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Macroscopical, Microscopical, and Laboratory Findings in Drowning Victims

A Comprehensive Review*

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CONTENTS

SUMMARY

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

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

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

1. Introduction

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

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

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

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

2. *Historical Aspects*

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

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

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

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

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

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

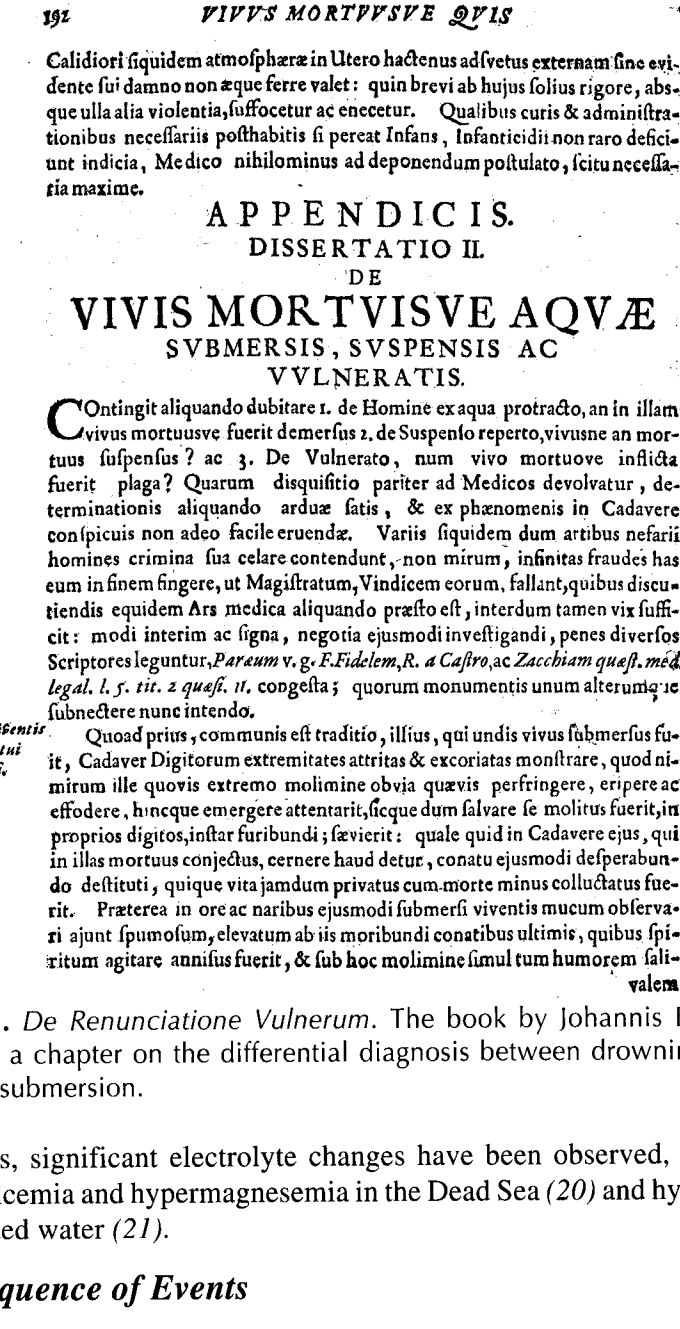
pus Iuris Medico-legalis (1722) also contains passages on drowning, including case reports on infanticide and suicide as well as correspondence between the author and Johannes Conrad Becker on drowning without inhalation or swallowing of water *(9)*.

3. Pathophysiology

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

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

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



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

APPENDICIS.

DISSERTATIO II.

DE

VIVIS MORTVISVE AQVÆ SVBMERSIS, SVSPENSIS AC VVLNERATIS.

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

Signa Viventis aut mortui ſubmerſi.

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

valem

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

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

3.1. Sequence of Events

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

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

3.2. Respiratory System

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

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

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

3.3. Cardiovascular System

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

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

3.4. Central Nervous System

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

3.5. Other Organ Effects

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

3.6. Delayed Complications

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

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

3.7. Dry-Drowning

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

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

3.8. Hypothermia

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

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

4. Macroscopical Findings

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

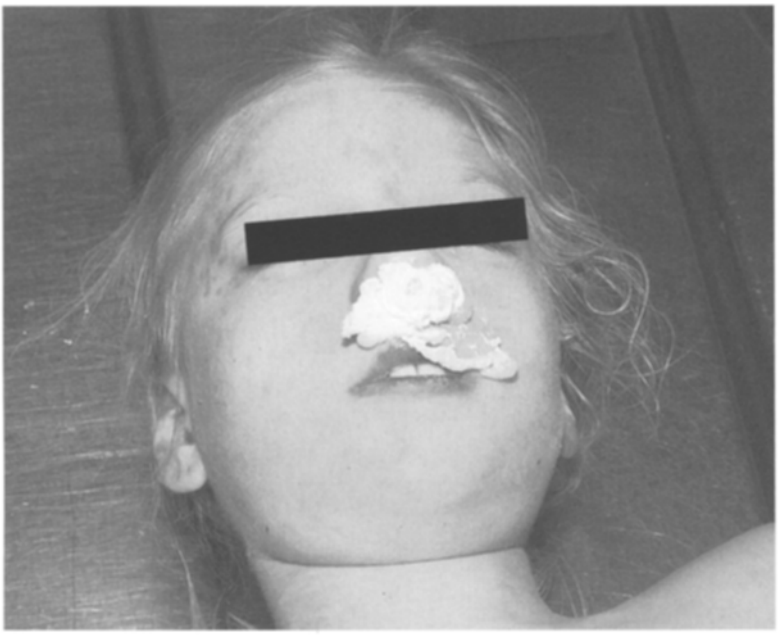

Fig. 2. Characteristic foam extruding from the mouth and nostrils of a drowned girl.

4.1. Upper Airways

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

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

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

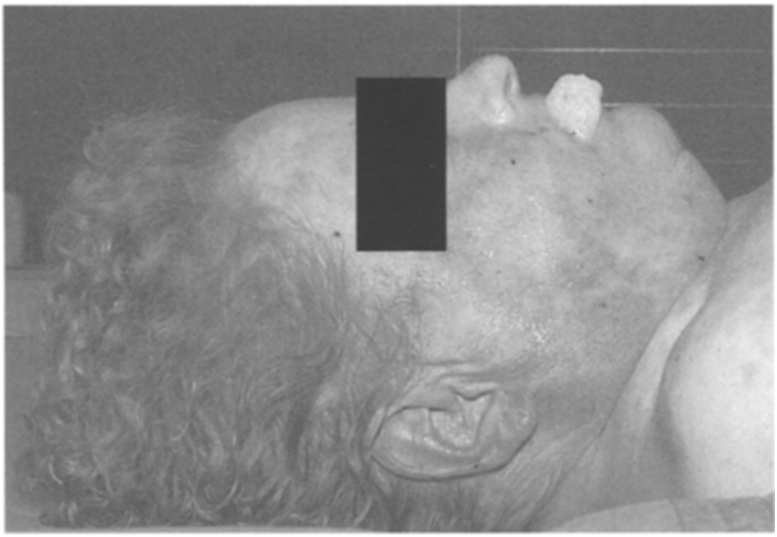

Fig. 3. Drowning victim. Characteristic foam extruding from the mouth and nostrils.

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

4.2. Lungs

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

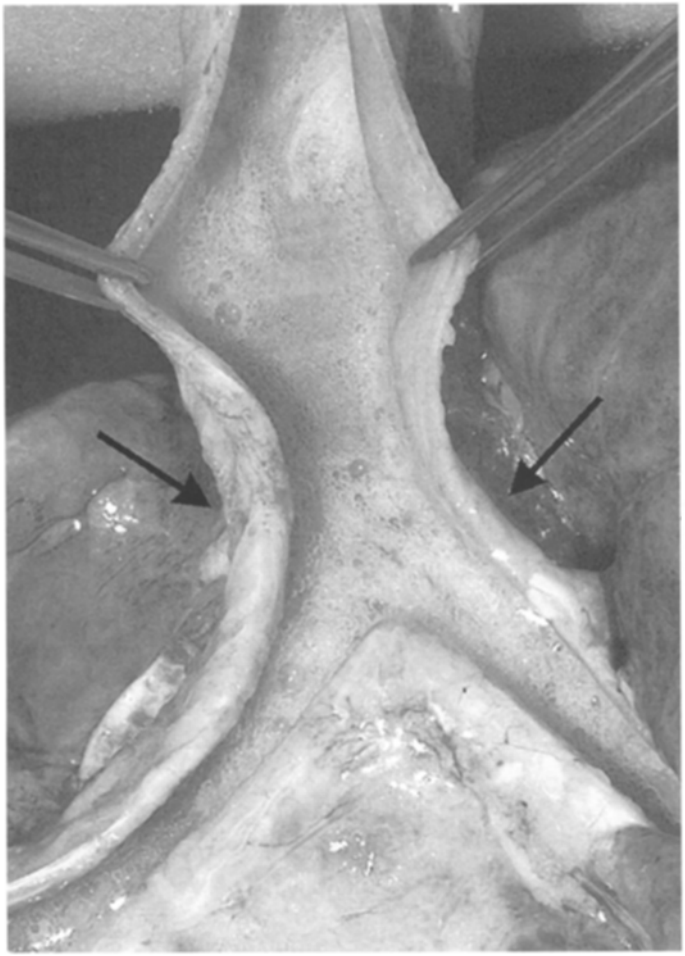

Fig. 4. Frothy fluid is seen in the trachea and bronchi of a drowning victim.

4.2.1. Lung Weight

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

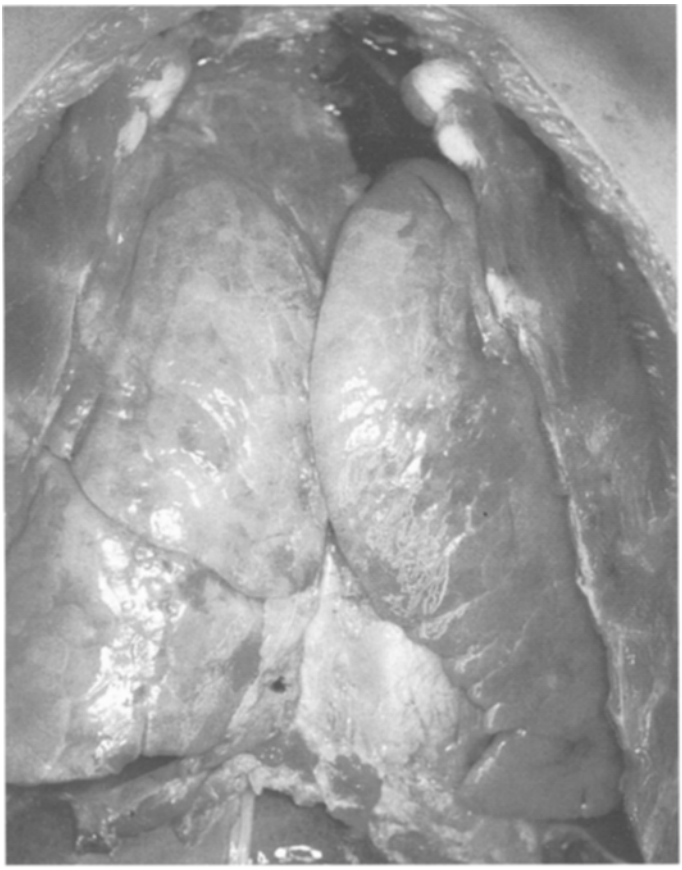

Fig. 5. Overdistension of the lungs with overlap of their anterior edges over the midline in a drowning victim.

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

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

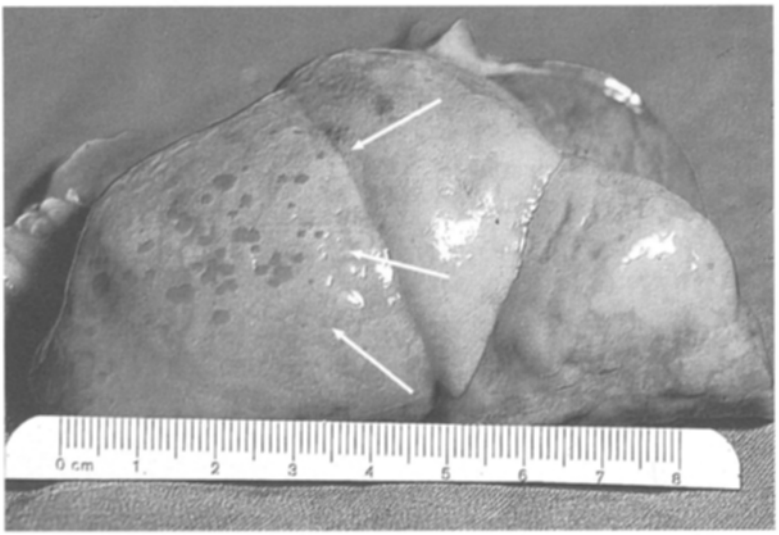

Fig. 6. Paltauf's spots (arrows) located in the upper lobe of the right lung. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

4.2.2. Dry Lungs

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

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

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

4.2.3. Drowning Lung vs Postmortem Hydrostatic Lung

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

4.2.4. Pleural Effusion

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

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

4.3. Temporal Bone

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

4.4. Sinuses

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

4.5. Other Organs

4.5.1. Spleen

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

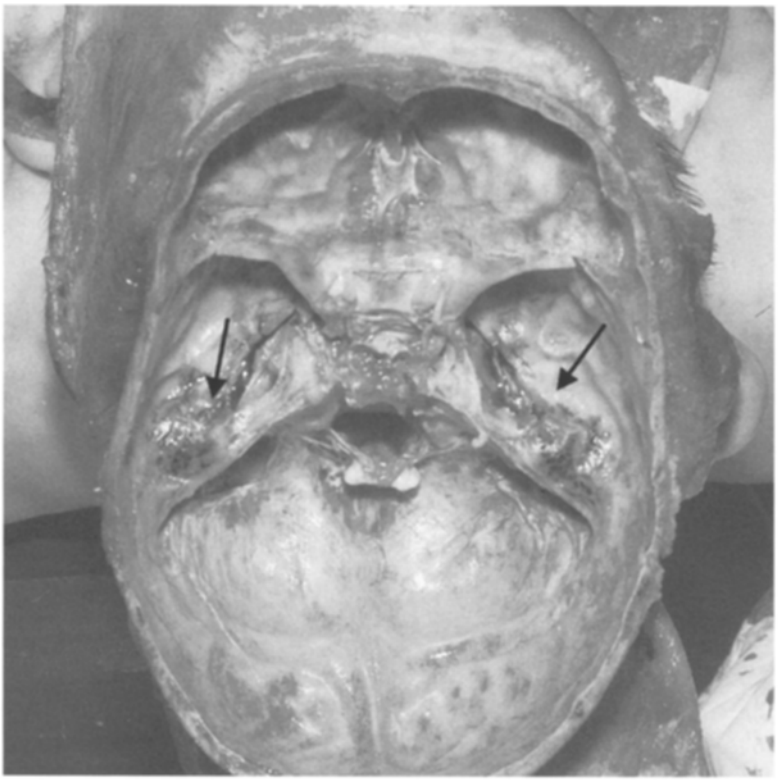

Fig. 7. Marked bilateral hemorrhages within the petrous region of the temporal bones (arrows) in a drowning victim.

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

4.5.2. Muscles

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

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

4.5.3. Gastroenteric Tract

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

5. Microscopial Findings

5.1. Light Microscopy

5.1.1. Lung

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

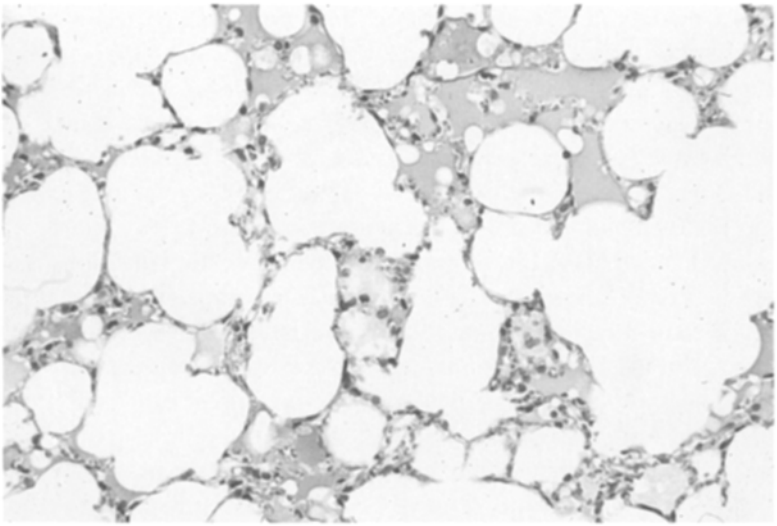

Fig. 8. Lung histology in drowning. Acute emphysema of the lung with edema liquid partially filling the alveoli (experimental conditions, mouse).

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

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

5.1.1.1. Surfactant

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

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

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

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

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

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

5.1.1.2. Alveolar Macrophages

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

5.1.2. Other Organs

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

5.2. Analytical Morphometry

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

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

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

5.3. Electron Microscopy

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

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

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

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

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

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

6. Laboratory Findings

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

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

6.1. Blood Properties and Composition

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

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

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

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

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

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

6.2. Exogenous Substances

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

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

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

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

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

6.3. Artificial Tracers

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

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

7. The Diatom Method

7.1. General Remarks

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

7.2. Diatom Biology

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

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

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

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

7.3. Sample Preparation and Analysis

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

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

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

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

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

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

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

7.4. Diatom Penetration in the Lung

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

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

7.5. Diatoms in Peripheral Organs

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

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

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

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

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

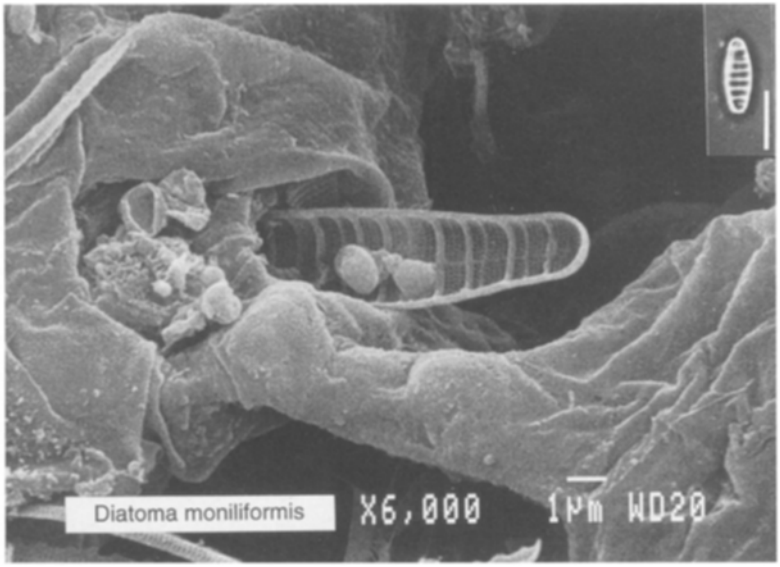

Fig. 9. *Diatoma moniliformis,* a diatom, penetrating the wall of a distal airway (scanning electron microscopy; experimental conditions, rat).

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

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

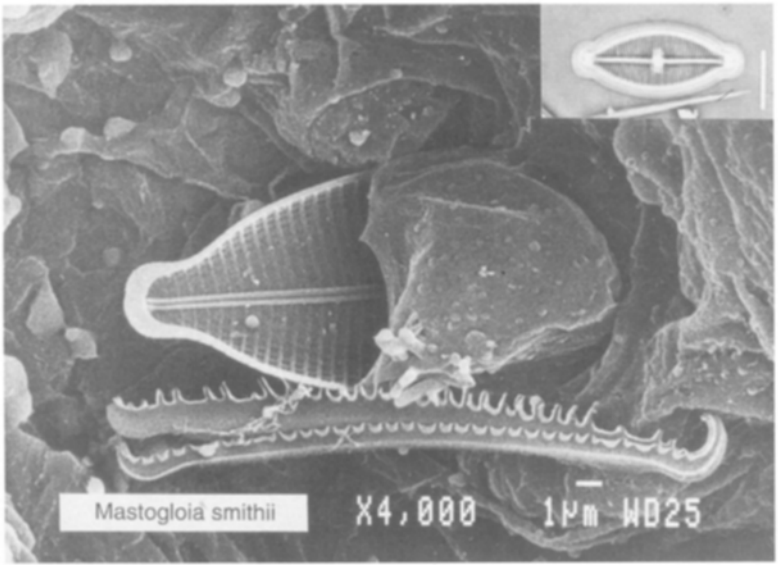

Fig. 10. *Mastogloja smithii,* a diatom, penetrating the alveolar wall through a clearly visible laceration (scanning electron microscopy; experimental conditions, rat).

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

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

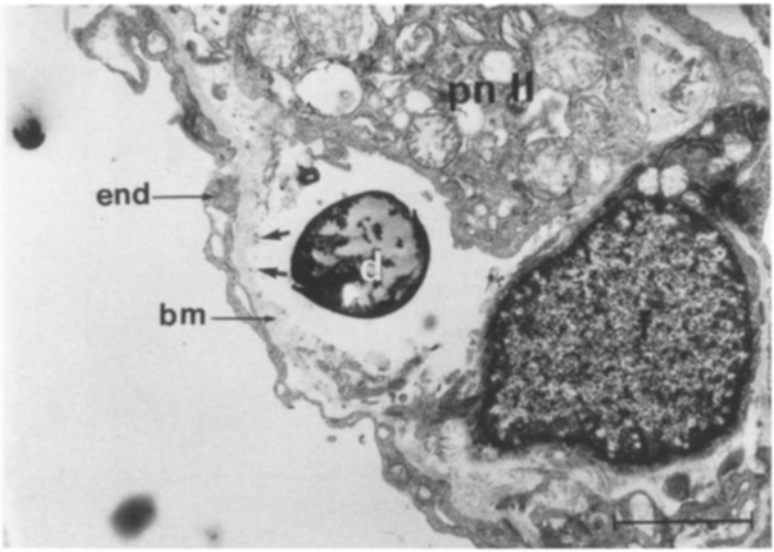

Fig. 11. *Phaeodactylum tricornitum*, a diatom, in the interstitial space with incipient ruptures of the endothelial membrane. End, endothelial lining; bm, basal membrane; pn II, pneumocyte type II; d, diatom (transmission electron microscopy; experimental conditions, rat).

7.6. False-Positive Results

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

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

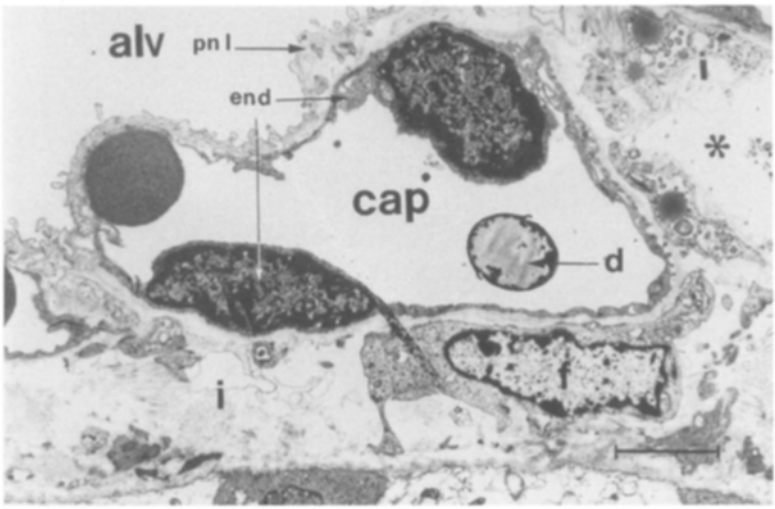

Fig. 12. *Phaeodactylum tricornitum*, a diatom, in a pulmonary capillary. Cap, capillary; alv, alveolar space; end, endothelial cell; pn I, pneumocyte type I; i, interstitial space (transmission electron microscopy; experimental conditions, rat).

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

7.6.1. Antemortem Contamination

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

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

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

7.6.2. Postmortem Penetration

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

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

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

7.6.3. Contamination During Sample Preparation

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

7.6.4. Other Sources of Contamination

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

7.7. False-Negative Results

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

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

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

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

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

8. Selected Issues

8.1. Identification

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

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

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

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

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

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

8.2. Postmortem Changes

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

8.2.1. Early Postmortem Changes

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

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

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

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

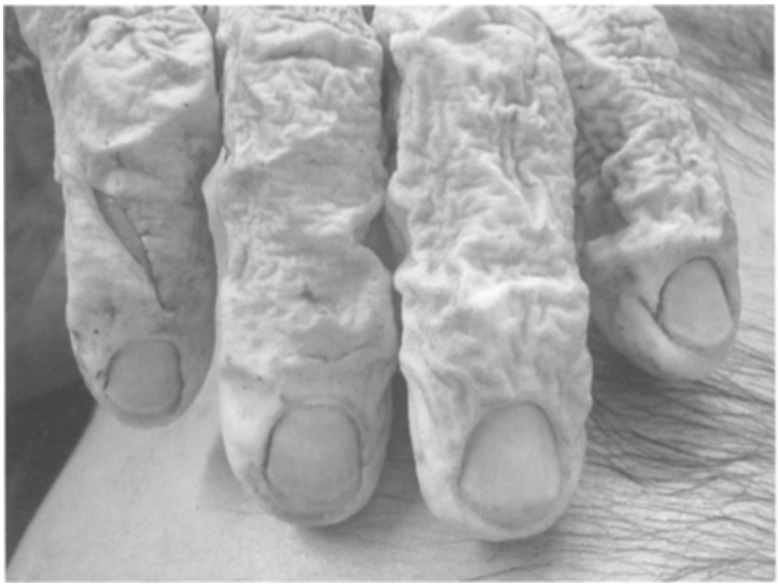

Fig. 13. Submersion. Typical maceration changes on the hand. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

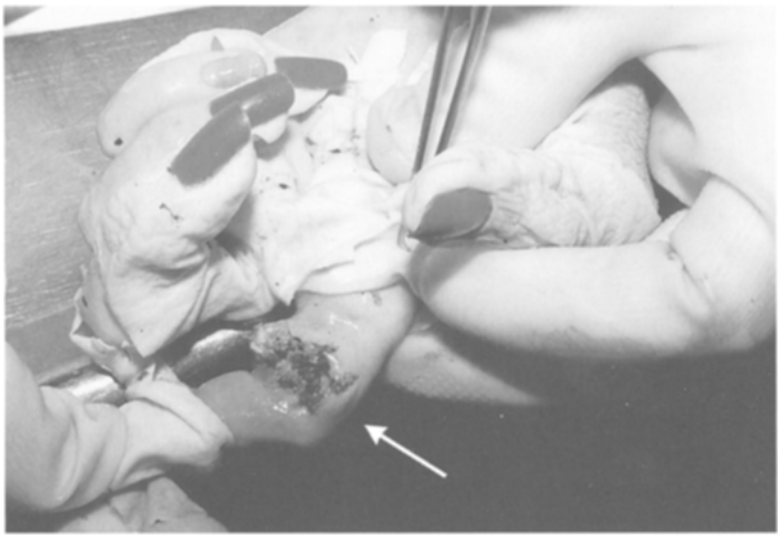

Fig. 14. Submersion. Maceration changes on right hand with partial peeling of skin and nails. Examination of the soft tissue under the peeled skin revealed small plastic fragments within irregular lacerations (arrow). Police investigations revealed that the victim was killed by firearm and that one bullet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

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

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

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

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

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

8.2.2. Putrefaction, Adipocere, and Late Decomposition

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

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

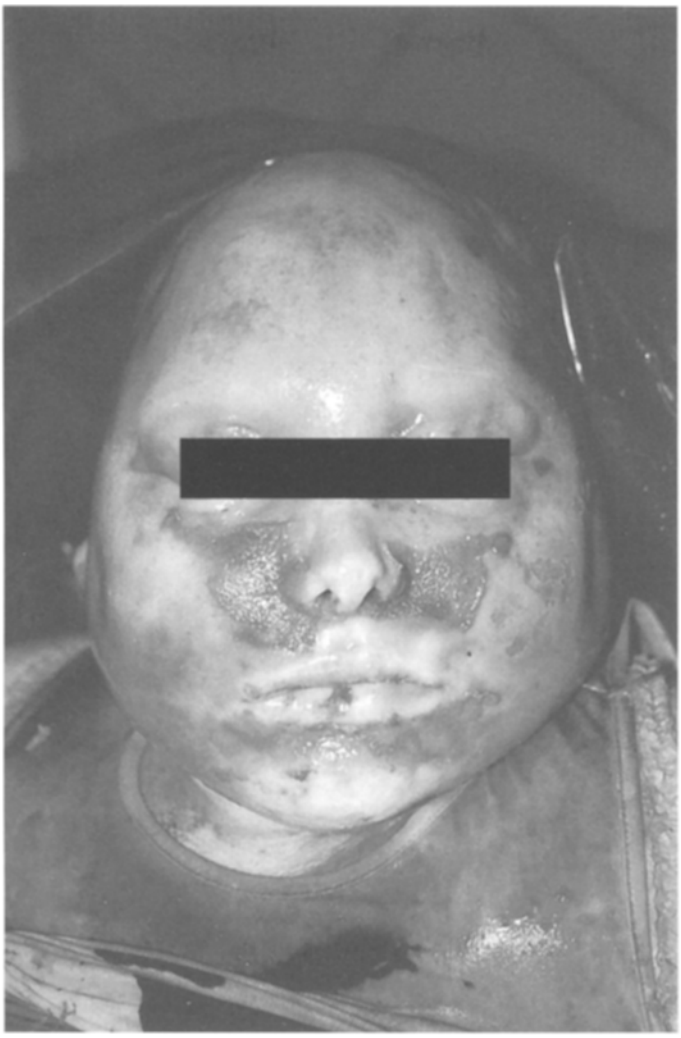

Fig. 15. Body found in water. Putrefactive and emphysematous changes on a young woman's face which hampered her visual identification. The dark grey changes on her cheek near the nose, arranged in a bilateral pattern, represent areas of algal colonization. Postmortem submersion time was 3 weeks.

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

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

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

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

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

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

8.3. Antemortem and Postmortem Injuries

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

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

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

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

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

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

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

8.4. Manner of Death

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

8.4.1. Unintentional Drowning

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

8.4.2. Suicide

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

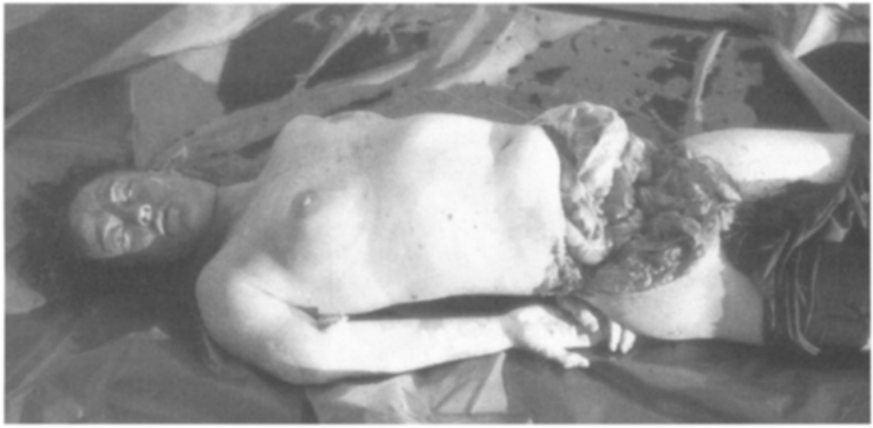

Fig. 16. Body found in water. Transversal postmortem propeller injury of the trunk with partial extrusion of abdominal viscera. (Courtesy of Dr. Michael Tsokos, Hamburg, Germany.)

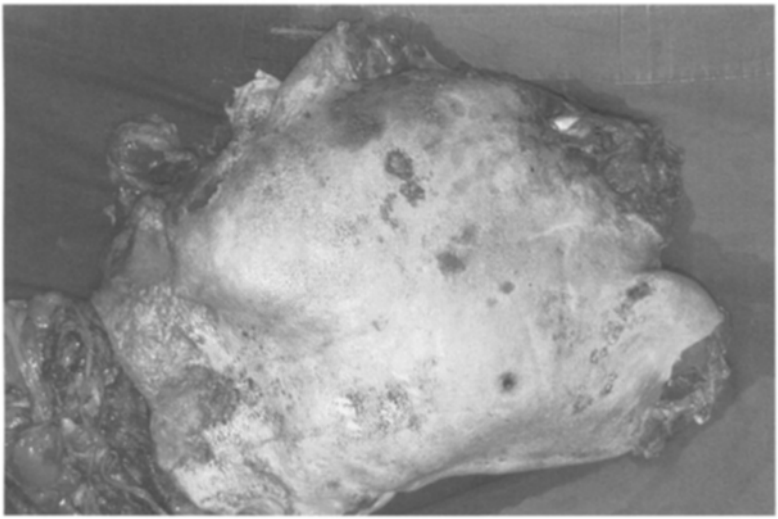

Fig. 17. Body found in water. Postmortem amputation of head and arms by motorboat propeller. Note the irregularity of tissue lacerations compared with the amputation surfaces shown in Fig. 18.

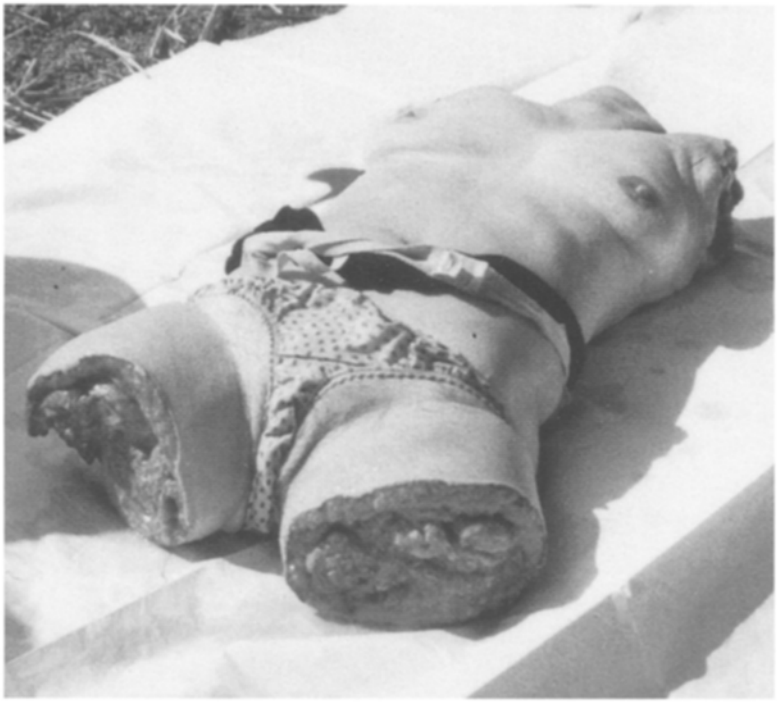

Fig. 18. Disposal of a body in water. Complete "defensive" dismemberment of the victim by the homicide perpetrator. Note the regular amputation surfaces compared with those seen in Fig. 17 and the lack of hemorrhages within the soft tissues.

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

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

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

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

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

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

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

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

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

8.4.3. Homicide

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

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

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

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

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

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

8.4.4. Undetermined

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

8.4.5. Natural Deaths

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

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

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

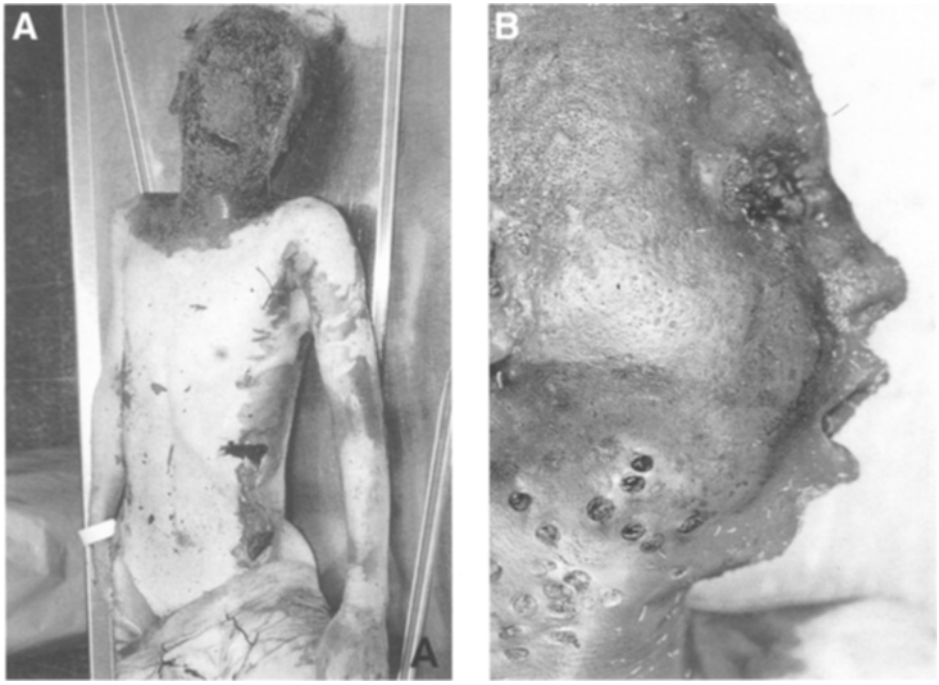

Fig. 21. Body found in shallow water. The young male victim's head and neck were submerged in a ditch and the corresponding regions showed advanced putrefaction and extensive fly maggots infestation (**A**,**B**). Police and medicolegal investigations ascertained the victim was killed by multiple stab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

8.5. Disposal of Corpses in Water

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

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

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

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

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

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

8.6. Bathtub Deaths

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

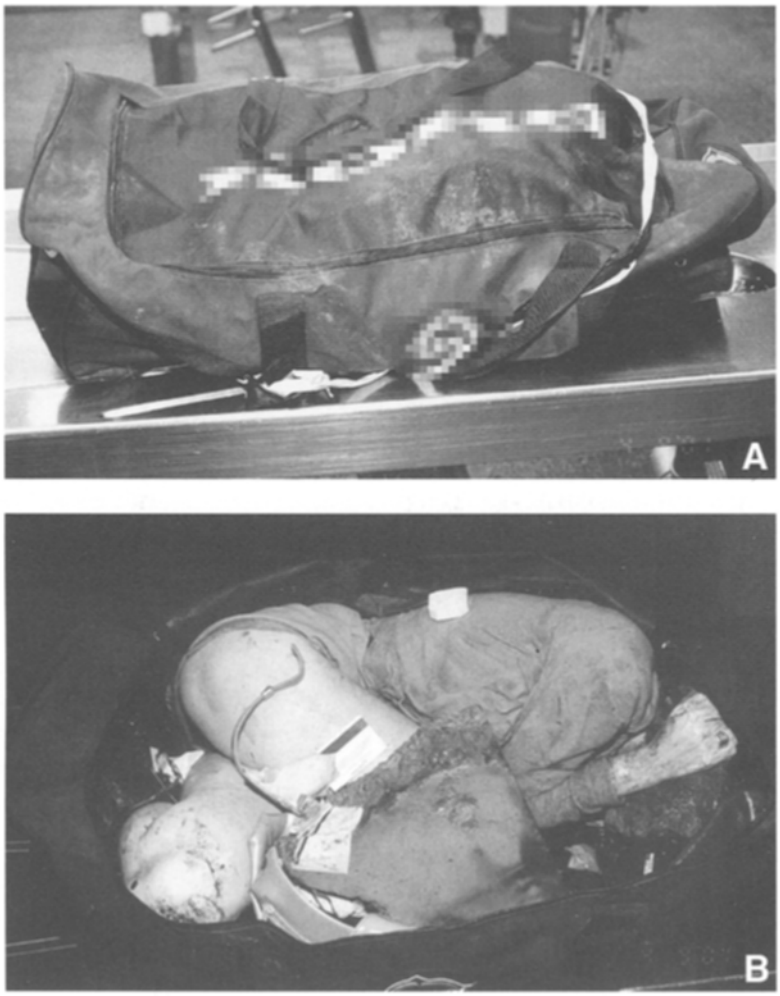

Fig. 22. Disposal of body. **(A,B)** A large bag that contained the corpse of a middle-aged woman was filled with stones and was submersed in the sea after a homicide by firearm (same case as Fig. 14).

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

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

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

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

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

9. Conclusion

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

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