3. Pathology of Sepsis

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Introduction

On various occasions, the pathologist is confronted with the question of whether a deceased suffered from a septic condition prior to death and thus whether sepsis caused or at least contributed to the fatal outcome.

Typical scenarios of infection-related deaths routinely encountered in autopsy practice are infection associated with vascular catheters, delayed diagnosis of Waterhouse-Friderichsen syndrome (WFS) in infancy and childhood, pseudomembranous colitis following the uncritical use of broad-spectrum antibiotics for minor infections, gas gangrene following surgical procedures, pyomyositis or necrotizing fasciitis resulting from introduction of pathogens into injured tissue (e.g., as a result of an assault), posttraumatic meningitis, and infection following intravenous drug abuse or infected decubitus ulcers.[1–13] The postmortem investigation and subsequent medical expertise in such fatalities often concentrate on a specific mode or portal of entry of microorganisms, respectively. Most of these cases are investigated against the background of an allegation of medical malpractice, nursing injury, or neglect, most often raised by close relatives of the deceased. In other, more rare instances, the accusation focuses on (grievous) bodily harm.

This brief overview of current perspectives of the postmortem elucidation of sepsis-related deaths provides a problem-oriented approach from the viewpoint of forensic pathology. The immense imponderabilities related to the postmortem diagnosis of infection are indicated, and aspects of diagnostic utility, interpretation of postmortem bacteriology, and the pathology of sepsis are discussed, focusing especially on the micromorphologic correlates of infection. Current developments in immunohistochemistry adding to the diagnosis are briefly surveyed.

In the present context, infection is defined as the presence of microorganisms, microbial debris, or products of microorganisms in organs and tissue leading to an inflammatory response in the host. It is not within the scope of the present chapter to discuss every possible scenario that may present when dealing with those dying of infection in autopsy practice.

Forensic Pathologic and Medicolegal Problems Arising in the Postmortem Elucidation of Infection-Related Deaths

In the clinicopathologic field with regard to fatalities occurring in-hospital, in at least in a relevant proportion of cases there often is acceptable evidence of an underlying infectious condition in a deceased prior to death, based upon the medical history and results of diagnostic procedures preceding death. Furthermore, there usually is good interdisciplinary communication between the physicians who cared for the deceased and the clinical pathologist performing the autopsy. The latter can make use of this communication to obtain additional information on the clinical course before starting with the postmortem examination. In sepsis-related fatalities of inpatients, the primary task of the clinical autopsy and the following investigations is not initially to establish the primary diagnosis of sepsis at autopsy but rather to obtain feedback on the accuracy of the clinical diagnosis, to search for underlying disease processes that could have been overlooked but nonetheless contributed to the onset of sepsis and fatal outcome, to verify a suspected or to uncover an uncontrolled focus of sepsis, and to demonstrate pyemic abscess formations or superinfection. In contrast, the postmortem diagnosis of sepsis is by far more difficult to establish in forensic pathology. In the majority of forensic autopsy cases, clear-cut information about the circumstances of death often is not available. Data on the medical history and the clinical course of a deceased or an individual's symptoms prior to death often are not reported or are not available at the time of autopsy, especially in outpatient fatalities. Similar difficulties may arise for the forensic pathologist in cases where the fatality occurred abroad, the patient was in no condition to give a history at admission, and/or the duration of hospital stay before death was too short to reveal any relevant diagnostic findings. Therefore, in a great number of fatalities there is hardly any valuable clinical information available for the forensic pathologist at the time of autopsy. Even in later phases of the postmortem investigation of death of individuals hospitalized for a longer period antemortem, problems can arise when the hospital/institutional documentation contains incomplete data on the deceased's clinical course.[1–3]

During the course of the medicolegal investigation, clinical expertise may be necessary to interpret the clinical data against the background of autopsy findings, histopathology, postmortem microbiologic results, and further analytical workups. All findings brought to light by the postmortem investigation may become evidence in later trial proceedings, and the forensic pathologist may testify as an expert witness against the deceased's physicians. Consequently, any personal or nonauthorized communication between the forensic pathologist and the deceased's physicians must be strictly avoided.

Attention must be given to the exceptional constellation of fatalities that often are subject to postmortem investigations in forensic autopsy practice. Special environmental conditions (e.g., low hygienic standards, low socioeconomic settings, indolent persons) accompany a great number of outpatient deaths. Representative groups of such fatalities are addicts (e.g., intravenous drug abusers, alcoholics), old, immobilized, and neglected persons, foreigners from countries with low hygienic standards, and members of social fringe groups (e.g., homeless people).

Alleged misdiagnosis or medical malpractice often is a matter of debate in the forensic autopsy setting. In this context, infection-related deaths may present as the following:

1. Death of an individual who had consulted a physician prior to death, but the correct diagnosis of a particular infection was not established because symptoms were misinterpreted for those of another disease and/or the applied diagnostic procedures were insufficient to achieve the correct diagnosis,
2. Death of an individual who had consulted a physician prior to death and the diagnosis of a distinct infectious disease was established, but treatment was inadequate, or
3. Death of an individual (most often occurring suddenly and unexpectedly outside of the hospital) as the result of a rapidly progressive course of a specific infection

Most recently, Bonds et al.[14] emphasized impressively the substantial discrepancies between clinical and autopsy diagnosis of infection. These authors investigated the discrepancy rates between clinical and autopsy diagnoses of infectious diseases and discovered that of a total of 182 adult autopsy patients, 137 (75%) had an infectious disease at autopsy that was unknown clinically in 59 cases (43%); of 94 fetuses and neonates, 45 (48%) had an infectious disease at autopsy that was clinically unsuspected in 26 cases (58%).

Autopsy is still the final word in quality control. Autopsy detection of an infectious agent responsible for death can have important clinical implications.[14–17] Research performed on specimens obtained postmortem is an important tool to improve our understanding of inflammatory organ changes and underlying pathophysiologic mechanisms[18–25] (*mortui vivos docent* [the dead teach the living]).

Autopsy Bacteriology

Determining the species and strain of a pathogenetic germ can be of evidential value in reaching etiopathogenetic conclusions about a causal relationship among portal of entry, infection, and fatal outcome. Therefore, the pathologist may have to decide on the value of obtaining samples for postmortem microbiologic investigations at autopsy. Although the diagnostic value of postmortem microbiology for the diagnosis of antemortem infection has been discussed controversially for decades, the literature is replete with examples demonstrating the importance of such investigations.[26] Postmortem microbiologic investigations have been successfully applied not only to the autopsy diagnosis of infection but also to a broad variety of epidemiologic subjects, such as evaluation of different bacterial, fungal, and viral species in contrastive autopsy populations[17,19,27,28] and drug-abuse related infections,[29,30] and to a number of divergent forensic questions such as proof of fatal food poisoning with respect to criminal offenses against hygiene regulations,[31,32] etiopathogenetic proof of fatal catheter-related infection,[1,33] and differential diagnosis between cutaneous hemorrhages as a result of streptococcal toxic shock syndrome versus their origin from physical child abuse.[34]

Despite advances in deoxyribonucleic acid (DNA) technology, the use of conventional microbiologic cultures for postmortem diagnosis of bacterial infection is still efficient. Advantages include easy access and high cost-effectiveness compared to DNA techniques. However, DNA technology may become indispensable in later stages of the medicolegal investigation for specific questions, for example, when subspecification of bacterial strains by polymerase chain reaction primers targeting bacterial gene sequences, with the aim of allocating a respective microorganism

toward its origin, is needed. Polymerase chain reaction methods, supplemented by immunohistochemistry, are frequently applied for the detection of viruses in forensic autopsy cases, usually on postmortem lung or myocardial specimens.[35–38]

In addition to their potential value for the medicolegal expertise concerning etiopathogenetic conclusions, postmortem microbiologic investigations can be of clinical relevance with respect to epidemiologic considerations related to hospital-acquired (nosocomial) infections.

In the following, general aspects of autopsy bacteriology are discussed, outlining the various parameters that may influence and limit the diagnostic utility of postmortem cultures.

Sampling Procedures and Selection of Appropriate Specimens for Culture

Obtaining swabs or blood at autopsy for postmortem microbiologic cultures, especially with the sampling procedure described by De Jongh et al.,[39] has proved useful in achieving reliable results in several autopsy studies. In brief, after the thoracic and abdominal cavity is opened and prior to evisceration, the surface of the organ from which the swab will be collected is seared in situ with a red-hot spatula, the prepared section is lanced, and a swab or a sterile needle for aspiration with a syringe is introduced for sampling. This method is widely accepted as the method of choice ("gold standard").[40–42] Other techniques, such as the "closed chest" method in which the specimen is obtained prior to opening of the thoracic cavity through the closed chest wall with a syringe[43] or modified surgical techniques using aseptic procedures,[44] have not had any particular impact on autopsy practice. However, in any technique used, special precaution is necessary to avoid contamination with body fluids on the surface of the organs chosen for specimen sampling. The swab or aspirate should be inoculated into a transport media or culture media immediately after collection to avoid contamination in the autopsy room or failure of survival of microorganisms.

Organ surfaces easily accessible for introduction of a swab or a needle for aspiration are the heart, spleen, and lungs. For specific questions (e.g., infection of the neurocranium), cerebrospinal fluid can be obtained by aspiration through the foramen magnum. In cases of meningitis, collecting smears directly from the brain surface immediately after the head cavity is opened has proved effective.[45]

The most promising media for postmortem bacteriologic cultures are spleen and heart blood.[41,46]

Lung cultures are the most difficult specimens to interpret in autopsy bacteriology. The results are widely accepted to be unreliable because of frequent false-positive cultures. About half of the swabs obtained from the lungs at autopsy grow bacteria without clinical or (histo)pathologic evidence of infection. The most obvious reason for this finding is the drainage of saliva into the lungs after death. As early as 1905, Norris and Pappenheimer[47] demonstrated that bacteria placed in the mouth of human cadavers can be detected in the lungs in approximately 50% of the cases at postmortem examination. Concerning the results of postmortem lung cultures, particularly the decision regarding which bacteria cultured represent true respiratory tract infections, commensalism without pathogenetic effect or pure postmortem growth is occasionally beyond the bounds of possibility based only

upon outcome of autopsy bacteriology. All results obtained must be supplemented by microscopic investigations. Especially in inpatient fatalities, the possible colonization of the respiratory tract by the patient's oropharyngeal flora and the changing intraindividual pattern of microorganisms to nosocomial flora without true infection in hospitalized patients[48–50] must be considered carefully.

When cultures are obtained from the liver and kidneys, keep in mind that positive cultures from these sampling sites may be the result of minor local biliary or urinary tract infections. In such cases, a careful correlation with autopsy and histologic findings along with postmortem culture results from other anatomic sampling sites, such as spleen or heart blood, is of considerable diagnostic benefit.

Urine is of no practical value because the possibility of postmortem spread of microorganisms into the urinary bladder from the bowel can never be fully ruled out, thus limiting its diagnostic utility.

The collection of specimens from at least two different sampling sites is a prerequisite and has to be the standard procedure in autopsy cases where an underlying infection is presumed. Multiple postmortem cultures from different sampling sites raise the probability of identifying the etiologic agent of antemortem infection, whereas cultures from only one sampling site are of no diagnostic utility.[42,46,51] If bacteremia occurred prior to death, postmortem cultures of all sampled specimens should yield the same results.[36,47,52]

Parameters Potentially Influencing the Diagnostic Utility of Postmortem Cultures

Time Interval Between Death and Sampling of Specimens

In potentially infection-related deaths, the forensic investigation should ensure postmortem blood sampling as early as possible to reduce the possibility of false-positive blood cultures as a result of postmortem bacterial invasion. Some authors recommend obtaining samples for microbiologic cultures as early as within the first 15 hours postmortem[53] (which is virtually impossible in most cases undergoing a forensic autopsy), whereas others point out that the time interval between death and sampling of postmortem cultures has little, if any, effect on the results and that false-positive postmortem culture results are a consequence of inadequate sampling techniques.[44] However, the extent of postmortem bacterial invasion in correlation to the postmortem interval cannot be generalized. In any given case the outcome of postmortem cultures must be interpreted on an individual basis independent of the time interval between death and collection of specimens.

Agonal Spread of Bacteria

According to Roberts,[46] certain bowel conditions, such as ulcers, infarction, or congestion, can be associated with bacteremia from the gut shortly before death or in the agonal period leading to multiplication of bowel flora organisms in internal organs, resulting in rapid putrefaction contrastive to the length of the postmortem interval. However, whether bacterial invasion prior to death or during the agonal period actually occurs is still a matter of debate. In my belief, in nonseptic individuals a translocation of bowel bacteria into the systemic circulation shortly before

death as a result of a generalized breakdown of the homeostatic barriers of the gut, even in the presence of the pathologic conditions mentioned, is highly doubtful.

Antibiotic Therapy Prior to Death and Outcome of Postmortem Microbiology

In larger autopsy series, no significant relationship between antemortem antibiotic therapy and the outcome of postmortem bacteriology could be established.[40,54] However, for forensic purposes, when interpreting the significance of bacteria isolated postmortem, the outcome of microbiology (especially the presence of multiresistant pathogens in hospital settings) should be related carefully on an individual basis to a preceding antibiotic therapy.

An interesting approach to this subject was performed by Roberts[51] who investigated the association between the duration of appropriate antimicrobial therapy and the outcome of postmortem spleen cultures compared to antemortem blood culture results. The postmortem spleen cultures yielded 96%, 55%, 41%, and 35% of the blood culture organisms in patients who received appropriate antibiotics for 0, fewer than 2, 2 to 4, and more than 4 days, respectively, suggesting that postmortem spleen cultures are helpful in assessing the value of antimicrobial therapy prior to death in patients with proven bacteremia.[51]

Contamination of Specimens Following Sampling

In this context, *contamination* is defined as the isolated growth or additional growth of microorganisms in postmortem cultures, not representing the authentic pathogenetic germ or veiling the true infectious agent. The result is an unproductive or adulterated outcome of postmortem microbiology that cannot be used for a comprehensive forensic argumentation regarding causality. Such contamination does not in any case categorically originate from improper sampling techniques, use of unsuitable transport media, or pure postmortem (over)growth of bowel flora. Contamination also may be the result of a transient and/or resident colonization of body surfaces, internal organs, or body fluids of a deceased prior to death, for example, in the hospital setting where colonization of body surface or mucous membranes with potentially pathogenetic germs is a frequent finding but may have no etiologic effect on fatal outcome in a given case.

The number of contaminated postmortem cultures increases when bowel manipulation, for example, during evisceration, occurs prior to sampling.[44] The possibility of contamination also depends on the anatomy of the sampling site. A higher contamination rate can be expected from specimens deriving from organs located deep in the body cavities, such as the kidneys or the inner female reproductive organs, which are easily contaminated by blood draining from mesenterial vessels opened during evisceration.

Careful sampling of specimens during autopsy is essential, but the samples also must be handled carefully afterward to avoid contamination (e.g., by airborne microflora) in the autopsy room.

The selection of appropriate media for transport is a prerequisite. Immediate processing of the samples or at least adequate storage in the microbiologic laboratory is obligatory, as is good communication between the forensic pathologist and the microbiologist in charge. Along with the samples for culture (a caption of the

sampling site and time of sampling on each specimen is self-evident), the microbiologist should receive a short report on the case history and autopsy findings and a brief synopsis of forensic relevant questions regarding the case.

Interpretation of Autopsy Bacteriology and Practical Aspects

Taking into consideration the various factors mentioned that can all influence and limit the diagnostic utility of postmortem cultures, a positive postmortem culture of a single pathogenetic germ from spleen and heart blood but not from the lungs has a similar significance as a positive blood culture obtained from a living patient.[46] Polymicrobial growth must be considered contamination in the majority of cases.[46]

For a concluding medicolegal expertise, a thorough histologic examination of inner organs and tissues potentially representing the site of primary infection toward the presence of inflammatory changes is a must. The histologic section must represent the sampling site for cultures. In addition, results of autopsy bacteriology must be correlated with all the information of the given case, including the previous history and symptoms prior to death (as much as available) and autopsy data on potential immunocompromise brought about by underlying debilitating diseases of noninfectious origin.

Different opinions concerning source and factors involved in positive cultures most often can be simplified in the practical setting. The existence of infection prior to death can be proved when postmortem bacteriologic cultures yield a single infectious pathogen (polymicrobial growth must be considered contamination in the majority of cases[46]), and a cellular response can be detected on microscopy in organ or tissue sections corresponding to the sampling site. This proof of antemortem infection ("proof of vitality") is reflected by an inflammatory host response, such as inflammatory cells demarcating clusters of bacteria (Figure 3.1), neutrophils or

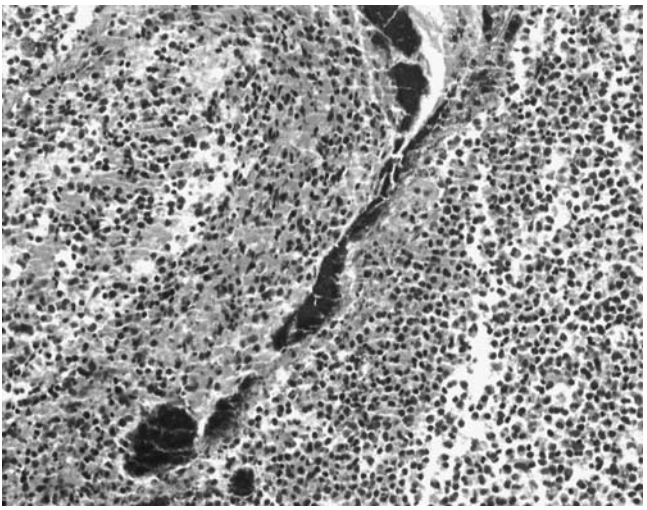

Fig. 3.1. Inflammatory cells demarcating clusters of bacteria in the lungs.

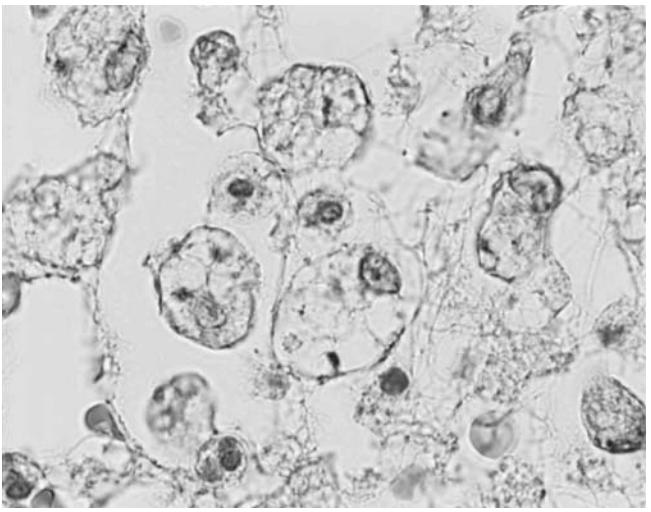

Fig. 3.2. Macrophages within an intragluteal syringe abscess showing intracytoplasmatic inclusions of Gram-positive cocci.

macrophages showing intracytoplasmatic inclusions of bacteria (Figure 3.2), lymphoplasmacytic infiltrates accompanying fungal hyphae (Figure 3.3), or fibrin aggregations adjacent to clusters of bacteria. On occasion, microscopic examination clearly points toward the route of infection (Figure 3.4), thereby enabling the forensic pathologist to distinguish between infection following airborne transmission of pathogenic germs and other routes of infection (e.g., indwelling catheter). In my personal experience, the clinical pathologist, often unfamiliar with the phenomenon of putrefactive organ changes and the pathologic features they display on the micromorphologic level, often tends to diagnose pure postmortem growth of bacteria in the lungs (Figure 3.5) too uncritically as vital respiratory tract infections.

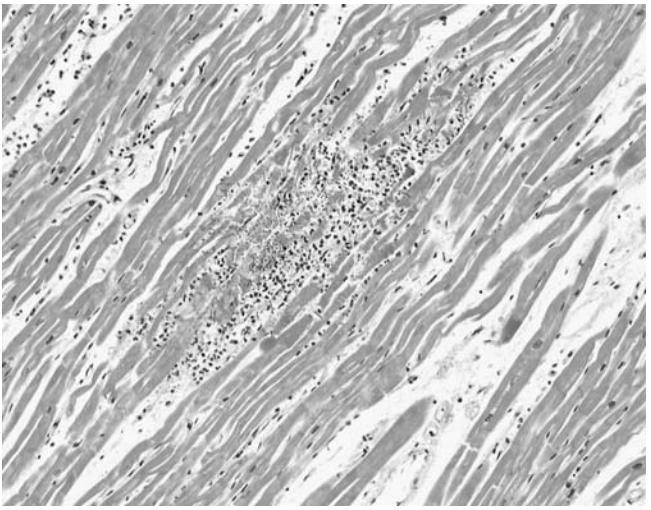

Fig. 3.3. Leukocytes sharply demarcating fungal hyphae in the myocardium.

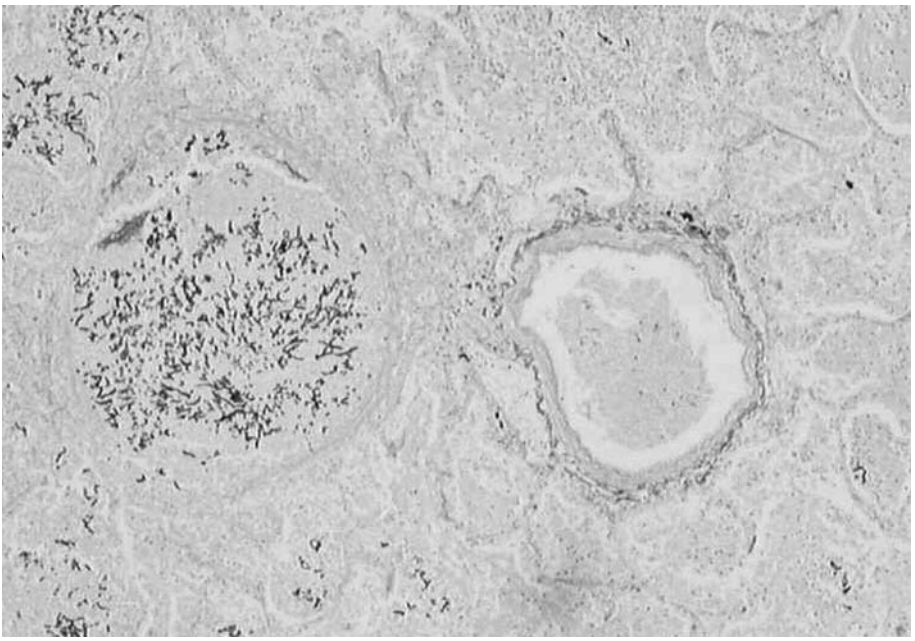

Fig. 3.4. Invasion of a small bronchi (left) by *Aspergillus* hyphae. Note the total absence of hyphae in the adjacent vessel (right), supporting the concept of a respiratory route of infection in this case.

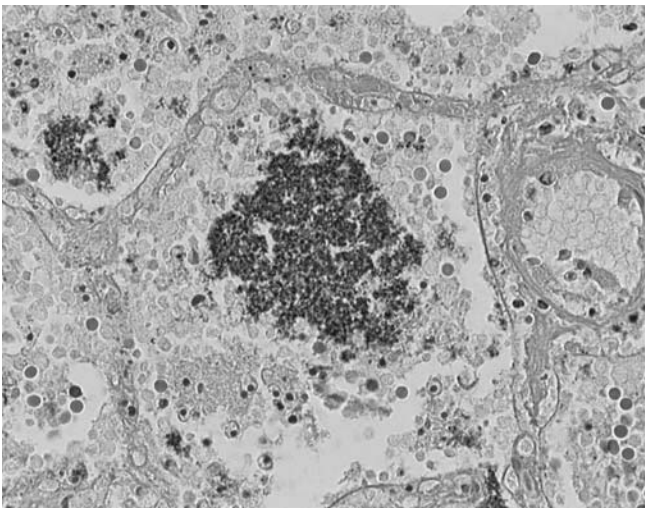

Fig. 3.5. Postmortem bacterial invasion into the lungs. Clusters of bacteria lacking accompanying inflammatory cell reaction are seen within the alveoli.

The following groups of bacteria isolated in postmortem cultures and their correlation with the histologic results must be differentiated:

1. Primary pathogenetic germs
2. Facultative pathogens
3. Postmortem contaminants

In cases of facultative pathogens, the course of the infectious disease process (with special reference to underlying predisposing factors for a fulminant course of infection, such as malignant diseases, immunocompromise, or emaciation) must be correlated carefully with its contribution to fatal outcome.

A comprehensive toxicologic workup is equally important in questioned cases because a relevant proportion of cases of fatal intoxications can have morphologic features similar to infection-induced organ and tissue alterations (e.g., considerable putrefactive skin alterations contrastive to the length of the postmortem interval, edema of the brain or lungs, circumscribed myocardial necrosis, cholestasis, foci of liver cell necrosis, acute tubular necrosis of the kidneys).

An example of medicolegal relevance that illustrates the practical value of postmortem microbiologic investigations is the forensic pathologic entity of "posttraumatic meningitis." In accordance with autopsy and histologic findings and the deceased's previous history, results of postmortem culture in most cases can determine the etiology of a leptomeningeal infection. For example, the culture results may indicate the infectious agent gained access to the intracranial compartment as a result of a previous iatrogenic procedure, such as a neurosurgical operation, or even as a result of a minor traumatic event that occurred prior to death.[13]

Postmortem bacteriology can provide valuable information regarding hospital-acquired infections as an additional indicator of nosocomial microorganisms within a specific hospital environment.[26,28,55,56]

Sepsis

Human sepsis is a spectrum of pathophysiologic changes in the host system resulting from a generalized activation and systemic expression of the host's inflammatory pathways in response to infection. Normally, proinflammatory mediators such as tumor necrosis factor-α, interleukin (IL)-1, IL-6, and IL-8, are released in response to infection, injury, and/or ischemia, to eliminate pathogens and to promote wound healing. This response then is down-regulated by the release of antiinflammatory mediators (e.g., IL-1 receptor antagonist [IL-1ra], IL-10), resulting in restoration of homeostasis. In sepsis, however, local defense mechanisms are insufficient to eliminate the infectious agent, and overstimulation of the host's immune effector cells occurs. This overwhelming systemic proinflammatory reaction frequently is followed by an overactive compensatory antiinflammatory mediator release. The severity of sepsis is proportional to the intensity of the host's immune and metabolic response to infection. When the balance between proinflammatory and antiinflammatory response is lost, immunologic imbalance and massive systemic inflammation result.[57–63]

Sepsis occurs in approximately 1% of all hospital inpatients and accounts for 20% to 30% of intensive care unit admissions. Despite modern techniques of resuscitation and organ support, septic shock continues to have a mortality rate of approximately 50%.

Definitions and Terms

Systemic Inflammatory Response Syndrome

In popular usage, the term *sepsis* implies a clinical response arising from infection. However, it is apparent that a similar, or even identical, deleterious generalized systemic inflammatory reaction can arise in the absence of infection in response to a variety of life-threatening clinical conditions, such as major trauma, burns, extensive surgical procedures, protracted hemorrhagic or cardiac shock, or pancreatitis.[62–68] A Consensus Conference of the American College of Chest Physicians/Society of Critical Care Medicine stressed this concept for practical use and recommended the term *systemic inflammatory response syndrome* (SIRS) to describe this generalized inflammatory process independent of its cause.[69] SIRS is clinically defined by two or more of the following clinical criteria: body temperature greater than 38°C or less than 36°C, heart rate greater than 90 bpm, tachypnea with more than 20 breaths/minute or P_{CO_2} less than 4.3 kPa, white blood cell count greater than 12.000 cells/mm^3 or less than 4.000 cells/mm^3, or greater than 10% immature neutrophils.[69]

Sepsis, Severe Sepsis, and Septic Shock

Systemic inflammatory response syndrome can result from either a noninfectious or an infectious condition. The presence of at least two of the SIRS components when triggered by infection is termed sepsis.[69] Infection leading to sepsis can be bacterial, fungal, parasitic, protozoan, or viral.

As mentioned earlier, sepsis and other critical illnesses produce a biphasic inflammatory (immunologic, hormonal, and metabolic) response. The acute phase is marked by an abrupt rise in the secretion of so-called *stress hormones,* with an associated increase in mitochondrial and metabolic activity. The combination of severe inflammation and secondary changes in endocrine profile diminish energy production, metabolic rate, and normal cellular processes, with potential multiple organ dysfunction.[70]

Sepsis, severe sepsis, and septic shock represent increasingly severe stages of the same disease.[71–73] *Severe sepsis* is defined as deterioration in the presence of hypotension, organ dysfunction, and hypoperfusion. The term *septic shock* is reserved for severe sepsis with hypotension despite fluid resuscitation and resultant perfusion abnormalities.[69] The progression from sepsis to severe sepsis and septic shock is a continuum reflecting the host's inflammatory response to infection. During this process, an increasing proportion of patients develop the acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC), organ dysfunction syndrome, and multiple organ failure. Figure 3.6 shows the possible reaction patterns of the host response after an initial event leading to injury and/or infection.

The clinical criteria mentioned (e.g., fever or hypothermia, tachycardia, tachypnea, and leukocytosis or leukopenia) are common clinical signs of systemic inflammation; however, these manifestations are neither specific nor sensitive for sepsis. Because of the wide range and variability of potentially sepsis-associated symptoms, physicians must be aware of the multiple differential diagnoses and the many ways in which an underlying septic condition may present.[71,73–75]

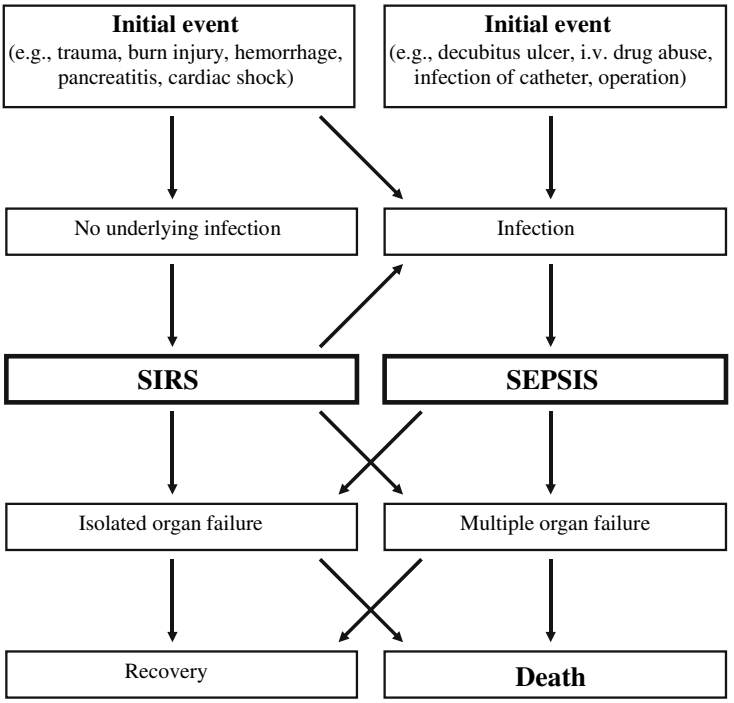

Fig. 3.6. Possible reaction patterns of the host response after an initial event leading to injury and/or infection. SIRS, systemic inflammatory response syndrome.

Postmortem Diagnosis of Death Due to Sepsis: A Substantial Challenge in Forensic Autopsy Practice

In clinical practice, sepsis currently is diagnosed by cardinal signs such as tachypnea, fever or hypothermia, tachycardia, and leukocytosis or leukopenia, clinical manifestations that are neither specific nor sensitive for sepsis.[69,71] Although diagnosing a septic condition may present a problem even in the living patient, the primary diagnosis of sepsis after death is far more difficult because the major limitation to a precise postmortem diagnosis of sepsis is the frequent nonspecificity of macroscopic and routine histologic findings encountered in such fatalities.

Postmortem microbiologic investigations occasionally are of little value in sepsis-associated fatalities.[76] The reason is the possibility of gut translocation of bacteria, which is defined as the passage of gastrointestinal microflora across the lamina propria to local mesenteric lymph nodes and from there into the systemic circulation. Three primary mechanisms that promote bacterial translocation in sepsis have been identified: intestinal bacterial overgrowth, increased permeability of the intestinal mucosal barrier, and deficiencies in host immune defenses. Migration of organisms across the bowel wall may occur by pinocytosis in epithelial cells. This mechanism has been proposed as the principal factor for translocation in the presence of an intact mucosal barrier. However, many studies have identified alterations

in intestinal permeability in critically ill patients. Under normal circumstances, bacteria reaching the mesenteric lymph nodes are phagocytosed by macrophages, but in immunocompromised septic individuals the normal defense mechanisms fail, permitting bacteria access to distant extraintestinal sites.[77,78] Therefore, heart and spleen blood obtained at autopsy from septic individuals often show polymicrobial culture growth of bowel flora.

Pathologic Features of Sepsis

General Approach

The majority of pathoanatomic textbooks and manuals devote little, if any, attention to pathomorphologic organ alterations in sepsis. The likely explanation is the fact that the clinical pathologist hardly ever is in the position to set up a primary diagnosis of sepsis postmortem. A thorough microscopic examination and toxicologic analysis are necessary to rule out concomitant diseases and/or intoxications, respectively, that may have contributed to fatal outcome in a given case.

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

Neither apoptosis nor necrosis are frequent morphologic features in sepsis. Many of the inflammatory organ changes found in sepsis-related fatalities also can be demonstrated in a large proportion of clinical conditions going along with SIRS or in individuals following prolonged ischemia, thus reflecting the nonspecific reaction pattern of organs and tissues to various endogenous and exogenous noxae.

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

Many sepsis-induced tissue alterations arise without symptoms and therefore may give rise to late clinical symptoms. Thus the pathologic age may be greater than the assumed clinical age estimated by the time elapsed from the onset of first symptoms.

As a consequence of DIC in sepsis, petechial or more extended hemorrhages can be seen on the skin, on mucocutaneous surfaces and serous membranes, or in parenchymal organs by gross examination. Despite the fact that cutaneous petechiae also can be found in a variety of forensic autopsy cases of death from various natural causes, such as acute right heart failure (e.g., asthma fatalities), and that these petechial bleedings are also a frequent finding in those who were subjected to cardiopulmonary resuscitation efforts prior to death, the presence of cutaneous

petechiae, especially on the facial skin or conjunctivae, should always focus the death investigator's attention toward the possibility of pressure applied to the deceased's neck prior to death. Such petechial skin bleedings are also a typical feature when in cases of mechanical compression of the chest antemortem, for example, as a result of accidents in the workplace.

In DIC, microthrombi formation may present histologically to various extents in capillaries, arteries, and veins of all sizes in each and every tissue and organ.[79–82] The frequency of fibrin thrombi is dependent upon the time between the onset of DIC and death. The postmortem finding of intravascular microthrombi is more common in individuals who died a few hours after the onset of septic shock than in those who survived the onset of septic shock for days.[83] Despite the obvious clinical manifestation of DIC in the living patient, fibrin thrombi may not be histologically detectable because of postmortem fibrinolysis.[84]

The internal organs may be both the focus of sepsis and the target of sepsis-induced tissue alterations. The investigator must distinguish between a primary infectious organ alteration (septic focus) and secondary lesions (septicopyemic abscesses) that are a direct result of bacterial spread from the initial focus on the one hand and tertiary organ alterations (unspecific shock lesions, inflammatory changes in internal organs far away from the initial focus) on the other hand. The latter are initiated by a variety of causes and triggered by a wide range of endogenous mediators in the sequela of the systemic inflammatory cascade.

The pathologist must bear in mind that sepsis, severe sepsis, and septic shock are complex pathologic conditions in which the overall morphologic picture depends on a variety of exogenous and endogenous factors and on the individual response to sepsis, with numerous most often unpredictable interrelations among all organ systems and tissues. Moreover, the clinical stages of sepsis cannot be differentiated based only upon their pathomorphologic features in most instances. However, some morphologic correlates of septic shock—the acute event of shock induced by bacteria or their endotoxins or exotoxins—can be discriminated postmortem by their micromorphologic appearance from sepsis and severe sepsis to a certain degree, and different stages of the ARDS can be determined based on their histopathologic features. Because all the findings presented here are facultative and can also arise in a broad variety of other pathologic conditions of forensic relevance, special attention has to be given to potential forensic differential diagnoses.

Morphology of Sepsis-Related Cell and Tissue Injury

Lung

The morphologic alterations of the lung in sepsis are a consequence of pathophysiologic changes defined by the term *acute respiratory distress syndrome* (ARDS). The development of ARDS is relatively rare in pure hypovolemic shock events without underlying infection or trauma.[85] At gross inspection, the lungs in ARDS usually display a gloomy bluish-red color. The organ weight is increased because of pulmonary edema, congestion, and pulmonary trapping of inflammatory cells. The cut surfaces of the lungs are commonly wet because of accumulation of protein-rich edema fluid in the alveolar spaces and interstitial edema. The amount of muddy-gray fluid draining from the cut sections is highly dependent on the quantity of intravenous infusions administered prior to death. Occasionally, as a consequence

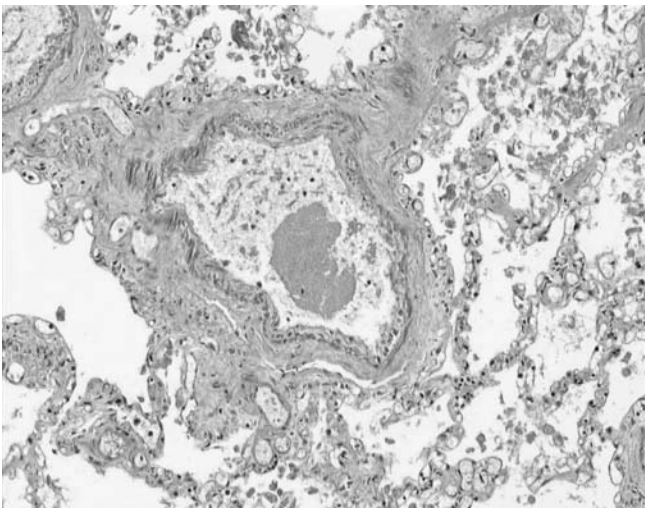

Fig. 3.7. Fibrin deposits in a pulmonary vessel in a case of septic acute respiratory distress syndrome.

of DIC, subpleural petechiae and parenchymatous hemorrhages are found in the lungs, and the forensic pathologist must be aware of possible differential diagnoses because these petechial hemorrhages are a hallmark of mechanical asphyxia.

At microscopic examination, especially in septic shock, there is often marked platelet aggregation with fibrin deposits in the pulmonary vessels (Figure 3.7). The occurrence of microthromboses (Figure 3.8) and megakaryocytes in the pulmonary microvasculature has been reported to appear more frequently in septic shock than in shock of other origin.[86]

Vascular congestion and more circumscribed hemorrhagic foci located in the alveolar spaces (both phenomena can appear in isolation or simultaneously during all stages of ARDS) are seen histologically in most cases. Interstitial (perivascular and peribronchial) edema and intraalveolar fibrin deposits, both indicative of earlier stages of ARDS, are followed by a protein-rich intraalveolar edema. Plasma

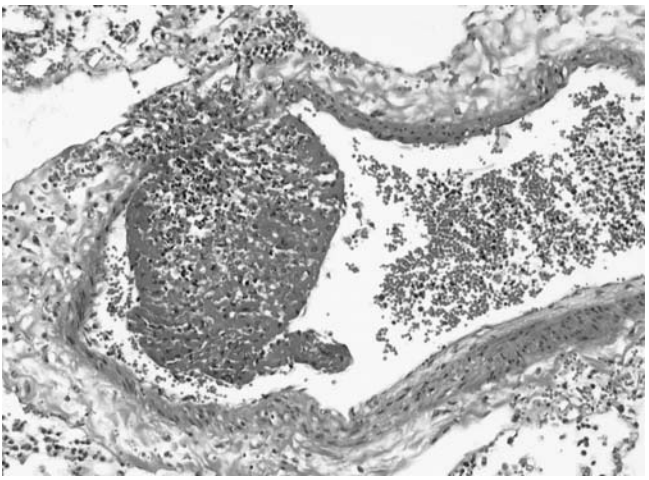

Fig. 3.8. Microthrombosis adjacent to a pulmonary vessel wall in a case of *Staphylococcus aureus* sepsis.

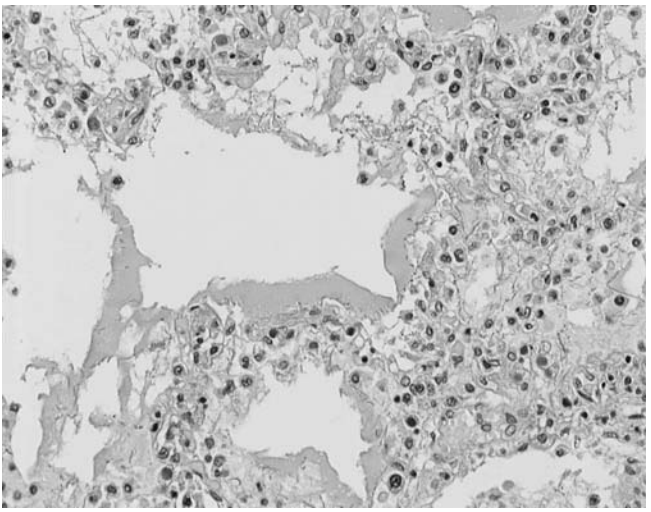

Fig. 3.9. Hyaline membranes covering the alveolar epithelial layer in a case of septic acute respiratory distress syndrome originating from cholecystitis.

proteins, cellular debris, and fibrin deposits covering the alveolar epithelium as hyaline membranes (Figure 3.9), interstitial deposition of inflammatory cells, and interstitial fibrosis are findings indicative of ARDS in advanced stages.[86–90] Surfactant secretion is impaired, and the coexistence of congestion and alveolar collapse ("congestive atelectasis") is another frequent finding seen not only in ARDS but also in septic shock with rapidly fatal course (Figure 3.10).

The histologic finding of pulmonary trapping of polymorphonuclear granulocytes (so-called "leukocyte sticking") reflected by vascular engorgement and extensive leukostasis, most often in the total absence of any interstitial or intraalveolar inflammatory reaction (Figures 3.11 and 3.12), is a striking phenomenon seen in cases of rapidly fatal septic shock. However, this finding may also be observed in hemorrhagic shock.

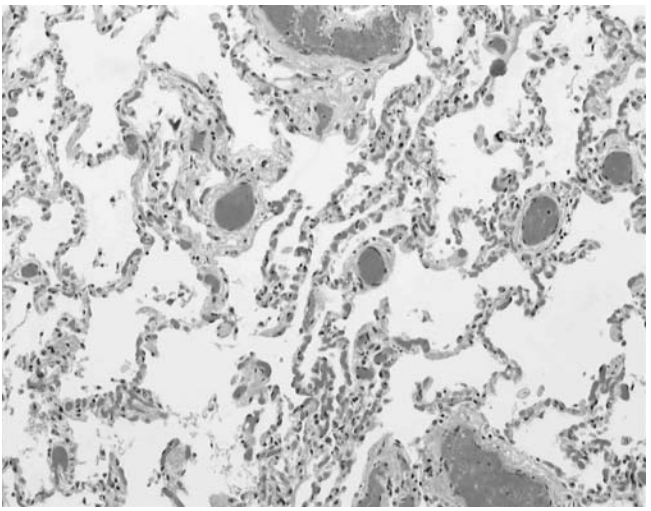

Fig. 3.10. Congestive atelectasis in a 13-month-old boy who died of *Salmonella enteritidis* sepsis.

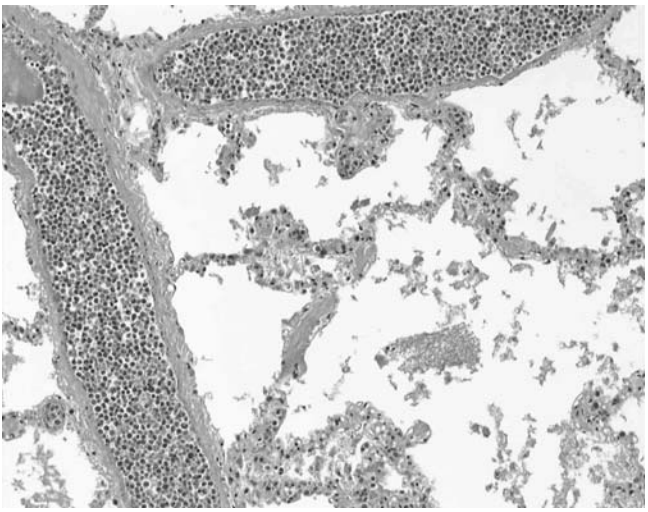

Fig. 3.11. Leukocyte sticking of the lungs in septic shock. Engorgement of pulmonary vessels and extensive intravascular leukostasis in the absence of interstitial or intraalveolar signs of inflammation are seen.

(Broncho)pneumonia frequently complicates the clinical course of sepsis, either due to septicopyemic abscess formation originating from a hematogenic spread of the underlying pathogenetic germ from its focus or in the sequela of artificial respiration as a secondary infection under conditions of intensive care. In some cases, spread of septic emboli in the vascular system can lead to vessel occlusion (Figure 3.13) with the possibility of subsequent pulmonary infarction.

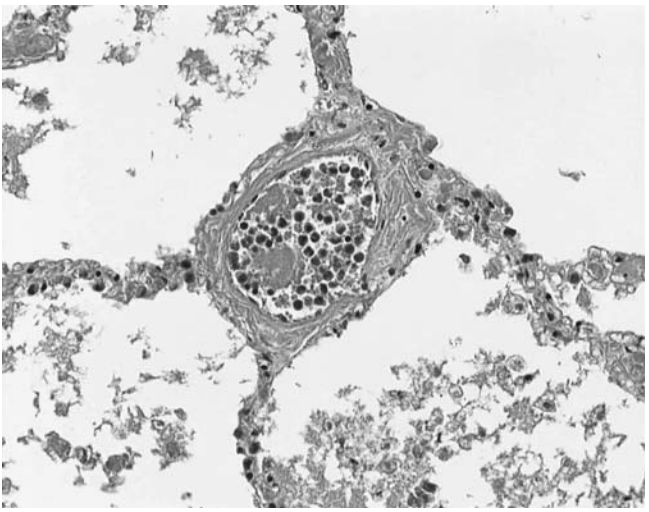

Fig. 3.12. Leukocyte sticking of the lungs in septic shock. Trapping of leukocytes (mainly neutrophil granulocytes) within a small pulmonary vessel are seen. Note additional intravascular fibrin aggregations within the vessel lumen.

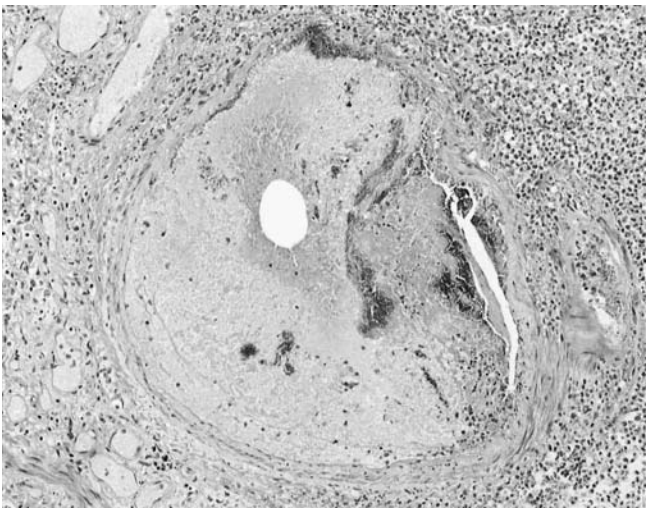

Fig. 3.13. Septic embolus leading to near-total pulmonary vessel occlusion in an intensive care unit patient who died of septic multiple organ failure.

Heart

Sepsis leads to depressed myocardial function. This situation is attributable to a number of mechanisms, including hemodynamic alterations, development of myocardial ischemia, changes in coronary vascular tone and myocardial contraction rate, and release of myocardial depressant factor.

Macroscopically, subepicardial hemorrhages, unevenly distributed and ranging from tiny little spots to more confluent hemorrhagic zones, are a frequent finding in sepsis due to DIC or other clotting disturbances. These hemorrhages may be intensified in cases where external cardiac resuscitation measures preceded death.

Most patients who survive the onset of severe sepsis for a few days present shock lesions in the myocardium. These uncharacteristic myocardial alterations, such as circumscribed (coagulation) necrosis, mural thrombi, and circumscribed hemorrhages (Figure 3.14), may vary in size, from lesions easily detectable at gross examination to alterations visible only by microscopy.

Subendocardial hemorrhages, also known as "Sheehan hemorrhages" (named after Harold L. Sheehan who studied subendocardial hemorrhages in cases of abortion and acute hemorrhage associated with pregnancy in the 1930s) are a striking feature seen on many occasions in forensic autopsy practice. Subendocardial hemorrhages are frequently seen following hemorrhagic shock, craniocerebral trauma, or stroke, preceding resuscitation efforts, and with intoxications (e.g., heroin, cocaine, or heavy metal poisoning such as arsenic). In these situations, hemorrhagic lesions beneath the endocardium are limited almost exclusively to the ventricle of the left heart. In contrast, subendocardial hemorrhages associated with severe infection not only are commonly seen in both chambers of the heart (in a great number of cases I have investigated), but the quantity of subendocardial hemorrhages in the right ventricle far exceeded that observed in the left ventricle (Figure 3.15).

Septic shock especially is often associated with severe left ventricular dysfunction. Therefore, in cases of septic shock, the left ventricle often is dilatated at autopsy, with the apex of the heart appearing rounded and the ventricular wall having a flaccid

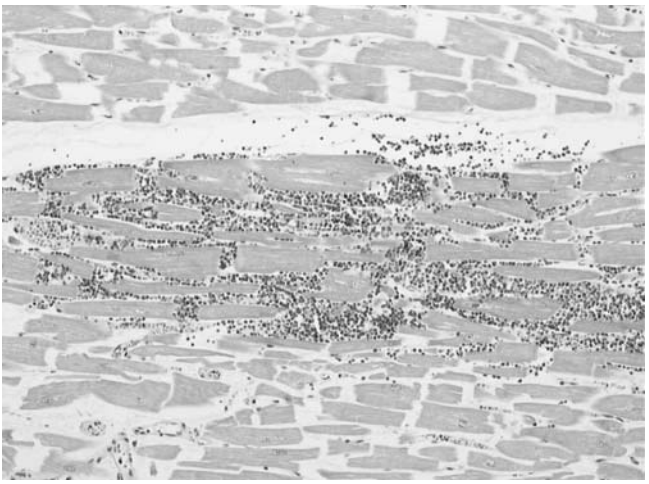

Fig. 3.14. Circumscribed hemorrhage in the myocardium in a case of septic shock.

appearance.[89] However, when postmortem rigidity (which also affects the myocardium) is strongly developed, the left ventricular dilatation may be fully masked.

Microcirculatory fibrin deposits are another not unusual finding in the septic myocardium. Hypercontraction bands and elongated and undulated cardiomyocytes, often separated by marked interstitial edema (extent depending to a certain

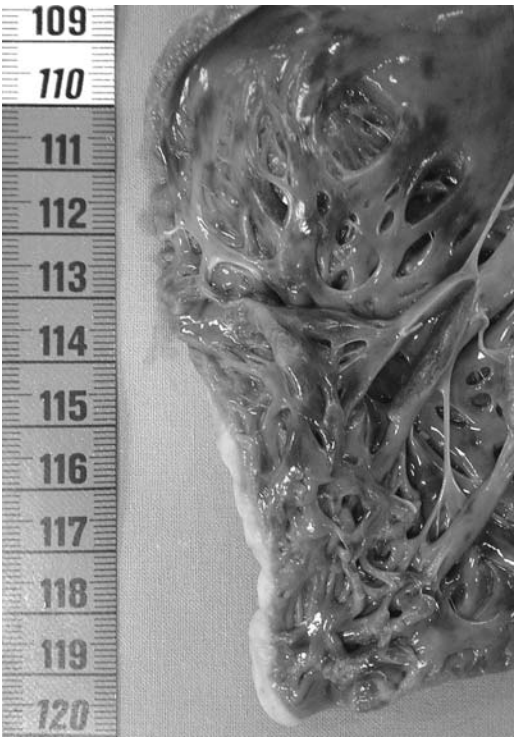

Fig. 3.15. Subendocardial hemorrhages of the right ventricle in sepsis. This patient died of multiple organ failure due to sepsis originating from an infected medullary pin of the tibia.

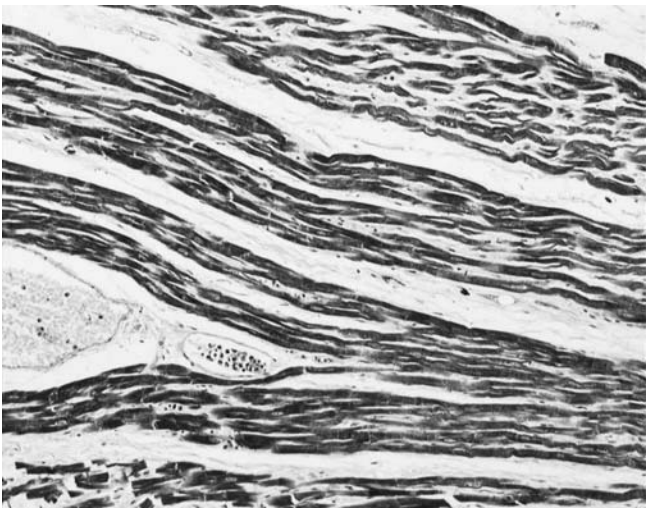

Fig. 3.16. Elongated cardiomyocytes separated by marked interstitial edema in a case of septic multiple organ failure.

degree on the amount of intravenous infusions administered prior to death; Figure 3.16) and occasionally showing a wavelike arrangement, are another characteristic but unspecific finding. The fixed waviness of the myocardium was theorized earlier to configure, on the micromorphologic level, the loss of ATP-dependent "plasticity" of myofilaments due to defective extrusion calcium pump mechanisms in a state of contracture. Hypercontraction bands and elongation of cardiomyocytes are also frequently observed in both sudden and prolonged deaths in the absence of infection.

An interstitial myocarditis can be found in nearly one third of sepsis-related fatalities by histologic means.[90] If present in the myocardium, septicopyemic abscesses (Figure 3.17) are most often located in subendocardial regions of the right ventricle.[91]

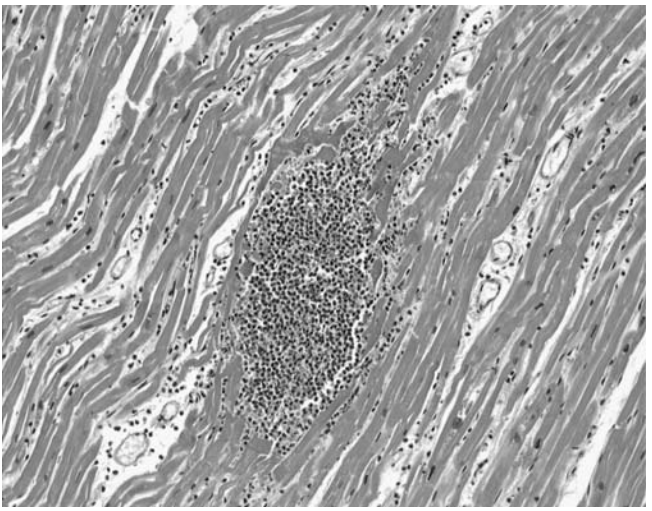

Fig. 3.17. Septicopyemic abscess in the myocardium.

An infective endocarditis may be both the source of sepsis and the effect of septicopyemic abscess formation during the course of an underlying sepsis. Endocarditis of the tricuspid valve in the sequela of intravenous drug abuse is not infrequently seen in the forensic autopsy setting.

Liver

The highly complex pathophysiology of the liver in shock is determined by a variety of overlapping inflammatory reactions. At gross inspection, the liver in shock is enlarged, showing a tense Glisson capsule and rounded edges. The weight of the liver in sepsis often is increased because of accumulation of leukocytes and interstitial edema. In septic shock complicated by DIC, spotty hemorrhages are a frequent feature on cut sections.

Despite the high incidence of cholecystitis, appendicitis, and diverticulitis (which are frequent sources of bacterial infection in the liver), pyemic abscesses of the liver (Figure 3.18) now are a relatively rare finding in developed countries. Pyemic hepatic abscess frequently is polymicrobial. Enteric Gram-negative bacilli, usually *Escherichia coli*, are cultured from the majority of pyogenic hepatic abscesses. The specific types of microorganisms that cause hepatic abscess probably vary with the underlying disease. In particular, *Staphylococcus aureus* abscesses in the liver often are associated with microabscesses in other organs as part of generalized hematogenous dissemination.

Morphologic changes of the liver parenchyma may reflect the pathophysiologic contributions with respect to the cause of shock, but the pathomorphologic changes hardly ever are sufficient to distinguish among the various possible causes of the liver in shock.[92] However, as a general rule, leukostasis of neutrophils in the liver

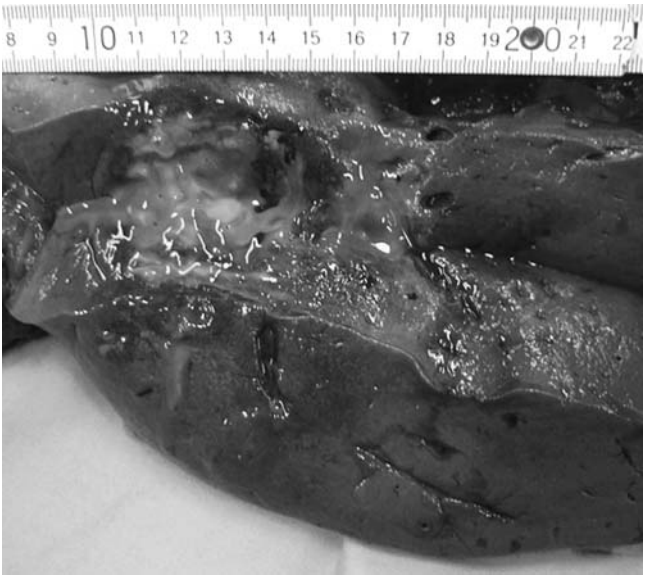

Fig. 3.18. Pyemic abscess of the liver that developed during the course of a missed diagnosis of appendicitis.

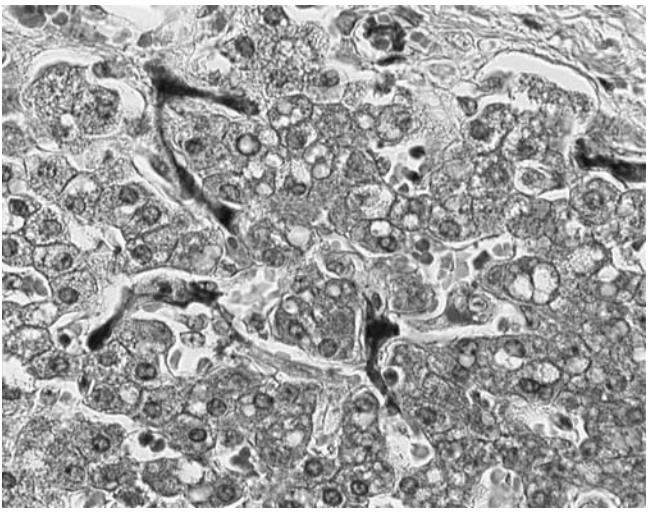

Fig. 3.19. Fibrin aggregations in the liver sinusoids. In this case sepsis was due to disseminated intravascular coagulation due to peritonitis as a consequence of bowel perforation during endoscopy.

sinusoids, formation of intrasinusoidal fibrin aggregations (Figure 3.19), and intraparenchymal hemorrhages are, more frequent and more pronounced in septic than hypovolemic/traumatic shock. Liver cell necrosis, a common sequela of prolonged hypovolemic shock, is rarely seen in sepsis.

Besides the spleen, the liver plays a central role in the resolution of bacteria that enter the bloodstream. Kupffer cells constitute the first macrophage population to come in contact with bacteria, bacterial endotoxin, and microbial debris from the gut transported to the liver via the portal vein. Kupffer cells constitute the largest compartment of tissue macrophages, representing 80% to 90% of total, fixed macrophages. They reside within the lumen of liver sinusoids and represent approximately 35% of the nonparenchymal liver cells. Enlargement of Disse spaces with swelling of Kupffer cells is a common finding in the septic liver; however, this finding may be masked by autolytic changes. For a long time, phagocytosis of bacteria and their products by Kupffer cells (Figure 3.20) was considered the principal

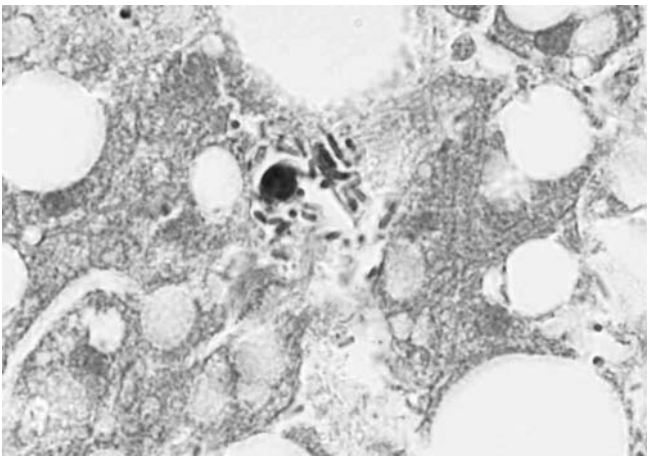

Fig. 3.20. Kupffer cell with phagocytosed rod-shaped bacteria in a case of clostridial gas gangrene. Note gaseous bullae formation of the highly autolytic surrounding liver tissue.

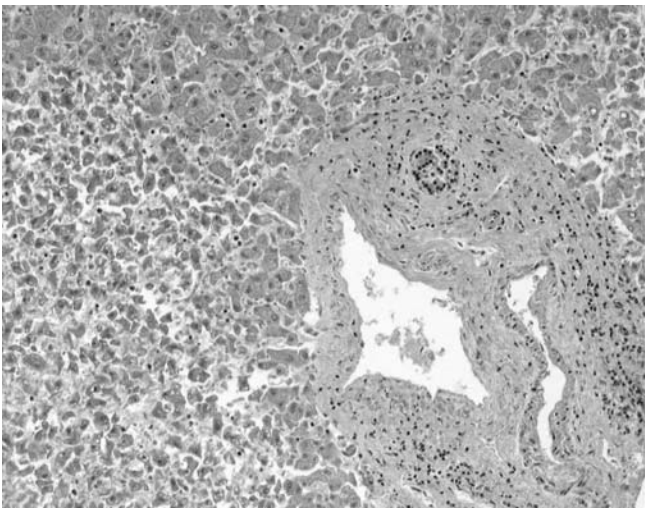

Fig. 3.21. Moderate leukocytic infiltration of the acinocentral areas of the liver in sepsis. Note absence of inflammatory changes in the liver parenchyma.

mechanism for elimination of organisms entering the liver circulation. However, evidence now suggests that the actual mechanism for eliminating bacteria taken up by the liver is dependent on a complex interaction of Kupffer cells and neutrophils, the latter accumulating in the liver sinusoids in endotoxemia and sepsis.[93] Kupffer and endothelial cells contribute to the proinflammatory response of the liver through the release of different mediators, thereby inducing immigration of neutrophils into the liver. Experiments indicate that immigrating neutrophils account for a large proportion of the bactericidal activity expressed in the liver during the course of infection.[94] Although neutrophils constitute only 1% to 2% of nonparenchymal cells in nonseptic individuals, a dramatic 10- to 20-fold increase in neutrophils occurs within hours after onset of sepsis. Therefore, especially in acute septic shock, extensive accumulation of neutrophils (leukostasis) in the liver sinusoids is regarded a characteristic histologic finding[92] and a distinguishing feature of endotoxemia by some authors.[93]

Leukocytic infiltration of the acinocentral areas (Figure 3.21) has been described as an infrequent finding in the liver in sepsis.[95,96] Cholestasis, occurring without demonstrable extrahepatic obstruction, is a common finding in the septic liver.

Spleen

In sepsis, the spleen usually is enlarged and swollen. The capsule has a tense appearance and is easily accidentally torn open during evisceration. The cut surface shows a soft and hyperemic parenchyma with a reddish-gray, sometimes muddy-brown appearance. At gross examination, the pathologist's attention should also focus on the splenic vein, because septic thrombophlebitis of this vessel can cause pyelophlebitic liver abscesses either by per continuitatem spread or via the hematogenous route.

The term *acute splenitis* ("septic spleen") refers to a soft, runny consistency of the splenic pulp draining from cut sections, histologically corresponding to an increased number of neutrophils and macrophages. The concept of acute splenitis as a postmortem marker of systemic infection is generally accepted, and in my experience it is the most frequent finding in sepsis-related fatalities, seen in more than 90% of cases. However, a study (investigating only a small number of cases) questioned the association of acute splenitis with sepsis, but the authors admitted that the presence of acute splenitis may be useful in distinctive sepsis cases with specific subsets of microbial infections or patient (newborn vs. adult) populations.[97]

Septicopyemic abscesses in considerable sizes were a frequent finding in the preantibiotic era but now are rare. However, various exogenous or endogenous factors, such as inappropriate or lack of specific antibiotic use, immunocompromise as the result of a severe underlying debilitating illness (e.g., malignant disease, metabolic disorder, immunodeficiency), or concomitant treatment with immunosuppressive agents prior to death, still may present as septicopyemic abscesses in the spleen.

Kidney

Gross pathology of the kidneys in sepsis includes bilateral swelling with tense capsules and a pronounced, dark-red (congested) medulla that contrasts with the paleness of the cortex. Septicopyemic abscesses, if present, usually are found bilaterally and located predominantly within the renal cortex.

As a consequence of DIC, microthrombi formation can be detected in the glomerular capillaries (Figure 3.22) in a large proportion of sepsis-related deaths,[83] especially in patients who die of septic shock.[91] Intravascular accumulation of blood and bone marrow cells in the vasa recta of the renal medulla, another frequent but unspecific finding, provides strong evidence of shock prior to death.[83]

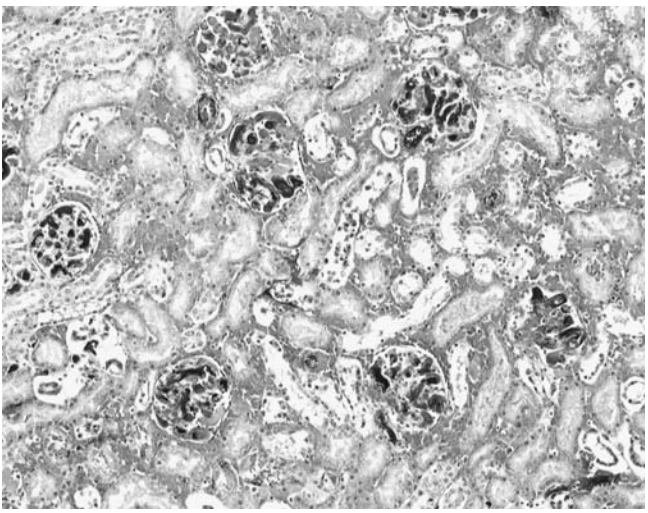

Fig. 3.22. Microthrombi formation in glomerular capillaries as a consequence of disseminated intravascular coagulopathy in a case of fatal Waterhouse-Friderichsen syndrome.

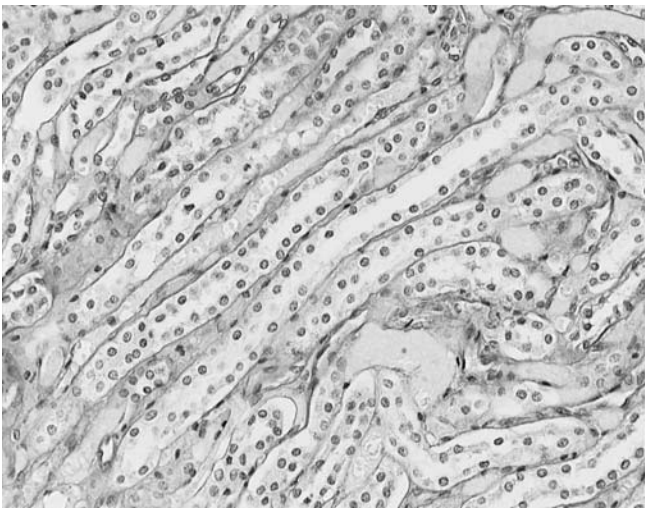

Fig. 3.23. Acute tubular necrosis with dilatation of proximal tubules and flattening of epithelium with loss of brush border and focal necrosis in a 3-year-old boy with *Haemophilus influenza* sepsis.

Acute tubular necrosis is the most frequent form of parenchymal acute renal failure. Acute tubular necrosis noted as dilated tubules with flattened basophilic and vacuolar endothelium (Figure 3.23) may be induced by sepsis, ischemia/reperfusion, and nephrotoxic drugs and therefore is frequently encountered in various forensic autopsy cases. However, with regard to septic shock, one study reported acute tubular necrosis occurred significantly more often in individuals dying under septic conditions than in individuals dying of shock as the result of primary cardiovascular or pulmonary failure, as observed in a large autopsy series of intensive care patients.[98]

In advanced stages of autolysis, the differentiation between antemortem shock-induced kidney changes and pure postmortem phenomena may be extremely difficult and at times impossible.[99]

Brain

Encephalopathy of variable severity, ranging from intermittent confusion to deep coma, is associated with septic shock in approximately one fourth of patients.[100] The severity of septic encephalopathy is positively correlated with mortality.[101] The pathogenesis of septic encephalopathy remains unclear. Various mechanisms have been proposed to contribute to the development of encephalopathy in sepsis (Table 3.1). These mechanisms may affect one other, and more than one may play a crucial role in individual patients.

At autopsy, cerebral infarction is an uncharacteristic finding in sepsis-related fatalities, presenting in 17% to 26% of cases. Unevenly distributed, round to cone-like hemorrhages are another frequent finding in sepsis. Their presence mainly depends on the manifestation of DIC or other clotting disturbances such as thrombocytopenia prior to death.

Table 3.1. Proposed mechanisms responsible for development of septic encephalopathy.

- Bacterial invasion or endotoxin effects on the CNS
- Ischemia due to reduced cerebral blood flow and/or increased cerebral oxygen consumption
- Blood–brain barrier breakdown with cortical neuronal injury
- Generalized inflammatory host response to infection, including release of proinflammatory and antiinflammatory cytokines, nitric oxide expression, and leukocyte activation
- Alteration of brain metabolism affecting CNS amino acid levels and noradrenergic and serotoninergic neurotransmission
- Systemic metabolic disturbances resulting from multiple organ failure and therapeutic drug administration during the course of sepsis

CNS, central nervous system.

Surprisingly, only a paucity of studies have investigated the neuropathology of sepsis, reaching discordant findings and reporting variable incidences of similar lesions.

Histologically, a circumscribed loss of neurons in the hippocampus formation has been described in humans dying of septic shock.[85] However, this finding simply reflects a protracted circulatory failure before death and therefore is highly unspecific.

In their retrospective study, Jackson et al.[102] investigated 12 fatal cases of encephalopathy associated with sepsis. The facultative histomorphologic features of septic encephalopathy were described as follows: cerebral infarcts 17%, brain purpura and multiple small hemorrhages 17% (Figure 3.24), septicopyemic microabscesses 67% (Figure 3.25), proliferation of astrocytes and microglia in the cerebral cortex 17%, and central pontine myelinolysis 17%.[102] Forensic pathologists agree that these findings, apart from septicopyemic abscesses, are highly unproductive for establishing the postmortem diagnosis of sepsis. Proliferation of astrocytes and microglial cells is a highly unspecific finding that may reflect various types of metabolic disorders, including ischemia. Central pontine myelinolysis, a rare neurologic disorder defined

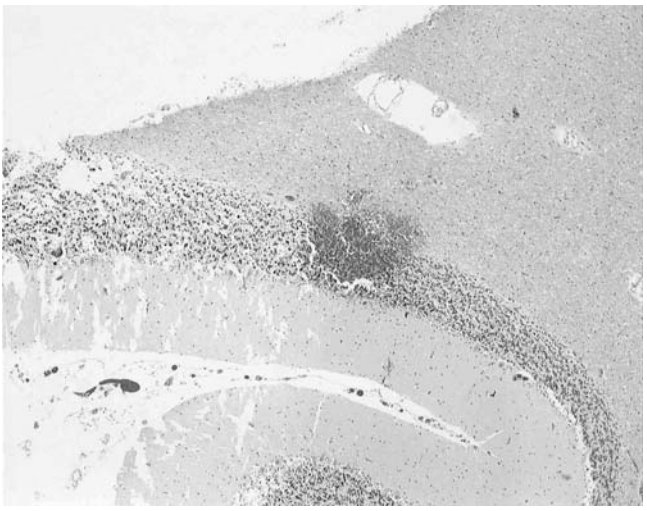

Fig. 3.24. Small hemorrhage in the cerebellum in sepsis.

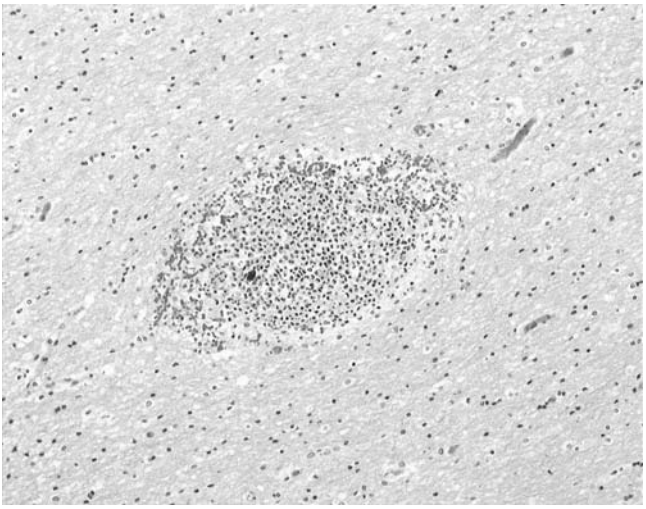

Fig. 3.25. Septicopyemic microabscess in the brain as a result of endocarditis lenta.

by symmetric demyelination in the central base of the pons, is sporadically found in autopsy cases of alcoholism and malnutrition.[103]

In a neuropathologic autopsy series of six patients who died of septic shock, changes in the brain similar to acute hemorrhagic leukoencephalitis were reported and interpreted by the authors as a manifestation of a generalized Shwartzman reaction.[104]

Pendlebury et al.[105] reviewed 2107 consecutive autopsies with neuropathologic examination and identified 92 cases with pathologic evidence for infection involving the central nervous system. Of these cases, 35 took the form of multiple microabscesses. An underlying sepsis was often present, and the lungs were the most frequent site of primary infection, with *S. aureus* and *Candida albicans* the most frequently identified causative organisms.[105]

Sharshar et al.[106] investigated the neuropathologic correlates of encephalopathy in 23 patients who died of septic shock in an intensive care unit and 13 nonseptic control individuals.[106] The grossly detectable abnormality in septic shock was cerebral infarction in 26%. The histopathologic abnormalities were DIC with multiple fibrinous microthrombi responsible for diffuse small microinfarcts (9%), microabscesses (9%), and multifocal necrotizing leukoencephalopathy (9%) (Figure 3.26), characterized by multiple small foci of necrosis in the white matter of the basis of the pons and ischemic changes in areas classically susceptible to ischemia (100%). The intensity of the ischemic lesions did not correlate with the duration of septic shock. In none of their cases could central pontine myelinolysis be identified despite great variation in the patients' plasma sodium levels.

When brain abscesses are detectable with the naked eye at autopsy or in the presence of purulent meningitis, the pathologist must carefully prepare the adjacent bony structures of the skull to search for a potential primary focus of infection, such as suppurative infection of the middle ear or the paranasal sinuses, which may have led to consecutive pyemic infection of the brain or the meninges by direct extension (per continuitatem spread) (Figure 3.27).

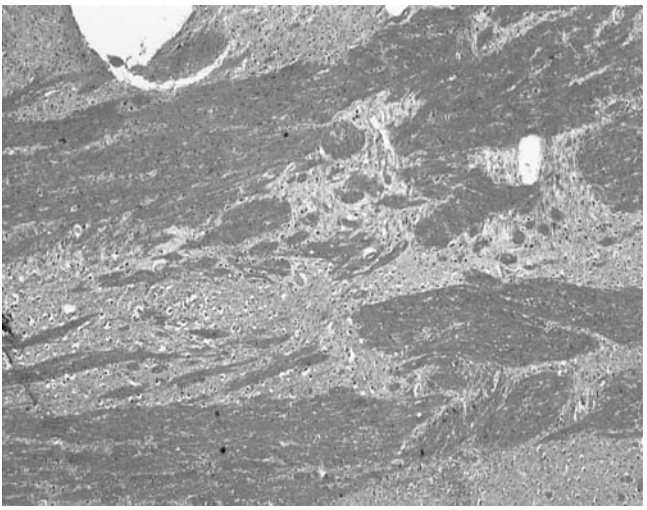

Fig. 3.26. Multifocal necrotizing leukoencephalopathy. Horizontal section of the upper pons with recent necrotic changes in the transverse pontine fibers. In this case, sepsis originated from a large bowel abscess (polymicrobial growth in postmortem cultures).

Adrenal Gland

One of the most characteristic morphologic findings in sepsis-related deaths is unilateral or bilateral bleeding in the adrenal cortex. Bleeding may vary in size from tiny focal hemorrhages visible at microscopy (Figure 3.28) to total hemorrhagic infarction ("adrenal apoplexy") easily detectable at gross examination. In my experience, adrenal hemorrhages are, in addition to acute splenitis, the most frequent finding in sepsis-related fatalities. Bilateral adrenal hemorrhage (Figure 3.29) in conjunction with skin bleedings due to DIC and meningitis is classically associated with WFS, most commonly associated with meningococcal or pneumococcal sepsis.

Lipid depletion of the cortex is an usual but highly unspecific finding in the adrenal glands in sepsis, reflecting stress of the affected organism during protracted

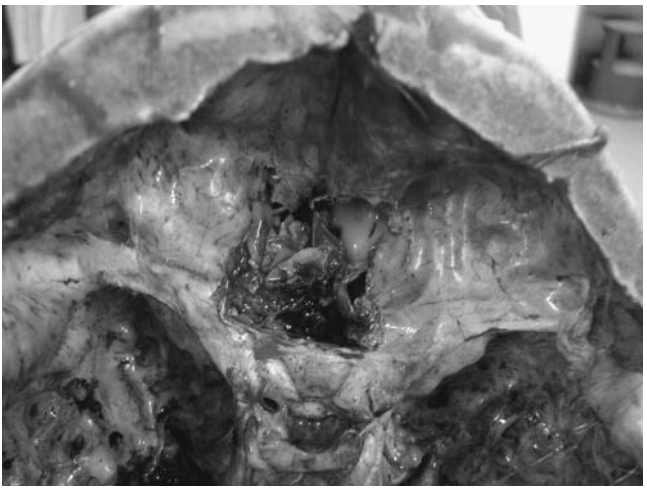

Fig. 3.27. Purulent exudate in the ethmoidal sinus. *Streptococcus pneumoniae* was cultured from postmortem swabs. Pyemic infection by direct extension from the ethmoidal sinus led to fatal meningitis.

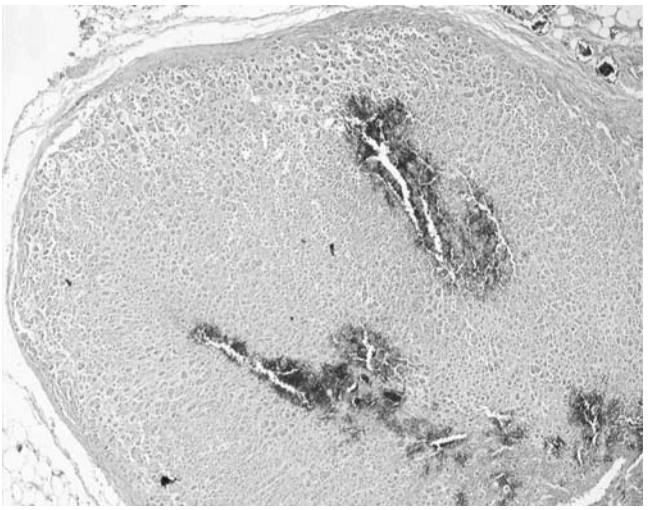

Fig. 3.28. Focal hemorrhages in the inner cortex of the adrenal gland in a case of ventilator-associated *Pseudomonas aeruginosa* sepsis.

agony. Therefore, this finding is observed more frequently in septic conditions with a long disease course than in septic shock with a rapidly fatal outcome.

Thrombi formation as a consequence of DIC can be observed more often in the adrenal cortex than in the medullary region.[85,107]

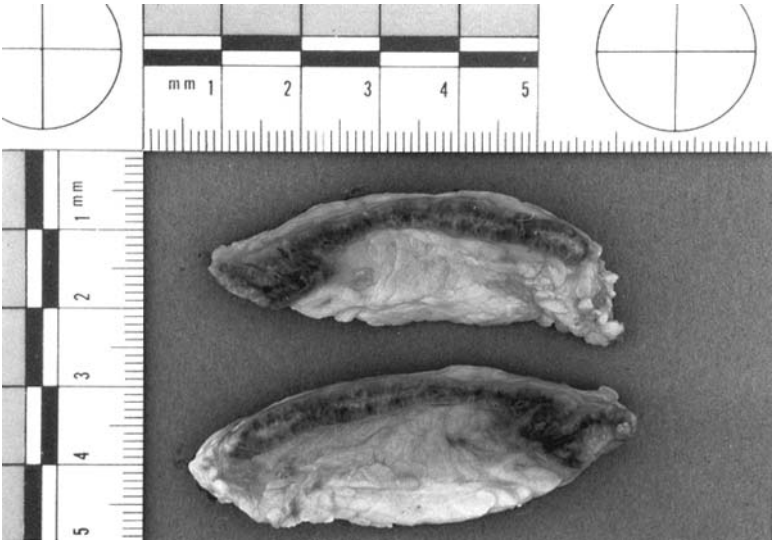

Fig. 3.29. Cut sections of adrenal glands showing intraparenchymal hemorrhage in a case of fatal Waterhouse-Friderichsen syndrome in an adult.

Gastrointestinal Tract

Subserous petechial hemorrhages, erosions, and acute ulcers visible with the naked eye at autopsy are the most common (nonspecific) shock lesions in the gastrointestinal tract. These gastrointestinal shock lesions have been reported to occur more often in patients dying of septic shock than in shock situations of cardiogenic or hypovolemic origin.[108]

Septic enterocolitis refers to necrotic and ulcerous changes of the gastrointestinal mucosa in sepsis (Figure 3.30) as a result of DIC.[99] Although the term *septic enterocolitis* implies the alterations are somehow specific, these lesions may resemble hemorrhagic gastrointestinal infarction during the course of arterial or venous occlusion.

Fibrin thrombi may be seen in smaller vessels of the bowel mucosa and submucosa, a finding detectable even on autopsy specimens that have undergone considerable autolysis (Figure 3.31). However, this is an uncharacteristic histologic finding, as vascular fibrin thrombi can be found on various occasions in autopsy histology, such as fatalities where the individual suffered from prolonged hypotension prior to death or in deaths with preceding vasoconstriction of the splanchnic vessels.

In a sepsis model in baboons, villous tip necrosis of the small intestine and submucosal edema of the colon have been described as typical for septic shock,[107] but these findings are of no practical value to the forensic pathologist because autopsy specimens of the gastrointestinal tract frequently have undergone advanced autolytic changes of the mucosal surfaces before they arrive for forensic pathology examination.

Pseudomembranous colitis, characterized by the gross appearance of pseudomembranes surrounded by hemorrhagic zones in the colonic mucosa, is not an infrequent finding in sepsis-related deaths. The disease can be found in individuals who initially were treated ambulatory with an unreasonable or uncritical use of broad-spectrum antibiotics for minor infections or can manifest during severe sepsis as a result of breakdown of cellular and humoral immune defense mechanisms of the gut.

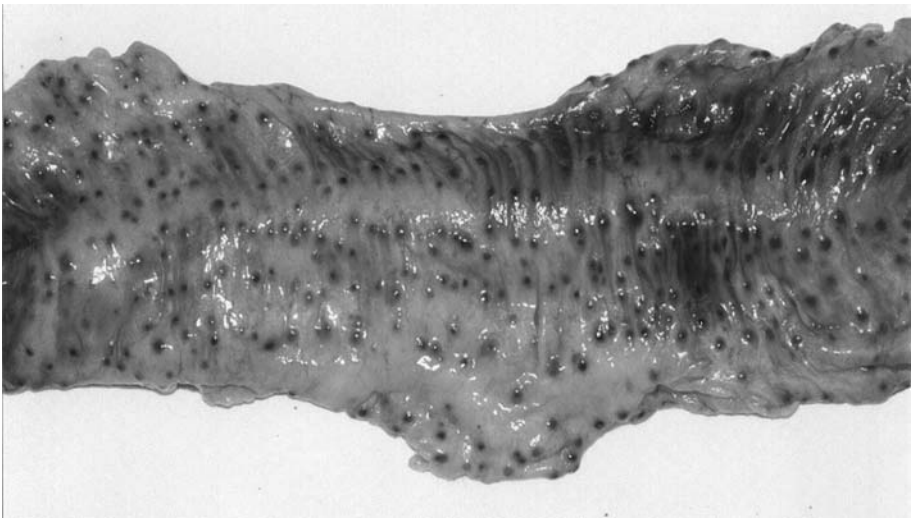

Fig. 3.30. Macroscopic appearance of so-called "septic enterocolitis."

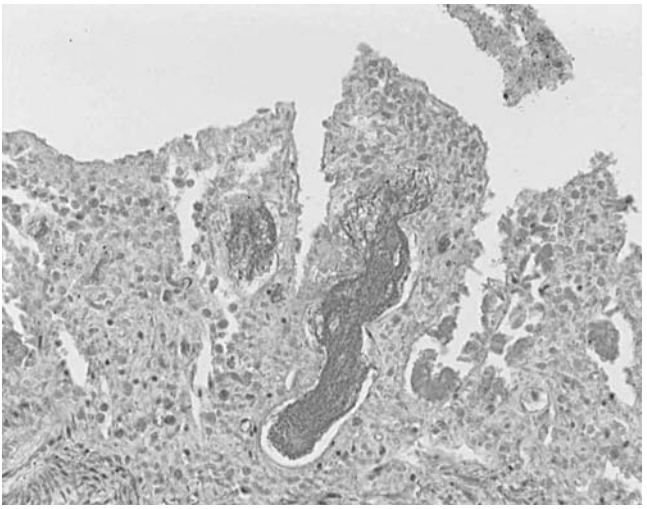

Fig. 3.31. Fibrin thrombi formation in the mucosa of the small intestine; considerable autolysis of autopsy specimen. This individual died of septic shock due to *Staphylococcus aureus* sepsis as a consequence of an intragluteal injection of diclofenac for treatment of lumbago.

Histologically, pseudomembranous colitis is characterized by partly or fully disrupted colonic crypts showing expansion by mucus and neutrophils. Mucosal erosions are covered by pseudomembranes consisting of fibrin, granulocytes, and mucus, giving the disease the typical "volcano-like" eruptive appearance (Figure 3.32). A short course of antibiotic therapy (e.g., perioperative prophylaxis in inpatients) seems to be sufficient to induce the disease in predisposed individuals. Although most cases of pseudomembranous colitis result from therapy with clindamycin and third-generation cephalosporins, the disease can occur as a complication of treatment with almost every antibiotic. Fatal complications of pseudomembranous colitis include shock due to volume depletion, toxic megacolon, massive lower gastrointestinal hemorrhage, or colonic wall perforation with subsequent peritonitis.

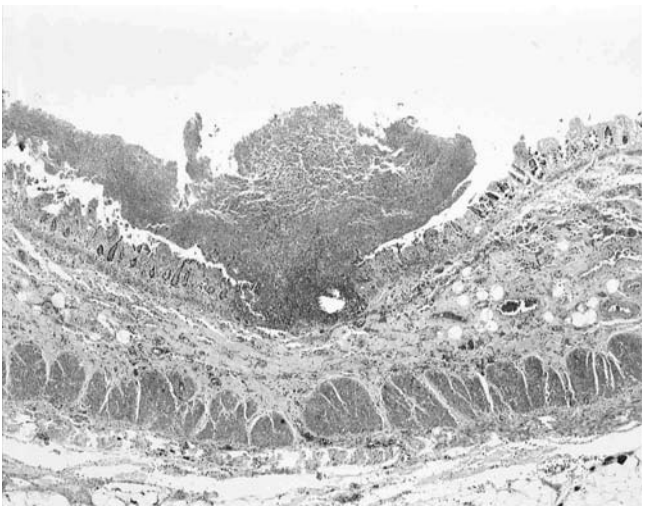

Fig. 3.32. Pseudomembranous colitis. Typical "volcano-like" eruptive appearance of colonic mucosa.

Fig. 3.33. Skin bleedings due to disseminated intravascular coagulopathy during the course of sepsis.

Skin

Circumscribed bleedings of the skin are a clinically well-known manifestation of DIC during the course of sepsis and are still apparent postmortem on the outer body surface (Figure 3.33). Sometimes these bleedings appear in a discreet petechial pattern, occasionally taking the shape of extensive, confluent hemorrhages.

Metastatic spread of septic microemboli may lead to circumscribed cutaneous bleedings in distinct locations (Figures 3.34 and 3.35) and must be differentiated from DIC-related skin bleedings that manifest usually over the entire body surface.

In sepsis-related deaths, jaundice of the skin, sclerae, and conjunctivae upon external examination of the body indicate liver failure complicating the septic disease state.

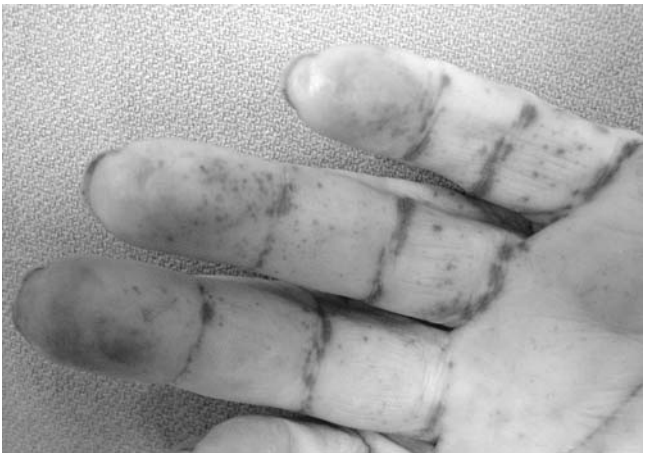

Fig. 3.34. Cutaneous bleedings on the inner aspects of the fingers due to metastatic spread of septic microemboli.

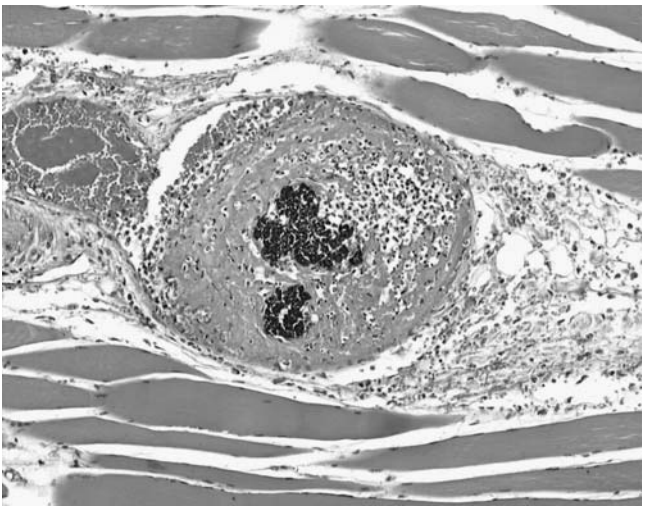

Fig. 3.35. Septic microemboli totally occluding a small artery. The emboli originated from endocarditis of the mitral valve. Strong inflammatory cell infiltration in all vessel layers are seen (same case as Figure 3.34).

A rapid onset of body decomposition (impressively contrastive to the length of the preceding postmortem interval and to the ambient temperatures to which the corpse was exposed after death) seen as putrefactive skin alterations with a greenish skin discoloration, skin slippage, and outlining of the superficial veins of the chest and upper extremities is frequently found in individuals who suffered from infection or sepsis prior to death. The most rapid onset and course of postmortem putrefaction is seen in gas gangrene (clostridial myonecrosis). The typical presentation of gas gangrene at external examination is a gloomy, violaceous to reddish-brown discoloration of the skin with hemorrhagic bullae formation (Figure 3.36). The skin appears tightened and shows palpable subcutaneous emphysema (crepitation). Clostridial gas gangrene is one of the most fulminant necrotizing infections affecting humans. Gas gangrene is not a disease of the past. Infection with *Clostridium perfringens* type A in devitalized tissue as a result of recent surgery or other trauma is the most common cause.

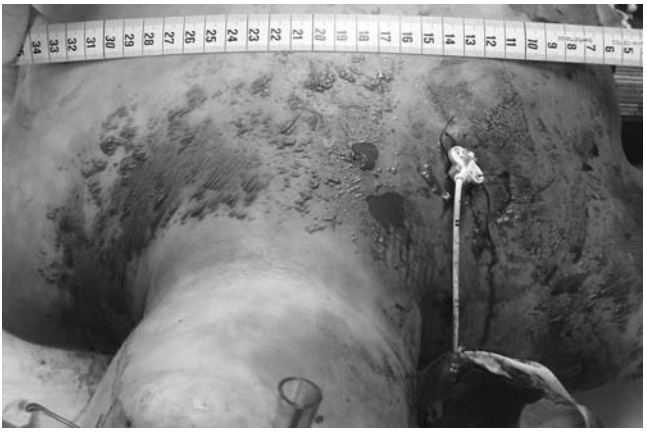

Fig. 3.36. Gas gangrene due to *Clostridium difficile* infection. Hemorrhagic bullae formation upon the skin is seen.

The proof of the portal of entry of the pathogenic organism often is difficult to establish because clostridial gas gangrene also may develop in the absence of trauma in individuals with underlying immunocompromise, malignancies, pancreatitis, cholecystitis, liver cirrhosis, diabetes mellitus, radiation colitis, or alcohol abuse.

Skeletal Musculature

The skeletal musculature is a rare site for septicopyemic abscess formations (Figure 3.37), a fact ascribable to the inherent resistance of the skeletal musculature to infectious agents. In a series of autopsied cases of staphylococcus sepsis, abscesses in skeletal muscle were found in fewer than 1% of cases.[109]

Skeletal muscle fiber alterations, such as the appearance of longitudinal striation, loss of cross-striation with homogenization of fibers, and the finding of a segmental and splinter-like fiber distraction, reflect the uniform but nonetheless unspecific reaction pattern of the musculature against exogenous noxae.

Muscle pathology attributable to gas gangrene is characterized by a brownish sooty discoloration and soft consistency of the affected musculature at gross examination, with crepitation during dissection due to gas formation by the anaerobic germs. Histologically, clostridial myonecrosis is characterized by Gram-positive, rod-shaped bacteria invading emptied sarcolemma and homogenization of adjacent muscle fibers with loss of nuclei. The histologic picture is remarkable for its absence of almost any inflammatory cell reaction next to clusters of clostridia (Figure 3.38), a picture distinctly different from all other forms of bacterial infections. Two reasons can be put forward to explain this phenomenon: (1) various extracellular toxins produced by clostridia, such as phospholipase C and

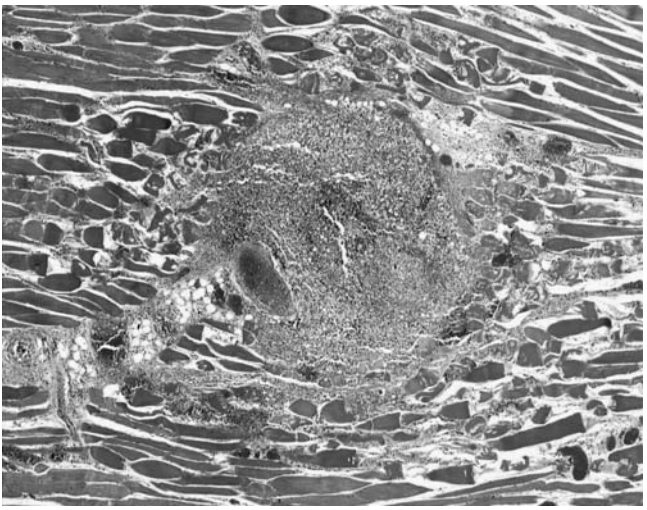

Fig. 3.37. Septicopyemic abscess formation in the psoas muscle that developed during the course of fatal *Staphylococcus aureus* sepsis following a burn injury.

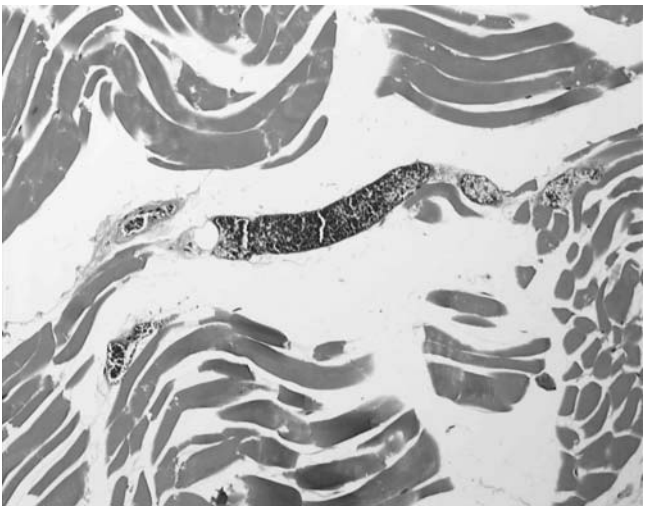

Fig. 3.38. Gas gangrene (clostridial myonecrosis). Bacteria are seen in an emptied sarcolemma. Loss of cross-striation with homogenization of fibers and segmental and splinter-like fiber distraction with occasional hypercontraction bands are observed. Note the total absence of inflammatory cells, which is typical for the disease. Postmortem cultures revealed *Clostridium difficile* as the responsible germ.

perfringolysin O produced by *Clostridium difficile,* are cytolytic for leukocytes[110]; and (2) what the forensic pathologist sees under the microscope most likely is the result of an extremely rapid postmortem overgrowth of the clostridia in most visual fields investigated.

Pyomyositis (primary muscle abscess) is an acute bacterial infection of the skeletal musculature. *Staphylococcus aureus* is the organism most commonly cultured from the abscess; it is seen in up to 90% of cases in tropical areas and 75% of cases in temperate countries. Group A streptococcus accounts for another 1% to 5% of cases. The term *pyomyositis* should be restricted to primary muscle abscesses arising within the skeletal musculature and should not be used to describe (1) intermuscular abscesses, (2) abscesses extending into muscles from adjoining tissues, such as bone or subcutaneous tissues, or (3) pyemic intramuscular abscesses secondary to previous sepsis. Fatal cases of pyomyositis seen in the forensic pathologic setting usually arise from a preceding trauma, such as a fall or assault.[10] Intravenous drug abuse is another important risk factor for pyomyositis.[10] The factors responsible are impaired cellular and humoral immunity, defective bactericidal capacity of neutrophils, increased bacterial colonization of skin, and direct injection of contaminated materials. Pyomyositis is increasingly documented in persons infected with human immunodeficiency virus (HIV). Mechanisms include muscle damage caused by HIV infection per se, zidovudine therapy, and infections caused by parasites and mycobacteria leading to impaired host defenses.[111,112] Abscess formations due to pyomyositis are characterized by extensive suppuration on cut sections. They are most often located in the thigh gluteal muscles but also involve muscles around the shoulder girdle, abdomen, pelvis, and around the spine. Histologic features of pyomyositis include Gram-positive cocci infiltration with surrounding granulocytic infiltration, edematous

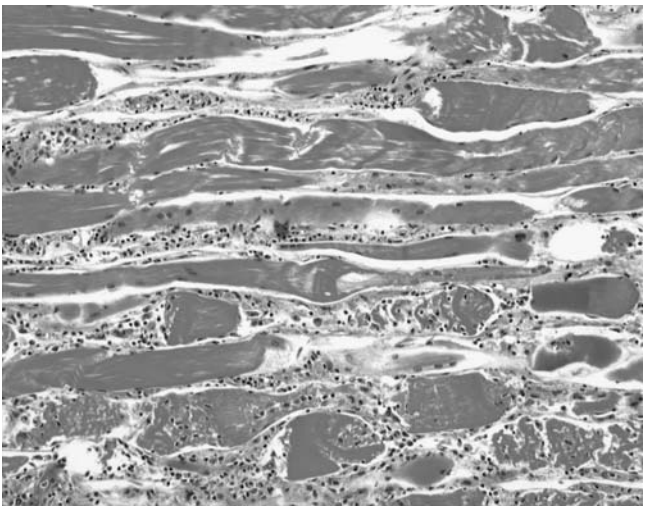

Fig. 3.39. Pyomyositis. Dense granulocytic infiltration and myocytolysis with disruption of muscle fibers. *Staphylococcus aureus* was cultured from native muscle specimens obtained at autopsy.

separation of fibers in early stages of the disease followed by patchy myocytolysis progressing to disruption of muscle fibers, and complete disintegration of the affected muscle (Figure 3.39).

The skeletal muscles are a relatively rare bleeding site in coagulation disorders such as septic DIC. However, iliopsoas muscle bleeding may be seen in septic individuals with severe deterioration of coagulation factors.[113] Relevant differential diagnoses of iliopsoas muscle bleeding (potentially leading to fatal exsanguination) include hemorrhage due to trauma, anticoagulant medication, and hypothermia.[114]

Fasciae

Fasciae are intrinsically resistant to bacterial infections under normal circumstances. Therefore, the fasciae usually present no pathologic abnormalities in sepsis-related deaths, except for cases where the septic condition originated from a primary infection of fasciae. One such example with considerable forensic relevance is necrotizing fasciitis, a rare but rapidly progressive and potentially fatal disease. Necrotizing fasciitis results from introduction of pathogens into injured or devitalized tissue and is most commonly associated with surgical procedures. Occasionally, the disease occurs in those who suffered minor trauma. In forensic pathology, necrotizing fasciitis leading to sepsis and death most often is determined to originate from liposuction or following assaults.[4] The disease usually is a polymicrobial infection, and the most common pathogens are aerobic Gram-positive cocci, Gram-negative bacteria, and anaerobes. At gross examination, infected areas show a bluish-brown discoloration of the skin, often well-demarcated by an erythematous zone and occasionally accompanied by purulent blister formation. Histologically, the disease is characterized by progressive inflammation and extensive necrosis of the

Fig. 3.40. Metastatic septic emboli spread originating from a large retroperitoneal staphylococcal abscess gave rise to suppurative inflammation of the knee joint in this case.

subcutaneous tissue and fascia, sparing the skeletal musculature. Necrosis of muscle or myositis is uncommon in necrotizing fasciitis.

Other

The morphologic features of the pancreas in sepsis are similar to pancreatic tissue injury of various etiology. The pancreas is not a typical site for manifestation of sepsis-induced tissue alterations.

In regional lymph nodes located in the vicinity of a septic focus, an increase of the average number of polymorphonuclear neutrophils, monocytes, and activated macrophages can be detected histologically in some cases.

Metastatic septic microemboli spread can give rise to osteomyelitis or suppurative inflammation in joint cavities (Figure 3.40).

Immunohistochemical Detection of Sepsis-Induced Lung Injury in Human Autopsy Material

The lung is the organ primary targeted for injury under septic conditions, and progressively impaired lung function is the major complication in sepsis. Attention has focused on the immunohistochemical detection of different markers of the inflammatory cellular response of the lungs to sepsis, and the pulmonary microvasculature has proved a worthwhile target for postmortem diagnosis of sepsis-induced lung injury. A brief survey of the pulmonary expression pattern of distinct cellular adhesion molecules in sepsis is presented.

Leukocyte Recruitment and the Acute Inflammatory Response

It has become clear that leukocyte recruitment (migration of leukocytes from the vascular system to sites of pathogenic exposure) is one of the key events in the inflammatory process. Entry of leukocytes into sites of injury or infection requires molecular mechanisms that enable the leukocyte to recognize the sites from within the vasculature and to come in contact with the vessel-lining endothelium in order to perform diapedesis through the vessel wall. Recognition and contact formation with the endothelium are dependent on the presence of both cytokines and adhesion molecules that mediate neutrophil–endothelial cell adhesive interactions. During sepsis, the lung is especially susceptible to injury, and a critically impaired lung function often leads to ARDS and death in the septic individual.[115–117] Extravasation and sequestration of leukocytes during acute lung injury are dependent upon a complex intercellular communication based on the following:

1. Activation of mononuclear cells and release of proinflammatory cytokines[118,119] with subsequent
2. Surface expression of endothelial adhesion molecules and neutrophil-derived adhesion molecules[120,121] resulting in
3. Enhanced rolling, adhesion, and transendothelial migration of leukocytes[122,123]

The adherence of leukocytes on the vascular endothelial cell surface and transmigration through the endothelial layer are regulated by at least three adhesion molecule families: the selectins (E-selectin, L-selectin, P-selectin), the integrins (e.g., LFA-1, Mac-1, VLA-4), and the immunoglobulin superfamily (e.g., ICAM-1, VCAM-1).[122,124,125] The selectins mediate the initiation of cell contact between leukocytes and endothelium. This selectin-mediated adherence of leukocytes to the blood vessel wall first leads to rolling of leukocytes within the bloodstream on the endothelial cell surface. The rolling leukocytes are able to sense signals from the endothelium, which stimulate them to adhere firmly to its surface. Stimulated by cytokines, leukocytic integrins bind to members of the immunoglobulin superfamily expressed on the endothelial cell surface. Firm adhesion via activated integrins is a prerequisite for leukocyte diapedesis through the layer of the endothelial cells. Little is known about the exact mechanism of diapedesis, namely, whether the leukocytes transmigrate through the junctions between adjacent endothelial cells or directly through a single endothelial cell.[126]

Expression Pattern of Endothelial and Leukocytic Adhesion Molecules in Sepsis-Induced Lung Injury

E-selectin (CD62E) is not expressed by unstimulated endothelium and requires activation by cytokines and bacterial lipopolysaccharides.[122,126,127] E-selectin, synthesized de novo and requiring several hours for its expression following activation,[127,128] is a strong primary adhesion receptor for neutrophils and provides rolling adhesion of circulating leukocytes.[121,129,130]

A study that investigated the immunohistochemical expression pattern of E-selectin in lung specimens from sepsis-related deaths and nonseptic controls showed strong, homogeneous E-selectin expression on endothelial cells of pulmonary arteries, arterioles, precapillaries, postcapillary venules, and veins in all lobes of

the lung in the sepsis cases, in contrast to a lack of immunopositivity for E-selectin in the nonseptic individuals ($p < 0.05$).[25] Local inflammatory lung alterations (e.g., bronchopneumonia, aspiration pneumonia) and other lung pathologies, such as blood aspiration or aspiration of soot, did not result in positive immunohistochemical staining reaction of the endothelial layer in the controls, a finding well in line with the previous observation that crucial differences seem to exist between the role of locally produced cytokines in pneumonia and systemic inflammation.[131] Therefore, a false-positive E-selectin immunoreactivity should not be expected in nonsepsis cases with merely localized inflammatory or mechanical pulmonary tissue alterations.

VLA-4 (very late activation antigen-4, CD49d/CD29) is a cell surface molecule that is expressed on monocytes, eosinophils, basophils, and lymphocytes.[131,132] VLA-4 is involved in leukocyte adhesion to activated endothelial cells with subsequent migration from the vasculature into pulmonary tissue and the alveolar compartment during inflammatory processes and sepsis.[132–135] ICAM-1 (intercellular adhesion molecule-1, CD54) is a cell surface protein that is expressed at very low levels on pulmonary endothelium, lymphocytes, and macrophages.[123] Expression of ICAM-1 is up-regulated upon stimulation by inflammatory mediators such as cytokines and bacterial lipopolysaccharides in sepsis.[123] ICAM-1 mediates inflammatory responses by adhesion of leukocytes to activated endothelium and subsequent leukocyte diapedesis through the pulmonary endothelial layer.[122,124,133]

The value of VLA-4 and ICAM-1 as micromorphologic postmortem markers for detection of sepsis-induced lung injury was evaluated in an immunohistochemical study.[136] Lung specimens were obtained at autopsy from 30 individuals divided into three study groups:

1. Sepsis group (n = 8; autopsy cases with a well-documented medical history and clinical diagnosis of death due to sepsis as confirmed by autopsy)
2. Nonsepsis group I (n = 6; death due to natural causes)
3. Nonsepsis group II (n = 16; death due to nonnatural causes, e.g., trauma, electrocution, drowning, hanging)

In all cases of the sepsis group, VLA-4 was strongly expressed on intravascular, interstitial, and intraalveolar pulmonary leukocytes. In the nonsepsis groups I and II, an irregular weak positive immunoreactivity was observed on interstitial leukocytes, whereas no immunopositivity could be detected on intravascular or intraalveolar leukocytes. In comparison to the nonsepsis groups I and II, VLA-4 expression in the sepsis group differed significantly ($p < 0.001$). In the sepsis group, the intensity of leukocytic immunoreactivity for VLA-4 was homogeneous in all lobes of the lungs irrespective of the length of the postmortem interval or the duration of the septic condition prior to death. Strong positive expression of ICAM-1 was detected on endothelial cells of pulmonary arteries, arterioles, precapillaries, alveolar capillaries, postcapillary venules, and veins in all cases in the sepsis group. In addition, immunoreactivity for ICAM-1 was strongly positive on pulmonary macrophages and lymphocytes. Both endothelial and leukocytic immunoreactivity for ICAM-1 was homogeneous, irrespective of the duration of the septic condition prior to death. In both nonsepsis groups, an infrequent weak immunopositivity for ICAM-1 was observed on pulmonary endothelial cells and leukocytes, the latter mostly located within the perivascular space. In comparison to the nonsepsis groups I and II, immunohistochemical expression of ICAM-1 in the sepsis group differed significantly for endothelial cells ($p < 0.001$) and leukocytes ($p < 0.001$).

Critical Appraisal of the Practical Value of Immunohistochemical Markers Applied to the Postmortem Diagnosis of Sepsis-Induced Lung Injury

The finding of intense endothelial E-selectin expression in the pulmonary microvasculature of septic individuals undergoing forensic autopsies[25] confirmed previous conclusions derived from animal models of sepsis that showed apparent sepsis-induced endothelial E-selectin expression.[137,138] The observed expression pattern of enhanced expression of VLA-4 and ICAM-1 on pulmonary leukocytes and endothelial cells in sepsis-induced human lung injury and the observed distribution pattern[136] are well in line with the results of laboratory studies performed on in vitro cell lines and animal models identifying the pivotal role of VLA-4 and ICAM-1 in the pathogenesis of systemic inflammation.[115,133,135] The value of determining the immunohistochemical expression pattern of different cellular adhesion molecules in the forensic pathologic studies[25,136] was assessed using well-documented sepsis cases and nonseptic controls in a scientific setting. A clinicopathologic autopsy study confirmed these results by demonstrating up-regulation of ICAM-1 and E-selectin in sepsis-induced lung injury in intensive care unit patients who died of septic shock as a result of infection with Gram-negative bacteria.[139] Accordingly, E-selectin, VLA-4, and ICAM-1 should be considered useful immunohistochemical postmortem markers of sepsis. Use of the presented immunohistochemical markers of sepsis-induced lung injury will be particularly helpful when autopsy findings and routine histology in cases of suspected fatal sepsis are unspecific or unconvincing, respectively. However, no single marker should be relied upon to establish the diagnosis of sepsis postmortem because such a practice may lead to misinterpretation and false conclusions. As a routine test, the immunohistochemical detection of cellular adhesion molecules is far from established in forensic autopsy practice. The development of a practical framework for the application of immunohistochemical sepsis markers that add to the postmortem differentiation between death due to sepsis and noninfectious causes is highly desirable for medicolegal practice.

For a more comprehensive overview of the immunohistochemical methods and markers currently available for postmortem diagnosis of septic ARDS, the reader is referred to a review on the topic[140] and publications focusing on the application of growth factors and glycoproteins to postmortem elucidation of death due to sepsis.[18,21,141]

Conclusions

Almost every infection is capable of causing death, some in a more rapid manner and some in a more prolonged manner, depending on various intrinsic and extrinsic risk factors such as poor nutritional status, immunodeficiency syndromes, drug- or alcohol-induced immunosuppression, or preexisting cardiopulmonary pathology that influence the disease course and outcome of the individual. These and other risk factors must be considered carefully in the concluding medicolegal expertise in light of the fatality in question. From the clinical point of view, a number of sudden, unexpected deaths occurring outside the hospital as the result of a rapidly progressive course of infection should be regarded as unavoidable. However, under medicolegal

aspects, the principal question to whether a causality between an exogenous noxa (e.g., occupational or traffic accident, sharp or blunt external force, decubitus ulcer, indwelling catheter, injection, surgical procedure) and infection can be proved.

To reach etiopathogenetic conclusions on the causal relationship between, for example, catheter-related infection and fatal outcome, proof that a given tissue injury (e.g., the insertion site of a peripheral venous catheter or a gluteal abscess following intramuscular injection) is the only and exclusive portal of entry must be established first. Next, the question of whether inoculation of microorganisms through the established portal of entry could have been avoided in all probability if the responsible medical staff had acted *lege artis* (e.g., in view of hygiene regulations), and therefore whether death as a result of infection can be ascribed to medical or nursing malpractice, will be the main focus of medicolegal interest.[1,3]

In fatalities resulting from WFS in infancy and childhood, the question almost inevitably arises as to whether the child could have been saved if the diagnosis had been made earlier.[9] Especially if a physician was consulted at the beginning of the disease, medical malpractice seems obvious to the parents. Because of the rapid clinical course of the disease and the rather unspecific findings at its beginning, even the clinical professional may not be able to distinguish WFS from a common cold or enteritis. Even if medical help is sought at an early stage of the disease, predicting the outcome in an individual case is impossible.[142] By the time DIC presents, it often is too late to save the child's life.

When advanced-grade decubitus ulcers are not mentioned on the death certificate but are observed upon external examination of the body or when decubitus ulcers seem not to be have considered sufficiently according to the death certificate (specifically regarding their potential causal relationship with the cause of death), questions regarding the cause and manner of death ("nonnatural death due to sepsis as a consequence of the decubitus ulcer?") may arise. The colonization of persistent open decubitus ulcers with microorganisms is connected with infectious complications such as bacteremia, osteomyelitis, and sepsis.[2,7,8] Sepsis related to decubitus ulcers is estimated to be associated with a mortality rate of up to 50%.[8] If the deceased required a nursing service or medical care prior to death, the forensic investigator also may be confronted with questions regarding neglect or the existence of an actual nursing injury. In addition, particularly in cases of decubitus-associated fatalities, an iatrogenic origin of the decubitus ulcer or even medical malpractice must be considered.[8] The development of sepsis as a consequence of decubitus formation should be regarded as a nonnatural cause of death when the responsibility for development of the decubitus ulcer can be attributed to neglectful care, incorrect nursing, or medical malpractice.[2,3,8]

These are just a few examples of the forensic pathologic and medicolegal problems associated with postmortem elucidation of sepsis-related deaths that forensic pathologists encounter in their practical work.

A close cooperation with the field of microbiology that should start as early as the time of autopsy is a prerequisite for a conclusive medicolegal expertise. The medicolegal expertise must meet the requirements of the legal authorities in judicial hearings demanding the highest degree of probability ("beyond any reasonable doubt") in criminal law.

Apart from the primary task, namely, the medicolegal elucidation of infection-related deaths, the forensic pathologist can contribute to the knowledge about the influence of specific pathogenic agents on fatalities that occurred outside of the hospital. Data obtained from such instances are valuable, as infections causing or

significantly contributing to deaths of outpatients probably are underestimated. In addition, the forensic pathologist can, in close cooperation with the field of clinical microbiology, make a significant contribution to one of the central public health issues, namely, the detection of highly infectious agents, which must be reported to the authorities.

Acknowledgment. This chapter is dedicated to my wife Jenny and my son Titus.

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