorganisms ubiquitously present on the corpse and its environment form a sufficient reservoir.

Basic investigations, for the most focusing on pathology, showed bacteria spreading within the human corpse *(168)*, which is a prerequisite for diverse metabolic processes. Table 2 displays typical postmortem bacterial distribution in various areas of the corpse *(169)*.

Quite a number of ubiquitous microorganisms are capable of forming or degrading alcohol. In 1940, Still provided evidence for alcohol degradation by *Escherichia coli (170)*. Gale *(171,172)* analyzed ethanol formation in a variety of putrefactive bacteria, yeasts, and molds.

Table 2
Growth of Putrefactive Microorganisms in Human Corpses as Based on Examination of 20 Corpses

Case no.	Peritoneum	Iliac vein	Axillary vein	Cardiac blood
1	*Clostridium bifermentans*	—	—	—
2	*Proteus* sp.	*Proteus* sp.	*Proteus* sp.	*Proteus* sp.
	Clostridium perfringens	—	—	—
3	*C. perfringens*	*C. perfringens*	*C. perfringens*	*C. perfringens*
	Clostridium pseudotetanicum	—	—	—
4	*Clostridium septicum*	*C. septicum*	—	*C. septicum*
5	*C. bifermentans*		—	—
6	*C. bifermentans*	*C. bifermentans*	—	—
7	*Clostridium tertium*	*C. tertium*	—	—
8	*C. bifermentans*	—	—	—
9	*C. perfringens*	*C. perfringens*	*C. perfringens*	*C. perfringens*
10	*C. tertium*	*C. tertium*		*C. tertium*
11	*C. tertium*	*C. tertium*	*C. tertium*	*C. tertium*
	C. septicum	*C. septicum*	—	*C. septicum*
12	*C. bifermentans*	—	—	—
13	*C. septicum*	–	—	—
14	*Clostridium noyi*	*C. noyi*	*C. noyi*	*C. noyi*
15	*C. perfingens*	*C. perfingens*	*C. perfingens*	*C. perfingens*
		C. tertium	—	— —
16	*C. perfringens*	*C. perfringens*	*C. perfringens*	*C. perfringens*
		C. septicum	—	— —
17	*C. perfringens*	*C. perfringens*	*C. perfringens*	*C. perfringens*
		C. bifermentans	—	— —
18	*C. tertium*	—	—	—
19	*C. perfringens*	—	—	*C. perfringens*
20	*C. perfringens*			
		C. perfringens	*C. perfringens*	*C. perfringens*

Adapted from ref. *169*.

Postmortem alcohol formation was attributed to microorganisms, such as *C. albicans*, *Saccharomyces* sp. and bacteria, e.g., *E. coli* and *Klebsiella* sp., by Gormsen *(173,174)*. Analyzing alcoholic dilutions of serum or distilled water, respectively, that had been inoculated with *Penicillium* sp., *C. albicans*, and *Aspergillus niger*, and stored at +4°C for more than 11 weeks, Gehm and Schmid *(124)* observed a strong decrease in alcohol concentration.

Chang and Kollman *(175)* inoculated human blood with *C. albicans* under different temperature conditions. Ethanol formation was analyzed over a period of 6 months. At 6°C, traces of ethanol could not be detected until after 182 days of incubation. At higher temperatures (22 and 37°C) concentrations of up to 0.05% were observed and ethanol formation commenced after 5 days.

Dick and Stone *(121)* detected decreasing ethanol levels in several blood samples stored for alcohol determination. *Serratia marescens* and *Pseudomonas* sp. could be isolated from these samples. As demonstrated in laboratory experiments, these microorganisms even grew and degraded alcohol in human blood to which 1% sodium fluoride had been added. Ethanol degradation seemed to be independent of temperature in *Pseudomonas* sp., whereas it occurred in *S. marescens* only at room temperature and not at 4°C.

Analyzing samples from 130 putrefied corpses, Zumwalt et al. *(176)* isolated *Proteus morganii*, *Clostridium* sp., and anaerobic streptococci.

Analyses carried out by Joachim et al. *(177)* using *Saccharomyces cerevisiae* showed ethanol levels in blood to be dependent on the microorganism count. Peak values were measured after 48 hours and, with decreasing ethanol levels, an increase in putrefactive alcohols was observed. According to the authors, most yeast species are incapable of forming ethanol, e.g., utilizing glucose, at 2 to 4°C.

Clark and Jones *(178)* analyzed samples of cardiac blood and vitreous humor that were stored at 4°C and were collected from 26 corpses with postmortem intervals of up to 28 hours. In 13 cases, microorganisms could be isolated from the blood samples. All ethanol levels were below 0.01% (blood glucose being significantly above 200 mg/dL in 7 instances). Based on these findings the authors ruled out (bacterial) ethanol neogenesis within short time intervals in corpses that were stored under cool conditions.

Davis et al. *(11)* compared putrefaction of normal and microorganism-free laboratory mice. Because ethanol formation could not be detected in microorganism-free laboratory animals, the authors attributed postmortem neogenesis to the intestinal flora. They were also of the opinion that storage at 4°C could inhibit bacterial growth for more than 50 hours.

Plueckhahn et al. *(33)* regarded ethanol concentrations measured up to 48 hours postmortem as unaltered, provided that storage and sampling were carried out properly. Within this period, they also consider cardiac blood to be usable. By contrast, improper storage might result in alcohol neogenesis of more than 0.2%. In their studies on blood from corpses, these authors isolated various *E. coli* strains, *Pseudomonas* sp., *Pullularia* sp., and *C. albicans*. Incubation of fresh blood samples inoculated with these microorganisms yielded an alcohol neogenesis of above 0.2%.

In another study on blood from corpses, Plueckhahn and Path *(179)* isolated *E. coli*, *Streptococcus faecalis*, *Klebsiella-Aerobacter* strains, *Proteus* sp., *C. albicans*, and *Staphylococcus* sp. Depending on the glucose concentration of the culture medium, ethanol concentrations of up to 0.212% (in *C. albicans*) were produced by these microorganisms. Glucose measurements showed that postmortem increase of glucose is highest in the right ventricle owing to agonal glycolysis in the liver. Femoral vein blood from corpses contained similar concentrations as that from living subjects.

Bonnichsen and Lundgren *(180)* described four autopsy cases in which they concluded postmortem ethanol formation because of the peculiar ethanol distribution in the corpses. The microorganisms isolated were *C. albicans*, *Saccharomyces* sp., unidentified Gram-positive and Gram-negative bacteria, *E. coli*, *Klebsiella* sp., *Candida welchii*, and *S. faecalis*.

In her survey on ethanol-producing microorganisms, Corry *(1)* detailed 58 bacterial, 17 yeast, and 24 mold species. The author regarded a body temperature of 5°C to be the lower limit for microbial spread within the corpse. *E. coli* was considered the most important ethanol-producing germ. In contrast, Gormsen *(174)* believed the fungus *C. albicans* to be of major importance in ethanol neogenesis. Table 3 summarizes the microorganisms isolated and identified in connection with studies on alcohol neogenesis in putrefaction.

5.1. Substrates of Bacterial Ethanol Neogenesis

In the strictest sense of the word, bacterial fermentation can be regarded as the anaerobic breakdown of carbohydrates by bacteria. With respect to type and concentration of the end products, this process is much more diversified than pure alcoholic fermentation by yeasts.

Therefore, ethanol is only a byproduct of bacterial decomposition processes. The reactions that take place are mainly influenced by temperature, pH value, concentrations of available carbohydrates, and the presence of other utilizable nutrients.

For alcohol formation solely from carbohydrates, several metabolic pathways have been described *(183–187)*. One pathway is analogous to yeast fermentation in that it proceeds via phosphorylation of glucose to glucose-6-phosphate, rearrangement to fructose-6-phosphate, and further phosphorylation to fructose-1,6-bisphosphate. Subsequent reaction steps are:

fructose-1,6-bisphosphate ⇒ glyceraldehyde-3-phosphate ⇒ 3-phosphoglycerate ⇒ 2-phosphoglycerate ⇒ phosphoenolpyruvate ⇒ pyruvate ⇒ acetaldehyde

The reaction acetaldehyde + glyceraldehyde-3-phosphate + H_2O then results in the formation of 3-phosphoglycerate + ethanol.

Table 3
Alcohol Neogenesis in Bacteria Isolated From Human Corpses

Microorganisms	Reference
Citrobacter freundii	Clark and Jones 1982 (178)
Clostridium sp.	Zumwalt et al. 1982 (176)
C. sordellii	Daldrup and Huckenbeck 1984 (181)
C. welchii	Bonnichsen et al. 1953 (106)
Enterococcus sp.	Clark and Jones 1982 (178)
Enterobacter cloacae	Clark and Jones 1982 (178)
Escherichia coli	Clark and Jones 1982 (179), Still 1940 (170), Gormsen 1954 (173, 174), Plueckhahn et al. 1967 (33), Bonnichsen and Lundgren 1953 (180)
Klebsiella sp.	Gormsen 1954 (173), Bonnichsen and Lundgren 1953 (180)
Klebsiella pneumonia	Clark and Jones 1982 (178)
Penicillium sp.	Gehm and Schmidt 1962 (124)
Proteus morganii	Zumwalt et al. 1982 (176)
Pseudomonas sp.	Dick and Stone 1987 (120), Plueckhahn et al. 1967 (33)
Pullularia sp.	Plueckhahn et al. 1967 (34)
Serratia marcescens	Clark and Jones 1982 (178), Dick and Stone 1987 (120)
Stapylococcus epidermidis	Clark and Jones 1982 (178)
Staphylococcus aureus	Clark and Jones 1982 (178)
Streptococcus sp.	Zumwalt et al. 1982 (176)
Streptococci (α-hemolysing)	Clark and Jones 1982 (178)
Streptococci (β-hemolysing)	Clark and Jones 1982 (178)
Streptococcus faecalis	Bonnichsen and Lundgren 1953 (180)
Aspergillus niger	Gehm and Schmidt 1962 (124)
Candida albicans	Gehm and Schmidt 1962 (124), Plueckhahn et al. 1967 (33), Bonnichsen and Lundgren 1953 (180)
Saccharomyces sp.	Gormsen 1954 (173, 174), Joachim et al. 1975 (177), Bonnichsen and Lundgren 1953 (180)

Another possible pathway is the following chain of reactions:

glucose ⇒ hexose-phosphate ⇒ bisoxyacetone-phosphate + glyceraldehyde-3-phosphate

Both products are then further metabolized:

bisoxyacetone-phosphate ⇒ α-glycerophosphate ⇒ ethanol + formate

glyceraldehyde-3-phosphate ⇒ 3-phosphoglycerate ⇒ pyruvate

A third possibility would be an anaerobic glucose-6-phosphate dehydrogenase shunt (so-called "Zwischenferment" shunt) that catalyzes the conversion of glucose-6-phosphate into CO_2 and phosphorylated pentose after prior oxidation to phosphohexonic acid. The pentose molecule could then disintegrate into C_2 and C_3 compounds. The two-carbon compound could then be the precursor for one ethanol molecule; the three-carbon compound could be converted into pyruvate, from which another molecule of ethanol could be generated.

Another possible pathway leading to ethanol formation would be the reduction of acetic acid to ethanol.

5.2. Contribution of Possible Neogenesis to Ethanol Levels

Medicolegal problems usually pertain to the actual ethanol level in blood before death. Thus, the overriding question for many groups of researchers was to what extent ethanol is produced during putrefaction.

Using blood from corpses, Schwerd *(188)* found increases in alcohol concentrations of more than 0.1% in samples kept under anaerobic storage conditions, whereas the increase amounted to only 0.048% if blood samples were accessible to air.

Using the alcohol dehydrogenase (ADH) assay method, Paulus and Janitzki *(189)* measured alcohol levels of up to 0.029% in blood samples from 29 of a total of 124 corpses examined, for which premortem alcohol uptake could be ruled out.

Weinig and Lautenbach *(190)* also estimated the possible neogenesis of ethanol to be as high as 0.1% (from 6 days and onward between death and date of determination).

Based on results from animal experiments, Mebs and May *(191)* concluded that ethanol neogenesis in blood from corpses can amount to 0.1%. Gilliland and Bost *(192)* were of the opinion that putrefactive ethanol can reach levels of up to 0.15%.

A postmortem ethanol formation of more than 0.15% has been described by Canfield et al. *(9)* in two airplane crash victims.

According to studies of this author, results on bacterial alcohol formation found in in vitro experiments using laboratory culture media cannot be applied to human blood *(193–200)*. Inoculating human blood with various anaerobic microorganisms, Gabriel and Huckenbeck *(201)* observed maximum ethanol concentrations of 0.15%, whereas more than 0.3% could be measured in culture solution. The quantity and quality of the formation of putrefactive alcohols was found to vary significantly. Table 4 gives an overview of different bacterial strains *(78)*.

Table 4
Neogenesis of Putrefactive Alcohols and Derivatives in Bacteria

	Acetaldehyde	Methanol	Acetone	Ethanol	2-Propanol	Ethyl methyl ketone	1-Propanol	Allyl alcohol	2-Butanol	Isobutanol	3-Methyl-2-butanol	1-Butanol	2-Methyl-1-Butanol	3-Methyl-1-Butanol	1-Pentanol
Clostridium species															
C. absonum	•		•	•	•		•					•	•	•	
C. acidiurici	•		•	•								•			
C. aminovalericum	•		•	•	•		•			•		•			
C. aurantibutyricum			•	•								•			
C. barkeri			•	•			•					•			
C. beijerinckii			•	•								•			
C. bifermentans	•		•	•			•			•		•			
C. bryantii			•	•											
C. butyricum	•			•			•					•			
C. cadaveris	•		•	•								•	•	•	
C. celatum	•		•	•			•			•		•	•	•	
C. cellulovorans	•		•	•			•					•			
C. clostridiforme	•		•	•								•	•		
C. cochlearium	•			•						•					
C. cocleatum			•	•			•							•	
C. difficile	•		•	•	•					•		•			
C. fallax	•		•	•											
C. glycolicum	•		•	•	•		•			•		•	•	•	
C. indolis	•		•	•								•		•	
C. innocuum	•		•	•	•		•								
C. irregularis	•		•	•	•		•			•		•	•	•	
C. lortetii	•		•	•	•										
C. magnum				•	•					•		•			
C. oceanicum	•		•	•			•					•		•	
C. oroticum	•		•	•	•					•		•			
C. papyrosolvens			•	•								•			
C. paraperfringens	•		•	•						•		•			
C. paraputrificum	•		•	•								•			
C. perfringens	•		•	•	•		•			•		•	•	•	
C. pfennigii	•		•	•											
C. polysaccharolyticum	•		•	•											
C. propionicum	•		•		•										

Table 4
(continued)

	Acetaldehyde	Methanol	Acetone	Ethanol	2-Propanol	Ethyl methyl ketone	1-Propanol	Allyl alcohol	2-Butanol	Isobutanol	3-Methyl-2-butanol	1-Butanol	2-Methyl-1-Butanol	3-Methyl-1-Butanol	1-Pentanol
Clostridium species															
C. puniceum	•		•	•								•			
C. purinolyticum	•		•				•						•		
C. quercicolum	•		•	•			•								
C. ramosum	•			•						•		•			
C. rectum	•		•	•				•		•		•			
C. saccharolyticum			•	•											
C. sardiniensis			•	•								•			
C. sordellii	•		•	•	•		•			•		•	•	•	
C. sporogenes	•		•	•	•		•		•	•		•	•	•	•
C. sticklandii	•		•	•	•					•		•			
C. subterminale	•		•	•	•		•			•		•	•	•	
C. symbiosum	•		•	•	•							•			
C. tertium				•											
C. tetanomorphum	•		•	•	•		•					•			
C. thermoaceticum	•		•		•										
C. thermodihydro-sulfuricum	•		•	•	•					•					
C. thermolacticum	•			•											
C. thermosulfurogenes			•	•											
C. tyrobutyricum	•		•	•											
C. villosum	•		•	•	•		•			•					
Proteus species/strains	•		•	•											
P. vulgaris DSM 2140	•		•	•											
P. vulgaris DSM 30118	•		•	•											
P. vulgaris DSM 30115	•		•	•			•							•	
P. vulgaris DSM 30119	•		•	•											
P. morganii DSM 30117	•		•	•			•								
P. morganii DSM 30164	•		•	•			•							•	
P. rettgeri	•		•	•											
P. mirabilis DSM 30115	•		•	•										•	
P. mirabilis DSM 30116	•		•	•			•							•	

(Table continues)

Table 4 (continued)

	Acetaldehyde	Methanol	Acetone	Ethanol	2-Propanol	Ethyl methyl ketone	1-Propanol	Allyl alcohol	2-Butanol	Isobutanol	3-Methyl-2-butanol	1-Butanol	2-Methyl-1-Butanol	3-Methyl-1-Butanol	1-Pentanol
P. inconstans	•		•	•										•	
Streptococcus species															
Streptococcus faecalis	•		•							•		•			
Streptococcus oralis	•		•												
Streptococcus faecium															
Streptococcus salivarius															
Streptococcus sp.	•		•	•	•		•					•			
Streptococcus sp.	•		•	•			•								
Streptococcus sp.	•		•	•			•								
Streptococcus sp.	•		•	•			•					•			
Staphylococcus species															
Staphylococcus xylosus	•		•	•			•					•			
Staphylococococcus aureus	•		•	•						•		•			
Staphylococcus haemolyticus	•		•	•											
Staphylococcus epidermidis	•		•	•	•		•								
Staphylococcus sp.	•		•	•			•								
Pepto-/Peptostreptococcus species															
Peptostrep. anaerobius	•		•												
Peptostrep. micris	•		•		•										
Peptococcus asaccharolyticus	•		•		•										
Peptococcus magnus	•						•								
Peptococcus prevotii	•			•			•								
Escherichia coli/strains															
E. coli DSM 9708	•		•	•											
E. coli DSM 9007	•		•	•											

Table 4 (continued)

	Acetaldehyde	Methanol	Acetone	Ethanol	2-Propanol	Ethyl methyl ketone	1-Propanol	Allyl alcohol	2-Butanol	Isobutanol	3-Methyl-2-butanol	1-Butanol	2-Methyl-1-Butanol	3-Methyl-1-Butanol	1-Pentanol
Clostridium species															
E. coli DSM 2607	•		•	•	•					•		•			
E. coli DSM 206	•		•	•			•			•		•			
Other microorganisms															
Bifidobacterium infantis	•			•	•										
Bifidobacterium adolescentis	•				•		•								
Lactobacillus animalis	•														
Lactobacillus acidophilus	•														
Lactobacillus fermentum	•		•		•										
Lactobacillus plantarum	•														
Eubacterium limosum	•			•			•					•			
Eubacterium lentum	•			•	•		•								
Fusobacterium nucleatum					•		•					•			
Ruminococcus albus	•		•				•								
Pseudomonas aerogenosa	•		•	•								•		•	

Adapted from ref. 2.

5.3. Time Course of Ethanol Neogenesis

In addition to the level of possible postmortem alcohol concentrations, the temporal sequence of metabolic reactions is of interest. Several—mostly German—research groups focused on this topic.

In 1936, Wagner *(202)* determined blood alcohol levels in 18 corpses over a period of 4 days. He used the method of Widmark and found that on average,

the alcohol level in blood from human corpses decreases by 5 to 6% in the first 2 days and by 20 to 25% up until the fourth day after death.

According to Lautenbach *(203)* the predominating compound of alcohol formation in anaerobic putrefaction is ethanol. Additional formation of 1-propanol is also observed; other alcohols occur only in trace amounts. At temperatures above 20°C, ethanol concentrations of 0.02 to 0.05%—or even above 0.1% under hyperglycemic conditions—could be produced. However, in aerobic putrefaction, other higher alcohols, such as 1-butanol, 1-propanol etc., would be formed from the start of putrefaction. Also under these conditions, ethanol levels of approx 0.1% may be present. The degradation of all alcohols, however, is assumed to commence within a few days *(204)*.

In animal experiments, Mebs and May *(15)* and Mebs et al. *(5)* analyzed postmortem formation of ethanol, methanol, acetone, and higher alcohols. Based on their results, the authors concluded that the occasional high concentrations of higher alcohols that had been described by other authors *(3,6,190,204,205)* are evidently only formed in stored blood samples.

In rat experiments, Winek et al. *(68)* observed that ethanol concentration peaked after 7 days and subsequently declined to zero by day 21. At 3.5°C, ethanol formation was not detected. Studies on putrefying rabbits reported by Takayasu et al. *(206)* found increasing ethanol and 1-propanol levels after 2.5 days.

The evidence for a slight decrease in alcohol levels during the initial hours after death as published by Wagner *(202)* was analyzed and confirmed by investigations on human corpses by Hansen *(207)*, who collected blood samples in duplicate. At a low storage temperature, ethanol neogenesis within the first 24 hours was not observed.

Using different bacterial strains, the Düsseldorf research group found large variations of measured values for individual putrefactive alcohols. This is shown for *Candida irregularis* in an anaerobically incubated culture solution in Fig. 4. Earlier findings of Osterhaus *(204)* on the subsequent decrease in ethanol concentration were confirmed by these experiments.

As mentioned earlier, results from test-tube experiments using culture solution cannot easily be transferred to the conditions found in human blood. Incubation of contaminated blood samples results in alcohol neogenesis, occasionally followed by a slight concentration decrease after 3 to 4 days. Thereafter, ethanol concentration remains more or less constant. This is shown for a selection of clostridia strains in Fig. 5; obviously, a “steady-state” is reached.

When assessing alcohol determinations in blood from corpses, the question as to whether ethanol neogenesis depends on ethanol concentrations that are present antemortem is relevant. According to Weinig and Lautenbach *(190)*

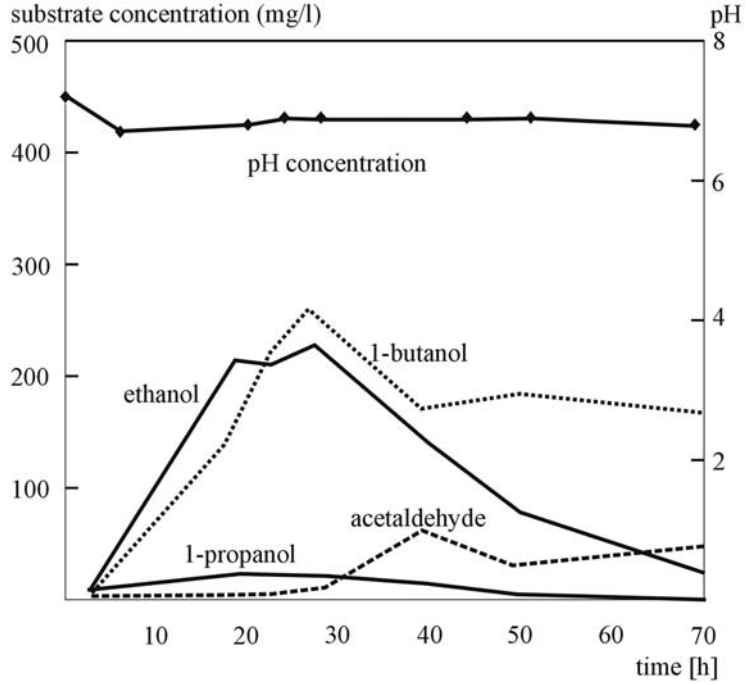

Fig. 4. Increase and decrease of putrefactive alcohols in *Clostridium irregularis* (according to ref. *2*).

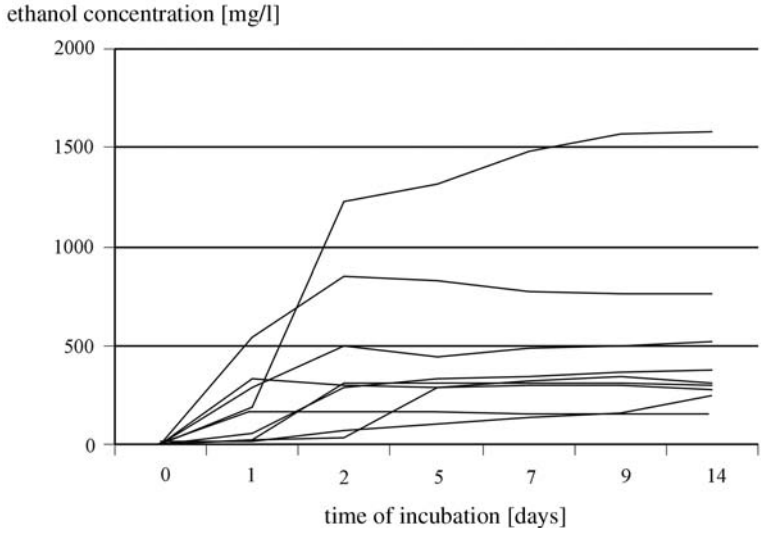

Fig. 5. Ethanol concentrations in different stains of *Clostridia* sp., grown in human blood (according to ref. *2*).

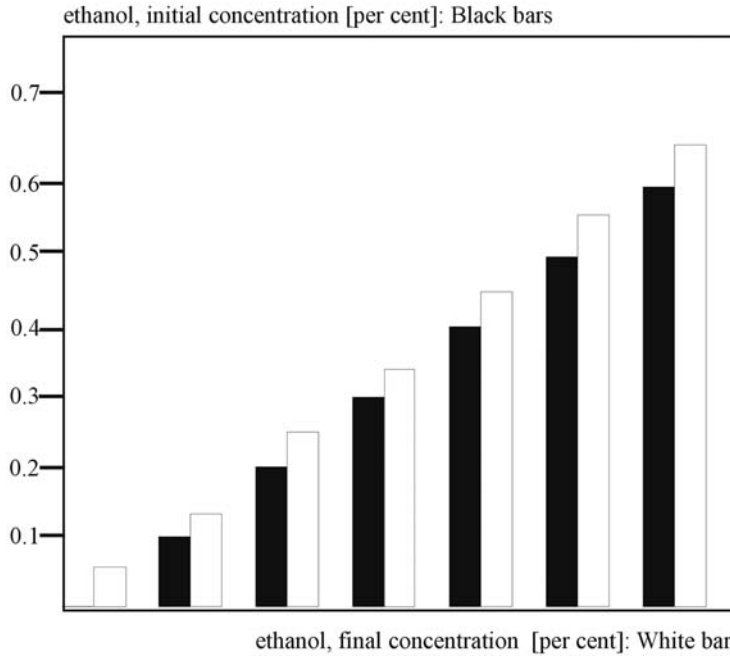

Fig. 6. Dependency of ethanol formation in *Clostridium irregulare* on initial ethanol concentration in the culture medium; incubation: 48 hours at 37°C. Black bars: ethanol concentration before incubation. (From ref. *2*.)

alcohol neogenesis is entirely independent of the initial concentration. This was confirmed by Huckenbeck *(2)* for a number of putrefactive bacteria; an example is given in Fig. 6.

6. Markers of Ethanol Neogenesis

Even though the putrefactive state can be assessed by inspecting the corpse, in some instances, significant differences between putrefactive changes of the outer surface of the body and internal organs can be noted. It is difficult to clearly define on a macroscopic scale the transitional stages of improperly stored or contaminated blood samples, up to obvious putrefaction. Moreover, the question whether the degree of putrefaction necessarily correlates with alcohol neogenesis has arisen. In this respect, several approaches were tested in order to find substances that are produced in parallel to putrefactive ethanol. The majority of studies focused on other (higher) putrefactive alcohols and their derivatives.

Table 5
Selection of Putrefactive Bacteria That Simultaneously Produce Ethanol, α-Aminobutyric Acid, γ-Aminobutyric Acid, and δ-Aminovaleric Acid

Bacterial species/strain	Ethanol	ABA	GABA	δ-AVA
Clostridium absonum	•		•	
Clostridium aminovalericum	•		•	•
Clostridium bifermentans	•	•	•	•
Clostridium glycolicum	•	•	•	•
Clostridium irregularis	•	•	•	•
Clostridium sordellii	•	•	•	•
Clostridium sporogenes	•	•	•	•
Clostridium sticklandii	•	•		•
Clostridium subterminale	•	•	•	•
Proteus vulgaris DSM 2140	•		•	
Proteus vulgaris DSM 30118	•		•	
Proteus vulgaris DSM 30115	•			
Proteus vulgaris DSM 30119	•		•	

ABA, α-aminobutyric acid; GABA, γ-aminobutyric acid; δ-AVA, δ-aminovaleric acid. (Modified from ref. 2.)

A completely different approach in the search for simultaneously produced marker substances of putrefaction is the detection of other bacterial metabolites—because putrefaction is the result of bacterial processes. Of course, in this case it has to be ensured that ethanol is actually produced by the bacteria identified by this indirect method.

This approach was again followed by the Düsseldorf research group *(2,194–198,201,208,209)*. In fermentation experiments with putrefactive bacteria it was shown that bacteria that produced the highest ethanol concentrations, usually clostridia and *Proteus* sp., are characterized by a specific fermentation pattern of amino acids, e.g., the production of α-aminobutyric acid, γ-aminobutyric acid, and/or δ-aminovaleric acid. The findings for some putrefactive microorganisms are given in Table 5. The authors recommend amino acid analysis of blood samples in case of doubt. This analytical method offers the advantage that presence of putrefaction can be determined by congener analysis, in which fusel oils are discriminated from putrefactive alcohols. If positive, the sample being tested can definitely be excluded from the evaluation. However, a quantitative correlation between putrefactive alcohols produced and putrefactive amino acids could not be established.

7. Alternative Sample Materials

Another approach utilized in order to clarify the issue of putrefactive ethanol in corpses is the simultaneous analysis of alternative sample material.

Because of uncertainties that occasionally occur during determination of alcohol levels in blood from corpses, several research groups have tried to develop alternative approaches *(210–212)*.

Bilzer and Kühnholz *(213,214)* recommended analyzing several substrates in order to assess whether the time of death fell into the initial perfusion or elimination phase.

Backer et al. *(215)* analyzed alcohol concentrations in vitreous humor, brain tissue, gallbladder fluid, spinal fluid, and urine and tried to correlate the concentrations to the BACs measured. The authors noted that conclusions with regard to actual BACs are subject to large margins of error.

Studies on blood, muscles, and inner ear fluid from 37 human corpses in an advanced state of putrefaction were carried out by Trela *(216)* and Trela and Bogusz *(217)*, who tried to determine correlation factors for BAC. In cases of advanced putrefaction and suspected putrefactive ethanol levels in blood, these authors recommended regarding ethanol concentrations above 0.1% found in inner ear fluid and/or muscle tissue as an indicator of alcohol consumption prior to death.

Gilliland and Bost *(192)* retrospectively examined 286 autopsies (covering all stages of putrefaction) for ethanol in various body fluids. Findings were classified as "postmortem ethanol formation" if ethanol was detected in more than one of the various body fluids analyzed, if ethanol distribution in various body fluids was found to be unusual, if relevant findings were present in the case history, and/or if higher alcohols were present in the body fluids.

Canfield et al. *(9)* examined 975 casualties from airplane crashes. BACs above 0.04% were detected in 79 corpses, of which 21 cases were classified as postmortem ethanol formation and 22 as antemortem ethanol uptake. The remaining 26 cases could not be classified. Assessment was based on alcohol determination in urine, vitreous humor, and organs.

Analyzing samples from 377 plane crash casualties, Kuhlman et al. *(4)* found the following values to be typical of samples showing "postmortem ethanol formation:" blood (mg/dL)—acetaldehyde less than 5, ethanol = 56, 1-propanol = 6; urine (mg/dL)—acetaldehyde less than 5, ethanol 5 or less, 1-propanol less than 5.

According to the studies of Weinig et al. *(218,219)* the previous recommendation given by several authors to use urinary alcohol concentrations (UAC) for assessing BAC values has to be doubted, because of the difficulty of obtaining information on alcohol dosage and micturition frequency.

As concluded by Trela *(216)*, the main problems in the assessment of ethanol concentrations in blood from corpses are water loss, autolysis, putrefaction, and postmortem glycogenolysis. His investigations included samples of blood, liquor, vitreous humor, perilymph, and urine from 160 fresh corpses, and urine and perilymph from 60 putrefied corpses. According to the author, the alcohol level in blood from putrefied corpses can be assumed to be 0.05% higher than that in perilymph.

Winek and Esposito *(64)* compared postmortem levels of alcohol in blood, bone marrow, vitreous humor, urine, and bile. The concentrations in blood and bone marrow were found to be significantly correlated (after correcting for lipids).

7.1. Determination of Vitreous Humor Alcohol Concentration

Rat experiments by Hentsch and Müller *(220)* showed that approx 3 hours after oral uptake alcohol levels start to decrease in blood and vitreous humor, albeit with different rates. The authors compared BAC and vitreous humor alcohol concentration (VHAC) following oral application of ethanol to test animals. Neglecting the water content, alcohol levels in blood were found to be roughly twice as high as those in vitreous humor. After 3 hours, levels slowly decreased in vitreous humor. According to the authors, sampling vitreous humor is preferable for alcohol determination in corpses because of the large distance to the stomach (hence the diffusional impact is low) and a higher resistance to putrefaction, at least on a temporal scale.

Analyzing blood and vitreous humor from 51 putrefying human corpses, Harper *(221)* found only a few bacteria and fungi in the vitreous humor samples. In contrast, more than 60% of blood samples were microbially contaminated to such an extent that they were classified as unacceptable for ethanol determination. Leahy et al. *(222)* and Leahy and Farber *(223)* described the high stability of glucose concentrations in vitreous humor.

The first analyses of alcohol concentrations in vitreous humor were carried out by Sturner and Coumbis *(224)*. Investigations by Leahy et al. *(222)*, Felby and Olsen *(225)*, and Coe and Sherman *(226)* followed. Further results with regard to alcohol determination in vitreous humor can be found in Norheim *(227)*, Raszeja et al. *(228)*, Jaklinska and Tomaszewska *(229)*, and Heumann and Pribilla *(230)*.

However, resistance of the vitreous body to putrefaction can only be assumed for a certain period of time. Zumwalt et al. *(176)* analyzed forensic samples from 130 putrefied corpses and recommended using vitreous humor as control sample. However, bacteriological tests of vitreous humor revealed positive findings in seven cases. *P. morganii*, *Clostridium* sp., and anaerobic streptococci could be identified.

Scott et al. *(231)* also recommended analyzing vitreous humor in cases of corpses that had already been embalmed. However, their results only allow for a rough assessment of BAC.

Alcohol determination in vitreous humor was also criticized by various researchers. Based on blood and VHACs in samples collected from 345 corpses, Pounder and Kuroda *(232)* found, in contrast to other researchers, vitreous humor to be of no practical use. Variability of measured values was found to be too high. Coe and Sherman *(226)* pointed out the differences in correlation factors between vitreous humor and blood that had been reported. Data published by Chao and Lo *(234)* also confirmed the uncertainties of the determination method. The BAC:VHAC ratio varied between 0.84 and 3.07 in samples collected from people who died during the early absorption phase. Therefore, the authors recommended using a correction factor of 1.29 for calculating BAC from VHAC in early absorption phase, and a factor of 0.89 in subsequent phases.

The relevance of alcohol determination in vitreous humor was investigated in a study by Caplan and Levine *(234)* on 347 corpses. BACs below 0.03% were often associated with VHAC values of zero. The presence of these low levels of blood alcohol was attributed to alcohol neogenesis. In virtually all cases, BAC values above 0.05% were associated with positive VHACs.

7.2. Determination of UAC

The opinion of Gormsen *(173,174)* and Sjövall and Widmark *(235)* that urine—even from corpses in a state of advanced putrefaction—could be used for BAC estimation, is regarded as having been disproved by the investigations of Joachim et al. *(177)*.

However, even before death many factors influence the UAC:BAC ratio and vice versa. Weinig et al. *(218,221)* analyzed alcohol concentrations in blood and urine collected from the urinary duct and urinary bladder of living subjects. They found that alcohol concentrations depended on the amount drunk, course of drinking, time of last micturition, residual urine amount, and course of diuresis. Because it is very unlikely that all these influencing factors are known, the authors concluded that it is impossible to derive precise BAC calculations from UACs. Further studies by Zink and Reinhardt *(236)* confirmed these findings.

Kaye and Cardona *(237)* also warned against drawing conclusions regarding BAC from UAC values. Too many influencing factors would have to be considered, such as the phase of alcohol uptake, degree of filling of the urinary bladder at the time of alcohol uptake, and micturition frequency.

Over a period of 3 days, Kissenkoetter *(238)* analyzed the course of ethanol concentration in urine from 14 corpses. In corpses with a positive BAC, an increase in UAC was observed in every case. The author, however, did not give an explanation for this finding.

The possibility of postmortem ethanol diffusion into the urinary bladder was investigated by Weinig and Schwerd, who regarded this as an unlikely phenomenon. *(239)*. A similar assessment was given by Hebold *(240)*. Based on experimental studies *(241–245)*, Weinig and Schwerd *(239)* estimated the permeability of the human urinary bladder to ethanol to be of minor importance. The same conclusions were drawn already in the 1930s and 1940s from a number of drinking experiments by Haggard et al. *(246–251)*. Years later, the permeability of the human urinary bladder to ethanol was reinvestigated by Weinig et al. *(218,219)*; in healthy subjects, the earlier findings could be confirmed, whereas permeability, and hence diffusion, was found to be increased considerably in some cases with inflammation of the wall of the urinary bladder.

After considering results of their own experiments with corpses, Kaye and Cardona *(237)* advised strongly against using UAC in forensic investigations.

7.3. Alcohol Determination in Gall Bladder Fluid

Because gall bladder fluid can easily be collected during autopsy, it is not surprising that several research groups have tried to establish a correlation with BAC; however, alcohol determination in gall bladder fluid is only recommended if additional evidence toward the underlying BAC needs to be obtained.

7.4. Alcohol Determination in Cerebrospinal Fluid

Hebold *(240)* concluded from his investigations on 68 corpses (postmortem interval up to 52 hours) that no alcohol is formed postmortem in cerebrospinal fluid. This finding is probably restricted to the postmortem interval because bacterial spread into the brain tissue and subsequent alcohol neogenesis in cerebrospinal fluid can occur within 72 hours postmortem as shown by Daldrup and Huckenbeck *(181)*. According to Gelbke and co-workers, it can generally be assumed that a diffusional equilibrium of alcohol between blood and cerebrospinal fluid has, in principle, been established at the time of death *(252,253)*.

7.5. Alcohol Determination in Testicle Tissue

Based on analyses of the correlation between alcohol levels in testicle tissue and blood in 633 cases, Piette et al. regarded this method as useful for postmortem intervals of up to 72 hours *(254)*.

7.6. Alcohol Determination in Brain Tissue

Krauland et al. *(255)* regarded brain and muscle tissue as the most suitable material for postmortem alcohol determination. Analyzing tissue samples from brain, muscles, spleen, lung, kidney, and liver, the authors measured alcohol concentrations that were usually lower than those observed in blood. Table 6 summarizes the data available in the literature. Findings of Forney et al. *(256)* relate to animal experiments (dogs) shortly after alcohol application.

7.7. Alcohol Determination in Synovial Fluid

Oshima et al. *(257)* analyzed synovial fluid from knee joints of 12 corpses for alcohol concentrations (synovial fluid alcohol concentration [SAC]) and compared these levels with that BAC and UAC. The following correlation factors were determined: BAC/SAC = 0.76 ± 0.12 (for values between 0.060 and 0.094%), and UAC/SAC = 1.03 ± 0.11 (for values between 0.090 and 0.121%). The authors recommend determining SAC for forensic purposes.

7.8. Alcohol Determination in Liver Tissue

Only a few research groups have focused on the ratio of BACs to alcohol concentrations in liver tissue. The number of cases investigated is not sufficient for a conclusive assessment, especially as the study of Jenkins et al. *(258)* is related to a comparison with alcohol levels in cardiac blood samples.

7.9. Alcohol Determination in Muscle Tissue

Determination of alcohol concentration in muscle tissue is certainly an interesting accessory assay. In this respect, Nanikawa et al. described a considerable resistance to putrefaction in muscle tissue *(259)*.

Because ethanol determination in muscle tissue is often the most reliable of applicable methods in cases of corpses with longer postmortem intervals or exsanguinated corpses, the procedure described by Iffland and Staak (*260*), which has become an accepted practice in Germany, is described here:

> Approximately 1 gram of tissue taken from the femoral extensor muscle is weighed in a 20-milliliter headspace tube. 3 milliliters double-distilled water is added to the muscle tissue, so that the tissue is completely submerged in water. The closed tube is incubated for a minimum of two hours at 60°C to ensure that equilibrium of alcohol levels between tissue and surrounding liquid is reached. It is then cooled down to room temperature, and centrifuged at 1,000 to 15,000 revolutions per minute. Using an adjustable pipette (e.g., Eppendorf) four aliquots of 200 microliters are taken, two for gaschromatographic and two for enzymatic alcohol determination. Standard ethanol

solutions of 0.02, 0.05, and 0.1% are used for calibration. For gaschromatographic determination of alcohol concentrations using the headspace technique, 200 microliters of the supernatant and calibration standards are measured. Then 200 microliters of a 0.04% solution of tertiary butanol are added as an internal standard. In order to measure the water content, the open headspace tubes used for alcohol determination and preparation of samples are weighed, incubated at 100–200°C in a drying oven subsequent to analyses, and weighed again. Alcohol concentrations are then calculated relative to the water content of the muscle tissue.

7.10. Alcohol Determination in Bone Marrow

Examining 42 corpses, Winek and Esposito *(64)* found an average ratio of BAC to bone marrow alcohol concentration of 1.94 ± 0.42.

7.11. Alcohol Determination in Other Organs

Handovsky et al. *(261)* analyzed ethanol distribution in 93 road casualties who were under the influence of alcohol and calculated correlation factors for tissue samples from heart muscle, spleen, and kidney.

7.12. Alcohol Determination in Stomach Contents

The importance of supplementary determination of alcohol concentrations in stomach contents has been discussed in connection with the issue of postmortem ethanol diffusion. In addition, based on investigations carried out on 21 corpses, Kühnholz and Bilzer *(262)* were of the opinion that when determining alcohol concentrations in stomach contents and other substrates from corpses it is often possible to discriminate between resorption and elimination phase postmortem.

The data in Table 6 includes further studies *(30,31,42,52,118,215, 216,222,224–227,231,233,234,237,252–254,256,261,263–276)* and gives an overview of ethanol determinations in various alternative other substances.

7.13. Alcohol Determination in Hematomas

Alcohol determination in hematomas is of special relevance in the medicolegal setting because it offers the opportunity to determine an alcohol concentration that can quite closely approach the actual blood alcohol level at the time the body was injured. This method is therefore applied to cases with longer survival times, e.g., after sustaining a subdural hematoma. A study of 43 corpses showed hematoma alcohol concentrations (HAC) above corresponding BACs in 21 cases. These findings could partially be used for assessing the time of death

Table 6
Ratio of Blood Alcohol Concentration to Alcohol Concentration in Various Alternative Materials

Ratio BAC/ alternative	Standard deviation	Number of cases	References
		Bile	
0.89	± 0.15	55	Handovsky et al. 1953 (ref. *261*)
0.7–1.04	—		Christopoulos et al. 1973 (ref. *30*)
0.86–0.99	—	42 (25)	Weiler and Klöppel 1976 (ref. *118*)
0.92	± 0.22	78	Stone and Rooney 1984 (ref. *263*)
0.9–1.4	—	350	Kraut 1995 (ref. *264*)
		Vitreous humor	
1.01	± 0.04	38	Sturner and Coumbis 1966 (ref. *224*)
0.92	± 0.03	20	Leahy et al. 1967 (ref. *272*)
0.75	± 0.05	27	Felby and Olsen 1969 (ref. *225*)
0.89	± 0.02	174	Coe and Sherman 1970 (ref. *226*)
0.87	—	20	Audrlicky and Pribilla 1971 (ref. *42*)
0.81–0.85	—	73	Norheim 1972 (ref. *227*)
0.91 ± 0.17	± 0.17	8	Scott et al. 1974 (ref. *231*)
0.85	—	40	Grochowska et al. 1974 (ref. *265*)
0.81	—	592	Gelbke et al. 1978 (ref. *266*)
0.95	—	110	Backer et al. 1980 (ref. *215*)
0.70	—	15	Budd 1982 (ref. *267*)
0.82 ± 0.05[a]1.07 ± 0.12[b]	—	43	Caughlin 1983 (ref. *268*)
0.66	—	75	Stone and Rooney 1984 (ref. *263*)
0.81 ± 0.16	—	75	Neil et al. 1985 (ref. *269*)
0.85	—	247	Trela 1989 (ref. *216*)
0.80	—	86	Yip and Shun 1990 (ref. *270*)
0.83	—	347	Caplan and Levine 1990 (ref. *234*)
0.84	—	115	Chao and Lo 1993 (ref. *233*)[c]
0.94	—	85	Chao and Lo 1993 (ref. *233*)[d]
		Brain tissue	
0.8	—	13	Forney et al. 1950 (ref. *256*)[e]
1.24	—	13	Forney et al. 1950 (ref. *256*)[f]
1.48	± 0.35	48	Handovsky et al. 1953 (ref. *261*)
0.8	± 0.4	25	Moore et al. 1997 (ref. *271*)[g]
0.7	± 0.4	25	Moore et al. 1997 (ref. *271*)[h]
		Testicle tissue	
1.18	± 0.33	43	Handovsky et al. 1953 (ref. *261*)
0.92	—	633	Piette et al. 1982 (ref. *254*)

Table 6 (Continued)

Ratio BAC/ alternative	Standard deviation	Number of cases	References
		Cerebrospinal fluid	
0.79	± 0.20	25	Handowsky et al. 1953 (ref. *261*)
0.84	—	22	Hebold 1959 (ref. *240*)
0.75	—	25	Van Hecke et al. 1951 (ref. *272*)
0.80	—	30	Maresch and Maurer 1962 (ref. *273*)
0.78	—	21	Schleyer 1962 (ref. *274*)
0.6–1.1	—		Christopoulos et al. 1973 (ref. *30*)
0.80	—	77	Prokop et al. 1974 (ref. *275*)
0.74	—	509	Gelbke et al. 1978 (refs. *252, 253*)
		Urine	
0.71	± 0.17	80	Handovsky et al. 1953 (ref. *261*)
0.72	—	148	Kaye and Cardona 1969 (ref. *237*)
0.79	± 0.20	42	Stone and Rooney 1984 (ref. *263*)
0.71	± 0.16	75	Neil et al. 1985 (ref. *269*)
0.5–0.7[i]	—	245	Mittmeyer and Blattert 1991 (ref. *276*)
0.5–1.0	—	230	Kraut 1995 (ref. *264*)
		Muscle	
1.28	± 0.31	57	Handovsky et al. 1953 (ref. *261*)
1.48	—	8	Garriott 1988[j] (ref. *31*)
0.94	—	19	Garriott 1988[k] (ref. *31*)
		Heart muscle	
1.30	± 0.25	51	Handovsky et al. 1953 (ref. *261*)
		Spleen	
1.50	± 0.32	33	Handovsky et al. 1953 (ref. *261*)
		Kidney	
1.32	± 0.27	60	Handovsky et al. 1953 (ref. *261*)

[a]Elimination phase.
[b]Absorption phase.
[c]With internal and external injuries.
[d]Without injuries.
[e]Saphenous vein (animal exp.).
[f]Cardiac blood (animal exp.).
[g]Occipital brain.
[h]Cerebellum.
[i]Depending on blood alcohol concentration (BAC).
[j]BAC below 0.1%.
[k]BAC above 0.1%.

Table 7
Blood Alcohol Concentration Compared With Alcohol Concentrations in Hematomas and Urine in 21 Corpses With Subdural Hematomas

Case no.	BAC	HAC	UAC	Case no.	BAC	HAC	UAC
1	0.01	0.05	0.09	12	0.21	0.26	0.33
2	0.11	1.30		13	0.00	0.09	
3	0.04	0.11	0.19	14	0.00	0.09	
4	0.08	0.13		15	0.23	0.26	0.33
5	0.02	0.07		16	0.00	0.15	0.19
6	0.02	0.07		17	0.22	0.27	0.64
7	0.06	0.07	1.30	18	0.01	0.21	
8	0.07	0.22	0.21	19	0.00	0.15	0.00
9	0.04	0.10	0.12	20	0.16	0.20	0.29
10	0.02	0.06		21	0.04	0.06	
11	0.00	0.01					

BAC, blood alcohol concentration; HAC, hematoma alcohol concentration; UAC, urine alcohol concentration. (Modified from ref. *277*.)

(277). Further considerations with regard to pharmacokinetics are discussed elsewhere *(278)*. Actual BAC at the time of an event may be "preserved" in a hematoma. Comparing HAC, UAC, and BAC sometimes gives reasonable assessments of resorption or elimination phase in a given case. Exemplary cases are given in Table 7.

8. Conclusions

The interpretation of postmortem alcohol levels is still problematic in the forensic pathological setting and in the field of legal medicine. The same uncertainty applies to alcohol levels in blood samples that are improperly stored or are contaminated with bacteria or fungi. Whereas advanced putrefaction of a blood sample can be recognized macroscopically and by its odor, the transitional phase into putrefaction presents difficulties. Certain bacteria, which can be recognized by their synthesis of putrefactive amino acids (e.g., production of α-aminobutyric acid, γ-aminobutyric acid, and/or δ-aminovaleric acid), play an important role in the postmortem neogenesis of alcohol. In case of uncertainty, an amino acid analysis can confirm suspected putrefaction.

Congener analysis can identify putrefactive alcohols. However, a discrimination of such alcohols from higher alcohols originating from alcoholic beverages consumed antemortem may be problematic. The postmortem identification of methanol is a definite indication of alcohol consumption before death.

Blood from a corpse should ideally be collected from the femoral vein. In cases of abdominal injury, it is recommended that blood is collected from the subclavian vein. In all cases where putrefactive changes are suspected, several tissues and body fluids should be collected. It has been shown that thigh muscles are especially resistant to putrefaction and are therefore well-suited for postmortem alcohol determination.

An important substrate for ethanol neogenesis is glucose. In the case of diabetics, a more significant level of error must therefore be taken into account. In this respect, a subject's medical history and also measurement of the blood glucose level as early as possible during the autopsy can provide important information.

The total contribution of microbially induced postmortem alcohol neogenesis to the blood alcohol level in a corpse is generally not more than 0.15%, regardless of the blood alcohol level at death.

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10

Agrochemical Poisoning

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CONTENTS

SUMMARY

A general increase in the use of chemicals in agriculture has brought about a concomitant increase in the incidence of agrochemical poisoning. Organophosphates are the most common agrochemical poisons followed closely by herbicides. Many agricultural poisons, such as parathion and paraquat are now mixed with a coloring agent such as indigocarmine to prevent their use criminally. In addition, paraquat is fortified with a "stenching" agent. Organochlorines have an entirely different mechanism of action. Whereas organophosphates have an anticholinesterase activity, organochlorines act on nerve cells interfering with the transmission of impulses through them. A

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kerosene-like smell also emanates from death due to organochlorines. The diagnosis lies in the chemical identification of organochlorines in the stomach contents or viscera. Organochlorines also resist putrefaction and can be detected long after death. Paraquat has been involved in suicidal, accidental, and homicidal poisonings. It is mildly corrosive and ulceration around lips and mouth is common in this poisoning. However, the hallmark of paraquat poisoning, especially when the victim has survived a few days, are the profound changes in lungs. Other agrochemicals such as algicides, aphicides, herbicide safeneres, fertilizers, and so on, are less commonly encountered. Governments in most countries have passed legislations to prevent accidental poisonings with these agents. The US government passed the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) in 1962 and the Indian government passed The Insecticides Act in 1968. Among other things, these acts require manufacturers to use signal words on the labels of insecticides, so the public is warned of their toxicity and accompanying danger.

Key Words: Agrochemical poisoning; rodenticides; insecticides; organophosphates; carbamates; organochlorines; fungicides.

1. INTRODUCTION

Early humans are believed to have started agriculture around 9000 BCE. As the knowledge of chemistry grew, so did the use of chemicals in agriculture. Today, chemicals are used in agriculture for three main purposes: to increase farm production (fertilizers and related chemicals), to kill pests (pesticides), and to preserve farm products (preservatives). Unfortunately, all three classes of chemicals can cause serious poisoning in humans, mainly through improper labeling, storage, or use.

Most poisonings with agrochemicals occur in predominantly agricultural economies where a lack of hygiene, information, or adequate control creates unsafe and dangerous working conditions. Cases of such poisonings also occur in small factories where pesticides are manufactured or formulated with little respect for safety requirements.

Accidental poisonings may also take place at home when pesticides are mistaken for soft drinks or food products, and often the victims are curious children who can easily reach pesticides if they are not kept safely away from them. Then, there are the intentional poisonings, where compounds, such as phosphorus, arsenic, paraquat, organophosphates, and strychnine, are used as agents for suicidal or even homicidal purposes. This may happen because these chemicals are easily available, relatively cheap, and almost certainly cause death.

Poisoning occurring as a result of improper use of chemicals used in agriculture has been termed "agrochemical poisoning." Agrochemical poisoning can be classified as shown in Table 1.

Agrochemical poisoning remains one of the major causes of morbidity and mortality around the world today *(1–14)*, and a review of this relatively untouched subject seems to be justified.

Experience has shown that above the wide range of chemicals a vast majority of poisonings occur because of pesticides only. The 2002 annual report of the American Association of Poison Control Center's (AAPCC) Toxic Exposure Surveillance System listed a total of 2,380,028 human exposures to poisons occurring in the United States during the year 2002 alone *(15)*. Out of these, there were 96,112 exposures to pesticides (4% of all exposures) and 10,632 exposures to fertilizers (0.5% of all exposures); a total of 18 fatalities caused by pesticides and one caused by fertilizers were reported. The break-up for 2002 pesticide exposure is shown in Table 2, and the 18 fatalities caused by pesticides are given in Table 3. Two categories in which deaths were not reported at all were fungicides and repellants. Most deaths (n = 7) were to the result of insecticides. Herbicides and rodenticides accounted for five deaths each, and one death was caused by fumigants.

A comparison of poisoning data for the years 1998 to 2002 *(16–19)* indicates that, although the absolute number of pesticide exposure has been increasing, it is more or less stable at around 4% of all exposures to poisons; fatalities owing to pesticide poisoning amount to 1.5 to 2% of all fatalities resulting from poisons (Table 4).

In the following sections, those agrochemical poisons that are important from a medicolegal and pathological point of view will be discussed.

2. Insecticides

2.1. Organophosphorus Insecticides

2.1.1. Short History

Organophosphorus insecticides are derivatives of phosphoric acid (H_3PO_4) or phosphonic acid (H_3PO_3) in which all H atoms have been replaced by organic moieties (Figs. 1–3). L represents the so-called "leaving moiety" and is the most reactive and most variable substituent. It is called so because this moiety "leaves" the organophosphate molecule after it is attached to the esteratic site of the acetylcholinesterase (AChE, also known as true cholinesterase type 1 ChE). R_1 and R_2 are less reactive moieties. Most commonly they are

Table 1
Classification of Agrochemical Poisons

1. Chemicals/plants used as fertilizers
   - (i) Nitrites and nitrates (methemoglobinemia).
   - (ii) Ammonium sulfate (hyperammonemia).
   - (iii) Anhydrous ammonia.
   - (iv) Fertilizers containing urea.
   - (v) Poisonous plants (used as green manure, e.g., *Ricinus communis*).
2. Chemicals used to kill pests (pesticides)
   - (i) Acaricides (used to kills mites and ticks, also known as miticides, e.g., avermectins, azobenzene, benzoximate, bromopropylate, dofenapyn, nikkomycins, tetranactin).
   - (ii) Algicides used to control growth of algae in lakes, canals, and water stored for agricultural purposes (e.g., cybutryne, hydrated lime [component of Bordeaux mixture]).
   - (iii) Aphicides (used to kill aphids, e.g., triazamate, dimethoate, and mevinphos).
   - (iv) Avicides (used to kill birds harmful to agriculture, e.g., 4-aminopyridine, 3-chloro-*p*-toluidine hydrochloride).
   - (v) Bactericides (e.g., bronopol, nitrapyrin, oxolinic acid, oxytetracycline).
   - (vi) Fumigants (gas or vapor intended to destroy insects, fungi, bacteria, or rodents, used to disinfect interiors of buildings, as well as soil, before planting, e.g., carbon disulfide, sulfuryl fluoride, methyl bromide).
   - (vii) Fungicides (e.g., sodium azide, various compounds of copper and mercury, thiocarbamates, Captan, Captafol).
   - (viii) Herbicide safeners (e.g., benoxacor, cloquintocet, cyometrinil, dichlormid, dicyclonon). These compounds basically protect crops from herbicide injury by increasing the activity of herbicide detoxification enzymes, such as glutathione-*S*-transferases and cytochrome P-450.
   - (ix) Herbicides/weed killers (e.g, paraquat, diquat, 2-4 Dichlorophenoxyacetic acid, Mecoprop).
   - (x) Insecticides (e.g., organophosphorus compounds, organochlorine compounds, carbamates).
   - (xi) Microbial pesticides (those pesticides whose active ingredient is a bacterium, virus, fungus, or some other microorganism or product of such an organism, e.g., *Bti* which is made from the bacterium *Bacillus thuringiensis* var. *israelensis* and used to control mosquito and black fly larvae, *Bacillus sphaericus* and *Laegenidium giganteum*, a fungal parasite of mosquitoes).
   - (xii) Molluscicides (used to kill molluscs, such as snails and slugs, e.g., metaldehyde).
   - (xiii) Nematicides (used to kill nematodes that feed on plant roots, e.g., 1,3-dichloropropene, 1,2-dibromoethane, ethylene dibromide, diamidafos, fosthiazate, isamidofos).

Table 1
(Continued)

(xiv) Ovicides (used to kill eggs of insects and mites).
(xv) Pesticide synergists (e.g., piperonyl butoxide, *N*-octyl bicycloheptene dicarbozimide, piprotal, propyl isome, sesamex, sesamolin).
(xvi) Rodenticides (used to kill rodent pests, e.g., Strychnine, Vacor, ANTU, Cholecalciferol, anticoagulants and Red Squill).
(xvii) Virucides (e.g., ribavirin, imanin).
(xviii) Miscellaneous chemical classes including contaminants and adjuvants of some pesticides which are toxic on their own (e.g., dioxins, present as contaminants of some herbicides produce toxicity of their own).

3. Chemicals used to disturb the feeding/growth/mating behavior etc. of pests, or used for other miscellaneous agricultural purposes
(i) Bird repellents (e.g., anthraquinone, chloralose, copper oxychloride).
(ii) Chemosterilants (e.g., 1,2-dibromo-3-chloropropane, apholate, bisazir, busulfan, dimatif, tepa).
(iii) Desiccants (chemicals which promote drying of living tissues such as unwanted plant tops or insects).
(iv) Defoliants (chemicals which cause leaves or foliage to drop from a plant, usually to facilitate harvest).
(v) Feeding deterrents or antifeedants (chemicals having tastes and odors that inhibit feeding behavior, e.g., pymetrozine, azadirachtin A).
(vi) Insect attractants (substances that attract or lure an insect to a trap, e.g. brevicomin, codlelure, cue-lure, dominicalure, siglure).
(vii) Insect growth regulators (chemicals which disrupt the action of insect hormones controlling molting, maturity from pupal stage to adult, or other life processes, e.g., hexaflumuron, teflubenzuron and pyriproxyfen).
(viii) Insect repellents (e.g., butopyronoxyl, dibutyl phthalate, diethyltoluamide).
(ix) Mammal repellents (e.g., copper naphthenate, trimethacarb, zinc naphthenate, ziram).
(x) Mating disrupters (e.g., disparlure, gossyplure, grandlure).
(xi) Plant activators (a new class of compounds that protect plants by activating their defense mechanisms, e.g., acibenzolar, probenazole).
(xii) Plant growth regulators (substances [excluding fertilizers or other plant nutrients] that alter the expected growth, flowering, or reproduction rate of plants through hormonal rather than physical action).

4. Chemicals used for preservation of grains
(i) Aluminum phosphide.
(ii) Nitric oxide.

Table 2
2002 Pesticide Exposures

Category of pesticide	Pesticide	Number of exposures	Total exposures by category
1. Fungicides (nonmedicinal)	Carbamate	181	1498
	Copper compound	25	
	Mercurial	2	
	Nonmercurial	60	
	Phthalimide	125	
	Wood preservative	480	
	Other/unknown	625	
2. Fumigants	Aluminum phosphide	97	680
	Metam sodium	10	
	Methyl bromide	4	
	Sulfuryl fluoride	458	
	Other	43	
	Unknown	68	
3. Herbicides (including algicides, defoliants, desiccants, plant growth regulators)	Carbamate	42	9562
	2,4-D or 2,4,5-T	455	
	Chlorophenoxy	1717	
	Diquat	355	
	Glyphosate	4472	
	Paraquat	75	
	Triazine	352	
	Urea	93	
	Other	1623	
	Unknown	378	
4. Insecticides (including insect growth regulators, molluscicides, nematicides)	Arsenic pesticide	422	50,911
	Borate/boric acid	3818	
	Carbamate only	3022	
	Carbamate with other insecticide	723	
	Chlorinated hydrocarbon only	1522	
	Chlorinated hydrocarbon with other insecticide	242	
	Insect growth regulator	160	
	Metaldehyde	199	
	Nicotine	15	
	Organophosphate	8031	
	Organophosphate/carbamate	189	
	Organophosphate/chlorinated hydrocarbon	42	

Table 2
(Continued)

Category of pesticide	Pesticide	Number of exposures	Total exposures by category
	Organophosphate/other insecticide	1338	
	Organophosphate/carbamate/ chlorinated hydrocarbon	22	
	Piperonyl butoxide only	30	
	Piperonyl butoxide/pyrethrin	1123	
	Pyrethrins only	877	
	Pyrethrin	4967	
	Pyrethroid	12,475	
	Rotenone	84	
	Veterinary insecticide	151	
	Other	7611	
	Unknown	3848	
5. Repellants	Bird, dog, deer, or other mammal repellant	205	12,954
	Insect repellant with DEET	5321	
	Insect repellant without DEET	1196	
	Insect repellant unknown	2183	
	Naphthalene	1883	
	Paradichlorobenzene	123	
	Other moth repellant	40	
	Unknown moth repellant	2003	
6. Rodenticides	ANTU	1	20,507
	Anticoagulant: warfarin type	462	
	Anticoagulant: long-acting superwarfarin		17,100
	Bromethalin	389	
	Cholecalciferol	27	
	Cyanide	2	
	Monofluoroacetate	2	
	Strychnine	124	
	Vacor	3	
	Zinc Phosphide	146	
	Other	791	
	Unknown	1460	
Total			**96,112**

2,4-D, 2-4 dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; DEET, *N*,*N*-diethyl-meta-toluamide; ANTU, α-naphthyl-thiourea.

Modified according to ref. *15*.

Table 3
Deaths Due to Pesticides

Category	Subcategory	Deaths	Total deaths by subcategory
1. **Fungicides**	-	0	0
2. **Fumigants**	Sulfuryl fluoride	1	1
3. **Herbicides**	Paraquat	2	5
	Chlorophenoxy	1	
	Glyphosate	1	
	Other	1	
4. **Insecticides**	Organophosphates	5	7
	Other	2	
5. **Repellants**	-	0	0
6. **Rodenticides**	Superwarfarin anticoagulants	3	5
	Strychnine	1	
	Other	1	
Total		**18**	**18**

According to ref. *15*.

alkoxy groups, but may be alkyl, aryl, alkylamino, or alkylthio. X can be an oxygen or sulfur atom. The extreme variability of L, R_1, R_2, and X gives rise to virtually hundreds of organophosphates; these can generally be divided into five broad categories *(20)* depending on the characteristics of these groups. Figure

Table 4
Comparison of Pesticide Poisoning Data From 1998 to 2002

Year	Total poisonings reported	Pesticide poisonings	Percentage of total poisonings reported	Total fatalities reported due to all poisons	Total fatalities due to pesticides	Percentage of total fatalities reported
1998	2,241,082	86,289	3.8%	775	16	2.06%
1999	2,201,156	78,853	3.6%	873	15	1.72%
2000	2,168,248	86,880	4.0%	920	17	1.85%
2001	2,267,979	90,010	4.0%	1074	17	1.58%
2002	2,380,028	96,112	4.0%	1153	18	1.56%

Data taken from refs. *16–19*.

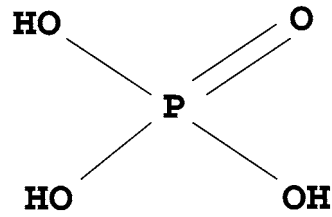


Fig. 1. Phosphoric acid (H_3PO_4).

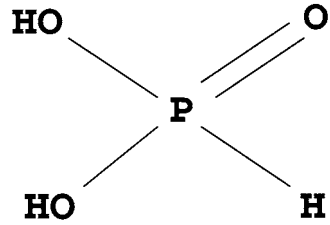


Fig. 2. Phosphonic acid (H_3PO_3).

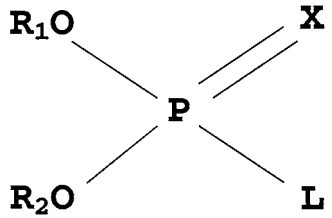


Fig. 3. General structure of organophosphorus insecticides.

4 shows five different categories of organophosphates depending on variations of different side chains.

Available as dusts, granules, or liquids, organophosphorus insecticides are among the most popular and widely used insecticides throughout the world. They began to be synthesized first around 1820 with the esterification of alcohols to phosphoric acid. The earliest synthesis of an organophosphate, tetraethyl pyrophosphate, was reported by Phillipe de Clermont at a meeting of the French Academy of Sciences in 1854 *(21)*. Many different organophosphorus compounds were synthesized in the early 1900s, but their toxicity was first recognized

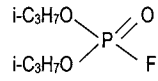
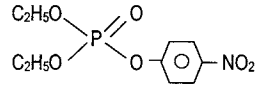
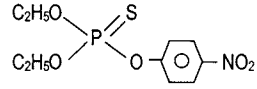
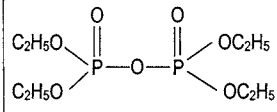
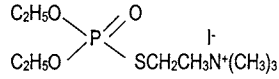
GROUP	DEFINING PROPERTY	REPRESENTATIVE MOLECULE	
A	L = Cyanide, Halogen or Thiocyanate		Diisopropyl fluorophosphate (DFP)
B	L = Alkylthio, Arylthio, Alkoxy or Aryloxy Group		Paraoxon (MINTACOL)
C	Thionophosphorous or Thio-thionophosphorous comporids X=S		Parathion
D	Pyrophosphates and similar compounds		Tetraethyl Pyrophosphate (TEPP)
E	L = Quaternary ammonium group		Diethoxyphosphinyl thiocholine Iodide (ECHOTHIOPHATE)

Fig. 4. Five different categories of organophosphates depending on variations of different side chains.

by Lange in 1932. Lange stated that inhalation of the vapor of dimethyl or diethyl phosphofluoridate produced a choking sensation and dimness of vision.

As nations started looking for lethal gases with the start of World War II in 1939, interest in these compounds was rekindled. By 1940, Schrader in Germany and Saunders in England and their study groups had synthesized a number of highly toxic organophosphates for possible use in warfare. Most notable among these were soman, sarin, and tabun.

Currently, about 50 organophosphorus compounds are in use as insecticides worldwide. Of these, parathion is the most effective for insecticidal use. Tetraethyl pyrophosphate enjoys two distinctions among organophosphates: it was the first organophosphate to be synthesized in 1854 and is the

most dangerous organophosphorus insecticide by either oral or dermal route of application.

2.1.2. Signs and Symptoms

Organophosphorus insecticides are basically AChE inhibitors allowing the accumulation of excess acetylcholine at various nicotinic and muscarinic receptors throughout the body including the central nervous system (CNS). This essentially results in acetylcholine toxicity. The main symptoms can be remembered by either of the two acronyms SLUDGE (salivation, lacrimation, urination, defecation, gastrointestinal distress, emesis) or DUMBELS (diarrhea, urination, miosis, bronchospasm and bradycardia, emesis, lacrimation, salivation). Rarely, there is chromolachryorrhoea (shedding of red or bloody tears) *(22)* because of a disturbance in porphyrin metabolism and its accumulation in lacrimal glands. LD_{50} (lethal dose; the amount of a material, given all at once, which causes the death of 50% of a group of test animals) of these compounds varies from 1 to 50 mg/kg (extreme toxicity) to more than 5000 mg/kg (slight toxicity). Compounds that are extremely toxic are chlorfenvinphos, diazinon, and methyl parathion, whereas those that are slightly toxic are malathion, acephate, and trichlorphon *(23)*. Most patients who have ingested a fatal dose will die within 24 hours of ingestion. Organophosphorus toxicity has recently been reviewed extensively by Rousseau and co-workers *(24)*.

2.1.3. Postmortem Findings

2.1.3.1. External Findings

Signs of asphyxia are commonly found in fatal intoxications with organophosphorus insecticides. There is congestion of the face and cyanosis of the lips, nose, fingers, and acral parts of the extremities. One of the most remarkable findings is the characteristic odor emanating from the corpse: it has been described as garlic- or kerosene-like and is due to the fact that organophosphates are dissolved on a kerosene base. There is often frothy, bloody staining at the mouth and nostrils, and the pupils may be constricted.

A coloring agent, indigocarmine, is added to parathion (E605®) to prevent its accidental ingestion or criminal use as a poison. This gives rise to a bluish-greenish discoloration of the lips and oral mucosa. The addition of indigocarmine, however, is not a general practice worldwide. For instance, in India and several other Asian countries, this practice is not followed. An interesting sign to be observed (albeit only in somewhat less modern mortuaries) is the death of bluebottles and others insects and flies dying immediately after they alight on an opened cadaver at autopsy *(25)*.

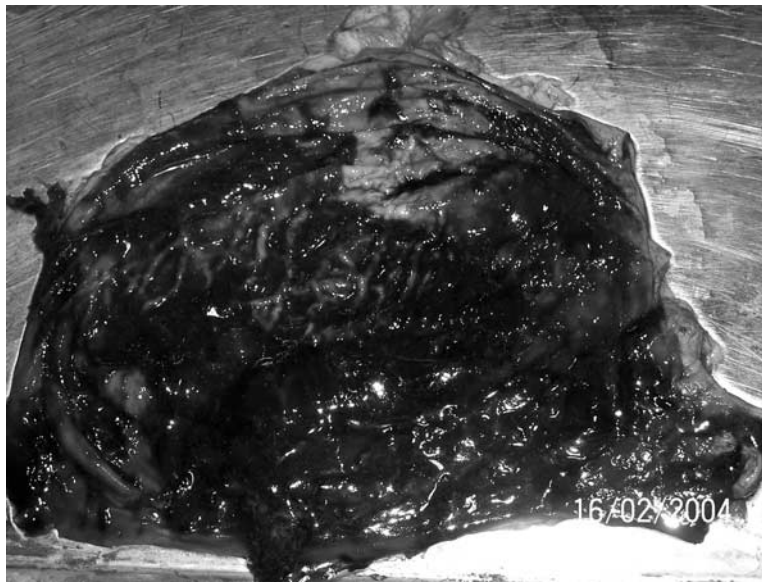


Fig. 5. Congested and hemorrhagic stomach mucosa in a case of fatal organophosphorus poisoning. (Courtsey of Dr. Avneesh Gupta and Dr. Puneet Setia, Maulana Azad Medical College, New Delhi, India.)

2.1.3.2. Internal Findings

The gastric mucosa is congested and may appear hemorrhagic (Fig. 5) and the stomach contents often contain an oily, greenish scum. The mucosa of the respiratory tract is congested and the airway passages contain frothy hemorrhagic exudate. The lungs show congestion, hemorrhagic pulmonary edema, and subpleural petechiae. The brain is swollen and there is generalized visceral congestion.

2.1.3.3. Histopathology

Parathion (E605) has been studied most extensively for histopathological lesions and these are considered to be representative of other organophosphorus insecticides, too *(26)*. In the kidneys, there is epithelial necrosis in the straight sections of the renal tubules. In the epithelia of the remaining renal cortical sections, there is pronounced plasma granulation, nuclear wall hyperchromatosis, and clumping and reduction in the chromatin and marginal nucleoli. Epithelia in loops of Henle and collecting tubules appear swollen.

The liver is more resistant to the effects of organophosphates, partly because of its ability to manufacture serum cholinesterase on its own. Hepa-

tocytes show opaque swelling and glycogen depletion; there are destructive changes in the liver cell strands, detached hepatocytes, and perivascular edema.

Myocardium, medulla oblongata, and vagal nuclei of the brain show fine, maculate perivascular hemorrhages. Limaye has described a type of toxic myocarditis that he had observed in 76 autopsy cases *(27)*. Kiss and Fazekas described focal myocardial damage with pericapillary hemorrhage, micronecrosis, and patchy fibrosis in victims of organophophorus poisoning *(28)*. Pimentel and da Costa *(29)* have described the following myocardial ultrastructural changes in fatal poisonings with organophosphorus:

1. Areas of partial or extensive lysis of myofibrils.
2. Mitochondria exhibiting decreased electron density, swollen forms and fragmentation or lysis of cristae.
3. Nuclei showing irregularity in shape and various degrees of disorganization of chromatin.
4. Z-line abnormalities of various degrees.

Multiple circumscribed necroses are found in the skeletal musculature. The oolemma is damaged and sometimes even necrotic. The glomus caroticum shows an increase in the number of dark-cell nuclei, perhaps as a consequence of increased nuclear metabolism owing to augmented demand.

2.1.3.4. Postmortem Biochemistry

AchE and butyrylcholinesterase (BChE, also known as pseudocholinesterase or type 2 ChE) levels are depressed in deaths owing to organophosphorus insecticides. The measurement of their levels can assist in the determination of the cause of death *(30)*. AChE is found mostly in red blood cells, motor endplates, and gray matter, whereas BchE is found mostly in plasma, white matter, liver, heart, and pancreas. The physiological function of BChE is unknown *(31)*, but it is established that BchE hydrolyzes suxamethonium (succinylcholine), and for this reason it is of interest to anesthesiologists as well. Postulated functions of BChE include its role in transmission of slow nerve impulses, lipid metabolism, choline homeostasis, permeability of membranes, protection of the fetus from toxic compounds, and degradation of acetylcholine and in tumorneogenesis *(32)*.

The plasma cholinesterase (pseudocholinesterase) is more sensitive and levels fall more rapidly than those of the red blood-cell cholinesterase. Red blood-cell cholinesterase levels are more satisfactory for the diagnosis of organophosphorus poisoning because they represent the true cholinesterase levels.

Sample collection and storage (time and temperature) are critical to the catalytic stability of ChE and thus influence the quality and interpretation of results of the toxicological analysis. Fluids and tissues that should be collected at autopsy are blood, cerebrospinal fluid (CSF), semen, muscle, brain, liver, heart, and pancreas. The recommended procedures for collection and storage of biological fluids are as follows:

1. Blood must be collected in heparinized tubes.
2. The samples must be collected and stored in glass rather than plastic containers to avoid contamination by leachates from plastic.
3. Sample contamination with acid or alkali must be avoided.
4. Samples must be immediately refrigerated because ChE catalytic activity is temperature dependent.
5. Fluid and cellular components of blood, CSF, and semen have to be separated.
6. Determine enzyme activity as soon as possible. If enzyme activity is not determined immediately, samples can be stored for several days at 4°C. If tissues are intended to be stored for longer periods, the storage temperature should be –20°C or below.
7. Tissue should be homogenized at pH 7.6 to 8.0 using a sonicator or nonmetallic homogenizer and then should be stored as indicated above.

ChE activity in blood, serum, and tissues can be measured by a number of methods. One of the most popular is the pH method by Michael *(33)*, whereby a change in pH is measured when ChE acts on acetylcholine. The principle is that cholinesterase hydrolyzes acetylcholine, thus producing acetic acid, which in turn decreases the pH of the reaction mixture. Electrometric determination of the change in pH from 8.1 for a definite period of time (e.g., 1 hour) at a specific temperature (e.g., 25°C) represents the enzyme activity. Normal values of ChE activity as measured by this method (in ΔpH/hour/0.02 mL red blood cells or plasma at 25°C, mean ± standard deviation) are given in Table 5 *(34)*.

In deaths owing to organophosphorus insecticides, the values will be much lower. A 25% or greater depression of the red blood-cell ChE level is a true indicator of poisoning. Death occurs when levels have decreased by more than 90%.

2.1.4. Toxicological Analysis

Blood and urine should be preserved for toxicological analysis of ChE levels. Samples from lung, liver, kidney, skeletal muscle, brain, and spinal cord, as well as gastric contents, must similarly be preserved for toxicological analysis of cholinesterase levels *(35)* according to the precautions detailed in Steps

Table 5
Normal Red Blood Cell and Plasma Cholinesterase Activity in Men and Women

	Men	Women
RBC ChE	0.766 ± 0.081	0.750 ± 0.082
Plasma ChE	0.953 ± 0.157	0.817 ± 0.187

RBC, red blood cell; ChE, cholinesterase.

1–7 in Section 2.1.2.4. Paranitrophenol is a metabolite of many organophosphates. It is excreted in urine and its presence in urine is characteristic of organophosphorus poisoning.

2.1.5. Organophosphates and Putrefaction

Organophosphates usually resist putrefaction and can be detected in the viscera for quite some time after death. Wehr *(36)* studied five exhumations where the decedents were suspected having been poisoned with parathion. He could detect the degradation products of parathion (aminoparathion and *p*-nitrophenol) up to 7 years after burial, but after 13 years, neither parathion nor any of its degradation products were detectable. Pohlmann and Schwerd found evidence of parathion in a corpse exhumed after 21 months *(37)*.

More recently, Karger and co-workers *(38)* described a case where they detected paraoxon, the main conversion product of parathion, from the abdominal cavity of a 9-month-old boy, 8 months after his death. His mother had poisoned him with parathion; her deed was detected when, several months later, her second child—a 3-year-old girl—also suffered the same fate and parathion was detected in her blood.

2.2. Carbamates

Carbamates (Fig. 6) are derivatives of carbamic acid. Their structure is similar to that of organophosphates (Fig. 7). The first recognized anti-ChE was in fact a carbamate, physostigmine (also called eserine), obtained in pure form in 1864 by Jobst and Hesse from the Calabar bean *(39)*. Some common carbamates used as insecticides today are aldicarb, carbaryl, γ-benzene hexachloride, triallate, propoxur, methomyl, carbofuran, and carbendazim. Like organophosphates, carbamates are inhibitors of AChE, but instead of phosphorylating, they carbamoylate the serine moiety at the active site. This is a reversible type of binding, and therefore, their toxicity is less severe and of

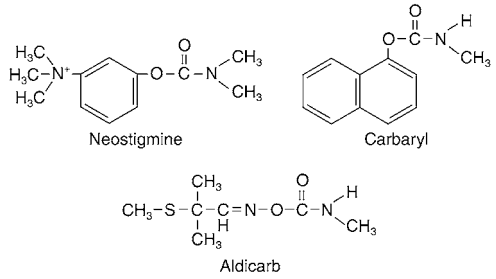


Fig. 6. Some representative carbamates.

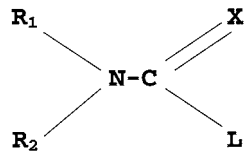


Fig. 7. General structure of carbamates. Note the similarity of the structure with that of organophosphates.

lesser duration *(40)*. Because they do not penetrate the CNS to any great extent, the CNS toxicity of carbamates is relatively low. Signs and symptoms are the same as those seen in poisoning with organophosphates/organophosphorus insecticides but they are milder in nature. Convulsions are not seen in carbamate poisoning.

Postmortem findings in carbamate poisonings are mostly similar to those found in organophosphates. A bluish discoloration of the mucosa of the mouth and stomach is not seen because the blue green dye indigocarmine is usually not mixed with carbamates. Determination of cholinesterase levels is not of much help because these are restored very rapidly in carbamate poisoning.

2.3. Organochlorines

Organochlorine pesticides are nonselective insecticides. They are cyclic in nature, have molecular weights between 300 and 550 D, are CNS stimulants, and have limited volatility. They are poorly soluble in water but readily soluble in organic solvents and fats, which is the way how they accumulate in the human body. They are very stable, both in the environment and in the body tissues, and can be demonstrated in the bodies of most people born since 1940.

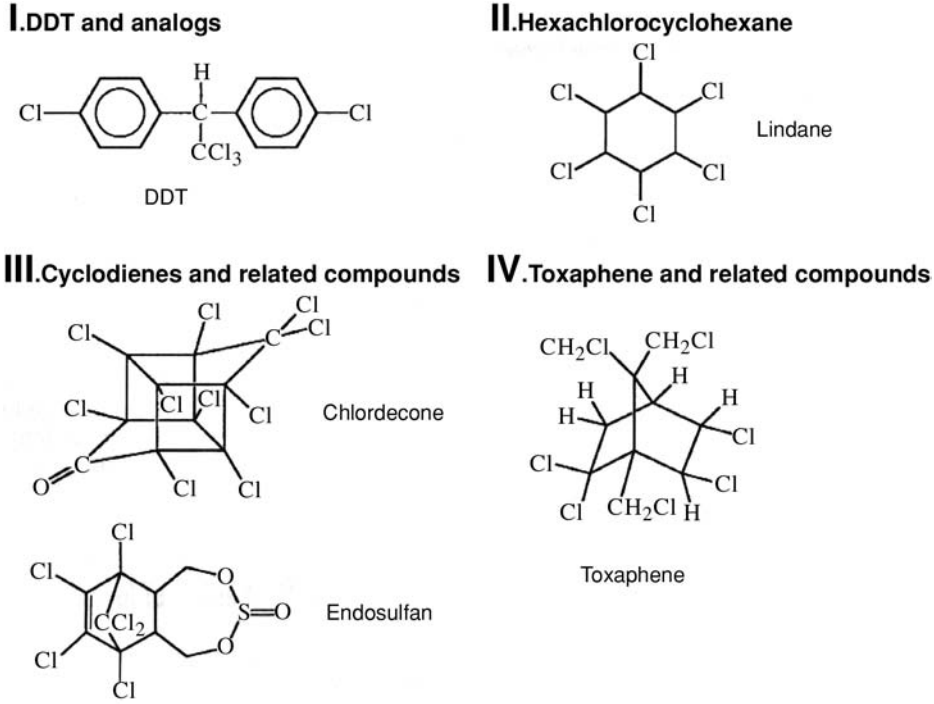


Fig. 8. Four general categories of organochlorine pesticides.

Based on their chemical structures, organochlorines can be divided into four categories (Fig. 8) *(41)*: (a) dichlorodiphenyltrichloroethane (DDT) and related analogs, such as methoxychlor, (b) hexachlorocyclohexane or lindane, (c) cyclodienes and related compounds (e.g., aldrin, dieldrin, endrin, endosulfan, chlordane, chlordecone, heptachlor, mirex, isobenzan), and (d) toxaphene and related compounds.

2.3.1. Short History

The best known organochlorine, DDT, was synthesized by the German chemist Othmar Zeidler in 1874, but he failed to realize its value as an insecticide. It was the Swiss Paul Hermann Müller (1899–1965) who recognized its potential as an effective insecticide. In 1939, DDT was tested successfully against the Colorado potato-beetle by the Swiss government. The United States Department of Agriculture used it successfully in 1943. In January 1944, DDT was used to quash an outbreak of typhus carried by lice in Naples, Italy; this was the first time a winter typhus epidemic could be stopped.

So revolutionary was his work that Müller was awarded with the Nobel Prize in Medicine in 1948. It is ironic that just 24 years later, in 1972, DDT was banned in the United States. It is perhaps a unique example in the history of science that a Nobel Prize-winning work was banned within such a short period of time. The main driving force behind this ban was the ecologists' concerns about the persistence of DDT in the environment and its resulting harm to the habitat—humans are equally affected by persistent DDT in the environment. It was Rachel Carson's (1907–1964) book *Silent Spring*, published in 1962, which brought the problem to everyone's notice.

Endrin, one of the cyclodienes, is chiefly used against insect pests of cotton, paddy, sugarcane, and tobacco. It is active against a wide variety of insect pests, and hence is commonly known as *plant penicillin*. It has been banned in most Western countries, but unfortunately continues to be used in several agrarian economies.

2.3.2. Signs and Symptoms

The mechanism of action of organochlorines is entirely different from that of organophosphates and carbamates. Organochlorines act on axonal membranes affecting the sodium channels and sodium conductance across the neuronal membranes. Organochlorines also alter the metabolism of acetylcholine, noradrenaline, and serotonin. Lindane and cyclodienes appear to inhibit the γ-aminobutyric acid-mediated chloride channels in the CNS.

Therefore, not very surprisingly, the main symptoms induced by poisoning with organochlorines are CNS-related and include vertigo, confusion, weakness, agitation, hyperesthesia or paresthesia of the mouth and face, myoclonus, rapid and dysrhythmic eye movements, and mydriasis (in contrast to organophosphates and carbamates, where miosis is found). Other symptoms include nausea, vomiting, fever, aspiration pneumonitis, and renal failure.

The fatal dose of DDT and lindane is 15 to 30 g, whereas that of aldrin, dieldrin, and endrin is 2 to 6 g *(42)*.

2.3.3. Postmortem Findings

2.3.3.1. External

The conjunctivae are congested and the pupils are dilated. There may be a kerosene-like smell emanating from the mouth and nostrils. This is because most organochlorines are poorly soluble in water and are dispensed as solutions in organic solvents that may have a kerosene-like smell. Fine white froth, which may or may not appear hemorrhagic, can be seen around the mouth and nostrils; this is a general effect of pulmonary edema coupled with respiratory distress and therefore, signs of cyanosis are seen on the face, ears, nail beds, etc.

2.3.3.2. Internal Findings and Histopathology

The mucosa of the respiratory tract appears congested and the respiratory passages contain frothy mucus which may or may not be tinged with blood. Subpleural and subpericardial petechial hemorrhages are common. The lungs appear large and bulky, showing pulmonary edema. The mucosa of the esophagus, stomach, and bowel is congested owing to the irritating effect of organochlorines on the gastrointestinal tract. The stomach contents smell kerosene-like. The visceral organs are congested. Hepatic necrosis may be found on cut sections of the liver.

In animals killed by DDT, vacuolization around large nerve cells of the CNS, fatty change of the myocardium, and renal tubular degeneration can be detected histologically *(43)*.

2.3.4. Toxicological Analysis

Feces, urine, and subcuatenous adipose tissue (placed in a glass-stoppered vial or a vial with a teflon-lined cap *[43]*) should be collected for toxicological analysis. Samples must be frozen before onward transmission to the toxicology laboratoy.

2.4. Nicotine

Nicotine salts, such as nicotine sulfate, were very popular pesticides in the 1920s and 1930s. These compounds generally contained 40% nicotine (Fig. 9). Now, because most countries have banned nicotine-based insecticides, less than 1% of home garden insecticides are nicotine-based. These are usually available in powder form. Main among these is Black Leaf-40 (manufactured by Black Leaf Products Company, Elgin, IL).

When nicotine-based insecticides come in contact with moist skin, fatal doses of nicotine may be absorbed through the skin *(44)*. Apart from occupational exposure to nicotine spray, other methods of fatal exposure include careless storage and inadvertent mixing with foodstuffs, fruits, and vegetables. These insecticides have also been used successfully with suicidal or homicidal intention.

2.4.1. Postmortem Findings

Brownish froth around the mouth and nostrils is a frequent finding in nicotine poisoning. There is a characteristic odor of stale tobacco emanating from the gastric contents. The esophageal and gastric mucosa is intensely congested, showing a brownish discoloration. Liver and kidneys show considerable acute congestion *(23)*.

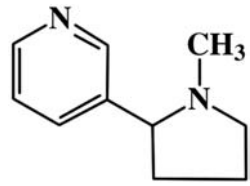


Fig. 9. Nicotine.

2.4.2. Histopathology

The liver shows plaque-like granulations in the cytoplasm of centrilobular and intermediary hepatocytes. Intrapulmonary hemorrhages and pulmonary edema are typical and there often is detachment of the alveolar epithelium. In the kidneys, there is necrosis and detachment of the epithelia in the straight and convoluted renal tubules. A variety of arterial wall lesions, including lacerations of the elastic interna, are seen that have been connected with extreme fluctuations in blood pressure from the effects of nicotine *(26)*.

3. Herbicides or Weed Killers

3.1. Short History and General Remarks

An estimated 10% of all plant species are weeds, with a total of some 30,000 species. Chemicals, such as common salt, have been used for centuries for weed control. The era of chemical weed control is generally recognized as starting in 1896. Bonnet in France found that the Bordeaux mixture, already being used on vines to control powdery mildew, also provided control of specific weeds. By the 1930s, farmers were still using simple chemicals for this purpose; for example, copper sulfate (Blue Vitriol), which was first used for weed control in 1821, was still in use at this time. In the early 20th century, scientists in Europe started using the salts of heavy metals to control weeds but when this was attempted in the United States, the low humidity in the Western states prevented these chemicals from being absorbed by the weeds. Other chemicals were tried, but most of them had drawbacks. For instance, carbon bisulfide used to control thistles and bindweeds smelled like rotten eggs and was, therefore, quite understandably unpopular. Most chemical weed killers of those times (such as sodium arsenate, arsenic trioxide, and sulfuric acid) were highly toxic to humans and had to be used in large quantities (several kilograms per hectare), which was another serious drawback.

The first synthetic organic chemical for selective weed control was introduced in 1932. Its chemical name was 2-methyl-4, 6-dinitrophenol, and it could control some broadleaf weeds and grasses in large seeded crops, such as beans.

More modern herbicides are now available. These have to be sprinkled in very low doses (grams per hectare) in order to kill weeds and the crop is spared.

Herbicides are categorized as *selective* when they are used to kill weeds without harming the crop and as *nonselective* when the purpose is to kill all vegetation. Killing of all vegetation is generally not intended in an agricultural setting. It is required more often in places such as recreational areas, railroad embankments, irrigation canals, fence lines, industrial sites, roadsides, and ditches.

Both selective and nonselective herbicides can be applied to weed foliage or to soil containing weed seeds and seedlings depending on the mode of action. The term *true selectivity* refers to the capacity of an herbicide, when applied at the proper dosage and time, to be active only against certain species of plants but not against others. Selectivity can also be achieved by placement, such as when a nonselective herbicide is applied in such a way that it reaches only the weeds but not the crop.

Herbicides can also be classified as *contact* or *translocated*. Contact herbicides kill the plant parts to which the chemical is applied. Translocated herbicides are absorbed either by the roots or the above-ground parts of plants and are then circulated within the plant system to distant parts.

Timing of herbicide application regarding the stage of crop or weed development forms another basis of classification. A *preplanting* herbicide is sprinkled on the farm before the planting of the crop. A *preemergence* herbicide is sprinkled after planting but before emergence of the crop or weeds. Finally, a *postemergence* herbicide is used after the emergence of the crop or weed.

Herbicides can be applied to weeds in a number of ways. A *band application* treats a continuous strip, such as along or in a crop row. *Broadcast application* covers the entire area, including the crop. *Spot treatments* are confined to small areas of weeds. *Directed sprays* are applied to selected weeds or to the soil to avoid contact with the crop. In the more recent *over-the-top-application*, herbicides are applied "over the top" of the crop and weeds shortly after germination. The crops in these instances are naturally tolerant to the specific herbicide or have been genetically engineered to be tolerant to the herbicide used.

From a toxicological point of view, the following herbicides are the most important.

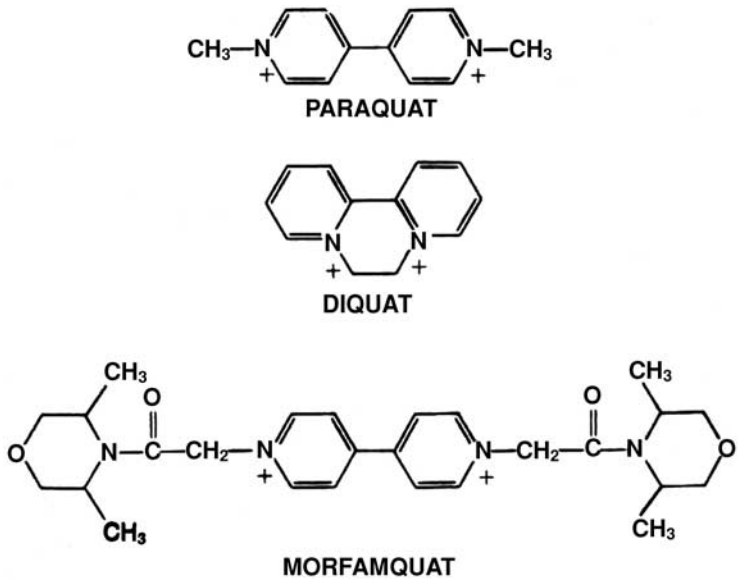


Fig. 10. The three most common dipyridyl weed killers: paraquat, diquat, and morfamquat.

3.2. Dipyridyl Weed Killers

Dipyridyl weed killers include paraquat, piquat, and morfamquat (Fig. 10). Paraquat is the most important of these three.

3.2.1. Paraquat

Paraquat (1,1′dimethyl-4-4′bipyridylium dichloride) is an important agricultural chemical from a toxicological viewpoint. Out of the 18 deaths caused by pesticides reported by the 2002 AAPCC annual report *(15)*, two were the result of paraquat poisoning.

Paraquat was first synthesized in 1882, but its herbicide activity was discovered very late. Its use as an herbicide was first reported in 1958, and paraquat was introduced commercially as a nonselective herbicide in 1962. The introduction of paraquat caused an agricultural revolution because it has some unique properties. It can be sprayed from the ground level or the air and is totally denatured when it comes in contact with the earth. Thus, it cannot harm the seeds or young plants that will be placed in the same ground a short time later. Indeed, the crop can be planted within days, if not hours, after herbicidal treatment with paraquat. An additional advantage is that plowing is unnecessary

in many cases with much less soil erosion. Paraquat is therefore of immense value in an economic sense *(45)*. In countries like Sri Lanka, its use has resulted in three crops, instead of two, per year being taken off the same field *(46)*.

Paraquat is highly soluble in water and is marketed most commonly as a concentrate containing 200 g paraquat dichloride per liter (20% wt/vol); this is an odorless brown liquid. A "stenching" agent (a pyridine derivative) is added to prevent accidental or criminal poisoning; a bluish-greenish dye is also added for the same reason, and an emetic may be added as well. Paraquat is sometimes sold in combination as a mixture with diquat and other herbicides. The liquid concentrate is known as Gramoxone (not to be confused with Gammexane, which is the trade name for lindane); a weaker, granulated preparation for horticultural use, known as Weedol, is also available (5% wt/vol). The solution may be decanted in soda bottles and left unlabelled. Because it looks like a cola drink, accidental ingestion may occur. It may be mistaken for vinegar as well; one patient is reported to have sprinkled it on his french fries.

Wesseling and co-workers *(47)* reported that paraquat is the pesticide most frequently associated with injuries among banana workers in Costa Rica; the injuries involve mostly the skin and eyes.

Although most fatalities caused by paraquat occur from ingestion, absorption through the skin can also cause fatalities. Wohlfahrt *(48)* reviewed paraquat poisoning in Papua New Guinea from 1969 to 1981 and found that out of 35 fatalities caused by paraquat, six were the result of transdermal absorption.

3.2.2. Diquat

Diquat (1,1′-ethylene-2,2′-dipyridylium dibromide) is less commonly used than paraquat. It has the same indications and mode of action as paraquat. Diquat is, however, used additionally for the control of aquatic weeds. Jones and Vale *(49)* compiled all cases of diquat poisoning published between the years 1968 and 1999 and found that only 30 cases were reported in detail in the literature, of which 13 (43%) were fatal. Conning et al. showed that out of the three dipyridyl weed killers, it was only diquat that produced bilateral cataracts *(50)*.

Diquat was introduced in 1957 as a fast-knockdown, contact herbicide and plant desiccant. Diquat-only formulations manufactured by Syngenta (formerly Imperial Chemical Industries) or its subsidiaries do not contain the dye, "stenching" agent, or emetic added to paraquat *(41)*.

3.2.3. Signs and Symptoms in Poisoning With Dipyridyl Weed Killers

The symptoms include intense pain in the mouth and pharynx, with inflammation and even ulceration of the oral mucosa. Esophageal ulceration

may lead to perforation with all its attendant risks. Renal and hepatic failure develop within 2 to 4 days. The most important effect is on the lungs (*pneumotropism*), where massive, irreversible pulmonary fibrosis is seen. Pulmonary fibrosis is thought to be the result of an increase in the pulmonary concentrations of prolyl hydroxylase, an enzyme which promotes collagen formation. Paraquat is one of the few poisons that may produce necrosis of the adrenal glands, possibly leading to hypotension. The fatal dose is 1 to 2 g (about a mouthful of Gramoxone). Subcutaneous injection of just 1 mL of Gramoxone has shown to be fatal *(51)*, with death occuring after 1 to 2 weeks as a result of respiratory failure caused by pulmonary fibrosis; greater doses can kill a human within 24 hours.

3.2.4. Mechanism of Toxicity of Dipyridyl Weed Killers

Why does paraquat show such remarkable pneumotropism? It has been postulated that inside the pneumocytes, the paraquat dication PQ^{2+} accepts one electron from reduced nicotinamide adenine dinucleotide and becomes the monocation $PQ^{+\cdot}$ (pyridinyl-free radical) (Fig. 11). The monocation $PQ^{+\cdot}$ is unable to cause any injury on its own, but in the presence of molecular oxygen (O_2) in the lungs, it is oxidized once again to its dication form (PQ^{2+}). In this process, it passes on its electron to the molecular oxygen (O_2), which, in turn, becomes the superoxide anion radical ($O_2^{\cdot-}$). This process, known as *redox cycling*, is sustained by oxygen in the lungs. The superoxide anion radical $O_2^{\cdot-}$ (reactive oxygen species) generated as a result of this cycle is responsible for cell death. This also explains why oxygen enhances the toxicity of paraquat and should never be administered during paraquat intoxication; by administering oxygen, one is supplying the "raw material" for the formation of the damaging superoxide radical.

Formation of free radicals is implicated in injuries caused by at least two other poisons—myocardial injury caused by doxorubicin and liver injury by carbon tetrachloride.

The related bipyridylium compounds, such as diquat and morfamquat, do not affect the lung as seriously, but rather cause liver damage *(52)*.

3.2.5. Postmortem Findings and Histopathology in Poisoning With Dipyridyl Weed Killers

There is ulceration around lips and mouth, although it is not as bad as is seen after ingestion of inorganic acids, such as nitric or sulfuric acids. The oral and esophageal mucosa is reddened and desquamated. A unique feature of paraquat ingestion is the formation of pseudomembranes in the pharynx resembling to that seen in diphtheria *(53)*.

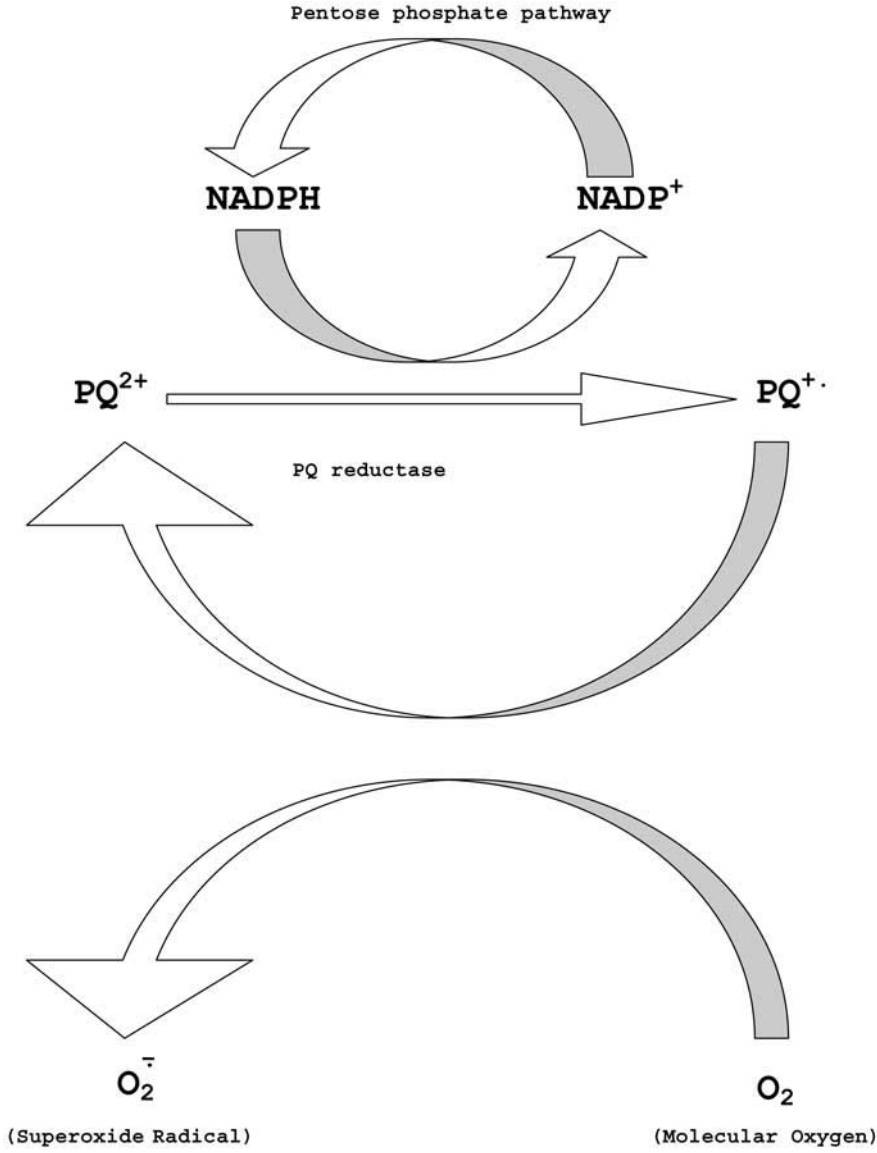


Fig. 11. Mechanism of paraquat toxicity.

Patchy hemorrhages in the stomach mucosa are a frequent finding. The liver is pale, showing fatty changes. The kidneys may exhibit pallor of the cortex. The most striking findings are found in the lungs. Both type 1 and type 2 alveolar epithelial cells accumulate paraquat and are thereby destroyed. This destruction is followed by inflammatory cell infiltration and hemorrhages; fibroblast proliferation then leads to fibrosis and impaired gas exchange. The lungs are congested, appear stiffened, and retain their shape during evisceration. Each lung is typically approx 1000 g or more in weight. Teare *(46)* reported a case of paraquat poisoning (a 44-year-old man dying of suicidal ingestion of paraquat after 17 days of illness), with the left lung weighing 1980 g and the right lung weighing 1920 g. Blood-stained pleural effusions and fibrinous pleurisy are other typical autopsy findings. Cut surfaces of the lungs reveal edema and fibrosis. Subendocardial hemorrhages may accompany the aforementioned pathological findings. The pathological features of paraquat poisoning have been reviewed in detail by Vadnay and Haraszti *(54)*.

At the beginning of the toxic process, severe degenerative changes appear in the pneumonocytes with fatty infiltration, desquamation, necrosis, and detachment *(26)*. Later, there is splintering of the basement membranes, fragmentation, aneurysma formation, and multiple ruptures. Fibrinous edematous fluid is seen in the interstitium and within alveoli and hyaline membranes can be observed. There is a large-scale dissolution of the pulmonary structure. There may be active proliferation of the bronchial epithelium, forming small adenomata within the pulmonary parenchyma. Marked proliferation of fibroblasts with an increase in macrophages in the alveoli (these two mechanisms obliterate the alveolar spaces) can be seen. Acute tubular necrosis is a frequent finding in the kidneys. Extensive renal cortical necrosis is also seen at times. In the liver, centrilobular hepatic necrosis, cholestasis, and giant mitochondria with paracrystalline inclusion bodies can be detected *(26)*. In the myocardium, there is edematous disaggregation of the sarcoplasm and sporadic fragmentation of the myofibrils.

3.2.6. Toxicological Analysis

Paraquat-type herbicides in aqueous solutions have traditionally been determined by colorimetric methods. These involve measurement of the complex formed with some chemical (α-dipicrylamine hexanitrodiphenylmethane). Plasma paraquat levels can be assayed by spectroscopy, high-performance liquid chromatography *(55)* or radioimmunoassays; levels greater than 0.2 µg/mL confirm death by paraquat intoxication. Urine paraquat levels can be deter-

mined using spectrophotometry, too; levels greater than 10 µg/mL confirm death by paraquat intoxication *(23)*. Berry and Grove introduced an ion exchange and colorimetric method in 1971 for the determination of paraquat in urine *(56)*.

Diquat (Reglone) is selectively concentrated in the kidneys and causes marked renal tubular damage. In a case of fatal diquat poisoning, McCarthy et al. found esophagitis, tracheitis, gastritis, and ileitis *(57)*. Autopsy findings and toxicokinetic data in diquat poisoning have been described in detail by Hantson et al. *(58)*.

3.2.7. Morfamquat

Morfamquat is used far less commonly than the other two bipyridyls, paraquat and diquat. Conning et al. have shown that rats that fed on morfamquat developed renal damage *(50)*.

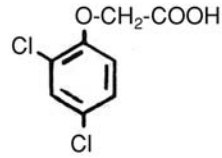
3.3. Chlorophenoxy Herbicides

Chlorophenoxy herbicides (Fig. 12) are growth regulators or auxins. They cause abnormal plant growth, thereby ultimately destroying the plant. Chlorophenoxy herbicides are commonly used for control of broadleaf weeds in cereal crops and pastures *(59)*. 2-4 Dichlorophenoxyacetic acid (2,4-D; Trimec) has been and continues to be one of the most useful herbicides developed; it is frequently applied to lawns to control broadleaf weeds and is often found in fertilizer products along with other phenoxy herbicides, such as dicamba, mecoprop, and (4-chloro-2-methylphenoxy)acetic acid.

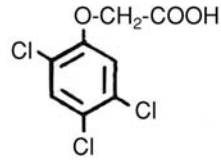
2,4-D is easily absorbed through the skin and lungs *(60)*. On ingestion, 2,4-D causes peripheral neuropathy, muscle weakness, Cheyne-Stokes respirations, hyperthermia, acidemia, and coma *(23)*. The patient is hypotonic, hyporeflexive, hypotensive, and comatose *(61)*, and nasogastric aspirate may be guaiac-positive *(62)*.

2,4-D earned a notorious reputation during the Vietnam war as an ingredient of Agent Orange sprinkled by United States troops over Vietnam (*see* Subheading 9.4). Suicidal ingestions of 2,4-D are occasionally reported *(61,62)*.

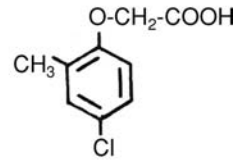
Postmortem findings in deaths caused by chlorophenoxy herbicides are nonspecific. The gastrointestinal mucosa may be intensely congested and/or hemorrhagic. All internal organs are usually congested. Confirmatory tests of suspected poisonings with chlorophenoxy herbicides are the demonstration of these herbicides in plasma and urine,which can be detected by radioimmunoassay *(63)* and gas liquid chromatography *(64)*.



2, 4-D



2, 4, 5-T



MCPA

Fig. 12. The three most common clorophenoxy herbicides: 2-4 dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, and (4-chloro-2-methylphenoxy)acetic acid.

3.4. Phenolic Weed Killers

This category comprises mainly dinitrophenol (DNP), dinitro-orthocresol (DNOC), and pentachlorophenol *(65)*. These substances are used in agriculture mainly as selective weed killers for cereal crops. The effects of DNP in stimulating metabolism have been known since 1885, and DNP was used at one time for "slimming." DNP (Fig. 13) is a potent "uncoupler" of oxidative phosphorylation, causing the energy obtained from the oxidation of nicotinamide adenine dinucleotide and reduction of O_2 to be released as heat.

It has been demonstrated that these compounds are dangerous to humans and thus, they are no longer used for medicinal purposes. The principal risk of poisoning is in the agricultural use of concentrated solutions for spraying crops

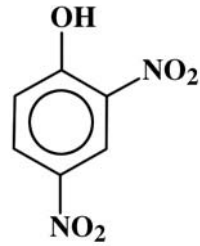


Fig. 13. Dinitrophenol.

(as weed killers). Dinitrophenol (DNP) is also used in agriculture for the control of mites and aphids *(66)*.

Absorption occurs by inhalation and thus, breathing apparatus are a must for those who are exposed to this poison. Absorption also occurs by ingestion and through the skin. Excretion of DNP is extremely slow, so the poison accumulates in the body gradually. The symptoms are fatigue, insomnia, restlessness, excessive sweating, weight loss, and thirst. Clinical signs include tachycardia, increase in the rate and depth of respiration, rise in temperature (up to 41°C and higher) and some yellow discoloration of the sclera. In severe cases, body temperature may keep rising and just before death, it may reach 44°C. When death occurs, the onset of rigor mortis is rapid.

3.5. Chlorate Compounds

Sodium chlorate is a nonselective herbicide. It acts as a soil sterilant at rates of 200 lbs/acre. It is also used as a foliar spray at 5 lbs/acre as a cotton defoliant. It was once avidly advocated as a weed killer, not only because it is effective, but also because it was considered safe. This fallacy was so prevalent that containers of sodium chlorate used to be marked as "nonpoisonous." However, chlorates cause methemoglobinemia. Severe hemolysis is a constant clinical feature in sodium chlorate poisoning, with presence of Heinz bodies in the red blood cells. Acute renal failure and anuria sets in later. Anuria occurs because of (a) a direct damaging action of chlorates on the renal tubular epithelium, and (b) mechanical obstruction of the renal tubules by the hemoglobin set free by hemolysis. The fatal dose of sodium chlorate is 20 to 35 g with death occuring within 4 to 5 days.

3.5.1. Circumstances of Poisoning With Sodium Chlorate

Poisoning with sodium chlorate can occur accidentally, suicidally, or even homicidally. Accidental poisoning is probably the most common. A 48-year-old

gardner was severely poisoned in a curious way. He was using a concentrated solution of sodium chlorate in an atomizer while a strong wind was blowing. Consequently, spray was blown onto his face and he inhaled and ingested some of the solution. Symptoms of poisoning started the same evening. He was saved with some heroic effort on the part of the doctors, yet he could only return to full-time work after about 1 year *(67)*.

3.5.2. Postmortem Findings

The skin has a distinctive chocolate-brown color. Blood smears may show evidence of hemolysis and Heinz bodies. The kidneys are enlarged and their principal change is a brown streaking of the cortex; microscopical examination reveals acute renal tubular degeneration with blockage of tubules by broken red blood cells and brown pigment granules (released hemoglobin owing to hemolysis).

3.6. Glyphosate

Glyphosate is an important agricultural chemical from the toxicological viewpoint. Out of the 18 deaths caused by pesticides reported by the 2002 AAPCC annual report *(15)*, one was caused by glyphosate. Glyphosate is a broad-spectrum, nonselective, systemic herbicide used for control of annual and perennial plants including grasses, sedges, broad-leaved weeds, and woody plants. It can be used on non-cropland as well as on a great variety of crops. Although glyphosate itself is relatively harmless, its chemical formulations (e.g., Roundup®, Rodeo®, Touchdown®, Gallup®, Landmaster®, Pondmaster®, Ranger®) have been used successfully for committing suicide. This is because glyphosate invariably is formulated in a surfactant (polyethoxylated tallow amine), which is quite toxic *(68,69)*.

Glyphosate is generally distributed as water-soluble concentrates and powders. Mild poisoning results only in gastrointestinal symptoms, such as vomiting, abdominal pain, diarrhea, and nausea, which usually resolve within a day or two. Severe poisoning results in intestinal hemorrhage and ulceration, acid base disturbances, renal failure, hypotension, cardiac arrest, pulmonary dysfunction, convulsions, coma, and death.

Postmortem findings are nonspecific. Glyphosate and the concomitant surfactant are demonstrated by toxicological analysis in the gastric contents and other visceral organs. Glyphosate levels of 1 mg/mL or more can be detected postmortem in blood, liver, and urine in less than a minute by using ^{31}P nuclear magnetic resonance *(70)*.

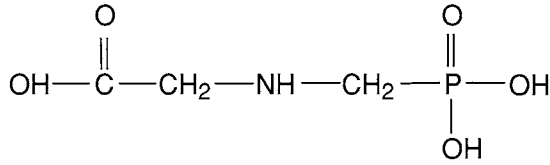


Fig. 14. Glyphosate.

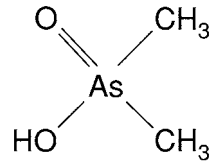


Fig. 15. Cacodylic acid.

3.7. Arsenical Herbicides

Among the several arsenical herbicides available are cacodylic acid, calcium hydrogen methylarsonate, disodium methylarsonate, hexaflurate (AsF_6K), methylarsonic acid, monoammonium methylarsonate, monosodium methylarsonate, potassium arsenite, and sodium arsenite.

Cacodylic acid (Fig. 15) is also known as dimethylarsinic acid. Cacodylic acid is a white crystalline substance, readily soluble in water and alcohol, and is still used as an herbicide. When it unites with metals and organic substances, it forms salts known as cacodylates. Cacodylic acid contains 54.3% of arsenic.

4. Fungicides

Fungicides, or antimycotics, are toxic substances used to kill or inhibit the growth of fungi that cause economic damage to crop or ornamental plants. Most fungicides are applied as sprays or dusts. Seed fungicides are applied as a protective covering before germination. Systemic fungicides, or chemotherapeutants, are applied to plants, where they become distributed throughout the tissue and act to eradicate existing disease or to protect against possible disease.

Bordeaux mixture ($CuSO_43Cu[OH]_23CaSO_4$) was one of the earliest fungicides to be used *(71)*. Bordeaux mixture is a liquid composed of hydrated (slaked) lime, copper sulfate, and water. It was accidentally discovered in 1882

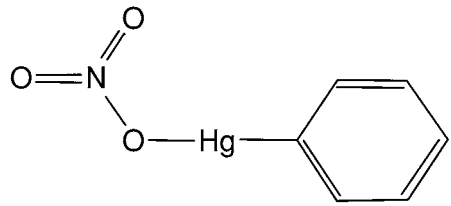


Fig. 16. Phenylmercury nitrate.

in the Modoc region of France, where farmers, tired of schoolboys pilfering their grapes, sprayed their grapevines with a poisonous-looking mixture of lime and copper sulphate; it was a desperate idea meant just to deter schoolboys from stealing their grapes. However, in 1882, PMA Millardet from the University of Bordeaux observed that the very same mixture effectively controlled the downy mildew of grapes as well.

Burgundy mixture is a mixture of copper sulfate and disodium carbonate. Both bordeaux mixture and burgundy mixture are still widely used to treat orchard trees. Copper compounds and sulfur have been used on plants separately and together. Synthetic organic compounds are now more widely used because they give protection and control over many types of fungi.

Cadmium chloride and cadmium succinate are used to control turfgrass diseases. Mercury(II)chloride, or corrosive sublimate, is used as a dip to treat bulbs and tubers.

Mercury salts used as fungicides include mercurous chloride, mercuric chloride, mercuric oxide, phenylmercury nitrate (Fig. 16), tolylmercury acetate, and ethylmercury bromide.

Organophosphorus fungicides include ampropylfos, ditalimfos, edifenphos, and fosetyl (Fig. 17). Carbamate fungicides include benthiavalicarb, furophanate, iprovalicarb, and propamocarb (Fig. 18); the toxicity of organophosphates and carbamates has been dealt with earlier.

Among the most important inorganic fungicides are potassium azide, potassium thiocyanate, sodium azide, and sulfur. Other substances occasionally used to kill fungi include chloropicrin, methyl bromide, and formaldehyde. Many antifungal substances occur naturally in plant tissues. Creosote, obtained from wood tar or coal tar, is used to prevent dry rot in wood.

The most important fungicides—from the toxicological viewpoint—aside from organophosphorus and carbamates, are sodium azide and compounds of copper and mercury. Copper compounds are also especially important because they are used in agriculture as insecticides and algicides.

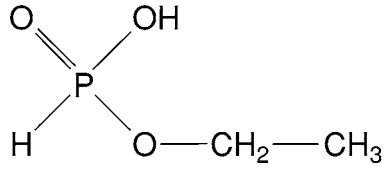


Fig. 17. Fosetyl, an organophosphate fungicide.

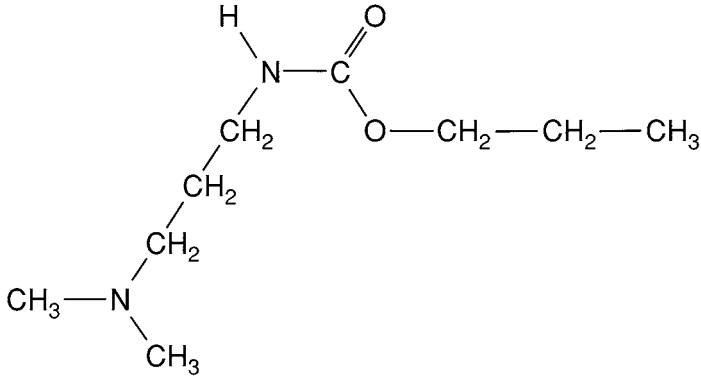


Fig. 18. Propamocarb, a carbamate fungicide.

Somerville discussed the metabolism of several fungicides including maneb, mancozeb, zineb, captan, chlorothalonil, benomyl, triadimefon, triadimenol, and cymoxanil *(72)*.

4.1. Sodium Azide

Sodium azide is important because it is a potential intentional or accidental poison. Aside from being used in agriculture, sodium azide is also used widely in hospitals where it is used as a component chemical in the fluid used to dilute blood samples. Sodium azide, like DNP, is an "uncoupler" of oxidative phosphorylation; it also inhibits the enzymes catalase and cytochrome oxidase.

Ingestion of sodium azide results in nausea, vomiting, diarrhoea, hypotension, and CNS symptoms, such as headache, hyporeflexia, seizures, and coma. Postmortem findings include edema of the brain and lungs. Edema of the myocardium with myocardial necrosis has also been reported *(73)*.

4.2. Copper

Salts of copper, although mostly used as fungicides, are used for a large number of other purposes in agriculture as well. Copper acetate, copper carbonate, cupric 8-quinolinoxide, copper silicate, and copper zinc chromate are used as fungicidal agents only; copper arsenate is used as insecticide and copper sulfate as algicide, fungicide, herbicide, and molluscicide; copper acetoarsenite is employed as insecticide and molluscicide; copper hydroxide is used as bactericide and fungicide; copper naphthenate is used as fungicide and mammal repellent; copper oleate as fungicide and insecticide; and copper oxychloride as bird repellent and fungicide.

Chronic exposure to Bordeaux mixture in vineyard sprayers causes the so-called "vineyard sprayer's lung." Observed mainly in Portugal, the disorder includes pulmonary fibrosis *(74)* and may lead to lung cancer *(75,76)*. Bordeaux mixture is the only other significant pesticide aside from paraquat that induces significant pulmonary fibrosis with organophosphates coming in a distant third *(77)*. The radiological picture in vineyard sprayer's lung resembles that of silicosis with micronodular features in the early stages of the disease *(76)*. Only in later stages does a picture of massive fibrosis emerge with continuing development of respiratory insufficiency.

Plamenac et al. *(78)* examined the sputum of rural workers engaged for years in spraying of vines. Sputum specimens were tested for copper by rubeanic acid. Macrophages containing copper granules in their cytoplasm were found in 64% of the workers engaged in vine spraying compared with none in a control group. Other abnormalities, such as eosinophils, respiratory spirals, respiratory cell atypia, and squamous metaplasia, were also found in the sputum. Atypical squamous metaplasia was observed in 29% of vineyard workers who were also smokers *(78)*. Eckert et al. *(79)* exposed mice to copper sulfate aerosol for a longer period of time and were able to replicate these changes in the animals' lungs. The authors concluded that the changes seen in vineyard sprayer's lung are a result of copper sulfate toxicity.

Pimentel and Menezes studied the liver of vineyard sprayers by percutaneous biopsy and also at autopsy *(80)*. They found histiocytic and noncaseating granulomas containing inclusions of copper as identified by histochemical techniques. They also found that the affected individuals were prone to liver fibrosis, cirrhosis, angiosarcoma, and portal hypertension *(81)*.

Copper sulfate is a popular suicidal poison in India *(82)* and copper sulfate was once a very popular homicidal poison *(83)*. Although no reports of suicide and homicide with Bordeaux mixture exist, this is certainly possible. Quite possibly such cases did, and still do, occur but have never been reported.

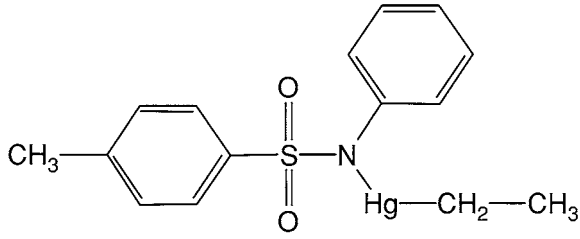


Fig. 19. *N*-ethylmercury-*p*-toluenesulphonanilide.

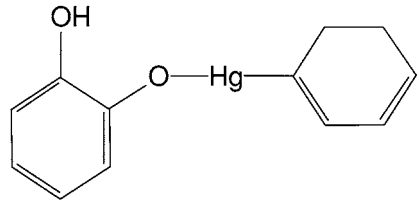


Fig. 20. Phenylmercury derivative of pyrocatechol.

4.3. Mercury

Mercury is widely used as a fungicide in agriculture. Both inorganic and organic salts are used. Inorganic mercury fungicides being used as fungicides include mercuric chloride, mercuric oxide, and mercurous chloride. Organomercury fungicides include (3-ethoxypropyl)mercury bromide, ethylmercury acetate, ethylmercury bromide, ethylmercury chloride, ethylmercury 2,3-dihydroxypropyl mercaptide, ethylmercury phosphate, *N*-(ethylmercury)-*p*-toluenesulphonanilide (Fig. 19), hydrargaphen, 2-methoxyethylmercury chloride, methylmercury benzoate, methylmercury dicyandiamide, methylmercury pentachlorophenoxide, 8-phenylmercurioxyquinoline, phenylmercuriurea, phenylmercury acetate, phenylmercury chloride, phenylmercury derivative of pyrocatechol (Fig. 20), phenylmercury nitrate, phenylmercury salicylate, thiomersal (Fig. 21), and tolylmercury acetate.

The ingestion of wheat and barley seed treated with methyl mercury fungicides for sowing by a largely illiterate population in Iraq led to a major poisoning with mercury in 1971 to 1972 with a high fatality rate *(84)*. The seed—about 95,000 tons of it—was intended for spring planting; there had been ample warning that the seed was unfit for consumption, but this warning was disregarded. There was a latent period of several weeks after which pares-

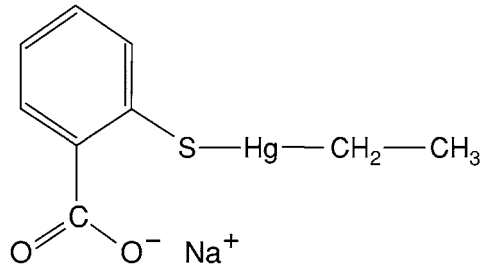


Fig. 21. Phenylmercury derivative of thiomersal.

thesias began to appear in several victims. Paresthesias involved lips, nose, and distal extremities. More serious cases progressed to ataxia, hyperreflexia, hearing disturbances, movement disorders, salivation, dementia, dysarthria, visual field constriction, and blindness. In the most severe cases, individuals remained in a mute rigid posture altered only by spontaneous crying, primitive reflexive movements, or feeding efforts. There were 6520 victims with 500 deaths *(85–88)*. Seven children remained permanently incapacitated both physically and mentally. This was the second major mercury disaster after the Minamata Bay disaster in Japan occurring between 1953 and 1960, when about 1200 people were poisoned and 46 died *(89)*.

Phenylmercury acetate has been found to be embryotoxic and teratogenic *(90)*.

4.3.1. Postmortem Findings in Mercury Poisoning

In deaths caused by acute mercury poisoning, the mucosa of the mouth, throat, esophagus and stomach is greyish in color showing superficial hemorrhagic erosions; a softened appearance of the stomach wall is characteristic. In cases where the patient survived a few days, the large bowel may show ulcerations. The kidneys appear pale and swollen owing to edema of the renal cortex. Microscopically, the kidneys usually demonstrate necrosis of the renal tubules *(23)*.

Sperhake et al. *(91)* reported the case of a 40-year-old chemist who died of mercury poisoning. An autopsy carried out 30 hours postmortem revealed unspecific signs of intoxication including severe edema of the lungs and brain, dilatation of the bowel, and marked congestion of the parenchymatous organs. The stomach contained 30 mL of a reddish fluid. Between the gastric folds, the mucosa appeared highly preserved with a brownish discoloration, but streak-like erosions in the exposed parts. The mucosal surface of the oral cavity and esophagus also appeared brownish and discolored. Histologically, the pre-

served areas of the gastric mucosa were totally unaffected by autolysis with an intact epithelial layer, whereas the eroded areas showed loss of mucosal lining with infiltrates of polymorphonuclear granulocytes and lymphocytes. Mercury was detected in the epithelial layer of the gastric mucosa *in situ* using 1,5-diphenylcarbazone staining (0.2% in 96% ethanol). Tubular necrosis was present in the kidneys.

4.4. Miscellaneous Fungicides

A case of chronic arsenic poisoning in a 75-year-old man has been described; the man used a sodium arsenite-based fungicide for cultivating his vine yard *(92)*.

5. Fumigants

5.1. Methyl Bromide

Methyl bromide (CH_3Br), also known as bromomethane, monobromomethane, embafume, or iscobrome, is mainly used as a gas soil fumigant against insects, termites, rodents, weeds, nematodes, and soil-borne diseases *(93,94)*. It has been used to fumigate agricultural commodities, mills, grain elevators, ships, furniture, clothes, and greenhouses. Its main advantages are its effective penetrating power and absence of danger of fire or explosion hazards.

Methyl bromide acts rapidly, controlling insects in less than 48 hours in space fumigations, and it has a wide spectrum of activity, controlling not only insects but also nematodes and plant-pathogenic microbes *(95)*. About 70% of methyl bromide produced in the United States goes into pesticidal formulations. Pure methyl bromide is a colorless gas that is heavier than air. Odorless and tasteless in low concentrations, it has a musty, acrid smell in high concentrations. Occupational exposure to methyl bromide also occurs frequently. It is estimated that about 75,000 American workers are occupationally exposed to this gas annually. Its toxicity is severe and, despite safeguards, cases of acute and chronic intoxication occur, mainly in the fruit and tobacco industries.

The maximum allowable concentration of methyl bromide is 15 ppm. Concentrations of 70 ppm or less are considered safe. Death has been reported to occur at 8000 ppm *(96)*.

Methyl bromide can enter homes through open sewage connections, thus causing fatalities. Lagard et al. *(97)* reported an interesting case of methyl bromide poisoning where methyl bromide caused toxicity in this manner. The sewage pipes serving two houses (one house was fumigated and in the other the

poisoning occurred) had been sucked empty only 1 to 2 hours prior to the start of fumigation.

Because it depletes ozone into the atmosphere *(95)*, methyl bromide has been banned in several industrialized countries, except for exceptional quarantine purposes. Phosphine, sulfuryl fluoride (*see* Subheading 5.2.), and carbonyl sulfide are considered viable alternatives.

5.1.1. Postmortem Findings and Histopathology

The mucosa of trachea and bronchi is congested and shows petechial hemorrhages. The lungs show subpleural hemorrhages and pulmonary edema. Bilateral bronchopneumonia may also be present.

The brain is edematous with necrosis of cortical cells, especially in the frontal and parietal lobes. Multiple perivascular hemorrhages may be detected throughout the brain and small subarachnoid hemorrhages may be seen in some cases.

Circumscribed hemorrhages may also be present in stomach, duodenum, myocardium, spleen, and retina. The kidneys are acutely congested and show tubular necrosis on the micromorphological level; the proximal tubules are most commonly affected. In severe cases, the loops of Henle and the distal tubules are also affected. The liver is also congested, but liver cell necrosis is not a common feature *(96)*. Methyl bromide can be detected and quantitatively determined in various biological samples by headspace gas chromatography *(98)*.

5.2. Sulfuryl Fluoride

Sulfuryl fluoride (F_2O_2S) is an important agricultural fumigant. According to the 2002 annual report of the AAPCC *(15)*, the only death that occurred as a result of fumigants was caused by sulfuryl fluoride (Fig. 22). It is an inorganic gas fumigant used in structures, vehicles, and wood products for control of drywood termites, wood-infesting beetles, and certain other insects and rodents. It is also used as a gas fumigant for postharvest use in dry fruits, tree nuts, and cereal grains. It is available under the trade name Vikane™ gas fumigant.

Because methyl bromide has now been graded as an ozone-depleting substance and is being gradually phased out, sulfuryl fluoride is taking its place. Because sulfuryl fluoride is an inorganic material, as opposed to the organic methyl bromide, it does not bind onto items being protected and therefore, less quantities of gas are required for the same insecticidal effect.

Sulfuryl fluoride is a colorless and odorless gas. It does not cause tears or immediately noticeable eye irritation and lacks any other warning property. Chloropicrin is added to products containing sulfuryl fluoride to serve as a

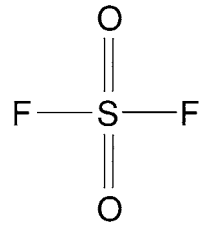


Fig. 22. Sulfuryl fluoride.

warning indicator; chloropicrin is a gas that causes eye and respiratory irritation and vomiting.

Sulfuryl fluoride acts as a CNS depressant. Symptoms of poisoning include itching, numbness, depression, slowed gait, slurred speech, nausea, vomiting, stomach pain, drunkenness, twitching, and seizures. Inhalation of high concentrations may cause respiratory tract irritation and respiratory failure. Skin contact with sulfuryl fluoride normally poses no hazard, but contact with liquid sulfuryl fluoride can cause pain and frostbite-like lesions owing to rapid vaporization. Occupational sulfuryl fluoride exposure may be associated with subclinical effects on the CNS, including effects on olfactory and some cognitive functions *(99)*. The oral LD_{50} for sulfuryl fluoride in rats and guinea pigs is 100 mg/kg.

Scheuerman has reported two cases of suicide by sulfuryl fluoride *(100)*. According to Scheuerman, toxicological analysis should include a plasma and urine fluoride level because the toxic effects of sulfuryl fluoride are probably related to this ion. Concentrations of fluoride in his cases were 20 and 50.42 mg/L, respectively. However, all values have to be interpreted in the light of all information available (kind and length of exposure, symptoms, autopsy findings, etc.) in a given case.

6. Agrochemical Poisoning Associated With Grain Preservation

6.1. Aluminum Phosphide

Aluminum phosphide (AlP) is an ideal grain preservative for a number of reasons. It is highly toxic to almost all stages of insects with remarkable penetration power. AlP dissolves well in water, oil, and fat. It is considered an ideal seed fumigant since the seeds' viability is not affected and is practically free from residual toxic hazards—provided the seeds have less than 20% water content. AlP is minimally absorbed and easily desorbed from the treated commod-

ity, such as wheat grains. It is inflammable at the prescribed dosage and devoid of tainting on fumigated stock. It has a distinct odor, which has been described as a fishy odor. Because of this and also because of delays in evolving, phoshine provides considerable safety in handling this fumigant. Safety in handling is due to both these reasons. Because it has an odor, it is difficult for handlers to accidently ingest it. Because the tablet generates the predetermined weight of gas, it is very convenient to administer the exact dose. Cost of fumigation is low and its effects on the fumigated stock last longer. AlP is easy to transport and handle. Unfortunately, no specific antidote to AlP is known.

AlP is used very extensively throughout agrarian economies like India. On exposure to moisture it releases the poisonous phosphine, which percolates through the grain: $AlP+H_20$ _ $Al(OH)_3+PH_3$.

As long as the grain is stored in airtight godowns, the liberated phosphine remains in the environment, repelling all pests. When the grain is to be used, it is brought out and aerated. This releases phosphine, leaving behind virtually no or only nontoxic residues.

AlP is generally available as tablets (Alphos®, Celphos®, Fumigran®), which are dark brown or grayish in color, 3 g in weight, and measuring 20 mm in diameter and 5 mm in thickness. They come in an aluminum container containing ten tablets. AlP is also available as 0.6-g pellets. The tablets are composed of pure AlP (the active ingredient) and ammonium carbamate/carbonate (the inert ingredient). The ratio of the active and inert ingredient is generally about 56:44. On contact with moisture, each 3-g tablet evolves about 1 g of phosphine along with carbon dioxide and ammonia, which prevents self-ignition of phosphine gas. This is why it is also called a "protective gas." Carbon dioxide and ammonia are liberated by combination of water with other inert ingredients in the tablets. The main function of the inert ingredients is to produce these gases, so phosphine may not ignite easily. The phosphine gas, once liberated, spreads quickly and kills insects and rodents almost in all stages of their development. After complete decomposition of the tablet, AlP is left behind as a harmless and nontoxic grayish white residue, which is less than 25% of the original tablet weight.

AlP is the leading cause of accidental and suicidal deaths in India *(101–105)*. It has been implicated in several homicides including dowry deaths (deaths of newlywed brides occurring in relation to dowry and covered under Section 304 B of the Indian Penal Code).

6.1.1. Postmortem Findings

The mortality rate for poisoning with AlP is almost 50% *(106)*. There is an intense garlic-like odor emanating from the mouth and after opening of the stomach at autopsy. All internal organs are congested and show petechial hem-

orrhages. Pericarditis may be present *(107)*. The stomach contents are hemorrhagic and the mucosa shows detachment. Residues of AlP may be demonstrable in the stomach contents, but rarely can AlP itself be detected because it readily reacts with acid and water within the stomach.

Misra et al. *(108)* described eight cases of AlP poisoning after ingestion of AlP tablets for attempting suicide; the mean age of the patients was 23 years (age range 14–25 years). Six of the patients died; the mean hospital stay was 19 hours (range 4–72 hours). An autopsy was carried out in two patients, revealing pulmonary edema, congestion of the gastrointestinal mucosa, and petechial hemorrhages on the surface of liver and brain.

Anger and co-workers *(109)* reported the case of a 39-year-old man who committed suicide by ingestion of AlP. Autopsy revealed signs of asphyxia with marked visceral congestion. The authors also toxicologically analyzed peripheral blood, urine, liver, kidney, adrenal, brain, and cardiac blood. Phosphine gas was absent in peripheral blood and urine but present in the brain (94 mL/g), the liver (24 mL/g), and the kidneys (41 mL/g). High levels of phosphorus were found in the blood (76.3 mg/L) and liver (8.22 mg/g). Aluminum concentrations were highly elevated in peripheral blood (1.54 mg/L), brain (36 µg/g), and liver (75 µg/g) compared with the reference values.

6.1.2. Histopathology

Histopathological findings in AlP poisoning have been described in detail by Chugh et al. *(106)*. Various viscera show congestion, edema, and inflammatory cell infiltration. In the myocardium, there are patchy areas of necrosis, whereas the liver shows fatty changes and the lung parenchyma displays gray/red hepatization. The adrenal cortex shows complete lipid depletion, hemorrhage, and necrosis. Chugh et al. assumed that the changes in the adrenal cortex could be both a sequel of shock and/or a cellular toxic effect of phosphine. In 20 out of the 30 patients studied by Chugh and associates, there was a significant rise in the plasma cortisol level (>1048 nmol/L). In the remaining 10 patients, the adrenal cortex was critically involved and the cortisol level failed to rise beyond normal levels (<690 nmol/L).

Pillay *(23)* noted that in AlP poisoning the heart shows features of toxic myocarditis, necrosis may be seen histologically in both liver and kidneys, and the lungs may demonstrate evidence of adult respiratory distress syndrome (ARDS). ARDS has also been reported by Chugh et al. *(110)*. The dose of the intoxicant in Chugh's cases varied from two g) to three tablets (corresponding to 6 and 9 g, respectively). All patients were in shock at admission and developed ARDS within 6 hours after ingestion of AlP. According to these authors, the exhalation of phosphine (which they detected by a positive silver nitrate paper test) was the possible noxious triggering factor in developing ARDS.

In Misra at al.'s series *(108)*, histopathological changes included pulmonary edema, desquamation of the lining epithelium of the bronchioles, vacuolar degeneration of hepatocytes, dilatation and engorgement of hepatic central veins and sinusoids, as well as hepatocytes showing nuclear fragmentation.

In Anger's single case *(109)*, microscopic examination revealed congestion of inner organs and pulmonary lesions that were attributed to asphyxia.

6.2. Silo Filler's Disease

Silo filler's disease is another disorder associated with agrochemical poisoning during preservation. Corn used for silage is usually grown under conditions of heavy sunlight and drought and its nitrate content is usually very high. When this silage is stored in a silo, the nitrates are fermented into nitrites, which in turn combine with organic acids to form nitrous acid. Nitrous acid decomposes into water and a mixture of nitrogen oxides. These are nitric oxide (NO), nitrogen dioxide, and dinitrogen tetroxide. The decomposition starts within approx 4 hours of putting the crops into the silo and continues for about 10 days. When entering these silos (which virtually turn into a kind of gas chamber), farm workers may suffer acute poisoning from these gases, and many such deaths have occurred. This type of death in a silo was first described in 1914, but at that time it was wrongly attributed to asphyxia *(111)*.

6.2.1. Pathophysiology

NOs, being relatively poor soluble in water, can reach the terminal bronchioles and even alveoli. Within the lungs, the NOs react with water to form nitrous and nitric acids, which cause extensive lung damage, resulting in chemical pneumonitis and profuse pulmonary edema. NOs trigger histamine release, which causes bronchoconstriction resulting in increased airway resistance.

6.2.2. Postmortem Findings

Douglas and colleagues *(112)* examined 17 patients of silo filler's disease between 1955 and 1987. All exposures had occurred in conventional top-unloading silos. Acute lung injury occurred in 11 patients, one of whom died. In the fatal case, autopsy findings included early diffuse alveolar damage with hyaline membranes, hemorrhagic pulmonary edema, and acute edema of the airway walls.

7. Fertilizers

Poisoning with and fatalities owing to fertilizers are rarely encountered but do occur. The 2002 annual report of the AAPCC Toxic Exposure Surveillance System reported one death caused by fertilizers *(15)* (Table 6).

Table 6
Exposures to Various Different Categories of Fertilizers and One Fatal Case

Fertilizer	Number of toxic exposures	Deaths
Household plant food	3533	0
Outdoor fertilizer	4554	0
Plant hormone	90	0
Other	1858	0
Unknown	597	1
Total	**10,632**	**1**

Data taken from ref. 15.

Used as a fertilizer, anhydrous ammonia is a respiratory irritant, which, in high doses, causes pulmonary edema (113). Exposure most often occurs during transfer operations. Ammonia reacts with water to form the strong alkali ammonium hydroxide, which causes severe tracheobronchial and pulmonary inflammation with bronchiolitis obliterans. Normally, the peculiar odor of ammonia warns the potential victim. During World War II, in London, a brewery cellar having ammonia-carrying condenser pipes was temporarily converted into a bomb shelter. During a bombing, a bomb fragment pierced one such pipe resulting in a mortality rate of the affected individuals as high as 63% (114).

Saito et al. (115) described the case of a 70-year-old male who presumably consumed water contaminated with a nitrate fertilizer. On admission to hospital, the man showed drowsiness, deep cyanosis, and dyspnea; the patient died 7 hours later. At autopsy, no particular morphological changes were noted except for the blood being a chocolate-brown color. Postmortem toxicology of the blood revealed a methemoglobin concentration of 78% and the concentrations of nitrate and nitrite were 1.50 and 0.76 µg/mL, respectively. In deaths caused by nitrate fertilizers, methemoglobinemia and the presence of appreciable quantities of nitrites and nitrates may be demonstrated in cardiac blood and gastric contents (stored at –80°C until toxicological analysis) (115). Capillary gas chromatography-mass spectrometry and capillary gas chromatography with a nitrogen–phosphorus detector can be used to detect nitrates and nitrites in blood.

Sato and colleagues (116) described the case of an 85-year-old woman who supposedly consumed agricultural fertilizer containing ammonium sulfate. She was found lying dead on the ground outside her house. A thorough autopsy could not determine the cause of her death. A beer can was found next to her, and when it was examined, it was found to contain ammonium sulfate. Subsequently, ammonium and sulfate ions were detected in her serum samples and

gastric contents. The cause of her death was determined as poisoning by ammonium sulfate. In order to further confirm that this death was indeed a result of an ammonium sulfate fertilizer, the authors administered a total dose of 1500 mg/kg of ammonium sulfate to three rabbits. The animals developed mydriasis, irregular respiratory rhythms, and local and general convulsions until they came into respiratory failure with cardiac arrest. Electroencephalogram showed slow, suppressive waves and a high-amplitude with a slow wave pattern that is generally observed clinically in hyperammonemia in humans and animals. There was a remarkable increase in the concentration of ammonium ions and inorganic sulfate ions in the animals' serum and blood gas analysis showed severe metabolic acidosis. The authors suggested that when the cause of death can not be clearly determined and the previous history is suggestive of ammonium sulfate intake, measurement of ammonium ions, inorganic ions, and electrolytes in blood, as well as in stomach contents, are a prerequisite for the diagnosis.

Villar and co-workers reported poisoning and death in animals who drank fertilizer-contaminated water *(117)*. The water had been hauled in tanks previously contaminated with a nitrogen-based fertilizer.

In Udaipur, India, chronic fluorotic lesions in cattle and buffalo have been described following consumption of fodder and water contaminated by the fumes and dusts emitting from superphosphate fertilizer plants *(118)*. Similar lesions have been reported from Australia where the main source of fluoride appeared to have been gypsum that was included in a feed supplement and also ingested from fertilizer dumps on paddocks *(119)*. Gypsum fertilizers have caused several deaths in animals *(120)*. Similar morbidity and mortality may be seen in humans who drink contaminated water either intentionally or out of ignorance as well. The latter situation is quite possible among the uneducated farmers of agrarian economies.

Adrian *(121)* drew attention to a very unique situation of poisoning related to fertilizers. In several countries, sewage sludges are used on farms as fertilizers because they do contain these materials. However the sewage—not surprisingly—also contains industrial wastes, such as chromium, lead, zinc, cadmium, and mercury. When this sewage is used as fertilizing material, plants tend to concentrate these heavy metals, especially chromium. Ingestion of such farm produce may lead to heavy metal poisoning. Several other cases of fertilizer poisoning, especially among animals, have been reported, too *(122–129)*.

In several countries, poisonous plants, such as castor, are used as green manure which can cause poisoning of both humans and animals. Soto-Blanco and colleagues from the University of Sao Paulo, Brazil, described a case of canine poisoning where castor bean (*Ricinus communis*) cake was used as a fertilizer *(130)*. The authors stressed that these cakes may be accidentally ingested

by humans as well, and recommended that cake production should include heat treatment to denature the poisonous proteins.

8. *Miscellaneous*

Nematicides can cause poisoning in banana plantations. Wesseling and co-workers, studying pesticide-related illness and injuries among banana workers in Costa Rica, reported that workers at highest risk per time unit of exposure were nematicide applicators *(47)*.

Slugs are major pests of oilseed rape that are poorly controlled by conventional bait pellets. Therefore, compounds, such as metaldehyde and methiocarb, are used as seed dressings to control slugs *(131)*. Metaldehyde is a popular molluscicide that can cause fatal poisoning; the 2002 AAPCC annual report *(15)* mentions as many as 199 cases of exposure to this agent. Kiyota *(132)* reported the case of a 55-year-old mentally retarded man suffering from pica, who ingested about 2.7 g of metaldehyde. Despite medical treatment, he developed acute lung injury and died after 33 days; he was found to have ascites and splenomegaly. High-performance liquid chromatography revealed 80.6 µg/mL metaldehyde in the serum. Jones et al. *(133)* developed a method to detect metaldehyde in samples of stomach contents by gas chromatography-ion trap mass spectrometry for forensic toxicology investigations. A suicide attempt using metaldehyde was reported by Hancock and co-workers *(134)*. A case of homicide using metaldehyde has been described by Ludin *(135)*. Detailed overviews of metaldehyde toxicity have been provided earlier by Booze and Oehme *(136)* and Longstreth and Pierson *(137)*.

Avermectins used as acaricides (avermectin acaricides), insecticides (avermectin insecticides), and nematicides have been used for suicidal poisoning. Chung and co-workers *(138)* from Taiwan studied the clinical spectrum of avermectin poisoning reported to a Poison Center from September 1993 to December 1997. Eighteen patients with abamectin (Agri-Mek; 2% wt/wt abamectin) exposure and one with ivermectin (Ivomec; 1% wt/vol ivermectin) ingestion were identified (14 males, 5 females; age range 15–83 years). Fourteen out of the 18 patients had been exposed as a result of attempted suicide; one patient died 18 days later as a result of multiple organ failure.

Algicides have not been reported to cause fatal poisoning in humans; minor ailments owing to algicide exposure include, e.g., contact dermatitis *(139)*.

Aphicides are known to persist in crops *(140)*; their toxicity in house sparrows has been described in detail by Tarrant and co-workers *(141)*.

Bird repellants are trigeminally mediated avian irritants *(142)*. Toxic effects to humans have apparently not been reported so far.

Chemosterilants are chemicals that aim at destroying the fertility of pests. 1,2-dibromo-3-chloropropane is used to induce infertility in rats *(143)*. The chemosterilant bisazir is extremely hazardous. Ciereszko and co-workers *(144)* have recommended that special safety measures are necessary when handling this chemical. However, toxic effects to humans have not been reported in the medical literature so far.

Antifeedants are chemicals having tastes and odors that inhibit feeding behavior. Several chemicals, such as silphinene sesquiterpenes *(145)*, 1,3,4-oxadiazoles *(146)*, and ryanoid diterpenes *(147)*, are used as antifeedants; again, toxic effects to humans have not been reported so far.

Herbicide safeners are compounds protecting crops from herbicide injury by increasing the activity of herbicide detoxification enzymes such as glutathione-*S*-transferases *(148–150)* and cytochrome P-450s. Several herbicide safeners are used in agriculture such as benoxacor *(151)* and dichloroacetamide *(152,153)*; there toxicity in humans has not been reported so far.

Insect attractants attract or lure an insect to a trap. Several of them are available, such as Boll Weevil Attract and Control Tubes® (Plato Industries, Houston, TX) *(154)*, imidacloprid *(155)*, and GF-120 fruit fly bait *(156)*. Their toxicity has been studied in detail by Beroza et al. *(157)*.

The secondary effects of conventional insecticides on the environment, vertebrates, and beneficial organisms have caused a move to the use of more target-specific chemicals, such as insect growth regulators (IGRs) *(158)*. IGRs are chemicals disrupting the action of insect hormones controlling molting, maturity from pupal stage to adult, or other insect life processes. Several IGRs are known, such as halofenozide *(159)*, *S*-methoprene *(160,161)*, buprofezin *(162)*, tebufenozide *(163)*, the chitin synthesis inhibitors teflubenzuron, diflubenzuron *(164)*, and hexaflumuron, as well as the juvenile hormone mimic pyriproxyfen *(165)*. Halofenozide (RH-0345) is a novel nonsteroidal ecdysteroid agonist that induces a precocious and incomplete molt in several insect orders *(158)*. The antifeedant 1,3,4-oxadiazoles also show a considerable amount of IGR activity *(146)*. The toxicity of these antifeedants to animals has been studied by Wright *(166)*.

Pesticide synergists are chemicals that, although they do not possess inherent pesticidal activity, they nonetheless promote or enhance the effectiveness of other pesticides when used combined (synergism). Synergists usually increase the toxicity of a pesticide so that a smaller amount is needed to bring about the desired effect. This may reduce the cost of application. An example of a synergist is piperonyl butoxide, often used with pyrethrin, pyrethroid insecticides, rotenone, and carbamate-containing pesticides. Piperonyl butoxide is a liver toxicant and a possible human carcinogen *(167,168)*; it also inhibits T-cell activation and function *(169)*.

Avicide 4-aminopyridine (4-AP) is a rapidly fatal nervous system toxin. It dramatically enhances transmission at the neuromuscular junction and other synapses. 4-AP has been employed clinically in the treatment of prolonged paralysis caused by antibiotics and muscle relaxants and in the Eaton-Lambert syndrome. Spyker et al. *(170)* reported on three men who were poisoned with 4-AP. Bischoff et al. *(171)* more recently again stressed the fact that 4-AP can cause poisoning in humans and may cause seizures.

3-Chloro-*p*-toluidine hydrochloride (CPTH) is an aniline derivative registered as a selective, low-volume-use (<45 kg/yr) avicide. Rice baits are treated with CPTH to cause poisoning in birds harmful to crops *(172)*. CPTH may be mutagenic. Stankowski et al. *(173)* conducted three in vitro mutagenicity tests of CPTH according to methods recommended by the United States Environmental Protection Agency, e.g., the Ames/Salmonella assay, the Chinese hamster ovary (CHO)/hypoxanthine-guanine phosphoribosyl-transferase mammalian cell forward gene mutation assay, and the CHO chromosome aberration assay. They found that CPTH did not display mutagenic activity using the Ames/Salmonella or CHO/hypoxanthine-guanine phosphoribosyl-transferase assays. However, CPTH induced statistically significant, concentration-dependent, metabolically activated increases in the proportion of aberrant cells. The authors concluded that the results were suggestive of minimal mutagenicity effects associated with exposure to CPTH *(173)*. Stahl and co-workers draw attention to the consumption of CPTH treated rice baits by nontargeted bird species, such as pigeon (*Columbia livia*) and house sparrow (*Passer domesticus*). CPTH can persist in the breast muscle tissues of both targeted and nontargeted birds which may be a potential secondary hazard to scavengers and predators *(174)*. Toxicity of CPTH both in humans and animals has been discussed by several other authors as well *(175–179)*.

If a particular agrochemical poison has been banned in a country, it is not necessarily that poisoning with this agent will not be seen in that particular country. For example, in Japan, production of Azomite emulsion (an acaricide) has been stopped since 1973. However, Moriya et al. in 1991 *(180)* described a recent Azomite-related fatality. Poisoning with Azomite was confirmed when aramite and azoxybenzene, two effective components of Azomite emulsion, were detected in the patient's serum when qualitatively analyzed with gas chromatography-mass spectrometry. The authors concluded that even if an agrochemical poison is banned, the pathologist must still keep the possibility of its ingestion in mind.

Many times, it is not the active agricultural chemical that is responsible for poisoning but impurities (such as dioxin), surfactants (e.g., polyethoxylated tallow amine used with glyphosate) and adjuvants used along with the chemical. These adjuvants, or "inert" ingredients, could be solvents, stabilizers,

preservatives, sticking or spreading agents, or defoamers *(181)* and may constitute petrochemical solvents, such as acetone, fuel oil, toluene, and other benzene-like chemicals. These could sometimes be *more* toxic than the active ingredient. Rubbiani drew attention to several of these adjuvants and clinical syndromes produced by them *(182)*. According to Harry *(4)*, toxicity is often due to solvents or surfactants included in the composition of a formula used as an agricultural chemical. When the obligatory declaration on the label about identity and concentration of some of these substances is not provided by the actual legislation in a particular country, the problem becomes more acute. It is also often difficult to determine if the cause of the poisoning is the actual agricultural chemical itself or its adjuvants.

Metabolites are breakdown products that form when a pesticide is exposed to air, water, soil, sunlight, or living organisms and often the metabolite is more hazardous than the parent compound.

9. Medicolegal Aspects of Agrochemical Poisoning

An estimated three million cases of agrochemical poisoning are reported from around the world every year, making it one of most serious toxicological problems of the present times. An overwhelming majority of these—more than 90%—are reported from developing countries, such as India, presumably because these are predominantly agrarian economies. In the United Kingdom, pesticides are responsible for only about 1% of deaths *(183)*, whereas in United States, as seen in Table 3, the figure varies between 1 and 2%. The equivalent figures in India have been reported to be as high as 70% *(23)*. Figure 23 shows some common pesticides used in India.

9.1. Accidental Poisoning

Accidental poisoning may occur in a number of ways. Accidental poisoning can occur if the insecticide is stored inadvertently with foodstuffs *(184)*. One of the most shocking cases of mass agrochemical poisonings occurred in the Indian state of Kerala in 1958 (known popularly as the "Kerala food poisoning case of 1958") when bags of foodstuffs, such as wheat and sugar, were inadvertently stored together with those of Folidol (parathion) in the same cabin on a ship *(23)*. The insecticide leaked and contaminated the foodstuffs; more than 1000 people were accidentally poisoned when they consumed these contaminated foodstuffs. Out of these, more than 100 people died.

Mixing of pesticides with foodstuffs may be intentional, albeit entirely because of ignorance and without any criminal intent. Such a case came to notice in the late 1970s in Lakhmipur in Kheri district, in the Indian state of

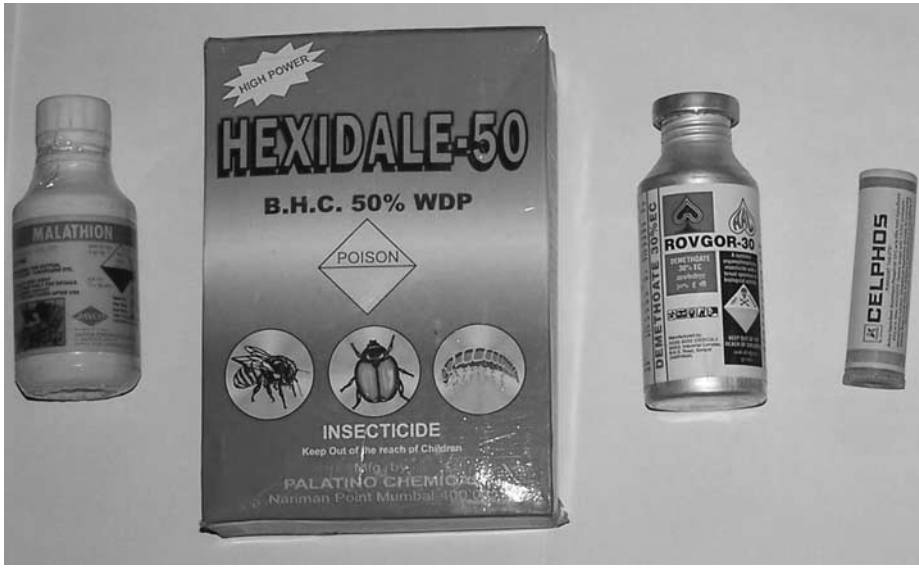


Fig. 23. A smorgasbord of pesticides used in India. (Courtsey of Dr. Avneesh Gupta and Dr. Puneet Setia, Maulana Azad Medical College, New Delhi, India.)

Uttar Pradesh. Farmers in this state were found to be preserving food grains with benzene hexachloride. A severe convulsive epidemic broke out among several hundred people because of this ignorance and more than 250 people died.

In 1997, improper use and application of benzene hexachloride in the town Sunser in the Indian state of Madhya Pradesh resulted in many people falling ill. Fortunately, no human died, but there were reports of several bird casualties.

In March 1999, a case of agricultural poisoning from India was reported where an entire family was poisoned owing to leakage of pesticides into cereal (sorghum/jowar) stored in the same room *(185)*.

The Indian state of Kerala is a major cashew growing region. There have been attempts at aerial spraying of this cash crop with endosulphan. Because these areas are close to local residential areas, deleterious effects occurring in humans have caused a major controversy in recent times *(23)*.

Pillay *(40)* suggests that accidental poisoning due to pesticides can occur in four different scenarios: (a) occupational exposure among agriculturists and those engaged in the task of pesticide spraying, (b) contamination of foodstuffs on account of negligence, (c) inadvertent ingestion by children, and (d) reusing

pesticide containers for storing food or drink (the latter is very common among third-world countries).

Instances of fatalities among agricultural workers due to accidental exposures have been reported from time to time *(186)*. Accidental poisoning owing to some pesticides, such as paraquat, occurs in a number of scenarios, e.g., when the mouthpiece of fumigation equipment is sucked by the operator while cleaning and it is suddenly cleared of obstruction, confusion under the influence of alcohol, consumption of contaminated water or foods, accidental ingestion by children, and accidental cutaneous exposure or oral topical application for toothaches by ignorant persons *(187)*.

Robert G. Book of Bloemfontein, South Africa, reported a unique case of accidental poisoning with paraquat: a young woman tried to "achieve a high" by spiking her Coca-Cola with paraquat. She died after a few days of hospitalization. At the time of her admission she had told the doctor that her husband had maliciously put paraquat in her drink a few days before; however, only 2 days later she changed her version as just mentioned *(188)*. It is noteworthy that in India it is very common for married women at the time of their death to shield their murderous husbands by making such statements. Whether the woman's first or second statement was correct is anybody's guess.

According to Harry *(4)*, accidental pesticide intoxications are mainly caused by ingestions of diluted fertilizers, low-concentration antivitamin K rodenticides, ant-killing products, or granules of molluscicides containing 5% metaldehyde, whereas voluntary intoxications are mostly by chloralose, strychnine, organophosphorus or organochlorine insecticides, concentrated antivitamin K products, and herbicides, such as paraquat, chlorophenoxy compounds, glyphosate, and chlorates.

9.2. Suicidal Poisoning

Suicidal poisoning with agrochemicals, especially organophosphates and AlP, is very common in countries like India. One of the main reasons is the easy availability of these agrochemicals. Many companies now add an emetic to dangerous agrochemicals, such as paraquat and AlP. Addition of a "stenching" agent to paraquat has apparently not deterred suicidals from consuming this poison.

9.3. Homicidal Poisoning

9.3.1. Organophosphorus and Organochlorines

Homicidal poisoning with organophosphorus compounds is possible and from time to time, one gets to hear or read about cases of a homicide commit-

ted with these substances. Svraka and colleagues have described four cases of homicide with organophosphorus compounds *(189)*.

However, homicidal poisonings with organophosphorus compounds are rare because of the unpleasant taste of most agrochemicals, especially of organochlorines, such as endrin, but they have been mixed with alcohol, especially Toddy (a strong liquor that is very popular in India), which masks its smell and has been used with organophosphorus compounds for homicidal purposes in this way.

Homicidal poisoning with parathion is much easier *(190–193)*. To prevent this, a coloring agent, such as indigocarmine, is added to parathion. This is, however, not a universal practice. In India for instance, addition of indigocarmine to parathion is not practiced.

9.3.2. Paraquat

The commonly used herbicide paraquat is odorless and gives rise to symptoms mimicking viral pneumonitis. These two properties—classically hailed as the properties of an ideal homicidal poison—make it very attractive as a homicidal poison. Paraquat is supposed to have a burning taste, but this can be masked in hot liquids or spicy foods *(194)*. Several homicide cases with paraquat undoubtedly must have gone unnoticed.

Teare and Teare and Brown *(46,195)* described five cases of paraquat poisoning, of which, two were homicidal in nature. The first is a well-documented case (*Reg vs Kenyon and Roberts*) in which a 22-year-old man, Keith William Kenyon, was killed by his wife Jennifer Kenyon and her friend, David Roberts, a consultant on the effects of agricultural chemicals. She purchased Gramoxone along with her friend Olive Hemming (who turned out to be the chief prosecution witness) from a farm shop, and most likely administered it to her husband in repeated small doses. Kenyon was taken ill on November 18, 1973 and died 13 days later, on December 1. During his illness, he displayed all the classical symptoms and signs of paraquat poisoning. Postmortem examination confirmed death by paraquat intoxication. Mrs. Kenyon was convicted of murder, whereas David Roberts was acquitted because of lack of evidence against him *(195)*. The second case occurred only 1 month later. After Christmas 1973, on the Falkland Islands, four local agricultural workers had been having a Boxing Day party when some Gramoxone was slipped for some unknown reason into one of their beers. The man died after displaying typical symptoms of paraquat poisoning. Autopsy confirmed poisoning by paraquat. Criminal charges against the other three laborers were contemplated, but eventually it was decided to drop them.

Paul *(196)* described the case of a 28-year-old woman who killed her husband by mixing paraquat in his steak-and-kidney pie twice. When he developed

a sore throat and was prescribed medicine for treatment, she mixed paraquat in the medicine as well. The husband died on June 27, 1981 after suffering a 24-day illness. The cause of death was attributed to cardiac arrest in combination with renal failure and bilateral pneumonia and it was only by a curious chain of circumstances that paraquat was detected in the young man's tissues preserved in the mortuary in a bucket, 8 months after the man's death. His wife and her paramour were found guilty and sentenced.

Stephens and Moormeister from the Medical Examiner's Office of San Francisco, CA, reported four cases of homicidal poisoning by paraquat *(194)*. Of these, the first three murders were perpetrated by one man against members of his immediate family, and the fourth case was equivocal—it could either have been suicide or homicide. The first three murders were committed by a man who had been married five times. His first three wives were alive and healthy. When the fourth wife threatened to divorce him, she found herself ill and died 24 days after the onset of her illness (19 days after hospitalization). Eight years later, when his fifth wife threatened divorce, she suffered the same fate, and a few months later, his 79-year-old mother also died. All three showed typical symptoms of paraquat poisoning. The postmortem findings seemed to suggest natural disease of the lungs. Although a suggestion of paraquat poisoning was made in all three cases, the concerned pathologist was reluctant to sign death certificates as paraquat poisoning. Toxicological analysis in the second and third cases revealed the presence of paraquat in the victims' tissues and this resulted in conviction of the murderer. It was found that the defendant worked as a mechanic on a large agricultural ranch and had easy access to paraquat; his thumb print was found on one of the opened paraquat containers, although he had earlier denied having to do anything with those containers. The fourth case involved a 27-year-old man, a registered herbicide and pesticide user, who had marital difficulties with his aggressive, "shrew-like" wife who also stood to benefit from a large insurance policy upon his death. While in hospital, the victim denied suicidal ingestion; he died 21 days after the start of his illness. No testing of toxic effects from the compounds he worked with was ever performed, nor was any consideration given to this possibility. The case did not result in court charges for anyone. Stephens and Moormeister concluded that the reason why such cases will often go unnoticed is because of the reluctance on the part of both clinicians and forensic pathologists to even think in the direction of paraquat poisoning when they see such a clear and typical picture of "viral pneumonia." In their opinion, the clinician should suspect paraquat ingestion in all cases in which there is progressive pulmonary involvement with no features of viral infection *(194)*. The pathologist conducting the postmortem would do well to go through the clinical history, if available, in detail to rule out

the possibility of paraquat poisoning. In all doubtful cases, a full toxicological analysis should be done and the tissues should be particularly analyzed for paraquat.

Daisley and Simmons from the University of the West Indies in Trinidad reported two cases of homicide by paraquat poisoning *(197)*. Both cases occurred in children and the common clinical presentations were gastrointestinal ulceration and acute respiratory distress with pneumomediastinitis. At autopsy, the most prominent finding was bullous lung emphysema. The authors stress that pathologists should be aware of this finding because they feel that if this autopsy finding is seen combined with the typical clinical presentation mentioned in Sections 3.2.1. and 3.2.3., it is almost diagnostic of acute paraquat poisoning. Da Costa et al. have dealt with the medicolegal aspects related to paraquat poisoning in detail *(198)*.

9.3.3. Sodium Chlorate

Another weed killer that has been used commonly for homicidal purposes is sodium chlorate. In *Reg vs Hargreaves*, Hampshire (Winchester) Assizes, April 1962, a 54-year-old woman was charged with the murder of a 78-year-old man whom she had known for the last 50 years as an uncle. In August 1960, he made his last will, written out by the accused in her favor. On January 10, 1961 the accused bought the weed killer sodium chlorate apparently for a friend who was a gardener. On January 19, 1961, the old man died and the postmortem examination showed signs of death from sodium chlorate poisoning. The victim had consumed beer and the remaining beer in the mug contained some 65 mg of sodium chlorate. The jury found the woman guilty of manslaughter and sentenced her to 18 months of imprisonment *(199)*.

9.4. Agent Orange

One of the biggest and most well-known medicolegal controversies in connection with herbicides has been that of Agent Orange. Agent Orange is the name given to a mixture of herbicides that United States military forces sprayed in Vietnam from 1962 to 1971 during the Vietnam War for the dual purpose of destroying crops that might feed the enemy and defoliating forest areas that might conceal Viet Cong and North Vietnamese forces.

The defoliant consisted of approximately equal amounts of the unpurified butyl esters of 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Agent Orange also contained small, variable proportions of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin—commonly known as dioxin—which is a byproduct of the manufacture of 2,4,5-T and is toxic even in minute quantities; dioxin is considered one of the most toxic compounds synthesized by humans.

Agent Orange was delivered in 55-gallon drums with an orange stripe to distinguish the drums visually from those containing other chemical agents (hence the name). About 50 million liters of Agent Orange were sprayed over Vietnam from low-flying aircrafts. Among the Vietnamese, it is considered to be the cause of an abnormally high incidence of miscarriages, skin diseases, cancers, birth defects, and congenital malformations (often extreme and grotesque).

Alterations in manufacturing procedures had reduced the dioxin content in Agent Orange later to minimal levels. Today, 2,4,5-T registrations have been cancelled and Agent Orange was voluntarily removed by the manufacturers in 1985.

Many United States, Australian, and New Zealand servicemen who suffered long exposure to Agent Orange in Vietnam later developed cancer and other health disorders. A class-action lawsuit was brought against seven herbicide makers that produced Agent Orange for the United States military. The suit was settled out of court with the establishment of a $180,000,000 fund to compensate some 250,000 claimants and their families. Separately, the United States Department of Veterans Affairs awarded compensation to about 1800 veterans.

Agent Orange has now been replaced by Agent White, a mixture of 2,4-D and picloram, which is longer lasting and more effective.

9.5. Pesticides and the Law

In the United States, The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) was passed in 1962 (amended in 1974, 1978, and 1988 *[200]*). This act divides all pesticides in four broad classes depending on their toxicity. The label of each pesticide has to contain a signal word depending on its toxicity. The criteria established by the FIFRA are given in Table 7.

According to the FIFRA, toxic category I pesticides must have the signal words DANGER and POISON (in red letters) and a skull and crossbones prominently displayed on the package label. The Spanish equivalent for danger, PELIGRO, must also appear on the labels of highly toxic chemicals. Toxic category II pesticides must have the signal word WARNING (AVISO in Spanish) displayed on the product label. Toxic category III pesticides are required to have the signal word CAUTION on the pesticide label. Toxic category IV pesticide products shall bear on the front panel the signal word CAUTION on the pesticide label. Pesticides formulated in petroleum solvents or other combustible liquids must also include the precautionary word FLAMMABLE on the product label. This was obviously done to prevent

Table 7
Criteria of Pesticide Toxicity, Established by the Federal Insecticide, Fungicide and Rodenticide Act of 1962

Category	Signal Word	Toxicity	Acute Oral LD50	Inhalation LC50	Dermal LD50
I	**DANGER** and **POISON**	High	0–50 mg/kg	Up to 0.2 mg/L	Up to 200 mg/kg
II	**WARNING**	Moderate	50–500 mg/kg	0.2–2 mg/L	200–2000 mg/kg
III	**CAUTION**	Low	500–5000 mg/kg	2–20 mg/L	2000–20,000 mg/kg
IV	**CAUTION**	Relatively safe	> 5000 mg/kg	> 20 mg/L	>20,000 mg/kg

LC/LD_{50}: concentration/dose which causes death in 50% of the exposed subjects.

cases of accidental poisoning, and similar acts exist in almost all countries. In India, a predominantly agricultural country, handling of insecticides is governed by The Insecticides Act 1968 and The Insecticide Rules, 1971 (amended in 1993) *(201)*. Section 19 of The Insecticide Rules, 1971 classifies insecticides on a similar basis. Section 19 also insists on affixing a label to the insecticide container in such a manner that it cannot be ordinarily removed. Among other things, it must contain a square, occupying not less than one-sixteenth of the total area of the face of the label, set at an angle of 45° (diamond shape). This square is to be divided into two equal triangles, the upper portion of which shall contain the signal word, and the lower portion the specified color. The classification of insecticides, signal words to be used, and the color of the identification band on the label according to The Insecticide Rules, 1971 of India are given in Table 8.

If a pesticide is misused in any way, the person who bought and stored the pesticide may be legally responsible. In the United States, The Food Quality Protection Act was passed in 1996 as a complementary set of regulations, which, among other important features, specifically recognizes the special situations and usages of pesticides for public health. These laws regulate the registration, manufacture, transportation, distribution, and use of pesticides. The regulations are administered by the Environmental Protection Agency.

Table 8
Classification of Insecticides, Signal Words To Be Used and Color of Identification Band To Be Used on the Label According to "The Insecticide Rules, 1971" of India

Classification of insecticide	LD50 (oral) in mg/kg of test animals	LD50 (dermal) in mg/kg of test animals	Signal word (to be incorporated in the upper portion of the square)	Color of identification band on the label (to be incorporated in the lower portion of the square)
1. Extremely toxic	1–50	1–200	*POISON*	Bright red
2. Highly toxic	51–500	201–2000	**POISON**	Bright yellow
3. Moderately toxic	501–5000	2001–20,000	**DANGER**	Bright blue
4. Slightly toxic	More than 5000	More than 20,000	**CAUTION**	Bright green

LD_{50}: lethal dose in 50% of the exposed subjects

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Apoptosis

11

Apoptosis in Tissue Injury

Barbara M. Aufiero, PhD, George C. Tsokos, MD, Maria Tsokos, MD, and Henry K. Wong, MD, PhD

CONTENTS

SUMMARY

Given that apoptosis is delicately regulated by a balance of apoptotic activators (e.g., Bax) and apoptotic inhibitors (e.g., Bcl-2) that are continuously being synthesized, the decision of whether a cell dies or lives is constantly reevaluated. Disrupting that balance leads to apoptosis. Surface receptors that activate diverse signaling cascades or traumatic insults that disrupt intracellular structural integrity are involved in this decision-making process. By understanding the pathway for apoptosis, specifically the mechanistic steps, we may be able to deduce the initiating event. Having an understanding of the specific mechanisms of apoptosis is essential in developing approaches to analyzing consequences of pro-

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grammed cell death. The consequences of apoptosis must be considered in forensic pathology as well because this may provide clues to the underlying pathological process. It will be important to determine whether the apoptosis is activated by receptor-mediated pathway or from an intrinsic pathway. The distribution of apoptotic cells will be important; for example, the level of apoptosis in a tumor may reflect whether it is a primary tumor or a secondary tumor. Thus, an appraisal of apoptosis may provide insight into the pathology of specimen.

Key Words: Apoptosis; necrosis; cell death; caspase; cytochrome *c*; nuclear condensation.

1. INTRODUCTION

Injury to tissues can be a consequence from various forms of insults, such as blunt trauma from a projectile, vehicular accidents leading to ischemia from loss of blood, or low perfusion. Other forms of trauma can direct injury from irritants and vesicant chemicals, or radiation damage. Frequently, a subset of these events resembles situations that are also encountered in the acute care setting in the hospital emergency room. Traumatic injuries can lead to incapacitation, and rapid medical care is necessary to limit further physical deterioration of the wounded individual to allow stabilization, healing, and repair. To develop novel approaches that enhance recovery in acute injury, understanding the molecular mechanisms and consequences of tissue injuries is critical to development of therapies focused on limiting tissue damage and stimulating rapid repair processes. It is therefore important to understand at the fundamental level the biochemical processes that occur during cellular injury. Understanding the types of injuries that lead to apoptosis is also important in forensic pathology.

Over the past decade, rapid progress has accumulated on the molecular events subsequent to injury at the cellular level that leads to cell death *(1)*. It has become evident that the demise of organs and cell death occurs by way of two molecular mechanisms, necrosis and apoptosis *(2,3)*. Traditionally, cell death was viewed as a passive event from increasing disorganization of biochemical processes to progressive exclusion from oxygen and nutrients, such as in ischemic injuries—defined as necrosis. However, it has become clear that active expenditure of energy, through specialized concerted biochemical programs, is also involved in cell death—defined as apoptosis (programmed cell death)—particularly during the earliest time following an injury *(4)*. Also, it is clear that apoptosis does not evolve solely in response to traumatic injury. Apoptosis is an integral part of normal development of the heart *(5)* and brain *(6)* during embryogenesis, and throughout development of the immune system *(7)*. In these systems, initial cells are generated in excess to permit and ensure the appropriate selection of functional cells that make up the mature organ. For

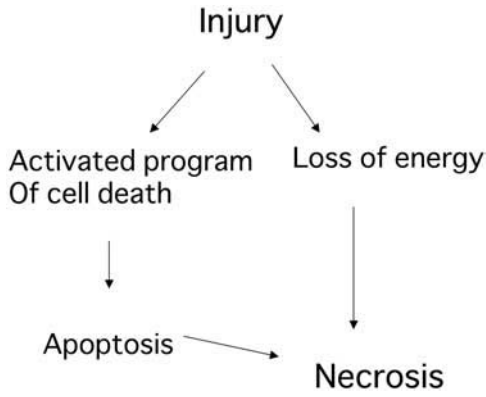

Fig. 1. Pathways that lead to cellular and tissue damage. Apoptosis is an energy-dependent pathway that can lead to cell death. Loss of energy can also lead to demise of the cell.

example, in the brain, many cells are needed to form the correct synaptic connections and those neurons that do not form appropriate connections are eliminated by apoptosis. In the immune system, only lymphocyte precursor cells that form the correct antigen receptor by gene rearrangement survive; the cells that fail to form functional antigen receptors are removed by apoptosis. The importance of apoptotic pathways as a mechanism for regulating development is noted by its conserved presence throughout evolution and across metazoan cells from the nematode worm *Caenorhabditis elegans* to humans.

Apoptosis is generally an early event that subsequently leads to necrosis. In many instances, both processes, apoptosis and necrosis, are involved in tissue damage (Fig. 1). For example, in severe cardiovascular collapse, necrosis is the late stage consequence of ischemia and anoxia, whereas apoptosis is an early stage process that is initiated following ischemia *(8,9)*. In autopsied organs of progressive damage, apoptosis very likely contributes to the observed cellular damage. Severe hypotension develops into hemorrhagic injury then hypoxia, a condition that leads to the activation of apoptotic pathways and subsequent necrosis. Murine macrophages subjected to experimental metal ion exposures, similar to those found in metal–metal hip replacement, first displayed apoptosis ensued by necrosis dependent on time and ion concentration *(10)*.

Cell death developing from necrosis is a disorganized process with increased entropy and loss of energy. There is release of cellular debris. Apoptosis serves to limit the total injury because it is a managed process that conserves energy by removing damaged tissue for the sake of survival of the greater organism. In ischemic tissue for example, there is less capacity to maintain tissue integrity. The damaged tissue removed by apoptosis does not lead to

disruption of surrounding normal tissue architecture and one may think of the process as walling off the area of damage. With modern resuscitative care, the ability to restore perfusion rapidly to injured tissues allows delivery of energy to the tissue to begin healing. Apoptosis under these circumstances may be excessive, leading to a greater loss of tissue than is necessary for survival. Being able to detect tissues that have undergone apoptosis is important in understanding the process that caused the damage. This chapter reviews the current understanding of mechanisms that are important in apoptosis.

2. Pathology

Injury leading to cell death is morphologically defined as either necrosis and/or apoptosis *(11)*.

Necrosis is characterized by oncosis, intracellular swelling with enlarged organelles, disruption of the nuclear membrane, plasma membrane, disintegration of nuclear structure and cytoplasmic organelles, cell shrinkage, random fragmentation of DNA, release of intracellular contents, and enzymes. Histologically, there is a loss of cellular organelles and degenerative changes with homogenization of structures, loss of distinct cell borders, and decreased intensity of staining. The process is disorganized and unpredictable, with collateral cellular and tissue damage and, often, inflammation is observed.

Apoptosis is a highly ordered process of cell death differentiated histologically by the presence of adjacent cells that are unaffected. One of the earliest changes in apoptosis is alteration of cell membrane with the movement of phosphotidyl-serine from the inner surface of the bilipid layer to the extracellular surface. This may mark the dying cell, and professional macrophages can phagocytize the cell and remove it in a controlled manner.

3. Assays: Detection of Apoptotic Cells

Apoptosis, originally described by Carl Vogt as early as 1842, is associated with shrinking of the cell, condensation of the nucleus, and little surrounding inflammation. Assays have been developed that utilize, detect, or visualize active components and features of apoptosis.

3.1. Nuclear Condensation

3.1.1. Terminal Deoxythymidine Transferase-Mediated dUTP Nick-End Labeling

Terminal deoxythymidine transferase-mediated dUTP nick-end labeling (TUNEL) assay takes advantage of an enzyme involved in DNA fragmentation.

Enzymatic staining, such as TUNEL, can identify cells in the process of apoptosis *(12)*. Surrounding damage to adjacent cells is minimal and inflammation is absent.

3.1.2. Propidium Iodide

Propidium iodide staining of DNA can identify a specific population of apoptotic cells by measuring the total DNA content within a cell. During apoptosis, there is cleavage of DNA between nucleosomes, where there is a reduction of the DNA content to less than the diploid amount of DNA. The population that has less than the normal level of DNA represents cells that have undergone apoptosis.

3.1.3. DNA Ladders and Direct Visualization of DNA Fragmentation

The detection of DNA fragments resolved on agarose gels appearing as "DNA ladders," with each rung growing in increments of 200 bp, distinguishes apoptosis from necrosis. DNA ladders result from specific cleavage at nucleosome (DNA/histone) junctions; hence, 200-bp increments by a Ca^{++}/Mg^{++}-dependent endonuclease.

3.2. Cell Shrinkage

Annexin V analysis is another useful test for measuring the progression of apoptosis. Annexin V is an anticoagulant that can bind phosphotidylserine (PS) in the presence of calcium. In living cells, there is more PS on the cytoplasmic surface than on the extracellular surface of the lipid membrane. Once apoptosis is initiated, this asymmetry is lost with increasing levels of PS appearing on the outer surface of the cell membrane where it is recognized by annexin V. This test is an early indicator of apoptosis and provides a way to identify apoptotic cells prior to the onset of DNA damage.

3.3. Caspase Activation

There are enzymes (i.e., caspases) that play a role in apoptosis. The detection of activated components of caspases by colorimetric assays or by detection of active subunits by immunoassays can provide hints to the activation of apoptosis.

4. Mechanistic Pathways in Apoptosis

The understanding of apoptotic mechanisms has progressed rapidly during the last decade, and these mechanisms vary in location from intracellular sensors, such as the cellular tumor repressor gene, *p53*, which is activated on

DNA damage to the cell surface receptor, Fas, which initiates apoptosis on cross-linking. The first indication that apoptotic mechanisms existed were suggested by early studies in *C. elegans* development that identified two proteins, CED3 and CED4, as being essential for appropriate development *(13)*. These gene products were important in the elimination of specific cells *(14)*. Loss of these genes led to an aberrant number of cells. Amino acid sequence comparison of these proteins revealed similarities to a special class of mammalian proteases (caspases), suggesting the importance of proteases in the regulation of apoptosis.

Apoptosis is mediated by a cascade of molecular events that is finalized by DNA cleavage, nuclear condensation, and specific protein degradation. One of the best understood and clearest examples of apoptosis occurs within the immune system. Throughout immune system development and maturation, there is ongoing selection of specific subsets of lymphocytes to either proliferate (cell survival) or contract (cell death by apoptosis) in an effort to maintain homeostasis during the immune response *(15)*. During T- and B-cell-development, those cells that do not rearrange their variable genes to form functional antigen receptors undergo apoptosis. For T-cells, the peripheral pool of circulating T-cells represents 10% of the former pool of cells undergoing selection. The remaining cells (90%) are eliminated by apoptosis. Thus, apoptosis is an ongoing process that is essential for normal function of the immune system. During an infection, antigen specific B- and T-cells proliferate to combat the invading pathogen, and on clearance of the foreign antigen, these cells are eliminated by apoptosis. Furthermore, the primary target cells of the ensuing infection are also destroyed by apoptosis. For example, *Staphylococcus aureus* infection of in vitro cultured keratinocytes causes 20 to 25% cell death by apoptosis (Young et al., unpublished results).

Regulation of apoptosis exists at many levels. Apoptosis is initiated by activation of two intersecting pathways: extrinsic and intrinsic, as defined by the source of the initiating apoptotic stimuli *(16)*. These pathways converge at the level of caspases, the enzymes that cleave hundreds of substrates and deliver the apoptotic phenotype (Fig. 2) *(17)*.

Apoptosis activation by the *extrinsic pathway* occurs through extracellular surface receptors, known as “death” receptors. This mechanism is important in immune surveillance of tumor cells and virally infected cells where abnormal proteins are localized on the cell surface. Death receptors via a protein interaction domain called the death domain (DD) interact with adaptor molecules at the plasma membrane. Adaptor molecules (Fas-associated death domain [FADD], TNF receptor-associated death domain protein [TRADD]) contain a similar DD responsible for death receptor binding, as well as a death

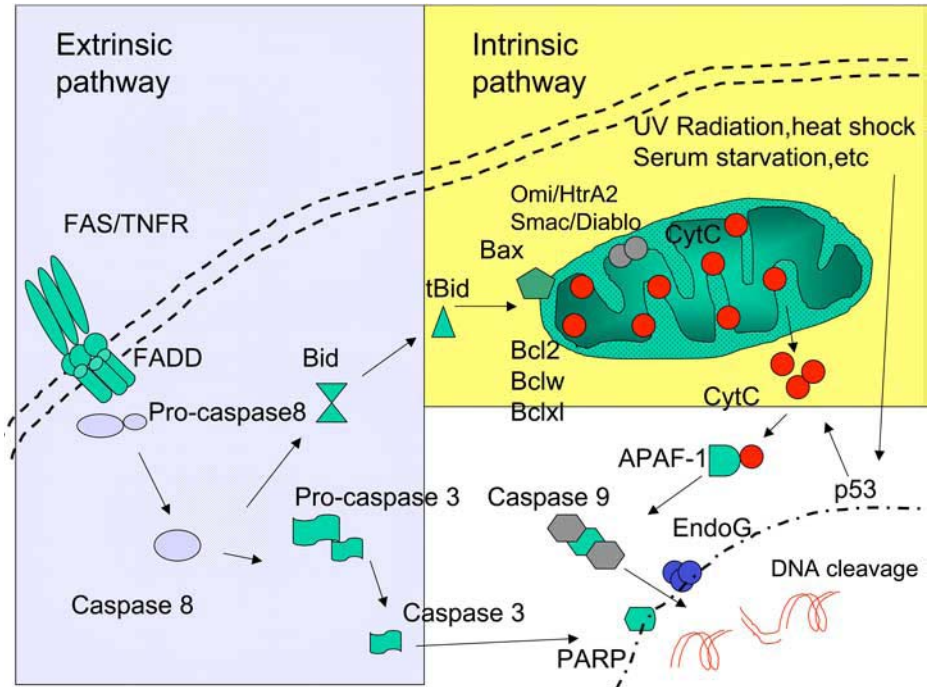

Fig. 2. Schematic overview of the intrinsic and extrinsic pathways of apoptosis.

effector domain (DED) that recognizes its partner domain found in procaspase 8 or 10.

The *intrinsic pathway* is comprised of proteins that can directly initiate apoptosis in response to excessive cellular stress from free radicals, ultraviolet (UV) irradiation, and γ irradiation. After initiation of apoptosis, the process does not require *de novo* gene transcription or protein synthesis and, as proof, enucleated cells can undergo apoptosis *(18)*. Induction of apoptosis through the intrinsic pathway occurs when the cell is deprived of survival factors or exposed to external toxins, intracellular damage, or stress signals. In this pathway, permeability changes occurring in the mitochondrial membrane release cytochrome *c*, which complexes with apoptotic protease activating factor (Apaf)-1. This complex termed "apoptosomes" activates caspase 9. Intermediate members of this pathway include the Bcl-2 family of proteins, which act in the outer membrane of the mitochondria to release cytochrome *c*.

Morphological changes associated with apoptosis occur through the biochemical actions of caspases. Caspases 8, 9, and 10, referred to as initiator caspases, enlist "effector" or "executioner" caspases 3, 6, and 7. The net balance

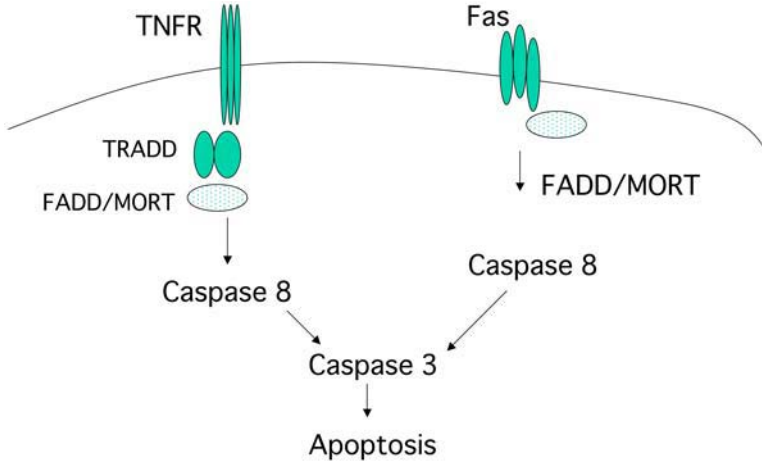

Fig. 3. Receptor-mediated mechanisms for the initiation of apoptosis. Examples of two well-characterized apoptotic pathways, the tumor necrosis factor receptor and Fas receptor pathways, are shown.

of proapoptotic vs antiapoptotic molecules determines the fate of cell survival or death.

5. *Extrinsic Signals: Regulation Through Extracellular Surface Receptors*

Death receptors are members of the tumor necrosis factor (TNF) superfamily and include Fas/Apo1/CD95, TNF-R1, TNF-R2, DR3, and DR4/APO-2/TNF-related apoptosis ligand (TRAIL)-R (Fig. 3). These receptors are characterized by the presence of immunoglobulin-like extracellular domains rich in cysteine residues (reviewed in refs. *19,29*) and share 25 to 30% amino acid identity. Other surface signaling proteins of the TNF family of receptors include lymphotoxin α and β, CD30, CD40, CD27 and TRAIL. The intracellular domains of this family of proteins share weak homologies and contain the DD that interacts with adaptor molecules. Each receptor acts through specific adaptor molecules that are responsible for initiating apoptosis. The unique combinations of receptor–adaptor complexes mediate receptor-derived specificity in the regulation of apoptosis.

Because apoptosis is regulated by specific cell-surface receptors, there is likely crosstalk between apoptotic receptors and other cell-surface receptors. For example, Fas has been shown to affect intracellular calcium release in Jurkat T-cells in response to stimulation through the T-cell receptor *(21)*. Furthermore,

our own experiments demonstrate that Fas signaling also affects transcription regulation, specifically of the nuclear factor (NF)-κB transcription factor that is important in the regulation of antiapoptotic genes (Wong and Tsokos, unpublished results). Thus, surface receptor initiation of apoptosis likely blocks multiple levels of intracellular pathways that exist normally to prevent apoptosis.

5.1. Fas/CD95/Apo-1

One essential receptor pathway for regulating homeostasis of T-cells is controlled by the Fas/Apo-1/CD95 apoptotic pathway *(22,23)*. Defects or abnormalities in the regulation of this pathway in the immune system have profound consequences, such as extensive lymphoproliferation and eventual disease *(24)*. Mutations in the Fas gene have been observed in both mice and humans. Murine mutations in the Fas ligand or receptor, the *gld* or *lpr* mutation, respectively, cause lymphoproliferation, and autoimmunity. These mutants have been models for the study of systemic lupus erythematosus and autoimmunity. In humans, a recently described syndrome, Canale-Smith, or autoimmune lymphoproliferative syndrome, have genetic defects in Fas *(25,26)*.

In the immune system, Fas is primarily needed for T-cell regulation. Fas, also known as Apo-1/CD95, is a receptor belonging to the TNF receptor family. The TNF receptor family members act by forming homotrimeric complexes with appropriate ligands to activate transmembrane signaling. Fas binds the Fas ligand, a type 1 membrane protein that exists as a trimerized complex that induces oligomerization of Fas on binding. During the development of the immune response, Fas is important in peripheral deletion of activated T-cells after resolution of an immune response. Additionally, Fas is involved in killing virally infected T-cells or cancer cells. Fas has a globular extracellular domain, a transmembrane domain, and an intracellular domain. The intracellular region contains the DD that is important for protein–protein interaction. When trimerized by Fas ligand, the oligomerized Fas receptor complex allosterically confers DD to recruit other proteins. DD, common to all TNF superfamily members, shares structural homology, and some have an affinity for other members of this family.

The structure of the DD is characterized by six helical domains that can mediate self association or association with other DD-containing proteins. The Fas DD recruits the adaptor protein FADD/Mort1 *(27)*. FADD through DED mediates interaction with the zymogen, Caspase 8/FADD-like interleukin 1β-converting enzyme (FLICE). Oligomerized Fas complexes draw the zymogens close together and promote autocatalysis *(28)*. The caspases are the effector enzymes that execute programmed cell death.

When caspase 8 is activated, a cascade of proteolytic activity is initiated that triggers the activation of additional caspases, of which all are interleukin-

Table 1
Caspases and Nomenclature

Caspase 1	ICE
Caspase 2	Nedd2, ICH-1
Caspase 3	CPP32, Yama, apopain
Caspase 4	ICE II, TX, ICH-2
Caspase 5	ICE III, TY
Caspase 6	Mch2
Caspase 7	Mch3, ICE-LAP3, CMH-1
Caspase 8	MACH, FLICE, Mch5
Caspase 9	ICE-LAP6, Mch6
Caspase 10	Mch4

ICE, interleukin-1 converting enzyme; ICH, ICE and Ced-3 homolog; CPP32, cycstein protease protein of molecular mass 32/KDA; TX, transcript x; TY, transcript y; Mch2, melanin-concentrating hormone; ICE-LAP3, ICE-like apoptotic protein; CMH, CPP32/Mch2 homolog 1.

1-converting enzyme-like proteases. These proteases cleave substrates, such as polyadenosine diphosphate ribose polymerase, lamin, actin, and others to transform the normal morphology to that of a shrinking pyknotic cell, nuclear condensation. The list of caspases identified, thus far, is listed in Table 1.

Although activated caspases are important contributors in the apoptotic signaling cascade, there are traditional signaling pathways that are useful and control self-destruction in an orderly, regulated manner without harming neighboring cells. Such precision is essential in developing organs, such as the brain, heart, and other fine structures, such as the digits of the hand. Fas has been found to signal through two pathways via DD proteins, the c-Jun N-terminal kinase (JNK) pathway mediated by death-associated factor (DAXX), and the caspase pathway mediated by FADD *(29)*. DAXX was isolated as a protein that bound to the DD of Fas. It was subsequently identified to activate the intracellular mitogen-activated kinase signaling pathway, the JNK pathway. The JNK pathway regulates the transcription factor c-Jun and upregulates new genes, which play a role in initiating apoptosis. Thus, multiple regulatory mechanisms of apoptosis, some of which are initiated by the expression of new genes, exist and display a complex system of execution.

5.2. TNF Receptors

TNF receptors are ubiquitously expressed, and their activation can lead to diverse cellular responses *(19)*. There are two TNF receptors, TNF-R1 and TNF-R2, which both bind to different ligands. Signaling by TNF-R1 leads to

activation of proinflammatory pathways, and in certain cells, leads to apoptosis. In order for TNF to initiate apoptosis, protein synthesis must be blocked. The role of TNF in mediating apoptosis is likely subjected to specific regulation by other signals and the outcome of TNF receptor activation remains complex. The TNF-R1 DD interacts with an adaptor molecule, TRADD, a prerequisite step in TNF-R1-mediated apoptosis. TRADD can interact with FADD through DED to activate caspase 8 and initiate apoptosis. In addition, TRADD binds to TNF receptor-associated factor (TRAF)2, a protein that activates transcription signaling cascades for activation of NF-κB and c-Jun. The interplay of TNF receptors and other receptors determine whether the response of a cell is to proliferate or undergo apoptosis.

5.3. DR3/DR4/DR5/DR6

Other members of the TNF receptor superfamily, DR3, DR4, DR5, and DR6, are best known for their ability to mediate apoptosis of tumor cells *(30)*. Unlike Fas, the expression of these death receptor proteins is ubiquitous and not restricted to the immune system. At the present time, there are six members of this family *(31)*. Common to this family of proteins is the presence of the cytoplasmic DD. DR3 (Apo3, lymphocyte-associated receptor of death [LARD], Ws1, TNF-related apoptosis-mediating protein [TRAMP]) is a protein with the ability to bind TRADD. DR3 can also activate NF-κB, a transcription factor associated with cell survival signaling. The ligand for DR3 is unidentified. DR4 and DR5 share structural similarities and bind TRAIL. Both receptors utilize signaling molecules, FADD, TRADD, and receptor interacting protein for apoptosis. DR6, most recently identified, mediates apoptosis when overexpressed in HeLa cells. In T-cells, DR6 is involved in the regulation of proliferation as determined by knock-out experiments. The ligand, as well as the effector molecule by which DR6 operates, are unknown. In summary, these death receptors have multiple functions and play a role in regulating proliferation, and differentiation in addition to apoptosis.

6. Intrinsic Signals: Mitochondrial Permeability and Apoptosis

The mitochondria are not only necessary for adenosine triphosphate (ATP) energy production through the electron transport chain, but they are also important for choreographing cell death. Mitochondrial proteins are intimately involved in apoptosis, and these components, when activated, can lead to apoptosis independent of other apoptotic proteins, such as caspases *(32)*. There are several mechanisms by which the mitochondria initiate apoptosis (Fig. 4). One mechanism is from the disruption of the electron transport pathway. Several inducers of

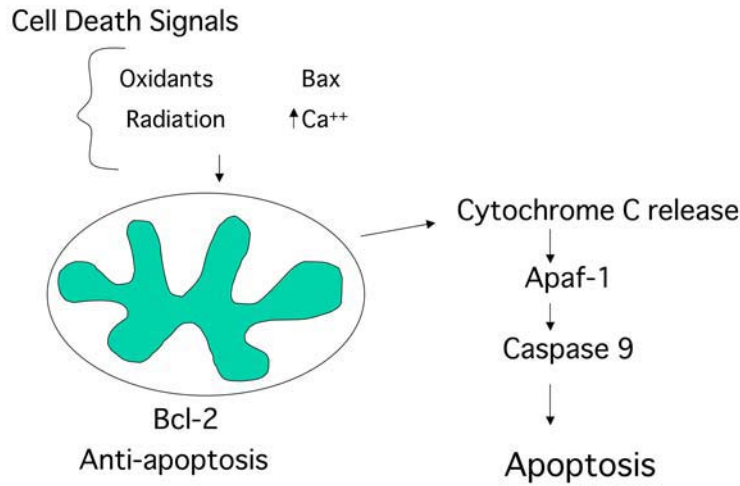

Fig. 4. Caspase structure. The general linear organization of caspase domains is shown. Proteolytic processing at specific sites transforms the inactive protein to active caspase.

apoptosis, such as γ irradiation, ceramide signaling, and receptor-mediated apoptosis via Fas, also disrupt electron transport by loss of mitochondrial membrane integrity. Disruption of this membrane inhibits electron transport and ATP generation. A second mechanism is by release of cytosolic cytochrome *c*, which is a potent proapoptotic component that activates caspases. Cytochrome *c*, when released into the cytosol, forms a complex with Apaf-1 and procaspase 9, which leads to the activation of caspase 9. Normally, cytochrome *c* (10 kDa) resides in the mitochondria by an intact membrane, which permits the movement of molecules less than 1000 Da in size. In situations of severe oxidative stress, another mechanism that can lead to apoptosis is by the generation of superoxide anions that have toxic effects. Caspases play an important part in regulating apoptosis and there is a large family of caspase members that are necessary for apoptosis.

Direct injury or depletion of energy distresses the mitochondria, which swell. Severe swelling of the mitochondria ruptures the outer mitochondrial membrane and releases mitochondrial proteins from the intracellular space. This collapse also disrupts the electron transport chain and therefore leads to loss of ATP. Under these situations, release of cytochrome *c* is associated with necrosis.

7. *Bcl-2 and Mitochondria*

Bcl-2 and cytoplasmic proteins with shared structural homology play an important role in regulating the functions of proteins of the mitochondria dur-

Table 2
Bcl-2 Family of Apoptosis Regulators

Antiapoptotic	Proapoptotic
Bcl-2	Bax
Bcl-xl	Bak
Bcl-W	BOK/MTD
Mcl-1	Bcl-XS
A1/BFL-1	BID
BOO/DIVA	BAD
NR-13	BIK/NBK
CED-9	BLK
	HRK
	BIM/BOD
	NIP3
	NIX/BNIP3

ing apoptosis *(33)*. Regulated release of cytochrome *c* occurs during apoptosis and is the end result of an activated biochemical cascade. The release of cytochrome *c* is controlled by the Bcl-2/Bax family of gene products. Bcl-2 family members were first identified as targets of rearranged genes in B-cell lymphoma. The *Bcl-2* gene, first identified as an oncogene, confers immortalization upon overexpression in primary rodent cells. Extensive studies have identified the importance of these proteins as antiapoptotic mediators *(34)*. The Bcl-2 family is large, with more than 20 members, and is defined by the presence of structurally related Bcl-2 homology (BH) domains (BH1–BH4). These domains mediate protein–protein associations in response to death-inducing stimuli (Table 2).

Proapoptotic members of the pathway are divided into two groups based on function and sequence homology; the "initiators" are capable of dimerization with antiapoptotic Bcl-2 family members (Bim, Bik, Bad, Puma, Bid, Noxa, Hrk, Bmf), and the "executioners" assault cellular substrates (Bax, Bak, Bok). The initiators are distinguished by containing a single BH domain (BH3). The executioners release cytochrome *c* by facilitating ion channel formation in the mitochondrial membrane *(35-38)*. Antiapoptotic Bcl-2 proteins (Bcl-2, Bcl-x_L, Bcl-w, Al, Mcl-1) prevent channel formation. Other Bcl-2-related proteins (Boo/Diva, Bcl-G, Bcl-B, Bcl-Rambo, BNIP3, BNIP3L) are currently under investigation.

The broad range of family members results in a diverse repertoire of responses to death signals. The Bcl-2 family is activated in response to cellular stresses, deprivation of growth factors, steroids, UV, and receptor-mediated

signaling. The exact mechanism by which Bax/Bak function is unclear, but their interaction with members of the Bcl-2 family by heterodimerization is an important function in apoptosis. Formation of heterodimers between pro- and antiapoptotic family members (e.g., Bax/Bcl-2) promotes apoptosis. Overexpression of Bax leads to the formation of Bax homodimers and induces apoptosis through the activation of interleukin 1-converting enzyme (ICE) and ICE-independent pathways. From knockout studies, it was determined that Bax or Bak serve redundant functions. Both genes must be eliminated to prevent apoptosis *(39)*. Bax and/or Bak deficiency prevents apoptosis initiated by other pathways, such as Fas, UV light, and caspase pathway *(40)*, suggesting that apoptosis is initiated through fewer signals. Bax and Bak promote the release of cytochrome *c* from the mitochondria through an unknown mechanism that is likely complex and controlled by proteins that are also tightly regulated.

The Bcl-2 family members function by forming interactions among partner proteins as homodimers or as heterodimers with Bax *(34)*. The domains (BH1–BH4) that mediate protein–protein interaction are α-helical structural motifs that are dependent on the formation of a hydrophobic pocket. Bcl-2 proteins are situated in the outer mitochondrial membrane, anchored by their hydrophobic C-terminus. This location is important in preventing apoptosis by preventing release of mitochondrial proteins. Members of this subgroup are capable of interacting with and inhibiting "initiator" and "executioner" Bcl-2 family members. Proapoptotic members (e.g., Bad) lack a membrane anchoring domain and heterodimerization of Bad with Bcl-2 results in loss of the membrane-bound Bcl-2 proteins. Other Bcl-2 family members, such as CED-9, inhibit apoptosis by binding CED-3 caspases in *C. elegans*, a mechanism not apparent in mammalian Bcl proteins.

Recent evidence points to Mcl-1 as a unique sensor among the antiapoptotic molecules capable of regulating apoptosis. Decreased expression of Mcl-1 triggers downstream apoptotic signals including Bax translocation and oligomerization with Bax, Bcl-x_L inactivation and Bim dephosphorylation *(41)*.

Another level of regulation of Bcl-2 related-proteins is through phosporylation of serine residues *(34)*. Phosphorylation of Bcl-2 destabilizes anchoring to the mitochondrial membrane *(42)*. Altered localization from the membrane promotes release of cytochrome *c* from the mitochondria. Similarly, phosphorylation of proapoptotic members, such as Bad, inhibits apoptosis. Phosphorylation of Bad prevents complex formation with Bcl-2 family members *(43)*. Bad is phosphorylated by Akt kinase in response to signaling from receptor growth factors, such as epidermal growth factor or insulin like-growth fac-

tor. Phosphorylated-Bad associates with the 14-3-3 protein, located in the cytosol, and prevents heterodimerization with Bcl-2.

Bcl-2 proteins also link surface apoptotic signaling to mitochondrial release of cytochrome *c*. Fas or TNF signaling leads to the activation of caspase 8, which cleaves Bid. The terminal fragment of Bid binds the mitochondria and leads to the release of cytochrome *c*.

8. Intracellular Molecules Involved in the Regulation of Apoptosis

8.1. p53

The *p53* gene product plays an important role in regulating apoptosis *(33)*. Cellular injury from UV irradiation, ionizing radiation, and excessive oxidative stress activates the *p53* mechanism of apoptosis *(44)*. Because *p53* resides in the nucleus, *p53* can be viewed as an intracellular sensor of cellular injury. *p53* is found in all mammalian cells and also plays an important role in cell cycle progression. From studies using DNA tumor viruses, *p53* was identified as a target of viral transforming oncogenes such as the SV40 T-antigen or the adenovirus E1B protein. The interaction of *p53* with viral proteins subsequently identified *p53* as a tumor suppressor gene. Thus, mutations in *p53* that disrupt its ability to repress cell proliferation are associated with malignant cell growth. Activation of normal *p53* arrests cells in the G1 phase of the cell cycle. *p53* induces the transcriptional upregulation of p21, cyclin-dependent kinase inhibitor, which plays a role in cell cycle progression and halts further cell division. *p53* directly mediates apoptosis by regulating members of the Bcl-2 family of proteins. *p53* antagonizes Bcl-2 function and activates *Bax* gene expression at the transcriptional level. Bax activation leads to its translocation from the cytosol to the mitochondrial membrane and the release of cytochrome *c*. The release of cytochrome *c* signals caspase activation and apoptosis.

8.2. Protease-Caspases

Caspases are a family of cysteine proteases that cleave target motifs beginning with an aspartic residue. This class of enzyme is the executioner of apoptosis *(45,46)*. Caspases were identified to be important in apoptosis by the discovery of CED-3, which was required for cell death in *C. elegans*. Subsequently, related proteins have been found throughout evolution. Members of this family of enzymes are defined by similarities in amino acid sequence, structure, and substrate specificity (Table 1). Currently, 13 members of the caspase family have been idenitifed in mamalian cells. They are expressed as proenzymes, 30 to 50 kD

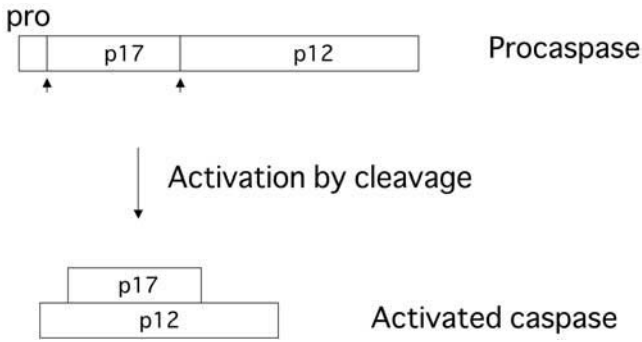

Fig. 5. Mitochondrial components involved in apoptosis. Examples of the role of different stresses that activate mitochondrial apoptotic pathways are shown.

in size, and they require enzymatic activation by cleavage at two sites for activation (Fig. 5). Caspases contain three domains between which the first two are cleaved on activation. Caspases autolytically cleave two target sequence sites embodied in their amino acid sequence and form two subunits. The cleaved subunits associate to form the activated enzyme. The N-terminal is the most variable in length and sequence and is involved in the regulation of activation.

Activation of the caspase cascade is dependent on an appropriate signal from the cell surface or from intracellular regulators in the mitochondria, such as Apaf-1/cytochrome *c*. However not all caspases are involved in apoptosis. Caspase 1 and ICE are involved in the regulation of inflammation. These proteases do not mediate apoptosis. Caspases 3, 7, 8, and 9 are specifically required for apoptosis.

Caspase 9 is one of the earliest effector caspases activated in response to altered mitochondrial membrane permeability disruption *(46)*. The disrupted mitochondrial membrane releases cytochrome *c*, which binds Apaf-1, and in the presence of ATP, associates with caspase 9. This association converts procaspase 9 to the active form, which then activates caspase 3 to initiate the cascade of steps involved in the caspase pathway.

Capase 8 activation occurs in response to cell surface apoptotic signals. Cell surface "death" receptors in conjunction with adaptor proteins launch caspase 8 activation. Adaptor proteins, such as FADD, have caspase recruitment domains that bind caspase 8. The activation of caspase 8 subsequently leads to the activation of the caspase casade and apoptosis.

Caspases lead to apoptosis presumably by degrading proteins essential for cell survival and inhibitors of apoptosis. Caspases directly disassemble cell

structures, such as nuclear lamins, and other cytoskeletal proteins. Apoptosis leads to DNA fragmentation, chromatin condensation, membrane changes, as well as other changes. Caspases cleave proteins in a targeted manner causing disintegration of structural and regulatory proteins. Apoptosis then proceeds by shrinkage of the cell with precise control of cellular content and debris without spilling cellular contents. This well-orchestrated process is executed by precise targeted cleavage of proteins. Some of the best characterized targets of caspases are polyadenosine diphosphate ribose polymerase, lamins, and U1 ribonuclear proteins. Others include proteins that regulate cell cycle progression and proliferation. Recognition by caspases of their cognate target sites requires four amino acids' N-terminal to the cleavage site. In fact, caspases are one of the most specific proteases. Because of the high specificity, caspases can be inhibited by the use of tetrapeptides that fit the active site of the enzymes.

8.3. Mitochondrial Proapoptotic Molecules

Proteins equipped with the ability to promote apoptosis have been discovered and reside in the mitochondria. These proteins are as follows: Diablo/Smac, Omii/Htr2, AIF, and endonuclease G. It remains to be established whether these proteins are essential for apoptosis.

Unleashed from the mitochondria, Diablo/Smac and Omi/Htr2 prey on the ability of inhibitor of apoptosis proteins (IAP) to inhibit caspase activity *(47–51)*. IAPs contain one or more baculovirus IAP repeat domains that encode the caspase inhibitory activity *(52)*. Smac has been shown to interact with the baculovirus IAP repeat domain 3 domain of xIAP *(53,54)*. Inhibition of IAPs is not sufficient to initiate apoptosis.

Capase-independent cell death through the degradation of DNA and chromatin condensation is attributed by the flavoprotein, apoptosis-inducing factor (AIF), and endonuclease G *(55)*, although it is controversial whether caspase-activated DNase is also required *(56,57)*. Caspase activity is crucial for AIF release from the mitochondria *(58,59)*.

8.4. Protease-Calpains

Calpains are a group of cytoplasmic cysteine proteases that are regulated by calcium and phospholipids *(60)*. There are two major calpains, I and II. Calpain I is more sensitive to calcium (3–50 μ*M*) than calpain II (200 μ*M*). Calpain activation is mediated by autolytic proteolysis. Disruption of membrane integrity triggers the activation of calpain, which is highly specific. Target substrates of calpain are numerous and include protein kinase C, calcium/calmodulin-dependent protein kinase II, microtubule associated protein 2, tubulin,

spectrin, calcineurin, and eukaryotic elongation factors 4E and 4G. The proteolysis of target substrates often leads to altered function rather than complete inactivation or degradation of the protein. Calpains play a role in cytoskeletal remodeling during migration and wound healing *(61)*. Calpains play a limited role in apoptosis.

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Imaging Techniques in Forensic Pathology

12

Recent Advances in Postmortem Forensic Radiology

Computed Tomography and Magnetic Resonance Imaging Applications

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Contents

Summary

The authors' personal experience of postmortem radiological imaging has revealed many uses for this technology. Not only does it allow planning of the subsequent autopsy, thus providing opportunities for specialist confirmatory dissection techniques, but it can also provide an investigative tool, such as the

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tracking of a projectile's passage through a body or confirmation of an identity. Postmortem radiological imaging techniques can also participate in ensuring protective aspects of health and safety. In such instances it is possible to diagnose pathology in high infectious risk cases without recourse to dissection, thus minimizing exposure. As demonstrated in this chapter, postmortem radiological imaging also allows for identification of fragments of metal in cases of blast injuries. Knowledge of the anatomical location of shrapnel diminishes the risk of receiving injuries during the dissection.

Key Words: Autopsy; less invasive; radiology; computed tomography (CT); magnetic resonance imaging (MRI); plain X-rays; fluoroscopy; angiography; ultrasonography; magnetic resonance spectroscopy (MRS); magnetic resonance microscopy (MRM); adult; children; anthropology; trauma; ballistics; drowning.

1. Introduction

The application of radiological investigations in cases of medicolegal interest originated within months of Wilhelm Conrad Roentgen's discovery of X-rays in 1895; a case of injury through negligence was pursued in a British court. Despite witness and medical testimony, the radiological evidence proved crucial in swaying the jury as to the extent of damage inflicted. Subsequently, the first description of radiological evidence's use in a murder trial was recorded the following year when the new technology revealed the presence of intracranial metal projectiles as a consequence of an ultimately fatal gunshot injury. The newly named "roentgenograms" had been taken in life, although each film required an exposure time of more than 1 hour *(1)*.

This new speciality of radiology developed over subsequent decades, with novel techniques being created and applied to all fields of medicine. Given the early history of forensic radiology, it is unsurprising that, as newer methodologies were created, these were equally applied to medicolegal scenarios. Thus, before considering the recent advances within the field, a brief overview of radiology is provided. For more in-depth information on the subject, specialist texts should be consulted.

2. A Brief Overview of Radiological Imaging Techniques

2.1. Plain X-Rays

The standard radiological assessment of the anatomical regions of the body (commonly referred to as a "plain X-ray") is produced by a collimated X-ray beam, being part of the electromagnetic spectrum and possessing a wavelength in the region of 10^{-8}m. The X-ray beam is produced through the

showering of a tungsten anode with free electrons, which emanate from a cathode. The resultant beam is focused on the anatomical region in question and the image created through the reaction of the beam with the silver emulsion present on a photographic plate, placed either beneath the body or on the opposing side, when the X-ray photons induce the chemical reduction of the exposed silver halide composite. The reduced silver particles produce black areas (areas of radiolucency) once the plate has been processed. Conversely, the inability to penetrate tissues results in an absence of reduction and, hence, a white (radioopaque) area. When the tissue overlying the plate is exposed as a whole, the result is a greyscale mosaic (the roentgenogram image) known commonly, although incorrectly, as an "X-ray" (Fig. 1).

Within the forensic setting, plain X-rays have been used for the purpose of confirming identities, identifying causes of death, fractures, evidence of nonaccidental injury in children, and locating foreign objects such as metal projectiles *(1–3)*. The benefit of such techniques includes the ability to create a permanent record, for the purposes of investigation and judicial usage. The radiographic units may also be mobile, allowing films to be exposed within the mortuary or mass disaster scenarios. Doctors within all fields are also familiar with the technique and examining the developed films through its numerous clinical applications, although definitive reporting of the film should be undertaken by a consultant radiologist with experience in examining roetgenograms of adult and childhood trauma, as well as postmortem changes.

The increasing disadvantage, certainly from a clinical view, is the request for archiving of these films, at least for the lifetime of the patient, although current UK legislation suggests that films should be retained for a further 5 years after death *(4)*. The modern answer is the development of the digital radiographic systems already installed with some UK hospitals *(5)*. The physical methodology is similar, although the resultant image is instead produced on a digital display, following image optimization and manipulation by the integrated computer system. This system, referred to as the picture archival and communication system, allows transfer of the image to separate departments or even remote links to separate hospitals for review through network systems, and eliminates the physical archival problem, thus reducing the potential for lost "films." Files may be accessed widely by numerous individuals and can be compared immediately with previous radiological investigations. The enhancement of the image ensures the majority of files are appropriately exposed and, for medicolegal purposes given the digital nature of the images, all enhancements or alterations are logged internally by the software. Multiple copies of the same image can be produced either on hardcopy, CD-Rom, or DVD formats. Computed tomography (CT) scan and mag-

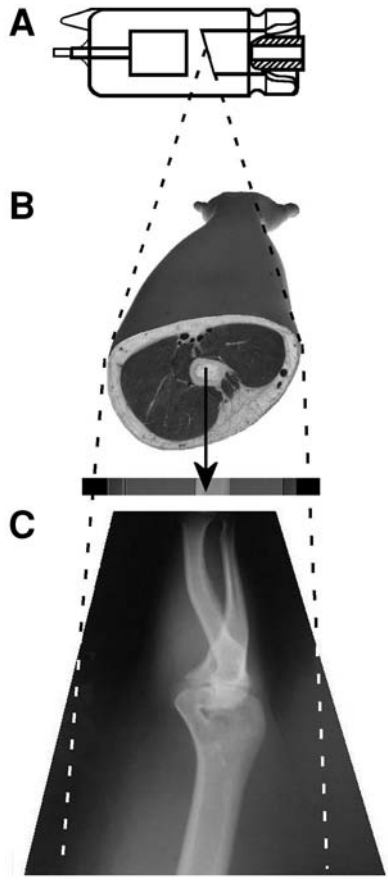


Fig. 1. A schematic diagram of a roentgenogram; the X-ray beam is produced by the cathode **(A)**, which is focused through the tissue in question. The differing densities of the tissue produces a greyscale mosaic **(B)**, which, when the plate is fully exposed, creates the roentgenogram, or "plain X-ray" **(C)**.

netic resonance imaging (MRI) systems already incorporate the picture archival and communication system, although increasingly, plain X-ray images may be created in this manner.

Another disadvantage to the medicolegal investigator is that the plain X-ray is two-dimensional. Although, it may reveal the presence of a projectile, the true anatomical location of the projectile may not be revealed (Fig. 2).

The forensic applications of plain X-rays have been extensively discussed in specialized textbooks elsewhere, such as the excellent *Forensic Radiology* by

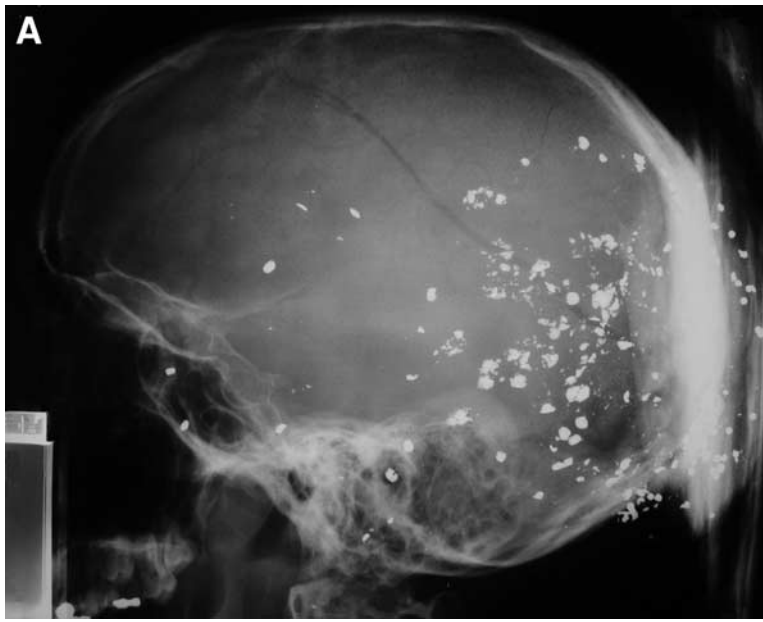


Fig. 2. (A) Postmortem radiology through plain X-ray examination reveals the presence of metal projectiles throughout the image. The appearance suggests intracranial fragments and whole lead shot, although its precise location is not discernible. Fracturing of the skull bones is also seen. *(Figure continues)*

Gil Brogdon *(1,2,6)*. As such, this chapter will instead review the recent advances in radiological techniques and their application to forensic medicine.

2.2. Fluoroscopy

The fluoroscope found many proponents of its use in the 1980s and 1990s as a mobile, rapid means of examining bodies or nonhuman material of medicolegal interest *(7)*. The units produce a continuous low-power X-ray beam focused on a region of interest. Rather than a photographic plate, the photon beam is detected by an input fluorescent screen and photocathode. Electrostatic lenses focus the image, which is outputted through a second fluorescent screen to the display monitor allowing real-time examination.

This technique is frequently employed in the postmortem period during the examination of cadavers for personal artifacts, bone trauma, metal projectiles, fragments of antipersonnel devices, or even needles where high resolution is not as important, although the latter may not show up on

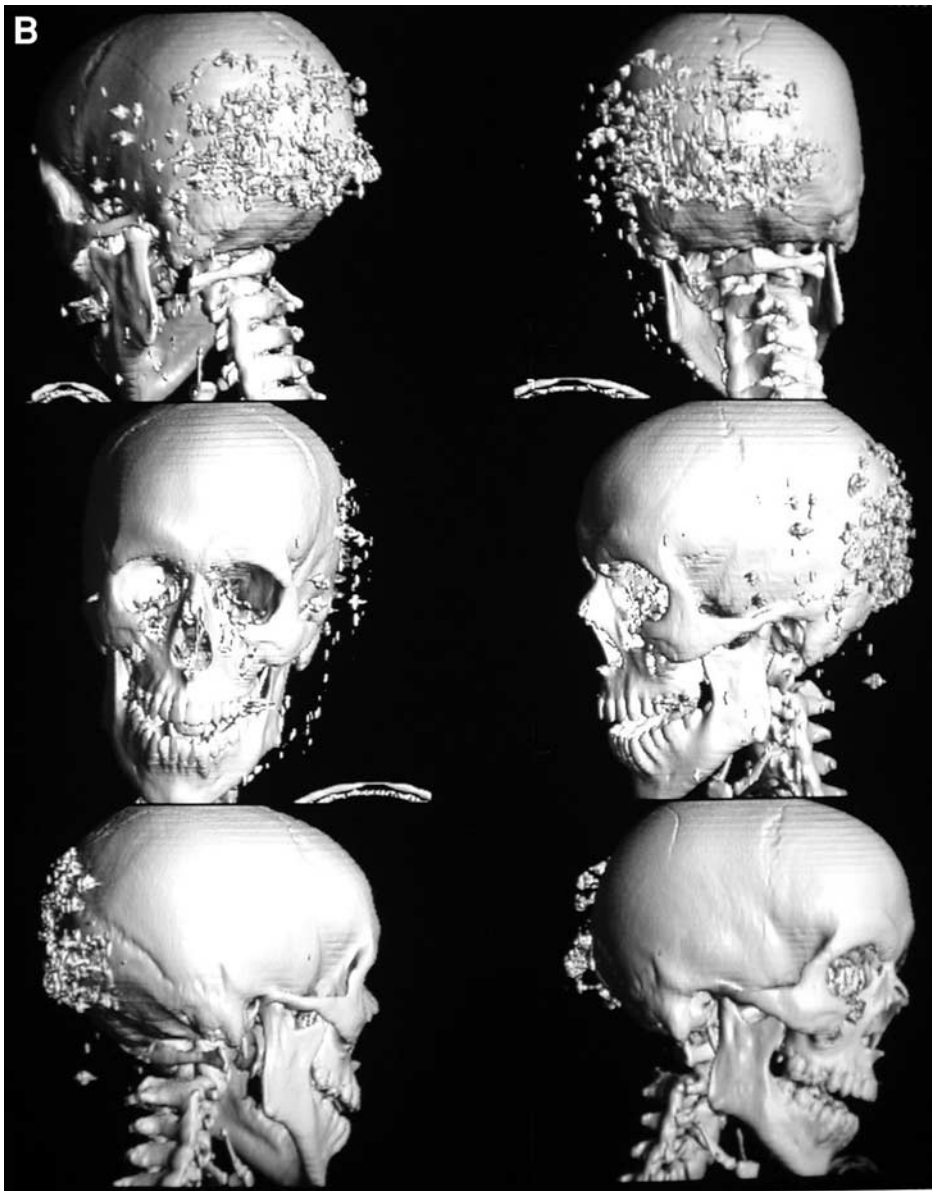


Fig. 2. *(continued)* **(B)** Postmortem computed tomography imaging with digital three-dimensional reconstruction identifies the presence of shot entirely extracranially, concentrated around the posterior and left lateral regions of the head. The fractures are seen radiating away from the mass of projectiles.

fluoroscopic images. The authors also have experience in its use when examining a large quantity of evidence bags containing a predominately clay-based soil infill from a clandestine grave for evidence. Through this, projectiles and items of clothing (notably buttons and studs) were located and recovered safely in a quick manner, whereas the alternative method of wet-sieving would have taken weeks.

However, the availability of newer technologies, such as CT/MRI, has resulted in fewer fluoroscopic investigations being performed outside temporary mortuary facilities. The fluoroscopic fields of view are narrow, have low resolution, and may not have the ability to create "hard copies" for evidential purposes.

2.3. Angiography

The injection of contrast media into vessels—for example, vertebral or coronary arteries—has remained, within the United Kingdom at least, a research tool within postmortem examinations, although many recommend vertebral artery angiography in all cases of subarachnoid hemorrhage to exclude vessel dissection or disruption *(8)*. Radio-opaque media may be injected into the coronary arteries, carotid vessels, or vertebral arteries through catheters, either *in situ* or *ex corpus* (Fig. 3). The technique is heavily operator-dependent, requiring a slow, steady injection of material to ensure that sequential images may be taken as the material passes along the length of the vessels in question. The results may be monitored either through the use of numerous plain X-rays or real-time through fluoroscopy. The images can be difficult to interpret and may not provide any additional information that cannot be gleaned through CT or MRI studies. The process is slow and time-consuming, and in the case of the coronary arteries, requires the heart to be removed from the body prior to injection, increasing the time until completion of the autopsy and producing issues regarding tissue retention and organ repatriation before release of the body for lawful disposal.

2.4. Ultrasonography

Like fluoroscopy, ultrasonography allows real-time examination of structures through the production of sound waves within the ultrasound range (between 3.5 and 7 MHz) from transducer arrays, or "probes," which pulse focused ultrasound. When the sound wave contacts an interface between tissues of differing echogenicity the wave is reflected, refracted, or absorbed depending on the laws of geometric optics. Reflected waves are received through the same hand-held probe unit and processed via scan conversion to form an image

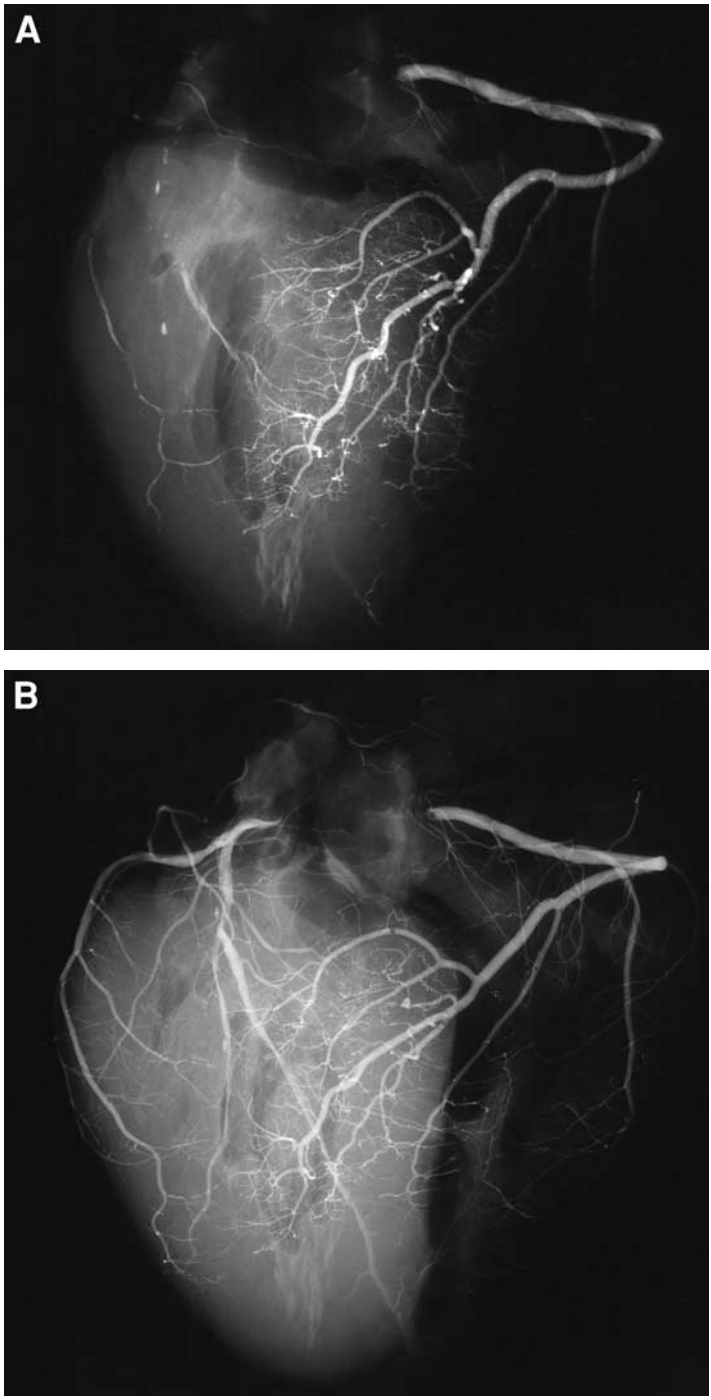


Fig. 3. (A,B) Postmortem coronary artery angiography. (Courtesy of Dr J. Bovee, Department of Pathology, Leids Universitair Medish Centrum, Leiden, The Netherlands.)

on a monitor screen, although it may be outputted to paper, videotape, or radiographic films. As it does not rely on the production of X-ray beams, ultrasound investigations have clear health and safety advantages for the user. Although portable and relatively inexpensive, the technique has only been regularly applied in research, notably the examination of the neonatal central nervous system (CNS) for the presence of germinal matrix bleeds or intracranial hemorrhages *(9,10)*. This technique has been superseded by the availability of MRI, which provides excellent views for neurological pathology and, therefore, ultrasonography will only occasionally play a role in forensic pathological practice under particular circumstances.

2.5. Computed Tomography

CT, then often referred to as "computed axial tomography" (a CAT scan), was developed in 1972 by Godfrey Hounsfield and Allan Cormack as a means of radiologically producing thin transverse sectional images through a body or an anatomical region. Instead of using a photographic plate, the technique requires a collimated X-ray beam to be passed through the body and detected using a circular array of photomultiplier tubes. The X-ray tube may rotate around the body, although more recent devices utilize numerous tubes to decrease the scan time required. The narrower the scan diameters, the greater the accuracy of the image (Fig. 4).

The scan results are analyzed for absorption patterns on a scale where maximum absorption is denoted as 100 HU (Hounsfield units) (such as bone) and minimum absorption is denoted as –1000 HU (such as air). The resultant image is outputted digitally upon a monitor, from which films may be produced, and is formed from a matrix of 2D picture image elements (pixels), which ultimately represents the three-dimensional (3D) volume elements of the tissue examined (voxels).

The result is a rapidly produced full body scan image (similar in appearance to a full length plain X-ray plate) from which it is possible to focus in greater detail (Fig 5). The disadvantages include the radiological exposure risk and cost, not only in setting up the devices, but also in frequently maintaining it. Although mobile units are in operation across the United Kingdom, the majority are currently immobile, thus requiring the establishment of either a dedicated unit within a mortuary (as may occur in the near future) or the need to liaise with radiology staff to ensure that the highly demanded devices used for investigations of living patients are not blocked by postmortem examinations.

Unlike plain X-rays, the data may be reconstructed digitally to represent the 3D tissue, bony or soft tissue in sagittal, coronal, or even a 360° "xyz" rotational field views, allowing location and tracking of projectile

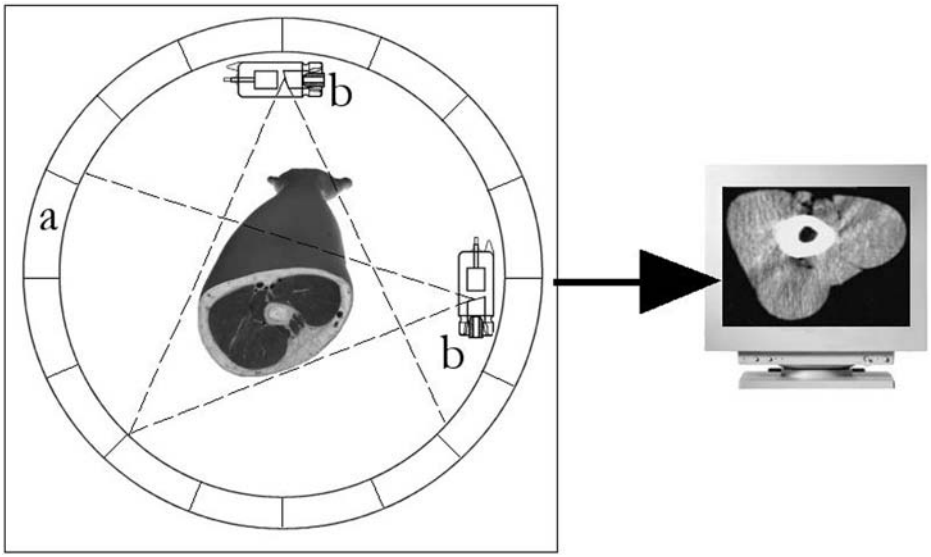


Fig. 4. A schematic diagram of computed tomography examination; the collimated X-ray beam is produced from the cathode **(a)**, and focused on the body. The passage of the beam is affected by the density of tissue through which it passes, and the density values are detected by the photomultiplier tube array rotating around the body **(b)**. The resultant image is then viewed on a video monitor and can be outputted to produce "hard copies" similar to roentgenograms.

paths or penetrating wounds prior to the autopsy examination. However, the presence of metal, such as projectiles, will often create artifactual digital image streaking or shadowing. The reason for this is that the density of metal far exceeds that of bone (>100 hμ), thus, accurate imaging may not at times be possible.

2.6. Magnetic Resonance Imaging

Developed in 1980 by Paul Lauterbur and Peter Mansfield, MRI utilizes the natural rotational behavior of hydrogen ions (H^+). By applying a strong external magnetic field it brings the H^+ into alignment, either in a parallel or antiparallel direction to the externally applied field. By far, the majority of ions align parallel to the overall field vector. The nucleus of the ions also demonstrates precession—the rotation of the nucleus around the field line—with the frequency being a property of the ion (named the "Larmor frequency").

A second magnetic field is applied externally, being perpendicular to the first at the same frequency as the Larmor frequency, known as the radiofre-

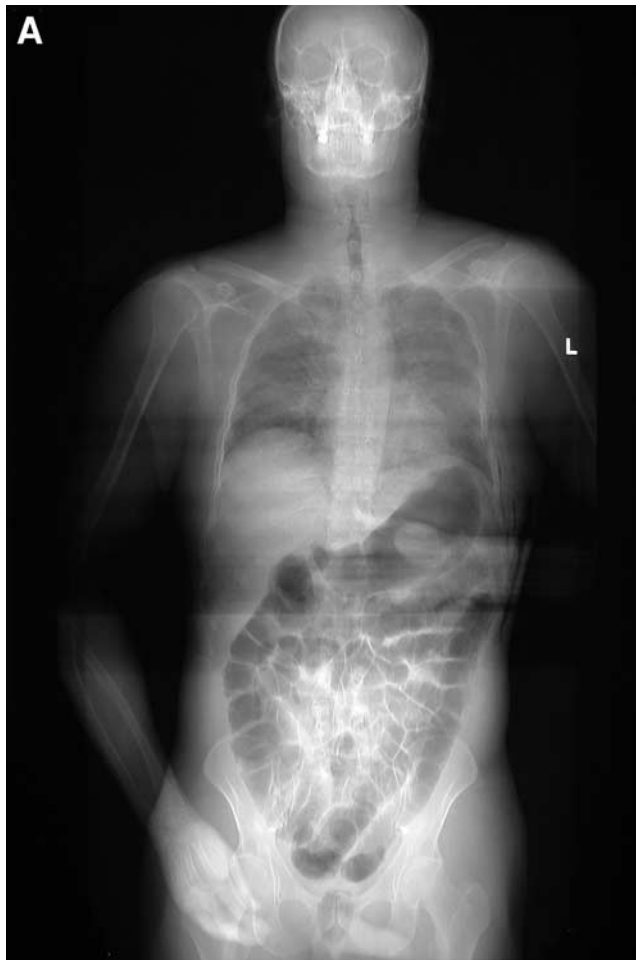


Fig. 5. (A,B) Scanograms refer to the rapidly produced body scan of large anatomical regions, produced through by the computed tomography scanner, to allow identification of the area requiring close radiological examination. *(Figure continues)*

quency (RF) pulse. A separate RF coil applies this field with the result that the H^+ realign 90° to their previous net direction with their precessations in phase. When the RF pulse ceases, the energy input through this pulse is dissipated to the surrounding molecules, known as T1 relaxation. The loss of temporary precessational synchrony results in T2 relaxation as the protons dephase. These

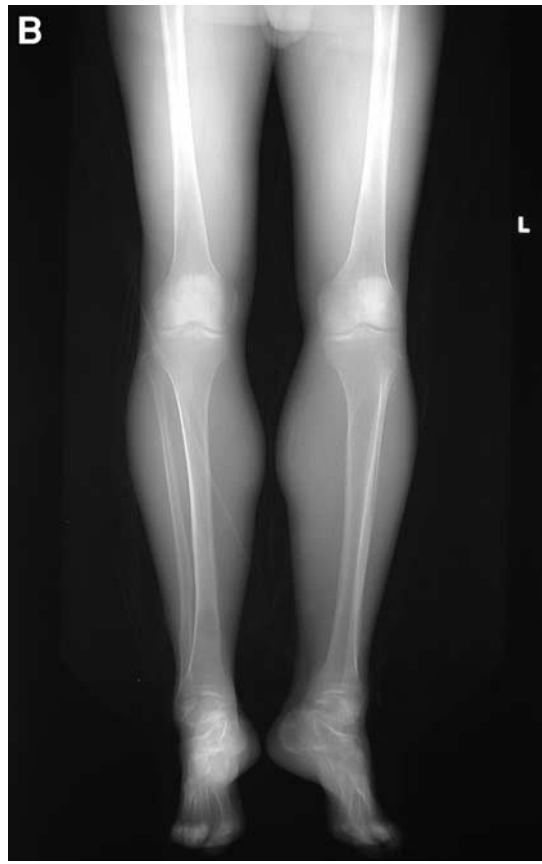


Fig. 5. *(Continued)*

phases are detected by the RF receiver coils, producing the magnetic resonance signal, which forms the basis of the image (Fig. 6).

The technique, therefore, holds no radiation exposure risk, although it must be certain that the body examined does not contain any metal including jewellery, pacemakers, projectiles, or prostheses. The presence of metal objects *per se* does not preclude the use of MRI but, because of the magnetic field generated during the examination, for example, projectiles may move within the body cavities or soft tissue and may even leave the body if they have only entered the skin or immediate subcutaneous tissues.

The degree of image resolution produced provides excellent views of the CNS and is highly recommended in pediatric cases, where the majority of lit-

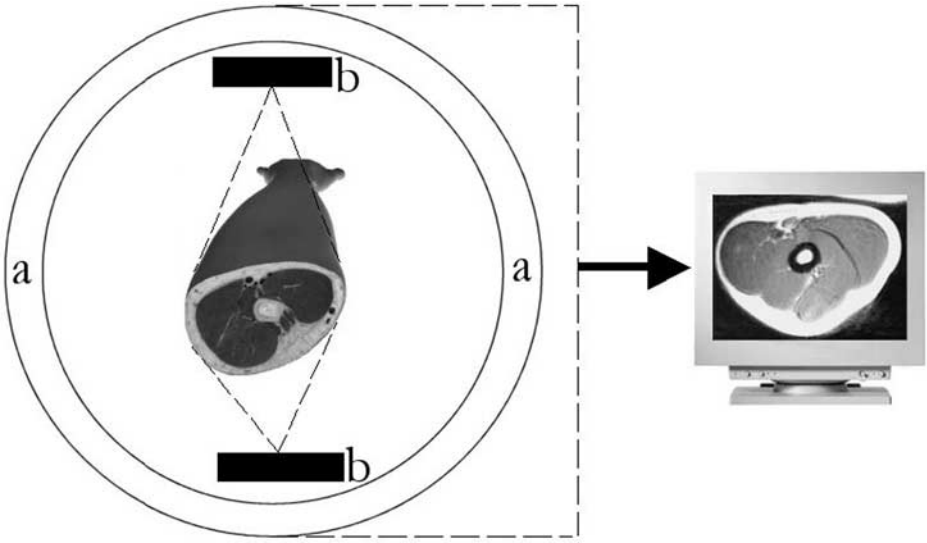


Fig. 6. A schematic diagram of a magnetic resonance imaging scanner; the surrounding magnetic field **(a)** results in the alignment of hydrogen atoms within the tissue. The production of a radiofrequency (RF) pulse from RF coils **(b)** alters the alignment and results in the release of energy from the atoms. This is detected by RF receiver coils and the data is decoded to produce a digital image on a video monitor.

erature exists to date on its application. However, little published data exists related to its potential use in adults and it cannot, to date, replace the full skeletal survey required for the investigation of child abuse.

3. Can Cadaveric Radiology Replace the Need for a Conventional Necropsy?

The literal meaning of the term "autopsy" means "to see oneself." It is synonymous with the terms "postmortem" and "necropsy," although neither of those terms have specific meaning *(11)*. Thus, under commonly held definitions, a postmortem examination may include any procedure performed for the purpose of defining the ultimate cause of death of the individual. As Benbow and Roberts highlight in their comprehensive review of techniques, this could constitute an external inspection (the "view and grant" system), cytology, or even laparoscopic biopsy-based histological examination *(12)*. Generally, the term is applied to both the external and internal surgical examination of a deceased individual, although recent legal issues have resulted in the number of

consented complete autopsies performed diminishing, often being replaced by a limited surgical examination of anatomical areas of interest. This decline in the number of necropsies is well documented, the reasons often ascribed to high-profile media cases, an increased desire to avoid litigation, heightened family concerns (possibly reflecting the alteration in ethnic population base seen in many regions during the last decades), and an increased reliance on noninvasive imaging and premortem diagnosis (be it correct or otherwise). The development of CT and MRI has vastly improved the abilities of clinicians to diagnose disease noninvasively allowing appropriate treatment. However, both anecdotal and published evidence would suggest that the reliance on such imaging may be unwarranted; subsequent postmortem examination may fail to reveal radiologically identified lesions *(13–15)*.

The extension of CT or MRI technology as a diagnostic tool applicable not only to diseases in the living but also in the dead was a predictable one. Given the noninvasive nature of the examination, together with the relative speed with which it may be performed, it has generated interest from certain religious denominations within society wherein standard surgical dissection is prohibited under religious doctrine *(16)*. However, the drawback to the use of this technology is ability of the pathologist to gain access to the imaging devices in the first place, the cost of the examination at present compared with conventional autopsy and the time that it takes to undergo MRI examination. For example, to study the heart on its own may take up to 1 hour of imaging time, generating thousands of images that all need to be examined and reported by the radiologist. Whole-body imaging, if required, could occupy many hours of valuable clinical scanning time. The answer would be to place a scanner in a mortuary, although to date there is no evidence-based research to justify this or to determine whether it should be a CT and/or MRI, as prescanning by CT prior to MRI is considered essential to date. It will also be seen from the remainder of this chapter that the literature to base the choice of which imaging modality is optimal for which purpose remains scanty, with publications based on availability of technology rather than evidence-based clinical assessment.

4. The Need for Postmortem Imaging

Postmortem radiology, as typified by CT or MRI scanning, can assist during the investigation of both natural and unnatural causes of death. Foremost, it can act as a means of autopsy planning, revealing possible areas of interest that may require an increased focus of attention. Alternatively, the imaging may reveal pathologies involving regions of the body that are not routinely examined in great detail at autopsy. Thus, it may identify natural or unnatural disease,

have a role in autopsy planning, have a health and safety role, or, in the future, may replace the necessity to undertake invasive autopsy in certain cases.

In unnatural causes of death, the radiological images can assist in evidence gathering; this is of great importance when considering deaths caused by projectiles or explosives, whereby fragments of foreign material can be identified and retrieved safely at subsequent autopsy examination (*see* Fig. 17). The images themselves can also be submitted for court purposes to detail injuries and deformities in a "sterile" and inoffensive manner for juries to consider. However, some have suggested that the digital format produced may be altered or enhanced, leaving the opportunities for evidence tampering open to complaint *(17)*. This is a time-worn argument recently aimed at the change from traditional photography to the use of digital images. It is naive to believe that a 35-mm photograph cannot be altered or tampered with and, in fact, because the software within digital cameras and memory cards record every alteration made to the use of the camera or image, digital imaging may in fact be more secure for medicolegal work than traditional laboratory-based imaging techniques. Thus, in the case of radiology, it is not anticipated that this would generate sufficient worry, especially given the subsequent surgical dissection performed to verify findings, although the need for an international agreed protocol related to the use of digital radiological imaging for medicolegal work can be raised: which machine, which fields, which radiological protocol, pricing, as well as how the images should be stored, accessed, and presented should all be considered.

5. *Health and Safety*

Two separate sources of hazards to the health of the operator need consideration when discussing postmortem radiological examinations: the risk of transmissible infectious diseases and the risks associated with ionizing radiation exposure.

5.1. Infectious Diseases

Although the examinations may take place within a separate clinical environment than the mortuary, notably radiological departments, a similar level of protective measures must be undertaken for the transportation, handling, and examination of the corpse. Appropriate risk assessment must be undertaken prior to the examination, especially in cases with body fluid leakage or the presence of known infectious agents. It is often acceptable, and recommended, that the examinations be performed with the body remaining in a sealed opaque body bag, thus minimizing the risk of exposure to infectious

agents or DNA contamination in forensic cases. Should the latter become an issue, samples and swabs should be taken prior to the removal of the body for radiological examination. Again, it remains unknown to date whether the use of radiological examination prior to autopsy examination affects trace evidence, specifically DNA that may be present on a body. It should also be remembered by those that examine the dead as a profession that the radiographic team may not be used to or prepared for such contact, especially in cases of violent death, and therefore, if the body remains concealed, one is more likely to gain the assistance of the radiology department. If manual manipulation of the body becomes essential to produce adequate views, the bag may be opened for this purpose and film plates passed beneath the bag (conventional plain X-ray). Again, appropriate care should be taken in the lifting of the "dead weight" to avoid work-related injury.

5.2. Radiation Safety

Because of their inherent design and purpose, radiological departments are better prepared to minimize the risks associated with ionizing radiation. They must comply with local and national radiation safety standards. Thus, whenever possible, cadaveric examination should be undertaken within the radiological department. Similar examinations performed within a mortuary facility could result in an increased risk of unnecessary radiation exposure, especially when using older mobile technology, which is often found in such establishments. The alternative of the installation of permanent imaging equipment within a mortuary facility, apart from the cost implications, will require a commitment to an adequate quality assurance program and regular maintenance to ensure minimal risk of radiation exposure. Risk assessment, adequate training, and compliance with standard operating procedures are essential. With improved technology and experience, the number of radiographic films that require exposing for each case should be minimized, thus reducing the radiation risk.

6. Postmortem CT and MRI Applications

The principle area of recent advances in the application of radiology to postmortem investigations is with the access to and use of CT and MRI. The application of CT and MRI to the postmortem period has resulted in the production of numerous published articles, be it single case reports or a series of observations. These are aimed to assist pathologists in their use of these technologies, as well as the introduction of new terminology, for example the Virtopsy® and the Necroradiologist *(18,19)*.

A review of the present literature allows division of the topics into three broad categories:

1. Postmortem changes.
2. Anthropological examinations and identification issues.
3. The ascertainment of the cause of death.

6.1. Postmortem Changes

The ability of the pathologist to recognize decomposition-related changes is paramount to the differential exclusion of not only pathological processes but also, in some instances, foul play. The time-related changes have been well documented by such authors as Knight and, although macroscopic and indeed microscopic alterations are well-described in pathological texts, it constitutes little of the published material in radiological-based examinations *(20)*. The absence of large numbers of higher quality comparative studies between macroscopic pathological appearances and CT/MRI images in children and adults for both natural and unnatural causes of death produces a wealth of possible future studies. This study of postmortem-related changes to the constituents of the body is essential if imaging replaces the need for invasive autopsy. At present, few radiographers/radiologists have sufficient experience in relation to changes that occur to the external and internal surfaces of the body after death, how these postmortem changes present on imaging, how they will alter the imaging process and how to distinguish postmortem changes from diseases that may have occurred in life.

One of the questions related to the investigation of a death is *when* did the person die? The examination for rigor mortis, postmortem lividity, body temperature, and biochemical changes that make up the standard armory that a pathologist can draw on in an attempt to answer this question cannot, to date, be assessed by radiological imaging alone. However it may be that other body tissue-related changes may be identified as experience grows with the use of CT or MRI to allow time-related changes to be assessed and used to estimate the time since death.

Pathologists recognize that after death artifacts can occur to the body that may mimic antemortem disease and can lead to misdiagnosis by the unwary. This area has been covered most recently by Rutty *(21)* and Tsokos *(22)*. To date, however, there are few publications related to such postmortem artifacts and how they appear or are interpreted using CT or MRI. The potential for a heat hematoma to be misdiagnosed as an antemortem head injury by a person who is unaware of such changes thus remains to date.

After death, even early decomposition-related changes might be readily and rapidly identifiable. The pressure within the subarachnoid space diminishes, resulting in the resting of the occipital lobe and the cerebellum against the occiput *(18)*. The effect is of little significance, although the erroneous diagnosis of raised intracranial pressure needs excluding when examining CT or MRI imaging alone.

Shiotomi et al. have demonstrated the early change of postmortem lividity (hypostasis) using CT imaging *(23)*. The authors examined the CT images for identifiable fluid levels within the intravascular spaces. In more than half of the cases examined (n = 126) lividity was demonstrable. This surprisingly low number, given the prevalence of such changes in clinical autopsy practice, may relate to a relatively short postmortem interval (2 hours) in their study group. The value of such a means of recognizing postmortem changes is, as pathologists are aware, to be able to differentiate lividity from diffuse areas of bruising; Thali and colleagues confirm this is possible *(18)*.

As putrefaction progresses, gaseous collections are noted initially within body cavities and solid organs. The result is the sponge, or "Swiss cheese," effect visible on CT as intravascular and intracardiac gas dissipates *(18,24)*. Its diffuse nature and the overall state of the body should exclude the possibility of air emboli in such cases, which forms the main differential diagnosis. Such gaseous extension is also visible in the soft tissues as a "surgical emphysema" type pattern with palpable crepitations in the subcutaneous tissues and muscle groups (Fig. 7).

Hepatic portal vein gas may often be observed in cases of small bowel infarction, intestinal mucosal damage, intraperitoneal injury, and sepsis. However, one small study revealed its presence in unexpected cases, such as ischemic heart disease, where imaging was performed within 2 hours of death for each case *(25)*. The cause of this venous gas collection is therefore unknown, given the short postmortem interval and the absence of established putrefaction, although a recent publication correlates its presence to gastric distension, suggesting it to be a postmortem pressure gradient effect *(26)*. Such changes require further research to avoid misdiagnosis or interpretational confusion.

The heart chambers also appear to alter radiologically in death. In a study of 50 live vs 50 dead individuals, maximum and minimum cardiac chamber diameters appeared to increase in the latter group *(27)*. Notably, the right ventricle dilates producing a spherical cross-sectional appearance. All such pathological causes of right ventricular dilatation which such alteration could mimic require exclusion during the assessment of postmortem imaging (Fig. 8).

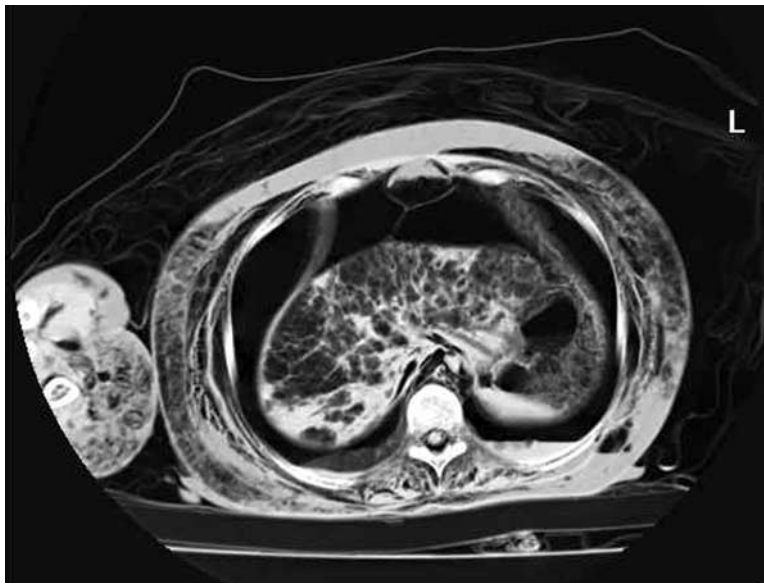


Fig. 7. Early decomposition is readily identifiable on postmortem computed tomography scanning as gas collects within the soft tissues of the arms and chest wall. Putrefactive gases accumulate within the abdominal cavity and the vasculature of the liver distends with gas, which is also visible in the portal and hepatic veins.

The aortic wall may also undergo alterations in its radiological appearance. Hyperattenuation was described in all 50 deceased cases (presumably the same cases described earlier given the same authorship), but similar changes were observed in only one case from the living subset *(28)*. The postmortem CT images were performed within 2 hours of death and the attenuation profiles across the central aortic wall were calculated. Such changes, previously described in the living in cases of atherosclerosis, severe anemia, dissection, and Takayasu's arteritis, were described in young patients, which generally excluded such possible causes. Closer examination of the aorta reveals that the vessel caliber was narrower compared with live patients, suggesting an element of smooth muscle contraction (postmortem stiffening of the musculature/rigor mortis). The authors raised other causes such as a lack of motion artifact and low fluid volume attenuation owing to infusions during failed resuscitation attempts. The authors in question examined the findings of this study further. A follow-up CT-based study of 12 deceased individuals, constituting both natural and unnatural causes of death, was undertaken *(25)*. The time from death to

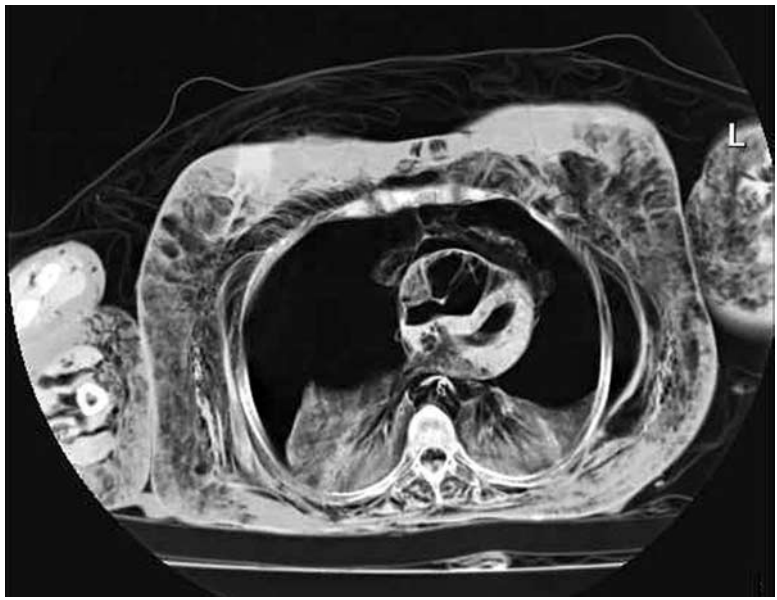


Fig. 8. Soft tissue decomposition within the chest, as detected through postmortem computed tomography scanning; the gaseous collections are visible in the chest wall and upper limbs, with collapse of the lungs and "pseudo-pneumothorax" development. Gas is also visible within the cardiac chambers, imitating a cardiac air embolus.

imaging was less than 48 hours in each case and was followed by formal necropsy. The finding of high-density attenuation of the aortic wall was again confirmed to be associated in the most instances with atherosclerosis, though the sample size was too small for accurate assessment.

The rapidly developing postmortem autolytic changes that occur within the CNS all too frequently effect in a semiliquid state that, at autopsy, results in the brain been disrupted by the removal of the skull cap and meninges (Fig. 9). Thali et al. described a technique developed in Belgium of dissecting the head from the neck, then deep-freezing the specimen *(24)*. This is later sectioned coronally and immersed in a formalin solution for fixation prior to the CT or MRI examination. The overall acceptance of this technique by relatives, the general public, or even the medical profession itself, who all too often consider the autopsy barbaric or mutilating in its usual state, may express severe concerns/reservations over such methodologies, certainly within the current political climate experienced in the United Kingdom, at least.

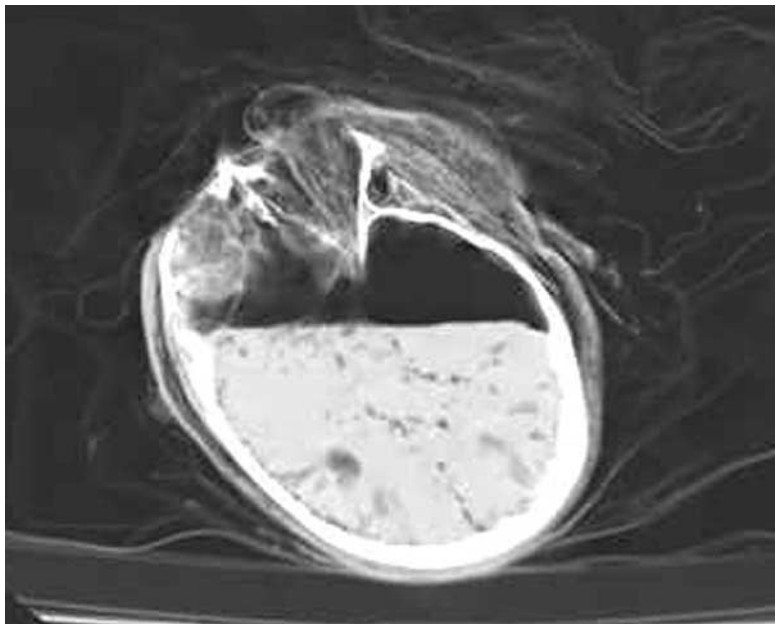


Fig. 9. Autolytic changes within the central nervous system result in the collapse of the cerebral hemispheres under gravity, such that a semifluid collection develops that may be disrupted on opening of the skull at autopsy examination.

6.2. Anthropological Examinations and Identification

The conventional autopsy starts with an external examination to document features from the body that can assist in answering the question *who* a person was. Color and length of hair, color of eyes, presence and description of tattoos and scars, as well as presence and color of nail polish, are all documented at autopsy, yet none of these basic identification features have been studied or can be identified on CT or MRI to date. Thus, for the unidentified body, an external examination will still, to date, remain a necessity.

Postmortem imaging techniques have been successfully applied to historical cases, although one could argue the specificity of diagnosis is less important in cases from 1500 BCE or earlier. "Oetzi," the Iceman discovered in the Tyrolean Alps in 1991, has been subjected to numerous radiological examinations during the last 10 years. The resulting images revealed an extensive pathological history including degenerative bone and joint disease, but also the presence of a stone arrowhead embedded within the upper body revealed by CT scanning. The need to differentiate antemortem from postmortem damage, and

even recovery damage, is paramount in the interpretation of all such disease processes *(29,30)*.

Similarly, the nondestructive nature of a radiological examination allows access to historical remains, such as Egyptian mummies, performed while still wrapped in ceremonial bindings. High-resolution CT and heli
cal CT at narrow
intervals (1 mm) in areas of interest allow not only identification of pathological processes of interest but also the potential to reconstruct the soft tissue and bony facial appearance of the individuals within a 3D digital format *(31,32)*. This technique has also been applied to forensic cases that used both CT and 3D laser color scans to reconstruct the facial features *(33)*. Rocha et al. experimented with five cadavers, each scanned and measured using data from agreed standard anthropometric points to produce a qualitative standard for quantitative analysis of images, both within the soft tissue and bone windows *(34)*. The result was the production of 3D facial reconstructions with sufficient accuracy to allow subsequent craniofacial measurements to be taken *(34)*. Such methods, although potentially useful, still remain dependent on the database inputted and possibly the artistic skills of the software developer.

The precision of the digitized results appears accurate enough for standardiszed measurements and comparisons. Hildebot and colleagues performed high-resolution CT scanning on five isolated skulls (including dysmorphic cases) *(35)*. The reformatted 3D images allowed digital measurements to be performed, which were subsequently compared with measurements taken from the dried skulls themselves. Although results were good, the authors state that callipers remain the most accurate means of producing measurements today. Precision may be improved through future imaging developments as it is reliant on the quality of data taken.

Rather than relying on visual cues, other means of using CTs for identification of deceased have been detailed. Simple comparative cases based on the identical radiological appearances of antemortem imaging and postmortem images have been published. These have recalled the ability to use lumbosacral bony landmarks or the cranial appearances on CT to identify a deceased *(36–39)*. Such techniques require the initial suspicion as to the identity and the ability to align the skeletalized remains to produce identical views to those demonstrated within the original films. Such changes may therefore result, albeit unconsciously, in subjective alterations to purposefully "fit match" remains to identities. Other supposedly person-specific morphological aspects may be examined; for example, the use of CT to examine antemortem and postmortem frontal sinus patterns *(38)*. It should also be remembered that up to 10% of adult individuals within certain populations may be lacking such sinuses, thus affecting this means of identification *(40)*.

Gender assessment has been reported by Rutty *(41)* and Swift and Rutty *(42)* by using the length of the external auditory canal measured from CT coronal section. The measurement of the area occupied by the mastoid air sinuses within the temporal bone has also been studied using CT images as a means of gender or personal identification (M. Viner, personal communication, 2004).

Another aspect of an anthropological examination includes the estimation of age at death based on bone changes and fusion of epiphyseal plates *(43)*. Similar approaches have been applied to imaging scans. Kreitner et al. analyzed CT studies of 380 live patients, all under 30 years of age, for the ability to identify the presence of medial clavicular epiphyses and its fusion to the clavicular shaft *(44)*. Such fusion was seen to be complete in all cases over the age of 27, although this may be seen beyond the age of 22 (partial fusion commences at 16 years). As with all age-dependent estimations, these are population- and gender-specific assuming equal nutritional and hormonal status throughout. At the very least it may assist in cases of decomposition or dismemberment where age estimation by other techniques is limited. Our personal experience of epiphyseal fusion assessment using CT is that traditional plain X-ray is, to date, superior for this investigation.

One of the established anthropological means of identifying age at death is the Suchey-Brooks phase analysis of the *os pubis (43,45)*. In such methods, casts are taken of the articular surfaces of the *os pubis*, the subsequent imprint being analyzed for ridge development. This is performed by visual comparison of the specimen cast against a series of manufactured cast "standards" for each age group. A CT-based method that aims at eliminating this stage by creating a regression algorithm to calculate interridge distances has been published *(46)*. The resultant equation produced, however, appears to be only male-specific, preventing age estimation in the other 50% of the population.

6.3. Cause of Death Ascertainment

6.3.1. Head and Neck Trauma

Deaths related to head and neck trauma continue to be a major aspect of either accidental or homicide investigations. Because the majority of publications related to the application of CT or MRI to autopsy practice have been generated from the forensic community, it is no surprise to find a number of publications in this area, especially related to injuries and death resulting from projectiles. The use of multislice CT with soft tissue and bony 3D reconstruction allows the identification and demonstration, for example, in the courtroom, of complex soft tissue and bony injuries to the skull and facial bones, congenital or acquired

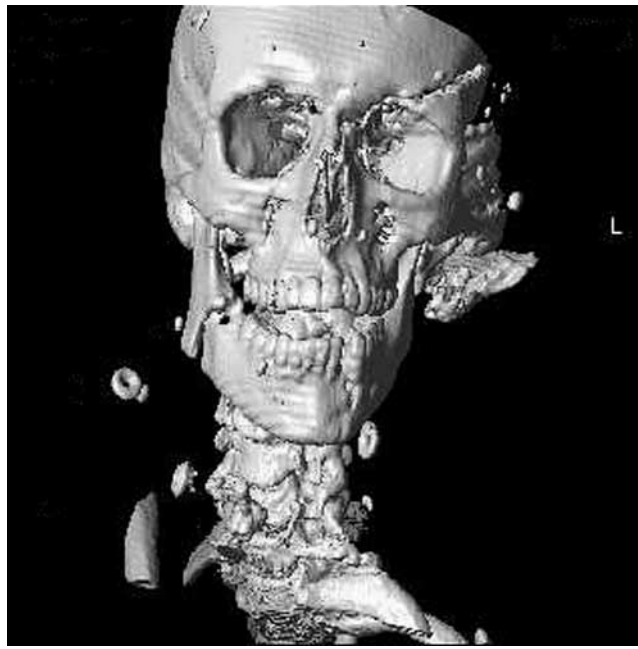


Fig. 10. Digital three-dimensional reconstruction from computed tomography data allows the production of "sterile" images acceptable for use within courts. The precision of the images allows identification of fractures across the left supraorbital region, extending into the nasal complex, with a fracture of the right angle of the mandible in a case of death caused by multiple blunt trauma.

periosteal markings or disease, wormian bones, or the marks left by nonpenetrating blunt trauma (Figs. 10 and 11).

Fracture examination and assessment has been examined by numerous authors, with a view to injury causation *(47,48)*. However, the manner in which CT scans are performed has been shown to affect the ability to produce accurate 3D image reconstructions. Levy et al. describe a study, performed on a dried skull and therefore *ex corpus*, whereby axial vs coronal datasets were retrieved and assessed by 10 independent observers *(47)*. The results indicate axial image data as the recommended means of examining zygomatic fractures, whereas Le Fort type I and III fractures are best examined using coronal CT sectioning. Knowledge of such differences is of great importance when reviewing a trauma scan and, hence, planning the optimal manner in which to examine anatomical regions at higher detail.

Craniofacial injuries are frequently encountered in both clinical and forensic practice. A CT study from Switzerland, based on a total of 377 fractures in 100 patients, proposed the creation of a new classification method for midfacial

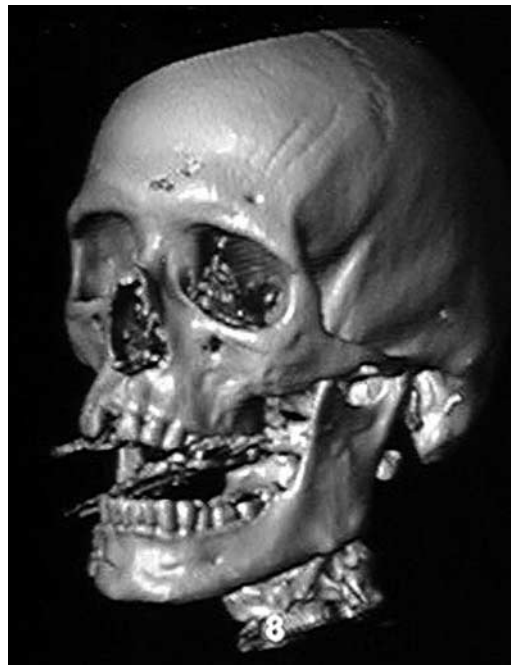


Fig. 11. The precision of scanning can be such that minute detail, such as vascular grooving to the supraorbital region, can be recognized prior to dissection of the head and facial regions. The scan time to produce such images is approx 15 minutes, and as such, does not unduly delay the investigation of suspicious deaths.

and craniofacial fractures relating to the Arbeitsgemeinschaft fur Osteosynthesefragen/Association for the Study of Internal Fixation (AO/ASIF) scheme, wherein the face is divided into three regions (lower midface, upper midface and craniobasal-facial unit) and graded on their type and severity *(49)*. The authors claim the results are an improvement over the Le Fort classification scheme and not only document the injuries, but also correlate to clinical aspects such as asymmetry and movement limitation and the need for bone grafting.

The MRI scanning of whole fixed brains has been recommended as a means of displaying intracerebral pathology in a manner that is acceptable for courtroom use *(50)*. Although the author describes its benefits, as would be expected, in cases of blunt force trauma and gunshot injuries, how this is an improvement over CT scanning of the cerebral hemispheres *in situ* is not discussed adequately. In fact, the use of MRI for *ex corpus* tissue, when the possibility of metal fragments remaining within the tissue is high (e.g., in gunshot injuries), may be contraindicated.

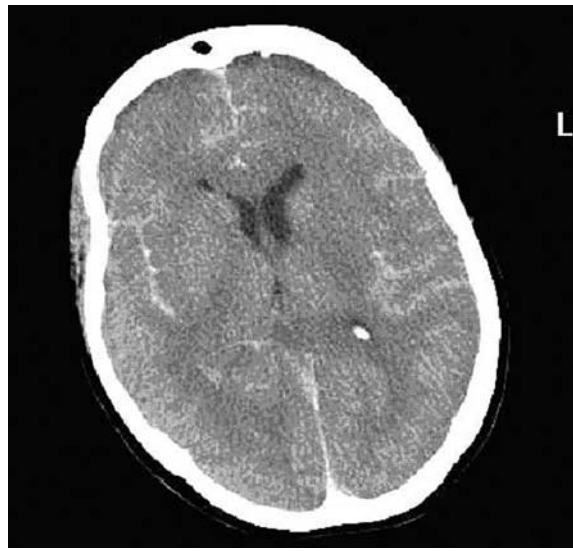


Fig. 12. The presence of small quantities of blood within the subarachnoid space at postmortem computed tomography scanning requires distinguishing between true subarachnoid hemorrhage and the "pseudo-subarachnoid hemorrhage" reported within the literature. (For further details, *see* ref. *53*.)

MRI has been used to confirm intravital skull fractures in a subsequently dismembered and burnt corpse *(51)*. The location of blood clot within the mastoid air spaces on MRI examination suggested that an adjacent temporal bone fracture occurred during life.

Intracranial hemorrhages of all types can be examined through CT scanning of a cadaver as they can in the living (Fig. 12). A study in France, based on 23 cases of sudden infant death syndrome, revealed not only pneurmatocoele collections relating to lumbar punctures prior to death and microcalcifications owing to congenital toxoplasmosis, but also inexplicable hyperdense regions within the subarachnoid spaces, despite normal ventricular systems *(52)*. This was not thought to correlate with traumatic subarachnoid hemorrhages. Similar findings of so-called "pseudo-subarachnoid hemorrhages" were described by Chute and Smialek *(53)*. The use of noncontrast enhanced films, such as would be experienced in postmortem practice, was identified as the possible cause for the apparent discrepancy between the CT and neuropathological findings, the majority of which showed hypoxic–ischemic changes. Recognition of this entity is important, given the articles published calling for pure noninvasive autopsy examinations in the future.

An interesting descriptive article detailed the appearances of cerebral edema relating to two fatal cases of cyanide ingestion *(54)*. Unlike hypoxic–ischemic insults, the authors describe a diffuse cerebral swelling with ultimate loss of the grey-white matter differentiation in a very short time frame (<3 hours) and hypothesized that this appearance on CT examination may provide a means of distinguishing such causes of collapse from other causes of cerebral hypoxic injury.

Other uses for CT/MRI include the assessment of the degree of head injury and its causation. The study by Besenski and co-workers assessed the relationship between the site of impact/trauma and the consequent distribution of cerebral injury *(55)*. A study of 21 cases from Germany in which a CT scan had been undertaken in life but the patient had subsequently died was able to demonstrate the site and distribution of linear or depressed fractures and associated coup/contre-coup injuries *(56)*. Based on the observations made, the authors proposed that it was possible to distinguish between an antemortem blunt trauma blow to the head and a fall and, in one case, superimposed a weapon onto the CT scan to demonstrate the causation of the skull fracture, although the published image of the superimposition leaves much to be desired *(56)*.

Considering the potential for the use of MRI in adults, very little has been published on its use in neck pathology/trauma. It is reported that in hyperflexion/hyperextension injuries, 80% of the injury is to soft tissue with only 20% of cases illustrating a bony lesion. A single study involving the imaging of the neck removed at autopsy (*ex corpus*) and imaged with a long acquisition time found poor correlation between the MRI and autopsy findings *(57)*. On the other hand, the effect of judicial hanging on the upper cervical vertebral column has been described *(58)*. Two individuals that had undergone state execution were subjected to a full range of cervical spine radiology, vertebral artery angiography, and CT and MRI scanning with subsequent full surgical necropsy examination. The results showed that both subaural and submental knots produce subarachnoid hemorrhage, presumably through traumatic vertebral artery injury, although the submental placement of the noose knot produced severe ligamentous damage and subluxation of the vertebrae; this was not observed in the subaural method *(58)*. The article describes the differences in each form of examination technique, confirming the benefits of MRI when viewing soft tissue damage to the neck. In our own practice we have found that CT can be used to gain excellent images of the upper cervical spine including the odontoid peg. In one case, complete collapse of the body of C2 as a result of lymphoreticular malignancy was clearly demonstrated prior to autopsy examination (Fig. 13).

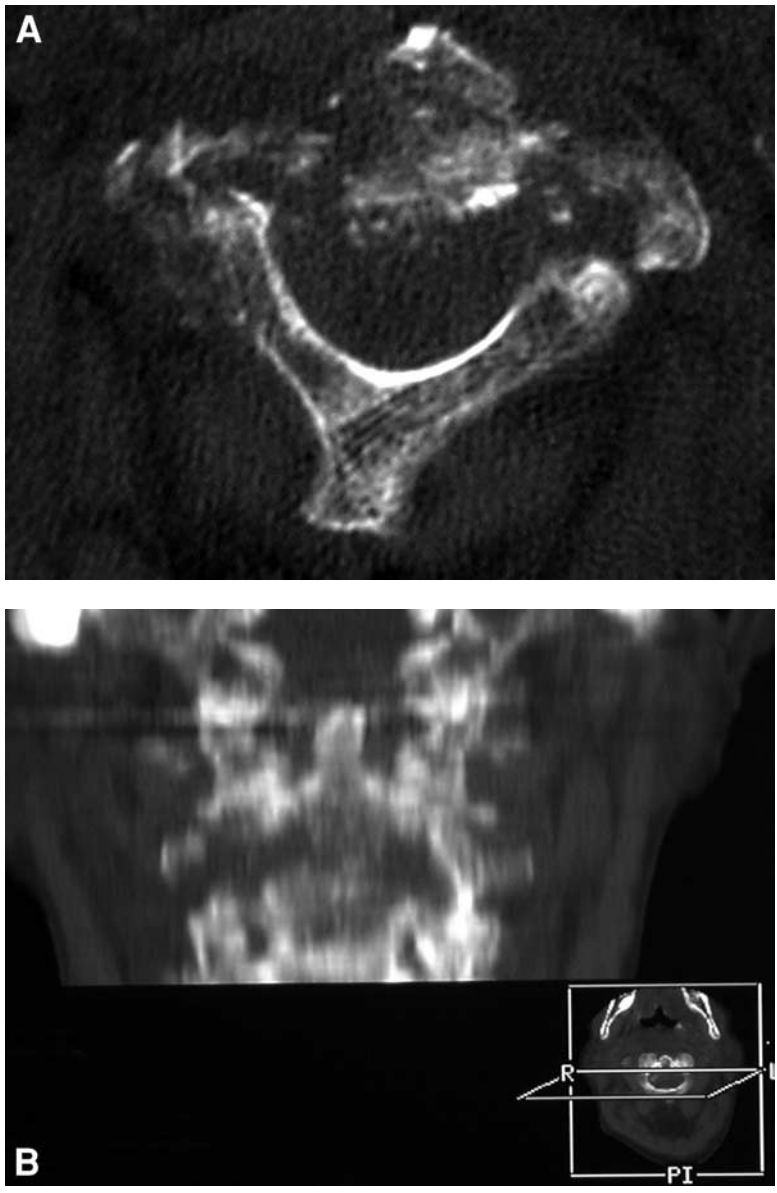


Fig. 13. (A,B) Computed tomography-based images of the axis vertebra (C2) in an individual with myeloma reveals the collapse of the vertebral body and odontoid peg, an area that may be difficult to visualize at postmortem examination without the use of specialist dissection techniques.

6.3.2. Pediatric Cases

The majority of studies regarding pediatric issues have focused on the application of MRI to cases of both natural and unnatural death. The largest perinatal study, by Alderliesten et al., examined 58 children and claimed a positive predictive value of up to 80% for MRI diagnosis *(59)*. The study was somewhat limited in that only 26 cases underwent MRI and autopsy examination, despite the latter being, by the authors' own admission, the gold standard in postmortem examination of perinatal deaths. The paper concluded that the results gained through the imaging examinations were insufficient to replace the postmortem examination by surgical dissection. Even major malformations were missed, including three abnormalities that would have been recorded during an external examination of the body after death (anal atresia, myelomeningocoele, and omphalocoele).

A more complete, albeit smaller, study was published by Brookes and colleagues, also concerned with the issue of perinatal mortality, although additional publications have shown the use of the technique also in focused anatomical regions, notably the fetal heart *(60–62)*. Twenty fetuses that were either aborted, miscarried, or stillborn underwent initial MRI studies prior to necropsy examination *(60)*. The major malformations and ascribed causes of death produced through each study were compared, revealing that concordance was reached in only 40% of cases. Necropsy examination proved more useful in a further 40%. Magnetic resonance imaging provided more information in the remaining cases, which generally fell into the category of CNS malformations, an area in which MRI is known to excel. This was shown through the publication by Griffiths et al., whereby 40 fetuses and stillborn neonates underwent postmortem MRI of the CNS *(63)*. The results claimed a sensitivity and specificity of 100 and 92%, respectively, although 11 of the cases were described subsequently as normal. It should also be noted that 20% of the cases examined ultimately proved too autolytic for diagnosis, either by MRI or necropsy. Such results are not unexpected given the unfixed nature of the tissue being assessed.

The issue of diagnosing child abuse by imaging technology is an area of interest. Hart describes his experience of neuroimaging of cases from New Mexico *(64)*. His review of MRI compared with necropsy findings revealed that few artifacts were produced in a postmortem period of less than 24 hours, although MRI proved less sensitive at diagnosing lesions outside of the CNS. Although gross vitreous hemorrhage was demonstrated, the equally important hemorrhage within the optic nerve sheath produced through shaking injuries went undetected radiologically. It was also noted that small intracranial

hematomas can be masked by the presence of massive brain edema. Overall, MRI proved important in its ability to illustrate the presence of intracerebral bleeds and both acute and chronic extracerebral hemorrhages, except for subarachnoid hemorrhages where CT scans appears to be much more sensitive.

The appreciation of aging changes within blood clots is, obviously, of importance to forensic investigations, whereby recognition of such alterations may suggest a history of repeated trauma, or even dating of the time of injury *(65)*.

The conclusion indicates that the combination of MRI with classical necropsy examination produces optimal results, indicating a role for imaging in all future medicolegal casework *(64)*. This dual examination technique has been demonstrated in a separate, though nonmedical, study. Two cases reported by Asano et al. detailed the digital 3D reconstruction of resected fetal laryngeal structures, confirming the presence of congenital laryngeal atresia *(66)*. Such studies may negate the need for serial sections from whole tissue autopsy preparations, a situation ideally suited to the current climate regarding tissue retention.

6.3.3. Natural Causes of Death

A number of studies have now been undertaken to assess whether or not CT or MRI could be considered as an alternative to the conventional invasive autopsy. These have been fuelled by the present general public attitude to the autopsy and organ retention, as well as from religious groups, particularly the Jewish population. Studies from Manchester (UK), Switzerland, Japan, Boston, and Israel have all considered the use of MRI as an adjunct or replacement to conventional autopsy and, to date, all agree that although CT and MRI are comparable to autopsy for the identification of certain pathologies and potential causes of death, the principle cause of death in the developed world (coronary artery disease) cannot be reliably diagnosed using these imaging techniques *(16,18, 67–70)*.

This work ultimately falls into two camps: imaging undertaken on the cadaver and imaging undertaken on specimens removed at autopsy. It has been shown that pathogenic gas collections, for example, cardiac and cerebral air embolus, subcutaneous emphysema, and hyperbaric trauma, can all be visualized, although false positive postmortem gas within the hepatic portal vein or heart within 4 hours of death or as a consequence of resuscitation effects remains a pitfall for the unwary as discussed previously *(71)*.

The assessment of metastatic cancer has been investigated with the use of MRI, although in one study of 37 cases, imaging was found to be less informative than autopsy in seven cadavers *(68)*. Elsewhere in the body, effusions,

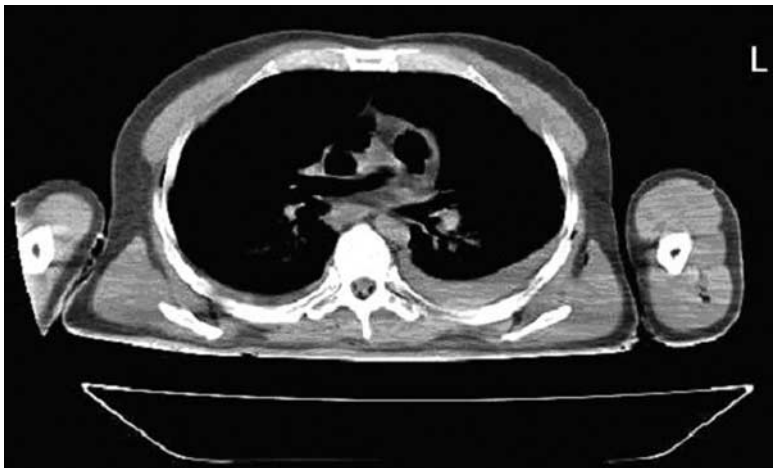


Fig. 14. Cardiac air embolism identifiable at postmortem computed tomography imaging in a case of multiple stab wounds inflicted to the neck region. Imaging was performed within 12 to 24 hours of death, and standard postmortem examination techniques were employed immediately after imaging was completed to confirm the presence of gas within the cardiac chambers.

pneumothoraces, air space disease, adult respiratory distress syndrome, aortic dissection, spleen and liver infarction, cerebral fat embolus, and pneumoperitonitis have all been reported *(70,72,73)*.

However, it is in the area of cardiac pathology that the major discrepancies between imaging and conventional autopsy are found. Although one group claimed to be able to issue a cause of death in 87% of their cases, this figure dropped to 30% in a later study with those dying of coronary artery disease making up the remaining 70% *(16,69)*. Other studies quote similar figures. Although intracardiac thrombus or air can be visualized, as well as fibrosis within the myocardial wall, calcium quantification of the coronary arteries or estimation of the right ventricular mass, to date the failure of the ability to image the pathology within the coronary arteries means that those dying of disease related to these vessels may still require conventional autopsy *(74,75)* (Fig. 14). The final consideration, as discussed by Rutty and Swift *(19)*, is that just because one identifies a pathology on a scan, is this automatically the cause of death? Without consideration for the need for other procedures performed at an invasive autopsy, for example, the external examination or ancillary tests, such as toxicology, then the possibility of a pure imaging autopsy missing a concealed homicide cannot be ruled out.

6.3.4. Musculoskeletal Injuries and Soft Tissue Trauma

Prior to commencing a review of the published literature, the benefits of the "trauma-scan" or "scanogram" should be described. This involves a preliminary head-to-toe CT scan producing, in effect, a full body skeletal survey allowing ready identification of gross natural disease, fractures, injuries, pathology subsequent to injuries (e.g., hemothorax), or the presence and location of projectiles. It is relatively fast, being produced in a couple of minutes, and, as with all postmortem CT imaging, can be performed with the body remaining within a sealed body bag. It allows the examiner to quickly focus on areas of potential interest that may then be visualized at higher resolution.

The examination of musculoskeletal and soft tissue injuries through postmortem imaging has followed two separate routes. Firstly, the study of injuries inflicted which have resulted in the death of the individual and secondly the creation of injuries in cadavers for subsequent assessment. This latter group falls into the research groups of those required to improve safety in vehicles, such as automobiles. An example of this work was published by Hines et al., whereby unembalmed human cadavers were exposed to low-speed impacts of the shoulder region within 48 hours of death *(76)*. Pre- and postimpact MRI images were compared, with the results indicating that such focused, low-speed impacts commonly result in clavicular instabilities and distal clavicular fractures. These results can therefore improve crash-test dummy data and alter car manufacturers' designs for passenger or pedestrian safety.

Alternatively, the injuries produced during fatal accidents have been assessed through the combined use of contact radiographs and MRI, compared with macroscopical and microscopical analysis. These imaging tests were performed "blind" to the pathological findings, although the study required the use of ex vivo cervical spine sections, apparently "carefully removed" during necropsy examinations of 10 cadavers and subsequently deep-frozen prior to assessment *(77)*. The results indicate that MRI is useful for demonstrating ligamentous damage, although it was unable to detect eleven of the 28 lesions identified by pathological examination alone. A further four cases (including a fracture case) were missed on initial MRI assessment. The overall findings were that, although useful, standard necropsy examination remains the method of choice.

A similar finding was reported by Donchin et al., whereby a CT-based study of 25 trauma victims revealed over 70% of soft tissue pathological findings were identifiable through radiological assessment *(78)*. The study was limited, in that only 13 cadavers underwent full autopsy examination to confirm the findings, in itself identifying more pathologies than CT alone. The

research article also confirms the anecdotal suggestion that fractures and skeletal pathologies are better detected radiologically than by autopsy alone. The results indicate that combined CT and autopsy examination produces the most accurate results, though where consent for surgical dissection is not forthcoming tomographical radiology may assist in identifying pathological conditions.

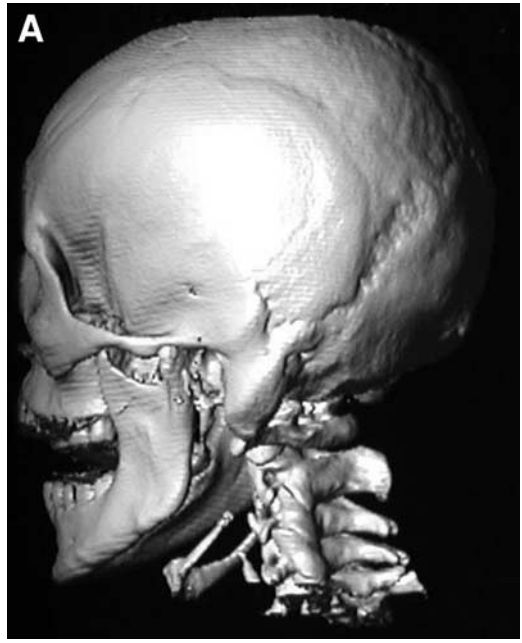
Meglin and colleagues examined the ability to evaluate laryngeal trauma *(79)*. Although none of the five cases detailed were postmortem studies, the paper is of medicolegal importance in the demonstration of the ability to, not only diagnose laryngeal fractures by 3D CT, but also to differentiate such injuries from natural anatomical variants that may simulate damage caused by manual strangulation. The hyoid and laryngeal cartilages can be reviewed within a 3D reconstruction format to display any fractures; however, laryngeal injuries are best identified when the structures in question have undergone a degree of calcification (Fig. 15).

6.3.5. Unnatural Death

In our own practice, wherever possible, we now examine all suspicious deaths or homicides with CT prior to autopsy for the reasons explained elsewhere in the text. This has led to the study of the causes and appearances of unnatural death including blunt trauma, projectiles (gunshot, shotgun, and explosive devices), sharp injury, vehicle trauma, burns, and dismembered and decomposed bodies. The literature also exists on barotrauma.

The presence of foreign material within the body, be it from gunshot, shotgun, or explosive devices can all be detected and imaged on CT, although the shadows created by the metal may make the interpretation difficult. The use of coronal, sagittal, and 3D reconstruction allows for the location of the projectile(s) prior to autopsy recovery, although in the case of MRI the projectile may move within the body cavity or even exit the body. Experimental and single case reports show how the CT can be used to yield information related to the entrance and exit wounds, angle of trajectory, extent of tissue damage, as well as the zone of temporary cavitation *(80–91)* (Fig. 16).

The role of CT in terrorist fatalities has been reported by Shaham et al. from Israel, where the use of alternatives to conventional autopsy has sped up the release and burial of bodies *(67)*. We have found that the examination of the head and hands from the detonation of homemade pipe bombs has assisted in the location of bomb fragments within the body prior to autopsy examination. It can also be used to demonstrate the extent of the injury sustained from the device to a jury without the need for the use of clinical or postmortem photographs (Fig. 17).



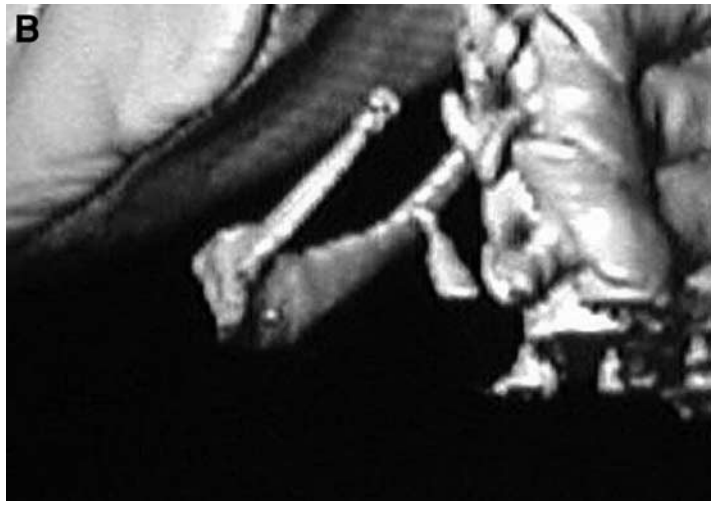


Fig. 15. (A,B) Digital three-dimensional reconstruction of computed tomography-based data allows close examination of the bony neck structures *in situ,* including the hyoid bone. This is of benefit when considering trauma to the region, as is seen in cases of manual strangulation.

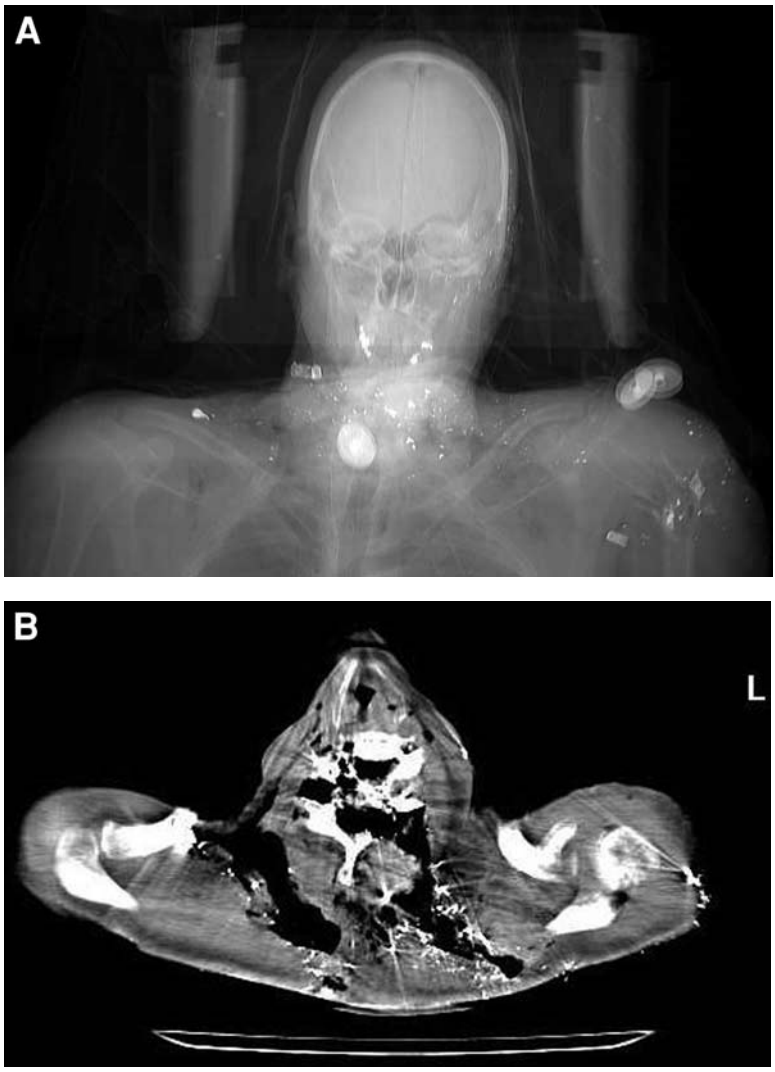


Fig. 16. A fatal case of multiple gunshot wounds to the neck, chest and abdomen. **(A)** The anterioposterior view scanogram image identifies the presence of casing fragments, together with more intact projectiles, and items associated with the body, although the exact location is uncertain. **(B)** Computed tomography (CT) scanning of the neck and upper shoulder regions reveals the extent of the permanent cavities left by the passage of projectiles through the body. The direction and angle may also be inferred through examination of such images. Also note the presence of fragmented projectile lodged within the left shoulder region. *(Figure continues)*

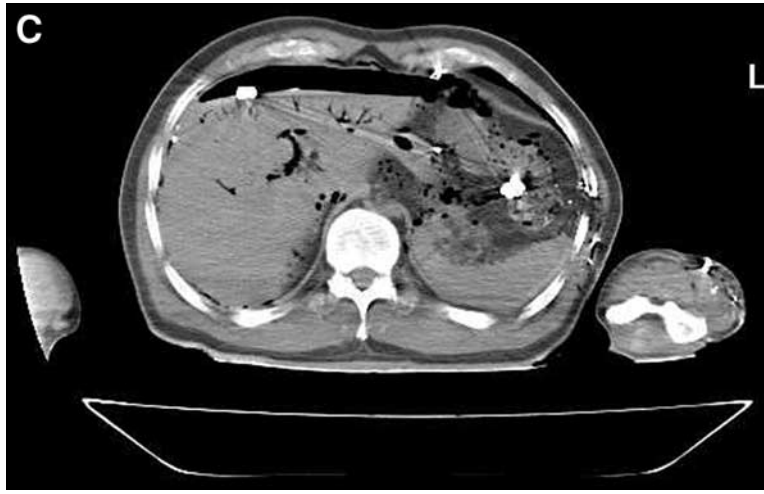


Fig. 16. *(continued)* (**C**) The location of additional projectiles is identified through CT scanning of the upper abdomen where early decompositional changes are also present.

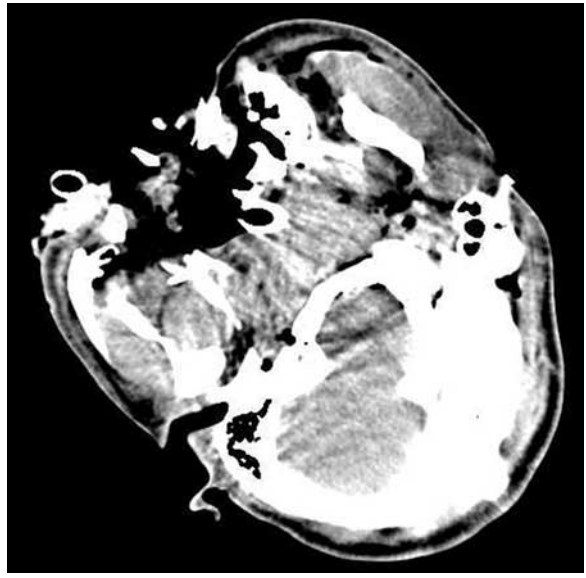


Fig. 17. The extensive facial damage related to the close-range discharge of a homemade pipe bomb device. Through such radiological examination potentially sharp fragments of metal casing can be identified and removed carefully, thus limiting the risk of injuries received during dissection of the head and neck.

The site of entrance as well as tissue and organ injuries can be visualized with knife injuries. Thali and co-workers reported a single case where CT was used to identify a sharp injury to the thoracic aorta caused by a knife *(92)*. We have found the examination of the neck with multiple wounds to be facilitated with CT, as has Keller et al. who reports the findings of the examination of the neck following a penetrating injury from a golf club *(93)*. The instrument can be used to measure the depth of penetration of the blade into the internal structures and may be more accurate than the crude measurements often recorded at autopsy examination.

The use of the trauma scan will allow visualization of bony trauma particularly to the limbs, which may remove the necessity to examine these areas at autopsy. This is particularly useful in vehicle incidents of blunt trauma (Fig. 18). Other examples of vehicle trauma, for example, transection of the aorta, have been reported.

A single case report illustrates the problems with examining the decomposed corpse *(23)*. In the case that Thali and colleagues illustrate the injuries to the head are, however, obvious externally despite decomposition. The imaging in the paper is used to illustrate the 3D reconstructive capability of the technology in relation to the underlying skull fractures. Taking this aside, the decomposed body will show a very classical intracranial appearance to the brain with putrefactive gaseous collections within the soft tissues and organs of the body, particularly the liver.

To date, a single case report of the examination of the charred body exists *(94)*. Again Thali and colleagues dwell on the use of CT to illustrate the changes seen to body caused by the effects of heat, such as fracturing of the skull, the heat hematoma, and long bone fractures, although the authors are unable to give a cause of death thus illustrating the need for conventional histology and toxicology in such cases.

Several papers illustrate the appearance in cases of fatal barotrauma. In these papers air can clearly be seen within the soft tissues, brain, and organs as a result of barotraumas *(95–98)*. In the paper of Oliver, not only do they illustrate the use of CT for such diagnosis but draw to the readers attention the differential diagnosis including postmortem change, the effect of external chest compression, and the event of postmortem decompression or "off gassing," an effect that occurs when the body is brought to the surface from the depths *(97)*.

6.3.6. Additional or Novel Applications

Computer Aided Design has been used in forensics investigations for several years, including the use of programs developed specifically for reconstruction of scenes or injuries. However, the combination of Computer Aided Design

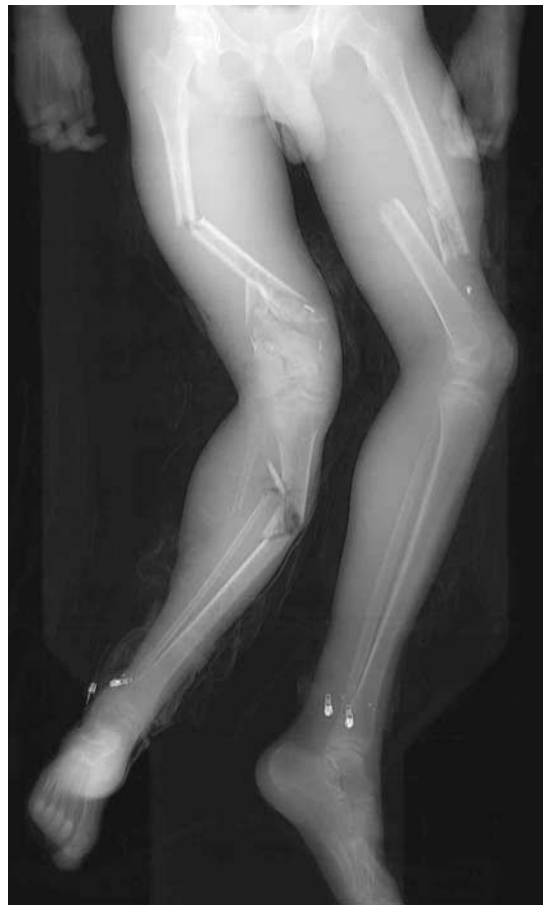


Fig. 18. Scanogram image allowing ready examination of multiple leg fractures in a victim of a road traffic incident. Identification of the number and site of fractures reduces the need to dissect the lower limbs for this same purpose. Note also the presence of zips at the distal end of the clothing worn by the deceased.

programming with photogrammetric images from the necropsy examination, and CT or MRI data allows overlaying of photographic representations of injuries onto a 3D digital reconstruction of the cadaver *(91)*. The result is an accurate digital representation of both the external and the internal aspects of a body, which may then be sectioned virtually to examine the passage or nature of injuries from any conceivable angle. The case report produced by Thali et al. details the use of photogrammetric images from a gunshot entrance wound over the torso of a male, which was subsequently combined with the 3D reconstruc-

tion produced from multislice CT imaging *(91)*. Such a procedure, albeit time-consuming and expensive, allowed digital confirmation of a muzzle imprint left following a close contact shotgun discharge and identification of the subsequent passage of the projectiles through the underlying tissues. Such new technology may produce images that are more "sterile" and, hence, acceptable to jurors; however, the clear advantages over "fit-matching" the muzzle of a weapon to an imprint is not clear in this publication (Fig. 19).

The technology of imaging can also provide novel "offshoots" that may benefit forensic investigators. Stein et al. published an article illustrating their experience with identifying gunshot residue, deposited on a single human cadaver, and experimentally with porcine skin models, through the use of CT *(99)*. All contact shots were shown to result in a circular deposition of radio-opaque material within the dermis and subcutaneous tissue which, on spectroscopic analysis, was determined as being lead together with traces of antimony and barium; metals found both within the projectile and the charge powder of the cartridges. Subjectively, the quantity of material appeared greater with unjacketed lead projectiles, although no material was detected for firing distances greater than 10 cm (5 cm for jacketed bullets), thus enabling a rough rule of thumb for reconstruction of events. However, as the authors themselves point out, the use of lead-free primer may limit the use of this technique.

Another such novel application is the development of stereolithography, which describes the creation of a 3D replica of a scanned structure, usually in resin or plastic. The result is an accurate rapidly manufactured rendering of aspects of the body, notably bony structures, such as the skull, which may be created from data created during digital radiological examinations. Current publications have focused on anthropological examinations, again exemplified by its use during the examination of the Tyrolean Iceman *(30,100,101)*. These could be used for the purpose of accurate demonstration in courtrooms or for the purpose of "fit-matching" tools to injuries *(102,103)*. It should also be noted at this stage that such technology can also be applied to the living and again can be a means of gathering evidence which may be utilized within the courtroom setting. Stereolithography is used regularly during the preoperative planning for surgical modifications, especially the correction of craniofacial malformations. Because the model is highly accurate to within 0.1 mm, it allows maxillofacial surgeons practicing on multiple models at a low cost. The result is an improved surgical outcome with reduced operating time *(100)*.

7. Conclusions and Outlook

The review of the published literature reveals two schools of thought as to the future of forensic imaging: those that consider it as a replacement to the

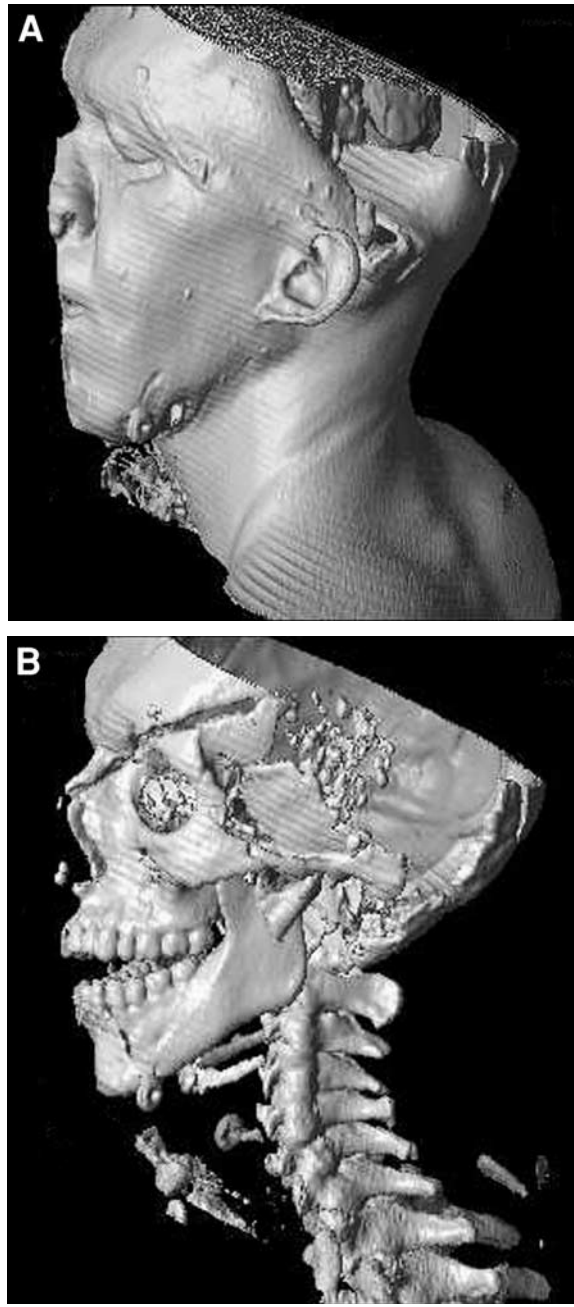


Fig. 19. (A) Digital reconstruction of soft tissue allows examination of the external surface of the body. In this case the effects of blunt trauma to the head are identifiable, with a defect in the left temporal region allowing visualization of the brain. **(B)** The extensive bony defect can also be displayed through examination of the skeletal structures, with associated supraorbital and mandibular fractures.

classical necropsy and those that view it as a valuable adjunct. The latter, it could be suggested, reflects the use of CT/MRI imaging in clinical scenarios such that, although these investigations may reveal the presence of a tumor, standard histopathological examination is still required to type the tumor for diagnosis, prognosis, and treatment. What is certain is that the role of postmortem CT and MRI in the future is assured in some form or another.

As with any new medical development, a novel scientific examination method requires stringent comparison against the recognized and established gold standard, in this case being the complete external examination and internal surgical dissection of all organ systems *(59,104)*. Only by such means could a new technique be verified. To date, few papers exist that compare radiological imaging to the standard necropsy, especially in difficult cases such as the decomposed scenario. Many of those that do are not performed blind to the imaging results and even fewer have compared CT with MRI to necropsy. Future publications should, therefore, address this issue before it becomes accepted as a standard accurate and reliable investigative tool.

The future for forensic radiological imaging appears bright and possibly even within the field of histopathology. Magnetic resonance microscopy is proving to be a valuable research tool, allowing visualization of the cellular structures constituting tissue, notably the CNS of mammal or insect models *(105–111)* (Fig. 20). The result is a 3D virtual microtome allowing digital sectioning of the tissue in any plane. It is therefore possible to suggest that future autopsy studies may also reveal a degree of histological architecture and pathological alterations identifiable using magnetic resonance imaging. Resolutions to approx 1.0 μm are possible, although practical limits, such as imaging time and magnetic field strength, may limit this. To date, however, publications have been produced revealing both the normal and the abnormal architecture of the human CNS in formalin-fixed specimens *(107,108)*, the appearance of magnetic resonance microscopy relative to chronic viral hepatitis *(111)*, and the visualization of perivascular lymphocytic cuffing in an experimental model of multiple sclerosis *(109)*.

An additional associated research tool is magnetic resonance spectroscopy, used to analyse the chemical constituents of intact biological tissue. The majority of in vivo magnetic resonance spectroscopy in humans relates to analyses of the CNS and have documented the presence of enzymatic deficiencies, mitochondrial defects, myopathies, and thyroid disease *(112–115)*. It is also suggested that earlier detection of primary brain tumors, demyelinating disorders, or epilepsy is possible *(115–117)*. The technique works by using the frequency gradient produced to identify chemical compounds, rather than for the use of spatial reconstruction information; the electrons around the nuclei

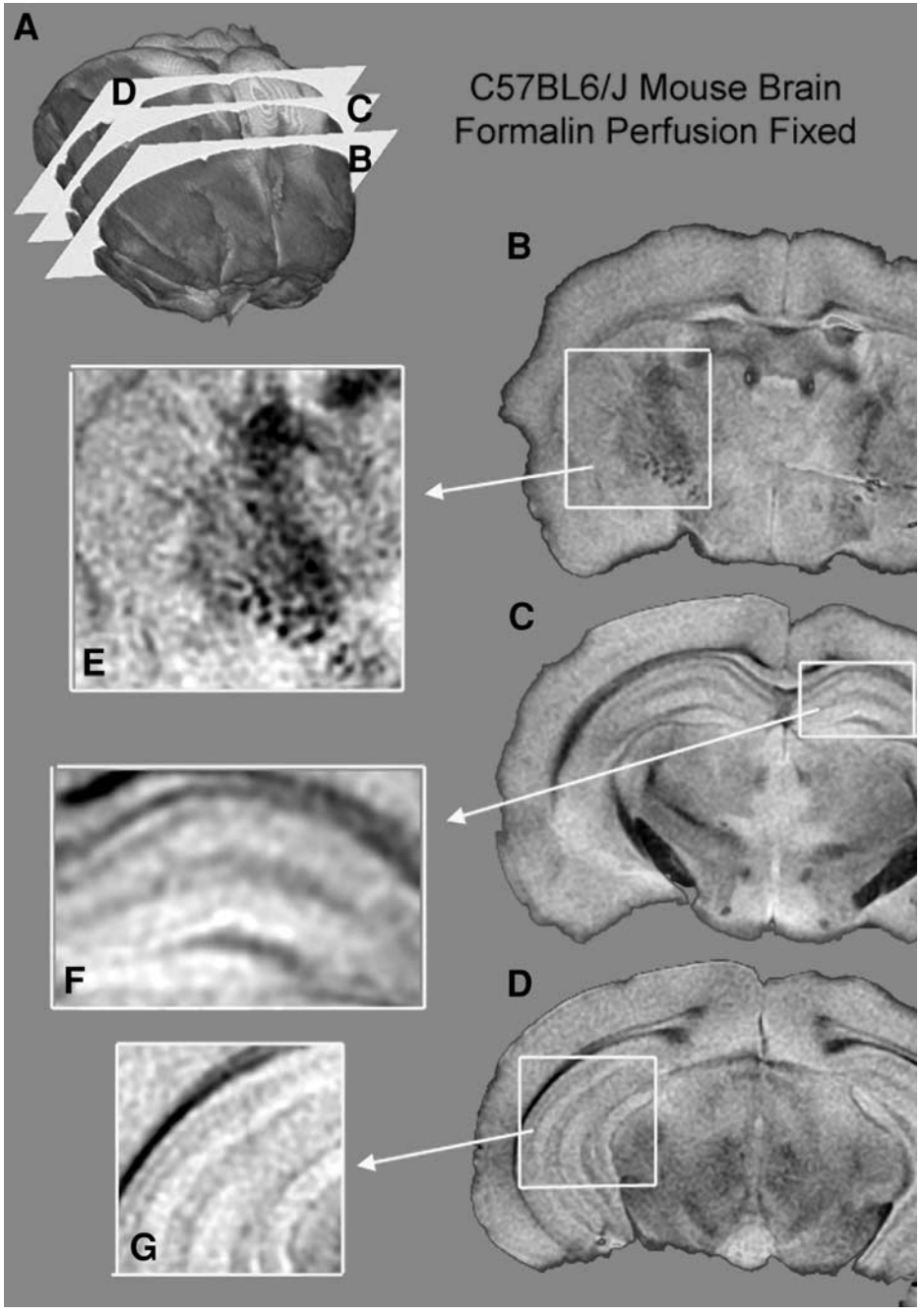

A
D
C
B
C57BL6/J Mouse Brain
Formalin Perfusion Fixed
B
E
C
F
D
G

Fig. 20. Magnetic resonance microscopy of the murine brain; the resultant three dimensional reconstruction allows the use of a virtual microtome, allowing repeated sectioning of the structures at multiple angles. The result is the production of recognizable architectural structures, which may, in humans, allow identification of pathological diseases that previously required histological examination. (Reproduced with kind permission from ref. *118*.)

shield the resonant atoms to a variable degree related to the specific molecular composition in a recognizable manner. Thus, the presence or quantity of neurotransmitter compounds or other chemicals may be inferred through such recognition. Its possible future application to postmortem toxicology is therefore evident.

With public opinion of necropsy examinations continuing to diminish it is unwise to respond in a "knee-jerk" manner by calling for pure radiology-based examinations when the field remains within its infancy and unable, at present, to sufficiently diagnose even the most common cause of death within the United Kingdom, that being ischemic heart and coronary artery disease. The next stage of the development of this technology within autopsy practice will be the acceptance of CT and/or MRI as an adjunct to clinical autopsy. This is as much a clinical acceptance from radiological departments for access to their machines to investigate the dead, as well as from the pathological and forensic communities to consider the use of this technology. The placement of scanners into mortuaries will occur one day but we are not yet at this stage in the evolution of this service. One still requires a minimal of an external examination, if only to satisfy the "view and grant" procedure, and the need for ancillary tests, such as toxicology, metabolic studies, or genetic tests, still remain. Until sufficient large-scale trials have been funded and undertaken to answer the question posed within this chapter in relation to whether or not CT and/or MRI can replace the need for a conventional autopsy, the continuing publication of single case reports on selected forensic issues unfortunately does not advance the issues related to whether or not CT and/or MRI will one day replace the need for conventional invasive autopsy.

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Postmortem Ultrasound Imaging in Forensic Pathology

Seisaku Uchigasaki, MD, PhD

CONTENTS

SUMMARY

Diagnostic imaging techniques, developing rapidly, are of great value in different medical fields. Some imaging techniques, such as conventional X-ray, computed tomography (CT), and magnetic resonance imaging (MRI), are applied also on a broad variety of occasions in the field of forensic medicine and pathology. At present, it is still difficult to carry out CT and MRI as part of the "routine work" in forensic pathological practice because special equipment, room, and technical staff are needed and these techniques are time-consuming and expensive. On the other hand, postmortem ultrasound imaging is easy to use and much cheaper than other imaging techniques. Using ultrasonography makes it possible to detect a variety of meaningful findings in corpses, as well as in living patients, in medicolegal practice. Application of ultrasound imaging to corpses is a new trial that needs further encouragement and research.

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Key Words: External examination; postmortem ultrasound imaging; ultrasonography; forensic medicine; forensic autopsy.

1. Introduction

Every nation and culture in the world has its own legal system regulating forensic autopsy practice, and forensic pathologists worldwide perform autopsies on the basis of their regional autopsy system in cases of murder, death by accident, and unexpected death. The frequency with which autopsies are conducted in some countries is not sufficient to meet the requirements associated with the clear-cut determination of the cause of death strived for by forensic pathologists and medical examiners, respectively. To get consent to perform an autopsy is, on occasion, impossible for social or religious reasons that prevail in some countries, although the need to conduct an examination of a deceased through a forensic autopsy is explained and emphasized to the legal authorities. In such cases, diagnostic imaging technologies can sometimes offer helpful and meaningful findings to determine the cause of death. Numerous publications have documented the beneficial application of X-ray, computed tomography (CT), and magnetic resonance imaging (MRI) to postmortem examinations *(1–3; see also* Chapter 12*)*, and these techniques are capable of providing valuable information, sometimes even in heavily decomposed corpses. One drawback of these technologies is that they require special equipment, room, and technical staff, and are quite expensive.

Ultrasound imaging is a common diagnostic imaging procedure in the clinical routine. The examination is easy to conduct and noninvasive to the patient. Moreover, there is no concern of contamination by radiation. Ultrasound imaging devices are usually much smaller than other diagnostic imaging equipments. Some devices, such as the SonoSite 180 Series (SonoSite Inc., USA) (Fig. 1) or the Logiqbook (GE Medical Systems, USA), are compact and portable and can be easily used at the scene of death by a trained investigator. In this author's opinion, there seems to be a misconception that it is impossible to obtain any significant ultrasound images from corpses. Surely, useful postmortem ultrasound images are impossible to obtain in the case of a highly decomposed body. However, it is possible up to 4 to 5 days after death to make ultrasound images that provide important forensic pathological findings from the inside of the corpse, thus giving valuable hints toward the most probable cause of death *(5–7; see also* Chapter 12*)*.

2. Ultrasound Imaging Devices

Commonly, an ultrasound imaging device is equipped with different types of probes (Fig. 2). The most common probe is probably the convex probe for

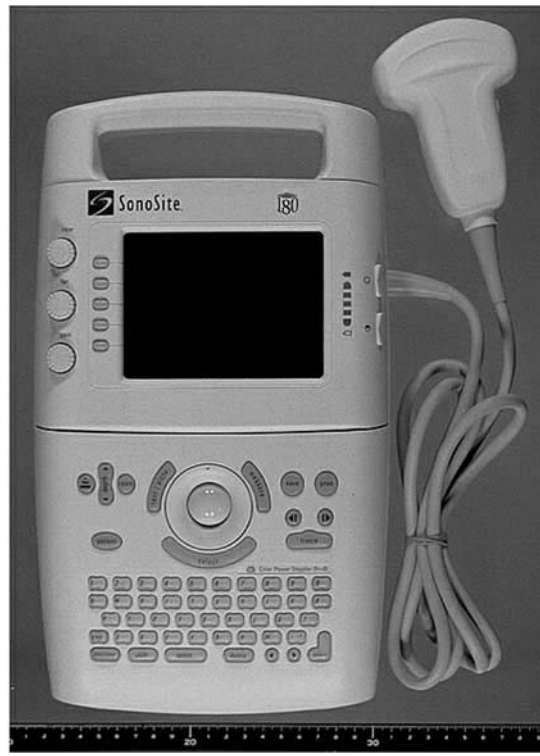

Fig. 1. The SonoSite™180.

the abdomen (2–5 MHz). This probe can be used to scan the abdominal and thoracic cavities but it may be too big to "view" through the thoracic cavity between the intercostal spaces. If a probe for both the cardiovascular system and abdomen is available, this is usually better than the conventional convex probe simply because it is smaller. The author recommends this type of probe to be used on corpses. To scan subcutaneous tissue or cervical organs, it is better to use a high frequency probe (7.5–10 MHz).

3. Ultrasonographic Examination of Corpses

Ultrasonography is relatively easy to learn and most devices are easy to handle *(5)*. The ultrasound findings of corpses are basically the same as findings from living patients. For example, a hyperechoic liver (appearing brighter than the kidney [Fig. 3]) signifies fatty changes of the liver parenchyma or a fatty liver. If the cadaver had been stored refrigerated, ultrasound examination

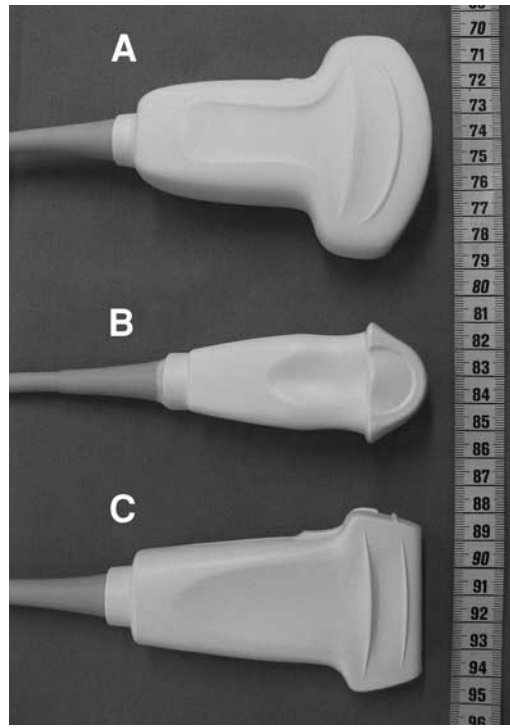

Fig. 2. Different types of probes for ultrasound imaging devices. **(A)** A convex probe for the abdomen (2–5 MHz). **(B)** A probe for both the cardiovascular system and abdomen (2–4 MHz). (C) A high-frequency probe (7.5–10 MHz) best used to scan subcutaneous tissue or cervical organs.

is preferably done not before 1 to 2 hours after the corpse was taken out of the cooling apparatus because hardening of adipose tissue owing to cool ambient temperatures may cause great differences in the permeation rate and reflection rate of postmortem ultrasound. Doppler mode is often unnecessary because of the lack of blood flow in corpses.

When performing a postmortem ultrasound scan of the thoracic and/or abdominal cavity, the most commonly asked question is where the probe should be placed onto the corpse. Basically, postmortem ultrasound scanning positions applied are the same as those used in clinical examination of the living. In many cases, however, the positions differ slightly (depending on the postmortem condition of the corpse) because the diaphragm shifts to the superior side when postmortem gas formation is present in the gut, whereas the liver also shifts a little to the superior side. High-quality ultrasound images are slightly more dif-

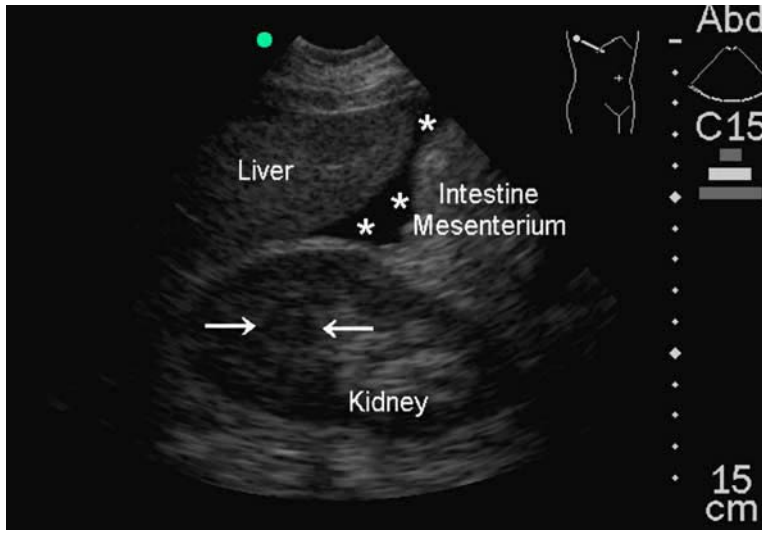

Fig. 3. Postmortem ultrasound finding of a fatty liver appearing hyperechoic. The presence of fatty transformation of the liver parenchyma is most likely when the liver appears brighter than the kidney ("liver–kidney contrast"). Ascites (asterisks) is observed between the intestine and other organs and a renal cyst (arrow) is present as well.

ficult to obtain from an obese corpse. It is impossible to obtain any image from the frontal approach in the case of profuse postmortem gas formation in the gut. In such case, a dorsal and/or a lateral approach may be helpful to take meaningful images by ultrasound. Fariña et al. reported a concordance rate of 83% in the cause of death and main pathological findings between "echopsy" (ultrasound diagnosis) and classical autopsy *(5)*.

4. Applications of Ultrasound Imaging to Forensic Pathological Practice

Examples of pathological findings detectable by ultrasound examinations of corpses are given in Table 1. These findings could be the cause of death itself or helpful hints to determine the underlying disease with respect to the cause of death. For example, some cases of pericardial tamponade can be diagnosed by ultrasound before autopsy (Fig. 4). If ultrasonography reveals bone fractures, such as cervical vertebral fracture in a deceased with no injuries to the outer body surface, this ultrasonographic finding could indicate the necessity to perform a forensic autopsy. In addition, the volume of urine retention in the uri-

Table 1
Pathological Findings Detectable by Ultrasound Examinations of Corpses

Cardiac hypertrophy	Bile stones
Pericardial tamponade	Renal cysts
Aneurysm of abdominal aorta	Diverticulum of urinary bladder
Pleural effusions	Hyperplasia of the prostate
Subphrenic abscess	Myoma uteri
Ascites or intraabdominal bleeding	Intracranial hemorrhage in infants
Liver metastasis	Bone fractures
Liver cirrhosis	Foreign substance in mammae (implant)
Fatty liver/fatty change of liver parenchyma	

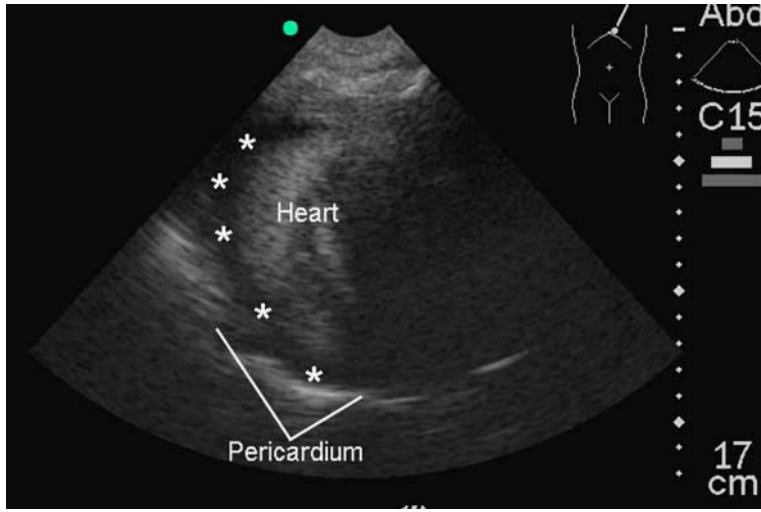

Fig. 4. Postmortem ultrasound image of pericardial tamponade. An echo-free space (asterisks) is seen between pericardium and epicardium, indicating the presence of blood or effusion in the pericardium.

nary bladder can be estimated from ultrasound images *(8)*. In the case of an infant, if the anterior fontanel is still open, it is possible to scan the brain and its structures through the anterior fontanel (Fig. 5), thus giving the possibility to detect intracranial hemorrhage (e.g., subdural hemorrhage as a result of shaking of the infant prior to death). A full autopsy is, without doubt, indispensable

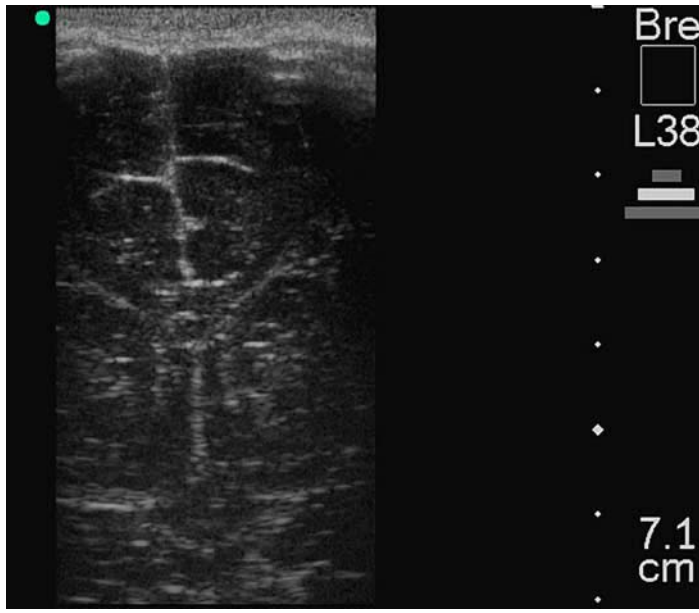

Fig. 5. Postmortem ultrasound image of an infant's brain viewed through the anterior fontanel.

for making a correct judgment about a given case, but scanning of the cadaver by ultrasound before autopsy can yield much helpful information.

Application of postmortem ultrasound imaging to forensic pathological practice is a new supplementary diagnostic technique and many questions remain to be solved. We encourage forensic practitioners to try and use ultrasound imaging in forensic pathological practice and report their findings to further establish this technique.

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Veterinary Forensic Pathology

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Veterinary Forensic Pathology

The Assessment of Injuries to Dolphins at Postmortem

Roger W. Byard, MBBS, MD, Catherine M. Kemper, PhD, Mike Bossley, PhD, Deborah Kelly, BVSc, and Mark Hill, BVSc

CONTENTS

SUMMARY

Injury assessment of nonhuman subjects may be performed for a variety of reasons, most often in a forensic setting to prove or disprove human activity. In Adelaide, South Australia, the killing of several dolphins from nearby waterways prompted the formation of a multidisciplinary Dolphin Trauma Group,

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among whose aims was the performance of rapid biological and forensic assessments of dead local dolphins. Necropsy and wound evaluation by group members utilizing standard forensic techniques aimed at determining as quickly as possible causes of death, whether injuries were inflicted ante- or post-mortem, whether injuries were responsible for, or had contributed to, the fatal episode, and whether human intervention was responsible for the wounds. Estimation of the type and dimensions of possible weapons was also undertaken, as well as attempts to determine possible time frames for the injuries. This information was then passed on to investigating officers. A series of necropsy findings in seven dolphins investigated by the group (four Indo-Pacific bottlenose dolphins, one southern right whale dolphin, one short-beaked common dolphin, and one common bottlenose dolphin) is described in this chapter. Two deaths were caused by exsanguination from stabbing/spearing, two to the sequelae of entanglement with fishing gear, one to blunt craniocerebral trauma from a boat propeller injury, one to probable sepsis, and one remained undetermined. All cases had extensive histological assessment of tissues with retention of blood and tissues for future toxicological screening if required. Examination of the brain is undertaken by a neuropathologist when tissues are not putrefied. Blood spots are signed and sealed and transferred to Forensic Science South Australia for storage in case comparisons are subsequently required with blood and tissue on possible weapons. In this way, each dolphin has an extensive forensic evaluation to provide as much usable information to courts and prosecutors if legal proceedings are ever undertaken. The skeletons and life history samples are held in the collections of the South Australian Museum.

Key Words: Inflicted injury; marine mammal; dolphin; death; stabbing; necropsy.

1. INTRODUCTION

The accurate assessment of wounds is a fundamental requirement for practitioners of forensic medicine and pathology. Most cases require careful description, documentation, and photographic depiction of injuries so that they can be presented to courts and selected experts for subsequent explanation and evaluation. Although forensic analysis usually involves injuries to humans, there are situations where injuries to animals may require examination to assist in the determination of the cause of death and the likelihood of possible legal proceedings. The following chapter details a range of injuries encountered in a group of dolphins in South Australian waters, and discusses difficulties that may arise in evaluating mechanisms and manners of death in such animals, and the potential legal consequences of such assessments.

2. Dolphin Trauma Group

Following a series of deaths of dolphins in South Australia owing to human activity, it was decided to formalize work being done in the investigation of these fatalities by individuals from the South Australian Museum, the Department for Environment and Heritage, primary industries (fisheries), Forensic Science South Australia, the Australian Dolphin Research Foundation (ADRF), and private veterinary practice. A Dolphin Trauma Group was therefore formed, the aim of which was to provide immediate biological and forensic assessment in cases of deaths of dolphins, particularly in the Port River area immediately adjacent to the South Australian state capital Adelaide, and to draw public attention to these events. The Port River estuary was the initial target site because it was an area frequented by groups of dolphins that had been the subject of study by the ADRF for a number of years, including the use of photo-identification to establish individual identities.

Every dead dolphin found in this area that was reported to police, the Department for Environment and Heritage, or fisheries officers was evaluated by members of the group. Autopsies were arranged through the South Australian Museum, with processing of specimens and tissues through both the museum and Forensic Science South Australia. In addition, members of the group also conducted a number of examinations of injuries to other marine mammals, such as whales and seals. The details of selected cases examined by members of the group over the 5-year period from 1999 to 2004 are provided in the following section.

3. Illustrative Cases

Case 1

The carcass of an Indo-Pacific bottlenose dolphin neonate (*Tursiops aduncus*, South Australian Museum 03.001) was found floating in the Port River estuary near Adelaide. The calf had been recently born to one of the dolphins studied by the ADRF and had been photographed swimming with its mother in the days before its death (Fig. 1A). Necropsy revealed two significant and lethal injuries. The first was an irregular, deeply incised wound of the left side of the head running downward, and slightly posteriorly from behind the blowhole in front of the eye (Fig. 1B). The injury was approx 300 mm in length with variably ragged and cleanly incised edges. It was associated with shattering of the underlying skull and facial skeleton with opening of the cranial cavity and exposure and loss of brain tissue. Recent hemorrhage into soft tissue and brain was demonstrated histologically. The second injury was an irregular, deeply

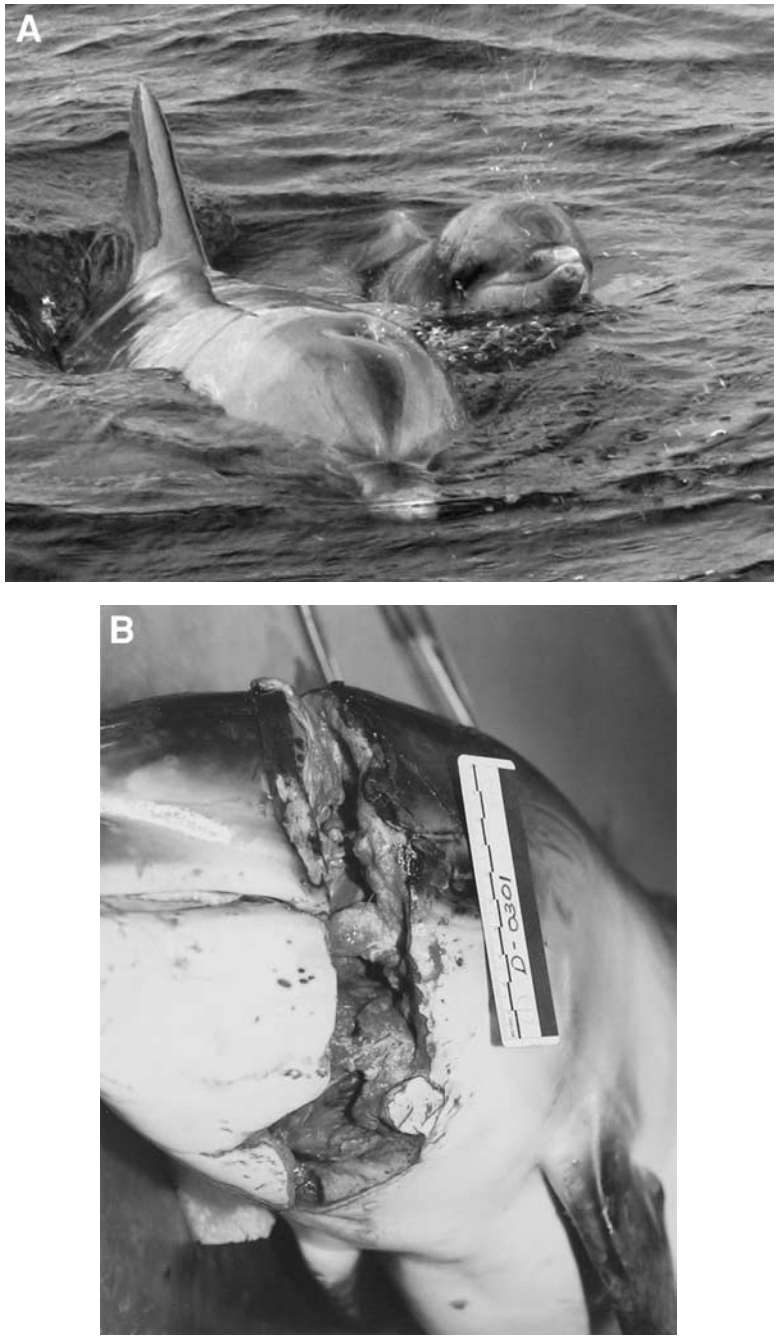

Fig. 1. (A) A young Indo-Pacific bottlenose dolphin calf who was seen swimming with its mother near power boats was subsequently found floating with a deeply incised lethal wound of the left side of the head. **(B)** The skull and facial skeleton were shattered with exposure and loss of brain tissue. *(Figure continues)*

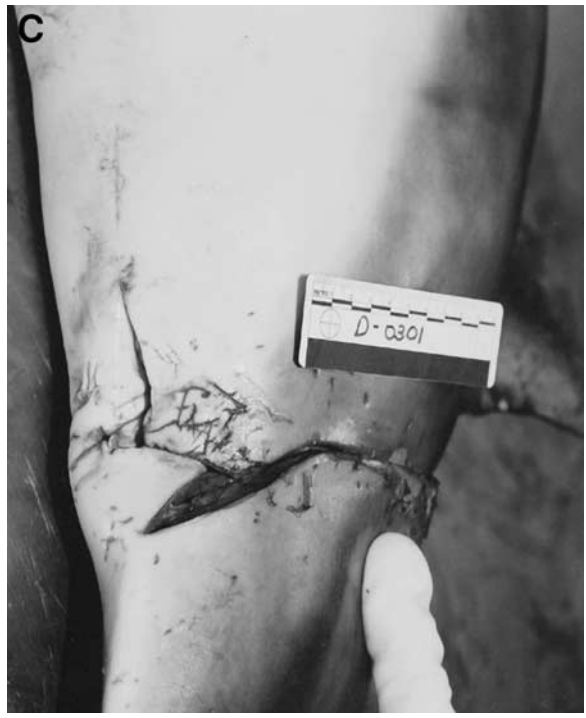

Fig. 1. *(continued)* **(C)** A parallel deep incised wound was also present on the left side of the tail. Both of these injuries were thought to be caused by a boat propeller.

incised wound of the left side of the tail (Fig. 1C), 170 mm in length extending to a depth of 40 mm into underlying vertebral bodies. The injury ran downward and slightly posteriorly, roughly parallel to the major injury to the head and 55.5 cm behind. Fresh blood clots were present within the wound. Recent hemorrhage was also confirmed histologically. Death was caused by severe craniocerebral trauma most likely inflicted by a boat propeller.

Case 2

The carcass of an adult male southern right whale dolphin (*Lissodelphis peronii*, 04.145), never previously stranded in South Australia, was found dead on Kangaroo Island about 100 km south of Adelaide. Reddish fluid initially thought to represent hemorrhage was emanating from the mouth (Fig. 2A) and what appeared to be a stab wound was present in the right flank. At necropsy, a "V"-shaped incised wound was present in the right flank, each arm of which measured 75 mm (Fig. 2B). The wound extended 40 mm into the epaxial muscles beside the

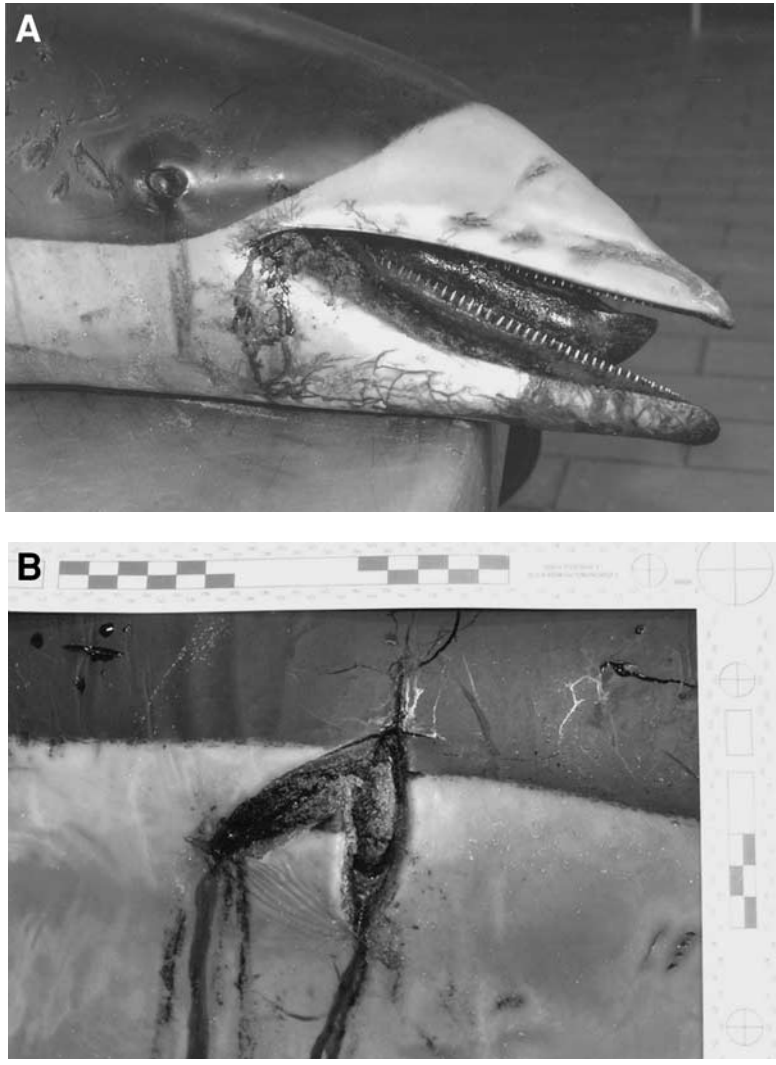

Fig. 2. (A) The head of southern right whale dolphin with purging of putrefactive fluids initially thought to represent hemorrhage from trauma. **(B)** A "V"-shaped incised wound was present in the right flank; this was found at necropsy to be a nonlethal injury that only extended into the paravertebral epaxial muscles and did not compromise major vessels or internal organs.

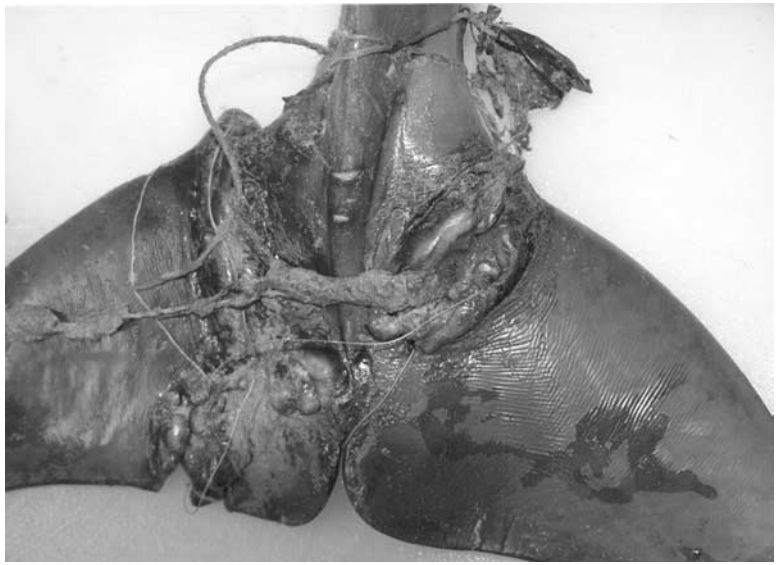

Fig. 3. The tail of a juvenile Indo-Pacific bottlenose dolphin with braided fishing line wrapped tightly around it. Movement of the animal's tail while swimming had caused the line to tighten and cut deeply into underlying tissues with resultant hemorrhage and abscess formation. The animal was emaciated, having been unable to feed itself adequately.

spine but did not involve any major vessels or organs. Examination of the stomach contents revealed that the animal had not fed on the day before death, and the presence of mesenteric lymphadenopathy raised the possibility of death resulting from infection. Advanced putrefaction had been responsible for the purging of fluids from the mouth and prevented further assessment. Death was attributed to natural causes and not to trauma. The lack of hemorrhage into tissues and the absence of a vital reaction would be in keeping with the wound being caused after death. The cleanly incised edges and lack of adjacent abrasions was against the wound being inflicted by another animal.

Case 3

The carcass of a juvenile Indo-Pacific bottlenose dolphin (*T. aduncus*, 03.085) was found dead on a beach south of Adelaide. Necropsy revealed an emaciated animal with braided fishing line wrapped around the tail associated with a 60-mm-deep wound extending into the underlying tissues (Fig. 3) associated with acute and chronic inflammation and microabscess formation. Moving

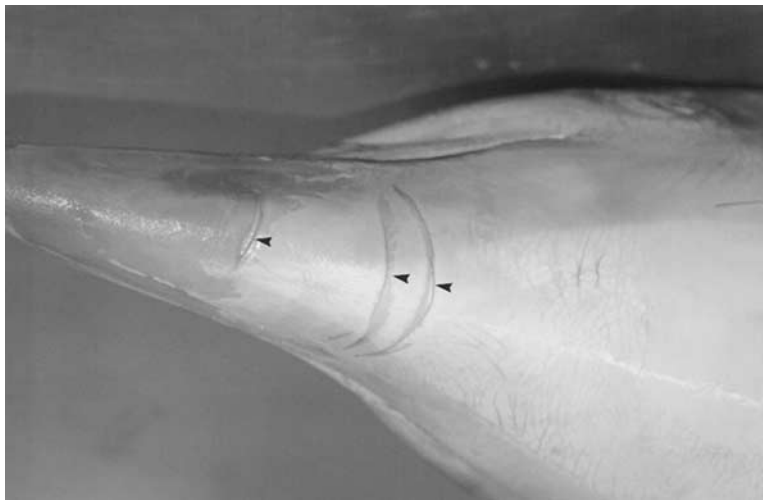

Fig. 4. Three curvilinear, superficial wounds on the under surface of the jaw of a juvenile short-beaked common dolphin, in keeping with injuries that could be sustained from a large mesh net.

the tail to swim would have caused the tightly wrapped mass of braided fishing line to saw deeper into the tissues. The extensive tissue reaction suggested that the twine had been in place for some time (weeks or probably months). The injuries would certainly have interfered with the animal's ability to swim and forage, and death was likely caused by sepsis and/or exhaustion associated with cachexia owing to the effects of the tail wound.

Case 4

The carcass of a juvenile short-beaked common dolphin (*Delphinus delphis*, 03.084) was found on a beach south of Adelaide. Necropsy revealed three curvilinear superficial abrasions on the under surface of the jaw (Fig. 4) associated with a linear mark behind the left eye. Whereas the features of these wounds were essentially nonspecific, the finding of interrupted circumferential markings under the jaw associated with a linear abrasion behind the eye would be in keeping with injuries from a large mesh net. Histological examination of tissues was complicated slightly by early tissue autolysis and putrefaction; however, no significant underlying organic diseases were detected microscopically. Although the cause of death remained undetermined, the absence of lethal trauma and organic disease with possible netting marks around the head made net entanglement a distinct possibility.

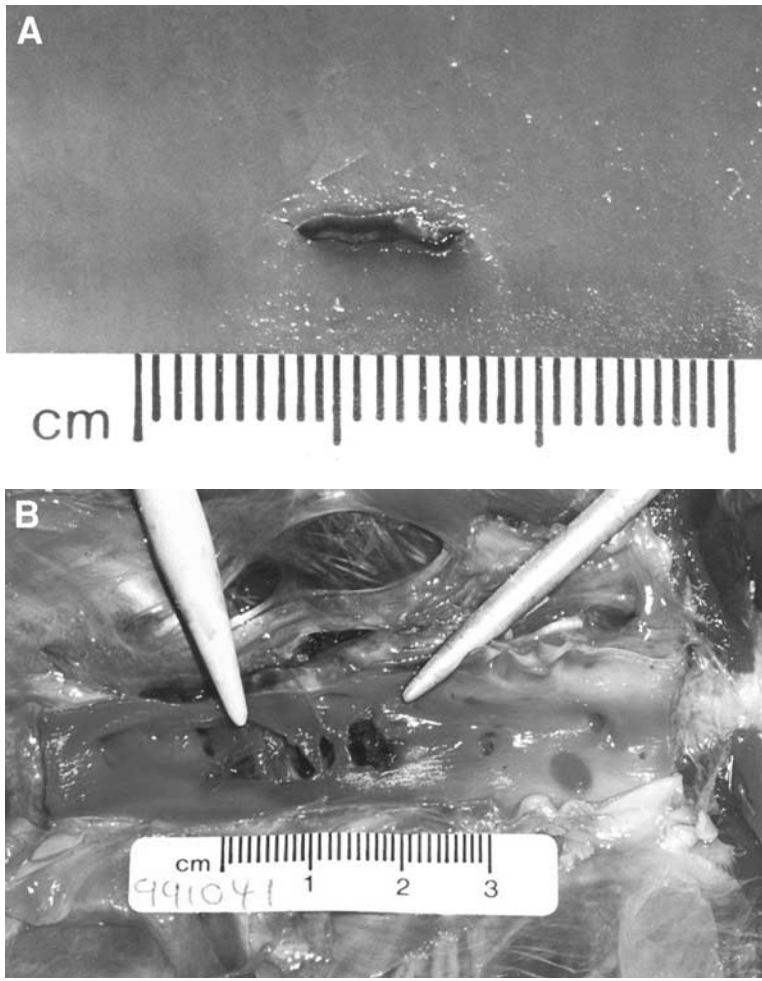

Fig. 5. (A) A stabbed Indo-Pacific bottlenose dolphin calf with a vertical exit stab wound present in the left flank. **(B)** Death had resulted from exsanguination from damage to the aorta.

Case 5

The carcass of a male Indo-Pacific bottlenose dolphin calf (*T. aduncus*, M20749), aged approx 4 to 6 weeks old, was found on a beach near Adelaide. Necropsy revealed a stab wound to the flank that had passed through the body incising the aorta (Fig. 5A,B). Based on the assessment of the wound, it was considered that the weapon used had been a single-edged blade approx 190 mm

Fig. 6. (A) A single lethal stab wound (arrow) to the ventral thorax of an adult Indo-Pacific bottlenose dolphin. The two linear wounds further towards the snout are probable rake marks from an attack by another dolphin, and the patchy areas of superficial loss of epidermis are the result of sea lice activity after death. *(Figure continues)*

long and 22 mm wide. Death was caused by exsanguination from the stab wound to the flank *(1)*.

Case 6

The carcass of an adult male Indo-Pacific bottlenose dolphin (*T. aduncus*, M21243) was found floating off the Adelaide coast. Necropsy revealed a single lethal stab wound to the ventral thorax that had penetrated the right ventricle of the heart and caused a left sided hemothorax (Fig. 6). Based on the assessment of the wound, it was considered that the weapon used had been a single-edged blade with a maximum width of 18 mm 85 mm from the tip. Death was caused by exsanguination from the stab wound to the thorax *(2)*. Extensive sea lice damage to the epidermis suggested that the carcass had been floating in the water after death, rather than the animal dying from stranding on a beach.

Case 7

The carcass of a juvenile male common bottlenose dolphin (*Tursiops truncatus*, M22411) was found on a beach in southeastern South Australia. On external examination, it appeared to have been stabbed in the thorax (Fig. 7)

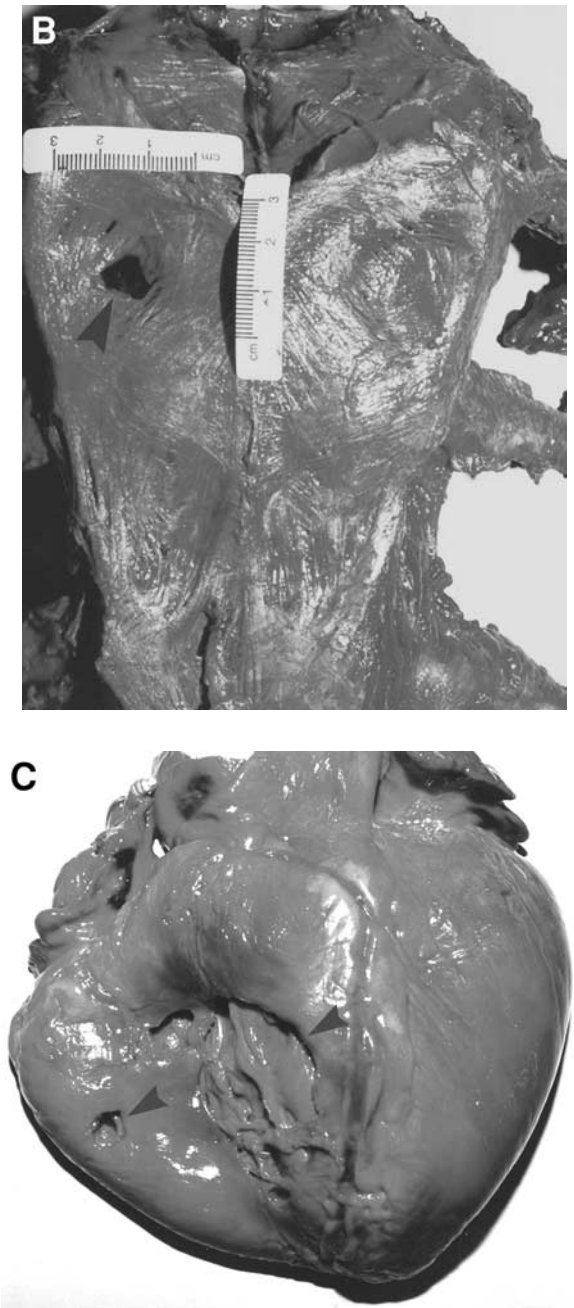

Fig. 6. *(continued)* **(B)** The weapon had penetrated the sternum (arrowhead) and **(C)** the right ventricle of the heart (arrowheads) and caused a left-sided hemothorax.

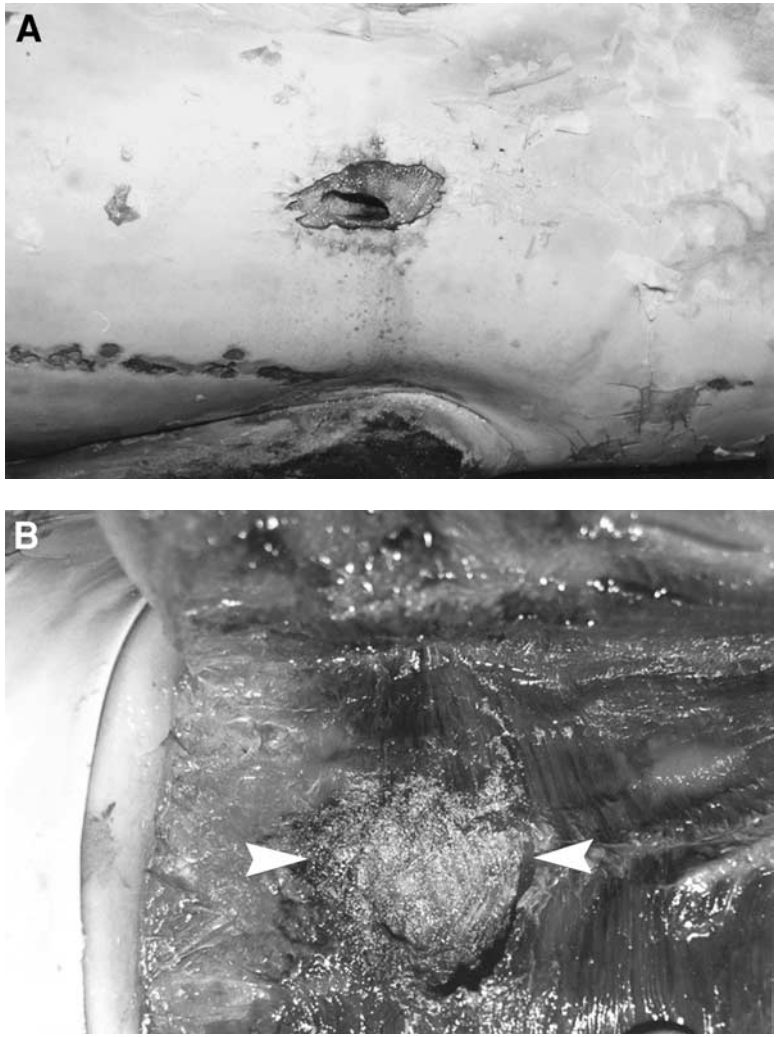

Fig. 7. (A) An apparent lethal stab wound to the ventral thorax of a juvenile common bottlenose dolphin. Dissection revealed that the wound was not lethal and extended only into superficial tissues, not involving any major vessels or organs. **(B)** Once overlying skin and blubber had been reflected, the shallow cavity within muscle was revealed (arrowheads).

Table 1
Summary of the Features of Seven Dolphin Necropsies in South Australia

Case no.	Animal	Injuries	Cause of death	Instrument
1	Bottlenose dolphin neonate	Deep incised wounds to head and tail	Craniocerebral trauma	Propeller
2	Southern right whale dolphin	Incised wound to flank	Probable sepsis	?
3	Bottlenose dolphin	Fishing line around tail	Sepsis/cachexia	Fishing line
4	Common dolphin	Curvilinear abrasions around head	Probable net entanglement	Fishing net
5	Bottlenose dolphin calf	Stab wound to flank	Exsanguination	Knife/ spear
6	Indian Ocean bottlenose dolphin	Stab wound to thorax	Exsanguination	Knife/ spear
7	Bottlenose dolphin	Wound to thorax	Undetermined	—

with a wound similar to that seen in Case 6. Necropsy, however, showed that the wound extended only into superficial tissues, had not involved major vessels or organs, and was inflicted postmortem. In this case death was not the result of trauma *(2)*.

A summary of the features of the seven dolphin necropsies is given in Table 1.

4. Medicolegal Considerations

The penalty for killing a marine mammal in South Australia is a $30,000 fine or 2 years in prison *(3)*. Given the significant consequences of such an act, cases have to be evaluated carefully in the knowledge that judicial outcomes will rely heavily on the quality of the medicolegal assessment. Similar penalties exist in other jurisdictions for killing dolphins, seals, and whales.

The purpose of initiating the Dolphin Trauma Group was to coordinate the evaluation of cases of suspected inflicted injury to local dolphins caused by human interference and to study the pathology of fresh dolphin carcasses. By involving Forensic Science South Australia, any injuries detected were examined and documented by a forensic pathologist following standard guidelines used in cases of assault/homicide in humans. This also enabled chain of evidence issues to be dealt with; for example, blood samples for DNA analysis are routinely taken to enable comparison with blood or tissue stains on possible weapons. DNA may also be used to assist with subsequent species and population identification *(4)*, and samples of tissue are stored at the South Australian Museum for this purpose. Samples of liver, kidney, muscle, and blubber are also frozen for possible toxicological analyses. The specific purposes of the forensic evaluations are to determine if an injury is likely to have been deliberately inflicted or is accidental, whether injuries are of human or animal origin, whether the injury occurred before or after death, whether the injury is responsible for, or has contributed to, death, and what the cause of death is. In addition, assessments are also made by members of the group of injuries that have been identified and photographed in living sea mammals to determine likely causes and whether human activities may have been involved.

5. Pathological Features

The cases discussed earlier demonstrate clearly some of the injuries that may be sustained by these animals and the issues involved. In four cases, it was considered that the injuries had been deliberately inflicted. The usefulness of an early forensic examination was illustrated in Cases 2 and 7 where stab and possible sharp implement injuries, initially thought to be causes of death, were shown to have been only relatively superficial, nonlethal and probably incurred postmortem. Unfortunately given poor tissue preservation, a feature often found with dolphin carcasses that are not collected fresh, it is often not possible to determine more precisely whether the injuries were inflicted ante- or postmortem. In two other cases, there was no doubt that the injuries had caused the deaths. In Case 5, a very young dolphin had been stabbed through the flank and had died from exsanguination when the aorta was incised. Examination of tissue sections revealed interstitial hemorrhage indicating that the injury had occurred during life. In Case 6, an adult dolphin had died after a stab wound to the thorax had penetrated the right ventricle of the heart and resulted in a massive hemothorax. Careful examination of the wound track enabled a possible scenario to be suggested for the terminal event, with the dolphin perhaps swimming along side a boat and rolling over. This enabled the perpetrator to stab the animal in the chest with a sharp-bladed weapon *(2)*. Early confirmation of these

results enabled the information to be passed on to investigating officers involved in searching for possible assailants. Another possibility may have been stabbing by a diver approaching the animal from beneath.

Injuries of human origin may also be nonintentional as was most likely the case in the young calf (Case 1) that had sustained two deep lacerations to the head and tail most likely from a boat propeller blade with almost immediate death from severe craniocerebral trauma. The mother of the calf was known to the ADRF and had an unfortunate habit of swimming around the stern of boats, and so the recorded behavior of the parent supported the findings of likely propeller injuries.

Curvilinear markings around the head of a dolphin in Case 4 strongly suggested entanglement in a fishing net with subsequent death, and fishing-line entanglement around the tail had led to the rather protracted and unpleasant death of the young dolphin in Case 3. Encounters with fishing apparatus are unfortunately common and ADRF members have on previous occasions had to catch and remove fishing line from some of the dolphins that frequent the Port River estuary. Identification of the potentially harmful effects of marine litter, such as fishing line, through the media in South Australia is another useful activity of the ADRF and the Dolphin Trauma Group. Publications are available on how to determine dolphin entanglement when carrying out autopsies *(5,6)*. Lesions, such as circumscribed abrasions of the fin, beak or tail fluke, or multiple evenly spaced parallel skin wounds, are characteristic. Apart from nonspecific bruising and fractures, serious injuries, such as atlanto-occipital fracture dislocations with damage to the underlying spinal cord, may be found at necropsy, most likely associated with violent struggling of the entangled animal *(7)*.

Thirty of 38 dolphins autopsied following strandings in South West England showed evidence of incidental capture in fishing gear *(7)*, and 86 of 108 common dolphins (*D. delphis*) (80%) and 66 of 176 harbor porpoises (*Phocoena phocoena*) (38%), in which causes of death had been established, were found to have died following entanglement in fishing gear around the coasts of England and Wales in another study *(8)*. Twelve of 18 Hector's dolphin (*CephaIorhynchus hectori*), two of three dusky dolphins (*Lagenorhynchus obscurus*), and a single common dolphin (*D. delphis*) in a study from New Zealand had skin lesions characteristic of net marks *(9)*. Ingested plastic bags are another form of marine litter that may be detrimental to marine mammal health *(10)*.

Other injuries that are often found on sea mammals arise from aggressive encounters with members of their own or other species. For example, parallel lacerations on a flipper and rake marks on a torso as shown in Fig. 8 are the

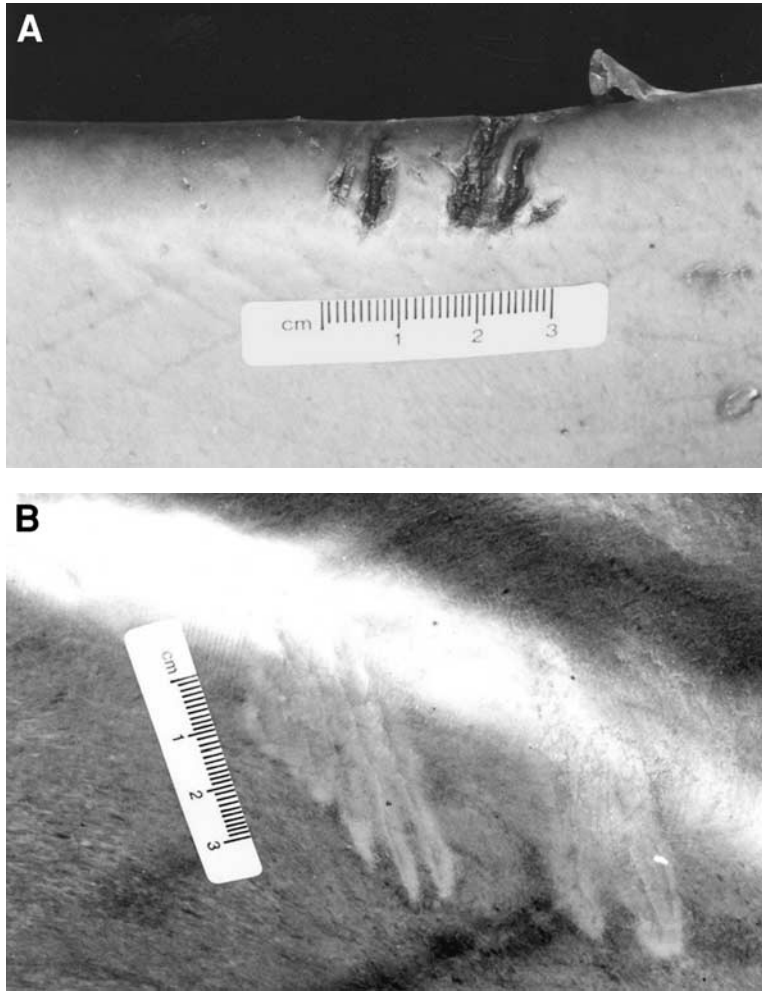

Fig. 8. (A) Irregular parallel lacerations on the edge of a dolphin flipper and **(B)** parallel superficial tooth rake marks on the trunk of a dolphin that are the results of fighting among dolphins and not human interference.

results of fighting and an attack from other dolphins, not from human intervention. Rake injuries may be significant, although only relatively superficial, as death has occurred from infection of these lesions by waterborne bacteria *(11)*. Inflicted injuries from adult dolphins have been reported in infant bottlenose dolphins that show similarities to inflicted trauma in human infants, with often bilateral injuries concentrated around the head and chest including bite marks, multiple rib fractures, lung lacerations, and soft tissue contusions *(12,13)*.

After death it is not uncommon for other marine animals to feed off a carcass resulting in the creation of, or modification of preexisting, wounds. Sea lice may be particularly voracious and can quickly strip surface epithelial layers (Fig. 6) *(14)*.

6. Causes of Death

A variety of causes of death have been reported in different series of beach-washed dolphins and porpoises from different parts of the world. In a study of 28 Indo-Pacific hump-backed dolphins (*Sousa chinensis*), 32 finless porpoises (*Neophocaena phocaenoides*) and four bottlenose dolphins (*T. truncatus*) from Hong Kong, 15 animals died as a result of human factors (6 from blunt trauma owing to boats and 9 with evidence of fishery-related mortality). Other factors contributing to or causing death were bacterial and parasitic infections, shark attacks, and uterine prolapse *(15)*. Infections were the most common cause of death in a series of 25 cetaceans found stranded on the Italian coast between 1990 and 1993, with a high incidence of pneumonia, enteritis, hepatitis, interstitial nephritis, and encephalitis *(16)*. Mass mortality may be caused by infections or algal toxin poisoning *(7)*.

The occurrence of outbreaks of disease among marine mammals by previously unrecognized infectious agents, such as morbillivirus, has raised the possibility of immunosuppression secondary to environmental contamination *(17)*. Analysis of dolphin tissues from many places has shown elevated levels of metals, such as mercury, cadmium, zinc, iron, and selenium, with the highest concentrations usually being in the liver *(8,18,19)*. Organic toxins from ingested fish have also been reported as causes of death in dolphins. These may be tested for using special techniques *(20)*.

Infections and entanglements were the most common causes of death in a series of 18 necropsied dolphins and 41 harbor porpoises (*P. phocoena*) beached on the coast of the United Kingdom *(21,22)*. It is interesting to note that just as causes of human mortality vary between countries, the same occurs for marine mammals, with a study of 68 beached marine mammals from Oregon in the United States revealing that gunshot wounds were the primary cause of death in almost 30% of the pinnipeds (10 of 23 harbor seals [*Phoca vitulina*] two of 12 Steller's sea lions [*Eumetopias jubata*] 5 of 10 California sea lions [*Zalophus californianus*] and 2 of 8 northern elephant seals [*Mirounga angustirostris*]) *(23)*. In a study of 17 bottlenose dolphins from Florida, the most common cause of death was infection, with two dolphins asphyxiating from whole fish lodged within their esophagi by dorsal and/or pectoral tail fin spines *(24)*. Asphyxiation from attempted ingestion of a prey that is obviously too large to swallow whole causing external compression of the upper airway, raises the possibility of an under-

lying neurological disorder, as is found in dementing humans who choke on food in the so-called "café coronary syndrome" *(25)*.

A study of 361 whale and dolphin carcasses in South Australia determined that 25% of deaths in which causes could be determined were intentionally or unintentionally caused by humans *(26)*. Entanglement in aquaculture and fishing apparatus accounted for many of the unintentional deaths. Illegal killings were from gun shots and stabbings.

7. Necropsy

Certain difficulties have been encountered in the assessment of these cases. The carcasses are often large, sometimes reaching more than 170 kg, and require an electric hoist to position them prior to necropsy, which is undertaken at the South Australian Museum's facility located near the local sewage treatment plant. The advantage of this location is that any extra smell associated with necropsying a large marine mammal will not be particularly noteworthy. Putrefaction is often present and this complicates wound evaluation and also the determination of whether underlying disease may have been present that caused or contributed to death.

8. Conclusions

Although cetaceans have been the focus of ongoing scientific study at the South Australian Museum for many years *(26)* it is only in recent times that there has been an interest in cause of death and collaborative work with agencies, such as Forensic Science South Australia. The initial aim of such work was to ensure that cases of human injury to marine mammals, and particularly dolphins, were documented as carefully as possible, so that they would survive scrutiny in a legal environment if convictions were pursued. Despite no charges having been laid in such cases to date, it appears that the group has had another effect, that of deterrence, e.g., since the formation of the group 2 years ago, with publicity regarding its activities, there have been no known further killings of dolphins reported in the Adelaide area. This may of course be completely coincidental to the group's activities; however, there may be a perception by possible perpetrators that the chances of being caught and prosecuted are much higher with the current system.

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Fixation Techniques for Organs and Parenchymal Structures

15

Methods of Lung Fixation

Roland Hausmann, MD

CONTENTS

SUMMARY

Traditional methods of tissue sampling at autopsy may occasionally be unsuitable for quantitative morphological studies. When examining lung tissue, special procedures are required in order to preserve the state of pulmonary expansion and the structural relationships within the parenchyma comparable to those presenting during life. For this purpose, different methods of lung fixation/preservation have been evaluated in numerous morphological and correlative radiological studies of the lung: (a) instillation of fixative solutions into the airways, (b) tissue preservation by vascular perfusion, and (c) *in situ* fixation techniques. The histological findings and measurement data reported in the literature demonstrate that none of these methods completely meets the aforementioned requirements, but rather outlines advantages, disadvantages, and limitations of the different procedures. Fixation techniques using an instillation or perfusion of fixative solutions usually result in excellent preservation of lung tissue. However, structural alterations and changes in the alveolar size have been observed in specimens fixed by such methods, in particular if they were

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performed with pressure application. To obtain more reliable data on the state of alveolar expansion, it has been proposed to use an *in situ* fixation of the lungs before opening of the thorax. As the artificial lung collapse at autopsy can be avoided, *in situ* fixation techniques could be of special forensic pathological interest, particularly in cases of suspicious infanticide, death by drowning or suffocation, and to differentiate between stillbirth vs live-birth.

Key Words: Lung; histopathology; morphometry; fixation methods; forensic pathology.

1. Introduction

Morphometrical analysis of lung tissue may occasionally be of considerable forensic pathological interest, particularly in cases of drowning, suffocation, and other types of fatal asphyxia. The histological diagnosis of an asphyctic death is to a high degree based on differences in the extent of alveolar expansion and the architecture of septal walls. Investigations on the aeration of the pulmonary parenchyma are also valuable to differentiate between still- and live-birth. On the other hand, the air content of the lung may be reduced in various pathological conditions, such as surfactant deficiency or hyaline membrane disease of premature newborns, in bronchial obstruction or compression of the lung parenchyma owing to pneumothorax, and in neoplasms. Although such diagnostic criteria are well-known and thoroughly investigated, it must be emphasized that quantitative studies on the state of pulmonary expansion may be limited by the collapse of the lung caused by the loss of the negative pleural pressure which usually occurs after opening of the thoracic cage at autopsy. Such an influence has already been described in the early 1960s by some pathologists *(1–5)* but obviously has not yet been sufficiently considered in forensic pathological issues. Furthermore, relevant changes in lung morphology may occur depending on the method of fixation, as described in several correlative radiological and pathological studies of lung tissue from animals and humans.

2. Fixation Techniques

It is a major objective of any fixation method that alterations in structural relationships within organs and tissue are avoided or are at least kept to an absolute minimum. Ideally, there should be no change in volume or shape of any individual component and, hence, it is of the greatest importance that all portions of the material are uniformly and adequately fixed. In examining lung tissue, special procedures may be required in order to obtain the state of pulmonary expansion and the air space volume comparable with that present during life.

Various methods of lung fixation have been reported using an inflation of fixative solutions through the airways *(6–18)* or vascular perfusion techniques *(19–23)*. However, such methods may be unsuitable for quantitative studies, particularly if they are performed with pressure application *(24)*. In order to obtain more reliable morphometrical data on the state of alveolar expansion, it has been proposed to use an *in situ* fixation technique of the lungs before the opening of the thorax *(2,4,5,25,26)*.

2.1. Airway Instillation Techniques

Instillation of suitable fixatives into the airways of collapsed lungs is a widely used fixation procedure for analysis of pulmonary structure–function relations. Reported advantages of the method are its easy performance, the excellent tissue preservation (including the cellular blood components within capillaries), and the homogenity of well-expanded lungs, assuring a representative sampling of all structures *(17)*. Furthermore, tissue samples of such preparations are thought to be well-suited for morphometrical evaluation, including estimations of pulmonary diffusion capacity *(10)*. It has been well-recognized that this method of lung fixation yields preparations that truly reflect the conditions in vivo *(16)*. However, by instillation of fixative into the airways and peripheral air spaces, the alveolar surface lining layer is removed, thereby altering the alveolar microarchitecture. These artifacts have been well recognized and defined and can appropriately be taken into account when analyzing the relations between structure and function of the lung *(10,17,20,27)*.

A number of inflation methods have been developed utilizing mainly *formalin vapor (7,8)* or *heated formalin (11)*. These methods require complicated apparatus and close surveillance during the procedure. Furthermore, none of these procedures have provided satisfying fixation results. Therefore, these inflation methods cannot be recommended to be used in forensic pathological practice.

In contrast, fixation of the lung with formalin solution and subsequent drying in vacuum *(6)* or by air inflation *(13)* requires little technical assistance and provides lung specimens suitable for morphometrical studies. A modified technique reported by Sutinen et al. is briefly described here because this method is considered simple and efficient enough for practical use by the general pathologist *(14)*:

> The lung is removed as intact as possible together with a long bronchus. Eventual tears are tied or sutured. The blood vessels are ligated, the bronchi are suctioned free of mucus and the lung is weighed. A cannula is placed into the main bronchus and the lung is inflated with air using a pressure of 15–20 cm H_2O. Using the cannula the lung is filled with formaldehyde-

polyethylene glycol-alcohol solution using a pressure gradient generated by elevating the surface of the fixative in the container approximately 55–65 cm above the organ to be fixed. The composition of the fixative is as follows: polyethylene glycol, 50%; 95% ethyl alcohol, 25%; 37% formaldehyde solution, 10%; distilled water, 15%. The inflation of the lung with fixative is continued until the pleura is unfolded and the organ appears filled. After filling, the bronchus is left unclosed and the lung is immersed in the fixative for at least 2 days, covered with cloth. The lung is then dried by blowing air through the cannula at a pressure of 20 mmHg. The fixation and drying are performed in a draught cabinet. In general, the drying of normal lungs is completed in 2 to 3 days.

The following advantages of this inflation-fixation technique have been reported by the authors *(13,14)*: (a) simplicity of the preparation methods and materials, (b) opportunity for close radiological correlatative studies, (c) excellent specimens for gross demonstration and teaching conferences, (d) easy handling and storage of the fixed lungs for future needs, and (e) excellent histological details. Thus, this method has been recommended for routine study of pulmonary diseases.

Further studies on the value of two different methods of inflation–fixation were performed by Javed et al. *(18)*. Morphometrical analyses were performed on normal adult human lungs from nonsmoking individuals fixed in inflation. One lung lobe was fixed by infusing 10% formaldehyde into the cannulated main bronchus and immersing the lung in a container of formaldehyde for 24 hours in a conventional manner (*wet fixation*). The second lung lobe was cannulated and placed in an inflation/fixation apparatus designed to inflate and fix lungs by means of a closed-loop pumping system. Initially, the lung was inflated with air to drain the blood from the vascular system (*semidry fixation*), and then 10% neutral formaldehyde fixative was perfused at a constant pressure at 23°C via the cannulated main bronchus with the lung suspended over a bath of fixative. When fixation was complete (after approx 48 hours) as judged by minimal collapse when the perfusion pump was turned off, the fixative was drained out of the inflation apparatus. The air pump was then reconnected to the cannula in the main bronchus and the pressure was gradually increased until the lung reached full inflation with air. After fixation, a computerized image analysis method of measuring the alveolar size in terms of the alveolar wall surface area per unit volume (AWUV) was performed in order to demonstrate differences in both fixation techniques. Statistical evaluation revealed evidence of significantly higher AWUV readings in the wet fixed lung than in the semidry method. The mean AWUV values were 39.37 in the *wet fixed* lung and 30.18 in the *semidry fixed* lung.

A modified technique of inflation has been reported by Butler et al. in order to investigate the relative capillary density and the alveolar diameter by a microscopical-morphometrical technique *(12)*. The study was carried out on 15 lungs from individuals who had died suddenly, usually as a result of trauma. After removal of the lungs at autopsy, the lungs were inflated through the main bronchi with air to a pressure of 20 to 25 cm water and were perfused via the pulmonary artery with neutral buffered suspension of either colored particulate dyes or India ink. After injection, the lungs were inflation-fixed with 10% neutral buffered formalin instilled within the bronchi at a pressure of 20 to 25 cm H_2O for 48 to 72 hours. The morphometrical analysis provided evidence that normal alveoli are somewhat smaller and have a denser capillary bed as one proceeds from apex to base. Furthermore, a trend toward a smaller capillary density to alveolar diameter ratio was noted in the lungs of older persons.

A special technique of immediate lung fixation *in situ* on patients who died from adult respiratory distress syndrome has been described by Tonczar et al. *(15)*. Immediately after death occured in these patients, a 10% formalin solution was instilled in an endotracheal tube while the intravital artificial pressure-supported ventilation was continued. The inflation was discontinued 10 minutes after maximum values of respiration pressure exceeded 4.26 kPa (45 cm H_2O). After fixation, the lungs were removed as usual at autopsy. Compared with other methods of lung fixation, the following advantages of the immediate post-mortem fixation have been pointed out by the authors: (a) Functional anatomical proportions are kept, (b) artifacts mainly caused by autolysis are avoided, and (c) simple performance and cost-effectiveness.

2.2. Vascular Perfusion Fixation

Tissue samples prepared by perfusion fixation of the lung have provided high-quality specimens for subsequent ultrastructural examination as well as for pulmonary morphometry. After briefly reviewing various technical procedures reported in the literature, the advantages and limitations of this method compared with other fixation techniques will be discussed.

Morphological studies on the alveolar septae in normal and edematous dog lungs fixed by vascular perfusion have been performed by DeFouw *(19)*. The lungs of anesthetized dogs were artificially ventilated with 5% CO_2 in humified air with tidal volumes of approx 18 to 20 mL/kg and at positive end expiration pressure of 5 cm H_2O. After opening of the thoracic cage, a segment of Tygon tubing was inserted into the pulmonary trunk and another segment was inserted through the mitral valve into the left atrium. The outflow connection led to a lower level reservoir from which the perfusate was roller pumped back to an upper reservoir, which was connected to the inflow arterial tubing.

The lungs were then removed from the thorax and placed on a platform that was suspended from a gram-sensitive scale. A hydrostatic (cardiogenic) edema was induced in three out of six isolated lung preparations by progressive elevations of outflow pressure and the presence of edema was verified by increase in lung weight. The controls and edematous lungs were fixed by vascular perfusion with 2% glutaraldehyde in a 0.15 M sodium bicarbonate buffer at pH 7.4 while they were artificially ventilated to prevent alveolar collapse throughout the fixation period.

The morphometrical results obtained in the reported study suggested that vascular fixation rather than tracheal fixation appears to provide a more appropriate model of alveolar capillary perfusion under stable and edematous conditions, whereas the alveolar dimensions were obviously not adequately preserved in lung specimens fixed by vascular perfusion.

The quality of tissue preservation in lungs fixed by vascular perfusion has been evaluated also by Bachofen et al. *(20)*. Excised rabbit lungs inflated to 60% of total lung capacity were perfused in different modalities using 1% solutions of glutaraldehyde (350 vs 510 mosM), osmium tetroxide (350 mosM) and uranyl acetate (350 mosM). The durations of perfusion ranged between 10 and 30 minutes. After fixation, the effects on the pulmonary microstructure were assessed by qualitative and morphometrical analysis of electron micrographs. Important results of this study include the following: (a) perfusion with isotonic glutaraldehyde at flow rates within the physiological range produce large increases of perfusion pressure and lung weight that reflect intracellular, interstitial, and intraalveolar edema, (b) no edema occurred if glutaraldehyde was added to isotonic buffer solutions, and (c) satisfactory results could be obtained by sequential perfusion with osmium tetroxide and uranyl acetate or glutaraldehyde (510 mosM) followed by osmium tetroxid and uranyl acetate. As reported by the authors, the latter combination yields optimal preparations to study the alveolar and capillary architecture but causes a hyperosmotic volume loss of lung cells resulting from cell shrinkage.

Further influence of the vascular perfusion on pulmonary morphology has been reported by Mooi and Wagenvoort *(21)*. The authors investigated rat lungs ($n = 6$), which were fixed in three different ways, e.g., by perfusion through the pulmonary vasculature, by immersing collapsed lungs, and by instilling the fixative through the trachea with intact (unopened) thoracic cavity. Subsequently, blood vessels were counted in histological sections. The lungs were perfused as follows: formalin was instilled through cannulae into the pulmonary trunk and trachea at pressures of 100 and 25 cm H_2O, respectively. In two animals, this high-pressure perfusion was maintained for 1 minute and in the other 5 animals for 10 to 15 minutes. After this perfusion, the lungs were further fixed by

immersion in a formalin bath for at least 4 hours. The *in situ* fixation was performed by cannulating the trachea without opening the thorax and subsequently instilling formalin at a pressure of 5 to 10 cm H_2O while the thorax was intermittently gently compressed, thus simulating breathing movements. In order to investigate the effect of the various fixation techniques on the numbers of pulmonary blood vessels, morphometrical analyses were performed on histological sections. The following results were reported: (a) the specimens fixed by immersion showed lung tissue with varying degree of collapse; (b) the specimens fixed by intratracheal formalin instillation *in situ* showed more expanded lungs, with very little variation in the degree of lung tissue expansion. Consequently, there was a more even distribution of pulmonary blood vessels in these specimens. In contrast to the specimens fixed by immersion, the pulmonary blood vessels appeared well expanded and the pulmonary arteries showed little or no crenation of the elastica laminae, and (c) in the perfused lungs, there was a varying degree of mechanical destruction of lung tissue, apparently resulting from the higher pressure gradients across the vessel walls and across the pleura. The larger blood vessels appeared very thin-walled with a diameter of more than 100 μm. The adventitial layers were swollen with accumulation of fluid, apparently formalin. Furthermore, many blood cells were situated in the adventitia, apparently having been pressed through the vessel's walls. In the lumina of veins, as well as arteries, only few blood cells were found. Occasionally, the vessel walls were ruptured and collapsed. Disruption of alveolar walls was found, especially near the pleural surface and the lung septae showed accumulation of fluid.

A vascular perfusion technique especially developed for ultrastructural investigations of large, freshly isolated tissue samples in experimental studies has been reported by Roberts et al. *(22)*. Perfusion of individual lobes of the lung was performed using a perfusion apparatus that is normally employed for whole-body perfusion of small animals. Single lobes of the lung were removed at autopsy within 3 minutes after death. A Gavage needle was inserted into a major blood vessel and saline solution was perfused at ambient temperature through the vasculature at a constant pressure of 25 mmHg. Clearance of blood from the tissue produced local tissue pallor within 10 to 30 seconds. Saline solution flow was then replaced by a fixative at the same pressure and temperature, which resulted in hardening of the saline-flushed tissue volume within 2 to 4 minutes. Tissue preservation by perfusion fixation was compared with that obtained by immersion fixation of thin slivers of tissue taken from the same lobe to compare both methods directly. The following results have been reported in this study: (a) the described perfusion technique proved as simple and quick to perform, (b) superior tissue preservation in all specimens exam-

ined was obtained by perfusion fixation compared with that obtained by immersion, (c) after perfusion fixation, the vasculature was patent, undistended, and free of blood, (d) variable cytoplasmatic staining as seen commonly in immersion-fixed tissue was largely avoided by this perfusion fixation, and (d) perfusion pressure and flow rates were important determinants of fixation quality and production of artifactual damage. Artifactual distension of the pulmonary vasculature was noted in early trials, which employed elevated perfusion pressures (40 or 60 mmHg) and could be avoided in later trials by using reduced flow rate and pressure (25 mmHg).

In order to examine alterations in lung structure that may be related to the stress bearing role of elastic elements, the lungs of different animal species and humans were investigated after vascular perfusion by Mercer et al. *(23)*. For their study of human lungs, lung lobes were obtained at the time of surgical resection and fixed within 30 minutes by vascular perfusion technique. By means of a tracheal cannula and pressure reservoir, the lungs were inflated with air that was maintained at a pressure of 5 cm H_2O during the perfusion of the fixative. For perfusion of the vasculature, the pulmonary artery was cannulated. The lungs were then inflated to 30 cm H_2O and deflated to 5 cm H_2O three times during a 2- to 3-minute period to establish the volume history. Blood within the lungs was then cleared by perfusion with 0.95 NaCl, 0.3% sodium nitrite, and 100 U/mL of heparin, and then adjusted to an osmolarity of 350 mosM with sucrose. The perfusion pressure was 15 cm H_2O. After perfusion of sufficient clearing solution, the perfusate was switched to a fixative solution. The initial fixative solution contained 2% glutaraldehyde, 1% formaldehyde, and 1% tannic acid adjusted to pH 7.4. The lungs were perfused with the fixative solution for 30 minutes, after which the perfusion line was clamped and the lungs allowed to mechanically harden for 4 hours while being inflated with air at a pressure of 5 cm H_2O. This fixation procedure was thought to be adequate for morphometrical determination of the architecture, quantity of connective tissue fibers, thickness of alveolar septal layers, and three-dimensional analysis to determine the number, size, diameter, and surface curvature of alveoli both in animal and human lungs.

2.3. In Situ *Fixation*

The first systematic studies on the state of pulmonary expansion using an intrathoracic *in situ* fixation technique were performed by Rahn *(2)* in animals. Tissue samples from 132 rabbits were obtained after removal of the thorax in toto at autopsy and fixation in formaldehyde solution for 4 to 6 days before opening. By comparing *in situ* fixed lungs with those fixed after retraction, it could be shown that atelectasis as seen in human lungs appeared only when the

lungs were able to retract *in situ* under pathological conditions with compression of the lung, such as pleural effusion, neoplasm, or inflammatory infiltrates. Otherwise, atelectasis was an effect of retraction caused by autopsy. Similar results were obtained from the investigations of lungs from infants *(4)*.

Further studies using the aforementioned *in situ* fixation method have been performed by Plank *(5)* who investigated nonretracted, nonaerated lungs of 27 stillborn infants. Histology revealed a uniform pulmonary structure in the sectioned parenchyma: the alveoli were expanded by fluid and the walls of the alveoli were tortuous. Numerous cushion-like and clavate projections of the alveolar walls have been described to protrude into the alveolar lumina, which are thought to be a characteristic sign of nonaerated lungs.

In addition to such pathological aspects, the method of intrathoracic *in situ* fixation can also be of forensic pathological interest, particularly in cases of drowning or traumatic asphyxia, as demonstrated in the following:

The lungs of six drowned and six nondrowned persons over 70 years of age were compared by Kohlhase and Maxeiner *(25)* in order to determine whether there is evidence of acute overinflation in drowning that can be distinguished from the pathological features of senile lung emphysema. All left lungs underwent inthrathoracic formalin fixation to preserve their state of insufflation, whereas all right lungs were sectioned as usual and then immersed in formalin to assess the effect of lung collapse. After histological processing, microphotography, and image processing of 12 specimens per corpse, 50 binary images of each specimen were measured by computerized morphometry. Intrathoracic fixation resulted in significantly less tissue area and more airspace in the left than in the right lungs of both groups. Comparing both groups' left lungs revealed that the interalveolar septae were thinner and the area occupied by connecting nodes smaller in drowning. These single nodes also tended to be smaller. Furthermore, there was a tendency for less alveolar tissue area per image in drowning than in control lungs and for narrow tissue structures to comprise a higher percentage of both the total tissue area and total tissue parameter per image. The authors concluded that (a) there is morphometrical evidence of acute overinflation even in senile lungs, but this is masked by postmortem lung collapse and (b) supporting the diagnosis of drowning in drowned elderly patients with senile lung emphysema is only possible in lungs that underwent intrathoracic postmortem fixation.

More recently, the degree of alveolar expansion in specimens from *in situ* fixed lung tissue from a 9-month-old, otherwise healthy infant who died from sudden infant death syndrome was examined in a morphometrical study by this author *(26)*. In order to differentiate the orthological expansion of the lung from artificial retraction, two different methods of fixation were performed: (a) *in*

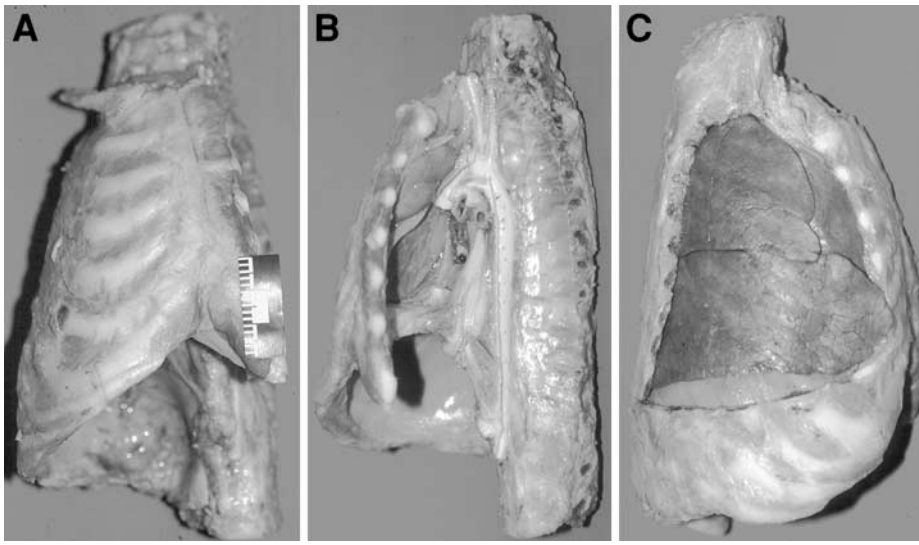

Fig. 1. Right hemithorax with the thoracic and upper lumbal part of the spinal column obtained at autopsy from a 9-month-old infant and fixed in a 4% formaldehyde solution for 3 days. **(A)** Before opening of the hemithorax, ventral aspect. **(B)** Before opening of the hemithorax, medial aspect. **(C)** After opening of the hemithorax (lateral aspect) showing the extended *in situ* fixed lung filling out the thoracic cavity similar to in vivo conditions.

situ fixation of lung, where the right lung was fixed *in situ* before opening of the pleural cavity. For this purpose, the right hemithorax with the thoracic upper lumbal part of the spinal column was removed in toto and fixed in a 4% phosphate-balanced salt–formaldehyde solution for 3 days (Figs. 1A,B). After that, the right hemithorax was opened and six peripheral, as well as two central tissue samples representing the complete cut section, were obtained for histological investigations and (b) *routine fixation of lung*, where the left lung was removed after opening the left hemithorax at autopsy. Tissue samples, obtained from peripheral and central locations as described for the *in situ* fixed lung, were fixed in a 4% phosphate-balanced salt–formaldehyde solution for 3 days. After fixation, the tissue samples from each lung were embedded in paraffin and sections (3–5 μm) were stained with hematoxylin and eosin. The data were compared with the findings in lung tissue specimens obtained at autopsy and routinely fixed after removal. The morphometrical analysis was performed using an automatic image processing and analyzing system (Leica QWIN®) and

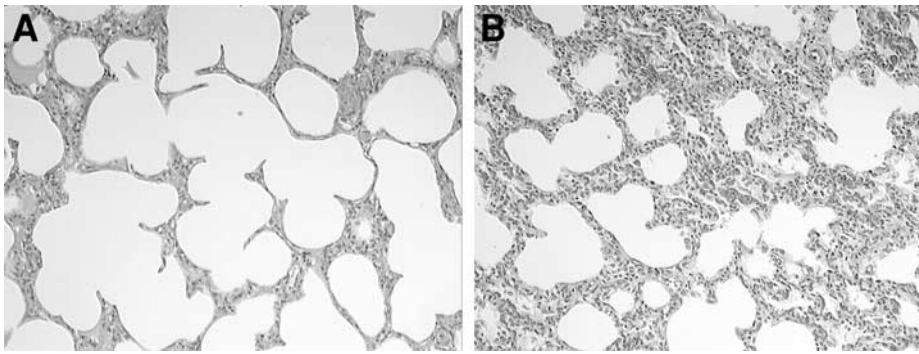

Fig. 2. Histological sections from the lung of the same 9-month-old infant using two different fixation techniques (hematoxylin and eosin). **(A)** *In situ* fixed lung with expanded alveoli (original magnification ×240). **(B)** Routinely fixed lung showing several microatelectases (original magnification ×240).

a Hitachi camera (3CC, HV-C20M). The size of the alveolar space, as well as the attendant thickness of the alveolar walls, was measured in 25 randomly selected microscopic fields of 0.135 mm^2 in size in each hematoxylin and eosin-stained preparation. The data presented in this comparative study revealed significant differences in both macroscopy and histology. The *in situ* fixed lung was totally extended and filled out the right thoracic cavity similar to the in vivo condition (Fig. 1C), whereas the left lung that was removed at autopsy was collapsed as usual. Histologically, the *in situ* fixed lung showed expanded alveolar lumina in all sections, the majority of septae appearing thin and stretched (Fig. 2A). In contrast, several microatelectases were found in the lung that was removed at autopsy by the usual technique and fixed using routine procedures (Fig. 2B). The results of the morphometrical analysis revealed a significant decrease (of about 27%) in the size of alveoli of the immediately fixed lung (Fig. 3, Table 1), whereas the differences in the average thickness of the alveolar walls were statistically not significant (Table 2).

3. Forensic Pathological Aspects

Considering the different methods of lung fixation reported in the literature, it has to be emphasized that in fact no tissue preparation technique for lung fixation reveals a perfect image of the condition in the living subject. The histological findings and measurement data do not point to a best recipe but rather outline advantages, disadvantages, and limitations of the different procedures.

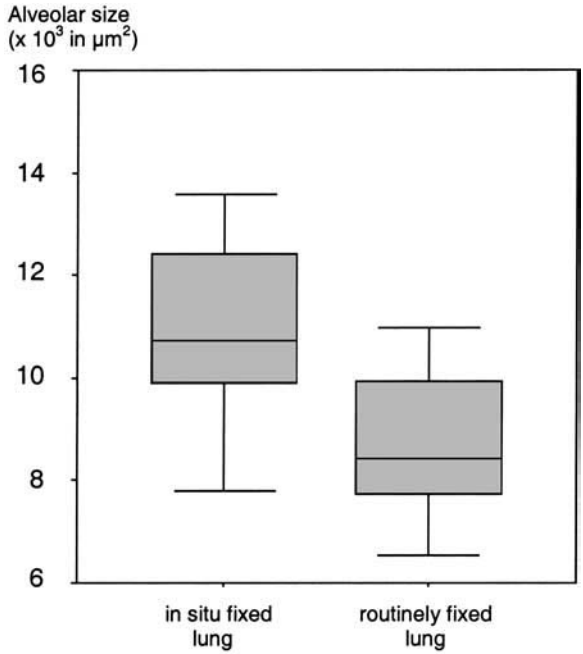

Fig. 3. Mean alveolar size in µm^2 of the specimens from the *in situ* fixed and routinely fixed lung. Note: box plot—the boxes contain 50% of the measurement values; the line in the box represents the median value.

Fixation techniques using an instillation of fixative solutions into the airways result in excellent preservation of lung tissue but alter the arrangement of septal tissue, as well as the alveolar microarchitecture, whereas the replacement of capillary blood by a fixative and changes of vascular properties are unavoidable artifacts in lung tissue fixed by vascular perfusion techniques. Fixation methods that are performed under considerable pressure application are, in general, of limited value for quantitative morphological investigations.

With regard to the state of pulmonary expansion and the air space volume, the *in situ* fixation technique may provide the most reliable information. The morphometrical findings obtained from specimens fixed by this method have been reported to be comparable with those existent during life *(4)*. As the artificial lung collapse at autopsy as well as the above mentioned morphological chances owing to other methods of fixation can be avoided, the *in situ* fixation technique can be recommended for use in forensic pathological practice, particularly in cases suspicious of infanticide, stillbirth or death by drowning or suffocation *(26)*.

Table 1
Alveolar Size

Alveoli (μm^2)		*In situ* fixed lung	Routinely fixed lung	*p* (*U*-test)
Total tissue	*n*	841	1.156	0.050
	Mean	10.9×10^3	8.7×10^3	
	Median	9.3×10^3	7.3×10^3	
	SD	6.5×10^3	8.3×10^3	
Central areas	*n*	218	329	0.333
	Mean	10.8×10^3	7.0×10^3	
	Median	10.2×10^3	5.4×10^3	
	SD	6.6×10^3	7.0×10^3	
Peripheral areas	*n*	623	828	0.310
	Mean	10.8×10^3	9.2×10^3	
	Median	8.9×10^3	7.9×10^3	
	SD	6.6×10^3	8.7×10^3	
	P (*U*-test)	0.857	0.71	

Mean, median, and standard deviation (SD) calculated from the morphometrical data obtained from the *in situ* fixed lung and the lung removed at autopsy by the usual technique and fixed using routine procedures.

n = number of alveoli measured in each lung. The mean values were compared with the Mann-Whitney *U*-test. (Modified according to ref. *26*.)

Table 2
Thickness of the Septal Walls

Septal walls (μm)	*In situ* fixed lung	Routinely fixed lung	*p* (*U*-test)
n	1.190	1.027	0.505
Mean	8.1	7.9	
Median	8.0	7.88	
SD	7.37	7.14	

Mean, median, and standard deviation (SD) calculated from the morphometrical data obtained from the *in situ* fixed lung and the lung removed at autopsy by the usual technique and fixed using routine procedures.

n = number of alveoli measured in each lung. The mean values were compared with the Mann-Whitney *U*-test. (Modified according to ref. *26*.)

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Dr. Michael Tsokos is a lecturer of forensic pathology and legal medicine at the University of Hamburg, Germany, and the Police Academy of the City of Hamburg, Germany. He is the primary or senior author of more than 120 scientific publications in international peer-reviewed journals and the editor of a number of books dealing with topics of forensic pathology.

In 1998 and 1999, Dr. Tsokos worked for a time with the exhumation and identification of mass grave victims in Bosnia-Herzegovina and Kosovo under the mandate of the UN International Criminal Tribunal for the former Yugoslavia. In 2001, he was honored with the national scientific award of the German Society of Legal Medicine for his research on micromorphological and molecularbiological correlates of sepsis-induced lung injury in human autopsy specimens. In December 2004 and January 2005, Dr. Tsokos worked with other experts from national and international disaster victim identification teams in the region of Khao Lak/Thailand for the identification of the victims of the tsunami that struck South East Asia on December 26, 2004.

He is a member of the International Academy of Legal Medicine and the German Identification Unit of the Federal Criminal Agency of Germany. Dr. Tsokos is assistant to the editor-in-chief of *Rechtsmedizin* (the official publication of the German Society of Legal Medicine), member of the Advisory Board of the *International Journal of Legal Medicine,* member of the Editorial Board of *Legal Medicine* and *Current Immunology Reviews* and European editor of *Forensic Science, Medicine, and Pathology.*