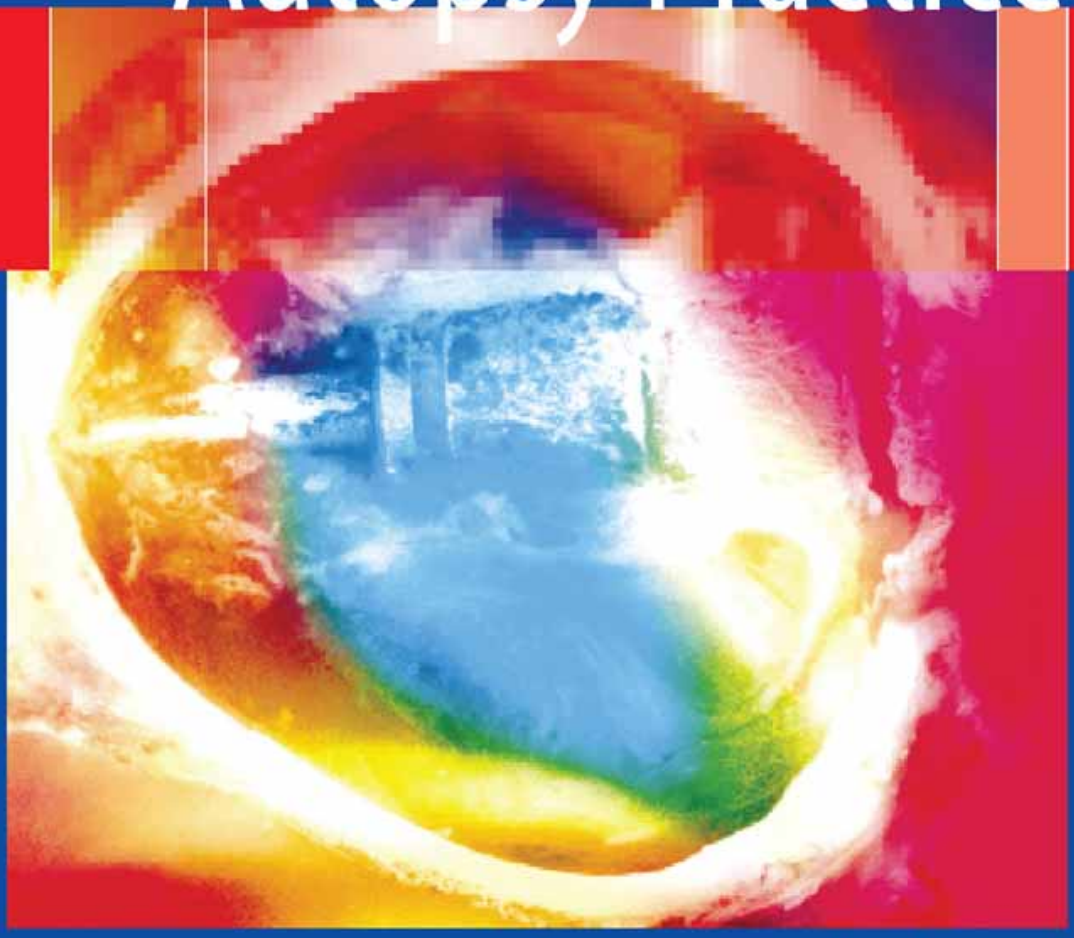
Guy N. Rutty *Editor*

Essentials of Autopsy Practice



Current Methods and Modern Trends

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With 88 Figures

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Preface

Since this third volume of *Essentials* has been compiled the arena of autopsy practice is undoubtedly changing. For the first time pathologists are beginning to opt out of autopsy training which will reduce the number of practitioners and result in centralisation of autopsy services. With the increase of knowledge, availability and use of genetic testing the nature of autopsy examinations are changing and the understanding of causes of death are evolving. New roles are evolving; new personnel are undertaking the jobs previously undertaken by the pathologist. Finally the very need for an autopsy examination is being questioned and an alternative technique for example axial radiography is now being researched to see if this may replace the need to undertake invasive autopsies. In this third volume in the series, subjects have again been chosen to assist all involved in post-mortem, crime investigation and bereavement work, specifically in areas where information may not be readily to hand, or in areas which may prove difficult for interpretation and where there have been advancements in practice over recent years. The book is again designed to be of use to the trainee and consultant pathologist alike, be they a generalist or specialist as well as to nurses, paramedical personnel, bereavement officers, lawyers and police, and to reflect the changing world of autopsy practice. As this series continues to evolves, each volume is intended for all involved with the dead and post-mortem work and hopes to assist with keeping one up-to-date with changing issues related to autopsy practice.

Guy N. Rutty
Leicester, 2005

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1. The Evolution of the Nurse's Role in Death Investigation

Jane E. Rutty

Introduction

This chapter begins by examining the general role of the nurse from a historical perspective to the current position in health care. The question as to whether the coronial service in England and Wales is in need of reform is debated, and comparisons to other death investigation frameworks being practiced around the world are discussed. The term *forensic nursing* is defined, and the emerging new nursing roles is analyzed. The chapter closes by suggesting there may be a gap in the provision of nursing care in the field of death investigation within the United Kingdom and Europe.

The Historical Role of the Nurse in Health Care

The word *nurse* originates from the Latin word *nutrire* meaning "to nourish."[1] The Western version of nursing has a long history and can be traced back as far as the Middle Ages.[2] In the mid-19th century, most nursing care was carried out at home as part of women's unpaid domestic duties. Hospital patients, who were mostly destitute, were attended by women in religious orders or by lay women trained informally on the job. However, they also had a reputation for drunkenness and immoral conduct.

Since the Reformation in the 16th century and the suppression of monasteries, the quality of nursing and hospitals suffered in all the Protestant European countries but most severely in England. When Henry VIII established the Church of England in 1534, he seized more than 600 charitable institutions and suppressed all religious orders. This seizure of church properties had a direct negative effect on women and nursing. Women lost political and administrative control of nursing operations. Inexperienced civil administrators took over from religious professionals who were steeped in a culture of care that had evolved since the beginning of the Christian church. As a consequence, women lost their voice in both hospital administration and nursing management, leading the whole medical system to begin a downward spiral of mismanagement, crowding, filth, and contagion.[3] It was these conditions that prompted Florence Nightingale's vocation.[4]

Nursing has its modern origins from the Crimean War of 1854–1856, when there was a need to care for wounded soldiers abroad. Florence Nightingale (1820–1910), a British nurse, fulfilled this role when she organized a group of women to deliver care under her supervision and that of the war surgeons in Scutari, Turkey.[5,6] Upon her return from the Crimean War, Florence Nightingale founded in 1860 one of the first nursing schools in the world, the Nightingale School and Home for Nurses at St. Thomas' Hospital in London.[2]

Perhaps the largest achievement in nursing history since Nightingale's reforms was The Nurses, Midwives and Health Visitors Act of 1979 (amended in 1992 and 2002), which set up the framework for the United Kingdom Central Council, the National Boards, and the Professional Register for Nursing, Midwifery and Health Visiting. Both the Council and Boards were dissolved in 2002 and replaced by the Nursing and Midwifery Council.

The Role of the Nurse in Health Care Today

Nursing is a complex activity that varies considerably according to where it is practiced. Defining what nursing is has always been difficult because its whole essence is its adaptability to varying needs and settings. Nightingale[7] stated in 1859:

> "Yet no man, not even a doctor, ever gives any other definition of what a nurse should be than this . . ." "devoted and obedient". This definition would do just as well for a porter. It might even do for a horse. It would not do for a policeman."

A definition of nursing adopted by the International Council of Nurses in 1960 and presently the most widely accepted definition in nursing was given by Henderson,[8] who proclaimed in 1966 that

> "The unique function of the nurse is to assist the individual; sick or well, in the performance of those activities contributing to health or its recovery (or to a peaceful death) that he would perform unaided if he had the recovery strength, will or knowledge. And to do this in such a way as to help him gain independence as rapidly as possible. This aspect of her work, she initiates and controls; of this she is the master."

Henderson's definition of nursing contains many elements that constitute the substantive nature of nursing today and is recognized all over the world as capturing the essence of what nurses do. However, in 2003 the Royal College of Nursing (RCN), after a 4-month consultation exercise that attracted the highest number of responses in the history of the RCN (160,000), redefined the core of nursing as follows[9]:

> "The use of clinical judgement in the provision of care to enable people to improve, maintain, or recover health, to cope with health problems, and to achieve the best possible quality of life, whatever their disease or disability, until death."

Despite these attempts to define nursing over a number of centuries, there is no common agreement within the profession. The problem lies in the difficulty in defining nursing, because nurses work in a wide variety of fields ranging from the largely preventative, advisory work of health visitors at one end of the scale to the highly technical, curatively orientated field of intensive care nursing at the other end. However, the advantage of the RCN definition is that it has the potential to

unite nurses across specialties but also to explain their differences, and it is a useful tool for explaining nursing values.

The Expansion and Advancement of Nursing Practice

The setting within which contemporary nursing works seems to be continuously changing with the introduction of new concepts and innovations, such as the nurse practitioner, specialist practice, advanced practice, consultant nursing, primary nursing, and surgeon's assistant. These concepts all increase the nursing role outside what historically were traditionally and established defined limits. However, this is not to state that this change is a bad thing. Following the publication of the documents "Junior Doctors: The New Deal"[10] in 1991 and "The Scope of Professional Practice"[11] in 1992, the issues related to the "expanding role of the nurse" have been at the forefront of both nursing and medical literature. These documents bestow nurses more independence, autonomy, and liberty to expand their roles than ever before while heightening their understanding of personal accountability.

Many see role expansion as an exciting opportunity to develop new subspecialties. However, expanding the nursing role has been controversial, and there has been disagreement within the nursing profession as to what approach should be taken. There is no national standard or catalogue of expanded roles, and practices differ from region to region and even within hospitals,[12] never mind country to country. Therefore the debate remains intense.

An example of where nursing is expanding and advancing outside the traditional boundaries of health care was suggested in 1993 by Dr. Mike Green,[13] Emeritus Professor in Forensic Pathology at the University of Sheffield. He believes that nursing staff in general are becoming increasingly more involved in legal processes, particularly with regard to their observations, records, and recall of events. Internationally, nurses have expanded and advanced their role more extensively within aspects of the legal profession, with the ultimate example of the ability of nurses to be coroners coming from the United States.[14–21] However, why has there been this major boundary shift and role advancement in providing nursing services within the coronial system in the United States and not in the United Kingdom or Europe?

The Coroner Service: A Relic in Need of Reform

In 1999, Derrick Pounder,[22] a professor of forensic medicine at the University of Dundee, suggested that the coroner service in England and Wales was a relic in need of reform, particularly when considering the implications of the Human Rights Act.[23] Pounder believed that the coroner's investigation is an enforceable intrusion by the state into what would otherwise be a private family matter, the death of a loved one. Striking the balance between the reasonable needs of the state to investigate and the rights of the next of kin to privacy and religious ritual is not easy, with present evidence suggesting this process is not done well in England and Wales. Among the rights in the European convention are the right to respect for privacy and family life (article 8) and freedom of thought, conscience, and religion (article 9). The new act in clause 6 provides that[1]

> "it is unlawful for a public authority to act in a way which is incompatible with a convention right."

This provided a starting point for questioning some current practices in the coroner service. According to the Home Office survey, 190,000 deaths, representing one third of all deaths in England and Wales, were reported to the coroner in 1996,[24] whereas in 1970 the number was 130,000, representing 20% of all deaths. The increase is largely accounted for by natural deaths voluntarily referred to the coroner by a doctor. Referrals by doctors now represent 60% of the coroner's case load.[25] Pounder[22] considered the directing of such a large number of natural deaths into the medicolegal investigative system to be both intrusive (for the families concerned) and costly.

Of the 190,000 deaths reported, 68% were subject to postmortem examinations under the legal authority of the coroner. However, among the 148 coroner districts the percentage dissected varied from 43% to 100%. Based on these figures, it was suggested that not all coroner districts can be striking an appropriate balance between the needs of the state and the rights of the next of kin. The prevalence of these legally enforced autopsies was considered to be of legitimate concern to everyone[26,27] but particularly to religious and ethnic minorities that do not approve of postmortem dissections.[28,29] The public inquest is another area of concern for Pounder,[22] who believed it necessarily conflicts with the right to privacy. Currently an average of 12% of reported deaths come to inquest, but the figures vary from 5% to 25% across the 148 coroner districts within England and Wales. It can be argued that many are unnecessary and serve only to increase the distress caused to the family, particularly when the death is by suicide.[22] Other areas of concern included a poor understanding of the circumstances surrounding such deaths by the investigating coroner's officer, who is either a police officer or a lay person.[30]

Other Death Investigation Frameworks Around the World

Following research into the practices of North America, Canada, Australia, New Zealand, western Europe (including southern Ireland), Malaysia, Japan, and South Africa, the death investigation frameworks that are practiced for the investigation of unnatural and certain other deaths can be summarized broadly into three types: the generic criminal investigation and judicial system; the medical examiner system; and the coronial system.

The Generic Criminal Investigation and Judicial System

Occasionally known as "civilian" systems, the generic criminal investigation and judicial system is found mainly in most European Union countries where an official is responsible for investigating crime and prosecutions. A prime example is France, where the "procureur" also investigates death. In most countries judicial involvement comes from the examining magistracy, who also takes responsibility for directing the investigation. Similarly, approaches based on forensic pathology services can be found in central and northeast European countries, for example, Finland.

In all countries that follow this particular system, there is a requirement for the authorities to undertake autopsies for which the next of kin have not provided consent, even though autopsies usually are performed only when there is suspicion

of a crime surrounding the death. In these cases, forensic autopsy specialists perform the autopsy. Several countries insist that two doctors participate. In contrast, medicolegal autopsies in Germany necessitate a court order and the consent of relatives. Nevertheless, if consent is not available, there is power to continue with the autopsy. The law in Germany makes a distinction between an external examination of the body, which is called a "postmortem" examination, and an internal examination, which is known as an "autopsy."

In countries that follow this system of death investigation, public judicial hearings into death are rare, except when investigations may lead to criminal prosecutions or if the decision not to prosecute is challenged in court. Deaths that occur in prison or detention typically do not have any special procedures. Self-inflicted deaths or deaths resulting from road traffic accidents also typically do not have any special procedures unless a prosecution is involved. Despite the fact that some countries have powers to investigate and autopsy deaths resulting from certain defined communicable diseases, this system routinely does not investigate deaths that are regarded as natural but rather deaths for which the causative disease is not known.

In summary, deaths that are suspicious in a criminal sense are the prime concern of this system of investigation. The system is concerned with deaths for which the specific causative disease is unknown as opposed to deaths for which the cause is natural. Furthermore, this system tends to have high rates of consented autopsies and for the most part includes public examinations involving prosecutions only.

The Medical Examiner System

The first medical examiner system was established in the state of Massachusetts (USA) in 1877. The system required that a physician known as a *medical examiner* replace the coroner. Using the system established in Massachusetts as a model, New York City developed an improved medical examiner system in 1915. Maryland soon followed in New York City's footsteps and in 1939 developed the first statewide medical examiner system in the United States.[31] The medical examiner system came about as a result of the high homicide rates and the public's general feeling that politically elected coroners were dishonest, crooked, and deficient in technical expertise, especially as the coroner could come from any professional or lay background.

The medical examiner system, which replaced many of the coroner systems in the United States, is unique in that it is directed by forensic pathologists who establish the cause of death but who generally do not inquire into the circumstances of that death. Within this system, forensic pathologists provide services to police and criminal investigation services and undertake in-house autopsies and other scientific investigation services. Many medical examiners employ specialist forensic investigators (not medically qualified but educated to degree standard) who manage cases and visit death scenes, such as forensic nurse examiners, the first of which were employed in New York City.

Medical examiners in the United States are public officials appointed by the state, city, or county. Their investigations are administrative, not judicial, but their determinations, like those of any other public official, can be contested through the courts. Currently the responsibility for death investigation in the United States lies not with the federal government but with individual states, which can assign the responsibility to individual cities and counties. Today, state medical examiners can be found in 22 states, coroner systems in 11 states, and a combination of the

two in 18 states. Similarly, Canada has medical examiner systems but in only four of its 12 provinces.

To discover the cause of death, whether by disease or injury, is the function of the medical examiner. However, medical examiners do not investigate the circumstances of individual deaths. Judicial inquests take place in a few US states, but they are infrequent. The decision to convene an inquest can be made by the district attorney, the elected official of the city, or the county administration. It is interesting that in Canadian medical examiner jurisdictions, inquests are held by the mainstream judiciary and are more common than in the United States. Nevertheless, inquests, when they occur, are presided by a judge from the state, city, or county bench. Medical examiners can and do make contributions to epidemiologic and preventative health literature.

The Coronial System Outside the United Kingdom

The coronial system was exported by early emigrants from England who settled in other countries, now known as those within the Commonwealth, and in the United States. The coroner system was brought to the United States in the early 1600s. The first recorded autopsy was performed in Massachusetts in 1647.[31] Today, elected coroners in the United States tend to be located where the state, county, or city has not been replaced by medical examiners. Many of the coroners are doctors, nurses, or lawyers who have been elected. However, the laws governing election do not impose any qualification, so even a plumber can be elected as coroner, as occurred in South Carolina (USA) in the 1980s.

Coroners' inquiries into deaths more likely occur in private than by public inquest as in England and Wales. However, traumatic cases tend to be published generally.

Two features that make the coroner systems distinctive in Commonwealth countries are as follows:

1. The coroner system is a specialist investigation service concerned only with the investigation of deaths. However, in Victoria and New South Wales, Australia, the coroner also investigates fires.
2. The coroner system, in addition to determining the medical or injury cause of individual deaths, undertakes judicial-style inquiries into the circumstances of the deaths,[32] unlike the medical examiner system.

Today, coroners are appointed rather than elected in most Commonwealth countries, and for the most part they must be a doctor or lawyer. British Columbia (Canada) is an exception to this rule, as lay people can be appointed there. Slightly different also is Quebec (Canada), where the service uses doctors for much of its work but uses lawyers to conduct the inquests. In Australia, death investigation is the responsibility of the individual states, and in Canada the responsibility lies with the provinces. Both Australia and Canada have made substantial reforms to the coroner system compared to most other countries. Statutes and structures were implemented throughout the 1990s in Australia and Canada, compared to some states in the United States, such as South Carolina, which still follows coroner's rules from the 1860s.

Despite the many variations within coroner systems around the world, the common development has been the advancement of a single coroner service for a state or province rather than multiple, locally appointed judicial-style city and

county coroners who hold a substantial number of public inquests. Instead, coroners today are involved in providing the following:

- Objectives, procedures, and standards set centrally for the service as a whole
- Chief coroner in charge who is supported by a headquarters organization and staff
- Training and quality control processes
- Service to the deceased and the family as opposed to a public act, ensuring that the process is private and administrative rather than public
- Public inquests that are held on a much smaller scale than in the past and that are discretionary in that they are chosen for their predicted ability to reveal general risks or system weaknesses and to produce recommendations that enhance public safety[32]

In summary, the literature has shown that, in certain instances around the world, nurses are playing important roles in death investigations. The next section begins by critically analyzing what is meant by forensic nursing as a lead-in to the role of the nurse in death investigation.

Forensic Nursing Defined

There is a preconceived misconception in the United Kingdom that forensic nursing involves only mental health/psychiatric nursing within institutional care units and prisons. This is not the case. In fact, women have been practicing forensic-type services as early as the 13th century, when they were involved in examinations confirming the virginity of women who were marrying into royalty or evaluations of sexual assault victims. Nevertheless, the 1990s saw a significant paradigm shift from considering forensic nursing as involved only in the mental health arena to today where it actually involves custody nursing, sexual assault nurse examiners, and nurses working in accident and emergency units. In addition, some nurses have pursued other careers and now are working as coroners' officers, forensic anthropologists, and lawyers.

To date within England, forensic nursing for the adult and pediatric branches of nursing is a very small but developing new subspecialty within the profession.[33] Custody nursing was developed, piloted, and implemented by the Kent Police Constabulary in 2000, Metropolitan Constabulary in 2001, Leicestershire Constabulary in 2002, and Humberside in 2003 and now is being considered by many other police forces, including Nottinghamshire and Derbyshire. The role of the sexual assault nurse examiner was developed in 2001 and currently is practiced in Greater Manchester, Lancashire, and London. Again many other police forces are considering the implementation of this development. All nurses working within accident and emergency units throughout the country have always examined the victims of trauma, but to date the role is unrecognized. Additionally, many registered nurses are making a major contribution to the coronial system and are respected by coroners and forensic pathologists throughout England and Wales for their work.[28] Despite these tentative and innovative developments, few members of other professions allied to the medicolegal system, including nursing at present, acknowledge, discuss, or analyze these roles to any great extent. Instead, forensic nursing, with the exception of forensic psychiatric nursing, remains somewhat unrecognized in the United Kingdom. However, a document from the Home Office in 2000 entitled

"Protecting Vulnerable People" has started to recognize the public's needs with regard to child protection, domestic violence, and elderly abuse.[34]

In contrast, advanced developments throughout the world and typified by the United States are widely accepted and grounded in evidence-based practice. Forensic nursing in the United States originally defined its role in the field of death investigation as a medical examiner's investigator.[35] The term *forensic nursing* was officially coined in the United States in 1992 when about 70 nurses gathered in Minneapolis, Minnesota, for what was billed as the first national convention for sexual assault nurses. Many years ago the United States recognized that nurses could bridge the gaps in many areas of medicolegal work resulting from a shortfall in medical staffing. Currently their principal roles include forensic nurse death scene examiner, custody nurse, sexual assault nurse examiner, clinical forensic nurse examiner in hospital emergency rooms, and nurse coroner. They have a national education program, national practice standards, quality assurance, and an association called the International Association of Forensic Nurses. Interestingly in the United States, often the forensic nurse rather than the forensic medical practitioner has the principal role, although the two work in partnership for the benefit of crime investigation and reduction, death investigation, and health care.[36]

In summary, forensic mental health nursing has a long history in the United Kingdom and is recognized in its own right as a nursing specialty. However, the literature demonstrates that the discipline of forensic nursing internationally is an umbrella term that encompasses not only forensic mental health nursing but also nursing within the arena of death investigation, sexual assault, legal nurse consulting, and clinical forensics.[36]

Death Investigation and Forensic Nursing in the United States and Canada

Nurses have served as field death investigators for Canadian medical examiners since the 1970s, when nurses in Alberta began receiving training. This trend moved across the Canadian provinces into the United States. In fact, the theoretical model of forensic nursing evolved from the role of the police surgeon (forensic medical examiner) in the United Kingdom and other European countries, as no such posts existed within the United States or Canada. Historically in the United States, a gap had existed among clinical practitioners, criminal justice system employees, and forensic science operatives, particularly because of a lack of communication and coordination. Previously this gap was either left open or filled by physicians and nurses who lacked forensic training, which resulted in misinterpretation and/or omission of valuable forensic evidence. Lynch[37] believed the failure of physicians and nurses to recognize the legal issues surrounding patient care robbed victims of the support and, in some cases, the evidence needed to validate their victimization. In order to understand the foundation upon which forensic nursing was built in the United States, it is necessary to examine the history of clinical forensic medicine, or "living forensics" as it sometimes is labeled today.

Living forensics is a field of inquiry that was brought to the attention of clinical nursing by Harry C. MacNamara, Chief Medical Examiner for Ulster County, New York (USA). Although the concept of clinical forensic medicine and its applications to the living was new to the United States, it had been widely practiced in the United

Kingdom and other parts of the world for the past 200 years. In 1988 MacNamara[38] defined clinical forensic medicine as

> "the application of clinical medicine to victims of trauma involving the proper processing of forensic evidence."

This concept stresses the importance of the health care team being sensitive to the legal issues surrounding their patients. It was believed that the resources available for help from law enforcement and the courts, as well as from the community and social service organizations, must include health care services. No longer could health care providers work in isolation from the legal issues previously delegated to law enforcement. Instead it was believed that the responsibility for maintaining a high index of suspicion and protecting victims belonged to health care professionals. It also was held that providing the necessary leadership in routinely identifying, treating, and properly referring victims of child, elder, and spouse abuse to appropriate authorities was the responsibility of health care professionals.

The practice arena included registered nurses, physicians, physician's assistants, paramedics, and emergency medical technicians. It also included community service professionals (e.g., police officers, court officials, and attorneys) interacting with victims. These professionals were expected to be able to recognize the problems in the existing system and alert other trained personnel to potential solutions. To this end, it was believed necessary to establish and train professionals in the philosophy of living forensics, beginning with emergency interventions.

In tandem with these beliefs was the recognition of an epidemic of violence and its associated trauma that resulted in a critical health care problem not only in the United States but throughout the world. In 1985 the US Surgeon General's Workshop on Violence and Public Health focused on sexual assault, physical assault, homicide, and spouse abuse. Victims from these scenarios constituted the target population already identified by emerging forensic nurses who were tenaciously maintaining a holistic view of intervention strategies.

Since then, the Joint Commission on Accreditation of Healthcare Organizations (JCAHO) has issued standards for emergency rooms and ambulatory services. The standards address all forms of abuse, including child abuse, sexual assault, domestic violence, and elder abuse. Meetings between staff members of the American Medical Association, the American Hospital Association, and the Education Development Centre, Inc., have been convened to develop model protocols, educational materials, and training programs that will assist health care providers to comply with the JCAHO standards.

Lynch[37] believed many cases of death encountered in the clinical setting fell within the jurisdiction of the medical examiner or coroner and believed it vital for clinicians to be skilled in the documentation and preservation of evidence related to those cases. Nurses, particularly emergency room nurses, often were the first personnel to come in contact with the victim and the evidence even before the police were notified that a crime had been committed. Additionally, with the advent of advised prehospital (paramedic) care, many trauma patients bypassed the emergency room and were admitted directly to the surgical or intensive care unit. This represented an opportunity for nurses to make a significant contribution to public health and safety as nursing embraced the continuum of the life cycle – caring for individuals at the time of death as well as the moment of birth. In fact, the events that immediately precede and occur at the time of death are crucial factors in the medicolegal investigation of death.

The focus of forensic nursing was clearly identified as a vital intervention by health care in advocacy and administration to victims of violent crime – the survivors, the deceased, and the families of both. The wide range of components that defined this focus may have been confusing to those without knowledge of the forensic sciences. Yet it was the body of knowledge in its entirety that provided its strength. The problems identified in US society were great and multifaceted and required education and expertise that were equally diversified. In truth, the combined efforts of forensic science, medicine, law, nursing, and public health were required to deal with the complex problems of violence.

Forensic nursing today in the United States provides health care responses to the sequelae of criminal and interpersonal violence. According to Lynch,[37] nurses are being challenged to share responsibility with the legal system in order to augment available resources for individuals with liability-related injuries and crime victims and for prevention of injury or death by early detection of potentially abusive situations, which are critical steps in stemming the effects of human violence. The responsibility of the forensic nurse in legal issues brings this new specialist in line with the concept of the nurse investigator, providing continuity of care from the acute care setting and/or crime scenes to the courts of law.

Forensic nursing within the field of death investigation as a discrete discipline was recognized in the first formal paper delivered on the subject at the American Academy of Forensic Sciences (AAFS) annual meeting in New Orleans, Louisiana (USA), in February 1986 following its innovation in Canada during the 1970s.[39] Despite this innovation nearly 20 years ago in the United States and more than 30 years ago in Canada, there is a lack of research on the nurse's role in death investigation. In fact, all published papers are discussion papers, which is understandable given that the subspecialty is so new. However, no published research has promoted knowledge and moved the forensic nurse forward despite the enormous achievements in the field in such a short period of time. One exception is the research performed by Rutty,[40,41] who explored the meaning of the nurse coroner's role in South Carolina.

Rutty[41] discovered through hermeneutic inquiries that nursing brings to the work of the coroner the encompassing roles of holistic family caregiver, researcher, manager, investigator, educator, health promoter, pathology assistant, and forensic nurse clinician. The nurse also brings to the role knowledge, clinical skills,and holistic experience in clinical care/intervention, anatomy, and physiology; psychology, sociology, health studies, and people skills/family dynamics; growth and development; and cultural/language interaction. The range of responsibilities practiced by nurses includes attending death scenes, photography, anthropology, fingerprinting, computerized facial reconstruction, and management of multidisciplinary forensic teams. As advocates for the deceased and family, nurses handle the issues of death certification, victim support, family notification, survivor grief resolution, media management, criminal proceedings, and probate. Nurse coroners believe they are making a difference in their community by promoting health and education (e.g., disaster preparedness and prevention of preventable deaths) and very interestingly by assisting in the return of stolen gravestones (a particular problem in South Carolina). Nurse coroners firmly believe they are providing holistic family health care within the legal framework of death investigation from initial presentation of a suspicious death through to family care and support that may continue for many years.

Death Investigation and Forensic Nursing in the United Kingdom and Europe

The assumption that nurses who perform their everyday work correctly will never be involved in any coronial matter is naïve. As discussed, Green[13] suggested that nursing staff in general are becoming increasingly more involved in legal processes, particularly with regard to their observations, records, and recall of events. In contrast, internationally nurses have embraced more extensively their role within aspects of the legal profession. The ultimate example of the ability of nurses to be coroners is seen in the United States, in Susan Chewning's office in Charleston, South Carolina. Sadly, this role of the nurse in the United Kingdom has been poorly addressed, and many nurses themselves do not consider this an area in which they should be involved. In addition, except for the research by Rutty,[40–42] no peer-reviewed published articles within the nursing or medical literature have evaluated, debated, or reflected on nursing practice in the United Kingdom within this area.

It seems that the final service to our patients and family care has not been considered in-depth. Death investigation is an area of great upheaval in people's lives, yet it seems to have been forgotten by the nursing profession in the United Kingdom. The Department of Health and Social Security in the 1970s stated that regardless of the changes in the nurse's professional role, the essence of the profession would always be about caring for people.[43] Henderson[8] in 1966 also referred to care when she said that nursing would never be seen as anything less than essential to the human race. The RCN in 1992 even stated that nursing is based on a combination of professional knowledge and skills, with the desire to care for others.[43] MacDonald[30] in 1999 inferred that coroner's officers who are either police officers or lay people often poorly understood circumstances surrounding death.

Henderson[8] believed that part of the nurse's role not only is assisting our patients to a peaceful death but also supporting all patients "in death." Davis[17] took this one step further by suggesting that the role of patient advocacy goes "beyond death." Rutty[28] showed that nurses in the United Kingdom are contributing regularly to the coroner's inquiry and death investigation, whether in a hit or miss fashion, particularly when compared to other expanding and advancing nursing roles.

After reviewing the literature, it appears that an area of health care for patients, clients, and families in the United Kingdom and Europe may have been missed or forgotten by the nursing profession compared with their international colleagues. Countries such as the United States and Canada are providing a nursing service within the death investigative process. In the year 2000, 201,000 deaths were reported to the coroner, which is 37% of all deaths in England and Wales.[44] Of note, associated with these reported deaths were 201,000 bereaved families involved in coronial inquiries and death investigations. Perhaps expanding and advancing the nursing role within this arena will provide valuable nursing care that currently is not being delivered and is leading to a gap in health care provision. Nurses need to take notice of the advancements in the field being made by international colleagues and evaluate the benefits, particularly of the new specialties emerging within forensic nursing, with respect to clinical expertise, education, research, career pathways, multiagency development, and policy/standards.

If nursing is to continue to successfully develop and advance its practice in the United Kingdom and Europe, it must be complementary to medicine as it evolves in response to society's changing demands. Medicine has always had a working

relationship with nursing, except in pathology and forensic medicine. It now may be time to extend the interdisciplinary support and assistance to include the forensic physician and forensic nurse as a cooperative team.

Summary

Forensic practice in nursing is not new to the United Kingdom, particularly with regard to mental health nursing. However, other branches of nursing involved in forensic practice provide new, expanded, advanced roles for the nurses of this century. Forensic practice has been making a difference in health care areas that traditionally have not always been part of the National Health Service, but importantly it is beginning to make a difference in the legal arena. Nursing has always been diverse. Nursing has always been about caring. The most vulnerable groups in our society have always needed nursing. Perhaps there is a need for the new forensic nurse examiner to be given the opportunity to be there for these individuals within the realms of death investigation.

It is time for nursing to take the lead in building multidisciplinary partnerships in clinical practice, education, and research, but it is imperative that the underlying intention of upholding and promoting patient advocacy and family health care remains. After all, caring is the central concern and essence of nursing.

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2. Postmortem Analysis for Inherited Ion Channelopathies

Kathryn A. Glatter, Nipavan Chiamvimonvat, Yuxia He, Philippe Chevalier, and Emanuela Turillazzi

Brief Overview

Coroners and pathologists commonly face the evaluation of young people who die in an unexplained fashion with an otherwise normal autopsy. Unfortunately such events are not rare. We begin this chapter by discussing how to collect blood and tissue samples for genetic testing of diseases causing unexplained death. We next outline the most common genetic causes of sudden death under the sections of "Disorders of Heart Muscle" (including hypertrophic cardiomyopathy and arrhythmogenic right ventricular dysplasia) and "Disorders of Ion Channels" (including the long QT syndrome, Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia). We describe the epidemiology, clinical findings, autopsy/pathologic findings, pathophysiology, and treatment options for each disease. Several tables summarizing the genes found, key features of some of the diseases, and actual electrocardiograms of patients with ion channelopathies are provided. Several excellent histologic and pathologic specimens taken from autopsies of affected patients are shown.

Introduction

In the developed world, sudden cardiac death (SCD) remains the major cause of death.[1–6] However, an increasing number of genetic causes of sudden death that may strike young, otherwise healthy people have been identified. It is important to be familiar with these genetic causes of SCD and the diagnostic difficulties they present so that they can be considered as possible causes of death if the autopsy is otherwise normal. We provide an overview for forensic pathologists on how to collect tissue samples for molecular testing of these entities. We discuss the clinical presentation; genetic, molecular, and cellular abnormalities; and diagnostic evaluation of these causes, including genetic cardiomyopathies and ion channelopathies.

The clinical pathologist should consider these diseases when the autopsy findings do not explain how the person died. There often is a genetic basis for these disorders, and such information could be given to surviving family members with the hopes that affected members can be offered treatment while they are still alive.

Autopsy Protocol in Sudden Cardiac Death

The pathologist has a unique opportunity to study SCD resulting from all manner of cardiac diseases. Myocarditis, cardiomyopathies, coronary artery and other congenital anomalies may be identified. However, there is occasional doubt as to the true cause of death in patients with SCD. There probably is nothing more frustrating for the pathologist than to perform an autopsy and come up with no significant findings.[7]

The four steps in the investigation of a sudden death are (1) obtaining the history and scene information, (2) performing a gross and microscopic autopsy, (3) performing appropriate laboratory tests, and (4) making the diagnosis. When examining the heart grossly, it is important to preserve the anatomic landmarks, section the coronary arteries closely, and recognize lethal abnormalities such as anomalous origin of the coronary arteries. Specimens useful for toxicologic analysis include whole blood, serum, vitreous humor, gastric contents, bile, urine, a purple top tube of blood, and frozen myocardium and spleen.[8]

A complete autopsy, including detailed neuropathologic and cardiovascular examination with toxicologic studies, must be performed in the context of all available clinical information and the circumstances of death. The dissection of the heart can be practiced in different ways, but we have adopted the inflow–outflow method: the cut follows the direction of the blood flow from the caval veins on the right side of the heart to the pulmonary trunk and pulmonary artery. On the left side, the atrium is opened by cutting the pulmonary veins, and the cut is continued with the dissection of the left side of the infundibulum and of the aorta. To examine the coronary arteries, different methods that are more or less complicated have been introduced.

We have adopted the following method to examine the heart in every case of sudden death. The heart is removed from the pericardium by cutting the great vessels, all cavities are cleaned, and the heart is weighed and examined on the surface. The heart is left in a large container containing a 10% formalin solution for 24 hours. Coronary arteries and each segment are cross-sectioned at 3-mm intervals along their whole course by carefully avoiding any damage. The lumen reduction of coronary arteries must be expressed as a percentage of the lumen diameter calculated from plastic casts of normal vessels. The whole heart is dissected into 1-cm slices parallel to the posterior atrioventricular sulcus, taking care to proceed from the apex to the base. The last upper slice is cut on the plane of the left ventricular papillary muscles. The heart slices, the atrioventricular valvular levels section, and the coronary segments are disposed in their anatomic sequence and photographed with a scale in color. Each histologic myocardial section (excluding epicardium and endocardium) should be measured by an image and analysis system. The numbers of both foci and myocardial cells with pathologic alterations must be normalized to 100 mm.[2,4]

Samples for full toxicologic analysis should be obtained. Blood, urine, liver, bile, ocular fluid, and gastric contents should be retained for potential analysis. In addition, samples of peripheral blood and tissue should be retained for future genetic studies.

Molecular Diagnosis of Sudden Death Diseases

With the explosion of molecular techniques, deoxyribonucleic acid (DNA) testing on peripheral blood and tissue has revolutionized the diagnosis of genetic causes

of sudden death. We describe the basic methods of tissue preparation and DNA analysis as a useful overview for the clinical pathologist and coroner.

Collection of Deoxyribonucleic Acid from Blood Samples

It is easiest to amplify DNA that will be used for genetic testing when the DNA is taken from blood samples.[9,10] Ideally, at the time of autopsy the coroner or pathologist collects 15 ml of blood in several tubes containing ethylenediaminetetraacetic acid (EDTA), which prevents coagulation and degradation of the DNA. The tubes are stored at 4°C until the DNA is extracted for analysis, which should be within 1 week, although sometimes we have extracted DNA 4 months after collection. If the blood samples are collected in tubes that do not contain an anticoagulant, the DNA should be extracted promptly (within days of the initial collection).

Collection of Deoxyribonucleic Acid from Tissue Samples

Extraction of high-quality DNA from tissue that can be used for polymerase chain reaction (PCR) amplification is much more problematic than use of blood samples. It often is difficult to amplify long fragments of DNA from formalin-fixed and paraffin-embedded tissue because of the fixation time in the formalin, the often long storage time in the tissue blocks prior to analysis, and the formation of formic acid in the sample.[11,12] Formic acid hydrolyzes the DNA and creates single-strand nicks in the DNA. In postmortem tissues fixed in nonbuffered formalin (usually in tissue preserved more than 20 years ago), DNA fragments longer than 90 base pairs cannot be amplified.

A variety of published methods for extracting DNA from preserved tissue are available.[13,14] Many involve a phenol-chloroform digestion and washing step. Commercial kits are available that may simplify the methodology. One article described a "pre-PCR restoration process" in which the single-strand DNA nicks are repaired with Taq polymerase prior to PCR amplification, which greatly improved the length of DNA pieces that could be amplified.[15]

An alternative method for obtaining usable DNA from tissue is to snap-freeze fresh myocardial tissue collected at autopsy in liquid nitrogen and store at –80°C until DNA extraction is performed. Tissue processed in this manner can be used many months later.

Clearly, collection and preservation of tissue or blood samples for future DNA analysis is cumbersome, time intensive, and costly. However, it is helpful for the pathologist or coroner to carefully preserve such biologic material for future DNA testing in cases where a genetic cause of sudden death is suspected, as outlined later. Affected persons could be identified and offered treatment while they are still alive.

Disorders of the Heart Muscle

Hypertrophic Cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is one of the oldest known causes of sudden death. It was first described in 1958.[16] It has been called hypertrophic obstructive

cardiomyopathy and idiopathic hypertrophic subaortic stenosis, despite the fact that 75% of affected patients do not have a sizable resting outflow gradient.[17,18] It is a polygenic, relatively common genetic cause of sudden death, particularly in young athletes.

Epidemiology

Hypertrophic cardiomyopathy is the most common genetically associated form of SCD. An estimated 1 in 500 people (0.2% of the general population) carries an HCM genetic mutation.[19,20] However, the phenotypic presentation or clinical penetrance of the disease is much lower. Most patients with an HCM mutation do not show signs of the disease during life.

Clinical Features

When present, the hallmark feature of HCM is myocyte disarray.[18,21,22] The clinical diagnosis of HCM during life is made most reliably by echocardiography. Severe ventricular wall thickening can be seen (Figure 2.1). Normal left ventricular wall thickness generally is less than 12 mm, and thicknesses greater than 30 mm are not unusual in cases of severe HCM.[23–28] This marked septal hypertrophy often is an age-dependent effect and may not be seen initially in young patients. In most cases, the left ventricle may be affected diffusely or may demonstrate asymmetric septal hypertrophy.[21–28] In contrast, in the Japanese variant of HCM, the apical left ventricle is primary affected and shows abnormal thickening.[21–28]

At autopsy using detailed pathologic examination, a frequently finding is hypertrophied myocytes with bizarre shapes, chaotic cellular alignment, and gross cellular disarray in the left ventricle (Figures 2.2 and Fig 2.3).[29–33] Patchy areas of myocardial scarring and fibrosis are sometimes noted and are believed to result

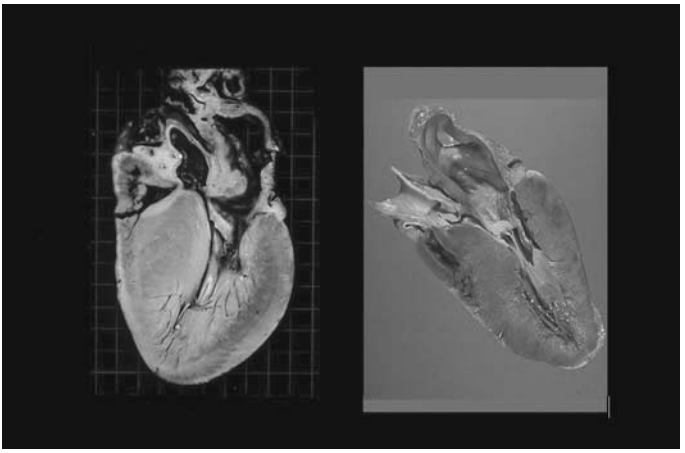

Fig. 2.1. Macroscopic view at autopsy of heart taken from two different subjects found to have hypertrophic cardiomyopathy. **Left:** Marked asymmetric left ventricular hypertrophy. **Right:** Symmetric left ventricular hypertrophy.

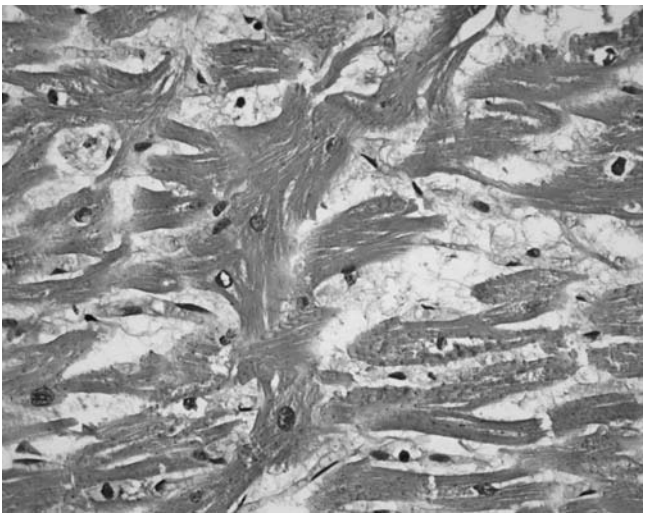

Fig. 2.2. Typical findings on autopsy of histologic examination showing myocyte disarray in the left ventricle of a patient with hypertrophic cardiomyopathy (hematoxylin and eosin, original magnification ×40).

from abnormal intramural coronary arteries.[34,35] From a practical standpoint, pathologic disarray can be diagnosed when it involves more than 20% of the myocardium in at least two tissue blocks.[36] In these cases, the detection of myocardial disarray is a highly sensitive and specific marker of HCM.[37]

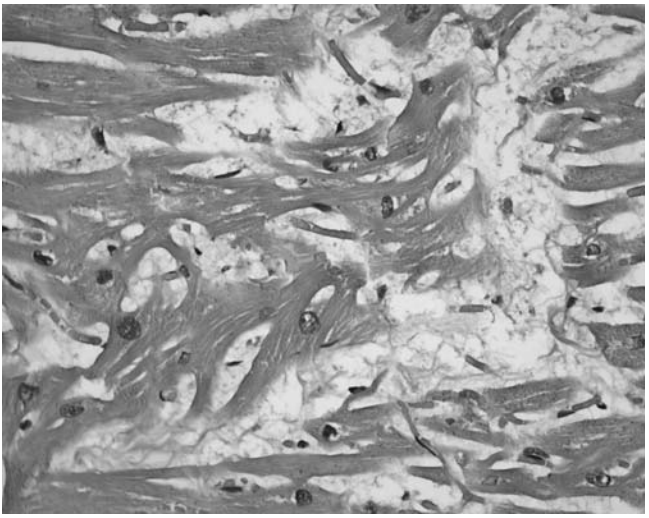

Fig. 2.3. Histologic examination of a patient with hypertrophic cardiomyopathy showing classic myocyte disarray in the left ventricle (hematoxylin and eosin, original magnification ×40).

Pathophysiology

Syncope in these subjects may be caused by arrhythmias or obstruction resulting from ventricular hypertrophy and cavitary obliteration.[18,23,24] Dehydration can trigger a syncopal event in such patients. Sudden death is thought to occur in HCM as a result of a primary electrical abnormality by ventricular arrhythmias.[18,23,38–41] In support of this view, one large study of HCM patients in whom defibrillators were implanted demonstrated that nearly 25% of the patients had documented ventricular arrhythmias over a 3-year follow-up period.[42]

The disease may be progressive in some patients.[18,23,24] The myocyte hypertrophy continues over years in a clinically silent manner and ultimately may lead to an end-stage, dilated cardiomyopathic picture. Depending upon the time frame during which the patient is evaluated, the HCM-affected heart could appear grossly normal, markedly hypertrophied, or even dilated, making the diagnosis difficult.

Genetics

The polygenic and multicellular nature of HCM makes it a frustratingly complicated disease to diagnose unless gross histopathologic abnormalities are found on echocardiogram or at autopsy. At least 10 different genes encoding the cardiac sarcomere have been implicated in HCM.[18,23,24,43] More than 150 unique mutations have been reported since the first genetic cause for HCM was identified in 1990.[44] Most such mutations are missense mutations found in the proteins of the cardiac sarcomere and are located in the β-myosin heavy chain, cardiac troponin T, or myosin binding protein C.[43–48] Although the disease is autosomal dominant, a family history of syncope or sudden death may be lacking, and the disease has widely variable clinical penetrance. Within the β-myosin heavy chain gene (*MYH7*), numerous mutations have been described as malignant mutations associated with a poor clinical prognosis.[47–50] These particular mutations seemed to be associated with a severe clinical phenotype, including progression to end-stage heart failure or sudden death, a relatively high penetrance of the disease, and extreme left ventricular wall thickness (Table 2.1).[18,23,24,41,46,47]

Treatment

There are no formal guidelines for treating asymptomatic patients with HCM.[18,23,24] In patients with symptoms of shortness of breath, medical therapy with drugs that reduce the outflow gradient remain the mainstay of therapy.[18,23,24,51,52] Such medications include beta-blockers and calcium channel blockers. In very symptomatic patients with a large (>50 mm) gradient, the outflow gradient can be reduced by surgical myomectomy or catheter-based alcohol ablation.[18,23,24,53,54] The latter is a

Table 2.1. High-risk features in hypertrophic cardiomyopathy.

- Family history of sudden death
- High-risk genotype (e.g., Arg719Gln)
- History of sustained ventricular arrhythmias
- Previous cardiac arrest
- Exertional syncope
- Massive left ventricular hypertrophy (≥30 mm)

relatively new technique that causes a controlled myocardial infarction and thus reduces the outflow gradient. In patients deemed at high risk for an arrhythmic event (see Table 2.1), an implantable cardioverter-defibrillator (ICD) may be implanted to avert sudden death.

Autopsy findings

The classic anatomic form of HCM described by Teare[16] involved thickening of the basal anterior septum, which bulges beneath the aortic valve and causes narrowing of the left ventricular outflow tract.[55] The characteristic gross morphologic feature is a hypertrophied and nondilated left ventricle.[20–22] It rarely is a "symmetric" or diffuse concentric hypertrophy of the left ventricle.[36] The majority of cases present as "asymmetric" hypertrophy that may involve any portion of the ventricle. Generally, the maximal increase occurs in the interventricular septum (interventricular septum/free wall ratio = 3:2), which, as a result, protrudes into the left ventricular cavity, drastically reducing the cavity size. This obstruction generally involves the mediosuperior portion of the interventricular septum. Frequently, the pattern of wall thickening is heterogeneous, and contiguous segments of the left ventricle may differ greatly in thickness.[32–35]

Arrhythmogenic Right Ventricular Dysplasia

Arrhythmogenic right ventricular dysplasia (ARVD) is a newly recognized disorder that is a cause of unexplained sudden death in otherwise healthy young adults, particularly young athletic men.[56–58] Affected patients may have grossly normal heart function, especially in the early stages.

Epidemiology

The true incidence of ARVD is unknown. In a prospective, autopsy-based study of the Veneto region of northern Italy, 20% of unexplained sudden deaths in subjects younger than 35 years had ARVD, including 22% of young athletic men who died suddenly in the region.[59] Whether northern Italy simply has an abnormally high incidence of the disease or if this finding reflects the true incidence of the disease is unclear. However, it likely is a much more common entity than initially appreciated given that most cases go undetected.

Clinical Features

Unfortunately, the initial clinical presentation of ARVD often is unexplained sudden death in a healthy athletic male. Patients experience ventricular arrhythmias from the diseased right ventricle (RV), ranging from benign premature complexes to ventricular tachycardia or even ventricular fibrillation and cardiac arrest.[60–63] It was first described briefly in 1961 and in greater detail in 1977.[64,65] The Study Group on ARVD/C has defined specific criteria to aid in the diagnosis of ARVD (Table 2.2).[66]

Electrocardiographic (ECG) findings include complete or incomplete right bundle branch block during normal sinus rhythm, with T-wave inversion on leads V_1 through V_3. An epsilon wave, a terminal notch in the QRS, may be present.[56–58,60–62] The signal-averaged ECG also is characteristically abnormal.[66]

Table 2.2. Diagnostic criteria for arrhythmogenic right ventricular dysplasia.

- Family history
  Confirmed at autopsy
- Structural findings
  Right ventricular global hypokinesis with preserved left ventricular function
- Arrhythmias
  Right ventricular tachycardia or premature beats
- ECG Findings
  Epsilon wave of QRS on leads V_1 through V_3
- Late potentials on signal-averaged electrocardiogram

Echocardiographic findings may be normal or reveal a variety of abnormalities in the RV, including RV wall thinning, dilatation, or dysfunction.[56–58,60–62,67] Cardiac magnetic resonance imaging (MRI) can be useful because it may reveal fibrofatty infiltration of the RV free wall.[68,69] Biopsy of the RV septum (done in the septum and not in the free wall because of free-wall thinning) often is not helpful because involvement of the septum in ARVD is sporadic.

Arrhythmogenic right ventricular dysplasia is one of the few genetically based causes of sudden death that can be identified at autopsy, at least in grossly abnormal cases. The pathologist may find diffuse or segmental loss of myocardium in the RV free wall, with concomitant replacement with fibrofatty tissue.[56–58] Two thirds of such patients have patchy, acute myocarditis-type of findings with lymphocytic infiltration and cell death.[60–62] Up to 50% of ARVD patients have RV aneurysms at autopsy.[60–62,66]

Patients can have progressive dilatation and failure of the RV over time, which also occasionally involve the left ventricle, leading to a diffuse cardiomyopathy. One study found that 76% of ARVD subjects had histologic involvement of the left ventricle.[66]

Pathophysiology

The pathophysiology of ARVD is unclear. It likely is a complex interplay among genetic predisposition, cellular mechanisms, and unknown environmental factors.[56–58,66] Several consistent features of ARVD can be noted: apoptosis (programmed cell death), a component of inflammatory heart disease (e.g., acute myocarditis), and myocardial dystrophy. The disease is progressive over decades in some patients, whereas for unknown reasons it is relatively quiescent in other patients.

Genetics

At least seven distinct chromosomal loci associated with ARVD have been located.[70–74] These loci include two on chromosome 10, two on chromosome 14, and one each on chromosomes 1, 2, and 3. One autosomal recessive form of ARVD called Naxos syndrome is associated with palmoplantar keratoderma and woolly hair.[75] It results from a mutation in the gene for plakoglobin. Another syndrome found in Ecuador involves a recessive mutation in the gene for desmoplakin.[76] Both proteins are components of desmosomes, which form the major cell adhesion junctions. Currently no commercial genetic tests for diagnosis of ARVD are available.

For most cases of ARVD, the genetic linkage is unclear. Up to 30% to 50% of cases have an associated family history consistent with ARVD (including sudden death).[60–62,66]

Treatment

No consensus exists on the treatment method for ARVD.[66] In patients who have survived cardiac arrest, placement of an ICD is generally recommended to prevent sudden death.[77] Pharmacologic therapy with beta-blocker or antiarrhythmic medications also has been suggested.[66] Radiofrequency ablation during electrophysiologic (EP) study of ventricular arrhythmias has been attempted.[78]

Autopsy Findings

Arrhythmogenic right ventricular dysplasia is characterized morphologically by the fibroadipose replacement of ventricular myocardium tissue.[79] The disease may be segmental or involve the RV diffusely. When the disease is focal, it most frequently is located at the angles of "the triangle of dysplasia": the pulmonary infundibulum, the RV apex, and the inferior wall of the RV.[80] Upon gross examination, the heart discloses little or no dilatation of the RV and transmural fibrofatty replacement of the RV musculature (Figure 2.4).[61] In ARVD the spectrum of the morphologic appearance may range from concealed RV myopathic changes to biventricular cardiomyopathy. In fact, the disease may be often overlooked even in autopsies. The RV should be extensively sampled for histopathologic analysis in all cases of sudden death, especially those associated with strenuous exercise and young age.[81]

Histologically, ARVD is characterized by substitution of the myocardium with adipose or fibroadipose tissue (Figures 2.5 and 2.6). Inflammatory infiltrates with focal myocyte necrosis are often present. The following pathologic features must be assessed and graded in histologic specimens: myocardial atrophy and fatty replacement; myocardial fibrosis; myocyte degeneration or necrosis; and interstitial cell infiltrates.[61]

Fig. 2.4. Macroscopic view of the heart at autopsy of a 27-year-old healthy young man who died suddenly. Subsequent histologic examination of the heart determined that he died of arrhythmogenic right ventricular dysplasia.

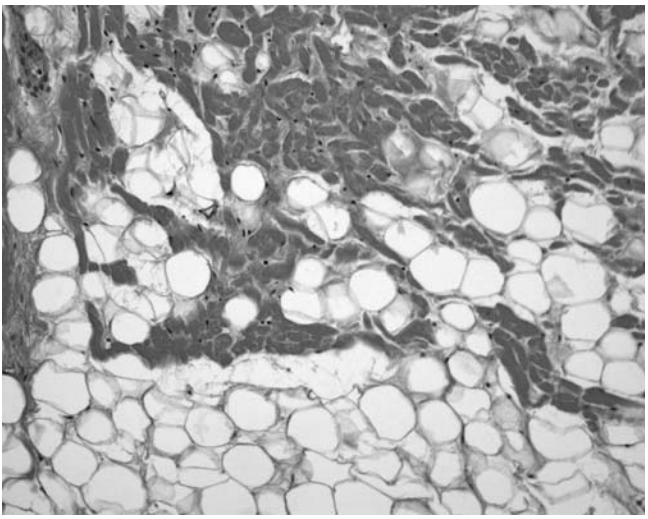

Fig. 2.5. Histologic examination of the right ventricle taken at autopsy of the young man shown in Figure 2.4. Note marked fatty infiltration of the right ventricle without fibrous tissue (trichrome, original magnification ×20).

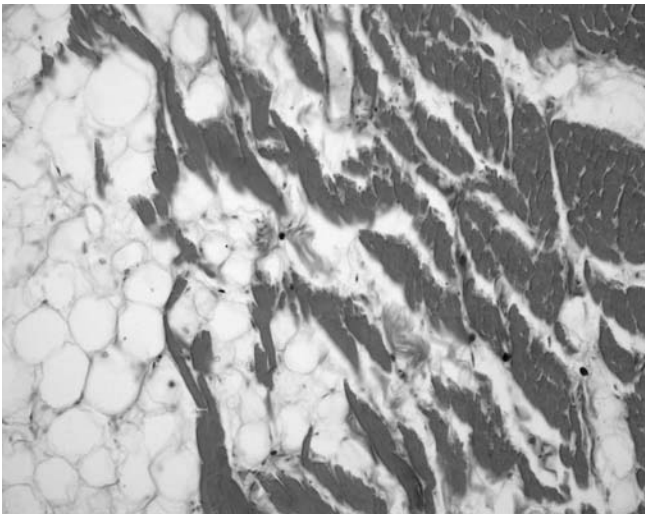

Fig. 2.6. Histologic examination of the right ventricle at autopsy of the young man with arrhythmogenic right ventricular dysplasia shown in Figures 2.4 and 2.5. Note other patchy areas with infiltration of the myocardium by both fatty and fibrous tissue (trichrome, original magnification ×40).

Disorders of the Ion Channel

Long QT Syndromes

Long QT syndrome (LQTS) is one of the more common and well known of the ion channelopathies.[82,83] It can be inherited as a dominant gene or can be seen in cases of acquired LQTS occurring after consumption of common drugs such as antipsychotics, antiarrhythmic agents, and allergy medications.[84,85]

Epidemiology

Currently an estimated 1 in 5000 persons carry an LQTS genetic mutation.[82,83] Including drug-induced or acquired LQTS cases, many of whom have the same genetic ion channel defects as those seen with congenital LQTS, some experts believe the true incidence of LQTS actually is 1 in 1000.[86,87] Certainly it is one of the more common genetic causes of sudden death and has been diagnosed with increasing frequency as more pathologists are educated regarding LQTS.

Clinical Features

At least 10% of affected LQTS patients present with sudden death as their first (and last) symptom.[88–91] However, most patients with an LQTS mutation never experience any symptoms. The majority of LQTS families are discovered when a young person tragically dies suddenly but has a normal autopsy and other family members are found to have a prolonged QT interval on ECG (Figure 2.7).

Each genetic subtype (described later) has its own trigger for events (Table 2.3).[88–92] Patients with the LQT1 subtype of LQTS usually experience symptoms (syncope, cardiac arrest, or sudden death) during adrenaline-driven type activities, such as exercise and running, or with strong emotion (e.g., during an argument) (Figure 2.8). An unexplained drowning of a person who is a good swimmer ultimately could be the result of an LQT1 mutation.[88,91,93,94] LQT2 (*HERG*) mutations may cause sudden death as a result of auditory triggers, such as an alarm clock or telephone ringing.[93–95] The rare LQT3 (sodium channel) subtype may occur during sleep or during periods of slow heart rates (Figure 2.9).[88,89]

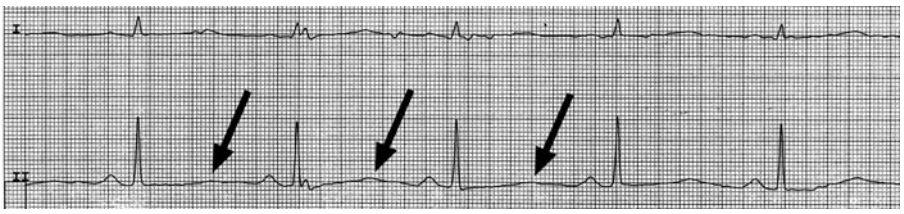

Fig. 2.7. Electrocardiogram recorded prior to death of a healthy 18-year-old girl who died suddenly. She was a volunteer firefighter who died when the fire alarm rang. Complete autopsy was negative. Other living family members subsequently were diagnosed and genotyped with LQT2. Note the flat, notched T waves consistent with the LQT2 (*HERG* gene) subtype (*arrows*).

Table 2.3. Long QT syndrome genes.

Disease	Chromosome locus	Gene	Gene product
LQT1	11p15.5	*KVLQT1*	I_{Ks}, α-subunit
LQT2	7q35–36	*HERG*	I_{Kr}, α-subunit
LQT3	3p21–23	*SCN5A*	Na channel
LQT4	4q25–27	*Ankyrin 2*	Ankyrin B
LQT5	21p22.1	*minK* (*KCNE1*)	I_{Ks}, β-subunit
LQT6	21p22.1	*MiRP1* (*KCNE2*)	I_{Kr}, β-subunit
LQT7	Chromosome 17	*KCNJ2*	I_{Kr}, α-subunit

Although LQTS is an autosomal dominant disease, females are far more likely to experience symptoms than are males.[88–92] In some cases LQTS can be diagnosed by noting a prolonged QT interval (>450 ms) on the ECG. However, up to 30% of gene-positive patients may have a normal or only borderline prolonged QT interval, making diagnosis difficult in some cases.[88–92,96] Exercise testing may reveal an otherwise concealed form of LQTS.[97,98]

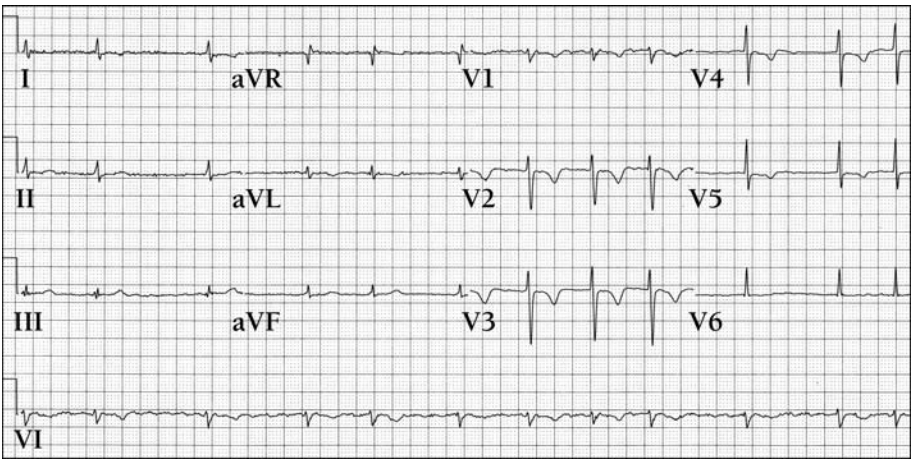

Fig. 2.8. Twelve-lead electrocardiogram recorded from a healthy 10-year old girl who collapsed and suffered cardiac arrest while she was playing on a playground. The QT interval is completely normal (QTc = 416 ms). She was genotyped with LQT1. She survived but was irreversibly brain-damaged from the arrest. There was no family history of sudden death or syncope.

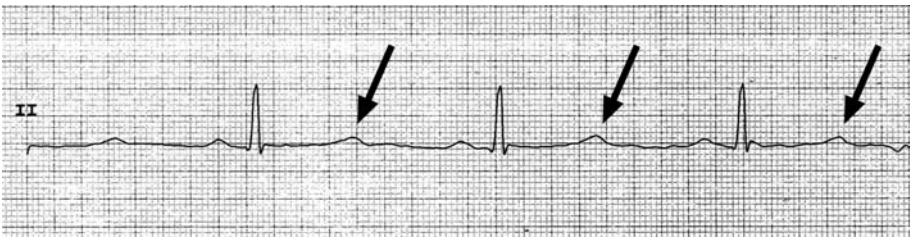

Fig. 2.9. Electrocardiogram recorded from a healthy 13-year-old girl with incidentally discovered long QT syndrome. The QTc interval (580 ms) is extremely long. There is no family history of sudden death or syncope, and the girl never had symptoms. She was genotyped with LQT3 (sodium channel defect).

A small association between sudden infant death syndrome (SIDS) and LQTS has been reported in the literature, although probably fewer than 5% of all SIDS cases result from ion channel mutations.[99,100] Other causes of SIDS likely are far more common, such as placing the infant prone, infants sleeping with adults, and inborn errors of metabolism.

Pathophysiology

The fundamental defect in LQTS is prolonged ventricular repolarization and a tendency toward torsades de pointes (polymorphic ventricular tachycardia) and ventricular fibrillation.[82,83] Beta-blocker medications (described later) do not shorten the QT interval; they are believed to act, in part, by blocking early afterdepolarizations, which initiate the ventricular arrhythmias.

Genetics

A total of seven genes causing LQTS have been identified.[101–104] The mutant ion channel that causes clinical LQTS is inherited in an autosomal dominant fashion with incomplete penetrance and originally was known as the "Romano-Ward syndrome." With the advent of genetic testing, it has become clear that each LQTS genetic subtype is a unique disease with different triggers to arrhythmias. The genes that encode the potassium channels *KVLQT1* (on chromosome 11) and *minK* (on chromosome 21) interact to form the cardiac I_{Ks} (inward slow potassium) current; mutations in each cause LQT1 and LQT5, respectively.[83,101,102] The potassium channels *HERG* (on chromosome 7) and *MiRP1* (on chromosome 21) interact to form the I_{Kr} (inward rapid potassium) current, and defects in each cause LQT2 and LQT6, respectively.[103] Mutations in the sodium cardiac channel *SCN5A* cause LQT3 (on chromosome 3).[104] The gene responsible for LQT4 on chromosome 4 has been identified as ankyrin 2.[105] The potassium channel mutations cause a "loss of function" in the channel (or a "dominant-negative effect" in the case of the *HERG* mutation), whereas defects in the sodium channel cause a "gain of function."[82,83] LQT7 is caused by a defect in the α-subunit of the I_{Kr} channel (gene product *KCNJ2*).[106]

In the unlikely event that a mutant copy of the I_{Ks} channel is inherited from each parent (mutations in the *KVLQT1* and *minK* genes), the child will suffer from a clinically severe form of autosomal dominant LQTS and from autosomal recessive congenital deafness. This condition is known as the "Jervell and Lange-Nielsen syndrome" (JLNS).[107,108] JLNS was first described in 1957 in a Norwegian family in which three congenitally deaf children died suddenly before age 10 years.[109] JLNS actually is quite rare, with an estimated incidence of 1.6 to 6 cases per million.[108]

Treatment

No consensus exists on treatment of LQTS.[82,91,110] Most physicians advocate an ICD for patients who have survived a cardiac arrest or possibly even in those with syncopal events.[111] Dual-chamber pacemakers, even with beta-blocker therapy, have been ineffective in symptomatic patients.[112]

Most physicians advocate beta-blocker therapy in asymptomatic LQTS patients.[89–92] The exact dose or type of beta-blocker medication to be used is unclear. In patients unable (or unwilling) to take medications, an ICD then may be recommended. Restriction from heavy physical activity is suggested for affected

patients. Sympathectomy to modify the effect of adrenaline upon the heart has been ineffective at preventing events.[112]

Autopsy Findings

As with many ion channelopathies that cause unexplained sudden death, autopsy findings are unremarkable. The presence of LQTS should be considered in all cases of SCD where autopsy is negative for anatomic and histopathologic findings. In these cases, after an accurate anamnesis, a genetic screening should ideally be performed.

Brugada Syndrome

The Brugada syndrome (BS) is another inherited ion channelopathy that causes unexplained sudden death, particularly in middle-aged men.[113–115] It is relatively common in southeast Asia and should particularly be considered in the autopsy of subjects with this ethnicity.[116]

Epidemiology

A BS consensus report published in 2002 estimated the worldwide incidence of the disease was up to 66 cases per 10,000 people.[117] In contrast to LQTS, it affects males more commonly than females (male/female ratio = 8:1), although it also is an autosomal dominant gene. However, the gene is much more prevalent in southeast Asia than in the United States or Europe. Brugada syndrome is thought to cause the entity known as *LAI TAI* ("death during sleep") in Thailand, a relatively common cause of sudden unexplained death among young healthy men.[116]

Clinical Features

Brugada syndrome was first described in 1992 in patients with right bundle branch block patterns on the ECG who suffered unexplained cardiac arrests.[118] Since then, more has been learned about BS, although much about the disease remains unknown.[119,120]

Why some Brugada patients become symptomatic whereas others do not is not known. However, once BS subjects experience a symptom (syncope or aborted cardiac arrest), the disease becomes highly lethal with a high clinical penetrance.[121–123] Several studies found that the recurrence rate following a resuscitated cardiac arrest was 62% at 5-year follow-up.[117,119,121–123] Most arrhythmic events occur for the first time when patients are in their early 40s, but episodes occurring over a wide age range (2–77 years) have been described. Symptomatic BS patients experience polymorphic ventricular tachycardia degenerating into ventricular fibrillation, leading to syncope or even death. The episodes occur most commonly during sleep but also may occur with exercise or at rest.

The ECG of the BS patient frequently is abnormal and is the best way to diagnose BS (Figure 2.10). A right bundle branch block-type pattern is often noted in the right precordial leads V_1 through V_3, with ST-segment elevation.[113–115,124] In many patients with BS, the ECG abnormalities normalize or are unmasked by pharmacologic challenge with a sodium channel blocking drug such as procainamide, flecainide, or ajmaline.[125,126] One study of more than 3000 ECGs of Finnish military

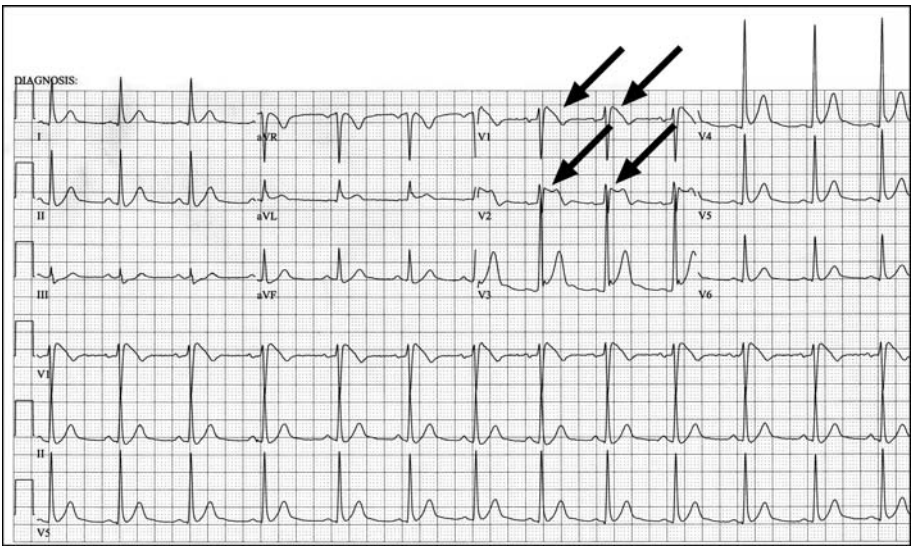

Fig. 2.10. Twelve-lead electrocardiogram recorded from a 46-year-old male Cuban boat refugee with a family history of sudden death among males. The patient originally was admitted for a stab wound to the neck and was found to have this ECG classic for Brugada syndrome. Note the pseudo-right bundle branch block pattern and ST elevation in leads V_1 through V_3 *(arrows)*. Electrophysiologic study easily induced ventricular fibrillation, and an implantable cardioverter-defibrillator was placed.

recruits with the Brugada-type pattern on ECG found that none of the recruits had episodes (syncope, SCD) in greater than 10-year follow-up, implying that this ECG finding is benign.[127] On the other hand, in another study of 547 patients with the Brugada ECG pattern (some did have syncope), 8% of the patients had SCD or cardiac arrest in only 2-year follow-up.[128] Interpretation of the Brugada ECG pattern probably depends on the population being studied.

Many BS patients have abnormal test results during invasive EP study.[129,130] Inducibility of malignant ventricular arrhythmias portends a worse clinical prognosis than for patients who have normal EP studies.[113–115,129,130] The results of the usual cardiac tests for BS are normal, including echocardiogram, cardiac MRI, and biopsy.

Pathophysiology

The mutation in the *SCN5A* gene results in either a reduced sodium channel current or failure of the sodium channel to express. The disease is caused by a defect in the α-subunit of the cardiac sodium channel gene *(SCN5A)*.[115,119,131,132] Numerous *SCN5A* mutations producing BS have been described, but most lead to a "loss of function" in the cardiac sodium channel. Interestingly, LQT3 (a completely different disease) also results from mutations in the *SCN5A* gene but leads to a "gain of function" in the sodium channel.[131–135]

The mutant sodium channel demonstrates more abnormal function at higher temperatures. Numerous BS patients experiencing symptoms during febrile illnesses have been reported in the literature.[113–115]

Genetics

Brugada syndrome is an ion channelopathy inherited in an autosomal dominant fashion. Only 20% of Brugada cases have been linked to the *SCN5A* gene; the precise ion channel mutations causing the remaining 80% are unknown.[118–120] The *SCN5A* gene is one of the largest ion channel genes known, with at least 28 exons identified thus far.[131–133]

Treatment

Medications are largely ineffective in treating BS.[117] Amiodarone, beta-blockers, and calcium channel blocking agents all have been tried but do not prevent sudden death in high-risk patients; sotalol and quinidine have been successfully used.[134] The recommended treatment for symptomatic patients with BS is ICD placement, particularly because the recurrence rate for these subjects is high. Patients who have not yet experienced an arrhythmic event but spontaneously exhibit the abnormal ECG findings are at intermediate risk for an episode and may benefit from prophylactic ICD therapy.[129,130,136,137]

Autopsy Findings

Autopsy findings of the hearts of BS patients are unremarkable. As in LQTS, in order to conduct postmortem molecular analyses, proper collection and storage of autopsy tissue are critical for possible DNA testing.

Catecholaminergic Polymorphic Ventricular Tachycardia

Catecholaminergic polymorphic ventricular tachycardia (CPMVT) is a newly described inherited disorder of cardiac calcium channels. It is another arrhythmogenic disorder characterized by sudden unexplained death associated with exercise.

Epidemiology

The disease has been characterized in several Finnish and Italian families to date.[138–141] The epidemiology of this disorder has not been fully described and is limited to case series. Its true incidence likely is much higher than is currently appreciated because most cases are undiagnosed.

Clinical Features

Catecholaminergic polymorphic ventricular tachycardia was first described in 1995 in 21 children.[142] This disorder is characterized by syncopal spells in childhood and adolescence that often are triggered by exercise or stress (catecholamines) as well as cardiac arrest and sudden death. The disease has a mortality of 30% to 50% by age 30 years in affected individuals.[138] Given the autosomal dominant nature of the disorder, there is often a family history of unexplained sudden death.

The resting ECG of a patient with this disorder usually is unremarkable, as are cardiac imaging studies (echocardiogram, angiogram, cardiac MRI).[138–141] Patients with CPMVT may experience bidirectional or polymorphic ventricular tachycardia with exercise stress testing, with emotional stress, or during infusion of adrenaline (isoproterenol).[143,144] Up to 30% of such patients initially were misdiagnosed as having LQTS in one study.[140] Autopsy findings in CPMVT subjects are generally normal.

Pathophysiology

Defective calcium channels that form as a result of mutations in the ryanodine receptor gene *RyR2* lead to abnormal conduction, which predisposes the heart to ventricular tachycardia and sudden death.[138–144,145] *RyR2,* the gene encoding the cardiac calcium channel, is responsible for mediating the coupling of the cell's electrical excitation and mechanical contraction. Cellular depolarization leads to release of Ca^{2+} from the sarcoplasmic reticulum via RyR2 and mechanical contraction. Sudden death is hypothesized to occur as the result of torsades de pointes or ventricular fibrillation due to abnormal calcium channel handling.

Genetics

Catecholaminergic polymorphic ventricular tachycardia results from a defect in the cardiac ryanodine receptor (*RyR2*) gene, which is inherited in an autosomal dominant fashion.[138–141,144] Ryanodine receptors are intracellular calcium channels that regulate the release of calcium from different cell sites. Three different isoforms of the ryanodine receptor are known, each encoded by a different gene. They are the largest ion channels yet described. RyR2 (encoded by 105 exons) is characteristically found in the heart whereas RyR1 is found in skeletal muscle.

Because this entity is newly described and the genes encoding the mutant calcium channel are so large, no commercial genetic screening is currently available for CPMVT.

Treatment

Beta-blockers are the mainstay of therapy in patients with this condition. An ICD is offered to patients who have survived cardiac arrest or are believed to be at particularly high risk for sudden death.[140,144]

Summary

In cases where the autopsy is unrevealing as to the cause of death, the pathologist or coroner should be alert to numerous genetic diseases that can lead to unexplained SCD, particularly in young people.

We reviewed a relatively complete autopsy protocol of the heart that we have found useful. We described the methodology for collecting biologic samples (including blood and tissue) for future DNA studies of these genetically based cardiac diseases. Although still largely limited to research laboratories, it is prudent for the

pathologist to be aware of how to collect blood or tissue at autopsy and those diseases that might be tested in the future.

Two important genetic channelopathies that cause disorders of the cardiac muscle and can lead to sudden death are HCM and ARVD. HCM is the most common genetic cause of sudden death, but the clinical penetrance is highly variable and can be difficult to make. Generally, the pathologist sees marked ventricular hypertrophy (sometimes in the septum) at autopsy. Myocyte disarray and scarring can be see microscopically with HCM. Arrhythmogenic right ventricular dysplasia is a newly diagnosed disorder characterized by fibrofatty infiltration of primarily the RV. It can lead to SCD, particularly in young, athletic men.

The common ion channelopathies that cause SCD include the relatively common LQTS, BS, and CPMVT. LQTS should be considered in patients who die by unexplained drowning, die during periods of strenuous exercise, or die in their sleep. Brugada syndrome is seen most commonly in Asian males who die in their sleep and is the result of defects in the cardiac sodium channel. CPMVT is a newly defined disorder in which patients die suddenly with exercise.

It is critical for the pathologist and coroner to be educated regarding this genetic causes of SCD so that they can be considered in subjects with the "normal autopsy." Such information could be disseminated to family members who possibly are affected with the same disease while they are still alive.

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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

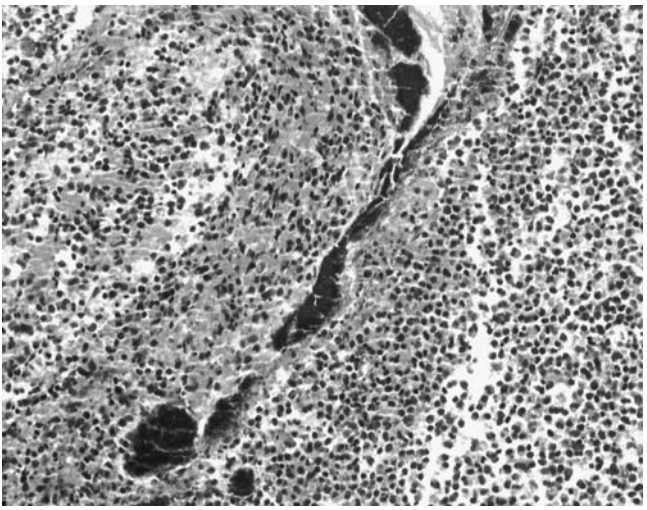

Fig. 3.1. Inflammatory cells demarcating clusters of bacteria in the lungs.

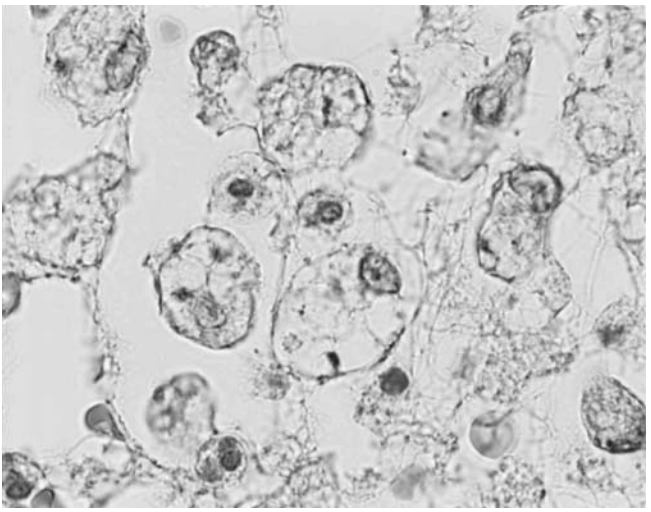

Fig. 3.2. Macrophages within an intragluteal syringe abscess showing intracytoplasmatic inclusions of Gram-positive cocci.

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.

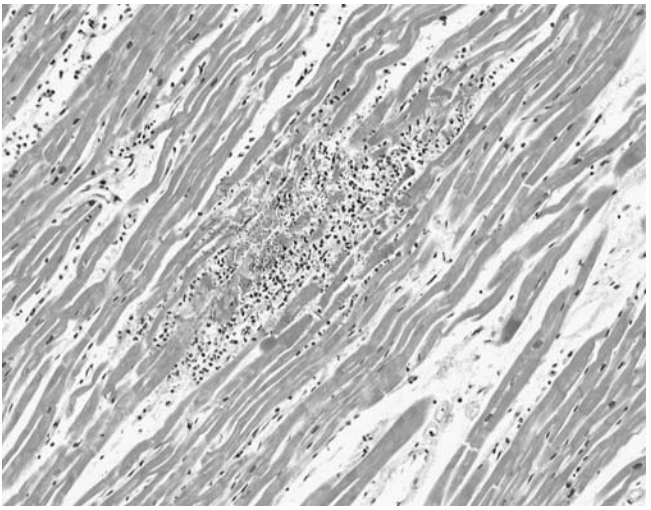

Fig. 3.3. Leukocytes sharply demarcating fungal hyphae in the myocardium.

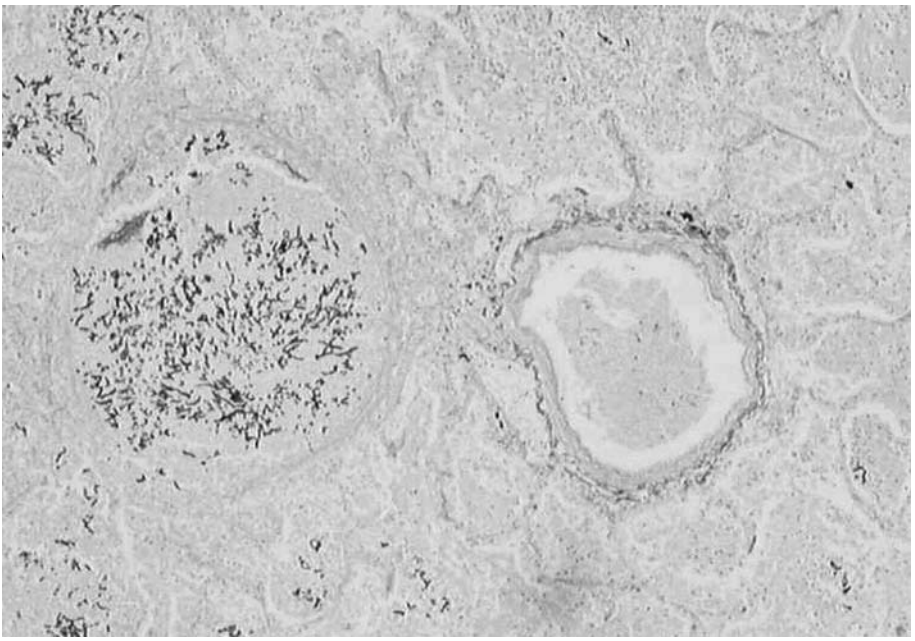

Fig. 3.4. Invasion of a small bronchi (left) by *Aspergillus* hyphae. Note the total absence of hyphae in the adjacent vessel (right), supporting the concept of a respiratory route of infection in this case.

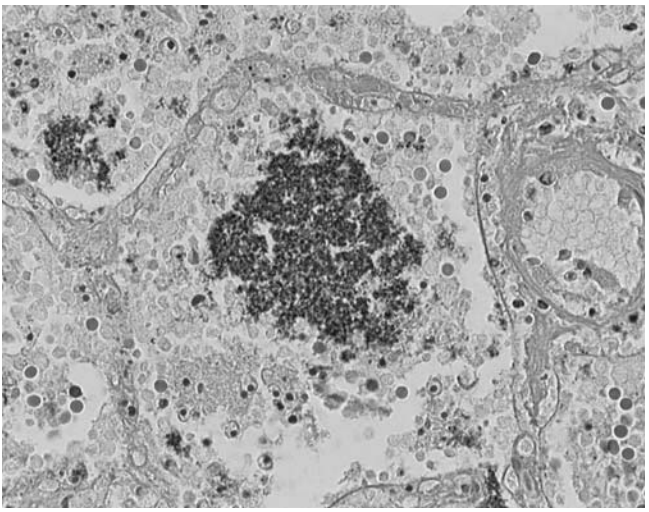

Fig. 3.5. Postmortem bacterial invasion into the lungs. Clusters of bacteria lacking accompanying inflammatory cell reaction are seen within the alveoli.

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]

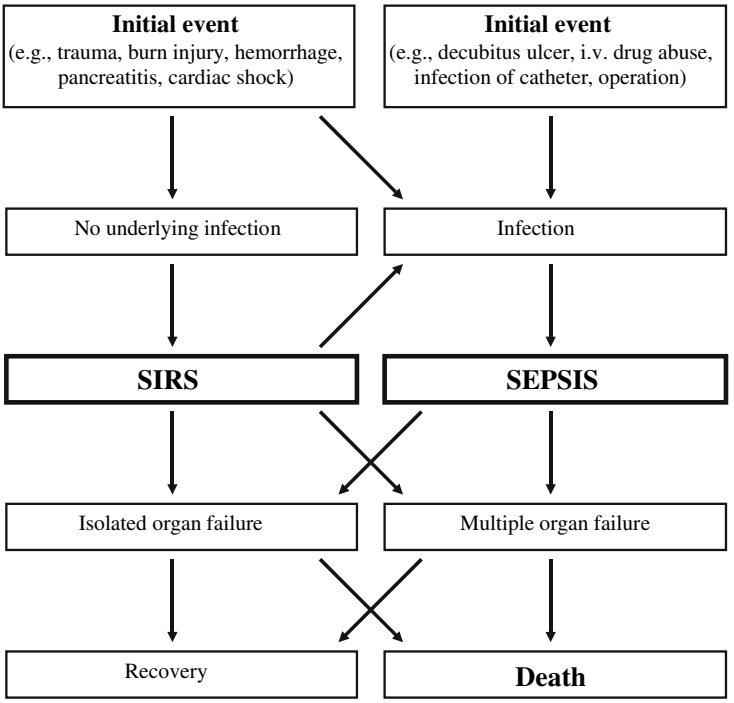

Fig. 3.6. Possible reaction patterns of the host response after an initial event leading to injury and/or infection. SIRS, systemic inflammatory response syndrome.

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

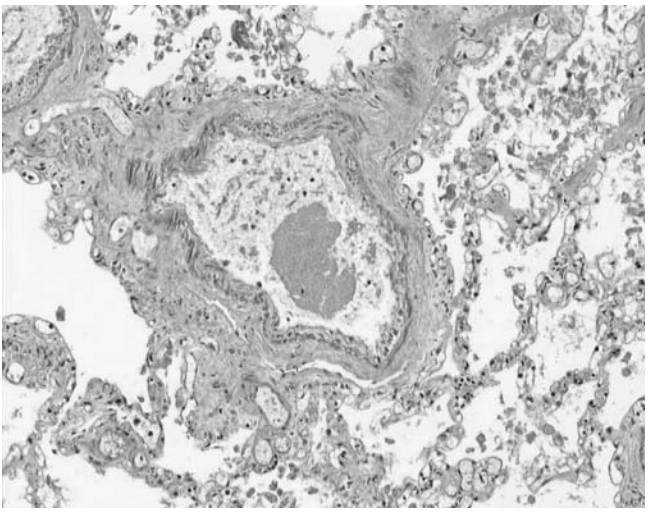

Fig. 3.7. Fibrin deposits in a pulmonary vessel in a case of septic acute respiratory distress syndrome.

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

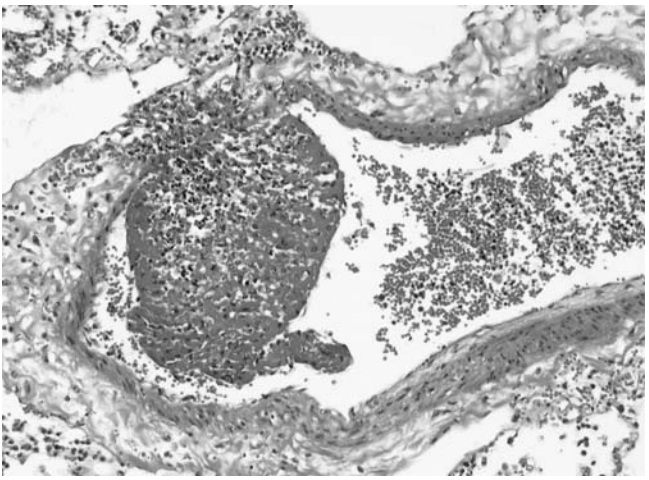

Fig. 3.8. Microthrombosis adjacent to a pulmonary vessel wall in a case of *Staphylococcus aureus* sepsis.

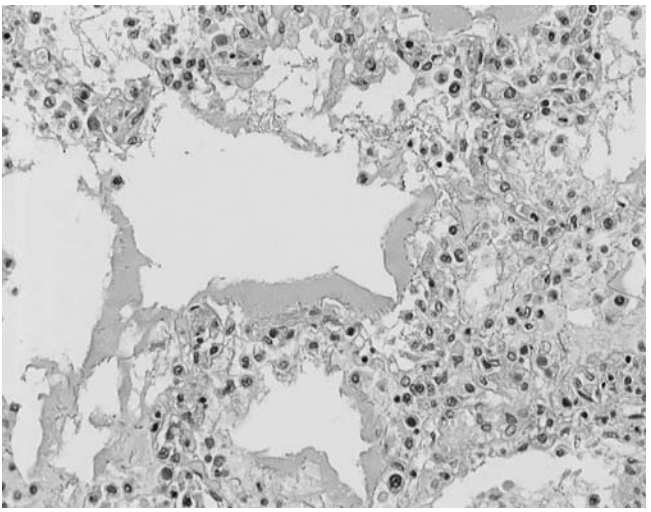

Fig. 3.9. Hyaline membranes covering the alveolar epithelial layer in a case of septic acute respiratory distress syndrome originating from cholecystitis.

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.

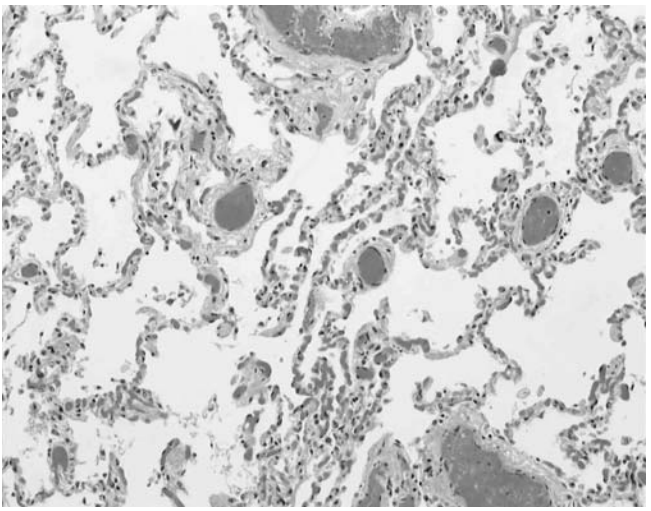

Fig. 3.10. Congestive atelectasis in a 13-month-old boy who died of *Salmonella enteritidis* sepsis.

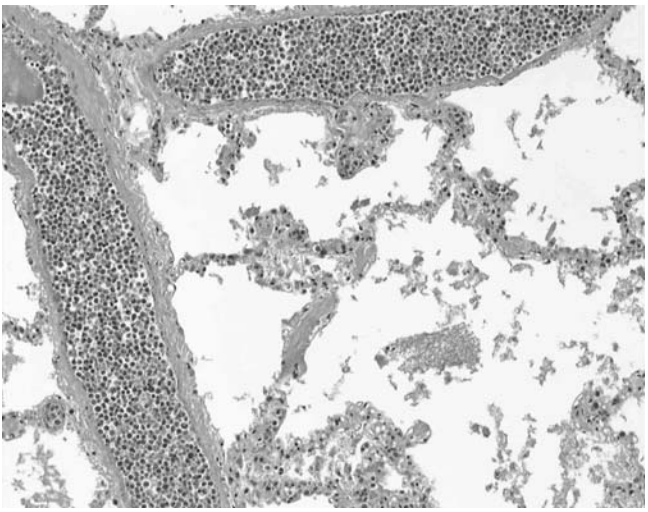

Fig. 3.11. Leukocyte sticking of the lungs in septic shock. Engorgement of pulmonary vessels and extensive intravascular leukostasis in the absence of interstitial or intraalveolar signs of inflammation are seen.

(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.

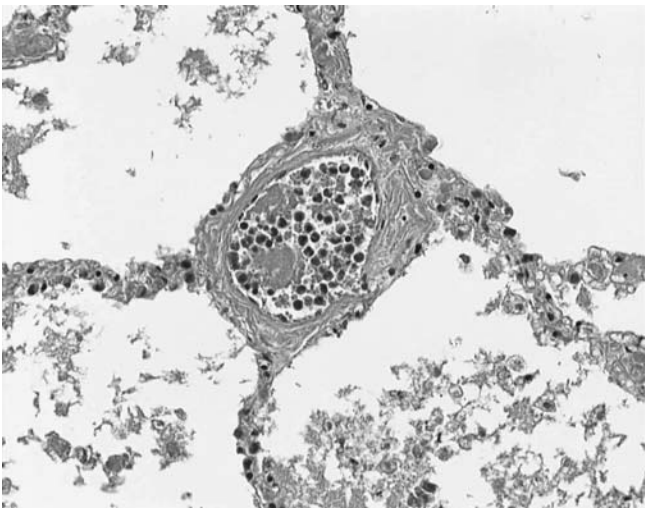

Fig. 3.12. Leukocyte sticking of the lungs in septic shock. Trapping of leukocytes (mainly neutrophil granulocytes) within a small pulmonary vessel are seen. Note additional intravascular fibrin aggregations within the vessel lumen.

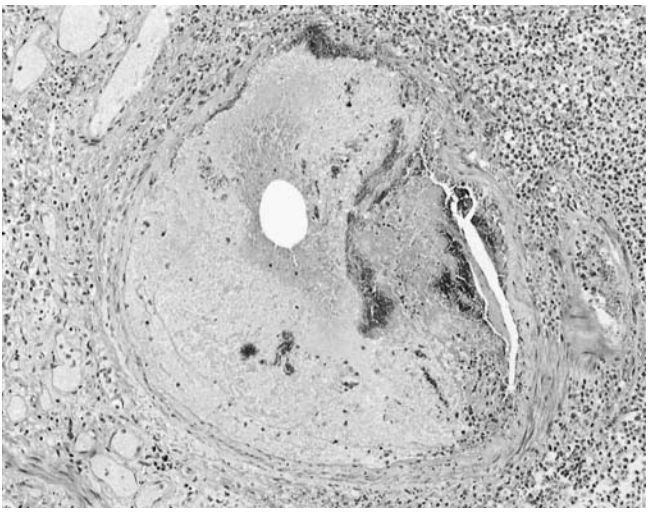

Fig. 3.13. Septic embolus leading to near-total pulmonary vessel occlusion in an intensive care unit patient who died of septic multiple organ failure.

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

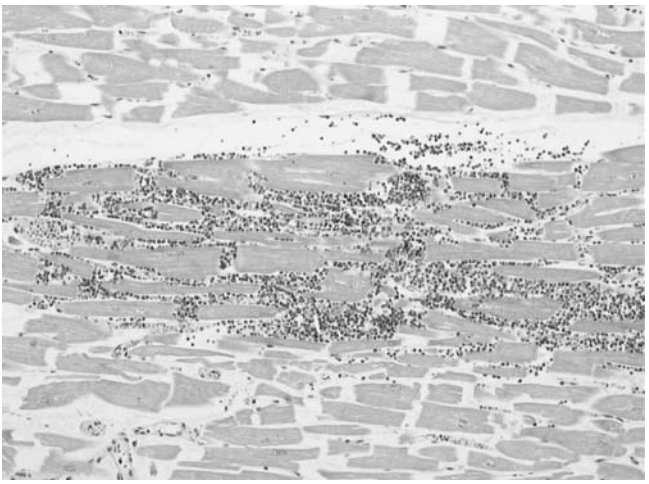

Fig. 3.14. Circumscribed hemorrhage in the myocardium in a case of septic shock.

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

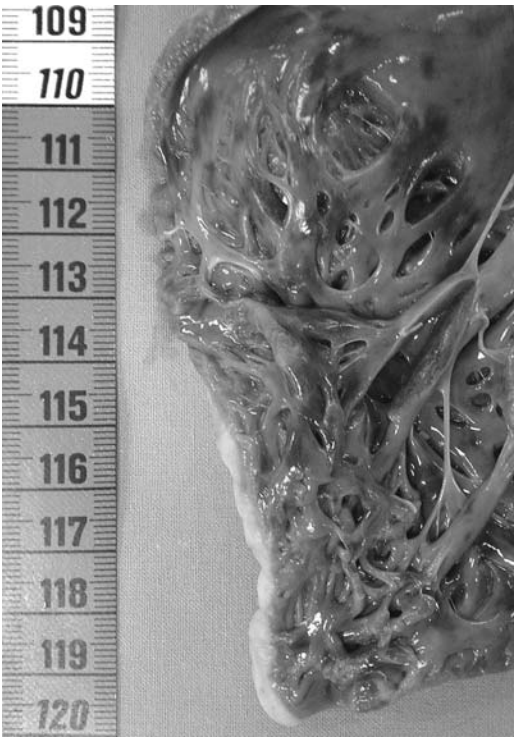

Fig. 3.15. Subendocardial hemorrhages of the right ventricle in sepsis. This patient died of multiple organ failure due to sepsis originating from an infected medullary pin of the tibia.

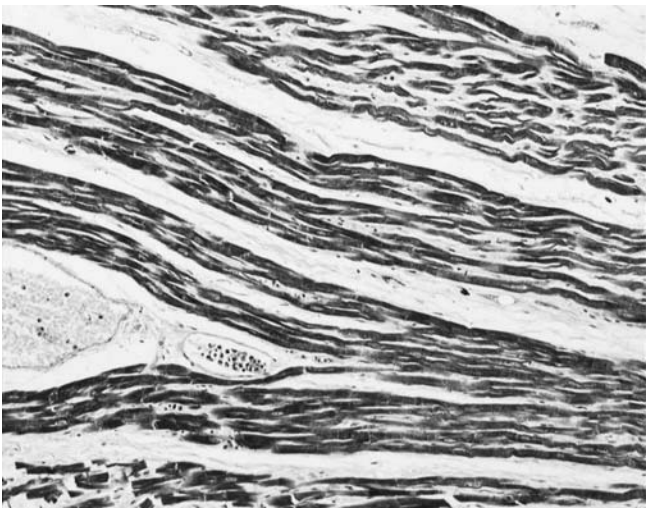

Fig. 3.16. Elongated cardiomyocytes separated by marked interstitial edema in a case of septic multiple organ failure.

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]

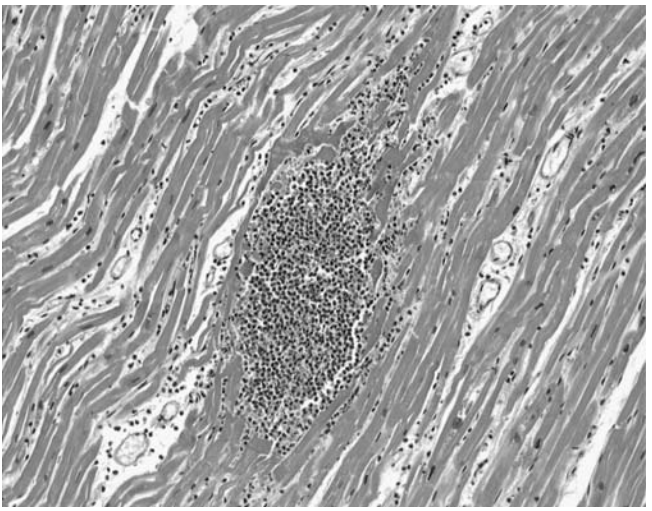

Fig. 3.17. Septicopyemic abscess in the myocardium.

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

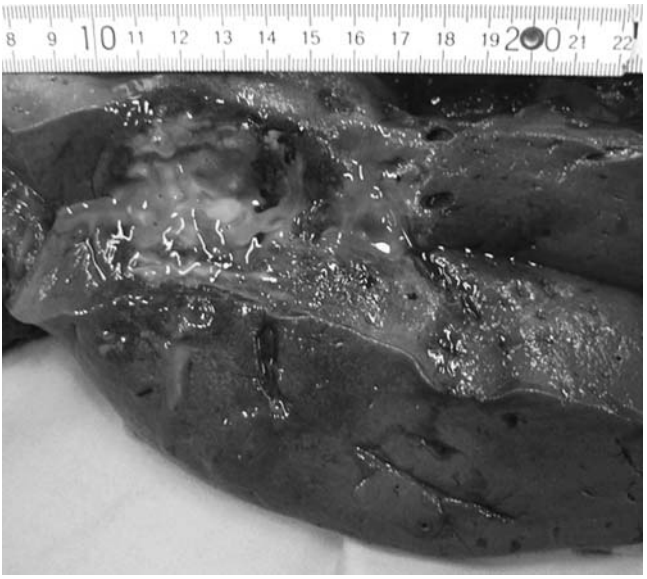

Fig. 3.18. Pyemic abscess of the liver that developed during the course of a missed diagnosis of appendicitis.

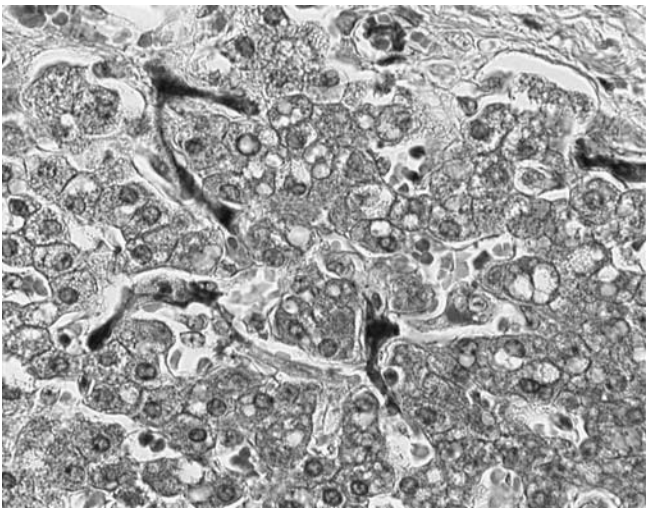

Fig. 3.19. Fibrin aggregations in the liver sinusoids. In this case sepsis was due to disseminated intravascular coagulation due to peritonitis as a consequence of bowel perforation during endoscopy.

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

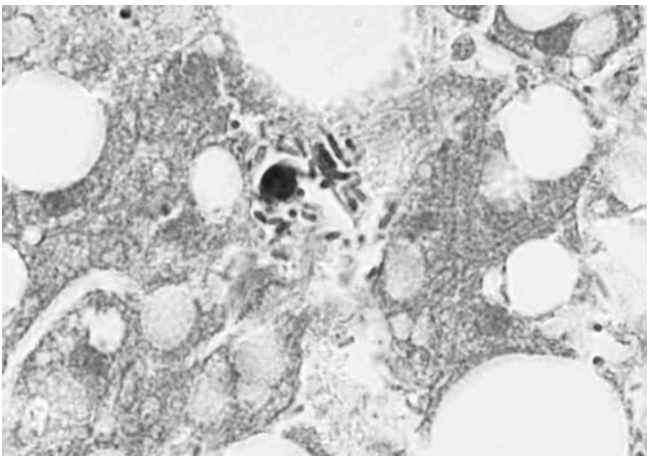

Fig. 3.20. Kupffer cell with phagocytosed rod-shaped bacteria in a case of clostridial gas gangrene. Note gaseous bullae formation of the highly autolytic surrounding liver tissue.

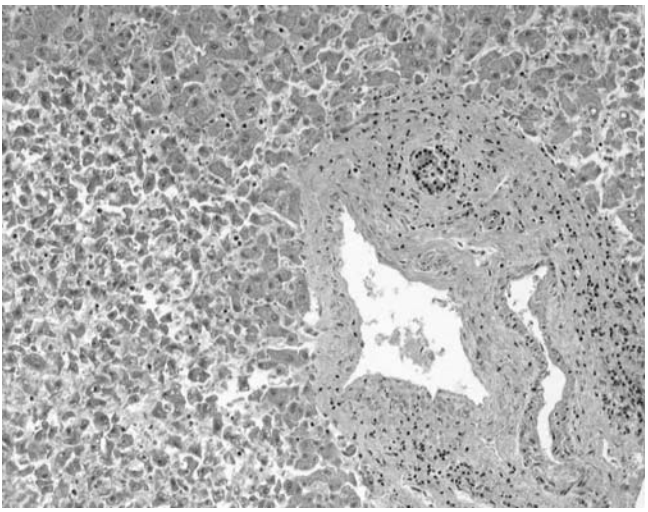

Fig. 3.21. Moderate leukocytic infiltration of the acinocentral areas of the liver in sepsis. Note absence of inflammatory changes in the liver parenchyma.

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]

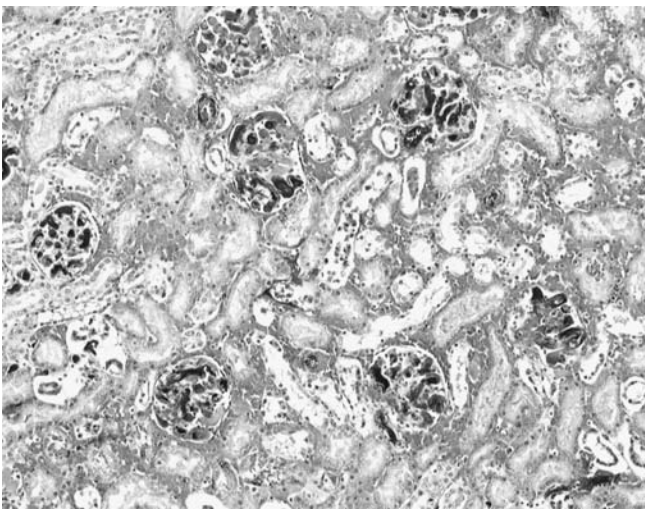

Fig. 3.22. Microthrombi formation in glomerular capillaries as a consequence of disseminated intravascular coagulopathy in a case of fatal Waterhouse-Friderichsen syndrome.

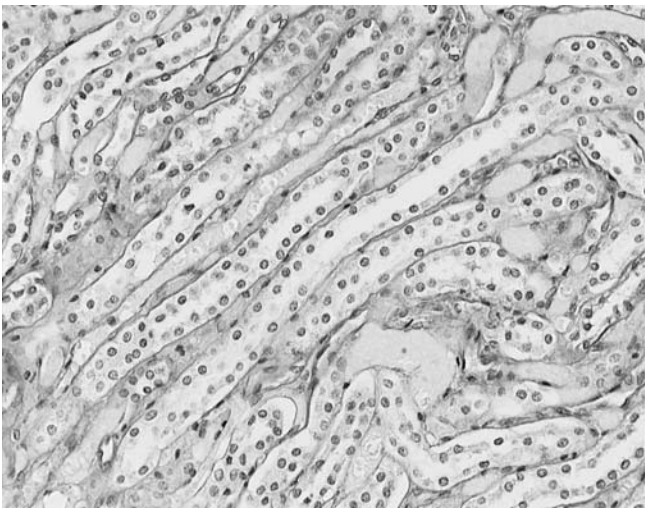

Fig. 3.23. Acute tubular necrosis with dilatation of proximal tubules and flattening of epithelium with loss of brush border and focal necrosis in a 3-year-old boy with *Haemophilus influenza* sepsis.

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

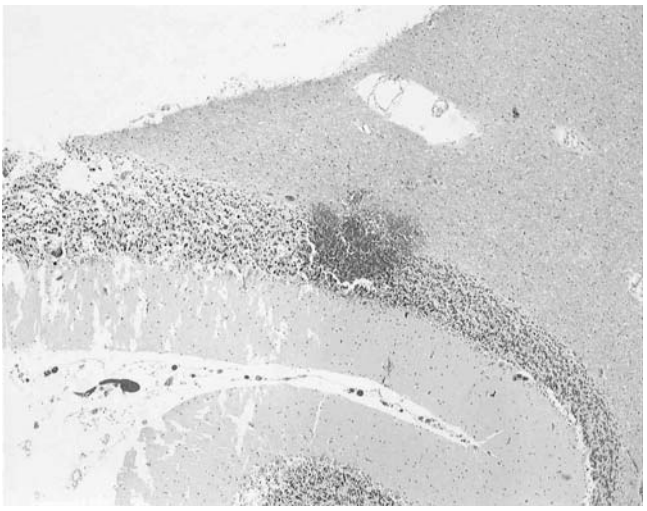

Fig. 3.24. Small hemorrhage in the cerebellum in sepsis.

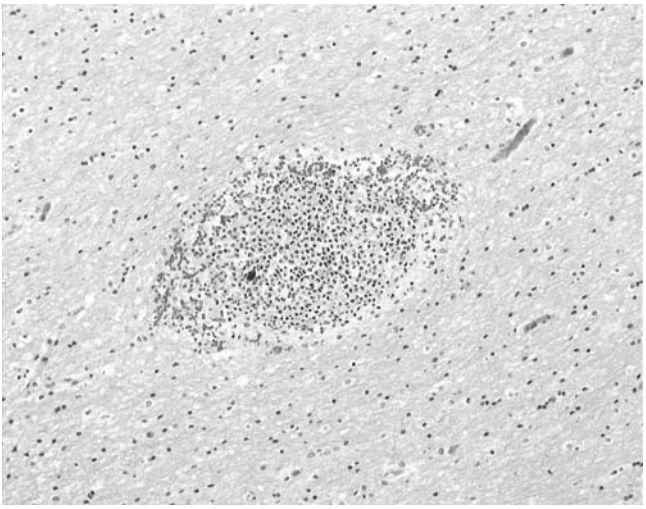

Fig. 3.25. Septicopyemic microabscess in the brain as a result of endocarditis lenta.

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).

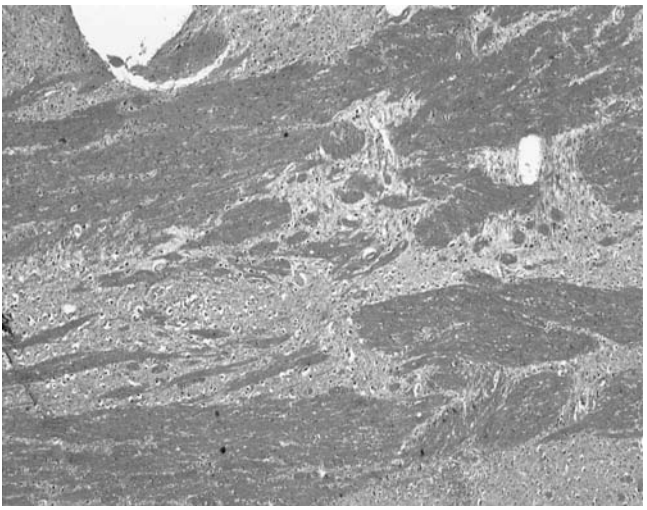

Fig. 3.26. Multifocal necrotizing leukoencephalopathy. Horizontal section of the upper pons with recent necrotic changes in the transverse pontine fibers. In this case, sepsis originated from a large bowel abscess (polymicrobial growth in postmortem cultures).

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

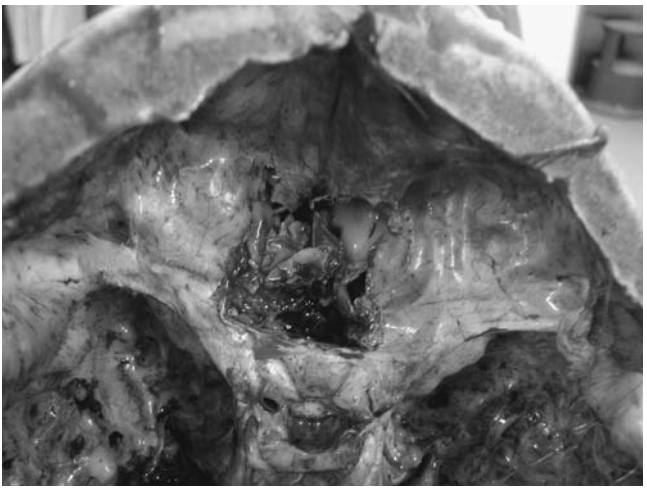

Fig. 3.27. Purulent exudate in the ethmoidal sinus. *Streptococcus pneumoniae* was cultured from postmortem swabs. Pyemic infection by direct extension from the ethmoidal sinus led to fatal meningitis.

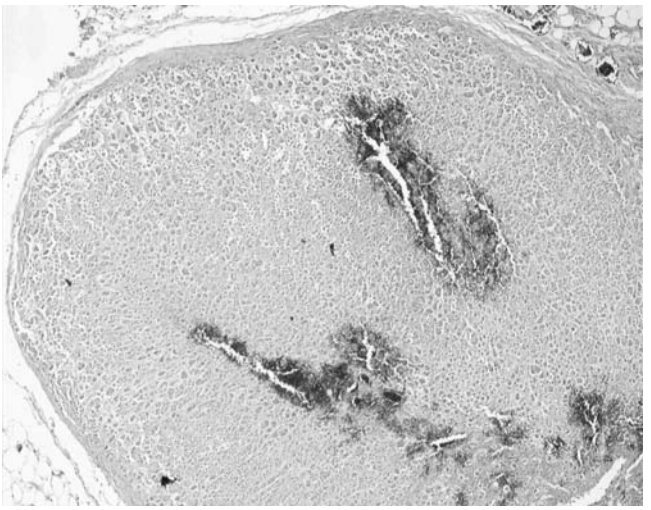

Fig. 3.28. Focal hemorrhages in the inner cortex of the adrenal gland in a case of ventilator-associated *Pseudomonas aeruginosa* sepsis.

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]

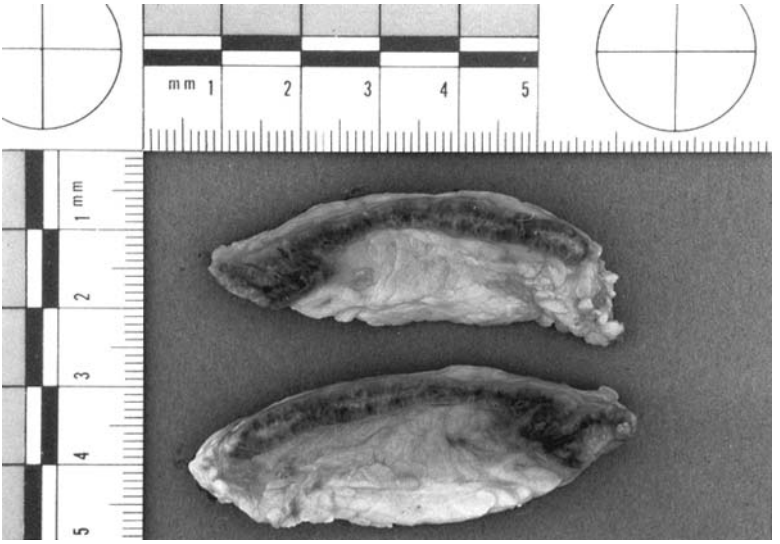

Fig. 3.29. Cut sections of adrenal glands showing intraparenchymal hemorrhage in a case of fatal Waterhouse-Friderichsen syndrome in an adult.

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.

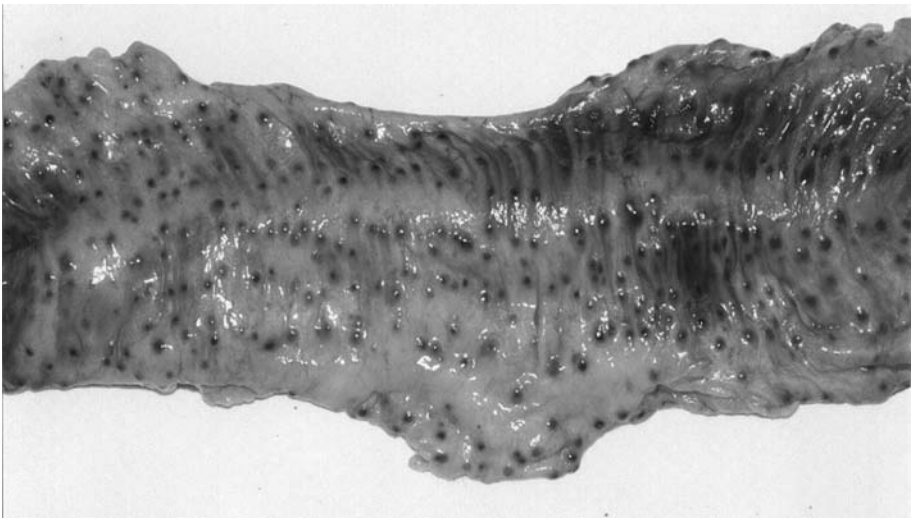

Fig. 3.30. Macroscopic appearance of so-called "septic enterocolitis."

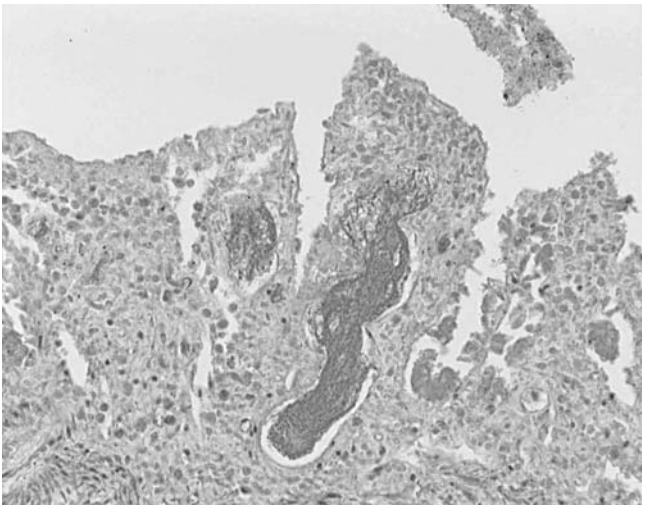

Fig. 3.31. Fibrin thrombi formation in the mucosa of the small intestine; considerable autolysis of autopsy specimen. This individual died of septic shock due to *Staphylococcus aureus* sepsis as a consequence of an intragluteal injection of diclofenac for treatment of lumbago.

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.

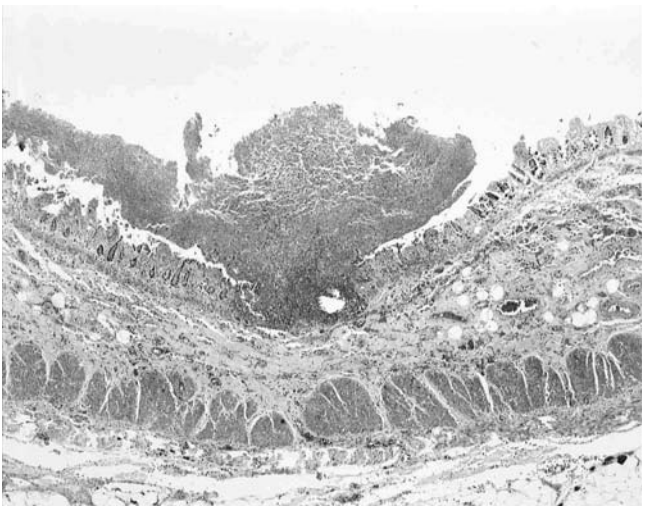

Fig. 3.32. Pseudomembranous colitis. Typical "volcano-like" eruptive appearance of colonic mucosa.

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.

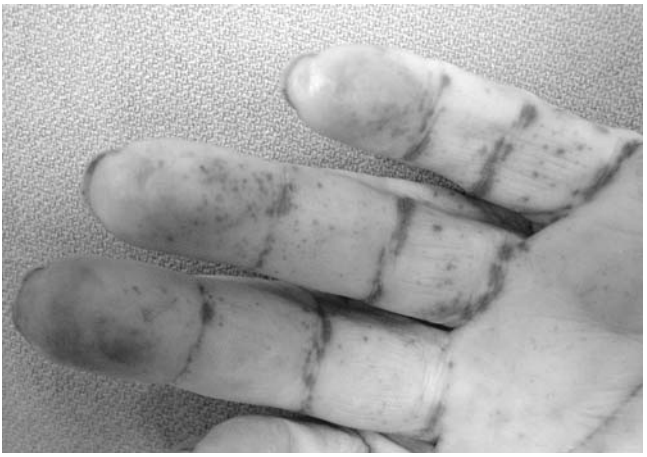

Fig. 3.34. Cutaneous bleedings on the inner aspects of the fingers due to metastatic spread of septic microemboli.

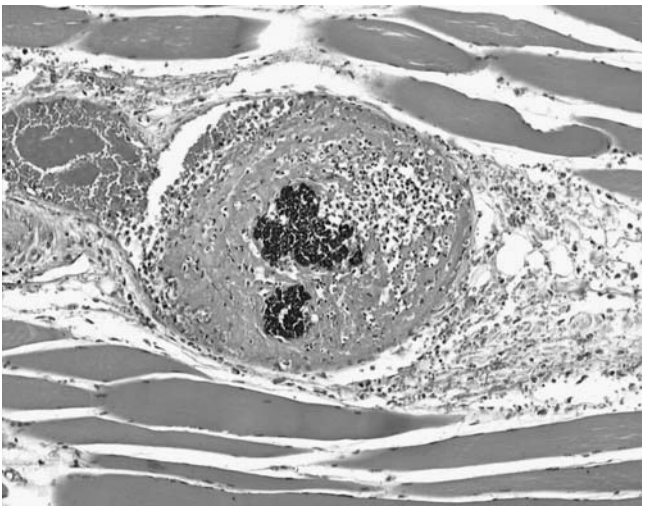

Fig. 3.35. Septic microemboli totally occluding a small artery. The emboli originated from endocarditis of the mitral valve. Strong inflammatory cell infiltration in all vessel layers are seen (same case as Figure 3.34).

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.

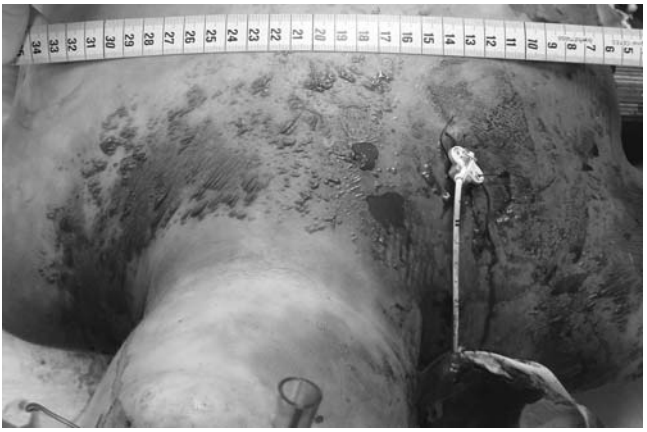

Fig. 3.36. Gas gangrene due to *Clostridium difficile* infection. Hemorrhagic bullae formation upon the skin is seen.

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

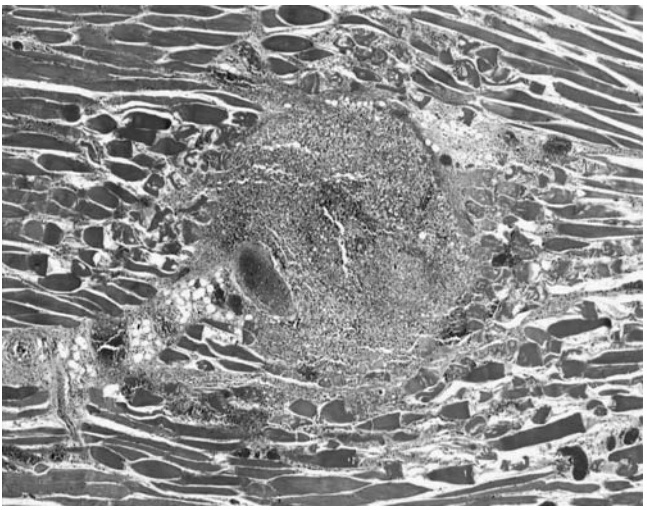

Fig. 3.37. Septicopyemic abscess formation in the psoas muscle that developed during the course of fatal *Staphylococcus aureus* sepsis following a burn injury.

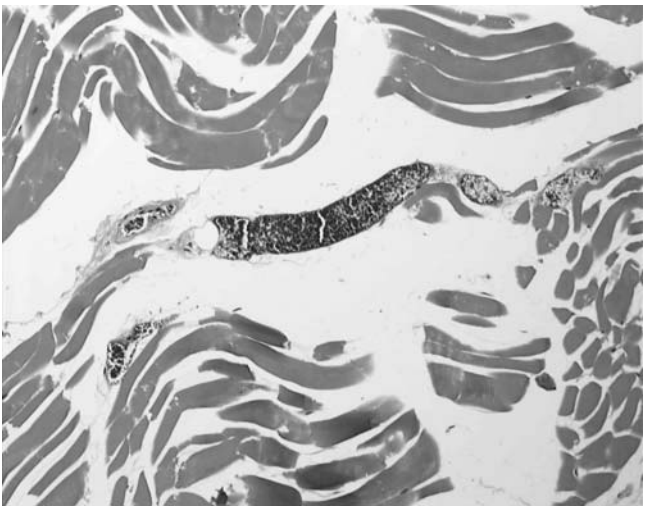

Fig. 3.38. Gas gangrene (clostridial myonecrosis). Bacteria are seen in an emptied sarcolemma. Loss of cross-striation with homogenization of fibers and segmental and splinter-like fiber distraction with occasional hypercontraction bands are observed. Note the total absence of inflammatory cells, which 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

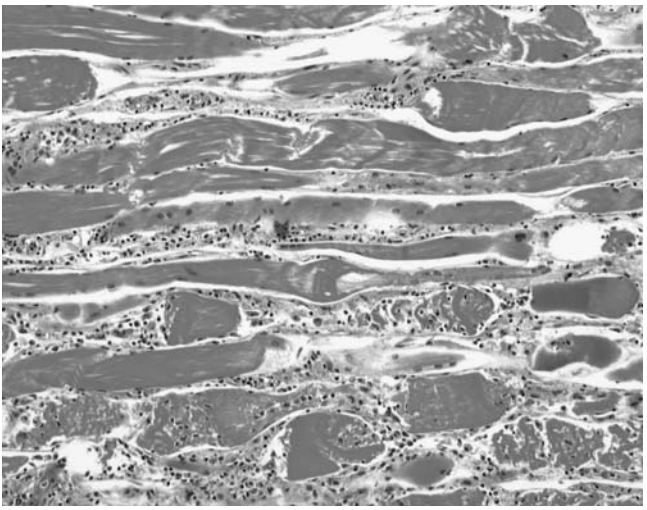

Fig. 3.39. Pyomyositis. Dense granulocytic infiltration and myocytolysis with disruption of muscle fibers. *Staphylococcus aureus* was cultured from native muscle specimens obtained at autopsy.

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.

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4. A Histopathologist's Guide to Ocular Pathology[1]

M. Andrew Parsons

Introduction

A great deal of important information can be obtained at autopsy of both adults and children by the pathologic examination of the eye and its adnexal structures. This information can be related to the cause of death (e.g., violent shaking trauma in physical child abuse) or pertain to disease processes affecting the eye, the orbit, and the surrounding structures outside the orbit.

In this chapter I review the different methodologies that are required to remove eye and related tissues and the indications for electing to use these different procedures. I describe the reconstruction methods that are used to achieve the perfect cosmetic result that is so important when dealing with facial structures. I describe how to orientate, examine, and dissect the eye once it has been removed and how to record the important macroscopic pathologic findings in the eye.

The chapter is directed primarily at histopathologists who do not have detailed knowledge of eye diseases, and it is intended as a basic guide to obtaining and safely securing the eye and related tissues at autopsy, for subsequent examination, perhaps by (or with) a specialist ophthalmic pathologist. Space does not allow me to cover the huge range of primary or secondary diseases affecting the eyes in children and adults, but I direct the reader to sources of this information. However, I do review one area – the ophthalmic pathology of child abuse – in more detail, because this topic has considerable forensic and medicolegal significance and because the detailed methodology and documentation at autopsy are so important and are prone to more than usual professional and public "scrutiny and interest."

It is important to recognize that the eye and adnexal ocular structures are "culturally sensitive" tissues, and for this reason their removal must be approached cautiously. Removing these tissues should be undertaken only for sound scientific reasons, with the fully informed consent of the relatives and/or the coroner (or equivalent authority) and with properly agreed procedures for the eventual

[1] This chapter has been adapted, reproduced, and expanded from Parsons MA, Start RD. Necropsy techniques in ophthalmic pathology. ACP Best Practice No. 164. J Clin Pathol 2001;54:417–427, adapted and reproduced with permission from the BMJ Publishing Group.

retention or disposal of the ocular tissues. For this reason, I review not only the scientific indications and methods for the removal of such tissues but also the legal and ethical issues that must underpin this pathology "best practice."

This chapter includes the guidelines originally published as "Necropsy techniques in ophthalmic pathology. Association of Clinical Pathologists Best Practice Guideline No. 164" in the *Journal of Clinical Pathology*.[1]

Background

The eye and its adnexal structures can be affected by primary ophthalmic disease and diseases outside the eye, by direct extension from adjacent structures, or by secondary involvement in malignant or nonmalignant systemic diseases. Some of these diseases are relatively common, and the pathology is well known. Other diseases are rare, or their pathology is poorly understood. In some common conditions there is little opportunity to examine human tissues, as the conditions may not require biopsy, surgical intervention, or enucleation of the eye. For example, the opportunity to examine eyes in the early stages of documented primary open angle glaucoma (the most common form of glaucoma) hardly ever occurs, except when

- the eye is removed for incidental disease, for example, primary ocular malignancy
- the patients die at a relatively young age as a result of accident or disease, and the eyes are removed at autopsy

A valuable research opportunity may be missed if the eyes are not removed for examination.

Many of the patients who die of nonophthalmic diseases and have autopsies have had ocular surgery, laser treatment, or other forms of management of primary or secondary ocular disease. Some patients may have systemic diseases that affect the eyes, or they may have unsuspected or unreported eye disease. Examination of the eye at autopsy provides potentially important feedback to ophthalmic practitioners as part of the process of clinical audit.[2,3] This examination allows assessment of the accuracy of diagnosis and organ imaging, detection of unsuspected diagnoses such as infection,[4] and determination of responses to medical or surgical treatment.[5–7]

Eye tumor tissue (e.g., primary malignant melanoma) and metastatic tumor tissue removed at autopsy from other organs, such as the liver, are important for research into the growth, progression, and treatment of eye cancer. An example of this is research into changes in the cytogenetics and molecular genetic changes in ocular malignant melanomas,[8] where useful research can be performed on both viable and nonviable tumor tissue.

Eye tissues removed at autopsy are an important source of viable cells for tissue culture (e.g., retinal pigment epithelial [RPE] cells, trabecular cells) in research into nontumorous conditions such as proliferative vitreoretinopathy (a proliferation of RPE cells that occurs after retinal detachment and can prevent successful surgery to reattach the retina). Eyes obtained at autopsy also have been used by ophthalmologists in the evaluation of new treatment modalities, such as determining whether the effects of YAG laser cryoablation can be detected by ultrasound biomicroscopy.[9]

Examination of the eyes can be vital in forensic investigations, as in the case of nonaccidental injury in infants, where examination of the eyes and optic nerves is required to demonstrate the characteristic distribution of hemorrhages and focal areas of retinal detachment.[10] Road traffic and other accidental deaths may be related to poor vision, and assessment of ocular pathology may help in the understanding of the cause of the accident. Sampling of vitreous fluid at autopsy is an important means of obtaining diagnostic biochemical and toxicologic information pertaining to the cause and time of death.[11–14] Care must be taken, however, in the identification of a body based upon eye color at autopsy, as postmortem clouding of the cornea and other changes tend to make the eyes appear more brown after the first 24 hours.[15]

The examination of the eyes at autopsy is often neglected or may be felt to be mutilation without good cause. Removal of the eyes is particularly emotive, although many patients who have suffered from defective vision are keen to have the causes investigated after their death, for the benefit of others. After appropriate training, it is easy for the pathologist to remove an eye, with or without the orbital contents, leaving no external signs that the procedure was done. In extreme and important cases, and with care, the orbits and large areas of the facial and skull bones can be removed and the face reconstructed, leaving an externally unchanged face for later viewing by relatives.

I would like to encourage pathologists to gain some ophthalmic autopsy experience so that they can contribute to scientific and clinical knowledge of important but rarely studied ocular diseases.

Technical Methods for Removal of Ocular Tissues at Autopsy

In this section I review the technical methods used to remove the vitreous (e.g. for biochemistry); the eye itself (anterior approach); the eye and orbital contents (posterior approach); the eye, orbit, and orbital walls; and the eye, orbital walls, and surrounding structures.

Before starting the practical work, the pathologist should consider the important legal, ethical, and health and safety issues related to removing the eyes at autopsy, as eyes are particularly emotive culturally. These issues are considered in greater detail at the end of the chapter.

Vitreous Fluid Samples

The most common reason for removing the vitreous at autopsy is for biochemical and toxicologic analysis to assist in determining the cause and time of death.[11,12] Vitreous may also be removed in a limited autopsy procedure for cytologic examination to determine the presence of infection or malignancy (if cloudy vitreous was noted in life). However, removing and examining the whole intact eye will be much more informative under these circumstances, as the retina and other ocular tissues are often involved in the disease process.

It is important that only "normal" vitreous be sampled for biochemical analysis, as disease affecting the vitreous may well markedly alter its biochemical and cellular composition and lead to misleading results. I recommend that vitreous not be sampled for biochemistry from eyes with a history of retinal detachment, surgical manipulation, or posterior chamber disease affecting the vitreous. Nonvitreous

fluids encountered in some eyes include blood, subretinal proteinaceous fluid, inflammatory exudates, and artificial vitreous (e.g., silicone oil) used in some vitreoretinal surgery. Note that the eye may be calcified or even ossified in some pathologic conditions, and vitreous sampling may be impossible. Vitreous sampling should be aborted in any eye that is small, wrinkled, and hard, or when there is firm (or gritty) resistance to puncture. A good practical test is to determine before puncture whether the eye is fluctuant, which is easily accomplished in the slightly flaccid postmortem eye by feeling transmitted pressure on the thumb and index finger of one hand placed medially and laterally on the sclera, when slight pressure is exerted on the superior sclera with the other index finger.

Method

The vitreous is obtained by puncturing the posterior chamber of the eye with a 15- or 17-gauge needle on a 5- or 10-ml syringe, taking care to avoid cosmetically unsightly disruption of the iris and the pupil.

1. Ensure that the eye is fluctuant (and therefore contains fluid) by putting slight pressure on the eye with the index finger and thumb of one hand. These fingers should then move apart slightly when you press the eye gently with the index finger of the other hand. Insert the needle into the sclera (Figure 4.1) at a point not less than 5 mm lateral (temporal) to the corneoscleral junction (limbus), in both the horizontal plane passing through the center of the pupil and 60 degrees lateral to the median (sagittal) plane (pointing to the center of the posterior

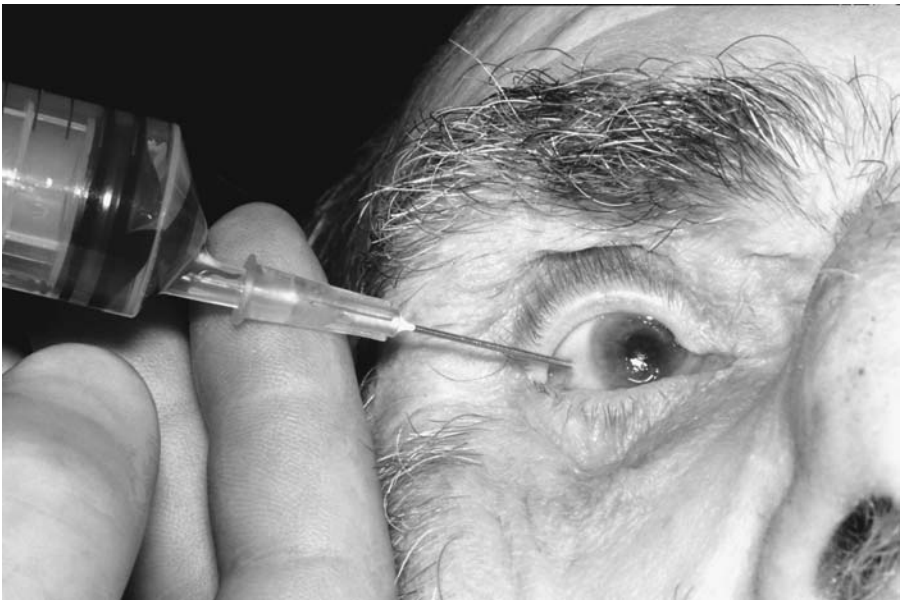

Fig. 4.1. Vitreous is removed from the left eye by inserting a needle (with attached syringe) at a point not less than 5 mm lateral (temporal) to the corneoscleral junction (limbus), in the horizontal plane, and 60 degrees lateral to the median (sagittal) plane.

chamber). Initially, point the needle slightly posteriorly so that it penetrates the sclera at an oblique angle (before final alignment as above). This step will help prevent leakage of fluid when the eye is reinflated at the end of the procedure.

2. Insert the needle about 10 to 15 mm into the eye before removing vitreous fluid. The needle can be withdrawn slightly while applying suction to help this process. You can remove up to 2 to 3 ml of vitreous, but frequently you will obtain less than this quantity. The eye collapses during this procedure (which requires only gentle suction), and fluid extraction is complete when you feel resistance.
3. Keep the needle in situ in the eye, and detach the syringe. Attach another syringe to inject clean water, saline, or fixative into the eye until there is resistance to flow and the eye is firm. It is important not to overinflate the eye, as this could cause iris/lens disruption or rupture of the eye.

For optimum results, you should place the vitreous that was removed for biochemical analysis into labeled 2-ml sample tubes containing fluoride and send them immediately for analysis (or keep them at 4°C until analysis can be performed).

Ophthalmic Factors in Interpretation of Vitreous Biochemistry

As stated earlier, only "normal" vitreous should be sampled for biochemical analysis, as disease affecting the vitreous may well markedly alter its biochemical and cellular composition and lead to misleading results. This important interpretational factor has hardly been considered in the extensive literature reporting biochemical and toxicologic findings in the vitreous.[16] An ophthalmic history taken from the family, clinical notes, or information recorded by the family doctor or optician probably would alert the chemical pathologist to the presence of eye disease.

In their consideration of vitreous alcohol concentrations, Jones and Holmgren[17] quote Sousa et al.[18] as reporting good agreement between the concentrations of ethanol in the left and the right eyes. Therefore it appears likely that major discrepancies between the concentrations of chemicals in the different eyes indicate that one eye is abnormal (or that one sample is contaminated, for example with blood). Abnormal vitreous color or viscosity may indicate contamination or the presence of silicone oil (which floats on water). Silicone oil may adversely affect the measurement of many analytes and make interpretation of those analytes more difficult. If its presence is suspected, you should examine a drop of vitreous with a hand lens and search for droplets of silicone oil. The vitreous sample should be centrifuged before biochemical or toxicologic analysis; this step will also separate the vitreous into aqueous and contaminating oil phases. The aqueous phase then can be carefully separated and used for the analyses. Note that the results should be interpreted with even more caution than normally used in the interpretation of such quantitative analyses. Despite this requirement, less than optimal vitreous humor samples may be extremely useful for qualitative toxicologic screening.

The likelihood of intrinsic ophthalmic disease is small but should be considered in the interpretation of biochemical and toxicologic findings from the vitreous humor. Awareness of this potential problem is the key to avoiding pitfalls, and attention to the color and viscosity of vitreous samples and to the assay of samples from both eyes likely will ensure that errors do not occur. If only one vitreous humor sample is available, and where the biochemical result is of particular forensic or

medical importance, you should obtain an ophthalmic medical history and make inquiries to exclude the presence of ophthalmic conditions that might affect the chemistry of the vitreous and the interpretation of the result.

The Intact Eye (Anterior Approach)

The simple and rapid anterior approach is the method of choice for removal of the intact eye (globe) and part of the optic nerve where the pathology is intraocular and the optic nerve is normal.

This method *should not be used*

- when the optic nerve is (or could be) abnormal
- in intraocular vascular disease affecting the central retinal artery or vein and/or ciliary vessels
- when intraocular tumors extend through the sclera into the orbit
- when orbital disease affecting fat and/or extraocular muscles is present

I also recommend that this method *not* be used to remove the eyes of children or adults when nonaccidental injury is suspected, as important orbital pathology can easily be missed unless the whole orbital contents are removed together.

Method

1. Retract the eyelids manually or using eyelid retractors, and keep the eyelids and eyelashes in direct vision throughout the procedure to avoid irreparable cosmetic damage.
2. Insert long, blunt-ended (and preferably curved) scissors between the globe and the eyelids (Figure 4.2) and use blunt dissection whenever possible to divide the conjunctiva as far posteriorly as possible in a circle around the eye, 10 to 20 mm behind the corneoscleral junction (limbus). This maneuver can be accomplished with sharp dissection, but with increased risk of damage to eyelids or the eye.
3. Use blunt dissection to move posteriorly and circumferentially to free the globe from the orbital wall, keeping as far from the eye as possible. Toothed forceps can be used to exert gentle traction on the globe by gripping one of the extraocular muscles (at this stage a suture inserted into an identified rectus muscle close to its insertion helps orientation later). The extraocular muscles can be divided during this procedure if desired, leaving approximately 5 mm of their insertions into the globe to aid orientation later.
4. Retract the eye laterally and dissect it from the medial wall of the orbit towards its apex, then cut the optic nerve with sharp scissors as far posteriorly as possible. This step allows the eye to prolapse forward and laterally, and any attached structures can be divided.

Unwanted orbital tissue can be trimmed off before fixation, taking care not to remove muscle insertions, which are needed to orientate the eye so that appropriate tissue blocks can be taken for histology.

Note: Some modifications of this basic technique involve more detailed dissection and division of intraocular muscles and orbital structures,[19] but these steps are more time-consuming and less suitable for general use by pathologists.

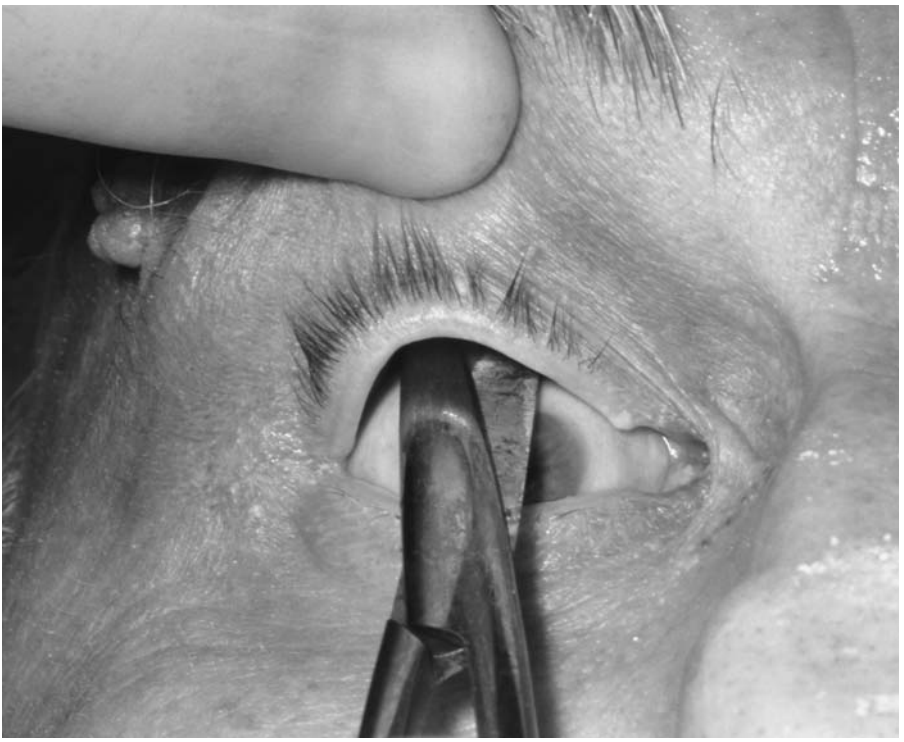

Fig. 4.2. To remove the right eye using the anterior approach, the eyelids are first retracted using the index finger and thumb to prevent eyelid damage. Curved scissors are inserted between the eyelid and the eye to divide the conjunctiva in a circle around the eye.

Reconstruction

Reconstruction is best done after the autopsy has been completed, the body and head incisions sutured, and the body washed.

1. Dry the face. Push any protruding orbital contents such as fat into the orbit.
2. Place a wad of wet, squeezed cotton wool (approximately 2-cm diameter) into the orbit and press firmly, then cover with dry cotton wool.
3. Insert a 2-cm-diameter, clear domed plastic eyecap (Dodge Chemical Company, Whitchurch, UK) into the orbit; this should approximate the normal position of the anterior (corneoscleral) surface of the eye (Figure 4.3). The final position of the eyecap can be modified by adding or removing the underlying cotton wool.
4. After ensuring that the eyelids can close normally over the plastic eyecap, dry the eyecap and the undersurface of the eyelids.
5. With the lids open, place superglue (Pacer RX-100 instant adhesive, Dodge Chemical Company, Whitchurch, UK, or an equivalent) on the front of the plastic eyecap (Figure 4.3), then rapidly position first the lower and then the upper eyelids, ensuring that the eyelids are closed with no gap.

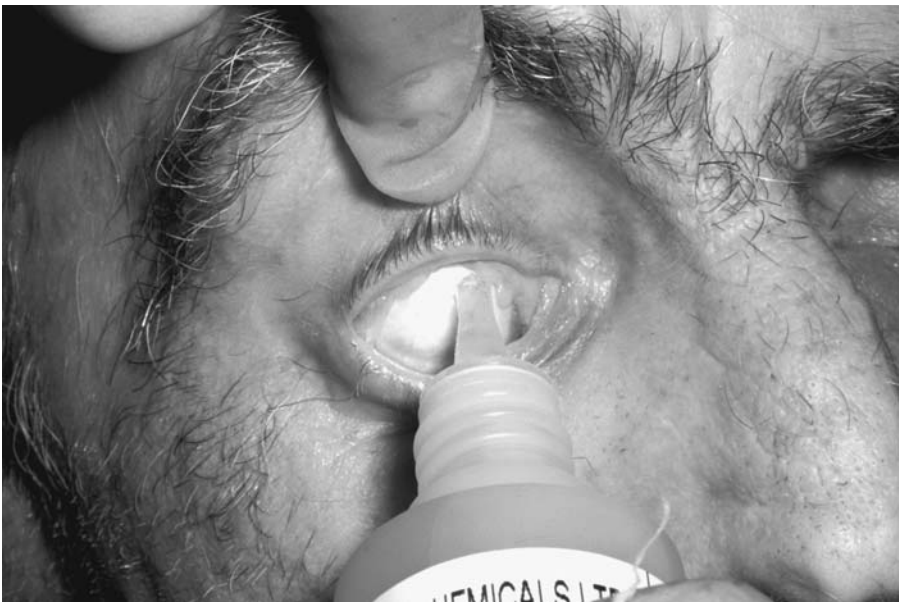

Fig. 4.3. Reconstruction of the left eye for cosmetic effect, after the body is washed and the face dried. A clear domed plastic eyecap has been inserted into the orbit after packing with cotton wool. The surface of the plastic eyecap and the inner surface of the eyelids are dried before superglue is applied to the surface of the eyecap, and the eyelids are placed in position (see text for details).

The Intact Eye and Orbital Contents (Posterior Approach)

The posterior approach via the cranial cavity is the method of choice for removing the eye and the contents of the orbit where there is

- ocular disease that extends into the orbit (ocular neoplasms with posterior invasion)
- vascular disease affecting the eye (where the ophthalmic and/or ciliary arteries and veins may be involved)
- optic nerve pathology
- orbital disease affecting orbital fat or extraocular muscles
- disease with eye and lacrimal gland involvement
- nonaccidental injury to the eye and/or orbit in children and adults (discussed later)

This method can be modified to remove the posterior part of the eye (behind the iris and lens) as part of a limited procedure, if this is requested (see The Posterior Part of the Eye Only).

The method *should not be used* if ocular or orbital disease extends into the bony wall of the orbit or beyond and into the frontal, ethmoid, or maxillary sinuses or the nasopharynx.

Method

1. Enter the cranial cavity and remove the brain by conventional means, then strip the dura from the anterior fossa of the skull.
2. Mark or identify the edges of the triangular piece of bone (Figure 4.4A), which forms the roof of the orbit; its posterior apex is formed by the optic nerve where it emerges from the optic canal. The medial edge of the triangle runs anteriorly from the medial aspect of the optic canal in a line that extends just lateral to the outer (lateral) aspect of the cribriform plate of the ethmoid bone. The lateral

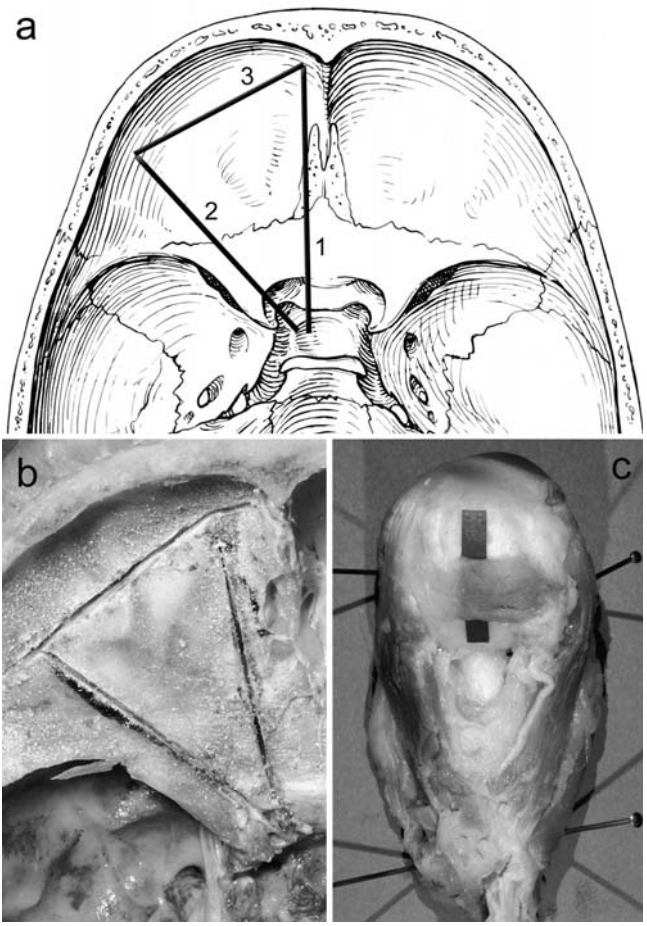

Fig. 4.4. Removal of right orbital contents through the base of the skull (posterior approach). **A, B:** After the brain and dura are removed, the triangle of bone forming the roof of the orbit is marked. Note the optic nerve is at its posterior apex. A mechanical saw is used to cut carefully into the orbit along cuts 1, 2, and 3 (see text for details). **C:** After removal of some fat, the right orbital contents (inferior surface) are pinned gently to a board for fixation. A piece of card lies under the muscular insertion of the inferior oblique muscle, on the inferior surface of the specimen (see Figure 4.10).

edge of the triangle runs anterolaterally from the lateral aspect of the optic canal just inside and parallel to the edge of the lesser wing of the sphenoid bone.

3. Using a pneumatic oscillating mechanical saw make cuts along the medial and lateral edges of this triangle (Figure 4.4A, cuts 1 and 2), taking care to withdraw pressure from the saw as soon as the orbit is entered to avoid damaging the soft orbital contents (the roof of the orbit is only about 2 mm thick, and there is sudden loss of resistance to the saw when the orbit is entered).
4. Extend the cuts anteriorly to a point approximately 10 mm from the inner original sawed edge of the frontal bone (take care not to extend the cut too far anteriorly, as the roof of the orbit is not far from the skin beneath the eyebrows at this point).
5. Saw into the orbit between the two anterior ends of the two cuts (Figure 4.4A, cut 3).
6. Insert a bone chisel into the cuts to break small points of attached bone, and lift the bony triangle by twisting the bone chisel, dissecting off any soft tissues. The orbital contents are now exposed.
7. Using blunt dissection wherever possible, separate the optic nerve and orbital contents from the bony walls of the orbit, working anteriorly to a point about half the distance between the apex of the orbit (at the posterior point of the triangle of removed bone) and the anterior base of the triangle.
8. At this stage it is vital to reflect the frontal skin of the face back to expose the eyelids and to dissect the eye from the eyelids using the anterior approach (Figure 4.2), carefully avoiding damage to the eyelids (which almost certainly would occur if this step was attempted from the posterior orbital route alone).
9. When the conjunctiva has been cut circumferentially, dissect toward the bony walls of the orbit, using blunt dissection, taking care to avoid the soft skin of the eyelids anteriorly. The anterior and posterior dissections along the bony orbital walls eventually will connect, and the eye and orbital contents can be delivered posteriorly, aided by a gentle push with a finger between the eyelids.
10. After removal, orientate the orbital contents, which then can be pinned gently to a cork board for fixation without distortion (Figure 4.4c). (For orientation details, see "Orientation of the Eye and Preparation for Histologic Examination.")

Reconstruction

1. Pack the orbit with a core of squeezed wet cotton wool surrounded by dry cotton wool. If desired, a 2-cm-diameter, plastic eyecap can be used in the posterior orbit, followed by packing with dry cotton wool and reattachment of the bony roof of the orbit using superglue.
2. Pack the cranial cavity with cotton wool, replace the skull cap, and close all skin incisions.
3. After washing the body (and as the last procedure performed on the body), the anterior orbit is packed, a plastic eye cap is inserted, and the eyelids are stuck down using instant adhesive (as for reconstruction following removal of the eyes via the anterior approach).

The Posterior Part of the Eye Only

Occasionally it is possible to obtain permission to remove only the posterior part of the eye, leaving the cornea, anterior chamber, and the iris-lens diaphragm undisturbed. The obvious reason is that the eye is left cosmetically normal for viewing by relatives, while important macular or retinal disease can be investigated by the pathologist. However, I advise that the eye be removed intact using other methods, if at all possible.

This method has been used in autopsies involving infants or children, when a good cosmetic result is particularly important. However, if there is the remotest possibility of nonaccidental injury, the transverse cut across the equator of the eye will either disrupt or leave behind (with the retained anterior half of the eye) peripheral retinal hemorrhages and focal areas of retinal detachment, which may be the earliest and/or the most characteristic diagnostic features of the shaken baby.[10] Therefore I advise that both eyes and orbital contents be removed and fixed intact in any such case and in any death of uncertain cause involving the central nervous system in children.

Method

This method is relatively difficult and requires some skill in order not to damage the iris and produce cosmetic defects. It also may disrupt the retina severely as a result of traction on the vitreous and direct trauma. For this reason, fixative (I prefer 0.5 ml of 3% glutaraldehyde) is introduced into the vitreous before the procedure, as soon as possible after death but a minimum of 30 minutes before the remainder of the procedure. This step makes the eye firmer and reduces the risk of artifactual retinal detachment.

1. At least 30 minutes before the remainder of the procedure, introduce a needle slightly obliquely into the vitreous, as in step 1 of the procedure for removing the vitreous (see Vitreous Fluid Samples), and inject approximately 0.5 ml of 3% glutaraldehyde into the vitreous.
2. Use the cranial posterior approach to remove the bone of the superior aspect of the orbit, as for steps 1 through 6 of th\e procedure for removing the intact eye and orbital contents (see The Intact Eye and Orbital Contents [Posterior Approach]).
3. Dissect and divide or remove the orbital muscles and fat in layers to expose the optic nerve from above, and divide the nerve as posteriorly as possible.
4. The eye can now be cut along the equator (in the coronal plane), superiorly initially with a sharp knife, then with scissors, to separate the posterior half of the eye. Ensure that the initial cut extends through the retina as well as the sclera and choroid. You may wish to place a small suture or V cut in the superior aspect of the cut edge of the sclera to aid subsequent orientation.
5. The inferior rectus muscle must be divided to free the posterior half of the eye.

Reconstruction

1. Gently place a pad of damp (not wet) cotton wool dyed with black ink behind the exposed iris-lens diaphragm, and pack the orbit gently with more cotton wool. Too much pressure at this stage could disrupt the iris and pupil.

2. Replace the bony roof of the orbit (stick with superglue).
3. Pack the cranial cavity, replace the skull cap, and suture all skin incisions.

The Intact Eye, Orbital Contents, and Bony Walls of the Orbit

If the eye and orbital pathology extends to involve the bony wall(s) of the orbit, it is possible to remove the eye and orbital contents within the intact bony wall of the orbit. Specific consent from relatives or the coroner (or equivalent authority) is advised, although there should be no cosmetic abnormality after this procedure.

Method

The best approach under these exceptional circumstances is as follows.

1. Use a conventional Y-shaped skin incision for dissection of the neck, combined with a conventional incision for entering the cranial cavity.
2. Open the cranial cavity and remove the brain. On the side from which the orbit is to be removed, extend the neck incision superiorly behind the ears to connect with the scalp incision.
3. Starting from this new incision, reflect a flap of skin and underlying soft tissue anteriorly off the skull, lifting the pinna and dividing the external auditory meatus where it enters the skull.
4. Dissect the flap medially over the frontal and maxillary bones to the lateral aspect of the nose, lifting the eyelids (with great care) from the anterior orbital rim, cutting the conjunctiva circumferentially at the fornix, and exposing the anterior aspect of the eye. This dissection also exposes the upper jaw and teeth (taking care not to cut the skin around the lips). The lower face (over the mandible) is reflected as little as possible.
5. With the cranial cavity opened and the brain removed, remove a large segment of the bone of the floor of the skull around the orbital wall using two cuts through bone (Figure 4.5). The resulting wedge of bone is bordered medially by the maxilla and frontal bone just lateral to the nose and laterally by part of the zygo-

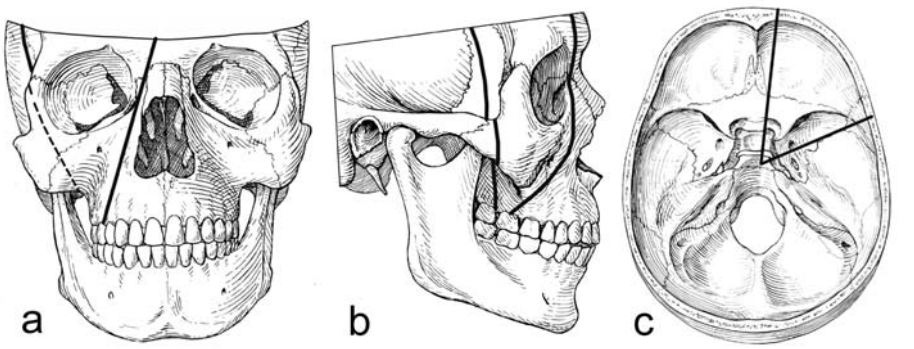

Fig. 4.5. Removal of the right orbit with intact bony orbital walls requires two mechanical saw cuts, seen from the anterior view **(A),** from the right side **(B),** and from above **(C).** The top of the skull and brain have been removed after reflection of soft tissues (see text for details). (Adapted and reproduced with permission from the BMJ Publishing Group from Parsons MA, Start RD. Necropsy techniques in ophthalmic pathology. ACP Best Practice No. 164. J Clin Pathol 2001;54:417–427.)

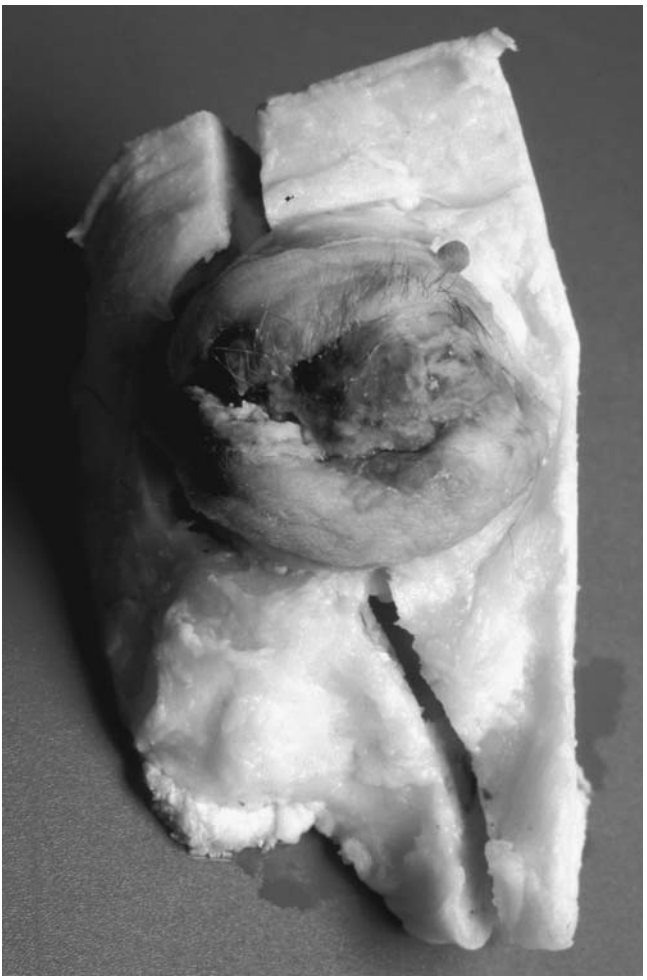

Fig. 4.6. The intact orbit, with bony walls and attached eyelids, has been removed and the bone bisected vertically. The patient died of causes unrelated to the extensive conjunctival malignant melanoma with infiltration of the eyelids and orbital extension. Dissection of the orbital contents from the lateral wall of the orbit exposed the lateral aspect of the orbital contents (and sinuses interiorly) to fixation. Specific permission was given by a relative for removal of the eyelids and orbit, and use of an eye patch was agreed in reconstruction (because the eyelids were attached, and the eye patch had been used in life). (Adapted and reproduced with permission from the BMJ Publishing Group Parsons from Parsons MA, Start RD. Necropsy techniques in ophthalmic pathology. ACP Best Practice No. 164. J Clin Pathol 2001;54:417–427.)

matic arch and greater wing of the sphenoid bone (from above the cranial cavity the cuts fall outside those made for the posterior approach to removal of orbital contents, with an additional coronal cut just posterior to the optic foramen). If desired, the maxilla can be cut in a horizontal plane at the level of the lower part of the zygomatic arch, which avoids removal of one or more upper teeth (molars).

6. The excised block of bone and orbit can be fixed intact, but further cuts can be made through the bone into the orbit to aid rapid fixation (Figure 4.6).

Reconstruction

The skull is stable after this procedure, and the cavity is easily packed with squeezed wet cotton wool alone or with orthopedic bandages impregnated with plaster of Paris. The eye is best reconstructed after all of the scalp and neck skin incisions have been sutured (when the eyelids fall into place), using the method described for the anterior approach for eye removal (see The Intact Eye [Anterior Approach]).

The Orbit(s), Nasal Passages, and Sinuses

Under very exceptional circumstances it may be necessary to remove one or both orbits with midline structures including the upper jaw and the palate. Examples include the investigation of malignant or granulomatous disease involving combinations of the eye or orbit, midline nasal or nasopharyngeal structures, the frontal, ethmoid, or maxillary sinuses, or the base of the skull. I recommend that you obtain specific consent from the relatives or the coroner (or equivalent authority; see "Ethical Considerations"), although with care there should be little or no residual cosmetic alteration. Discussion with involved clinical colleagues and careful planning are required before undertaking such procedures, and modifications can be planned according to the extent of the disease process. It may be very valuable for the surgeon to attend (or assist with) this part of the autopsy, always assuming that the coroner (or equivalent authority) has approved in cases under their jurisdiction.

Method

1. The approach requires a Y-shaped neck incision, extended superiorly behind the ear(s) to connect with the scalp incision(s), as for removal of the orbit with bony walls (see The Intact Eye, Orbital Contents, and Bony Walls of the Orbit).
2. Reflect the skin on the affected side, as for removal of one orbit (see The Intact Eye, Orbital Contents, and Bony Walls of the Orbit), and lift the skin carefully from the nose. If both orbits are to be removed, reflect the tissues inferiorly from the whole of the upper face, including the nose (retaining attachments over the mandible).
3. Remove the block of skull and facial bones (Figures 4.7 and 4.8), including most of the frontal bone and all of the upper jaw and both orbits in one piece (one uninvolved orbit can be retained, as in Figure 4.7), and the block modified according to the known extent of the disease process.

Reconstruction

Careful reconstruction is accomplished using orthopedic bandages impregnated with plaster of Paris and wet/dry cotton wool. The remaining skull is stable, and replacement of the top of the skull provides the shape of the front of the face when skin sutures are inserted. In practice the shaping of the nose with plaster of Paris is not particularly difficult, and the bandage impregnated with plaster of Paris are easily removed when wet if the first attempt does not produce a satisfactory result. The final cosmetic effect is likely to be excellent.

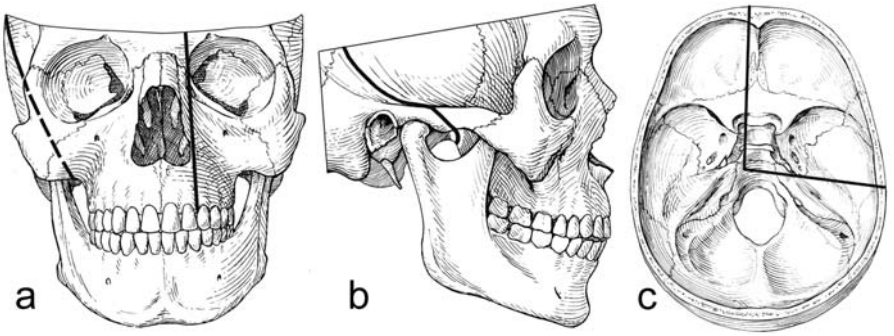

Fig. 4.7. The right orbit, with nasal passages and sinuses and part of the maxilla, can be removed using left paramedian and right temporal mechanical saw cuts, seen from the anterior view **(A),** from the right side **(B),** and from above **(C).** The top of the skull and the brain have been removed after reflection of soft tissues (see text for details). (Adapted and reproduced with permission from the BMJ Publishing Group from Parsons MA, Start RD. Necropsy techniques in ophthalmic pathology. ACP Best Practice No. 164. J Clin Pathol 2001;54:417–427.)

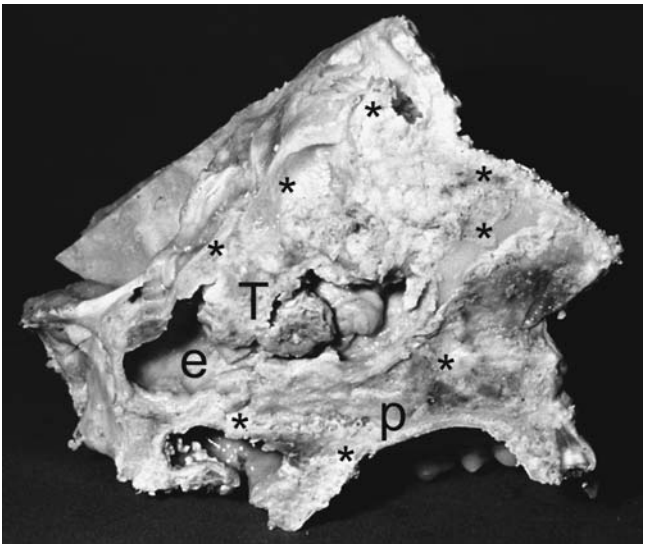

Fig. 4.8. Midline view (nose right) of the left nasal passages, sinuses (*e,* ethmoid sinus), nasal passage, maxilla, and palate *(p)* removed in a block. A large fungating ethmoid adenocarcinoma *(T)* originates from the ethmoid sinuses and invades the surrounding structures as far as the areas marked by *asterisks.* The tumor had entered both orbits, fixing eye movements and blinding the patient. (Ethmoid adenocarcinoma as the result of occupational hardwood dust exposure.)

Removing Individual Adnexal Ocular Structures

The eyelids are easily removed but results in obvious cosmetic defects, thus requiring specific consent from the relatives or the coroner (or equivalent authority), with attendant ethical considerations. An eye patch will be required if the body is to be viewed. In practice it is best to involve an ophthalmic surgeon if individual lacrimal glands, nasolacrimal ducts, meibomian glands, and other adnexal structures need to be removed. The ophthalmic surgeon can perform the procedure for you (this is the ideal scenario) but at least can advise about likely cosmetic effects so that arrangements can be made accordingly.

Obtaining Fresh (Unfixed) Intraocular Tissues

In some circumstances (e.g., in case of intraocular tumor), it can be worthwhile to sample the tumor fresh, accepting that this will inevitably cause unwanted damage to the eye and perhaps unwanted contamination of intraocular and extraocular tissues with tumor cells. If this is the case, the eye should be orientated (see below "Orientation of the Eye and Preparation for Histologic Examination"), and the position of the tumor determined by transillumination before opening the eye by removing a calotte adjacent to the tumor (see below "Transillumination" and "Dissecting the Eye"). After a tumor sample has been removed, the eye can be gently lowered into formalin (the freshly opened eye retains its shape without further treatment).

Fixation of Ocular Tissues

I find that in ideal circumstances, it is best to fix the intact globe in 3% buffered glutaraldehyde for 12 hours, followed by 10% formalin for at least 24 hours, both at room temperature. Use of formalin alone tends to discolor the specimen, but, more importantly, it may cause artifactual retinal detachment in the intact eye during fixation. However, use of glutaraldehyde also has disadvantages (e.g., if immunohistochemistry is required), and this step can be omitted without major detrimental effect.

Do not inject fixative into the eye without thought of the consequences, as this can cause serious displacement of intraocular tissues. However, injection of 0.5 ml of 3% glutaraldehyde into the vitreous may be justified if

- there is likely to be a delayed autopsy (to prevent autolysis)
- the vitreous is essentially normal or is of little interest
- there is a particular interest in the pathology of the retina or macula
- only the posterior half of the eye is to be removed (see The Posterior Part of the Eye Only).

For larger specimens, such as the whole orbital contents with the globe, I advise fixation in 10% formalin. I find that orientation and pinning to a cork board before fixation (floating upside down) prevents distortion of the orbital anatomy. Specimens with the eye surrounded by bony orbit can be fixed intact before further dissection or partially dissected to aid fixation of the orbital contents (possibly changing the buffered formalin for optimal effect).

Eyes from patients with known or suspected spongiform encephalopathies (or high-risk cases) must be fixed using the formalin-formic acid or formalin/hypochlorite procedure[20,21] before subsequent treatment for histology.

Orientation of the Eye and Preparation for Histologic Examination

A detailed description of the examination of the eye and adnexal structures and their preparation for histologic examination is beyond the scope of this paper, and the reader is referred to more detailed published texts for further information on technical methods[22,23] and interpretation of macroscopic and microscopic findings.[24]

For most purposes a horizontal section is taken for histologic examination, as this is the only plane in which the center of the pupil and the optic nerve appear in the same histologic section (note that the fovea centralis, at the center of the macula, lies in this horizontal plane approximately 2 mm inferior to the center of the optic disc). The eye is oriented using the attachments of the extraocular muscles (Figures 4.9 and 4.10), but an easier approach is to place a suture in the insertion of the superior rectus (or another) muscle before removing the eye (with the attached suture).

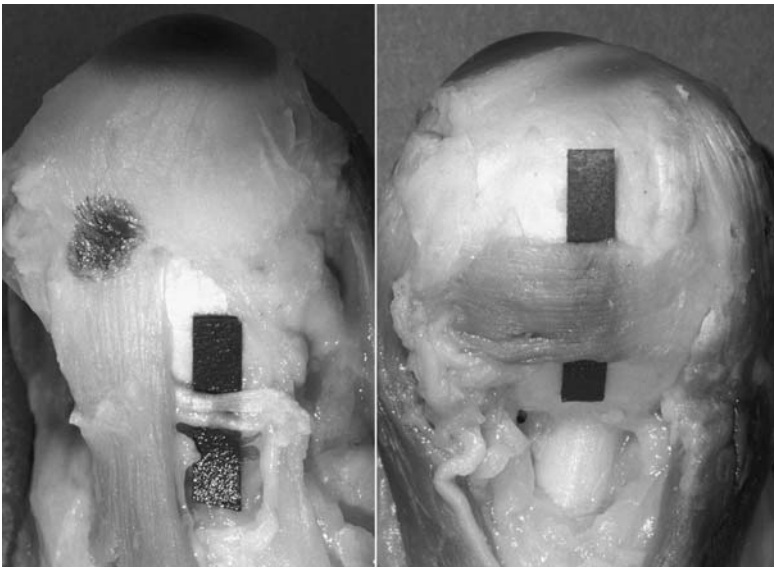

Fig. 4.9. Orientation of the removed right eye. **Left:** The superior aspect of the right globe showing the superior oblique muscle forms a tendon (over a piece of card) as it runs towards its insertion beneath the superior rectus muscle. The insertion of the superior rectus muscle (marked with ink dot) orientates the eye. **Right:** The inferior aspect of the right globe showing (in contrast) the muscular insertion of the inferior oblique muscle running over a piece of card close to the optic nerve (below it). The point of insertion of the inferior oblique muscle, close to the lateral rectus muscle border, forms the external scleral marker of the macula within the eye. (Adapted and reproduced with permission from the BMJ Publishing Group from Parsons MA, Start RD. Necropsy techniques in ophthalmic pathology. ACP Best Practice No. 164. J Clin Pathol 2001;54:417–427.)

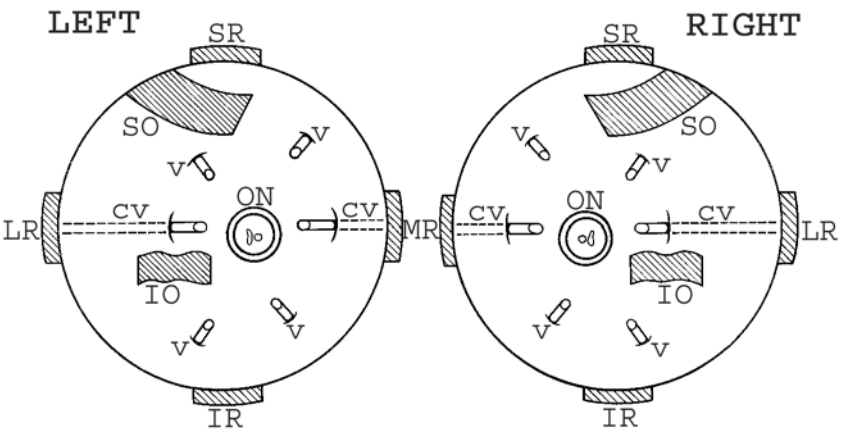

Fig. 4.10. Diagram of the posterior aspects of the eyes showing just the rectus muscles (*IR,* inferior; *LR,* lateral; *MR,* medial; *SR,* superior). The superior oblique muscle tendon *(SO)* inserts close to the superior rectus muscle (SR) and is further away from the optic nerve *(ON)* than is the inferior oblique muscle *(IO).* Note that, from the back, the inferior oblique muscle is to the right of the optic nerve in the right eye and to its left in the left eye – the best way to determine which eye you are examining. The posterior ciliary blood vessels *(cv)* form a useful blue horizontal line on the sclera. Note the four vortex veins *(v)* in each eye, sampled to determine if posterior uveal melanomas have invaded through the sclera.

The superior oblique muscle has a tendonous insertion beneath the anterior part of the superior rectus muscle (Figure 4.9). By contrast, the inferior oblique muscle is muscular to its insertion. This point of insertion lies close to the lateral rectus muscle, and the muscle insertion is the external scleral marker of the position of the macula within the eye (Figure 4.9). The posterior margin of the lateral rectus muscle lies close to the optic nerve, whereas the superior oblique muscle has a more anterior insertion (Figure 4.10).

There is a useful rule for distinguishing between the right and left eyes when both unlabeled eyes are put into a fixative container together or if doubt exists about labeling of an eye. Identify the superior oblique muscle and the insertion or the superior rectus muscle, and then orientate the eye (Figure 4.10) so that the superior aspect of the eye is uppermost. The posterior view of the eye will now have the optic nerve just off center medially (nasally). Thus the left eye has the insertion of the inferior oblique muscle to the left of the optic nerve, and in the right eye it is to the right of the optic nerve.

Transillumination

You can use this simple technique before dissecting the eye, to discover whether there are any dense focal lesions (such as tumors or collections of blood) in the posterior chamber. The isolated eye normally glows like a Chinese lantern when a bright light is directed through the pupil into the posterior chamber (a fiberoptic source is ideal, shielded by the fingers). Shadows produced, for example, by tumors, can be outlined with indelible ink on the sclera (if it is dried first) to aid subsequent cutting of the eye.

Retroillumination of the eye (by placing the light source on the posterior sclera and directing it forward) is an excellent method for highlighting abnormalities of the pupil and holes (such as produced by focal surgical iridectomy) in the iris. This is particularly useful if the cornea or anterior chamber contents are semiopaque.

Dissecting the Eye

As a good general rule, the eye is *never* cut through its center, as this disrupts the delicate zonules of the lens, causing dislocation. Two parallel horizontal cuts are made (Figure 4.11A and 4.11B) approximately 1 mm below the limbus superiorly and above the limbus inferiorly,[22] removing the two calottes (or "caps"). The resulting central block, incorporating the whole lens and the optic nerve (Figure 4.11C), is processed into wax intact for later sectioning to the midline. Some pathologists would make a possible exception to this rule and cut a tissue block of "normal" thickness in disorganized and phthisical eyes, when the lens has been lost or is fused in a mass of fibrous or bony tissue (often caused by osseous metaplasia of RPE cells). In inexperienced hands, however, I recommend great care and a two-stage approach under such circumstances. First open the eye by removing a calotte to ensure there is nothing in the eye (e.g., a plastic intraocular lens) that will be damaged at the next stage. After decalcification (if required), a central thin block can be cut for subsequent processing.

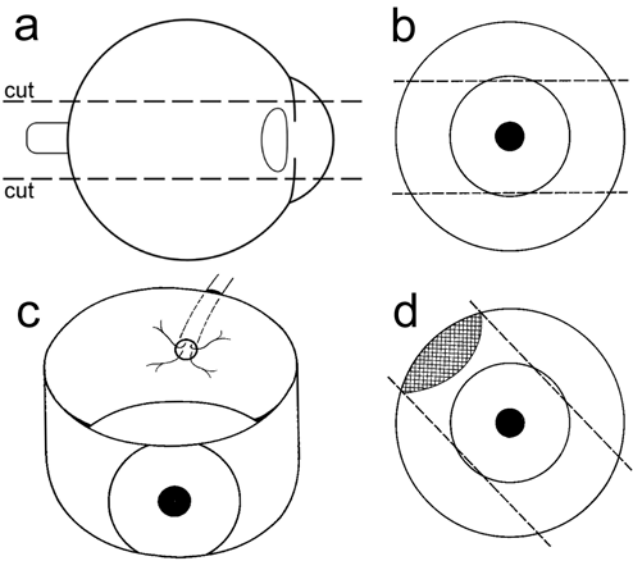

Fig. 4.11. **A:** Lateral view of the eye showing the two "standard" horizontal cuts that avoid the lens and just enter the anterior chamber. **B:** Anterior view. **C:** The resulting horizontal eye block is processed into wax before sectioning to the midline (superior to inferior direction). **D:** If a tumor is found in the eye, the cuts are rotated as indicated, again avoiding the lens (see text for details).

Vertical cuts may be necessary if an artificial lens was inserted in life, to avoid the lens haptics, the small pieces of plastic that extend from the lens and hold the lens in place in the anterior or posterior chamber. These plastic lenses dissolve during tissue processing, and do so without the damage you almost certainly would inflict if you attempted to remove them mechanically. If a tumor is present, as determined by transillumination of the eye through the pupil, mark its edges on the sclera with a pen and cut vertically into the tumor at 90 degrees to the underlying sclera (Figure 4.11D), in a plane that also passes through the center of the pupil (again, plan to cut either side of the lens).

If you have localized a lesion in the posterior chamber, you may elect to dissect the eye so that a perpendicular section is produced (for histology) through that lesion and the sclera. This is particularly important when dissecting tumors such as malignant melanomas (in adults) or retinoblastomas (in children), as such a cut will provide optimum information about invasion of the choroid and sclera. Under these circumstances, rotate the eye so that the cuts through the tumor avoid the lens but pass through the limbus (Figure 4.11D). Note that the final position of the optic nerve is of secondary importance under these circumstances (you will need to take transverse sections of the optic nerve posterior resection margin to determine whether the tumor is present at this prognostically important site).

In my hands the best instrument to cut open the eye is a disposable low-profile microtome blade (e.g., Accu-Edge, Sakura Finetek U.S.A. Inc., Torraine, CA, USA) held in a microknife blade holder (Cell Path plc, Newtown, Powys UK). I use a fresh blade for each eye, as the fixed sclera is extremely hard, and it blunts any blade rapidly.

Note: After cutting the eye, take care that the gelatinous (or liquid) vitreous does not prolapse from the eye (if it is filled), as this could detach the retina. If the vitreous touches paper tissue (or similar material), moving this material also could detach the retina.

Fetal Eyes

Isolated eyes from a small fetus may be tiny and are best processed intact (and sectioned anteroposteriorly through the cornea and optic nerve) if their diameter is less than 3 or 4 mm. Slightly larger eyes may be more difficult to treat in this way, and you may consider removing one horizontal calotte only, before processing the eye. Still larger eyes (e.g., from a third trimester fetus) are processed "normally," as for adult eyes.

In a small fetus, the eyes can be processed with the head and sectioned horizontally in situ.

Gilliland and Folberg[25] described a technique for examining the eyes of children in suspected child abuse by removing the anterior segment of the eye (cornea, anterior chamber, iris, lens, and pars plicata of the ciliary body) in the coronal plane. The aim was to expose any retinal hemorrhages in these children so that photographs would show the fundus (to a jury) as it would be seen in life by an ophthalmologist. This technique is fraught with potential dangers for all but experienced ophthalmic pathologists. However, I still do not use this method because it destroys the continuity between anterior and posterior segment findings in nonaccidental blunt traumatic injury.

Macroscopic Examination and Recording of Pathologic Findings

It is important that you develop a careful systematic approach to the examination of the eye, regarding it almost as a "mini-autopsy" in its own right. This approach is necessary because it is easy to overlook important diagnostic features in many different parts of the eye, and because dissection and processing of the eye rarely leave enough tissue for meaningful reexamination. For these reasons, it is vitally important to record the examination findings correctly, ideally supplementing written findings with photographs of important features.

I strongly advise that you use a dissecting microscope to examine the eye, both externally and internally. After just a little practice, you will find that this step allows you to appreciate diagnostic detail, which you will miss without magnification. It also considerably aids subsequent interpretation of histologic sections. Use a good fiberoptic or "ring" light source while examining the eye.

The following is a brief account of the external and internal examination of the eye. Because of limitations of space this section is by no means comprehensive, and you should refer to detailed ophthalmic pathology textbooks for more detail. I recommend Lee's "Ophthalmic Histopathology" for both his illustrations and practical approach to diagnosis, which is particularly suitable for histopathologists with little background in ophthalmology.[24]

External Examination

Clean the eye carefully, removing any attached blood clot with saline-soaked paper tissue and blunt forceps, before making some vital basic measurements.

1. Measure the dimensions of the eye
   - Anteroposterior (normal 22–23 mm)
   - Horizontal (normal 22–23 mm)
   - Vertical (normal 22–23 mm)

   The anteroposterior dimension is increased in myopia (short-sightedness) and decreased in hypermetropia (long-sightedness).

   Measure the horizontal and vertical diameters of the cornea (normally 12 mm and 11 mm, respectively) from the limbus (the corneoscleral junction).

   Record the length of the attached optic nerve.
2. Record the appearance of the surface of the cornea (opaque, ulcerated?) and the corneal stroma (general opacity; size and nature of focal opacities). Record (if visible) the contents of the anterior chamber and note whether they obstruct view of the iris (e.g., hypopyon [pus] or hyphema [blood] in the anterior chamber).
3. Record abnormalities of the shape and appearance of the iris and pupil (if visible). Are there any defects (e.g., iridectomy) in the iris? (Retroillumination, using a fiberoptic light source touching the posterior sclera and directing it anteriorly, highlights pupil and iris defects, if the posterior chamber is translucent.)

4. Record any abnormalities of the shape and appearance of the episclera and sclera.
5. Measure the length, diameter, and appearance of the optic nerve.

Internal Examination

After making cuts in the eye (see "Dissecting the Eye"), examine the individual structures inside the eye, working systemically from the anterior to the posterior.

Cornea: Surface, shape and opacity
Anterior chamber: Shape (deep or shallow), content (pus, blood, exudates)
Anterior chamber (iridocorneal) angle: Shape open (normally 45 degrees), narrow (5–30 degrees), or closed; presence of artificial lens
Iris: Pupil, surface, defects
Ciliary body: Ciliary processes (color, focal abnormalities), pars plana (scars, exudates, focal abnormalities)
Lens: Position, size, shape, transparency; position of opacities (cortex, nucleus, focal, etc.); presence or absence of artificial lens
Vitreous: Opacity; position of detachment, if present; presence of blood, pus, or oil (e.g., silicone oil); localized opacities (e.g., lymphoma)
Retina: Attachment (local, total); presence, position, and pigmentation of focal/diffuse lesions; presence and appearance of subretinal material (if retina detached)
Macula (located just below the horizontal line, lateral to the optic disc, often with a central yellow macula lutea): Localized swelling, exudates, detachment or pigmentation
Optic disc: Size, blurring of edges, swelling, cupping (glaucoma), atrophy
Choroid: Position (?detached), thickening (diffuse or local), presence and pigmentation of diffuse or local lesions (e.g., malignant melanoma, lymphoma)
Sclera: Shape (e.g., staphyloma [bulging and thinning; thickening]), calcification pigmentation
*Abnormal masses: P*osition, size, attachments, color, consistency (hard, soft, cystic)

X-Ray Films of the Eye

You should consider taking x-ray films of the eye (and associated tissues, if present) if there has been penetrating trauma, particularly if a foreign body is suspected. This is particularly important if a clinician has not been able to examine the eye because of hyphema, and where the clinical history or circumstances are unclear, unknown, or suspicious. I once found an air-gun dart on x-ray film of an eye enucleated after trauma from a patient with an (until then) vague clinical history (Figure 4.12).

If a foreign body is found, take more x-ray films with additional position markers (e.g., metal pins), particularly because small foreign bodies may be very difficult to find.

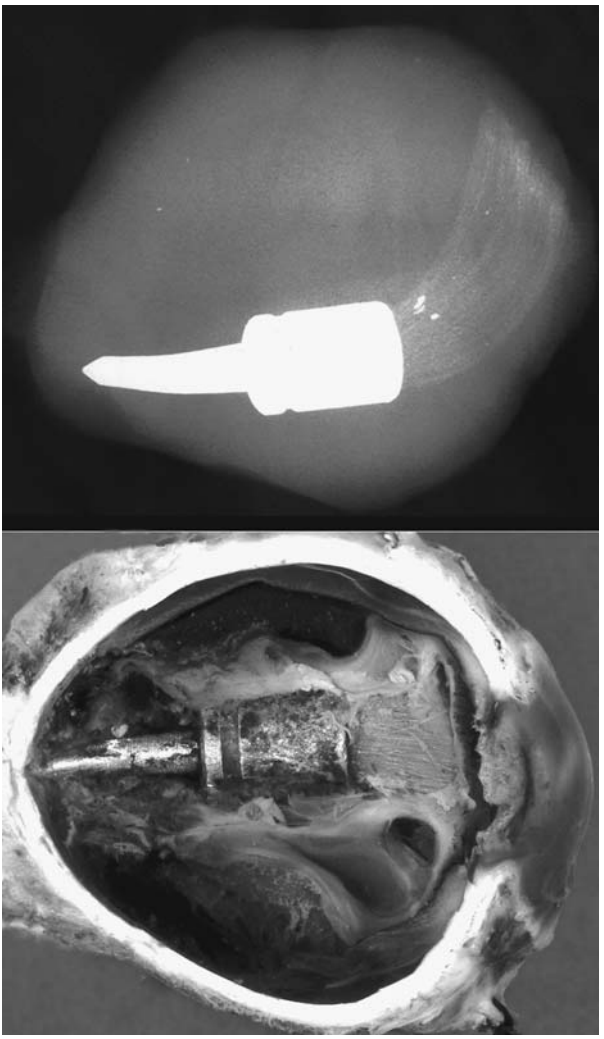

Fig. 4.12. X-ray films of eyes can be an important part of the examination. An unsuspected air-gun dart found in the posterior chamber on x-ray film **(top),** which aided specimen dissection (macroscopic pathology; **bottom**).

Photography of the Eye

Photography of eye specimens, well described by Eagle,[26] is a vital form of documentation, particularly for purposes of comparison with clinical ophthalmic images (slitlamp/fundus photographs, fluorescein angiography, and in forensic examinations, e.g., deaths from child abuse, see "Diagonostic Ophthalmic Pathology of Suspected Child Abuse"). Because the eye is small, good magnification is required from camera systems. Lighting can be difficult, as the eye has wet and translucent surfaces that cause reflections of necessary good even lighting. The inside of the eye is (in effect) a "deep dark hole," and small camera apertures (f32–f16) are required for good depth of field (range of focus).

To eliminate reflective highlights, photograph the eye under normal saline (not water, the absorption of which may make the vitreous opaque) or 70% alcohol, preferably after fixation.

The eye can be suspended on a plastic cylinder or pins and can be tilted to obtain required views. If the eye contains oil (which floats to the surface and blurs the photograph), the oil must be removed (by gentle agitation of the whole *fixed* eye in weak household detergent, if otherwise appropriate) before attempting photography.

Laboratory Processing

Note that is much easier to cut sections of a wax-embedded eye (particularly when it has been fixed in glutaraldehyde) if the eye is softened during a tissue processing regimen that includes stages in phenol, as follows.

Eye Processing Schedule

1 hour	10% neutral buffered formaldehyde
1 hour	70% phenol (80 g phenol in 100 ml 70% ethanol)
1 hour	95% phenol (80 g phenol in 100 ml 95% ethanol)
1 hour	99% ethanol
1 hour	99% ethanol
1.5 hours	99% ethanol
1.5 hours	99% ethanol
2 hours	ethanol/xylene (50:50 v:v 99% ethanol and 50% xylene)
2 hours	xylene
2 hours	xylene
2 hours	wax
3 hours	wax

Diagnostic Ophthalmic Pathology of Suspected Child Abuse

Aims

The intention of this section is to show how an ophthalmic pathologist is involved in the detailed investigation of infant deaths, which may be the result of physical child abuse. The scope of ophthalmic pathologic examination of these infants covers a range of diagnoses, including accidental falls with head injury, abusive head injury, abusive shaking injury ("shaken baby syndrome"), smothering, sudden infant death syndrome, accidental and abusive blunt trauma, and others. The discussion in this chapter cannot include all important differential diagnoses, and its main aim is to show the importance of obtaining the eyes as soon as possible after death, as a mandatory component of such investigations. In the hands of experts, the eye pathology can provide important positive and exclusive evidence for the investigation of infant deaths.

The Role of the Ophthalmic Pathologist

The starting position is a dead child and the investigation of the death of that child. The duty of the ophthalmic pathologist engaged to assist in that investigation is to determine what abnormalities are present in the tissues and to record the findings clearly using photographic images and words so that nothing is lost to any other investigator who may follow, for example, medical experts employed to assess the ophthalmic findings and conclusions drawn from them. Detailed and careful examination of the eyes is vitally important. In my consideration, high-quality macroscopic pathology of the outer and inner aspects of the eyes in particular is mandatory to preserve evidence that is lost to other experts by subsequent tissue processing.

Once the eye has been examined macroscopically and microscopically, it is vital that the ophthalmic pathologist interpret the ophthalmic findings with full knowledge of other autopsy findings and in the context of the medical history and the multidisciplinary investigation findings (police, social services, etc.) into the wider circumstances of the child. The duty of the ophthalmic pathologist is to the truth and to any court involved, and not to "the prosecution" or "the defense" who may have engaged them. If investigating a fatality, the desired "medical outcome" is the prevention of injury to any other children in the same social circumstances, and not the prosecution of the alleged perpetrator (although the evidence obtained by the ophthalmic pathologist may be used for this purpose). Numerous pitfalls await pathologists and other medical experts at all stages of the investigation and preparation of their report, the latter documented and discussed wisely in a review by David.[27] For this reason, expert examinations and opinions are vital, and no pathologist should proceed with such investigations (other than obtaining the necessary samples) without this expertise.

Much media attention has focused on medical evidence in alleged child abuse. In recent times, medical theories have tended to be released to the press, and discussed in court cases, well before they have been properly assessed scientifically, a practice much to be deplored and not to be practiced. Controversy certainly exists about some aspects of diagnosis in this field; some have even questioned the value of histologic examination of the eyes under such circumstances.[28] One theory suggested that only very gentle shaking of infants can cause the severe (and often fatal) head injury and eye trauma[29,30] although now this theory is being seen as a minority view based on highly inadequate data.[31]

In reality, a large body of medical evidence is well established[10,32–35] and is reviewed by Levin.[32] Papers such as those produced by the Ophthalmology Child Abuse Working Party[33,34] are attempts to find consensus views on these important issues, to guide clinical practice. This is not in any way to say that there are not difficult and poorly understood aspects of the precise mechanisms by which infants are physically abused. My aim in this chapter is to highlight the pathologic features that must be looked for and properly documented in the eyes. Once you and the ophthalmic pathologist have secured this "primary evidence," other experts and lawyers will have the opportunity to see the pathologic material on which to base their discussions and opinions. If allowed by the court, juries also will be able to see the material.

Methods

The expert ophthalmic pathologist is often presented with the eyes in fixative solution but ideally should examine the face and eyelids before the eyes are removed for distribution of postmortem lividity, signs of head and eye trauma, and hemorrhages (including petechial hemorrhages of the face and conjunctiva). The pathologist removing the eyes should obtain extensive high-quality photographs of the head, neck, face, and eyes before they are removed, preferably according to a protocol that ensures no important views are missed (see Practice Points). You should remove the eyes with the orbital contents; I use an anterior dissection combined with dissection via the roof of the orbit (see "The Intact Eye and Orbital Contents (Posterior Approach)" above). The fixed eyes should be examined in detail using a dissecting microscope, taking high-quality color photographs of any significant findings, both at low power to indicate context and at sufficiently high magnification to show the abnormality clearly (i.e., to another ophthalmic pathologist expert witness appointed by the defense, or to a jury in court). Because the whole of both eyes are processed for histology, the macroscopic findings are not available for later examination.

Microscopic examination of both eyes is performed using conventional hematoxylin and eosin stains and Perls stain for hemosiderin (a breakdown product of blood in life, helps to time the hemorrhage with respect to time of death).

Note that if hemorrhages are present in or around the eyes at death, the pathologist should determine whether and when such hemorrhages were present in life (from medical notes and photographs, family photographs, etc.).

For the histopathologist removing the eyes in these cases, it is vitally important to record whether or not any procedures were undertaken on the eyes after death. These include injections to remove vitreous (which may cause external or internal hemorrhage). Injection of fluids such as water (to replace removed vitreous) may cause artifactual displacement of internal structures, and other artifacts such as episcleral and focal internal ocular hemorrhage may be possible.

Pathologic Findings In and Around the Eyes

The ophthalmology and ophthalmic pathology of the eyes in physical child abuse is a massive subject, and I can cover only some aspects superficially in the space available. In this section I consider some important conditions that can easily be missed (or inadequately considered) in a child with apparent "shaken baby syndrome," particularly birth-related hemorrhages and nonaccidental direct blunt trauma to the eyes.

Abusive Head Trauma/"Shaken Baby Syndrome"

Approximately 80% of children who die of "shaken baby syndrome" have retinal hemorrhages, often with optic nerve sheath hemorrhages.[10,32–35] The retinal hemorrhages may be very extensive (Figure 4.13) and often extend to the periphery of the retina. Histologically they often involve all layers of the retina (Figure 4.13), often accompanied by preretinal (and vitreous) and subretinal hemorrhage. There is a

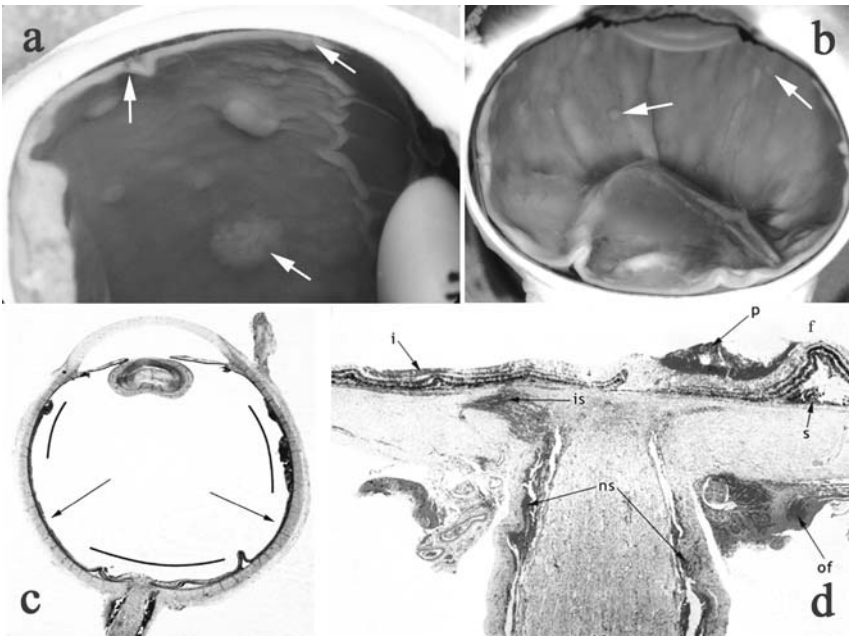

Fig. 4.13. The eye in abusive head injury ("shaken baby syndrome"). **A:** On macroscopic examination, there are extensive hemorrhages in the retina. *Arrows* indicate a large domed hemorrhage **(center bottom),** a subretinal/intraretinal hemorrhage **(top left),** and a peripheral retinal hemorrhage **(top right). B:** In another eye, there are also diffuse retinal hemorrhages *(arrows),* but the most obvious feature is the large round "perimacular" retinal fold, with a crater-like central depression. **C:** Histologically, these infants often have hemorrhages concentrated at the peripheral retina and posterior retina *(curved lines),* sparing the equatorial areas *(arrows),* although frequently they are universal (note that 20% of such infants have no retinal hemorrhages at all) (hematoxylin and eosin, original magnification ×2). **D:** The posterior aspect of the eye shows the retinal fold *(f),* and hemorrhages that are preretinal *(p),* intraretinal *(i),* subretinal *(s),* intrascleral *(is),* and in the optic nerve sheath *(ns)* and orbital fat *(of)* (hematoxylin and eosin, original magnification ×12).

good correlation between the severity of these eye injuries and the number and severity of intracranial injuries, particularly subdural hemorrhages.[10,32] This type of violent shaking/impact injury is associated with almost immediate onset of clinical indicators of acute encephalopathy, such as unconsciousness, floppiness, apnea, and cardiac arrest.[32,36]

Retinal folds ("perimacular folds"; Figure 4.13) at the posterior pole of the eye are a frequent (but not pathognomonic) finding in shaken baby syndrome.[33–35] They often consist of a rounded elevated fold of retina, with a central crater (nonelevated retina). The internal limiting membrane of the retina is often stripped from the central "crater" and not infrequently stretches between the raised edges of the fold (like a tent), with blood filling the crater beneath it. I have observed that the vitreous is detached over the central crater, sometimes with a conical elevated area of vitreous detachment, while the vitreous remains attached on the crest of the retinal fold. This complex of findings appears to suggest that perimacular retinal folds are caused by vitreous traction on the perimacular retina during violent shaking.

Birth-Related Hemorrhages

In the neonatal period, conjunctival and retinal hemorrhages (and some intracranial hemorrhages) can be the result of nonaccidental injuries (violent shaking, direct trauma) or birth trauma, and careful consideration should be given to this differential diagnosis.

Birth-related conjunctival hemorrhages may be documented in delivery or neonatal medical notes/charts, or they may be seen on family photographs of a newborn child. Note, however, that their absence may be the result of sampling problems and may not exclude birth trauma (I once looked through more than 60 family photographs of one child to find the single photograph that showed a conjunctival hemorrhage that I had seen microscopically).

Birth-related retinal hemorrhages are extremely common but usually are not seen or documented because nobody looks for them. They are not seen on family photographs. The RetCam (a wide-angle "contact" eye fundus camera) was used in a prospective study (revised manuscript submitted to J American Association Pediatric Opthalmology and Strabismus: Hughes et al. "Incidence, distribution and duration of birth-related retinal hemorrhages: a prospective study.") of retinal hemorrhages in neonates (in our hands RetCam is safe and very easy to use in unsedated neonates, and it is well tolerated by the parents watching or holding the infant). In 50 full-term normal neonates, retinal hemorrhages were very common (32% overall) and often were extensive (Figure 4.14). Their incidence in normal vaginal deliveries was 33%, ventouse deliveries 88%, forceps deliveries 14%, and cesarean sections 7%. The hemorrhages can be unilateral or bilateral, and are intraretinal. Most resolved very rapidly (in a few days and certainly within 2 weeks after birth). However, denser and are intraretinal hemorrhages persisted up to 10 weeks after birth.

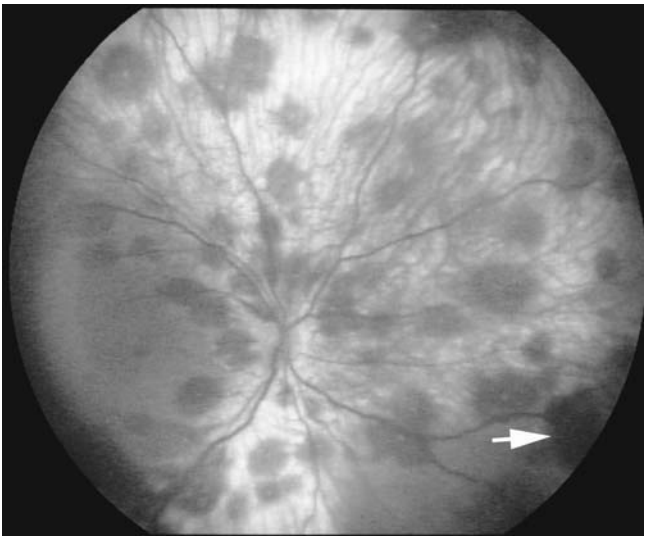

Fig. 4.14. Wide angle fundus camera (RetCam) picture of the retina of a child 1 day after normal full-term delivery. Widespread superficial retinal blot hemorrhages extend from the central optic disc toward the periphery, where more dense hemorrhage is seen *(arrow)*. Approximately one third of newborn infants have retinal hemorrhages, which usually clear very rapidly.

In a living infant (if a shaking injury is suspected), I would have an increased suspicion of "shaken baby syndrome" (bearing in mind the general clinical history and examination) in an infant presenting at 2 weeks with extensive flame-shaped hemorrhages and at 4 weeks with extensive intraretinal hemorrhages. However, even at 12 weeks an isolated hemorrhage can be related to birth trauma. In a dead child, "fresh" retinal hemorrhages with no demonstrable hemosiderin could not have occurred more than 48 to 72 hours before death (using the timing usually quoted by forensic pathologists, although care is always needed when quoting precise timing of pathologic processes). In a child born 2 weeks previously, it is extremely unlikely that such a "fresh" hemorrhage was caused by birth trauma.

Abusive Blunt Trauma to the Eyes

Blunt trauma to the eyes may be accidental (e.g., caused by a fall onto a protruding object) or abusive (e.g., caused by poking or gauging, with or without instruments). For diagnosis, documentation of facial and eyelid injuries is absolutely vital.

In my experience, I have found it extremely important to look for indications of direct blunt trauma to the eyes in dead children, even though such direct blunt traumatic injuries are extremely unlikely to have caused the death of the infant. The ophthalmology and/or pathology of accidental blunt injuries to the eyes is well known, but such injuries can also be caused by nonaccidental (deliberate) trauma to the eyes. If the ophthalmic pathologist finds severe injuries attributable to blunt trauma in one eye, the injuries could be explained by accidental blunt trauma to that eye. However, if such severe injuries are found in both eyes, then simultaneous (or consecutive) severe injuries to both eyes are extremely unlikely to be accidental (as the eyes "point" in different directions, and they are protected by being sunk in the orbits) and are extremely likely to be deliberate. In a dead child, these findings are extremely important medicolegally, either with or without another nonaccidental cause of death.

Blunt trauma to the eyes caused by abuse is recognized in clinical ophthalmic practice but has hardy ever been recorded in fatalities. However, a small number of fatally injured infants have had eye injuries similar to those documented in accidental blunt eye trauma, which emphasizes the importance of looking for, and documenting fully, such changes in infants in whom abuse is suspected or possible.

Note that some methods of removing or dissecting the eyes at autopsy may cause artifactual changes that suggest blunt trauma (particularly subluxation or dislocation of the lens, rupture of the lens capsule, and vitreous detachment). These are avoided by removing the eye and orbital contents en bloc "Intact Eye and Orbital Contents (Posterior Approach)," and by opening the eye by removing a calotte, the conventional means used by ophthalmic pathologists.

Nature and Timing of "Highly Specific" Direct Blunt Traumatic Injuries

In the context of trauma, some injuries are highly specific for direct blunt trauma to the eyes. However, others may be the result of trauma elsewhere on the face, other factors such as hemorrhagic diseases, or even (in the case of conjunctival hemorrhages) respiratory illness with violent sneezing or "spontaneous" isolated hemorrhage.

Highly specific blunt traumatic (but not necessarily abusive) eye injuries include the following.

Lens Displacement and Traumatic Cataract

The lens may be displaced as a result of blunt trauma, producing dislocation (completely displaced lens) or subluxation (partial displacement, although part of the lens remains attached to the zonules (suspensory ligaments). Note that lens displacement also may occur in homocysteinuria and Marfan syndrome, in the absence of significant trauma.

A cataract is present when the lens becomes opaque, and in extreme cases the lens may appear totally white. In acute traumatic cataract the opacity of the lens may be very slight and may be difficult to see. In some children I have seen marked swelling of the lens almost entirely as the result of increased clear fluid beneath the posterior lens capsule (posterior lenticonus). Such changes should be fully documented, but their traumatic significance is disputed by some.

Cataract formation is a well-recognized and common consequence of blunt ocular trauma, and in blunt trauma it results from contusion or concussion to the lens.[37,38] Traumatic cataracts often develop immediately after injury and are evident when the patient presents to the hospital,[37] although some traumatic cataracts develop over weeks or months. Some cataracts have a rosette/petal-shaped form of opacity and appear within a few hours of the injury.[38] After concussion of the lens, the capsule of the lens may be severed from the "mass of the lens,"[38] which appears to be the case in several of these children (in whom there is fluid between the capsule and the body of the lens). The lens has a marked capacity to take up fluid very rapidly, as can be seen in experimental digital manipulation of the lens capsule, which simulates the effects of concussion.[38] Lens swelling appears to increase markedly with capsular rupture but also occurs without this rupture.[38] These changes are clearly distinguishable morphologically from the relatively very small multiloculated "transient lens vacuoles" seen in some neonates.[39] Note that it is important to determine whether water has been injected into the eye after death, as this may raise the question of artifactual lens swelling.

Posterior Lens Capsule Rupture

Rupture of the lens capsule is a common consequence of direct blunt trauma to the eye[37,38,40] and is seen clinically by alteration of the red reflex.[39]

Posterior Vitreous Detachment

Detachment of the vitreous after concussion (direct trauma) is not uncommon, and detachment of the posterior half of the vitreous is well recognized.[41] It is important not to dissect the eye such that recognition of posterior vitreous detachment is prevented.

Commotio Retinae (Retinal Contusion)

Macroscopically, commotio retinae is seen as yellowish areas of retinal edema (Figure 4.15). The microscopic features are of retinal edema, but the most specific change of commotio retinae consists of disruption of outer segments of photoreceptors (Figure 4.15). The earliest of these changes have been produced experimentally in pigs within 1 minute of injury[42] and in monkeys within 4 hours.[43] Similar changes were found in one human examined 24 hours after injury.[44] The changes were asso-

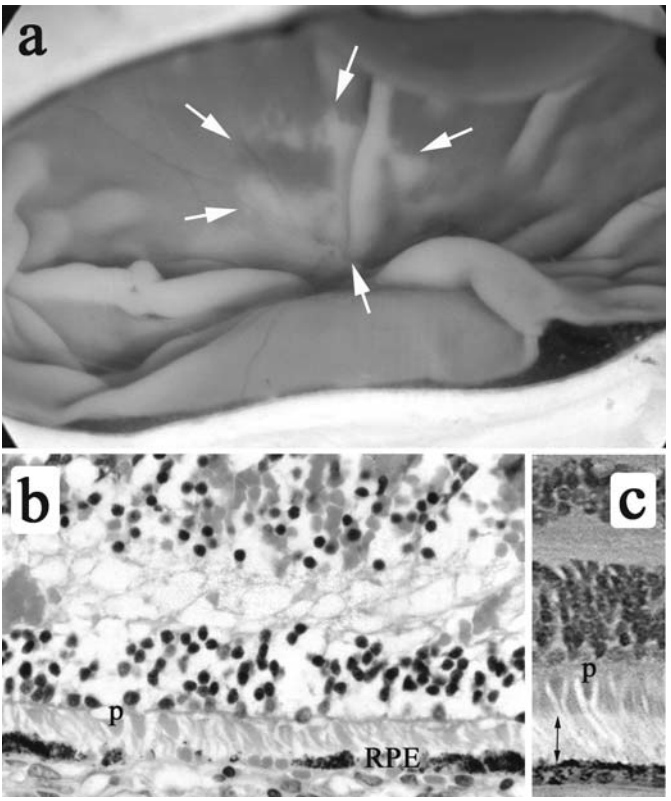

Fig. 4.15. Blunt trauma to the eye can cause commotio retinae (retinal contusion), seen macroscopically **(A)** as yellowish areas of retinal edema with variable pigmentation (outlined by *arrows*). These noncircular retinal folds are common artifacts of tissue fixation. Histologically, commotio retinae **(B)** is compared with normal retina **(C)** (both outer retina; hematoxylin and eosin, original magnification ×200). In panel B, the retinal edema separates the cells with clear fluid. Only the inner parts of the photoreceptors (note the triangular cones) remain *(p)*. The outer segments, indicated by the *arrow* in the normal retina in panel C, are lost. Note the retinal pigment epithelium *(RPE)* is swollen and fragmented in panel B, another result of contusion.

ciated with some RPE changes (see "Retinal Pigment Epithelial Contusion"). RPE cells may appear to migrate into the retina approximately 48 hours after injury in experimental commotio retinae in monkeys.[43] These changes are subtle and must be distinguished from the effect of photoreceptor autolysis.

Retinal Pigment Epithelial Contusion

Retinal pigment epithelial contusion, as manifested by intracellular edema, is first seen within 1 minute of experimental injury in pigs,[42] although only at the electron microscopic level. By 24 hours in experimental animals[42] and humans[44] RPE damage appeared greater. Functional changes of traumatic RPE edema have been described 48 hours after injury experimentally[43] and in a human.[45] Histologically the RPE is edematous, with later reactive changes. Again, autolytic change should be excluded.

Less Specific Changes Seen in Blunt Trauma

Less specific changes of blunt trauma (which can be seen in other pathologies) are described in some of the following papers on blunt traumatic injuries:

1. Eyelid bruises[45]
2. Conjunctival/episcleral hemorrhage[44,45]
3. Retinal hemorrhages[42,45]

"Composite Diagnosis" of Abusive Blunt Trauma

The diagnosis of severe blunt trauma to the eyes does not depend on a single macroscopic or microscopic finding (e.g. a particular feature such as "isolated" changes in the lens). The diagnosis actually depends on the concomitant presence of a number of different injuries within the eye, each one of which is caused by direct trauma to the eye. These injuries occur as a "diagnostic cluster" or "syndrome." Thus each separate direct traumatic injury in the eye forms one element of the diagnosis. When the severe direct trauma injuries are brought together they support each other in a firm composite diagnosis of direct trauma injury to the eye. In the appropriate clinical or forensic context, such injuries to both eyes are a strong indicator of nonaccidental blunt trauma.

Practical Points

It is becoming increasingly clear that the nature of nonaccidental injuries to the eyes of infants may be complex, perhaps involving more than a single mechanism, and that pathologists and clinicians need to consider this situation when they are investigating and collecting evidence of injuries in living or dead children. In an ongoing qualitative study of autopsy photographs taken as evidence by police and forensic pathologists, I have found that most do not provide adequate contextual or high-magnification views of the face or eyelids. Such photographs are vital (in living and dead children) to providing factual/visible evidence for civil and criminal court proceedings that result from episodes of physical child abuse. Therefore we have developed a simple "photographic protocol" for use in all suspected cases of possible child abuse. Using this protocol ensures that all positive (and negative) evidence is documented – in a matter of only a few minutes – as soon as possible after the abusive event (see The Sheffield Head Photographic Protocol, pp. 123–131).

All practitioners need to be alert to the possibility of several possible coexisting pathologies in apparently abused children. Although some are not life threatening (e.g., blunt trauma to the eyes), they are vital in establishing a composite diagnosis of child abuse and may have some bearing on the assessment of criminal intent.

Legal and Ethical Background to Ophthalmic Autopsy Practice

Legal Considerations

You may remove the eyes legally at autopsy in the United Kingdom under three circumstances[46]:

1. In any autopsy performed under the provisions of the Human Tissue Act,[47] where there is a validly completed written consent form and where there is no objection to the removal of the eyes. It is vitally important, however, to obtain specific written consent both for the removal of the eye and/or adnexal structures and for the retention of these tissues, in accordance with the Royal College of Pathologists "Guidelines for the retention of tissues and organs at post-mortem examinations."[48]
2. In an autopsy performed by the coroner or equivalent authority, when the eyes are required for diagnostic purposes (e.g., in suspected cases of nonaccidental injury in infants) or to determine the possible contribution of suspected eye disease to the cause of death (e.g., retinitis pigmentosa or diabetic retinopathy in a road traffic accident). Again, the coroner and pathologist should have a policy on the eventual treatment of the eye tissues that reflects the Royal College of Pathologists guidelines for the retention of tissues and organs at post-mortem examination.[48]
3. From patients who have donated their eyes for corneal transplantation. These eyes usually are removed by ophthalmologists after arrangement through eye banks, and pathologists are rarely involved. Specific authorization must be confirmed before the eyes are removed.

As a pathologist you have an ethical duty to ensure that the wishes of the patient when alive (if known) and the wishes of the family of the patient have been fully investigated. Under the Royal College of Pathologists guidelines,[48] it is important that you obtain fully informed specific consent both for removal of ocular tissues and for the retention and eventual means of disposal of these tissues. Note that a considerable portion, or the whole, of an eye will be retained and used to prepare tissue sections, using conventional ophthalmic pathologic processing methods.

It might seem that more stringent procedures to obtain specific consent to remove and retain ocular tissues would inevitably lead to reduced availability of these tissues. In my experience, however, the relatives of even very young patients readily give their consent for such procedures. It is extremely important for clinicians and pathologists to be very sensitive and take time to fully inform the relatives of the good reason for the procedure to be undertaken. Under such circumstances, relatives may feel that at least some benefit may derive from the death of their family member.

Note that, under the specific provisions of the Human Tissue Act, 1961 (as modified by the Corneal Tissue Act, 1986[49] and the National Health Service and Community Care Act, 1990[50]), an eye (or part of the eye, e.g., the vitreous) must be removed by a registered medical practitioner, who must be satisfied that life is extinct by personal examination of the body (or based on a statement by a registered medical practitioner who has satisfied himself by personal examination of the body that life is extinct). Removal of an eye (or part thereof) also may be performed by an appropriately trained person (e.g., an anatomic pathology technician) acting on the instructions of a registered medical practitioner (who has examined the body, as described). However, such a trained delegated person must be "in the employment of a health authority [or NHS trust]"; therefore technical staff employed solely by universities or local councils cannot legally perform this task.

The law relating to the removal of tissues after death varies considerably in different countries around the world. Pathologists working in countries outside the United Kingdom should contact their professional organizations and institutional legal advisers for specific written legal advice before they contemplate removing any tissues from the eyes or ocular adnexa.

Ethical Considerations

I believe that pathologists have an ethical duty to avoid any procedure that likely will add to the distress of relatives of the deceased, and removal of "culturally sensitive" ocular tissues should be considered to be more than usually emotive under these circumstances. Ocular tissues should be removed only for sound scientific purposes, which I believe include audit of clinical practices, continuing medical education, and sound scientific research. This reflects the recommended policy for the uses of these tissues issued by the Royal College of Pathologists and the Institute of Biomedical Science.[51]

Whenever the removal of tissues is likely to alter the facial appearance of the deceased, specific signed consent from relatives or consent from the coroner or equivalent authority *must* be obtained for any procedure, and ethically the reasons for the procedure must be sufficiently important to warrant the possibility of further distress to relatives. Arrangements may be necessary to ensure that the body is only viewed by the relatives before the procedure. Removal of tissues to this extent must be viewed as most exceptional, and discussions with the coroner or local ethical committee may be advisable before relatives are consulted or informed. Under such circumstances it probably is advisable for a senior ophthalmologist (possibly accompanied by a senior pathologist) to explain the circumstances to the relatives to ensure truly informed consent. It must *always* be assumed that a close relative will view the body after the autopsy procedure has been performed, unless specific arrangements have been made with the consent of the relatives to prevent this occurrence.

Health and Safety in Ophthalmic Autopsy Practice

The same health and safety considerations apply to ophthalmic autopsy practice as to any other autopsy.[52] You may create microdroplets and aerosols of blood and tissues when you cut orbital/skull bone with a mechanical saw, and jagged edges of bone may cause glove puncture and injury. Sharp dissection (using a scalpel) is not required for most ophthalmic autopsy procedures and can be dangerous if two-handed methods are used (e.g., when the orbital contents are removed via the base of the skull; see The Intact Eye and Orbital Contents [Posterior Approach]). Even removing the vitreous carries a small risk of needle-stick injuries, as the vitreous is known to harbor pathogens such as human immunodeficiency virus.[53]

In addition to the usual infectious agents potentially encountered at autopsy,[4] the eye is one of the tissues with the highest titers of infectivity for the human and animal transmissible spongiform encephalopathies, and infectivity is present in unfixed or routinely fixed tissues.[54] In view of the small risk of infection of autopsy workers, the Advisory Committee on Dangerous Pathogens and Department of Health have issued guidelines for the United Kingdom.[54,55] These guidelines, which are considered to represent "good practice," state that, "as an extreme precaution," the eyes should not normally be removed from humans or animals known or suspected to have suffered from transmissible spongiform encephalopathies, such as Creutzfeldt-Jakob disease (CJD) and Gerstmann-Straussler-Scheinker syndrome (GSS), or who fall into one of the following groups:

- Recipient of hormone derived from human pituitary glands, such as growth hormone and gonadotrophin

- Recipient of human dura mater grafts
- Member of recognized CJD or GSS families

If, under exceptional circumstances and after consideration of the guidelines and ethical points, the eyes are removed from such patients, extreme precautions are mandatory. I advise adoption of the precautions devised by Bell and Ironside.[56] After the eyes are removed, they must be fixed using the formalin-formic acid procedure of Brown et al.[20] or the formalin/hypochlorite method[21] before undergoing treatment for histology.

Summary

Ophthalmic pathology at autopsy can seem a daunting business to the uninitiated. However, it has much to offer scientifically and diagnostically once the initial fears are overcome. The procedures are no more difficult – once learned – than those in many other branches of pathology. As ever, the "secret" is in good preparation and thoughtful and careful execution of all of the technical procedures. Therefore I encourage you to consider working in this extremely interesting area of pathology.

The most important component of autopsy ophthalmic pathology practice remains that of ensuring that all appropriate permissions and informed consents have been obtained for taking and retaining tissues. It is vitally important that the family of the patient be fully consulted and informed about the procedures, that they can be reassured that the reasons for performing the procedures are valid, and that there is no possibility that their relative will be "mutilated" by the procedure. The pathologist's role here is to ensure that family members can spend important time with their deceased relative *after* the procedures have taken place, should they wish.

Specialist ophthalmic pathologists are available for consultation in individual cases. Most of us are very happy to receive case material from other pathologists should they wish to refer it and obviously to provide feedback of the results to both pathologists and clinicians.

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The Sheffield Head Photographic Protocol

R. Farr, P.L. Wheeler, and M.A. Parsons

Introduction

High-quality photographs have become the "gold standard" method for documenting and presenting both medical and nonmedical physical evidence for medical education or for the civil and criminal courts. This is particularly the case within forensic medical practice, when documenting accidental and nonaccidental injuries in children and adults. In our experience, however, the photographs that are taken to document such injuries (or to record important negative findings) are often inadequate or missing, for a variety of reasons.

We have devised a photographic protocol to ensure that positive and negative findings in the head and neck are recorded optimally. Following the sample images in this protocol will ensure that all the important medical and forensic evidence is recorded in a simple photographic "shoot" taking only a few minutes, which can be applied to either living or deceased subjects.

The Sheffield Head Photographic Protocol has been developed for use by photographers, pathologists, and clinicians when dealing with infants who have sustained head injuries, an unexplained collapse, or unconsciousness, or in cases where nonaccidental injury is a possibility. The protocol can be downloaded from our web site for free use by anyone, worldwide.[1]

Background

Infants subjected to physical child abuse may sustain serious head injuries from direct trauma, violent shaking, burns, bites and other mechanisms. These injuries produces a range of physical findings on the head and face and in the mouth, but sometimes the absence of external marks is important to record (e.g., head injury resulting from violent shaking alone). Our recent practice in fatally injured infants indicates physically abused children may have additional (or isolated) direct eye trauma (e.g., poking in the eyes). In these groups of children, good-quality photographs of any skin bruises are vital, but it is also very important to photograph the skin (particularly the eyelids) to record when bruising is absent.

In practice, however, it soon became clear that there were serious inadequacies in the autopsy photographs being taken by various agencies around the country. Therefore we conducted a retrospective study of all the photographic evidence that we had received over the previous 3 years. These images mainly were from police scene of crime officers (SOCO) or were taken at autopsies by medical photographers or pathologists. The results showed conclusively that there is no standardized method for documenting a subject (alive or dead). In 80% of the cases we reviewed, the supplied photographs provided a minimal amount of information that would be useful not only to ophthalmic pathologists but also to other pathologists and clinicians. The reasons for this finding, despite the presence of a photographer, often relate to the failure of the pathologist or clinician to direct the photographer to take the correct photographs at the correct magnification (this is easier to do with increased use of digital cameras, when images can be checked instantly).

To remedy this situation, we developed the Sheffield Head Photographic Protocol with the specific aim of creating a standardized repeatable method for photographically documenting a subject (either alive or dead) who may have been abused or where the cause if injury or illness is unclear.

Aims of the Protocol

The overall aims are to ensure high-quality photo documentation of the physical evidence in the shortest possible time. With this in mind we designed the protocol for the following reasons:

- Standardize the photographic documentation of positive and negative medical findings in every case for later use by other medical and scientific experts

- Produce a repeatable series of images taken in as short a photographic "shoot" as is reasonably practical
- Allow the photographer to take a rapid series of relevant photographs without direction from a clinician or pathologist, whose presence is not necessarily required
- Produce photographs that are of sufficient quality and magnification that are optimal for presentation of evidence in court (i.e., to jury members).
- Produce photographs to place the higher-magnification photographs in their wider body context
- Use in life or at autopsy
- Ensure a clear and precise record of the photographs that have been taken
- Inform photographers, pathologists, and clinicians of the reasons why individual photographs are included within the protocol

Protocol Features

The protocol requires that the photographer take a series of predefined images that are designed to offer maximum coverage of the subject while using the minimum amount of photography (especially useful when photographing young children).

The protocol illustrates the series of images that should be taken, showing the suggested subject framing and magnification of each image, with explanations and technical hints for each image. Because of constraints of space and format, only the outline if the protocol is given here. The full protocol with explanation and documentation is available to view and download free of charge (as a PDF file) from our web site.[1]

The images fall into three distinct categories: core images, high-power images, and autopsy photographs. The images are recorded in these categories on the Record Card (Protocol Figures 4.1 and 4.2).

The core images are ideally taken first and cover the entire patient's body in 23 photographs, concentrating on the head (Protocol Figures 4.2 and 4.3). It is important to take *all* of these photographs as "default practice," recording the reason for each one that is not possible. The photographer then documents important negative findings and any specific injuries individually at "high power" (Protocol Figure 4.4). Finally, if there is an autopsy, there is a series of defined additional photographs covering the opened skull, orbits, and eyes (Protocol Figure 4.5). As the "Protocol" images are taken, they are recorded on the two-page Record Card (Protocol Figures 4.1 and 4.2).

This protocol covers the head and neck only, but important findings elsewhere on the body should be fully documented according to standard forensic or clinical practice.

Conclusion

We present a protocol that allows photographers to take a rapid series of standardized views to document important positive and negative physical findings on the head and neck. The protocol is specifically designed to record findings in child

Sheffield Head Photographic Protocol

SUBJECT DOCUMENTATION RECORD CARD

OFFICE USE ONLY
LEAVE BLANK

PEOPLE FILLING OUT THIS RECORD CARD MUST HAVE READ AND UNDERSTOOD THE SHEFFIELD HEAD PHOTOGRAPHIC PROTOCOL DOCUMENT AND SHOULD COMPLETE THIS RECORD CARD IN ACCORDANCE WITH SAID PROTOCOL. ONLY TO BE USED IN CONJUNCTION WITH SHPP PROTOCOL EDITION 1

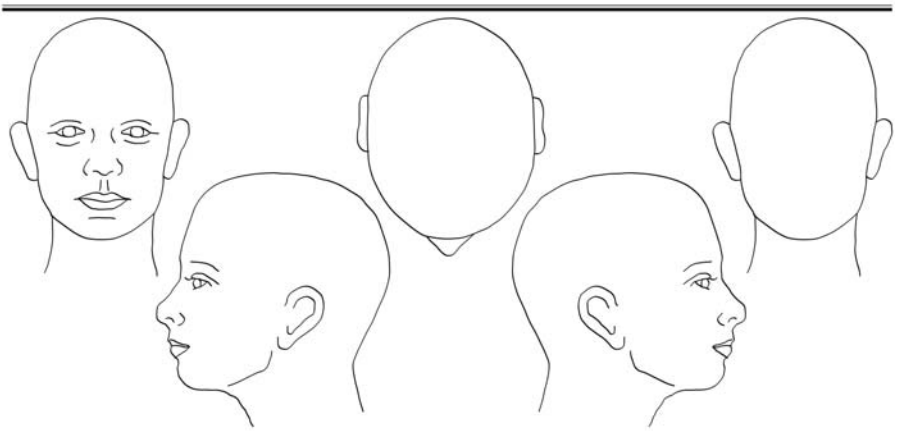
Subjects Details & Specific Information

Surname: _____
Forname: _____
Date of Birth: _____
Hospital/Unit Number: _____
NHS Number: _____

Sex Male ☐ Female ☐

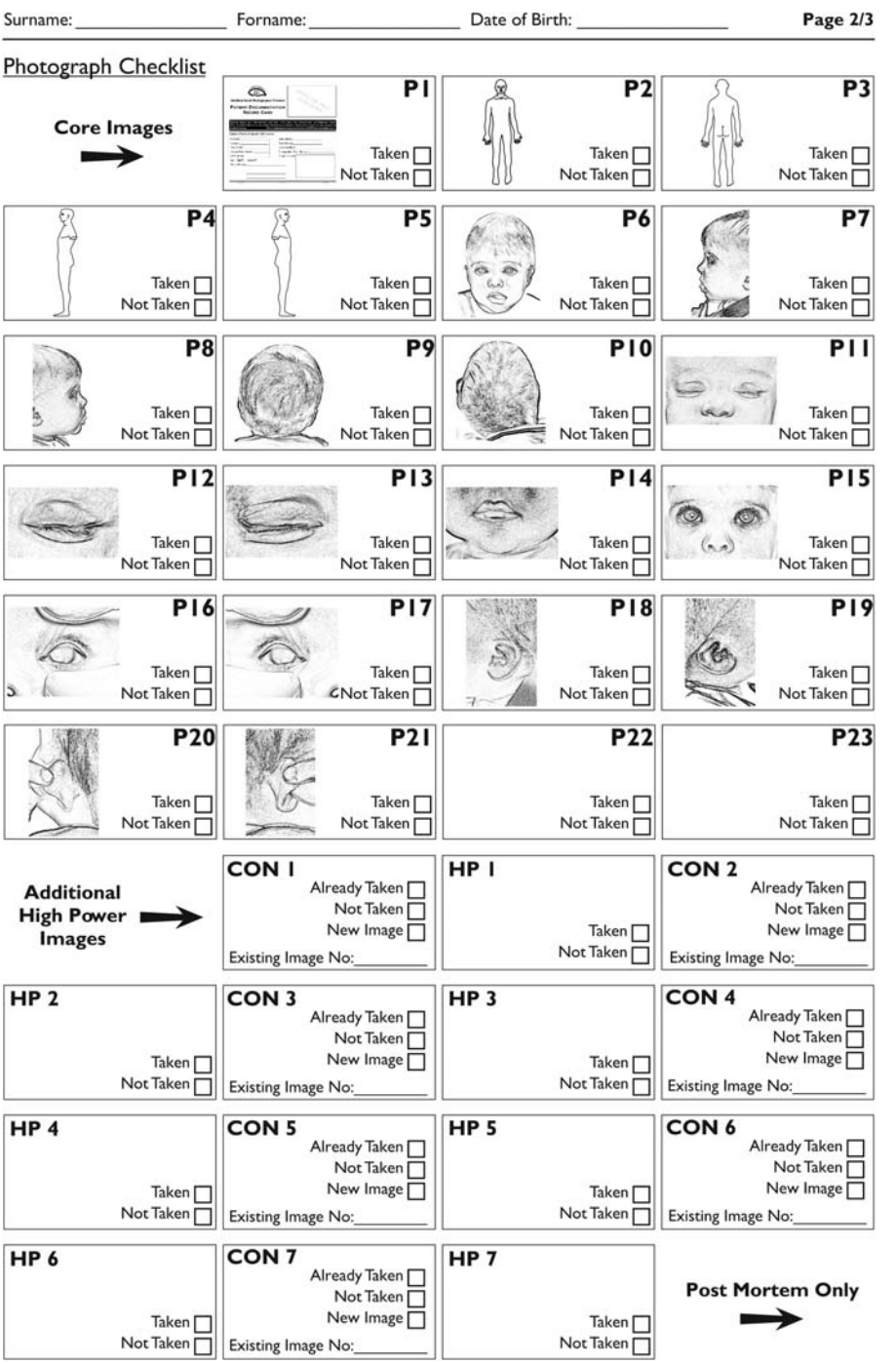
Others Present: _____
_____
_____
_____

Date of Shoot: _____
Time of Shoot: _____
Location of Shoot: _____
Photographer (Your Name): _____
Equipment Used:



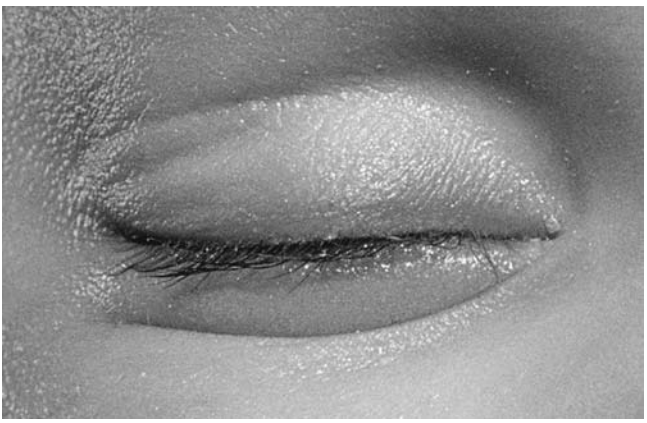
Complications & Other Information

PROTOCOL Fig. 4.1. Protocol Documentation Card (front) for recording subject details and specific sites of injuries. Note that this is the photographer's record card and would be in addition to any documentation or drawings of injuries made by the clinician or pathologist.

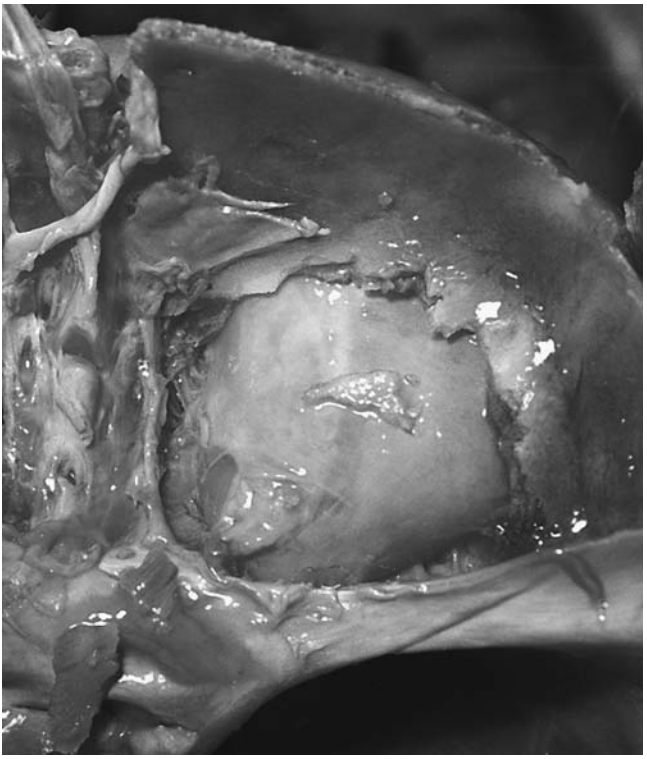


PROTOCOL Fig. 4.2. Protocol Documentation Card (back) showing "thumbprint" images of core photographs, with "tick" recording boxes. The **top images** are "Core Images." The "Additional High Power Image" area **(bottom four rows)** allows the high-power photograph *(HP)* and its related lower power "context" *(CON)* image to be recorded.

PROTOCOL Fig. 4.3. Context image of the front of the head and neck.



PROTOCOL Fig. 4.4. "High-power" picture of important negative findings (right eyelids).



PROTOCOL Fig. 4.5. Autopsy image of the in situ right eye, which has been exposed by removing the roof of the orbit.

abuse, but it can be used for a range of applications where photographs of injuries or medical conditions around the head are required. The protocol is copyrighted but is available for free downloading and use (by individuals or institutions worldwide) from our web site.[1]

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5. Subdural Hematoma in Children

Guy N. Rutty and M.V. Waney Squier

Introduction

There are few areas within medicolegal practice at present that generate as much controversy as the interpretation of findings upon which mechanisms of injury to children, living and deceased, are based. High-profile cases within the English legal system highlight the difficulty of diagnosing the cause of pathologic findings "beyond reasonable doubt." The pathologist may be faced with a panel of experts clinging to "mainstream thinking" backed by anecdotal and personal experience rather than considering peer-reviewed research and literature. This is one area where evidence-based medicine is an absolute requirement, particularly when evidence is being put before a court.

In recent times the question of who should be undertaking these examinations has been raised. Should it be a forensic pathologist who may have little, if any, formal pediatric training, or a pediatric pathologist who equally may have little, if any, forensic training? The solution seems obvious: have both pathologists present at the autopsy, especially when the pathologic findings may have both a natural and/or an unnatural causation. Ultimately, the pathologist's duty is to the court, as an unbiased witness to present and interpret the findings within his or her limits of expertise.

This chapter addresses a single pathology from an area of contentious medicolegal practice, that is, the subdural hematoma (SDH), which is regarded by some as highly indicative of nonaccidental injury. Whereas most SDH in infants is caused by trauma, many other natural and unnatural causes have been described. We review all causes that could be identified from the world literature as possible causes of SDH and consider the ages when they occur, the associated medical or pathologic findings, and the hypothesized mechanisms of causation. Only by considering all of the causes examined within this chapter can we start to plan the investigations required to determine the causation of SDH in childhood.

Incidence

Subdural hemorrhage occurs in 10.9 per 100,000 infants aged 0 to 2 years, 20.8 per 100,000 infants younger than 1 year.[1] Most SDH occurs in infants aged 0 to 4 months.[2] The age distribution is similar for all causes of traumatic SDH, indicating that pathogenesis is dependent on age and independent of cause.[3]

Nature and Distribution of Subdural Hematoma

Subdural hemorrhage results from bleeding into the potential space just beneath the free edge of the dura. A layer of cells the *dural border cells* form a loosely adherent junction with the arachnoid membrane, and it is into this layer that subdural bleeding occurs.[4] The cells are clearly very loosely adherent, if not a genuine space in life, as the dura is very readily lifted from the arachnoid at autopsy without evident attachment. Further, SDH usually is very extensive[5] and, particularly in infants, frequently forms a widespread thin film over the cerebral hemispheres. Subdural hematomas may connect between all subdural compartments, and bilateral subdural hematoma can be drained with a shunt placed on only one side of the head.[6,7] In older children SDH is more likely to form a thick, space-occupying unilateral hematoma, similar to the pattern in adults following trauma.

Natural History

In the natural history of subdural hematoma, it resolves by formation of a granulating membrane.[5,8,9] In the first days after bleeding, macrophages enter the clot, and release of enzymes causes clot lysis. Hemoglobin is converted to hemosiderin which stains blue with Perls reaction. This process is thought to take 48 hours from the time of bleeding. However, neither this nor other histologic reactive processes can be precisely timed, especially if the infant has been nursed on a ventilator for the last hours or days of life when reactive processes are altered by impaired or absent cerebral blood flow (respirator brain).

After 3 days the infiltrating macrophages take up red cells and contain brown breakdown products within their cytoplasm. Fibroblasts and capillaries grow into the clot in the first 3 days and form many large vascular channels that are particularly numerous in two bands, at the junction of the healing clot with the free edge of the dura and at the free deep border of the membrane. Although the healing adult subdural membrane is described as granulation tissue, Friede[9] has suggested that in infants the reactive membrane can be distinguished by the very large size and number of in-growing thin-walled sinusoidal capillary vessels that he termed *macrocapillaries*. By 10 days the membrane becomes visible to the naked eye. It may be extensive and thin, often no thicker than the dura, and certainly beyond the resolution of routine computed tomography and magnetic resonance imaging (MRI) brain scans. In time the numbers of capillaries are reduced, and the membrane becomes predominantly fibrotic, with calcification or even areas of ossification. In very chronic cases there may be proliferation of arachnoid cells in the arachnoid villi resulting in impaired drainage of cerebrospinal fluid (CSF).[10]

Chronic Subdural Hematoma

There are two forms of chronic SDH. In a few cases the original blood clot becomes liquid and is encapsulated by a fibrous membrane. The resulting "subdural hygroma" has a similar shape and location to the original hematoma.

In most cases there is more widespread accumulation of fluid in the subdural space. Transition of acute infantile subdural hematoma into chronic SDH has been

recognized for many years.[11] This is a delayed process. In the series reported by Hwang and Kim,[12] 3 of 16 acute SDH in infants younger than 1 year old became chronic, after intervals of 68, 90, and 111 days after initial trauma. The development of chronic SDH may be recognized by clinical signs of enlarging head or raised intracranial pressure or by the scan appearance of fluid collections in the subdural space. Subdural hemorrhages may present clinically with rebleed after minor trauma or even spontaneously.[13]

There are four pathologic mechanisms for development of chronic SDH:

1. The most important is repeated hemorrhage into the granulating membrane of healing acute SDH.[14,15]
2. Leakage of CSF through an opening in the subarachnoid allows CSF to enter the subdural space. This can occur after shunt placement in hydrocephalic babies. The CSF mixes with blood and results in a thin xanthochromic subdural fluid collection.[7]
3. Babies with brain atrophy may be more susceptible to development of chronic SDH as are result of tension on the dural border layer as the arachnoid collapses with the shrinking brain.[4]
4. After an infection or inflammatory process; subdural empyemas can result from sinusitis or otitis media.[16,17]

The pathogenesis and mechanisms of development of chronic subdural fluid collections are far from well understood. Although rebleeding into subdural membranes is very well recognized by pathologists,[9,11] the relationship between subdural and subarachnoid spaces is complex. Vinchon et al.[18] performed serial imaging in infants with accidental traumatic SDH. They noted that initial bleeding into the subarachnoid space preceded subdural fluid collections. They proposed that this process resulted from impaired CSF absorption at the arachnoid villi, and in one case they noted an extending tear in the arachnoid membrane. The pathophysiology of CSF accumulation in infantile SDH appears to be age specific. Hwang and Kim[12] described the evolution of acute to chronic SDH in three cases. In these cases, small acute subdural hematomas disappeared, followed by widening of the subarachnoid space and then by accumulation of subdural fluid at a much later date (68, 111, and 90 days). An MRI study of nine birth-related subdural hemorrhages failed to demonstrate persistent SDH at 4 weeks of age.[19] The experience of Hwang and Kim[12] suggests that early radiologic improvement does not exclude later development of chronic SDH in infancy. A further point of note is that imaging studies only recognize fluid accumulation; a thin granulating membrane would not be identified by routine scans without contrast enhancement.

Nontraumatic Causes of Subdural Hematoma in Children

Fetal

The most common form of antenatal intracranial hemorrhage is SDH that can be diagnosed by ultrasound or MRI.[20] Fetal SDH carries significant morbidity and mortality and is associated with arteriovenous malformations, tumors, disorders of coagulation, drugs (aspirin and warfarin), cholestasis of pregnancy, fetal distress, and hypoxic events, or it may be considered idiopathic.[21,22] Trauma as a cause has

been reported from traditional massage techniques to encourage cephalic version of breech presentations or from true maternal injury such as assault (so-called "battered fetus"), falls, and motor vehicle incidents[23].

Neonates

The incidence of birth injuries is estimated at 2 to 7 cases per 1000 live births.[24] They include soft tissue and skeletal injuries as well as subdural and retinal hemorrhages, all of which can be misinterpreted as nonaccidental injuries. Subdural hemorrhage is reported in one series as the most common form of intracranial birth related pathology, accounting for 73% these findings.[25] Many SDHs are clinically silent, small, and completely resolve within 4 weeks of the delivery.[19]

An MRI study of asymptomatic neonates has shown a 9% incidence of SDH.[19] Other studies have shown SDH in up to 50% of normal deliveries.[26,27]

Mode of Delivery

A number of maternal and fetal factors have been implicated as the cause of SDH related to the birth of the infant. These factors include cephalopelvic disproportion, prematurity, both maternal primiparity and grand multiparity, and both precipitous and prolonged labor.[28,29] The mode of delivery of the infant also must be considered, as this factor affects if and where the SDH may occur. However, there does not appear to be a higher incident of occurrence of SDH whether forceps or vacuum was used to assist the delivery.[30] Having said this, in the case of forceps delivery, the angle of rotation may be of importance. Hankins et al.[31] studied forceps rotation and found that a single case of SDH occurred with rotation greater than 90 degrees.

Tentorial and interhemispheric SDH have been described with normal vaginal delivery.[28] It is hypothesized that occipitofrontal compressive forces stretch the internal cerebral veins, the basal veins of Rosenthal, and the mobile aspect of the vein of Galen, resulting in tearing injuries either to the veins themselves or to the falx or tentorium.[28] Subtentorial posterior fossa SDHs have been reported in unassisted vaginal deliveries but more frequently in forceps and vacuum assisted deliveries.[32,33] Although osteodiastasis associated with forceps delivery has been considered the cause of posterior fossa SDH, others have rejected this theory. An illustrated theory to support tearing injury of the deep veins as the cause of SDH during vacuum extraction has been put forward by Hanigan et al.[34] The operative procedure of cesarean section itself is not reported as a cause of SDH.

When considering the location of birth-related SDH, Whitby et al.[19] found only one SDH in the supratentorial compartment alone, 6 of 9 were in the posterior fossa, and 2 of 9 were in both supratentorial and infratentorial compartments. These findings correspond with other reports in which no isolated supratentorial hemorrhages were identified. This does not support the contention that these subdural bleeds result from tearing of the draining veins that insert over the frontal vertex but suggests that these bleeds are the result of tentorial tearing or bleeding from the dural folds, which are prominent in the posterior fossa. Bleeding from dural folds is more frequent than from less vascular dura over the convexities.

Perinatal Hypoxia

Perinatal hypoxia has been considered a risk factor for SDH during birth. However, in a paper on risk factors for intracranial hemorrhage in full-term infants, Jhawar

et al.[35] draw attention to the earlier publication of Wigglesworth and highlight that this relationship should be considered with caution because the hemorrhage may be secondary to birth trauma, which in turn may precipitate a respiratory crisis and hence the hypoxic event, rather than vice versa, that is, the hypoxic event having been caused by the SDH. Coexisting cerebral trauma as the cause of SDH is also proposed and supported by Chamnanvanakij et al.[27]

Cerebral Infarction

Subdural hemorrhage is described in association with cerebral infarction in the neonate.[36,37] The mechanism is unknown. It has been suggested that the blood leaks from the infarcted brain tissue into the overlying meninges.[38] An alternative hypothesis is that subdural hemorrhage resulting from some other cause, such as torn blood vessels, displaces the brain and compresses the arterial supply, leading to infarction.[37]

Total Parenteral Nutrition

Intracranial complications of total parental nutrition (TPN) are rare and the underlying mechanisms unclear. Although subdural collections, whether they consist of transudates or fat effusions, are reported in the literature, subdural hematomas or blood-stained collections are not.[39] Tuthill et al.[39] draw attention to the hypothesis that retrograde venous flow occurring in response to raised pulmonary vascular resistance results in rupture of the bridging veins. The TPN fluid, which has been infused into the superior vena cava, then can enter the subdural space via the injured intracranial vessels. Retrograde flow is also postulated to occur as a result of high pulmonary pressures and reduced venous return caused by mechanically assisted ventilation.

Neuroendocrine Syndromes

Prader-Willi syndrome is a neuroendocrine disorder with chromosomal abnormalities (characterized by deletion of the proximal part of the long arm of chromosome 15 among other abnormalities) first described by Prader, Labhart, and Willi in 1956.[40] The diagnostic features are those of obesity, short stature, and developmental delay, with the child presenting with profound muscular hypotonia, swallowing difficulties, and hypogonadism. A single case has been reported of a 10-day-old neonate with bilateral SDH of the posterior falx with associated extradural hemorrhage, which was hypothesized to be caused by a genetically related abnormality of homeostasis, although the specific homeostatic abnormality was not stated.[41] The infant had been delivered by cesarean section following breech presentation, although these delivery related mechanisms were excluded as the cause of the SDH.

Other Causes

Other causes of neonatal SDH include thrombocytopenia, vitamin K deficiency, hemophilia, hepatic disease, infection, and disseminated intravascular coagulation (DIC). Maternal use of aspirin may result in SDH because of placental crossing of the drug.[42]

Infants

Coagulation and Hematologic Disorders

By far the largest literature on natural causes of SDH in children relates to hematologic and coagulation disorders. These disorders can affect the fetus, newborn, infant, and juvenile but are all considered in this section. Because of the shear number of papers, particularly in relation to hemophilia, only selected references are given to relevant conditions.

Severe hemophilia A (factor VIII deficiency) can present with intracranial hemorrhage in neonates. This condition can result from congenital hemophilia or transplacental transfer of acquired factor VIII:C inhibitor from maternal circulating immunoglobulin G class antibodies[43]. Despite the pressures involved in vaginal delivery, intracranial hemorrhage of all types is estimated to occur in only 1.0% to 4.0% of infants with hemophilia A. It is associated with traumatic and instrument-assisted deliveries but also has been reported to be spontaneous in nature, occurring several days after birth[44]. There is no specific distribution for the SDH, although in several reported series the bleeds were all supratentorial and unilateral in location.[45,46] The mortality from SDH has dropped in modern times to approximately 30%.[47]

Hemophilia B (factor IX deficiency) also may present with SDH in the neonatal period.[48] As with hemophilia A, the most common site for infant bleeds is intracranial, with SDH the most common type.[49] The mean age for intracranial bleeds in hemophilia B is younger than that for hemophilia A: 4 to 6 years as opposed to 10 to 10.5 years.[50]

Hemophilia, afibrinogenemia, leukemia, and thrombocytopenia (various types) have all been reported to cause SDH in school-age children. Although minor trauma has been put forward as a common cause, SDH may be spontaneous in nature.[51,52]

Four-factor deficiency is a very rare, autosomal recessive, inherited bleeding disorder involving the vitamin K-dependent coagulation factors. A single case of a 3-month-old male child with the deficiency who presented with a right-sided SDH has been reported.[53]

Another rare factor deficiency is factor V deficiency. A single case report of a male with postdelivery SDH and three more SDHs in the first 10 months of life was reported by Salooja et al.[54] Factor V deficiency is also associated with other congenital anomalies, including cardiac failure, transient hypertension, and renal tract and intracranial anomalies.

Congenital deficiency of factor VII, an autosomal transmitted disorder, usually is asymptomatic. Although it has been reported to be associated with arterial and venous thrombosis, only a single case of a homozygotic male infant 7 months old presenting with a left occipitoparietal SDH has been reported.[55] The child presented after an episode of minor head trauma.

Factor XIII deficiency is an autosomal recessive disorder in which bleeding occurs in less than 1% of affected individuals. A single case of a 38-month-old male child with factor XIII deficiency who had a left-sided SDH was reported by Larsen et al.[56]

Hermansky-Pudlak syndrome (HPS) is an autosomal recessive inherited disorder characterized by oculocutaneous albinism, tissue accumulation of choroid pigment, and bleeding diathesis as a result of platelet disorder. A single case of a 7-week-old male child with HPS who presented with a left-sided occipital SDH has been reported.[57]

Disorder of the function of fibrinogen, as highlighted earlier, may result in SDH. Autosomal dominant congenital dysfibrinogenemia has been recorded in several hundred families. Although they may be clinically silent, severe forms may present with bleeding, thrombosis, or abnormal wound healing. Occasional case reports, such as the 2-year-old male child reported by Al-Fawaz and Gader,[58] illustrate the complication of this disorder with SDH.

Fatal intracranial hemorrhage in sickle cell disease, although rare, is more common in children than in adults. The site of the bleed may be subdural.[59] The mechanism behind the SDH of sickle cell disease remains unproved, although it has been speculated to result from vascular abnormalities. In sickle cell patients, venous capillary saccular dilatations and ectasias, which are known to occur, may rupture and result in SDH.

Infections

A number of reports of the association of cerebral or systemic bacterial, viral, and parasitic diseases that can affect children and be complicated by SDH appear within the literature, but care must be taken to differentiate those diseases that cause effusions rather than hematomas.

Congenital toxoplasmosis can result in neonatal central nervous system damage. Subdural hematoma has been reported in association with this infection. Toxoplasmosis has been postulated to cause toxic damage to the dural blood vessels, resulting in increased permeability and rupture and thus formation of SDH.[60]

Malaria is another parasitic disease that can be listed as a cause of SDH. However, malaria has been reported to result in subdural effusions in children aged 11 to 66 months rather than hematomas.[61]

Bacterial meningitis has been reported to result in both subdural effusions and hematomas.

A Polish-language publication reported seven cases from a series of 97 children with toxic diarrhea who had associated SDH.[62] The SDH was reported in those cases with central nervous system signs and symptoms. None of the cases were reported to have hypernatremia or dehydration, although vascular manifestations and vitamin K abnormalities were reported.[62]

Although there is a single case report in Japanese of herpes simplex encephalitis (HSE) associated with SDH, Kurtz and Anslow[63] in their series of 13 infants with HSE draw attention to the fact that none of their cases had SDH. In fact, they use the presence of SDH to differentiate HSE from nonaccidental head injury.[63]

Finally, a single case of SDH following chronic otitis media is reported in the literature.[64]

Hemorrhagic Shock and Encephalopathy Syndrome

Hemorrhagic shock and encephalopathy syndrome (HSES) was first reported by Levin et al.[65] in 1983. This acute, frequently lethal syndrome usually occurs at about 3 to 4 months of age.[66] The cause remains unknown.[67] There is a prodromal period lasting on average 2 to 3 days during which the child exhibits fever, irritability, diarrhea, or signs of an upper respiratory tract infection. It then deteriorates into profound shock, seizures, coma, DIC, and oliguria. Cerebral edema, hypoxia, and boundary zone infarction may be seen. Rarely, SDH and retinal hemorrhages are seen in these cases and are attributed to the coagulation disorders present. The

authors have seen one case mimicking nonaccidental injury that ultimately was clinically diagnosed as consistent with HSES.

Hydrocephalus and Benign Extraaxial Collections

Hydrocephalus can be classified as internal, external, or communicating, depending on whether the abnormal CSF accumulation is within the ventricles, over the surface of the brain, or both.

Hydrocephalus is well recognized following SDH, and this complication represents the primary indication for surgical drainage of SDH.[68] The blood collections impede drainage of CSF via the arachnoid villi, leading to CSF accumulation.

Benign extraaxial collections of infancy are a complex and incompletely understood group. This is a self-limiting disorder, sometimes with a family history of dominant inheritance. Affected children present under 1 year of age with macrocrania (defined as an occipital frontal circumference at or greater than the 98th percentile) or rapid head growth and can show vomiting, irritability, seizures, and failure to thrive. Psychomotor development usually is normal.[69] Original reports considered the fluid collections to be located in the subdural space.[70] However, modern neuroimaging suggests that in many cases the fluid is within the subarachnoid space.[71] There seems to be no consistency, and the term *benign extraaxial collections* encompasses fluid collections in either compartment. These collections may be either protein-rich effusions (protein content ≥40 mg/dl) or gross blood (red cell count >1,000,000/ml^3). The cause of the collections is unknown, although typically there is no history of previous intracranial infection or trauma (see next paragraph). It has been hypothesized that the collection interferes with normal CSF absorption, leading to communicating hydrocephalus.[72]

Piatt[73] updated the controversial debate on benign extraaxial collections, considering them to be a form of external hydrocephalus. He suggests that that as many as 11% of infants with external hydrocephalus may develop SDH.[73,74] It is thought that the craniocerebral disproportion increases the distance that the bridging veins must traverse, rendering them vulnerable to shearing forces that make the infant susceptible to SDH either spontaneously or after otherwise "inconsequential" trauma. Papasian and Frim[75] developed a mathematical model that supported the hypothesis. Piatt describes a child with SDH and external hydrocephalus in association with retinal hemorrhages. He warns of the potential for making the mistaken diagnosis of child abuse in these cases, which is further discussed by Pittman.[76]

Space-Occupying Lesions

Subdural hemorrhage is a recognized complication of intracranial tumors in children. This section describes a number of intracranial space-occupying lesions associated with SDH.

Meningioma is infrequent in the first decade of life. A single case of chronic SDH has been reported in a 5-year-old male child with a frontal chordoid meningioma associated with bilateral SDH.[77]

A single case in Japanese reports a lesion described as a "neuroepithelial cyst" with associated SDH overlying the left temporal tip on histology in an 8-year-old male child.[78]

Two cases of SDH and intracranial sarcoma of childhood have been identified.[79,80] In each case, a separate mechanism for SDH is proposed. In the first, SDH is attrib-

uted to bleeding from tumor vessels within the subdural space. The second suggests that SDH is the primary pathology, possibly of traumatic origin, with the sarcoma developing several years later at the site of the previous SDH cavity. It is hypothesized that a chronic inflammatory reaction to SDH with proliferative membrane formation ultimately leads to the development of sarcoma. This parallels the occurrence of chronic pleural inflammation with the ultimate development of pleural malignancy. It is also hypothesized that surgical intervention or repeated intracranial taps amplify the risk.

A single case of an 8-month-old child with juvenile xanthogranuloma was reported by Labalette et al.[81] On presentation the infant had a chronic subdural hematoma attributed to spontaneous hemorrhage.

A 2-year-old male child with extramedullary hematopoiesis (EMH) of the cranial dura with associated SDH has been reported.[82] The EMH had formed a space-occupying lesion within the cranium and was attributed as the cause of the SDH.

Hypernatremia

Whether hypernatremia (defined as serum or vitreous sodium in excess of 150 mEq/L) is the cause or the result of a subdural hematoma has been discussed in the literature since the 1950s. Prior to Kempe's article on "The Battered Child Syndrome," it had been theorized that the dehydration associated with hypernatremia resulted in brain shrinkage, which in turn stretched the bridging veins, predisposing to rupture and SDH.[83,84] However, others have considered that it is the cause of the dehydration (e.g., severe intracranial disease, surgical procedures, or encephalopathy leading to central diabetes insipidus) and not the primary hypernatremia that results in SDH.[86] Handy et al.[85] review several series of cases where children died of nontraumatic deaths with evidence of hypernatremia, although SDH was not found at autopsy. In their own series, single cases were identified where hypernatremia and SDH coexisted; however, clinical documentation indicated that hypernatremia followed the onset of SDH. Finberg et al.[86] demonstrated that peritoneal administration of hypertonic sodium chloride solution to kittens could result in SDH, but the methodology and model have been criticized. One mechanism that could be considered from the observations of Finberg et al. in relation to infant accidental salt poisoning is that venous sinus thrombosis, a recognized complication of hypernatremia, is itself associated with subdural haemorrhage.[86] However, other papers reporting both accidental or intentional salt administration to children do not report the presence of SDH.[83,85]

Although the overwhelming evidence reported in the medical literature indicates isolated hypernatremia does not cause SDH, in extremely rare cases hypernatremia has been reported to be the cause of SDH.[87,88]

Connective Tissue Disease

Two connective tissue diseases have been reported to present with SDH in childhood: Ehlers-Danlos syndrome (EDS) and osteogenesis imperfecta (OI).

Ehlers-Danlos syndrome is a heterogeneous group of inheritable connective tissue disorders characterized by skin laxity, joint hypermobility, and tissue fragility. There are six types classified according to their signs and symptoms. The vascular type (type IV) has an associated shortened life expectancy because of rupture of

vessels and organs. A single case of EDS has been reported as simulating nonaccidental injury of child abuse.[89] In a similar manner, a single case of recurrent SDH in a 15-year-old child with EDS type IV was reported by Ortiz Remacha et al.[90]

Osteogenesis imperfecta is an inherited connective disuse disorder where the basic defect is the abnormal synthesis of the α_1 and α_2 chains of type I collagen. There are four types (with subclasses), with each type defined by its genetic abnormality or clinical features.[91] Osteogenesis imperfecta is a recognized diagnostic pitfall in the investigation of apparent nonaccidental injury, especially when considering the interpretation of recurrent skeletal fractures.[92] Scanty publications report a number of cranial pathologies, including intracranial bleeds, which have been associated with OI.[93] Tokoro et al.[94] reported a single nonfatal case of chronic SDH in a baby girl, which they attributed to rupture of the bridging veins during normal molding of the skull bones during delivery. They hypothesize that the chronicity of their cases may have been associated with other factors associated with OI, including coagulopathies and increased vascular fragility. This vascular fragility is caused by abnormal collagen support around the vessels and can be demonstrated by a positive Rumpel-Leede capillary fragility test. In a series of 79 patients with OI presented by McAllion, vascular fragility was also considered a significant factor in the causation of intracranial hemorrhage of all types, at all ages.[95] Osipenkova and Trakhtenberg[95] presented a single fatality in a 6-month-old infant with OI, SDH, and subarachnoid hemorrhage that was attributed to the trauma from bumping of the child's head against a wall.[95]

Minor trauma as a cause of SDH and retinal hemorrhages in individuals with OI was presented in a series of three children by Ganesh et al.[96] All three children had type I OI and, following through investigation, all three cases were considered accidental in nature. The principle author (G.R.) has encountered a fatal case of type I OI that presented with recurrent rib and long bone fractures, skin bruising, SDH, and retinal hemorrhages. Following full police investigation, this case ultimately was considered nonsuspicious in nature. These cases illustrate the diagnostic dilemma facing the investigating authorities as to whether these cases are patients with spontaneous or accidental pathologies in association with OI or cases of abuse of patients with OI.

Vitamin Deficiencies

Subdural hematomas may occur in childhood with vitamin C, and K abnormalities. No reports exist of SDH with vitamin A, B, or E abnormalities. A single case related to vitamin D deficiency is discussed in the following.

The majority of papers on the association of SDH and vitamin deficiency/abnormalities relate to vitamin K. This finding may be the result of either maternal vitamin K deficiency with SDH arising in the developing fetus[97] or postdelivery neonatal vitamin K deficiency known as *hemorrhagic disease of the newborn* (HDN). As reported by Rutty et al.,[98,99] HDN may be delayed beyond the immediate postnatal period and may present with both SDH and retinal hemorrhages, mimicking nonaccidental injury.

Only one paper related to vitamin C deficiency could be identified to date. In this report, both SDH and retinal hemorrhages are hypothesized to result from a combination of ascorbate depletion and injection of foreign protein (from vaccinations) leading to exceptionally high blood histamine level. This situation in turn was hypothesized to result in capillary fragility and venous bleeding.[100]

To date no definite cases of subdural hematomas occurring in children in association with vitamin D deficiency have been reported. However, a single case of a 2.5-year-old Bengali male child with rickets who underwent bilateral exploratory burr holes to both the frontal and parietal bones because of clinical suspicion of SDH has been reported, although only 15 ml of clear fluid to the left frontal burr hole was found.[101]

Therapeutic Drugs

A case of an 8-month-old male infant who had severe hepatotoxicity from phenobarbital toxicity was reported by Roberts et al.[102] This child presented with a severe seizure-like episode and collapse, the cause of which was attributed to bilateral chronic SDH. Unfortunately this paper does not enlighten the reader as to the clinical cause of the SDH but rather concentrates on the complications related to phenobarbital treatment.[102]

Vascular Disease, Malformations, and Flow Abnormalities

Subdural hematomas may occur in children as a presenting symptom of vascular disease or vascular malformations.

Kawasaki disease is a multisystem disorder characterized by vasculitis of small and medium arteries. Neurologic complications, including meningoencephalitis, monocyte-predominant pleocytosis in the CSF, facial nerve palsy, seizures, hemiplegia, and sensorineural hearing loss have been reported to occur in 1.1% to 3.7% of affected children.[103–105] Two papers detailing seven cases of SDH in children as young as 6 months have been reported.[106,107] The SDH in these cases was hypothesized to be caused by vasculitis of dural vessels. All cases reported to date have been in living subjects with no fatalities or for whom postmortem findings had been reported.

Intracranial arteriovenous malformations (AVM) and cavernous hemangioma are rare causes of neonatal intracranial haemorrhage.[108] Neonatal subdural hemorrhage in association with AVM appears to have been reported only four times in the literature, although the principal author (G.R.) has dealt with a nonreported fatal case of SDH associated with a subtentorial meningeal AVM that initially was suspected to be a nonaccidental injury.

Cerebral aneurysms have a distinct male predominance (12:1), occurring most frequently in the distal middle cerebral artery distribution or the posterior circulation.[109] They also have a higher incidence of large/giant aneurysms compared to adults. They are associated with head and birth trauma, infection (mycotic), fibromuscular dysplasia, moyamoya disease, coarctation of the aorta, subacute bacterial endocarditis, collagen vascular disease, EDS, Marfan syndrome, syphilis, sickle cell anemia, and tuberous sclerosis. Rupture of the aneurysm may occur, resulting in intracranial hemorrhage. The case of a 7-month-old boy with SDH in association with a cluster of six basilar artery aneurysms was reported by Plunkett.[110]

Congenital Heart Disease

Despite technical advances, neurologic complications of open heart surgery are estimated to occur in up to 24% of cases.[111] The association between SDH and congenital heart disease (CHD) in infants is related to surgery and the postoperative period rather than a complication of untreated CHD. Humphreys et al.[112] drew

attention to intracranial hemorrhage occurring in children following open heart surgery for CHD, most frequently valve or congenital cyanotic heart operations. In their series of 16 cases, 11 had SDH. Bleeds varied from "small localised area of haemorrhage" to "a clot occupying an entire hemicranium." They then postulated that the SDH resulted from low arterial perfusion pressure and brain shrinkage with tearing of the surface bridging veins. Other authors have favored preoperative coagulopathies as the cause of SDH.

Complications of Medical/Surgical Treatment

The best-known association between SDH and medical/surgical treatment is noted with shunting for hydrocephalus. This complication was first reported by Anderson[113] in 1952. It occurs more frequently in children with normal-pressure hydrocephalus (20%–46%) than in those with hypertensive hydrocephalus (0.4%–5%). Those with hydrocephalus secondary to vein of Galen malformations have an incidence up to 10%.[114]

The cause of the SDH has been related to excessive drainage of CSF resulting in a combination of collapse of the brain and opening of the subdural space[115] and tearing of the bridging veins. Fifty-eight percent of all cases are asymptomatic.

There is a single report of SDH complicating endoscopic III ventriculoscopy in a 2-year old-male child. In this case report, Maeda et al.[116] hypothesize that the blood arose from the wound site or from bridging vein injury resulting from acute decompression of the hydrocephalus.

Finally, a case of a 2.5-year-old female child who developed SDH following encephalo-myo-synangiosis (EMS) was reported by Sonobe et al.[117] The girl had right hemiparesis as a result of a very narrow left carotid artery bifurcation and small vessels as a result of moyamoya. EMS led to improvement of her hemiparesis, but she subsequently represented with ipsilateral SDH attributed to a complication of the EMS.

Metabolic Disease

Garrod[118] was the first in 1909 to use the term *inborn errors of metabolism* to describe a spectrum of genetically inherited disorders characterized by deficient activity of an enzyme in a metabolic pathway. The subject was visited by Olpin and Evans[119] in *Essentials of Autopsy Practice* (Volume 2), in which neurologic complications of a number of metabolic disorders were described. Of these disorders, glutaric aciduria type 1 (GA1) is the most commonly misinterpreted as nonaccidental injury because one of its presenting features may be SDH.

GA1 is an autosomal recessive inborn error of metabolism that can produce mild-to-moderate macrocephalus.[120] In the presence of frontotemporal atrophy the bridging veins are abnormally elongated and vulnerable to damage during minor injury, leading to both SDH and retinal hemorrhages (via subarachnoid effusion).[121–123] The most common presenting age group consists of those 7 to 18 months old, but presentation in the immediate postnatal period has also been reported. Examination reveals multiple SDH of differing ages[124] that may be unilateral or bilateral, with the latter being more common.[125]

Another neurometabolic condition that has been reported in association with SDH is D-2-hydroxyglutaric aciduria (D-2-HG). The genetic transmission and pathway abnormality for this disorder remain poorly defined.[126] In a single case report, a 7-month-old male child with macrocephaly and cerebral atrophy presented

with a unilateral right SDH with normal retinas. Again, the cause of the SDH is attributed to stretching of the bridging veins because of the increase in head size with associated cerebral atrophy.

A single case of a 5-week-old female child who had SDH and retinal hemorrhages in association with methylmalonic aciduria and homocystinuria has been reported. The cause of this abnormality is cobalamin C deficiency. These cases suffer from microcephaly and not macrocephaly, so the authors rejected a traumatic bridging vein abnormality. Rather they hypothesized that the raised levels of homocysteine cause direct vascular endothelial damage resulting in spontaneous haemorrhage.[127]

In 1962 Menkes[128] described a lethal neurodegenerative disorder. Menkes disease is an X-linked recessive disorder located on chromosome X13.3, which encodes for a copper-transporting P0type ATPase. The resulting abnormality of copper absorption results in a number of systemic abnormalities and is associated with SDH.

Vaccines

In recent years, particularly in the United States, vaccinations, specifically the diphtheria, tetanus, and pertussis (DTP) combined vaccination, have been postulated as a mimic of shaken baby syndrome. An autoimmune mechanism for cerebral injury has been speculated but, unlike pathology that may occur with rabies vaccination, no such mechanism has been demonstrated or proved with the DTP vaccine.[129]

A combined vaccine that has been reported to have a causal relationship with idiopathic thrombocytopenic purpura (ITP) is the mumps, measles, rubella (MMR) vaccine. Miller et al.[130] estimate that the absolute risk of developing ITP within 6 weeks of receiving the MMR vaccine is 1 in 22,300 doses. However, none of the cases presented in their paper had evidence of SDH.[130]

Thus, to date no peer-reviewed papers have demonstrated a categorical causal link between vaccinations and SDH. In the paper by Clementson (discussed in the section on vitamin deficiencies), the author hypothesizes a link between vaccinations, vitamin C deficiency, abnormal histamine levels, and SDH but presents no research-based evidence supporting the theory. A second theoretical paper on the subject, also authored by Clementson,[131] again postulates that inoculations cause a variant of infantile scurvy (Barlow disease). Although he again argues that vascular fragility may led to SDH and retinal hemorrhages during the normal handling of a child following inoculations, he presents no raw data or peer-reviewed research supporting his theory.

Calcified Subdural Hematoma

Calcified SDH has been reported in association with postmeningitic SDH effusion and as a long-term complication of ventriculoperitoneal shunts. It can be unilateral or bilateral and may undergo ossification. It may produce the so-called *armored brain appearance.* It rarely is symptomatic and more usually is asymptomatic.[132]

Alagille Syndrome

Alagille syndrome is a congenital absence of bile ducts in the liver. It is quoted as a cause of SDH, and yet no peer-reviewed paper could be identified to associate the two conditions.

School Age and Juveniles

Arachnoid Cysts

Arachnoid cysts are congenital lesions resulting from meningeal maldevelopment.[133] They occur most frequently within the middle fossa, usually unilaterally, with left-sided predominance (left to right 1.8:1) and male predominance (3:1). They are associated with SDH, subdural hygroma, and intracystic hemorrhage in teenage children, although cases in children as young as 5 years have been reported. Although the exact source of the SDH remains unknown, attention has centered on the unsupported bridging veins that traverse the cyst and fragile leptomeningeal vessels found within the cyst wall.[134] Two mechanisms have been hypothesized for the SDH: traumatic and spontaneous. The majority of SDH follows minor head trauma, when either traumatic cyst rupture or bridging vein injury is hypothesized to occur.[135,136] During the recovery phase, formation of unstable internal capillaries and sinusoids within the cyst wall may lead to chronic SDH as the result of repeated bleeds from these fragile vessels.[137] Spontaneous cyst rupture with associated SDH has been hypothesized to be caused by two mechanisms:

- Raised intracranial pressure, as may occur with a Valsalva maneuver
- Raised intracystic pressure resulting from increased flow of CSF into the cyst because of traumatic valve flap communication with the subarachnoid space such that it distends and ruptures[138]

Bone Marrow Transplant

There is a recognized significant risk of morbidity and mortality from neurologic complications following bone marrow transplant (BMT). Subdural hematoma has been reported to occur in up to 12% of patients (all ages), with 75% diagnosed at autopsy without antemortem clinical symptoms.[139] Although more commonly occurring in adult BMT patients, SDH has been reported in children as young as 4 years.[140] They usually are unilateral in nature, randomly distributed, and variable in size.[141] Although the mechanism underlying the cause of the SDH remains unknown, there is an association with pretransplant thrombocytopenia and/or coagulopathy.

Traumatic Causes of Subdural Hematoma in Children

Several studies have shown that the majority of SDH in infants is the result of trauma.[142,143] Within this group, more cases result from inflicted than accidental trauma. Feldman et al.[144] identified inflicted trauma in 59% and accidental trauma in 23% of their series. Figures in a report from the Royal College of Paediatrics and Child Health estimated that 51% of infantile SDH results from inflicted trauma.[1] The association of SDH and abuse in infants has been known for a long time, first recorded by Tardieu in 1860.

The distinction of accidental from nonaccidental traumatic causes is of great importance in forensic work and frequently is demanded of forensic pathologists. This distinction depends on a number of factors, including social investigations. It is impossible for the pathologist to make this distinction, particularly when only

intracranial injury is present. In other words, no pathologist or neuroradiologist can diagnose "intent."

Pathogenesis of Traumatic Acute Subdural Hemorrhage

There are four possible origins of subdural hemorrhage:

1. Traumatic subdural hemorrhage usually results from tearing of the draining veins as they cross the subarachnoid and subdural spaces to enter the sagittal sinus.[5] These veins are large, and traumatic rupture leads to a thick subdural blood clot. This pattern is typical of infants older than 9 months and adults.[144,145] The majority of SDH in young infants is thin fluid, which is readily tapped, rather than dense clot.[18]
2. Subdural hemorrhage may result from tentorial tearing, which is associated with molding of the head and movement of the skull bones, usually during delivery.
3. Thin-film subdural hemorrhage may result from oozing of blood from dural and arachnoid vessels in conditions where there is hypoxic endothelial damage together with raised intracranial vascular pressure. This is consistent with the recognized vascularity of the dura and the frequency of intradural bleeding in infants following asphyxia.[9]
4. Bleeding may into the subdural space from a bleed in another intracerebral compartment, after rupture of an aneurysm,[5,146] or following arachnoid tear.[18,147]

Causes of Traumatic Subdural Hematoma

As noted at the beginning of the chapter, the majority of infant SDH is the result of trauma, of which inflicted injury is the most frequent cause. Proposed mechanisms of such injury are highly contentious. Although severe high-velocity impact, such as motor vehicle accidents and falls from great heights, undoubtedly can cause SDH, very little is known about the effects of much lesser forces.

Direct trauma or impact causes deformation of the skull, with or without fractures, contusion, and epidural and subdural hemorrhage. Angular or rotational acceleration or deceleration of the head on the neck occurs in whiplash and shaking injuries, although there also is a rotational element in most falls as a result of hinging of the head on the neck. These forces are more likely to cause shearing injuries of the intracranial structures.[148,149] However, all mechanisms – translational, rotational, and angular – can cause subdural hematoma.

Shaking

More than 30 years ago a hypothesis was proposed to explain subdural hemorrhages in infants thought to have been abused.[150] This hypothesis proposed that shaking could cause brain damage and tearing of the draining veins of the brain leading to subdural hemorrhage. However, biomechanical studies using animals and models have indicated that the forces required are enormous, about 20 times those attainable by fit young adult volunteers.[151] Further, and more recently, model studies indicate that impact generates far more force than shaking alone[152] and that impact is required to produce SDH.[147] The degree of force (or violence) required to damage an infant is unknown, but it is noteworthy that healthy adult volunteers fatigued

after shaking an infant model for 10 seconds.[153] These authors suggested that impacting of the infant head/chin against the chest or the upper back during shaking might produce a contributory impact. In any infant who may have been violently shaken, injury to the ligaments, muscles, and nerves of the neck and of the cervical spinal cord would be expected.[144,153,154]

However, there is one further suggested mechanism by which a baby's brain may be damaged by shaking or whiplash. The pathologic studies of Geddes et al.[144,145] showed that most infants with inflicted traumatic brain injury had suffered lack of blood or oxygen supply to the brain. One third had torn nerve fibers in the part of the brainstem (craniocervical junction) where the respiratory control centers are found. The authors suggested that damage here would cause a baby to stop breathing, which would lead to a cascade of events resulting in brain swelling and retinal and possibly subdural hemorrhages. However, this mechanism depends on producing apnea (cessation of breathing) and subsequent severe brain swelling. This hypothesis has been rejected by some[155] and further emphasizes the importance of careful examination of the spinal cord and craniocervical junction in all infants with SDH. Readers are drawn to the Court of Appeal transcripts related to R v Harris, Rock, Cherry and Faulder where consideration is given to the controversies surrounding the pathology and causation of SDH in so-called shaken baby syndrome.[158a]

Low-Level Falls

Low-level falls can, albeit extremely rarely, cause SDH in infants and young children. Absolute height is not as important a criterion for injury as the following:

- *Exact nature of the fall* (What was the fall distance? Was it a free fall?)
- *For a particular infant* (What was the weight? Was the infant standing at the start of the fall? Which part of the body hit the surface?)
- *In a particular circumstance* (What was the nature of the landing surface? Did the body roll and dissipate the force?)

The effects of twisting, rotation, or crushing of the structures of the neck are crucial to outcome. It is well recognized that infants frequently tumble and fall, and fortunately in the majority of cases they suffer little ill effect. However, many studies show that low-level falls *can* cause serious intracranial injury, so they cannot be dismissed out of hand.

Biomechanical studies show that falls even from low levels of 3 to 4 feet can generate far greater forces in the head than impulsive action (or shaking).[150] Plunkett[156] and Kim et al.[157] have reported series demonstrating that children may suffer considerable intracranial damage after falls from levels as low as 6 feet. Patients in Plunkett's series were all older than 1 year. However, other papers have recorded intracranial bleeding in infants younger than 5 months who fell from less than 5 feet.[158] Schloff et al.[159] included four babies older than 6 weeks who fell less than 8 feet and developed intracranial hemorrhage. Single cases also have been reported. Aly-Hamdy et al.[160] described a baby who fell 3 feet onto a carpeted floor and developed subdural and retinal hemorrhages. Thus, although most children and infants may suffer little from an apparently trivial fall, it is clearly well recognized that minor low-level falls can cause intracranial bleeding, including subdural and retinal hemorrhages.

Localization of Subdural Hematoma

Most traumatic SDH is found in the parafalcine region close to the sites of insertion of draining veins. Localization depends on age and, as noted earlier, in infants the hemorrhage often is widespread over the convexities and thin. Postnatal SDH is seen far more commonly in the posterior fossa. Ewings-Cobbs et al.[161] found interhemispheric hemorrhage in most of their cases and always in association with convexity hemorrhage, never in isolation. Vinchon et al.[3] found a similar high incidence of parafalcine bleeding, especially in infants with signs of brain swelling. The falx is commonly hemorrhagic in asphyxiated infants,[9] indicating that, in some cases at least, imaging may be showing intrafalcine congestion and bleeding rather than parafalcine subdural hemorrhage.

Neuroradiologic studies have further suggested that localization of SDH can distinguish between inflicted and accidental SDH.[146,147] These studies state that posterior interhemispheric parasagittal and posterior fossa SDH are more likely to result from inflicted injury. However, the figures reported by Ewings-Cobbs et al.[161] demonstrate that the location of SDH is related to age. In infants younger than 1 year, there is no difference in localization of intracranial hemorrhage between infants with inflicted or accidental trauma. Vinchon et al.[18] reinforced this finding and stated that only the presence of retinal hemorrhages was of any assistance in distinguishing inflicted from accidental trauma.

It is hard to explain posterior fossa hemorrhage by shaking. The draining veins of the cerebellum would not likely be torn by rotational forces given their anatomy, as they run deep at the base of the cerebellum, close to the fulcrum of the craniocervical junction, not at its distal most equator. Nor would the close confinement of the cerebellum within the posterior fossa permit much movement to generate great stresses on its draining veins.

Summary

Although abusive head trauma remains the most common cause of SDH in children, the mechanisms remain under investigation and the subject of debate within the medical literature. When investigating a child with SDH, remember that trauma is not the only cause of SDH, and an extensive, often time-consuming, exhaustive investigation into all the causes considered in this chapter must be undertaken if the real cause is to discovered at the end of the day.

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6. Chest and Abdominal Injuries

Kenneth Shorrock

Introduction

Injury to the trunk is the major cause of nonnatural death resulting from trauma. Survival after severe chest or abdominal injury is more common than after severe head injury. Consequently the proportion of cases that are seen in forensic pathology practice is lower, and this fact should be borne in mind when interpreting published series. The nature, severity, and prevalence of any type of injury varies considerably, depending upon whether the pathologist is examining data derived from autopsy studies or from clinical practice. In the published literature the overwhelming majority of statistics derive from clinical series.

The autopsy provides valuable information on the severity and pattern of injury, both in the individual case and in epidemiologic studies. This can be useful in evaluating outcomes and planning strategy in the management of trauma services.[1,2] However, any such utility is dependent upon the quality of the examination and the report. There is evidence that in jurisdictions where only a broad statement as to the cause of death is required for certification and registration, the autopsy will add nothing useful to what has already been established clinically.[3] Therefore it is the responsibility of the pathologist to carefully look for and to describe in detail the nature, location, and severity of all the injuries.

The range of possible injuries and their causative mechanisms is enormous. Despite this large range, a number of basic patterns of injury are commonly seen. Knowledge of these basic patterns should enable the pathologist to determine the likely mechanism of causation in a particular case. For example, a number of injuries, seen in an appropriate context, including myocardial contusion, aortic disruption, sternal fractures, multiple rib fractures, and tracheobronchial disruption, are commonly associated with severe decelerational thoracic injury.[4] The range and complexity of visceral injury make accurate assessment of the prognosis of injury to any particular organ difficult. Almost all reported series comment upon the worsening prognosis with higher grades of injury, but this inevitably is affected by concurrent injury to other structures. Knowledge of the likely outcome of these injuries is obviously required by those treating the victims of trauma. However, the pathologist also should be able to assess the importance of the various injuries and their interaction, particularly when asked to provide an opinion regarding the role of competing

causes. Questions may be asked about the likelihood of survival if more rapid or alternative modes of resuscitation and definitive treatment had been provided.

A theme that soon becomes apparent when studying the published literature is the overwhelming preponderance of severe blunt injury that is caused by road traffic accidents. Sporting injuries are seen occasionally, and novel patterns of injury occasionally are associated with emerging pastimes.[5] Although assaults are an important part of forensic practice, death resulting from assaults is much less common than road traffic accidents. Penetrating injuries most commonly result from stabbing or gunshot wounds. The proportion of these wounds depends upon the nature of the jurisdiction from which any particular series is derived. For instance, British and mainland European experience has been of large numbers of victims of stabbing injuries and relatively few resulting from firearms. The opposite applies to urban populations throughout most of the rest of the world. To date, pathologists in civilian practice have had relatively little experience with injuries resulting from explosive devices, but this may change. Severe visceral injury, in both the chest and abdomen, can follow the effects of blast or other sudden pressure change.

Thoracic Trauma

Rib Cage

The thoracic viscera are protected to a certain extent by the ribs and sternum. Consequently, any significant blunt injury to the chest involves fractures of these bones. The ribs are flexible and resist bending to a variable extent without fracturing. This ability is more marked in infancy and early childhood. Consequently, compressive forces applied to the chest in younger individuals may not result in rib fractures. However, this does not necessarily mean that there will be no consequent injury to the thoracic or abdominal viscera. This relative immunity from rib fractures in children is reflected in the observation that their presence indicates that severe trauma has been sustained and that they are associated with a disproportionately high mortality compared to adults. In one pediatric series, 70% of rib fractures resulted from road traffic accidents and another 21% from nonaccidental injury.[6] In infants younger than 3 years, 63% of cases were the result of nonaccidental injury. Conversely, rib fractures are common in the elderly as a result of progressive osteopenia and ossification of the costal cartilages. As expected, mortality in adults rises with an increasing number of fractures,[7] due in part to the increasing risk of associated visceral injury. The relative risk of splenic, and to a lesser extent hepatic, injury has been shown to be increased when three or more ribs are fractured.[8] Rib fractures in the elderly (older than 64 years) have a mortality about twice that in younger patients.[9] Intrathoracic complications, particularly pneumonia and late development of pleural effusion, are much more common in this age group than in younger adults. This is an important consideration when determining cause of death and the assailant's culpability when elderly victims of physical assault who sustain rib fractures die after a period of hospitalization.

Ribs fracture at points where stresses tend to be concentrated, which often are at points of maximum curvature. A common pattern of rib fractures is symmetrically arranged bilateral fractures following anteroposterior compression of the chest. The frequently observed fractures that follow vigorous cardiopulmonary resuscitation often are anterior and may involve the costochondral junctions. A large autopsy series identified rib fractures in 32% and sternal fractures in 21% of patients who

had undergone cardiopulmonary resuscitation (CPR). The number or site of the fractures was not described, and cases of circulatory collapse following trauma were not separately identified.[11] Another study reported rib fractures in 14 of 130 cases, of which 11 patients also had sternal fractures. Interestingly, they also identified bone marrow embolism in five of these cases.[11] In any case of suspected assault where CPR has been performed and there are anterior rib and sternal fractures, it is prudent to state that these injuries likely are the result of the resuscitation unless there is good objective evidence to the contrary.

In cases of child abuse, particularly where there has been shaking, rib fractures are characteristically situated posteriorly and may be close to the costovertebral joints.[12] These fractures are thought to arise as a result of forcible gripping of the chest, with side-to-side compression. In this context, it often is suggested that any visceral injury in children who have undergone CPR may result from this compression rather than any antecedent trauma that caused the initial collapse. A large study showed no difference in the incidence of visceral injury between those who had and those who had not been resuscitated. Of more than 300 children who had received CPR and in whom there was no antecedent trauma, no significant injuries were found at autopsy.[13]

In a particularly severe form of rib injury, multiple adjacent ribs are fractured bilaterally, leading to the development of a "flail chest." Here, the instability of the thoracic cage causes impairment of respiration and interference with coordinated diaphragmatic and intercostal muscular action. In severe cases, this instability leads to paradoxical inward movement of a substantial part of the chest wall during inspiration. In cases of severe compressive chest injury, it is not uncommon to find fractures of most of the ribs bilaterally, with sparing only of the lower ribs that do not articulate with the sternum. It has been shown that, as expected, mortality is significantly greater when rib fractures are bilateral than unilateral. The presence of radiologically detected pulmonary contusions was also shown to adversely affect prognosis.[14] Fractures of the first ribs are uncommonly seen in clinical practice, although they are frequently seen at autopsy in those who have sustained overwhelming chest trauma. Occasionally, isolated fractures of the first ribs occur and are considered to be associated with a high incidence of severe local complications.[15] However, a number of case reports highlight the relative frequency with which this rib is fractured as a result of repetitive minor injury, with no consequential problems except for local pain.[16]

At autopsy, rib fractures are usually found in adjacent ribs and are generally arranged in lines. The discovery of apparently randomly arranged fractures suggests multiple episodes of trauma, as would be seen following an assault, with multiple kicks or stamps to the chest. The presence of bruises that are visible beneath the parietal pleura discloses the likely site of any rib fractures. If fractures are sustained close to the time of death, however, little or no bruising may be seen because of the absence of an effective circulation. Rarely the subpleural bruising is sufficiently severe that it produces a radiologically visible hematoma. This hematoma may cause diagnostic difficulty and in extreme cases contribute to a poor outcome.[17] Individual fractures can be adequately identified and demonstrated only if the chest wall muscles are dissected off the ribs as far as possible posteriorly, after which the intercostal muscles are divided in order to separate the individual ribs. Attempting to examine the ribs without prior dissection leads to errors because of mutual splinting, rendering it impossible to fully determine the mobility of individual ribs. However, bear in mind that there is a surprising degree of mobility at the costovertebral joints of the ribs that have

been separated in this way, and this should not be misinterpreted as indicating the presence of a posterior fracture.

Sternum

Sternal fractures result from severe blows causing anteroposterior compression of the chest. They are rarely displaced and require no specific treatment. In clinical series they are identified in approximately 8% cases of blunt chest trauma. Their significance is that they indicate severe force has been inflicted. This finding may be important in determining culpability in cases of alleged assault. They also raise the possibility of likely injury to underlying viscera.[18] Sternal fractures commonly result from road traffic accidents, particularly in drivers and front seat passengers, if intrusion into the vehicle occurred, or if the victim was not restrained. They also are seen following violent assaults. They must be differentiated from fractures resulting from resuscitation attempts, particularly in individuals with osteoporosis. If this issue is important, as in the investigation of a suspected assault, details of the nature of any resuscitation must be obtained. Confusion can occur in the case of single-vehicle road traffic accidents where the driver is the sole occupant. In one of my own cases, death resulted from a coronary thrombosis. The sternal fracture was caused during resuscitation. My first suspicion, that the fracture resulted from a front-end collision, with the driver being thrown against the steering wheel, was shown to be erroneous when witnesses came forward who had seen the car slowly leave the road, after which it came to rest on the grass verge. Police reports confirmed that there was minimal damage to the vehicle.

Heart

Blunt Chest Trauma

Blunt injury to the chest, particularly if there is compression in the anteroposterior direction, can cause direct injury to the heart. The degree of injury can vary from minor myocardial contusion to complete disruption of one or both ventricles. The range and nature of possible injury was originally described by Parmley et al.[19] in 1958, and this still provides the benchmark for classification. In addition, it has been postulated that there is a condition of "myocardial concussion" that can cause transient contractile dysfunction in the absence of any anatomic abnormality.[20] This is also commonly referred to as *commotio cordis*. It often is postulated as a cause of death in those who are witnessed to collapse and die after sustaining a severe blow to the front of the chest, but who are subsequently found to have no identifiable fatal injury. An experimental study of controlled blunt chest trauma in rats supports this finding.[21] The authors demonstrated that arrhythmias were induced every time and that 31% of fatal cases demonstrated no objective pathologic abnormality. Myocardial contusion appears histologically as extravasation of red cells, associated with necrosis of myocytes. This finding can lead to confusion with myocardial infarction caused by ischemic damage. The mechanism of causation is thought to be direct compressive damage to the myocardium, although it is suggested that coronary artery spasm or thrombosis consequent upon intimal disruption may cause secondary myocardial ischemia and infarction.[22] Unlike myocardial infarction resulting from atherosclerotic coronary artery disease, however, it commonly affects

the wall of the right ventricle. As an isolated abnormality, myocardial contusion probably is relatively benign if there is no consequent dysrhythmia or hemodynamic disturbance.[23] More severe compressive injury may give rise to damage to the valvular apparatus. This situation may manifest itself as rupture of papillary muscles or chordae tendineae, tears to the cusps, or partial rupture of the annulus. Full-thickness rupture of the myocardium affects the right ventricle more commonly than the left ventricle, probably because of its more anterior position and the relative wall thickness of the chambers. The interventricular septum also may be ruptured.

Penetrating Chest Trauma

The heart is commonly injured by penetrating injuries, such as stabbing. In most jurisdictions where firearms are widely available, gunshot wounds are increasingly common, although they still are greatly outnumbered by stab wounds.[24] This is not the case in the United States, where many series of penetrating thoracic trauma contain a preponderance of cases of gunshot wounds.[25] In the United Kingdom, the overwhelming proportion of penetrating chest injuries are caused by knives or other bladed weapons. Because of the anterior position of the right ventricle, behind and immediately to the left of the sternum, the right ventricle is damaged by penetrating injuries more often than the left ventricle,[26] particularly when the assailant deliberately aims for this typical site of election. Many such injuries also penetrate the interventricular septum and may exit posteriorly through the free wall of the left ventricle. Death often results from cardiac tamponade, despite the inevitable presence of a defect in the parietal pericardium. It is less common for loss of blood into the mediastinum and pleural cavities or externally to cause exsanguination. It must be remembered that stab wounds that involve the heart and in which the direction of thrust is downward may penetrate the diaphragm and cause concurrent injury to abdominal viscera or blood vessels.

Surprisingly, penetrating cardiac injuries are not uniformly fatal, and impressive survival rates are recorded from centers that have extensive experience with their surgical management.[27,28] However, these results should be interpreted with caution because they derive from trauma center statistics. Some of their patients were moribund upon arrival at the hospital, but no data are presented showing how many cases were certified to be dead at the scene and were transferred directly to the mortuary. Right ventricular injuries are more favorable than those to the left ventricle, and the prognosis is made much worse when multiple chambers are involved. Penetrating injuries that involve one of the major coronary arteries carry a much higher mortality, largely because of the additional burden of ischemia in the myocardium supplied by the vessel distal to the point of transection. Some injuries can give rise to septal defects, damage to valves, or interruption of the conducting system.

Respiratory System

Blunt Chest Trauma

Tracheobronchial injuries caused by blunt trauma are seen rarely in clinical practice but are seen more commonly in autopsy practice because of their association with other lethal injuries, such as aortic transection. The majority are situated within

2.5 cm of the carina or involve the right main bronchus. Occasional cases involve avulsion of a lobar bronchus.[29] The mechanism of injury is thought to be distraction consequent upon tissue deformation caused by anteroposterior crushing force. Because of their protected position in the midline behind the sternum, the intrathoracic part of the trachea and the main bronchi are infrequently damaged by penetrating injuries, although this is common in the cervical part of the trachea.

The lungs can be injured by any blunt or penetrating injuries of sufficient severity. In the absence of preexisting pulmonary disease there is a large functional reserve; consequently not all such injuries are fatal or even contribute significantly to causing death. Cases of blunt pulmonary injury carry a much higher mortality than do penetrating injuries.[30] This finding results largely from the greater magnitude of associated injuries in the former group as reflected in their higher average injury severity scores.[31] It is common to find varying degrees of pulmonary injury, even to the extent of localized parenchymal disruption, when death has clearly resulted from injury to other structures. The most common manifestation of pulmonary blunt injury is parenchymal contusions. These contusions are caused by a combination of tissue deformation, acceleration/deceleration injury, and hydrostatic forces within the vasculature. The relative contribution of each of these factors depends upon the circumstances in individual cases. A single case report of contrecoup pulmonary contusions[32] highlights the role of transmitted forces in their causation. In survivors, pulmonary contusion is diagnosed based on the radiologic identification of diffuse opacities within the lung fields. At autopsy the diagnosis can be more difficult, particularly if death was delayed such that secondary pulmonary complications, such as edema, infection, or infarction, supervene. Macroscopically, affected tissue appears engorged and hemorrhagic. This is confirmed histologically by the presence of widespread interstitial and intraalveolar extravasation of blood. Late pulmonary consequences of injury, such as the development of the adult respiratory distress syndrome, often cause delayed death. This results partly from primary pulmonary injury and partly from the remote consequences of injury to other organs. Pancreatic trauma or the development of septicemia is particularly potent in this respect. It seems almost inevitable when performing an autopsy on anyone who has died after a period of treatment on the intensive care unit to find very heavy and consolidated lungs. Severe crushing chest injuries can cause damage to the major pulmonary vessels in the mediastinum as a result of distraction of the lungs laterally. Such injuries commonly accompany major injury to other thoracic and abdominal viscera so that their specific contribution to causing death is impossible to determine.

Penetrating Chest Trauma

Direct injury to the lungs is extremely common in cases of fatal stabbing or other penetrating injuries. Displaced rib fractures can cause laceration of the underlying lung. Frequently a weapon passes through the lung parenchyma in addition to other viscera, such as the heart, or the major vessels. Death then results from injury to these structures. However, cases where the only injury is to the peripheral lung parenchyma, resulting in exsanguinating hemorrhage into the pleural cavity, are encountered occasionally. In some of these cases there is a history of considerable delay between infliction of the injury and subsequent collapse and death. In these instances, death results from hypovolemic shock and the slow accumulation of blood in the

pleural cavity. Many such fatalities could have been prevented if the deceased or other parties had sought prompt access to emergency medical facilities.

The development of pneumothorax or hemopneumothorax as a result of pulmonary injury often contributes to mortality by impairing respiration, particularly if the air is under tension, with significant mediastinal shift or impairment of venous return to the heart. It is easily missed at autopsy if the condition is not specifically sought. The presence of a pneumothorax can easily be demonstrated by producing a lateral pocket between the subcutaneous tissue and the chest wall musculature. This pocket is then filled with water and the chest wall incised so that any escape of air bubbles can be observed. Alternatively, if radiologic facilities are available, the presence and size of any pneumothorax can be determined from a plain chest x-ray film. Any mediastinal displacement also can be demonstrated. Radiology also has the advantage of providing a permanent record that can be used as evidence in any litigation.

Thoracic Aorta

The thoracic aorta is commonly injured as a result of severe acceleration/deceleration forces, generated either during transportation accidents or following falls from a height onto a solid surface. In about half of all cases the resulting aortic tear is found at the junction of the arch and the descending part, immediately distal to the origin of the left subclavian artery. The remainder are distributed approximately equally between the arch and the ascending and the descending aorta.[33,34] A small proportion of cases have injuries at multiple sites within the thoracic aorta. The major branches arising from the arch also may be affected.[35] In a large series, approximately 20% of road traffic accident fatalities had severe aortic injury, of which more than half were complete transections. The remainder were tears involving part of the circumference. In one of my own cases resulting from a high-speed motorway accident that involved multiple vehicles and resulted in six fatalities, two individuals recovered from the same vehicle had identical transections at the junction of the arch and descending aorta. The characteristic pattern of injury probably results from the fact that the descending aorta is almost completely immobile and is firmly attached to the prevertebral tissues, whereas the arch is capable of significant movement, lying within the superior mediastinum. Consequently, shearing forces are established at the point at the junction by sudden movement of the heart and proximal aorta relative to the prevertebral tissues. Experimental studies performed on human cadavers suggest that sudden compression of the lower sternum causes the thoracic viscera to rise, a mechanism referred to by the authors as *upward shoveling*.[36] This causes distraction of the tissue of the aortic wall. Other factors, such as a sudden rise in intraaortic hydrostatic pressure consequent to severe compression of the trunk, also may be implicated.[37] These factors may be responsible for injuries seen at other sites within the thoracic aorta. Lesser degrees of injury may present as delayed rupture or may be demonstrated by progressive mediastinal widening, as seen on imaging. This finding is caused by progressive accumulation of blood around the aorta. Under these circumstances, surgical correction and repair are possible. With the advent of modern imaging techniques, minor areas of aortic damage that may involve only the intima and the inner part of the media are being recognized.[38] These cause no extravasation of blood and probably require no treatment, but they may be identified at autopsy in those who died as a result of more severe injury elsewhere. Severe aortic injury is often seen in cases where there

is overwhelming injury to other viscera or to the head. In some of these cases, there is little or no hemorrhage in relation to the aortic injury, indicating that death resulted from the effects of the other injuries. Care must be taken in removing the thoracic viscera at autopsy if the aortic tear is to be demonstrated reliably. The tissues should be removed without excessive traction, preferably by sharp dissection between the aorta and the prevertebral fascia. Forceful distraction, by pulling the thoracic viscera anteriorly and downward toward the diaphragm, can cause artifactual aortic tears to develop or can extend a preexisting traumatic tear. The thoracic aorta is frequently incised as a result of fatal penetrating wounds. Injury is seen at any point, depending upon the site of entry and the path of the causative object. In one large autopsy series the overwhelming majority of such injuries affected the ascending aorta.[35]

Blunt injury to the abdominal aorta is much less common than in the thoracic aorta. In many cases it is seen in conjunction with fracture-dislocation of the lumbar vertebrae, causing distraction and angulation of both the aorta and the inferior vena cava.[39] In a series of 46 patients with abdominal aortic injury, almost half had other injuries. Unlike thoracic aortic injury, the mechanism appears to be direct pressure, possibly in association with the generation of high intraluminal hydrostatic pressure.[40]

Diaphragm

The diaphragm is commonly ruptured as a result of compressive blunt trauma to the abdomen, causing a rise in intraabdominal pressure. It is most commonly encountered in drivers and front seat passengers of motor vehicles that are involved in head-on collisions at speed or in which there is lateral intrusion into the vehicle.[41] The abdominal viscera, particularly the liver, are forced upward, rupturing the diaphragm and prolapsing into the thoracic cavity. This almost always is associated with injury to other structures, particularly rib fractures and disruption of the hepatic or splenic parenchyma. Data relating to the side of the diaphragm that is most commonly affected and the common patterns of injury are contradictory.[42] However, an extensive review of the English language literature identifying 1000 cases demonstrated a marked left-sided predominance of injury.[43] They found an overall 17% mortality and confirmed the high incidence of associated injury to the spleen and thoracic viscera. Occasionally, diaphragmatic rupture presents later, with complications of herniation of abdominal viscera.

Abdominal Trauma

Blunt Abdominal Trauma

Blunt injury to the abdomen often results from severe accidents, particularly those involving motor vehicles, following falls, or when a hard object strikes the abdomen. In cases of homicide it usually results from blows, kicks, or stamping. Abdominal injuries frequently form part of the catalogue of injuries seen in cases of severe widespread trauma, regardless of their mode of causation. In some cases they are the primary or sole cause of death, but less commonly than in cases of head or chest injury. Abdominal trauma often results in death after an interval, either as a result of progressive uncontrollable hemorrhage or infection resulting from perforation of the hollow viscera. Specific late complications also develop. For example, injuries

to the pancreas may give rise to secondary diabetes or biliary obstruction. Urinary tract trauma may result in chronic renal failure or secondary hypertension. Many victims of abdominal injury undergo surgery prior to death, or they die after a period of prolonged hospital treatment. Consequently the pathologist may have to differentiate the consequences of treatment from those of injury. Careful scrutiny of the medical records in these cases is often vitally important because it may offer the only real evidence of what injuries were inflicted, as the offending viscera have been removed, repaired, or subjected to extensive replumbing.

Liver

Injury to the liver represents one of the major causes of death in the early period after abdominal trauma. Severe blunt trauma and rapid acceleration/deceleration both cause extensive parenchymal disruption, with consequent hemorrhage. There also may be disruption of the hepatic veins at their confluence with the vena cava. The latter injury often results in an inability to control hemorrhage at operation, despite resection of damaged parenchyma and packing. The Organ Injury Scaling Committee of the American Association for the Surgery of Trauma (AAST) grades injury to the liver and other abdominal organs according to severity. These classifications are used primarily for assessing prognosis and evaluating outcomes, but they do provide the pathologist with a convenient and reproducible benchmark against which to assess and describe visceral injury. It is worthwhile to have a broad understanding of the classification for each of the major abdominal organs. Liver injury is graded from I to VI. At the lower end of the scale are minor subcapsular hematomas and limited degrees of parenchymal laceration. Grade V lesions involve disruption of more than 75% of one lobe or severe injury to major hepatic veins. Grade VI refers to hepatic avulsion.[44] Severe blunt hepatic injury often accompanies injury to other viscera. The presence or absence of significant hemorrhage within the abdominal cavity in these cases indicates whether or not the liver injury should be considered the primary cause of death. Penetrating injury to the liver is very common, partly because of the liver's large size and location. It is also immediately adjacent to the chest cavity and lies within the rib cage. Consequently it is easily penetrated as a result of homicidal stab wounds to the chest. The resulting injury is rarely life threatening in itself. Death usually results from concomitant injury to other viscera, either as part of the same wound or from others.

Mesentery

A very characteristic consequence of blunt abdominal trauma is hemorrhage into or disruption of the mesentery or the transverse mesocolon. Large full-thickness tears of these structures are often encountered, and there may be severe bleeding from branches of the superior mesenteric artery or the mesenteric vein. If diagnosis is delayed and hemorrhage is not immediately fatal, localized infarction of the intestine can occur. As the mechanism of injury, the mesentery becomes compressed between the anterior abdominal wall and the midline promontory produced by the lumbar vertebral bodies. This situation can be demonstrated at autopsy by the fact that, in the anatomic position, the site of injury usually lies in the midline. The hemorrhage often extends into the retroperitoneal tissues at the root of the mesentery. If retroperitoneal hemorrhage is identified but no mesenteric injury is obvious, careful examination of the retroperitoneal tissues may reveal injury to the proximal

pancreas and/or duodenum or distraction of the root of the superior mesenteric artery. This latter injury results from forceful downward displacement of the intestine by pressure applied in the center of the abdomen. These injuries may coexist because of their similar mechanism of causation. They frequently present late and may manifest as failure to improve or as progressive deterioration and death after apparently successful surgery to control bleeding arising elsewhere or to repair damage to more obviously visible structures.

Intestines

The intestine is only infrequently injured by blunt abdominal trauma, although intramural hematomas or subserosal hematomas are seen on occasion. The relative immunity of the intestines to injury results from their mobility and from the fact that any damage frequently affects the mesentery. The possible mechanisms of causation of intestinal injury, as described by Vance,[45] continue to be useful in evaluating abdominal trauma. He described injuries caused by crushing, deceleration, and bursting. In addition to clinically overt acute injury, delayed perforation or stricture formation can develop as a result of localized ischemia caused by disruption of the intestinal vasculature. A disproportionate number of injuries to the abdominal hollow viscera, particularly the small intestine, are a consequence of road traffic accidents in which the victim was wearing a seat belt.[46] However, this must be viewed in the context of the consequent reduction in the number of head injuries.[47] The tradeoff perhaps can be justified by the fact that intestinal injury is much more amenable to treatment and cure. Overall, 85% of intestinal and mesenteric injuries are caused by vehicular accidents involving either the occupants or pedestrians and cyclists.[48] The ascending and descending parts of the colon are retroperitoneal, but they are protected to a certain extent by their position in the paracolic gutters. Penetrating injuries of the intestine, however, are relatively common. Such injuries rarely cause death, but they are seen at autopsy when the causative implement has penetrated other structures, particularly the major retroperitoneal vessels. It is common to find recently repaired perforations of the intestine in those who have undergone emergency surgery but who have died as a result of uncontrollable hemorrhage from other structures. In those who have been profoundly hypotensive for a prolonged period as a result of blood loss from major trauma, ischemic damage to the intestines may be encountered at autopsy. This damage presents as dusky discoloration of the wall, sometimes in a geographic pattern. Altered blood and exudate will be present in the lumen. Their presence commonly affects the splenic flexure of the colon most severely, in the watershed between the part of the colon supplied by the superior and inferior mesenteric arteries.

Stomach

Blunt injury to the stomach is encountered infrequently. A study concluded that blunt injury to the stomach was present in only 0.5% of cases of blunt abdominal trauma.[49] This finding probably results from its relatively protected situation. It is perhaps not surprising that it often is associated with injury to the spleen and the chest.[50] Gastric injury occurs more commonly when the stomach is full, after a heavy meal. The Heimlich maneuver, in which sudden pressure is applied to the upper abdomen in order to expel an obstructing bolus from the trachea or glottis, was first described in 1974.[51] It has been associated with rupture of abdominal

hollow viscera, including the stomach and duodenum.[52] Injury to other structures, including the mesentery and abdominal aorta, have been described.

Duodenum and Pancreas

It is appropriate to consider injury to the duodenum and pancreas together because of their close anatomic relationship and the fact that damage to both structures may coexist. This is reflected in the AAST classifications of duodenal and pancreatic trauma, each of which classifies combined pancreaticoduodenal disruption as grade IV.[53,54] Because of their position in the upper part of the abdomen, in the retroperitoneal space, they are relatively well protected. Consequently, injury to them is often associated with severe injury to other structures. This circumstance is important when assessing the consequences of pancreatic and duodenal trauma.[55] The position of the neck and body of the pancreas and the third part of the duodenum immediately in front of the vertebral bodies does, however, give rise to the most characteristic injury, which is contusion or splitting of these structures caused by anteroposterior crushing injuries.[56] Injury to the distal pancreas has a better prognosis than proximal injury, particularly when the latter breaches the major ducts.[57] A commonly encountered problem and one that contributes to a relatively poor prognosis is delayed diagnosis of duodenal injury.[58]

Overall, the incidence of blunt injury to the abdominal hollow viscera ranks third, after that to the spleen and liver. The small intestine, excluding the duodenum, is most commonly injured, in approximately two thirds of cases. Approximately 20% of injuries involve the colon and another 8% involve the duoudenum.[59]

Spleen

Splenic rupture is commonly encountered during the surgical treatment of those who have suffered blunt abdominal injury. Often the spleen is consequently found at autopsy to have been removed in those who later died of the effects of injury to other organs. There has been a trend toward splenic conservation and nonoperative treatment of splenic injury, such that up to 90% of patients now are treated conservatively. The overall mortality of approximately 20% is related to the severity of the splenic injury, the presence of associated injuries to other organs, and the patient's age.[60] The AAST classification grades splenic injury from minor degrees of subcapsular hematoma or parenchymal laceration (grade I) through to a completely shattered or devascularized organ (grade V).[61] Compressive injury to the left upper quadrant of the abdomen is responsible for most injuries. The spleen is often partly adherent to adjacent structures and to the parietal peritoneum laterally, so distraction of the viscera in this region often gives rise to capsular tearing. The ease with which it can be injured in this way is illustrated by the frequency with which it is damaged during its removal at autopsy, particularly if the abdominal viscera are removed in a separate block from the retroperitoneal tissues. The most common finding in cases of splenic trauma is one or more tears of the capsule, with varying degrees of disruption of the underlying parenchyma.

Kidney

Severe crushing injury can injure the kidneys but is much less commonly encountered than with hepatic or splenic injury. In many such cases, the renal injury is

overshadowed by widespread and severe bony and visceral injuries, all of which have contributed to causing death. Minor degrees of renal injury manifest as parenchymal contusions or lacerations (grade I–II).[62] More severe injury (grade III) communicates with the collecting system (pelvis and calyces). Occasionally blunt abdominal trauma, with compressive forces applied in the anteroposterior direction, causes distraction and rupture of renal vessels (grade IV injury), with consequent severe retroperitoneal hemorrhage. A small amount of retroperitoneal hemorrhage in the region of the renal hila is commonly encountered at autopsy, even in cases with no antecedent trauma. It is important that this possibility be recognized and that significant abdominal injury not be incorrectly concluded. Injury to the renal vasculature may cause late complications such as infarction or renovascular hypertension.[63]

Pelvic Cavity

Bladder injury is rare. It is caused by bursting or laceration as a result of intrusion of bony fragments from a fractured pelvis. Regardless of the mechanism, most vesical injury is accompanied by severe pelvic fractures and other injuries. The bladder is ruptured as a result of blows to the lower abdomen, particularly if the bladder is distended with urine. Approximately two thirds of such injuries are extraperitoneal.[64] Severe pelvic injury, particularly in men, can cause transection of the membranous urethra, with upward dislocation of both the prostate and the bladder.

Rupture of the uterus as a consequence of blunt abdominal trauma is occasionally seen during pregnancy. It is associated with a maternal mortality of approximately 10%.[65] Fetal loss is much more common.[66] The loss results from a number of factors, including placental abruption, fetomaternal hemorrhage, and fetal hypoxia consequent to maternal hypotension.

Fractures of the pelvic ring are common following severe injury to the lower abdomen. They are classified according to the site of fracturing and the likely mechanism of causation. The most commonly used classification defines fractures caused by anteroposterior compression, lateral compression, and vertical shearing forces.[67] Pelvic fractures can be missed at autopsy, particularly if they are undisplaced. In order to perform a thorough examination of the pelvic bones, the dissection must be more extensive than in routine cases; mere inspection for bruising and palpation of bones that normally are accessible is not sufficient. Incision of the peritoneum anteriorly and the anterior abdominal wall muscles at their insertion into the pubis allows palpation of the pubic and ischial bones. The blade of the ilium and the sacroiliac joints can be examined by incision of the psoas muscle medially where it originates from the lumbar vertebrae. This part of the dissection demonstrates fractures of the transverse processes of the lumbar vertebrae, which are commonly present following severe injury but are often overlooked. The psoas is then reflected laterally while the iliacus is dissected off the anterior aspect of the ilium. Fractures can then be easily visualized and photographed. If a single displaced fracture is identified in the anterior part of the pelvic ring, a corresponding fracture or dislocation must be present elsewhere. This is commonly represented by a dislocation of one of the sacroiliac joints. This injury is not easily visualized radiologically, so it may not have been identified prior to death. Superficial examination of the pelvis also would fail to demonstrate the characteristic bruising around the joint.

Blast Injury

Blast injury affects predominantly the thoracic and abdominal viscera. It is rarely seen in civilian forensic practice, but the pathologist should be familiar with the basic physiologic principles and the pathologic consequences. The lungs, airways, and to a certain extent the intestine contain gas. This gas is compressible, and the most severe tissue injury resulting from effects of pressure usually occurs at the gas–liquid or gas–solid interface. A number of adverse effects on the body result from proximity to explosions.[68] In the lungs, the effects of energy transfer give rise to a variety of injuries, ranging from minor parenchymal petechiae and ecchymoses to extensive parenchymal contusion and disruption. Rupture of alveolar membranes and microvascular trauma cause progressive exudation into the alveolar and interstitial spaces, resulting in consolidation (hepatization).[69] The effects of dust inhalation may exacerbate this condition. Pneumothorax, hemopneumothorax, or pneumomediastinum may result from escape of air from disrupted pulmonary or bronchial tissues.[70] Passage of air into the vasculature also may cause fatal air embolism. The biomechanical effects on the abdominal hollow organs are similar to those in the lungs. Injuries here range from mural contusions to areas of mucosal ulceration and full-thickness perforations, with consequent peritonitis. Vascular damage can give rise to intestinal infarction.

Summary

Injury to the chest and abdomen is common in clinical and autopsy practice. Although the range of injury to both the body wall and the viscera is enormous, constant patterns and associations are encountered. Knowledge of these patterns and association is essential to the accurate interpretation of their causation, which then assists greatly in the reconstruction of events that caused the injury. Identification and precise description of the pattern and severity of individual injuries enable comparison with published data. Reference to the clinical literature provides models for such an approach. A vague and imprecise description of the injuries should no longer be considered sufficient in the pathologic reporting of such cases.

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7. Injuries and Death Resulting from Restraint

Noel W.F. Woodford

Introduction

Article 2(1) of the Human Rights Act 1998 (UK) states that everyone's right to life is protected by law[1]; however, this is not an absolute right. Exceptions under Article 2(2) provide qualification so that there is no contravention if loss of life results from defense of a person against violence or in order to effect a lawful arrest, so long as the force that is used is *no more than is absolutely necessary*. A peculiarity of the language sees the word *restraint* applying simultaneously to the physical act of restricting or curtailing movement, the means or apparatus used to cause the restriction, and the moderation of behavioral excess. All of these factors are of concern to the pathologist with regard to the approach to what often are emotive and controversial cases. Numerous factors conspire to complicate the analysis of cases where death has occurred during restraint, some of which include the relative paucity and occasionally contradictory nature of empirical physiologic data concerning cardiorespiratory functioning in situations of severe stress, the difficulty in determining the significance and effects of drugs (both therapeutic and illicit) identified in postmortem specimens, preexisting natural disease processes, determination of the sequence of events and methods used to enforce restraint, and the effect of complex neuropsychiatric syndromes.

Fundamentally, restraint is used to prevent persons from harming themselves or others. In any robust physical interaction between human beings there is the risk that one or the other party will suffer injury, but the aim of restraint should be an acceptable balance between minimization of that risk and effectiveness and rapidity of control. The high mortality associated with firearm use has stimulated the search for so-called less-lethal technologies, which simultaneously provide adequate protection for police or other parties with responsibility for public/patient safety. This chapter reviews some of the commonly used restraint modalities and the injuries that may ensue. Consideration is given to the concepts of restraint asphyxia, excited delirium, and psychiatric factors involved in some restraint-related deaths. Finally, a suggested approach to the autopsy and pathologic investigation is presented, along with a discussion of the formulation of the cause of death.

Restraint Modalities

Methods of restraint can be considered physical or chemical and further subdivided according to the actual mechanism involved (Table 7.1). Some of the various types are discussed here, with greater emphasis on the newer entities.

Taser

Taser is an acronym for Thomas A. Swift's Electric Rifle. The Taser is designed for relatively close-range incapacitation by the delivery of a high-voltage, low-energy electric impulse that causes involuntary skeletal muscle contraction but is below the threshold for induction of ventricular arrhythmias. These weapons have received increased publicity of late in the wake of police-related handgun fatalities in Australia[2] but have been in use in Los Angeles since 1982. The latest model of this weapon is the Taser X26, a 26-watt device. This model is an updated version of the M26 Taser, which replaced an earlier, lower-power (7-watt) version thought to provide less than optimal incapacitation of particularly violent and focused people.

The Taser is a handgun-size and handgun-shaped weapon that uses compressed nitrogen to fire two barbs from a replaceable cartridge (velocity 55 m/s) over a range up to 7.0 m. The barbs, which are 4 mm long, are connected to the gun by thin wires and are designed to spread out (at an angle of 8 degrees) and contact two different areas of the body (Figure 7.1). When contact is made with either skin or clothing, up to 50,000 volts is discharged in pulsed delivery over a 5-second period at 151 mA.[3] The cycle of pulses can be repeated, and the manufacturer claims the device is effective even if only one barb contacts the offender so long as the other barb contacts a satisfactory earthing point. Small, round paper disks (each bearing the individual cartridge serial number) are discharged at the time of firing, thus facilitating reconstruction of scene events. In addition, a data port within the Taser enables subsequent interrogation of the device as to the number and timing of pulses delivered.

Table 7.1. Restraint modalities.

Physical methods
- Manual
  - HProne positioning (with or without supplemental ligature application)
  - Neck holds and other incapacitation holds
- Implement
  - Restraint suits (violent person emergency restraint system, emergency restraint belt)
  - Handcuffs and ligatures
  - Batons
- Firearm
  - Impact rounds
  - Kevlar nets
- Electrical
  - Taser, stun gun

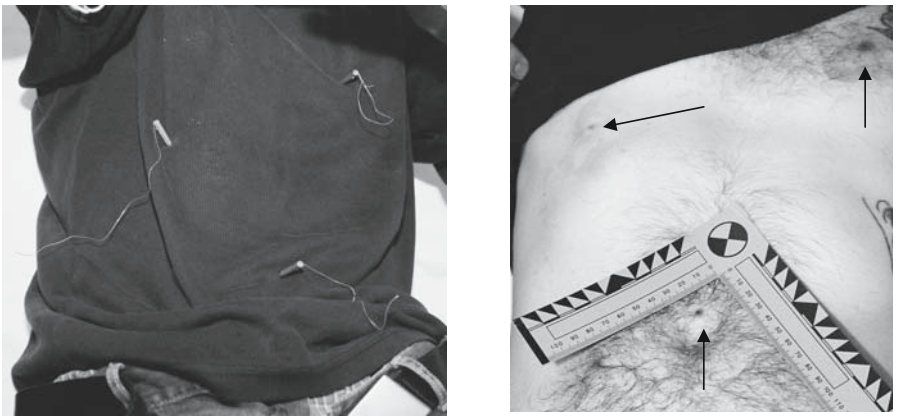
Chemical Methods
- Lacrimation agents and chemical irritants

Sedative drugs

Fig. 7.1. The barb of a Taser with attached wire connected to the gun. (Image courtesy of Dr. J. Bond, Northampton Police, UK.)

Although there is considerable literature related to the safety of the first-generation Taser, reports of fatalities associated with its use are infrequent. In a 1986 study of 218 "tasered" people, the mortality rate was 1.4%, although in each case high levels of phencyclidine (PCP) were detected, which the coroner determined was in fact the cause of death.[4] A later retrospective study investigated 16 deaths and concluded that the Taser may have been a contributory factor in only one in which there was also evidence of PCP use and significant cardiac disease.[5] In a response to this study, one author has asserted that identifying the temporal relationship between Taser use and collapse is important in the analysis of these deaths and raises the possibility of cardiac depolarization effects.[6] However, other authors state that the particular characteristics of the Taser electrical pulse, "akin to rapid fire, low amplitude DC shocks," result in the current staying close to the surface of the body and not interfering with internal organs.[7] The manufacturer claims that the energy is well below the threshold for induction of ventricular fibrillation or interference with cardiac pacemaker function. There appears to be little, if any, independent peer-reviewed literature on the electrical effects of the M26 or X26 Taser on the body.

Injuries caused by the Taser may be the result of barb impact or the effect of an uncontrolled fall following loss of skeletal muscle control, diaphragmatic paralysis, or, theoretically at least, cardiac rhythm disturbance, particularly if there is significant preexisting cardiac pathology. The barbs have the potential for mechanical trauma to vulnerable areas such as the orbital contents, but otherwise the risk of deep penetration is considered low. Ordog et al.[4] reported that greater than 75% of barb strikes were to the chest, abdomen, or lower limbs and 1% were to the face.[4] The barbs may require minor surgical incision for removal. Kornblum and Reddy[5] have described the pathologic appearances of barb penetration sites. The macroscopic findings tend to be minimal and compose small puncture wounds with occasional evidence of surrounding erythema and bruising (Figure 7.2). Histologically, the changes in the area of electrical discharge are those of thermal/electrical



(A) (B)

Fig. 7.2. **A:** Three barbs from a Taser in the clothes of an individual. **B:** The corresponding skin injuries from the Taser. (Image courtesy of Dr. J. Bond, Northampton Police, UK.)

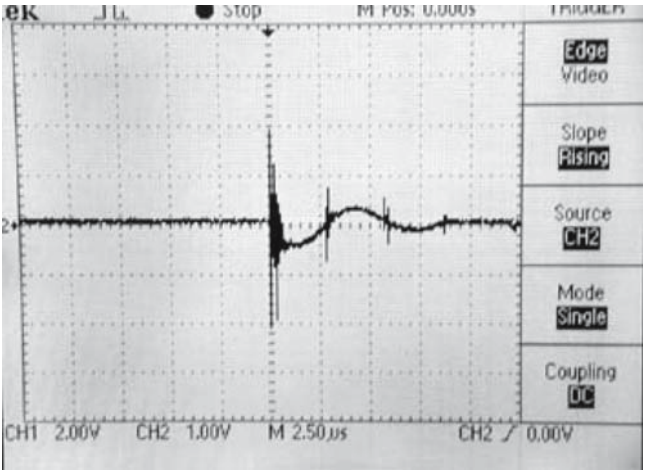
injury, including hyalinization of dermal collagen, hyperpigmentation and polarization of basal epidermal nuclei, and subepidermal blister formation. The cutaneous injury has been described to a depth of approximately 6 mm. Because discharge results in complete loss of motor control, blunt force injuries may be sustained during a fall onto or against hard surfaces, and these collapse-type injuries may need to be distinguished from other inflicted blunt force trauma sustained during attempts at restraint.

Stun guns (Figure 7.3) operate on the same electrical principles as the Taser. In fact, the Taser can be used in the manner of a stun gun when the replaceable cartridge is removed from the front of the device. These guns are designed for close-quarter use by the direct application of electrodes to the skin of an offender, with the potential for cutaneous electrical injuries as described for the Taser.[8]

Lacrimation Chemicals

Lacrimation chemicals commonly take the form of synthetic agents (ortho-chlorobenzylidene malonitrile [CS], 1-chloracetophenone [CN[) or naturally derived substances (oleoresin capsicum [OC]). They are used in the form of liquid or aerosolized sprays delivered to the face and are designed to cause intense pain, blepharospasm, and lacrimation. They are extremely irritating to exposed mucous membranes, and the effects may last for up to 30 minutes. Because of the nature of delivery they are useful only for close-range deployment. According to one study, most were sprayed within 1.5 mm, and the maximal effective range is approximately 3 to 4 m.[9] Concerns exist about their effectiveness in incapacitating people who are particularly violent and focused, although in some instances the ineffectiveness may be related to ineffective delivery (e.g., onto clothing instead of the face). There have been several reports concerning safety issues with these chemicals, with most concerns centered on the potential for bronchospasm and respiratory compromise, particularly in susceptible individuals.[10–12] A further safety concern that has been identified is the

(A)



(B)

Fig. 7.3. **A:** Stun gun discharge. **B:** Oscilloscope representation of stun gun discharge showing brief voltage peak. (Images courtesy of Dr. M. Odell, Victorian Institute of Forensic Medicine, Australia.)

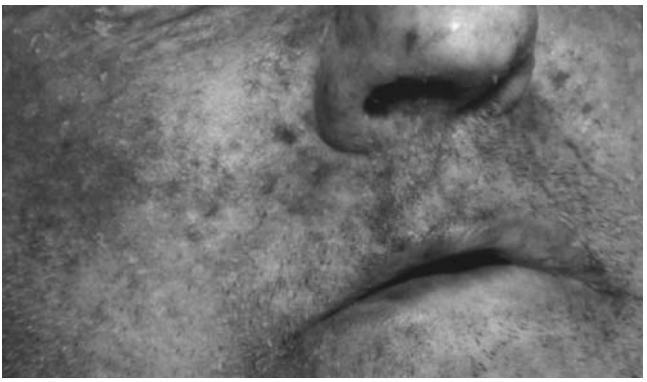


Fig. 7.4. Cutaneous chemical burn injury to the nose and mouth area resulting from use of CS spray. (Image courtesy of Professor C.M. Milroy, Medico-Legal Centre, Sheffield, England.)

potential for the solvent used in these preparations to contaminate clothing and pose a fire risk with subsequent use of a Taser.[13]

The risks of significant respiratory injury appear to increase with higher concentrations of the inspired chemical, particularly in confined, poorly ventilated spaces.[10] The studies on respiratory functioning in people who have been sprayed have been inconclusive, but the effect on respiratory functioning appears to be relatively insignificant. Because OC exposure may result in histamine release from mast cells, there is an identifiable mechanism by which bronchospasm may occur. However, one study showed no differences in the duration and severity of bronchoconstriction among asthmatics, smokers, and controls.[14] Another study showed no significant alterations in respiratory parameters compared with placebo in either the sitting or prone maximal restraint position.[15]

The clinical and pathologic findings depend on the concentration and duration of exposure. Not surprisingly, the eyes are the most commonly affected area. Findings may include erythema, blistering, conjunctival congestion, and corneal edema and ulceration. Methyl isobutyl ketone, the solvent used in CS spray, may also cause cutaneous irritation, erythema, and chemical burn injury (Figure 7.4). Airway findings range from mild bronchorrhea to laryngotracheobronchitis. Pulmonary parenchymal effects appear unlikely when exposure is not prolonged or excessive.

Although some formulations of these sprays may contain fluorescent dyes, identification of the chemical on the skin and clothing of a subject may be problematic and requires specialized techniques. One paper advocates using saline-soaked swabs in the living and methanol-soaked swabs on cadavers.[16]

Baton Rounds and Other Impact Devices

A number of different types of these weapons are in use around the world. They are designed to deliver a blunt object with high speed (and therefore kinetic energy) to a subject, with diminished risk of integument penetration and visceral injury compared with conventional firearm projectiles. They are fired at ranges from approximately 20 to 50 m. These impact devices can be deployed from specially designed firearms or comprise modified cartridges designed for use in standard weapons such as 12-gauge shotguns. Variants that have been tested by the United Kingdom (UK)

police include bean bags (a square or rectangular fabric bag containing lead shot), sock rounds (which are similar to bean bags but have rounded edges), baton rounds (manufactured from foam, rubber, or plastic and varying from cylindrical to slug shaped in profile; the type currently in use in the UK is the L21A1), and so-called ball rounds, which compose one or multiple rubber or plastic balls.[13]

The injuries inflicted vary from nonspecific bruises and abrasions to patterned wounds such as targetoid bruises. Because of the high velocity and energy of some of these devices, there is a risk of cutaneous penetration and/or visceral trauma. The bean bag (which has been used by Australian Police Forces) is designed to open up prior to impact, but in UK studies, some failed to unfold or hit the target edge on. One retrospective study into the bean bag documented penetrating injuries to the eye, chest, abdomen, arm, and leg, as well as blunt injuries to the spleen, liver, and heart.[17]

Conventional Mechanisms (Handcuffs, Batons, and Neck Holds)

Most injuries caused by handcuffs are the result of forceful application, overtightening of the ratchet mechanism, or continued resistance by the offender after the handcuffs have been applied. More pronounced injuries may result if the handcuffs are used to forcefully drag the offender after the handcuffs have been applied (Figure 7.5).

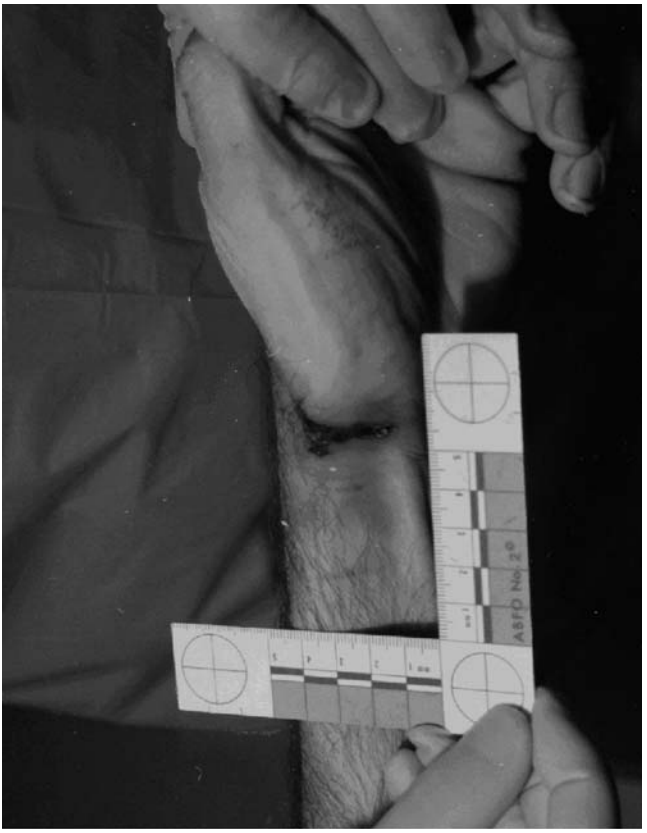


Fig. 7.5. Bruising and abrasion caused by dragging of offender after application of handcuffs.

Cutaneous injuries encompass the spectrum of blunt force injury from erythema and bruising (which may be parallel and linear depending upon which part of the cuff is contacting the skin; Figure 7.6) to abrasions and lacerations. Bony injuries are uncommon. Compression neuropathies have been documented, with the risk increasing with duration of application and the degree of neurologic impairment, as in acute alcohol intoxication.[18]

The cutaneous appearance of baton-related injuries depends upon which part of the baton strikes the offender (nonspecific or targetoid bruises inflicted by the tip, or the classic tramline bruise-abrasion inflicted by the curved profile of the shaft; Figure 7.7). In individuals who are particularly heavily pigmented, these injuries may be inconspicuous, thus reinforcing the need for a low threshold toward subcutaneous dissection. A comparative study between an old-style wooden police baton and a new, expandable (ASP) baton demonstrated that the latter produced approximately twice the impact pressure. Both types were considered capable of inducing skull fracture.[19]

Vanezis[20] and Reay[21] reviewed the various types of neck holds (including the "carotid sleeper" and "choke" holds) and their potential for injury and death. Because forceful manipulation of the neck structures may result in vascular compression, airway obstruction, or carotid sinus stimulation, careful layered neck dissection is mandated in all cases to identify strap muscle bruising and laryngeal skeletal fracture. If circumstances suggest pressure on the neck is a possibility, then consideration should be given to retaining the larynx, obtaining radiographs, and taking multiple sections to identify less obvious injuries that may indicate pressure has been applied, such as subepithelial hemorrhage and intralaryngeal muscular haemorrhage.[22] It should be remembered that artifactual injuries, including laryngeal bruising and petechiae of the face and conjunctiva, can arise because of resuscitative maneuvers such as endotracheal intubation.[23]

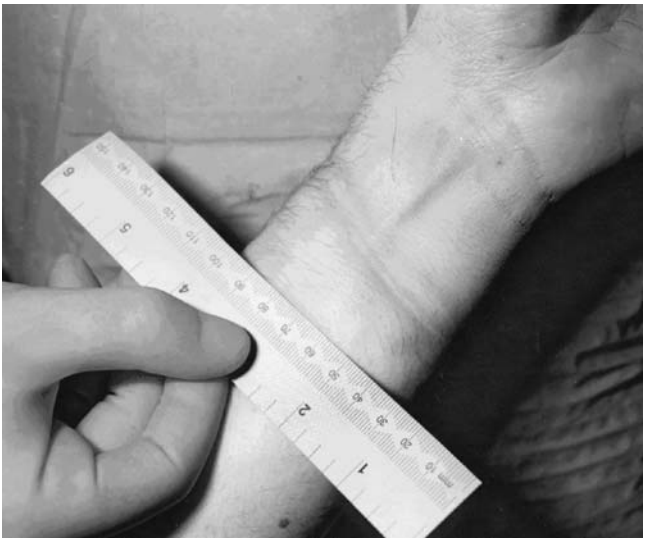


Fig. 7.6. Erythema and intradermal bruising associated with application of police handcuffs.

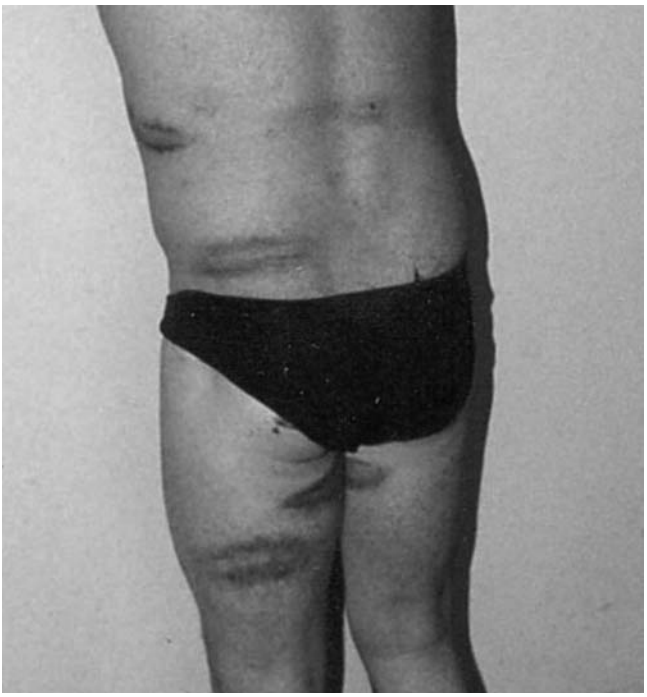


Fig. 7.7. Tramline bruising caused by baton use. (Image courtesy of Dr. M. Odell, Victorian Institute of Forensic Medicine, Australia.)

Other Methods

Sundry other restraint or area-denial modalities have been experimented with or deployed, some of which may be encountered in the setting of civil disturbances or riots. Space prohibits an in-depth discussion of these devices here, but they include various types of explosive disorientation devices (with the potential for visual or auditory injury and blast injury if explosion is proximate), water cannons, Kevlar net rounds, smoke grenades, foam, glue, and grease deployment weapons, and electromagnetic shock wave generators. In addition, two types of physical restraint apparatus have been introduced in the United Kingdom: VIPERS (violent person emergency restraint system) and ERB (emergency restraint belt), both of which are designed to restrict violent movement. Testing of these devices is ongoing.[24]

Deaths Occurring in the Setting of Restraint

Injuries and death occurring in the setting of restraint provide some of the most challenging and contentious cases likely to confront the forensic pathologist, not least because a number of different factors require assessment and weighing in the

balance before conclusions can be reached about the cause and mechanism of death. These factors include the method and duration of restraint, the effects of psychiatric disorders and other natural disease processes, the effects of superadded trauma (particularly to the head), and an understanding of the physiologic processes likely to have been operating at the time. To this list must be added the effects of drugs, although equating postmortem levels with particular outcomes must be undertaken cautiously because of issues of postmortem redistribution, degradation, and individual characteristics of the user.[25] Clearly in many instances, none of these factors occurs independently of the others, and assessment of the relative contribution of each to the outcome can be extremely problematic. Nevertheless, before the pathologic approach to the autopsy and formulation of the cause of death are discussed, it is useful to consider some of these factors individually.

Restraint Asphyxia

The concept of restraint asphyxia as a distinct pathologic entity has not met with universal agreement, but sudden death occurring in the setting of restraint is a scenario not uncommonly encountered by most, if not all, forensic pathologists. Typically a restrained individual is extremely agitated and violent, and a number of people are required to secure the individual in a position such that the risk of violence to bystanders and the individual is minimized or so that some type of restraining device can be applied. The individual usually is held in the prone position with pressure applied to the back of the arms, shoulders, or torso. Variants include hog tying (with the wrists shackled to the ankles) and the "basket hold," where the arms are crossed over under the chest and the wrists are held tightly. A major problem immediately evident with the prone position is that monitoring of the clinical status of the individual during a period of potential vulnerability is significantly impaired. In addition, the arms or hands of the restrainers may be placed across or around the front of the neck, leading to possible vascular occlusion, upper airway constriction, or carotid sinus stimulation. However, asphyxia as a result of pressure on the neck, although a potentially important causal or contributory factor, is not the focus of the present discussion.

The mechanism of death during prone positioning with violent resistance has been ascribed variously to positional asphyxia, mechanical asphyxia, and catecholamine-induced cardiac rhythm disturbance. But if asphyxia (defined as interference with adequate oxygen delivery to peripheral tissues) is the ultimate mechanism, is there evidence supporting this mechanism? Much of the evidence appears to be circumstantial and intuitive, and the matter is further complicated by the observation that some asphyxial deaths (e.g., irrespirable atmospheres and smothering) show little in the way of the so-called classic asphyxial signs: petechiae, cyanosis, congestion, or edema. In fact, one review contends that "no relationship exists between the development of petechiae and the presence of absence of asphyxia."[26] "Burking," the favored method of the Edinburgh murders Burke and Hare, is reprised as evidence that death can occur as a result of a combination of pressure on the chest and smothering. Reports exist of the restrained party complaining of inability to breathe shortly before collapse.[27] Use of the term *positional asphyxia* may not be entirely appropriate because it has connotations of self-induced impairment of respiration, often as a result of alcoholic intoxication. *Mechanical* (or *traumatic*) *asphyxia,* on the other hand, might be more apt but typically is applied to situations where heavy weights crush the chest, as occurs in industrial accidents.

There is a degree of overlap with all the aforementioned entities (and others such as "crush" asphyxia), and each has potential relevance to the situation of prone restraint inasmuch as they entail interference with adequate chest expansion and pulmonary ventilation. One other factor in particular, truncal obesity or protuberant abdominal panniculus, may result in splinting of the diaphragm in the prone position. Despite the number of qualifying terms with varying degrees of applicability, in the view of the author, the relatively stereotypical history of cardiorespiratory arrest during prone restraint is best encapsulated in adoption of the qualifier "restraint," reflecting as it does the mechanics of the particular situation: "compression, restriction and position."[21] But what of other evidence for asphyxia?

One study into prone positioning (with hog tying) showed delayed recovery of oxygen saturations,[28] but these findings have been refuted by other authors who conclude that no clinically significant deteriorations in measurable respiratory variables or recovery time occur as a result of restraint positioning following exercise.[15] Furthermore, a study using the placement of weights to the back showed a restrictive ventilatory pattern but no evidence of hypoxemia or hypoventilation.[29] One problem with extrapolation from these study findings is that they cannot accurately reproduce all of the complex factors operating in the typical restraint scenario, including extreme exhaustion, biochemical and metabolic derangements, cardiorespiratory disease, and drug use. Disappointingly, no comment is made regarding the development of petechiae in the test subjects.

Identification of florid petechiae on the conjunctivae, face, and neck at autopsy is strong evidence of impaired venous return (and, by extension, an "asphyxial" mode of death), which may be the result of prolonged neck or chest compression. However, more commonly, "in contrast to the anatomic findings encountered when neck holds have been used, findings to support the diagnosis of restraint asphyxia can be meagre to non-existent."[21] Compounding these difficulties is the fact that petechiae, particularly if relatively sparse, are nonspecific findings that may result from resuscitation attempts and some natural disease processes.[26,30] Despite the conflicting physiologic studies, intuition suggests it may not be unreasonable to postulate that extreme, prolonged physical exertion could result in a degree of hypoxemia, lactic acidosis, catecholamine surge, and hyperkalemia to which restricted ventilation is added, resulting in a potentially lethal combination alone or in association with other factors such as cardiovascular disease. Parenthetically, critical cardiac disease may not be evident macroscopically, thus reinforcing the need for thorough histologic examination of the heart to identify conditions such as small vessel disease.[31,32] Genetically determined vulnerability also may be important,[33] and in this respect obtaining the family history is clearly indicated. Whether the confluence of all these factors justifies the term *asphyxia* has implications for how a case is certified, particularly if the cause of the agitation is a condition such as cocaine-induced excited delirium, where the high mortality of the condition itself makes problematic the assessment of other possibly contributory factors such as the act of restraint.[16]

Excited Delirium

Variously referred to over the years as lethal catatonia, acute exhaustive mania (described by Lewis Bell in 1849), delirious mania, and agitated delirium, excited delirium is now the commonly accepted name for a relatively stereotypical constellation of psychomotor signs associated with high mortality.[34] The cause may be related to an underlying psychiatric illness or ingestion of stimulant drugs such as

methamphetamine or cocaine. However, when associated with cocaine the levels detected tend to be low to moderate, that is, not dissimilar to levels detected in recreational cocaine users.[35] Four phases apparently occur in sequence, usually over a time frame of less than 6 hours[36]: hyperthermia, delirium/agitation, respiratory arrest, and death.

The violent and bizarre behavior associated with this disorder often results in police attendance with subsequent attempts at restraint. When death occurs during such attempts, the inevitable questions arise as to the degree to which the restraint, the injuries resulting from the violent behavior, or the condition itself contributed to the mechanism of death. Similarities have been noted between excited delirium and neuroleptic malignant syndrome, leading some authors to propose that the processes are related to similar abnormalities of dopamine receptors. However, Karch[16] suggests that different processes are responsible because excited delirium can occur in schizophrenic patients and in those with bipolar effective disorder, even when they are not taking dopaminergic agents. It is interesting to note that some of the features of neuroleptic malignant syndrome, including altered consciousness, sweating, hyperthermia, and autonomic instability, overlap with the serotonin syndrome.[37] Abnormalities of dopamine receptors in the brains of cocaine abusers who develop excited delirium are thought to be the basis of the psychotic behavior (no increased cocaine recognition sites on the striatal dopamine transporter), and hyperthermia (depletion of hypothalamic D_2 receptors). An increase in κ_2 opioid receptors in the amygdala may explain the psychotic symptoms and paranoia seen in some of these individuals.[16]

Complicating the pathologic assessment of these cases is the fact that stimulant abuse may lead to significant cardiovascular abnormalities. Because cocaine blocks the presynaptic reuptake of dopamine and noradrenaline, it acts as a sympathomimetic and vasopressive agent. It is possible that cocaine use sensitizes the myocardium to the effects of endogenous catecholamines, which become elevated at times of high emotional or physical stress. Other reported cardiovascular complications include intracerebral hemorrhage and acute aortic dissection.[38]

Cocaine and other stimulants may be associated with a number of arrhythmia types (ranging from relatively benign supraventricular tachyarrhythmias to ventricular fibrillation and asystole), left ventricular hypertrophy with systolic dysfunction, coronary artery spasm (and angiogram-negative acute myocardial infarction), accelerated coronary arteriosclerosis (small and large vessel disease), and endocarditis (with injecting use).[39,40] Histologically the myocardium may show evidence of contraction band change and small foci of fibrosis.[16]

Schizophrenia and Neuroleptic Drugs

An association with excited delirium is but one manifestation of the increased mortality associated with schizophrenia. Patients with this often devastating disorder suffer an increased incidence of natural disease, particularly cardiovascular disorders, as well as deaths by unnatural means (including suicide and accidents).[41,42] However, attention has increasingly focused upon abnormalities of cardiac rhythm, particularly prolongation of the QT interval. Several antipsychotic agents (phenothiazines, haloperidol, and clozapine) have been implicated, as well as tricyclic antidepressants and other drugs including some antihistamines and antibiotics.

Antipsychotic drugs may exert a quinidine-like effect of causing sodium channel blockade and a delay in ventricular repolarization.[43] Newer antipsychotic agents

such as olanzapine are reputedly less likely to be associated with this phenomenon at therapeutic doses. A number of other factors, such as electrolyte imbalance (particularly hypokalemia and hypomagnesemia), chronic liver disease, and genetically determined abnormalities of cardiac ion channels, also have implicated.[44] These factors theoretically could act alone or increase cardiac vulnerability to the superadded effects of the drugs. Although antipsychotic drugs may be associated with abnormalities of the autonomic nervous system, Rosh et al.[42] have suggested that schizophrenia itself (in the absence of neuroleptic drugs) may be associated with autonomic dysfunction causing increased resting heart rates and lower heart rate variability, thus providing a possible explanation for sudden unexpected death where the autopsy and ancillary investigations are negative. A similar mechanism, that of autonomic dysfunction, has been proposed for sudden unexpected deaths in some diabetic patients.[45]

In addition to the possible deleterious cardiac effects of neuroleptic drugs, infrequent reports have linked haloperidol use (particularly in young males) with a localized extrapyramidal reaction, namely, acute laryngeal dystonia.[46] Typically these cases have been of relatively sudden onset, arising within a few days of haloperidol therapy in which anticholinergic agents have not been used. The symptoms include stridor and a hoarse voice, presumably because of laryngospasm, and resolve rapidly with appropriate therapy. No autopsy findings have been reported for these cases, but the potential for lethal upper airways compromise with little in the way of subsequent findings is obvious.

Pathologic Investigation of Restraint-Related Death

The pathologist must assess several issues, including the following:

- Contribution of drugs (both licit and illicit), including drug and alcohol withdrawal
- Contribution of preexisting natural disease processes, both physical and psychiatric; family history may be relevant
- Contribution of excited delirium or related disorders
- Precise details of the restraint: timing, body position, areas of body contacted, etc.
- Timing and description of circumstances preceding collapse or cardiorespiratory arrest
- Causation of any injuries identified at autopsy (i.e., consequent to the violent behavior prior to or during restraint)
- Contribution of any injuries (particularly to the head) to the outcome
- Identification of occult injuries
- Identification of features potentially attributable to resuscitation endeavors (e.g., petechiae or laryngeal bruising) or agonal events (e.g., aspiration of vomitus)

Scene and Preliminaries to Autopsy

Several authors have emphasized the vital importance of maximizing the information available to the pathologist so that meaningful interpretation of subsequent autopsy findings can be attempted. Ideally this should include visiting the scene to

note possible causes of blunt or sharp force trauma, evidence of hyperthermia (towels, discarded clothing), patterns of blood staining, ambient temperatures, and restraint devices used. Reenactments may also be used at a later date.[47] If the body is still in situ at the scene, then core temperatures should be taken at that time. In addition to the information available from the scene, attempts should be made to obtain all relevant medical records, including lists of prescribed medications.

The Autopsy

External

These cases should be treated as potential homicides when the cause of death likely is to be unascertained following autopsy and as such warrant as comprehensive and thorough an approach from the outset as circumstances and facilities allow. The following is a suggested protocol, adopted with revisions from the Police Complaints Authority.[48]

Consideration should be given to moving the body to a better equipped mortuary if the facilities are considered suboptimal. The autopsy should be performed as rapidly as possible after death.

- Radiologic examination of the body is advisable if facilities are available
- Full photography
- Height, weight, and description of body build
- Documentation of the type and state of clothing
- Core temperature measurement (if not performed at the scene, then as soon as practicable afterward and certainly before refrigeration of the body); record the time taken and the ambient temperature
- External examination with documentation of injuries and distinguishing characteristics (e.g., abdominal obesity, new and old injection sites, stigmata of natural disease processes)
- Collection of trace evidence if appropriate, including nail scrapings and anogenital swabs; consider specific swabs for lacrimation agents if they were used
- Collection of other specimens pertaining to possible recent or long-standing drug use (nasal swabs, plucked head hair, fingernails)

Internal

- Layered neck dissection (include dissection of posterior cervical musculature)
- Subcutaneous dissection of limbs, back, and face
- Routine internal examination including documentation of injuries, visceral petechiae, and natural disease processes
- Weigh all organs; use standardized heart weight comparison tables to assess degree of cardiomegaly[49]

Ancillary

- Routine histologic sampling of all organs
- Consider retention of heart and brain for expert examination; if the heart is not retained, remove cardiac conduction system (atrioventricular node area) for fixation and later dissection

- Full toxicology: include vitreous humor (for biochemistry), blood (preserved and plain, central and peripheral specimens; the latter ideally from the femoral vein), urine, and entire stomach contents
- Consider freezing 20 g of brain white matter and specimens of skeletal and cardiac muscle
- Blood in an ethylenediaminetetraacetic acid (EDTA) tube for possible gene analysis; a Guthrie (dried blood spot) card may be useful
- Blood or muscle for deoxyribonucleic acid (DNA) determination
- Other specimens (e.g., microbiology) as indicated

Determination of the Cause of Death

Consider the following scenario.

> A 49-year-old chronic schizophrenic male became acutely agitated and extremely violent. His past history included alcohol abuse and cigarette smoking, and his medications included clozapine. There was no other significant medical history. He was restrained in the prone position by four carers and held in this position for a period of 15 minutes, all the while demonstrating persistent, strenuous resistance. His forehead was noted to contact the floor "firmly" several times prior to and during the restraint. He was noted to suddenly go limp and was found to be in cardiorespiratory arrest. Resuscitation attempts were made by the carers and ambulance staff, but he was declared dead at the scene.

At autopsy he was noted to have an obese abdomen. Scattered, sparse conjunctival petechiae were observed (no other facial petechiae identified). Cutaneous injuries included bruising and abrasions to the forehead and further discoid bruises to the upper arms. Internal examination showed mild single vessel coronary artery disease and mild pulmonary emphysema. There was no evidence of skull fracture. Thin-film, liquid subdural hemorrhage was identified over the cerebral convexities, but there was no space-occupying hematoma. The brain was mildly swollen and heavy, but there were no cortical contusions or evidence of internal herniation. Subcutaneous dissection showed bruising corresponding to the areas of externally evident injury but no other significant findings. Toxicologic analysis showed therapeutic levels of clozapine.

Arriving at a conclusion about the cause of death may be a very difficult and contentious undertaking. The philosophical and pathologic approach ultimately adopted no doubt will be informed by the individual pathologist's experience and equanimity and the particular characteristics of the case. Clearly, if the autopsy reveals florid asphyxial signs and injuries suggestive of a particular etiology, such as neck injuries or back bruising, then a strong argument exists for ascribing death to these mechanisms, that is, neck compression or restraint asphyxia, respectively. More typically, as in this case, the autopsy findings are minimal or nonspecific, and consideration must be given to all the identifiable factors (circumstantial and pathologic) that may *reasonably* be argued to have *significantly* contributed to the death. Although attempting to single out one factor of all those operating at the time would be subjective and potentially misleading, the indiscriminate grouping of multiple factors under part 1a of the death certificate most probably is just as unsatisfactory and unhelpful. One approach might be to leave the cause as Undetermined or even Unascertainable, indicating that one cannot be certain as to what exactly happened.[20] On the facts of the case described, there is no obvious patho-

logic (physical) or toxicologic cause for the death, so this approach may not be unreasonable. However, what *is* known can be summarized as follows:

- The deceased was extremely agitated and struggling forcefully for a prolonged period of time.
- Prone restraint was used.
- There was a temporal relationship between the restraint and death.
- The deceased had a protuberant abdomen that likely impeded ventilation in the prone position.
- Only small numbers of conjunctival petechiae of uncertain significance *(vide supra)* were found.
- The deceased suffered from schizophrenia and was receiving neuroleptic medication, both of which may be associated with cardiac rhythm disturbance.
- There was evidence of relatively minor cardiorespiratory disease and head injury.

Of course, much more information needs to be evaluated, including the precise details of the restraint, the timing of collapse, any evidence of hyperthermia, the results of any previous electrocardiographic examinations, the possible presence of hypersensitivity myocarditis, biochemistry results, consideration of the heart weight (i.e., whether it is abnormally elevated for a male of the deceased's height and weight[49]), and examination of the cardiac conduction system. The mild coronary and respiratory disease are unlikely to have been significantly contributory factors, and although the head injuries appear minor and in all likelihood inconsequential, careful examination of the brain is warranted to refute later assertions of contribution.[40]

A number of factors have, however, been identified in this case that are likely to be of relevance to the outcome. Thus an alternate approach would be to group these factors in a narrative format, as follows: Cardiorespiratory arrest in the setting of prone restraint of an agitated schizophrenic male treated with clozapine. Abdominal obesity also might be cited as a contributory factor. This method has been favored by some authors.[50,51] Although an argument could be made that it is too broad in scope, it does have the advantage of acknowledging that although a number of factors could reasonably be considered to have conspired to cause death, the circumstances, pathologic findings, and current state of medical knowledge does not allow one to be given preeminence over the others.[51]

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8. The Timing of Death

Benjamin Swift

Introduction

Death is inevitable, although it occurrence is unpredictable. Decedents are often discovered at intervals of variable length after the actual event, depending upon the mode of death and the location of death and social factors, such as the geographical and psychological proximity of relatives. Despite the almost supernatural abilities of fictional pathologists, the capacity to estimate the time of death remains limited in forensic practice. In the absence of documentary evidence of fatal collapse, such as closed-circuit television surveillance footage, investigators must rely upon additional information derived from three main sources:

- Evidence provided by the body itself,
- Environmental and artifact-based evidence, which is evidence in association with a body, and
- Anamnestic evidence, which is based upon the knowledge of the individual's movements and day-to-day activities.[1]

Although the majority of police investigations rely upon the latter two sources, the literature is constantly expanded by methods extolling the vast number of identifiable alterations that may occur during the postmortem interval (PMI), to which a temporal value for the time since death can be ascribed.

Requirement for a Dating System

"When did they die?" continues to be one of the major questions during a death investigation and is one of the main questions posed during an inquest into the fatal events presided over by Her Majesty's Coroner.[2] Therefore the chronologic dating of events is of great importance to forensic investigators (as with ancillary scientific studies such as geology and archaeology), that is, the ability to create a theoretical timeline upon which the social interactions and events of a person's last days assists in either eliminating or incriminating others from an inquiry.

Of the methods published, the majority can be divided into two main categories: those occurring within the early postmortem period and those during the late

postmortem period. The arbitrarily defined early postmortem period relates to the soft tissue phase of decomposition, whereas the late period refers to the phase of skeletalization and subsequent taphonomic alterations to the bony matrix itself.

Early Postmortem Interval

The literature relating to PMI estimation within the soft tissue phase of decomposition is extensive, being constantly updated each year with novel or adapted techniques. Although it is not practical to discuss all methodologies within this chapter, reference is made to dedicated texts where in-depth information can be found.[3]

Algor Mortis

Algor mortis relates to the postmortem cooling of a body and relies upon the knowledge of a steady-state core body temperature during the antemortem period, presumed to be approximately 37°C. Davey's experimentations with cadavers in 1839 resulted in one of the first scientific investigations into the issues of dating time since death.[4] The basis lay in Newton's Law of Cooling, which states that for a body cooling by forced convection the rate of heat loss is proportional to the differences in temperatures between the body and its surroundings. This law, however, is applicable only to regular inorganic spherical objects. Although it formed the basis for much early research, the law fails to account for the irregularity of the human form, the effects of clothing, ventilation, or physical positioning of the cadaver. In fact, during Davey's study while in British-occupied Malta, the high ambient temperature frequently resulted in the temperature initially *rising* in cadavers during the early postmortem period.

The anatomical sites from which temperature readings are taken vary, the principal locations being the brain, skin surface, nasal cavity, axillae, rectum, and individual internal organs, as cooling occurs at different rates for each site.[3] Given the variables relating to the human body, experimentation has confirmed that Newton's law fails to hold. Rainy first described a recognizable anomaly in 1868, referred to as the lag phase or plateau.[5] Rather than the expected single exponential curve of temperature loss, a sigmoid-shaped curve is produced. With the lag phase varying between 30 minutes and 3 hours in duration, another unknown is factored into any such assessment of time since death. The causation remains unknown but indicates either continued heat production as a result of anaerobic metabolism or the prevention of heat loss from tissues, possibly through the insulating effects of clothing or bed linen.

Further research has even suggested the presence of double or triple exponential curves, depending upon the anatomical site of reading. In order to overcome this anomaly, algorithms and nomograms particular to each anatomical site examined have been developed.[6–10] The most frequently referenced nomogram was described by Henssge et al.[3] for use with rectal temperatures.

The assumption that the individual's core temperature is at a "normal" level at death also may not hold true. Stress, fevers, cold exposure, metabolic disorders (e.g., thyroid disease), peripheral vascular disease, and the individual's age all could affect the temperature in the antemortem period.

The cooling rate in a nonexperimental environment also is dependent upon on numerous factors, such as the variation in ambient temperature over the time frame

that the body has lain exposed, the presence of wind passing over the body, precipitation, body posture (as a result of altered surface area exposed), the presence (or absence) of clothing, and the body mass index of the deceased.[3]

More recent advances have utilized infrared digital thermometers placed within the external auditory canal. The underlying concept is that, in life, the internal carotid artery perfuses the tympanic membrane; thus the temperature is identical to the core body temperature. The results indicate that single temperature measurements recorded from this site can provide accurate methods of estimating the time since death up to 16 hours postmortem when used in collaboration with a standard algorithm such as Henssge's nomogram for the brain.[11] Also of note, unlike other sites where double or triple exponential cooling curves resultant from lag phases are well documented, there appears to be no lag phase associated with estimations based on the external auditory canal, resulting in a single exponential curve.[11,12] External auditory canal-based estimations are, however, affected by head position, the presence of wind passing over the cadaver, and natural circadian temperature alterations during the antemortem phase.[11]

Rigor Mortis

Rigor mortis, or postmortem rigidity, commences after a 3- to 6-hour period of muscular flaccidity at death and lasts up to 36 hours, after which it diminishes.[13]

Ultrastructurally, skeletal muscle is composed of two main components: actin and myosin. The interaction between the two proteins produces contraction of individual sarcomeres, resulting in the contraction of muscles in their entirety. The release of the contraction, and hence the commencement of muscular relaxation, is a process that is dependent upon the concomitant binding of adenosine triphosphate (ATP). The subsequent hydrolysis into adenosine diphosphate (ADP), via an ATPase, releases energy that frees the actin–myosin protein complex.[3] The ATP is resynthesized to allow subsequent contractions, with energy for this chemical conversion originating from glycogen. After death, glycogen resources are rapidly depleted, thus preventing the energy-dependent "breaking" of sarcomere contractions and resulting in rigor mortis. Although previously disputed, true sarcomere shortening similar to antemortem muscle contraction has now been proven to cause rigor mortis.[14–16] As autolysis commences and the ultrastructural cellular components lose their integrity, the rigor is "released".

In 1811 the first descriptive study was reported by Nysten, who considered that rigor occurs predictably first within the temporomandibular joint passing inferiorly to the limbs in a descending and ordered manner. Although it was assumed that rigor progresses simultaneously in all muscles, the causation of the apparent sequence was suggested to result from the difference in muscle volume at these sites.[17,18] However, later work has disproved this theory, instead explaining the difference through the varying proportion of red and white fibers in individual muscles, the dynamic characteristics of each joint (e.g., the elbow is inherently more mobile than the jaw), and the differences in temperature of each muscle.[14]

By recognizing the status of rigor mortis within a cadaver, estimates for time since death can be provided. Unfortunately, many factors affect the onset of rigor, notably the temperature of the environment, the degree of muscular activity prior to death, and the age of the deceased.[13] Therefore, despite numerous publications claiming to demonstrate accurate estimation of the PMI based solely upon the nonsubjective assessment of rigor mortis, little progress has occurred in the field.

Livor Mortis

Livor mortis, or hypostasis, describes the red-blue hue created in the absence of a cardiovascular circulation by the gravity-dependent accumulation of blood within small cutaneous or visceral vessels. The spectrum of coloration is great, depending not only on premortem conditions but also on the time since death.[13] Attempts have been made to divide lividity into categories: (1) beginning, (2) confluence, (3) maximum intensity, (4) slight pressure displacement, (5) complete shifting, and (6) incomplete shifting.

It is through the recognition of color changes that quantitative analysis can assist in PMI estimations. Working independently, Kaatsch et al.[19] and Bohnert et al.[20] examined photometric measurement of livor mortis as a means of assessing PMI, with similar work by Vanezis and Trujillo[21] using a tristimulus colorimeter measuring system, although intervals of only up to 48 hours could be estimated with confidence. Indeed, the rate of occurrence, as well as the coloration, distribution, and possible redistribution of hypostasis, is so variable that its use may remain purely within the realm of experimental trials, being unsuitable for the intense scrutiny of medicolegal cases.

Morphologic Changes

Although the recognition of morphologic changes requires less specialized means of assessment, such methods remain heavily reliant upon the abilities and experience of the individual assessing the changes. The timing of decompositional changes may assist in narrowing the PMI estimate but is fraught with variations, many based upon unknown factors such as the health of the deceased prior to death, the ambient environment (including temperature and humidity), the effects of pharmacologic agents, the extent of animal activity, and the presence of perimortem trauma.[22]

Of these factors, the environmental conditions in which the deceased laid are the most important. Although animal-based studies continue, little work using human bodies has been performed. Published work tends to focus upon the Decay Research Facility in Knoxville, Tennessee. Although this small center remains the nidus of much research, the results cannot be applied internationally and may even vary within the same locality.[23,24] Reflecting upon experience within the United Kingdom, only rough indicators of postmortem changes related to time since death can be produced (Table 8.1). Caution is advised, however, in giving any definite time since death estimation based solely upon such descriptive findings.

Table 8.1. Descriptive alteration to bodies in the early postmortem interval.[11]

Days	Decompositional changes to the external surface
1	Green staining of abdomen and flanks
2–3	Initiation of bloating
3–4	"Marbling" of the skin
5–6	Gaseous expansion, skin slippage, "bleb" formation
14	Marked body swelling
21	Vesicles burst, eyes expand, tissues soften
28	Extensive skin liquefaction and blackening

(Source: Rutty GN. The use of temperatures recorded in the external auditory canal in the estimation of the time since death. Medical Doctorate Thesis, University of Sheffield, 2001.)

A more specialized morphologic method, although only useful for premenopausal women, requires the estimation of menstrual cycle based upon the histologic changes within the endometrium.[25] This method is also reliant upon a regular, uninterrupted cycle of known commencement (based upon witness descriptions) and the absence of autolysis that would limit recognition of the uterine phase.

Muscle Excitability

Professor Luigi Galvani first established the ability of isolated muscle groups to contract under external electrical stimulation during experimentation with frog legs in the 1780s. As the principles became understood the technique was refined, resulting in numerous publications on the subject of postmortem muscular excitability. By subjectively assessing the degree of contraction of specific muscle groups during electrical stimulation, it has been suggested that rough estimates of the PMI can be reached.[3,26,27]

Gastric Contents Emptying

This method is of limited value, being based upon the assumption that gastric clearing of ingested contents occurs at a predictable rate. Therefore, in the presence of a known time for the last meal, the postprandial period subsequently elapsed can be estimated, providing a time of death.[28] However, these estimates have many confounding variables and are dependent upon not only the type of food ingested but also the physiologic and psychological status of the deceased in the period prior to death.

Ophthalmologic Changes

Numerous methods for analyzing the eye have been suggested, ranging from tonometry (measurement of intraocular pressure), which may assist only up to 6 hours after the death of the individual, to direct visualization of the contents of the retinal blood vessels via an ophthalmoscope.[11] The latter requires the observer's recognition of "trucking," the situation in which the blood separates into distinct units. However, this feature has been noted within 15 minutes of death, rendering it of limited value.[11,13]

Reflex contraction of the iris may persist in the early postmortem period in response to the application of an electrical charge or pharmaceutical stimulation. Localized injection of catecholamine solutions may induce this reflex up to 46 hours following clinical death,[13] although electrical stimulation beyond this time frame may result in asymmetrical "drawing out" of the iris toward the electrodes, as only select muscle fibers react.

Assessment of the chemical constituents of the eye are considered in the following.

Biochemical and Hematologic Changes

Postmortem blood samples are notoriously difficult to assess because of the redistribution of electrolytes and chemicals dissolved within, the loss of cellular integrity, and the absence of energy-dependent transmembranous transportation. The extensive animal-based work of Querido[29–31] has been followed-up in human cadavers by

Singh et al.,[32] who demonstrated a means of PMI estimation based upon alterations in the serum concentration of potassium and sodium, although external influences continue to affect its accuracy. Later work by the same author also aimed to demonstrate a double logarithmic linear relationship between plasma chloride concentration and time since death, although numerous intrinsic and extrinsic factors appear to alter these findings significantly.[33]

The blood cells themselves may also assist, with morphologic alterations of leukocytes described in the early postmortem period by standard histochemical staining techniques.[34] Eosinophils, neutrophils, and monocytes displayed the earliest recognizable pyknotic degenerative changes (at 6 hours), with lymphocytes becoming altered after 24 hours. Use of this technique appears limited up to 120 hours postmortem. However, these changes are subjective and therefore open to interobserver variation. The study was biased in that none of the tests were performed "blind" to the PMI.

The history of analyzing vitreous humor chemistry dates back more than 40 years and remains subject to controversy.[3,13,35–37] The basic principle underlying the test is that the vitreous humor within the eyeball forms a "closed environment" separate from the rest of the body, although it still may be influenced by ambient temperatures.[13] Therefore, the postmortem biochemical changes of the contents may assist, if found to be predictable in their alterations, in estimating the time since death. The most investigated is the potassium concentration, although the hypothesis is somewhat reliant on premortem data, which may be limited given the difficulty with which such biochemical information can be obtained from healthy living patients. Studies have suggested that concentrations increase as potassium leeches out of intraocular cells because of a loss of energy-dependent transmembranous transportation, creating an exponential increase. There are also suggestions that interhumoral differences exist within the eyes of the same individual, although a study of 24 individuals found these differences occurred significantly in only one case.[38] The possibility of intrahumoral pathology producing spurious results also requires consideration, as variations in concentrations throughout the vitreous have been reported.[3]

The subsequent analytical methods described have become increasingly technical, including the creation of an artificial neural network in collaboration with capillary zone electrophoresis to assess vitreous chemistry that may improve the predictive value of the test.[37]

Additional studies of vitreous humor have investigated the concentrations of zinc and nickel, although these experiments were within an animal model and no follow-up has confirmed its use in human subjects to date.[35]

A similar method has been advocated for both cerebrospinal fluid (CSF) and synovial fluid biochemistry. Like vitreous humor, CSF and synovial fluid reside within closed environments.[3,39] Alterations in potassium, glucose, and lactate concentrations progress in a similar manner.[3] A methodology involving synovial fluid may be of benefit in cases where severe trauma, heat exposure, or decomposition has destroyed the integrity of the globes. However, synovial fluid itself is more viscous, rendering analysis more difficult.[39]

Additional studies have analyzed bone material for total lipids, proteins, triglycerides, and free fatty acids, although only the logarithmic values of protein and triglyceride concentrations correlated to the time since death over a 20-year period.[40]

Biochemistry has been suggested in a novel method of analysis. As early postmortem decomposition progresses, the breakdown products leak from the corpse

into the soil, where concentrations of volatile fatty acids and specific cations or anions may be detected.[41,42] Unlike previous studies, Vass' method involves the application of the principles of "accumulated degree-days," devised by entomologists to account for fluctuations in ambient temperature, to the recorded chemical concentrations. The work fails to address several points, such as the effect of altered pH, the difference in soils, exposure to different conditions of burial, or environmental aspects such as temperature alterations, wind exposure, or the presence of excess water. Although results have been encouraging, the test remains experimental and only applicable to human remains present upon or within the soils of the University of Tennessee's Decay Research Facility in Knoxville.

Additional research within this field is predicted with the identification of the location of murders possible through the recognition of blood products within soils, even in the absence of the body. Such chemicals have been shown to be present up to 10 years after death (Hugh Tuller, Personal Communication, International Commission on Missing Persons 2003).

Molecular Techniques

Molecular techniques may provide information to assist in early PMI estimation. The molecular stability of calmodulin-binding proteins was assessed on rat skeletal muscle through the use of autoradiography.[43] Lung calmodulin content was also measured by Immunoblot analyses. The results indicated a steady concentration of Ca^{2+}/calmodulin-dependent kinase II over a 96-hour time frame while additional binding proteins underwent alteration, possibly indicating future use over such short periods of time.

Similarly cardiac troponin I, a protein involved in the stimulation of muscle contraction, has been examined through denaturing gel electrophoresis and Western blot via specific monoclonal antibodies.[44] The results show a correlation between cardiac troponin I degradation and the log of time elapsed since death, which, when assessed against a standard reference material, provides an estimate of PMI but only over periods up to 5 days.

Immunohistochemical staining for thyroglobulin has been proposed, with a positive reaction described up to 13 days postmortem, although this would be dependent upon the type of antibody used, the technical capabilities of the laboratory, the thyroidal status of the deceased during the antemortem phase, and the subjective opinion of the observer.[45]

Deoxyribonucleic Acid and Ultrastructural Changes

Following cessation of adequate perfusion and the onset of cellular death, the ultrastructural components of cells undergo autolytic alteration. Nuclear deoxyribonucleic acid (nDNA) degenerates into discrete fragmentary lengths, with their molecular weight altering as time progresses. The theory follows that assessment of DNA denaturation, by means such as hybridization probe analysis, could provide a method of early PMI assessment.[46–51] Similarly, messenger ribonucleic acid (mRNA) degradation has been reviewed with success.[52]

Flow cytometry may produce an alternative means of quantifying this degradation and has been applied to both human splenic samples and dental pulp tissue, although with somewhat contradictory results.[47,49,53]

Although the ultrastructural degradation process does not appear to be individual specific, the rate, like other methods, appears to be dependent upon ambient temperature and humidity.[46]

Entomologic Methods

Tzou Sung[54] first described the forensic application of entomology in the 13th century. Bridging the transition between the early and late postmortem periods, the study of insect life cycles may assist through two main approaches: (1) recognition of the time-dependent maturation of blowfly (Calliphoridae spp.) larvae, and (2) recognition of species succession over an inferred time frame.[55,56]

Blowflies, such as the commonly encountered green and bluebottle flies, have a predictable life cycle, passing through larval maturation phases known as instars prior to pupation and subsequent emergence as an adult fly. Likewise the faunal succession by additional species, such as beetles or spiders, which feed upon the primary insect species present, provides additional means of estimation through the knowledge of the average period elapsed for each species to enter the region of the corpse. All such assessments require through sampling and the assistance of a forensic entomologist (Figure 8.1).

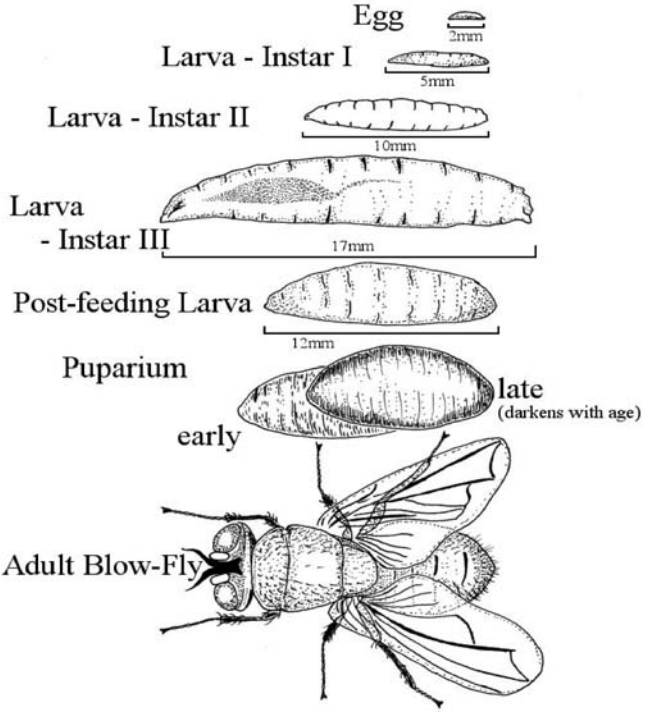

Fig. 8.1. Illustration depicting the life cycle of the blowfly. The time interval between each stage is dependent upon ambient temperature and is species specific, thus requiring expert entomologic opinion when assessing the postmortem interval.

The periods of time for both of these elements vary depending upon temperature, humidity, manner of death, and even the presence or absence of narcotic drugs within the body. The use of "accumulated degree-days" calculations assists by diminishing the effects of the ambient temperature, although expert advice is always recommended to provide accurate PMI estimations. The principle is that the product of the average daily ambient temperature against the number of days exposed allows evaluation between two different environments. For example, the maturation of a population of insects after 10 days at 10°C will be the same as a population after 20 days at 5°C.

Late Postmortem Interval

When decomposition has advanced, entering the late PMI and resulting in only skeletal elements, dating of the PMI becomes much more difficult. Few methods have published over the years but include use of plant growth and the changes within the remaining skeletal matrix.

Botanical

Palynology (the study of pollen) and botany can assist in forensic investigations.[57] By recognizing pollen species adherent to articles of clothing or parts of the body, seasonal exposure can be indicated, especially within interred remains. Alternatively, rootlet infiltration of remains can similarly aid in estimation through the counting of concentric annual growth rings within perennial plants, similar to that used for dendrochronology. Such methods allow assessment of periods of growth and subsequent dormancy by measuring the cell sizes between each ring, with larger cells indicating a period of increased growth.[58] However, growth ring sizes vary between the same species plants, and irregular growth may introduce eccentric rings that produce complications in time assessment. Exposure of rootlets to light, as may occur following the creation of a so-called "shallow grave," also inhibits plant growth.[58] Unlike perennial plants, annual plants do not produce growth rings and, as such, their association with human remains indicates only that the deceased was present before the start of that plant's season of growth.

Although many case studies have been cited where botanical studies assisted criminal investigations, these methods rarely provide accurate answers. Growth rings only indicate the minimal time since death, although such techniques may suggest a season or year in which the individual died.[58]

Bone-Specific Methods

To compound the problem of PMI estimations within the late period, human skeletal remains are regarded as separated into two distinct groups: those of "forensic interest" and those of "historical interest."

The rather arbitrary difference between these two groupings is the PMI, itself the very point under investigation; for this, a period of 75 years PMI is taken as the cutoff between the two groups. Police forces within the United Kingdom consider that if a criminal act has been perpetrated and attempts were made to conceal a body, then it remains feasible that the individual(s) who committed the crime may still be alive up to 75 years after the fact. The skeletal remains of those who died

after the 75-year time frame are thus considered "historical," as the chances of convicting anyone diminishes greatly beyond this point. Therefore, for any such PMI dating method to be of forensic use, it must reliably and accurately differentiate between these two categories, and herein lies the problem.

The method most commonly used by pathologists previously was based purely upon the morphologic appearances of the bone. Many authors claim that such an assessment continues to rely heavily upon the experience of the individual examining the bones. Yet unless the individual is identified by additional means, such as DNA extraction or by the presence of corroboratory evidence interred with the remains, these investigators will fail to learn from their mistakes.[13,59,60]

Morphologic Appearance

As in the early PMI, during which the soft tissue decays, the appearance of the skeleton alters as time progresses. Within temperate climates such as that of the United Kingdom, skeletalization is expected within 5 to 8 years in dry soil conditions, being dependent upon the surrounding environment.[13] Following the decomposition of the skin and subcutaneous tissue, muscles that originate from or attach to the underlying bone begin to decompose. The rate is somewhat delayed compared to the former tissues because of the lack of inherent exposure to microorganisms, unlike the skin or gastrointestinal tract.

The periosteum and dense connective tissues that are rich in collagen, such as tendons, decompose later and often remain closely adherent for many months or even years after death. The presence of a constant air draught passing over the remains within an enclosed space may, although not invariably, induce mummification, thus drying the connective tissue to the bone surfaces.

The presence of animal scavengers within the environment (enclosed or exposed) in which such material remains also will affect the bone. Although rodents may gnaw exposed bone in situ, larger animals such as canids may physically remove elements in a predictable sequence, often dragging these elements far from the body over time.[61] The epiphyseal ends are then chewed open, exposing the metaphysis and medullary cavity, allowing access to the nutrient-rich bone marrow within.

The residual soft tissues are removed by fungal, bacterial, and insect activity, notably beetles. The bone then undergoes additional changes, although alterations that are recognizable to the naked eye may take decades or even centuries to progress.

The organic components of the bone are degraded further through continued microorganism action, with bone itself being destroyed, potentially in as little as 25 years.[62]

Time-Dependent Morphologic Alterations to the Bone Matrix

Initially bone remains relatively fresh, possibly with blood product evidence still identifiable. Adipose tissue from the medullary cavity leeches out under exposure to warm temperatures, resulting in the "greasy-to-the-touch" feeling to recent remains described by Knight[13] (Figure 8.2). The continued presence of organic components results in a "heavy" feeling to the bone and, upon exposure to the frictional heat experienced during sectioning of the bone, the smell of burning organic material is appreciable, although somewhat subjectively.

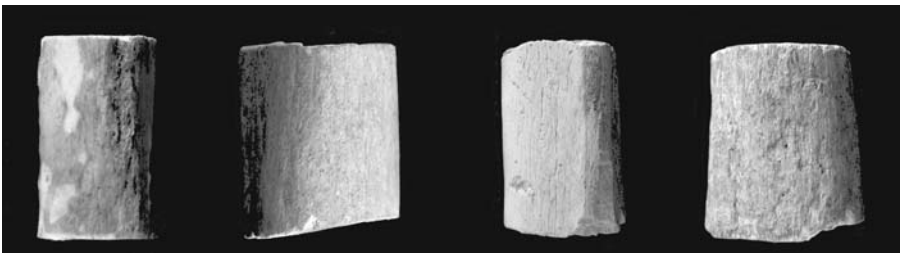

Fig. 8.2. Aging of bone (from **left** to **right**). Initially bones continue to have a greasy appearance. Loss of protein and blood product content over the centuries results in an increasingly "chalky" texture and appearance.

In the majority, the organic stromal component is composed of collagen. Like any other protein material, the α-helical polymers of collagen are broken down through enzymatic action and heat exposure, with the resultant protein fragments metabolized by organisms associated with the remains. The result of the continued degradation of collagen is the loss of the organic component of bone, visible on sectioned bone as the concentric loss of the normal appearance on the internal (medulla cavity) and external (subperiosteal) aspects. Organic-poor bone appears "chalky," and given that the surfaces may be exposed to environmental degradation, loss of their collagen content tends to occur here first. Therefore, a cross-section of material in which protein degradation of the organic phase of bone had commenced demonstrates a concentric ring of remaining minimally altered bone deep within the compact bone, separated by organic-depleted matrix on either side[13] (Figure 8.3).

The type of bone itself affects the rate of postmortem alteration. Large, dense, compact-rich bones, such as femora, tibia, and humeri, are slower to decompose than those that are trabecular bone-rich or possess larger surface area to volume ratios, such as phalanges, vertebrae, and calvarial bones. These issues are discussed in more detail later when considering isotopic analyses. It should be noted, however, that these morphologic alterations are far from diagnostic. In Knight's experience bones of several millennia antiquity have been noted to retain an excellent mor-

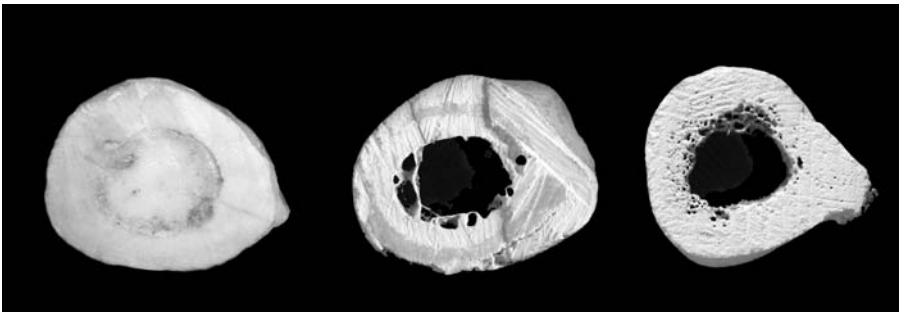

Fig. 8.3. Transverse sections of aging bone showing the change from a recent appearance, with bloodstaining and persistence of periosteum and bone marrow, to that of "chalky" organic-poor bone matrix. The intermediary stage is represented by a "sandwich" effect of organic matter.

phologic appearance, yet the skeletons of those recently deceased that have lain exposed to peat (within which low pHs are experienced) have almost completely decomposed.[13]

Despite the fact that the majority of estimates continue to be performed on morphologic grounds, the inherent inaccuracies of this method reinforce the requirement for a reliable and accurate method for estimating the time since death in human skeletal material.

Microscopic Methods

The microscopic alterations in human bone during the late postmortem period have been assessed.

In their seminal paper detailing methods of PMI estimation, Berg and Specht[63] concluded that histologic changes were evident only after a PMI of 10 years, seem as erosive changes identifiable initially within the Haversian canals and the interstitial lamellae. However, it appears that quantification of these changes does not allow assessment of the time since death.

Nokes et al.[64] analyzed five specimens, dating from 11 to 3000 years PMI. Sections of compact bone were removed from each specimen, polished flat, and enzymatically treated. Following appropriate preparation, the material was examined within a scanning electron microscope. Despite the marked differences in time since death, Nokes et al. concluded that the degeneration of the matrix into an appearance of particulate aggregates between 30 and 50 nm in diameter was recognizable in all specimens; thus, the technique was unable to distinguish ancient from modern material. There was no subsequent attempt to identify the constituents of these aggregates, nor was there any evidence that the investigators had excluded the possibility that these changes were actually artifacts possibly introduced by excessive enzyme treatment. A subsequent publication by Yoshino et al.[65] attempted to reexamine the initial findings of Berg and Specht, by investigating the issue of erosive tunneling. Yoshino et al. used compact bone specimens (humeral shaft segments) placed within three different environmental conditions: within soil, within the sea, or exposed to the air. The PMIs varied between 0 and 15 years, with all specimens analyzed through microradiography and scanning electron micrography.

Microscopic analysis of air-exposed bones showed alterations only after 15 years, seen as the presence of erosive fungal and bacterial tunnels forming characteristic labyrinthine spaces. Prior to this time, few changes were recognizable. The samples within soil showed similar tunneling after 30 months within both the internal and external circumferential lamellae, with tunneling recognizable histologically after 5 years when fungi or bacteria infiltrate the midzone of the substantia compacta. The result is loss of both organic and inorganic content, evidenced by the microscopic recognition of erosive changes within both collagen fibrils and the calcified matrices.

Although these results, unlike those reported by Nokes et al., demonstrate structural alteration of bone postmortem, they are reliant upon the environmental conditions to which the bone has been exposed and, hence, the subsequent activity of microorganisms. Like Berg and Specht, no attempt is made to provide a calibration means based upon the quantitative changes recognizable, instead purely stating a minimum time of exposure to soil, air, or seawater in which such changes have been observed. This in itself also fails to take into account the length of the preceding early PMI, as defleshed bone was used by Yoshino et al. Thus, the conclusion reached

with this method fails to address the additional variations that exist within this time frame, which in turn are themselves also dependent upon ambient environmental temperature, soil and burial conditions, and the humidity of the surroundings.

Analytical Methods

As previously mentioned, analytical methods for estimating the PMI are few and often based upon small studies involving numerous techniques. These techniques may themselves be divided into

- those measuring physiochemical or serologic changes, and
- those measuring alterations in radioisotope concentrations

Physiochemical and Serologic Changes

Berg and Specht, 1958

The first formal investigation into the dating of the PMI in human skeletal remains was published in 1958 by Berg and Specht,[63] who applied morphologic, histologic, and physiochemical tests to bones of known PMI. Berg later refined this work by considering a range of techniques from the simple, such as specific gravity, to the complex, including supersonic conductivity.[66] However, his conclusions were that the macroscopic examination by an experienced pathologist remained more accurate than any of the tests.

Knight and Lauder, 1967

Despite Berg's findings, Knight and Lauder,[67,68] in a study of 68 dated samples, aimed to produce a method that was less time consuming but still relatively accurate. By using several predominately physiochemical methods to test bones of known PMI, the research was aimed at identifying which tests would provide the most information regarding the time elapsed since death. Although no one individual test was advocated, a series of separate methods were highlighted to aid forensic investigators in their judgments:

- Nile blue and dichloroindophenol staining
- Reaction with mineral acid
- Nitrogen content
- Amino acid content, including "free" amino acids.
- Benzidine reaction
- Ultraviolet (UV)-induced fluorescence
- Antihuman sera immunologic reaction
- Fat estimation

The authors concluded that many of these techniques failed to discriminate accurately between bones of antiquity and bones of forensic interest. Of the tests described, only four showed a direct correlation with the PMI:

1. Loss of nitrogen: retention of greater than 2.5% by weight nitrogen suggests the time of death is less than 350 years; results greater than 3.5% suggests less than 50 years

2. Overall reduction or loss of particular amino acids from the organic phase of the bone: Fewer than seven remaining amino acid types suggest a PMI of more than 100 years. However, the specific loss of proline, or hydroxyproline, suggests the bone is ancient.
3. Progressive loss of inherent fluorescence pattern of the cut bone surface. The fluorescence diminishes after 100 years PMI, with concentric loss up to 800 years since death.
4. Loss of immunologic activity or benzidine staining after 5 years and 150 years, respectively.

Given the extent to which the work of Knight and Lauder on the dating of human skeletal remains is referenced in the literature, each of these recommended tests is discussed and critiqued in detail.

Nitrogen Loss

Amino acids constitute the major nitrogen-containing molecules within the organic phase of bone tissue. Therefore, progressive loss of proteins through decomposition results in loss of nitrogen content. This can be estimated through assays with concentrated sulfuric acid and subsequent titrations to allow calculation of original nitrogen content, although automated methods are used more frequently in modern laboratories. Knight[69] assessed several bone types of known PMI, ranging from "limb bones" to skull fragments, using a micro-Kjeldahl method, with digestion through a potassium sulfate-copper sulfate-selenium dioxide catalyst system.

As suggested, decomposition of the bone results in a steady reduction in nitrogen content, although, as with previously described methods, humidity, exposure to air, and both the acidity and the temperature of the ambient environment alter this rate. Despite collagen being the major bone protein type, some authors consider protein identified within bones of antiquity to originate from the preservation of noncollagen sources.[70]

The nitrogen content of bone in a live individual, which is prone to variation between persons, is regarded as approximately 4.5% by weight.[13] Therefore, the difference between time zero and 350 years PMI is, potentially, only 2% by weight nitrogen content, allowing little possibility to improve the accuracy of this estimation. The surface area to volume ratio also influences these changes, and, as expected, the protein content of smaller bone fragments experiences decompositional alterations earlier than do larger, intact bones.[71] Overall decompositional changes result in an unpredictable linear decrease in protein content, the rate of which is influenced by numerous external factors. The possibility of fertilizers added to the soils in which remains lie, thus potentially influencing intrinsic nitrogen content, has yet to be examined. As such, the accuracy and reliability of this method for use within forensic investigations is questionable.

Amino Acid Content

The amino acid content was extracted by heating bone samples in a hydrochloric acid solution and analyzed, in the case of Knight and Lauder, by two-dimensional chromatography.[67,68] The results of their work suggested that, of the 15 amino acids

naturally present in human bone, proline and hydroxyproline provide the most useful information even though other amino acids such as glycine and alanine are present in higher concentrations.

Depending upon the storage environment, proline and hydroxyproline are absent in specimens of PMIs greater than 50 years, making them useful in forensic investigations. Other amino acids are lost over subsequent decades, although glycine may persist for millennia.[13] However, the authors admit that their method was "poor," resulting in technical difficulties in identifying the presence, or absence, of particular amino acids. Four of the samples from the "forensic interval" failed to demonstrate any proline and/or hydroxyproline content.[69] Conversely, three of the 16 archaeologic bones contained measurable proline/hydroxyproline content.

How can these results be explained? Proline and hydroxyproline are water soluble and therefore vulnerable to the effects of water passing through bone, decreasing the internal concentrations. In addition, quantities of hydroxyproline may be present within ground water itself, which theoretically could wash into bones and produce false-positive results.[72] The degree of acid hydrolysis, including the ability to lyse all peptide bonds and thus release all amino acids present, may have affected the resultant profiles produced by Knight and Lauder.

Additional studies have shown a virtually normal amino acid profile in a 2000-year-old sample, suggesting that it may not be purely the presence of the amino acid types but the percentage content compared to that within living bone. A total amino acid concentration greater than 10% of that present within modern (time zero) bone maintains the normal ratio and profile of amino acid types; below this percentage the profile is altered significantly.[73]

Zinc concentrations are said to decrease during this period, presumably as the metal ion binds numerous biologic proteins. Thus the loss of zinc purely reflects the loss of protein content.[40]

Although amino acid content may naturally alter in a somewhat recognizable manner, it is prone to too many unknown variables. As such, it should not be recommended as a sole means of estimating the time since death.

Bone Fluorescence

The blue-white fluorescence of the cut diaphyseal bone surface seen under UV light is said to alter with time. Knight describes the concentric loss of fluorescence, beginning around the medullary cavity, between 3 and 80 years PMI with subsequent loss of the subperiosteal fluorescence. The result is a "sandwich" of residual fluorescence that diminishes over subsequent centuries.[13] Japanese publications confirmed this effect, observing fluorescence at 460-nm UV, and quantification suggested that loss of 20% intensity occurs within 15 years of death.[65]

Facchini and Pettener[74] furthered Knight's original research, but they used a different specific UV source than Yoshino (366 nm). They confirmed the "sandwich effect" of fluorescence in bone material from medieval periods and, to a lesser extent, material more than 2000 years old. They concluded that fluorescence could be extended up to 350 years, attributing the physical attribute to the organic phase of bone in contrast to a previous publication suggesting the attribute resulted from the mineral component.[75] If the latter were the case, then diagenetic recrystallization could affect the fluorescent properties of bone, rendering the test prone to further inaccuracies.

Immunologic and Benzidine Stain Reactions

Benzidine reactions detect the presence of blood components, notably hemoglobin, within the bone matrix. The survival of blood proteins over variable periods of interment is unknown, although Knight suggests it occasionally may be demonstrable by this method up to 150 years after death.[69] However, the method is fraught with false-positive results. The chemicals now are considered carcinogenic, so the method has been altered to used aminobenzidine instead.[62] However, negative results have been obtained with bones of forensic interest, that is, less than 50 years PMI.[67,68]

Knights' use of the antihuman serum (AHS) reaction produced negative results after only 5 years PMI.[69] It was thought that the result was highly reliant upon the quality of the antisera available and the concentration used.

Introna et al.[76] investigated the use of luminol, an alkaline reagent commonly used for the identification of blood stains; the reagent is altered through the peroxidase activity of the heme portion of hemoglobin. The objective was to identify blood remnants in skeletal remains, but, unlike the methods described earlier, the reaction of luminol creates visible light so that it can be photographed for evidential purposes.

Introna et al. performed the test upon 80 bones (PMIs ranging from 1 month to 60 years) and graded the intensity of the bioluminescence by both naked-eye observations and gray-scale video image analysis. The inevitable intraobserver and interobserver bias variations failed to be addressed, although the investigators claimed to be able to identify a decrease in percentage activity after 10 years PMI using the image analysis software. The test appeared to be ineffective after 50 years. Follow-up studies currently underway aim to reduce the observer bias through the use of high-resolution computerized image analysis. Initial unpublished results show some promise, although the experimental methodology requires attention to ensure minimization of the risk of false-negative or false-positive results.

Physiochemical testing methods may assist in estimating the PMI but only when used in conjunction with additional methods. No single reliable method has yet been identified. These methods are reliant upon specific environmental conditions; deviation from such conditions alters the results in an unpredictable manner, which cannot be accounted for after the fact. For example, the water table may rise, affecting bone chemistry. However, the water table rises only during specific seasons and, being dependent upon rainfall, may not occur every year of the PMI. The forensic investigator usually will not be aware of these temporary changes in soil environment.

The geographic location affects rates of decomposition, as alluded to previously. Therefore, the results from a temperate climate cannot be applied to the conditions within a tropical country or to an arid climate. Note, however, that variations might occur within the same locality.[77]

Radioisotope Methods

The ideal method for estimating the PMI of human skeletal remains is one that is not affected by the surrounding environment to which the remains lie exposed. Such a means could be seen as resembling a stopwatch, being internal to the bone itself and therefore present in all bone types. The inability to "reset" the stopwatch is of great importance, allowing quantitative assessment and therefore producing an accurate, reliable, and reproducible method based upon existing accepted scientific principles. One such methodology would be analysis for radioisotope activities,

Table 8.2. Atomic differences and prevalences of the main isotopes of carbon.

Carbon	Protons	Neutrons	Proportion	Half-life (t½)
^{12}C	6	6	98.89%	Stable
^{13}C	6	7	1.11%	Stable
^{14}C	6	8	0.0000000001%	5730 years

which accumulate within human bone and are present in measurable concentrations. Of these isotopes, by far the most recognized is one of the least useful in forensic investigations: radiocarbon dating.

Radiocarbon (^{14}C) and Radiocarbon Dating in Forensic Practice

Radiocarbon is the naturally occurring radioisotope of carbon that decays through the beta emission of an electron to form stable nitrogen (Table 8.2).

Radiocarbon is rapidly oxidized within the atmosphere to form $^{14}CO_2$, which enters the oceans via atmospheric exchange forming dissolved carbonates and into plants and phytoplankton through photosynthesis. Hence radiocarbon can enter animals via the progression of the isotope through the subsequent food chains, where it is incorporated into organic biochemicals. Plants and animals may exist during their lifetime in equilibrium with atmospheric ^{14}C. At death, metabolic function ceases, resulting in a steady decay of the radiocarbon content present and the loss of this equilibrium. ^{14}C Testing is somewhat limited in the archaeologic time frame (up to 50,000 years) and provides wide age estimates that are acceptable in archaeology but may be too broad for forensic application.[59,62]

In developing the technique, Willard Libby made the incorrect assumption that environmental radiocarbon has remained constant over millennia, such that modern values can be correlated to initial concentrations in historical material.[78] This is not the case. The magnetic fields of the sun and the earth result in global variations in atmospheric production, and the global fractions vary, being the quantities of radiocarbon within the atmosphere, the biosphere, and the oceans.

Also, when Libby created his system, the half-life of radiocarbon was considered to be 5568 years, again incorrect (t½ = 5730 years). These errors have resulted in the recognition that any material produced between 1650 AD and modern day will produce inaccurate carbon-based dating results.[62] Given that the system had in-built errors, it required the development of a correction calibration system to counteract the errors.

By accurately analyzing samples from the annual growth rings in trees of known age, it was possible to establish atmospheric ^{14}C concentrations through knowledge of the isotope's true half-life. In such a manner a correction curve was created, represented as calendar years against radiocarbon dated years.[1] By calculating the radiocarbon date from material of unknown origin, using Libby's incorrect assumptions, cross-reference with the established correction curve allows easy estimation of the calendar year value.[2]

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[1] It should be noted that the latter is always expressed as BP (Before Present), which is taken as 1950.

[2] Given as CalBP, CalAD, or CalBC to indicate the fact that the date has been corrected.

Since the industrial revolution in the late 1800s, the use of fossil fuels as an energy source has resulted in the release of large quantities of carbon, predominately as carbon dioxide, into the atmosphere over a relatively short period of time. Given the age of the fuel source, (being millions of years in creation), the ^{14}C content of these materials has decayed to virtually zero. The result was a large increase in the overall carbon content of the atmosphere, but one that was radiocarbon depleted. Thus, up until the mid-1940s, the radiocarbon signal became diluted by up to 2%. For material created between 1890 and 1950, the estimated ages based on radiocarbon dating were inaccurate.

Atmospheric nuclear weapon detonations after 1945 have rapidly increased the radiocarbon burden. This is represented as a sudden peak, often referred to as the "bomb peak," when large quantities of artificial carbon isotopes were created through nuclear fission. The result has been a doubling of the ^{14}C activity in terrestrial organisms.[79] The geographic distribution of this peak also varied, being higher in the northern hemisphere where the majority of nuclear weapons testing occurred. Since the signing of the atmospheric nuclear test ban treaty by the majority of nations in 1963, this radiocarbon has rapidly redistributed into the biosphere.[80]

Several publications have used this recognizable increase in atmospheric radiocarbon content when attempting to differentiate bones of forensic interest from bones of historical interest. Taylor et al.[81] detailed several case studies in which the ^{14}C content was analyzed and remains were placed in one of three categories:

1. Nonmodern period (prior to 1650 AD) and of no forensic interest
2. Premodern period (1650 AD to 1950 AD), of possible forensic interest
3. Modern period (1950 AD to the present), of definite forensic interest

Despite the apparent success of this methodology in these cases, the limitations are obvious. The second category covers three centuries that at present include cases that may warrant police interest. Delineation between these cases and ancient material is not possible using the method described.

Researchers have recently focused upon the bomb peak in forensic casework. Wild et al.[80] used the peak as a calibration curve based on the recorded atmospheric ^{14}C values to allow assessment of different human biologic material to produce accurate radiocarbon-based dates.[82] Ubelaker[83] also recognized the use of the peak, although the estimate produced is wide ranging and potentially of little practical use compared to macroscopic examination-based estimations (the results indicated a "95% probability of death occurring between 1670 AD and 1955 AD").

One of the potential problems raised by Wild et al.[80] is the biologic half-life of the carbon content of human tissue. Radiocarbon, biologically identical to ^{12}C, is incorporated into the organic phase of bone matrix. Therefore, the majority resides within collagen. Estimates based upon previous work suggests collagen turnover may occur every 15 to 30 years, depending upon the nutritional status and the chronologic age of the individual during life.[80,84] Therefore, in an individual who is 50 years old and who died in the early 1990s, the collagen phase of the individual's bone potentially retains the radiocarbon profile from when the protein was created in the early 1960s, when atmospheric radiocarbon was high. The result is an inaccurate estimate of time since death. This may be corrected by testing several tissue types in combination, such as bone marrow or hair, which have faster production rates and hence quicker turnover rates. However, in the event of the discovery of human skeletal remains devoid of soft tissue, the applications of the technique may be limited.

Artificial, or Man-Made, Radioisotopes

An interesting approach was suggested within the concluding remarks of Knight and Lauder's publication.[68] It was considered that the measurement of man-man (artificial) isotopes might provide information of value. Unlike the methods that depend upon chemical changes, radioisotopes are less affected by changes within the physical environment to which bones have lain exposed. Many of these isotopes are also known to accumulate within the calcified matrix of bones.

Exposure of the population to radiation has increased in the last century through fallout from nuclear weapons testing. The isotopes of the alkaline earth metals, for example, strontium and barium, are similar in their biochemical properties to calcium. Although they have no apparent metabolic function, they are absorbed across the intestinal mucosa and preferentially incorporated in to the matrix of the skeletal system.[85] Therefore, man-made fission products that entered the biosphere following atmospheric nuclear weapons detonations should be present in the bone matrices of those who died after 1945 and remain absent in those who died before 1945. The criteria that must be met by any radioactive isotope dating technique to be of forensic interest are defined as follows:

1. To have a half-life commensurate with the time scale of investigation that is required (i.e., <40 years)
2. To be abundant enough to be detected easily by conventional analytical techniques
3. To have some biologic function so as to be incorporated into the human skeleton[60]

MacLaughlin-Black et al.[85] measured the ^{90}Sr concentrations in contemporary femora (from postmortem examinations) and compared them with concentrations in medieval femora. Although initial results seemed encouraging, the archaeologic samples possessed significant concentrations of radiostrontium. The authors of the study explained the process through diagenesis, the process whereby radionuclides in solution with groundwater percolate through the soil, ultimately being passively absorbed and adsorbed by the bones buried within it. Obviously this process applies to both bones of antiquity and bones of forensic interest, with isotopes exchanged between the hydroxyapatite matrix and the soil. MacLaughlin-Black et al. noted this potential postmortem variation; however, soil samples were unavailable for comparison. To determine the PMI, it was suggested that future investigations into the viability of using ^{90}Sr dating must relate the concentrations of radionuclide in the samples to the location and condition of the burial with respect to the process of diagenesis. It also would be essential to map the concentrations of isotopes present against the time table of atmospheric nuclear detonations, which peaked in the 1960s, although isotope concentrations may vary nationwide.

For their study, MacLaughlin-Black et al. followed an analytical technique from a publication in 1965.[86] The method was a form of precipitation extraction requiring long periods of time for the in-growth of ^{90}Y and the establishment of an equilibrium, which would be unacceptable for forensic investigations. This process occurred after the extraction of the strontium nitrate precipitate from the calcium matrix. The method, though not described by the authors within the published article, was

altered; concentrated nitric acid (70%) was substituted for fuming nitric acid (95%–96% w/w). Prior to the in-growth, however, purification of the precipitate is essential; many impurities and contaminants remain present that, if not completely removed, may produce false-positive results. Because of time constraints, the pilot study by MacLaughlin-Black et al. may have inadequately purified the resultant precipitates (Joanna Norris, Personal Communication 1998). Therefore it is likely that the beta-activity detected in the specimens tested was not purely ^{90}Sr but a mixture of radiostrontium and contaminate beta-emitters. These include not only man-made isotopes but potentially also naturally occurring radioisotopes, such as ^{226}Ra and ^{40}K, both of which possess long half-lives. It is the probable presence of these isotopes that may account for the beta-activity (detected by liquid scintillation counting) measurable within the ancient bone material and not necessarily the assumed diagenetic in-growth of ^{90}Sr.

Neis et al.[87] continued the work using ^{90}Sr, claiming it was an ideal means of estimating the PMI. The results of the study revealed the expected lower concentrations of ^{90}Sr in older bones, although no significant activity was demonstrated in bones from the 1930s. These claims appear to contradict the findings of MacLaughlin-Black et al., possibly confirming the flawed analytical method. However, the use of occipital bone as a standard prevents accurate comparison with other studies. Conversely, should any diagenetic in-growth have occurred, it would be expected to be greater in bone such as calvarial material, which possesses a high trabecular content and hence a greater surface area to volume ratio exposed.

A possible limitation inherent to the methods measuring man-made radioisotopes is the irregular creation of such nuclides. Between 1945 and 1998 more than 2040 nuclear weapons tests were performed at irregular intervals around the world. Each produced different isotope ratios, dependent not only upon the warhead design used but also on how the warhead was detonated. For example, atmospheric detonations resulted in rapid cycling of isotopes into the biosphere and food chains compared with subterranean detonations. Ultimately these isotopes became globally distributed. The relative determinations of isotope concentrations must be plotted against the intermittent creation of new concentrations, each "topping up" the existing quantities present within the environment. Nuclear accidents, such as the Chernobyl disaster, added to the worldwide isotope burden. Therefore, it is possible that bones of greatly differing PMIs will have similar concentrations of fallout isotopes as the decay versus creation times change over the decades.

Naturally Occurring Radioisotopes

Every population is exposed to significant concentrations of radiation through inhalation of naturally occurring radioisotopes, such as radon and lead 210 (^{210}Pb), and through ingestion of isotopes present within food and water supplies. These primordial elements are unrelated to nuclear explosions, and their uptake should remain constant throughout an individual's life. Naturally occurring radioisotopes have a regular and predictable background concentration, one that has remained relatively unchanged over millennia.[88] Although localized increases have been observed around certain industrial facilities, they have not been shown to produce identifiable increases of ingested concentrations.[89,90]

The nuclides lead 210 (^{210}Pb) and polonium 210 (^{210}Po) are members of the uranium 238 decay series and are widely distributed within the environment. As such they have been recommended as a means of estimating the time since death in skeletalized remains.[59,60,91] They enter the body from two main sources: direct ingestion in food and decay of ingested radium 226, which is retained in the bone and bony tissue. Direct inhalation of these isotopes is negligible, although inhalation of a short-lived parent isotope (radon 222) can result in elevated concentrations of ^{210}Pb and ^{210}Po.

Previously the concentrations of ^{210}Pb and ^{210}Po were determined in human bone mainly to evaluate the contribution to internal radiation doses. ^{210}Pb has also been used successfully to date sediment depositions over the last four centuries, and it has proved useful in the dating and hence the verification of the authenticity of works of art.[92–94] Little work has been undertaken into the potential for using these isotopes as detection tools for dating human skeletal remains, despite appropriate radioactive half-lives (notably 22.3 years and 138.4 days for ^{210}Pb and ^{210}Po, respectively). The potential for using such isotopes as predictable biologic clocks has been shown through pilot studies.[60,91]

The results of these studies have shown that the creation of a time-dependent analysis, although feasible, requires the creation of a population-specific calibration scale, given the variation in isotope exposures across countries relating to the underlying geologic strata and, hence, dietary-based differences (Figures 8.4 and 8.5). By knowing the levels within a living population, it would be possible to produce a calibration curve against which the time since death can be identified, knowing the activities within discovered remains and the half-life of the isotope in question. This method may provide a novel and accurate tool in estimating the date of death in skeletal remains within the forensic interval.

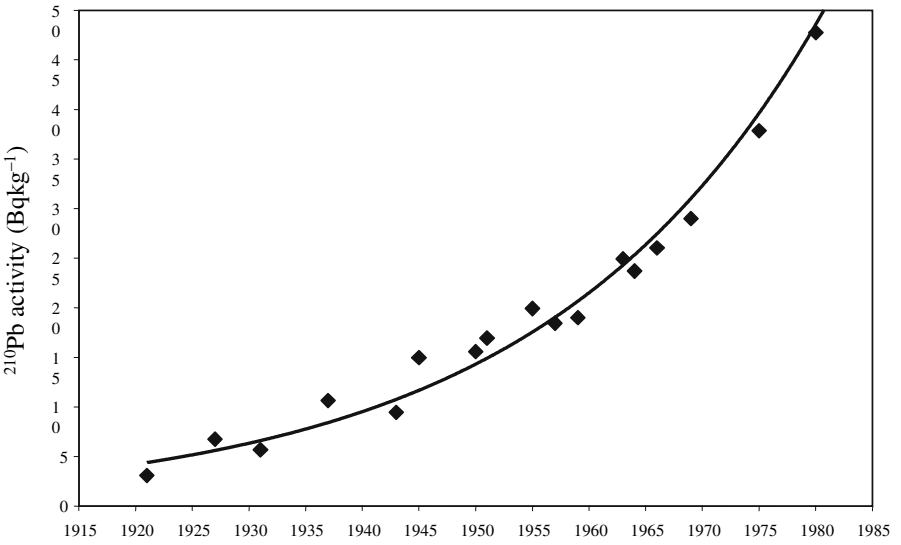

Fig. 8.4. Relationship between ^{210}Pb activity and the time since death shows a steady loss of activity over the period of time presenting the forensic interval. The radioactive decay of ^{210}Pb accounts for the exponential loss.[91]

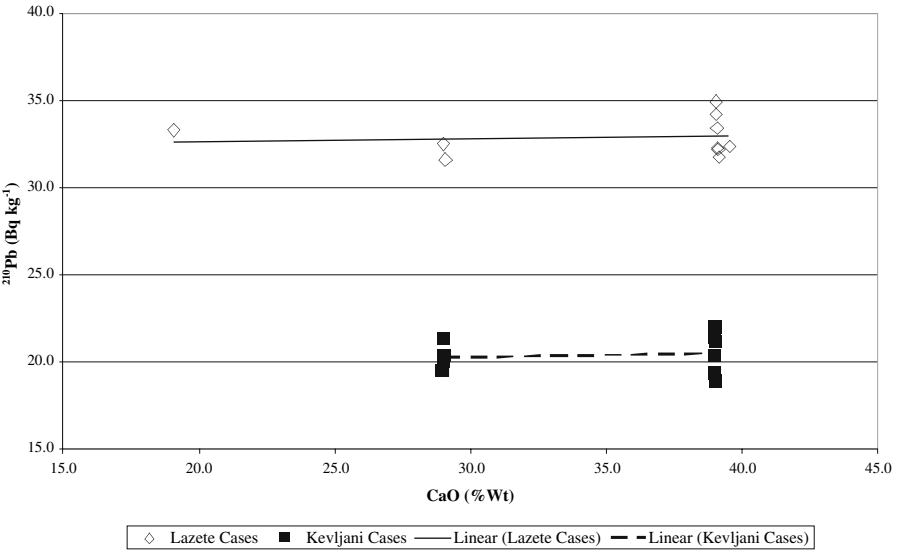

Fig. 8.5. The ^{210}Pb activity measured within bones exhumed from two mass graves from the former Yugoslavia. These cases from Kevljani (year of death 1992) and Lazete (year of death 1995) reveal an appreciable difference in activity (statistically different means at 95% confidence; Mann-Whitney test confirms statistical significance, $p < 0.0001$ at the 95.5% interval), confirming the presence of a time since death decay correlation. The figure also graphically demonstrates the potential degree of accuracy to which an estimate of a postmortem interval can be given, that is, to a single year.[91]

Summary

Although numerous studies encompassing both the early and late forensic interval have been published throughout the international forensic community, little useable information can be applied to casework. Temperature-based analyses remain the most commonly used because they are less reliant upon subjective assessments of change, so use of such techniques continues to be recommended.

Numerous opportunities remain for continued work within the field of PMI estimations, either revisiting published work for evidence-based validation of findings or application of novel technology to the seemingly eternal problem of fulfilling the question frequently posed, not only by police officers but also by the Coroner during his/her investigation.

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9. Burn Injury

P. Nigel Cooper

Introduction

Burn injury is defined as an area of tissue damage caused by the effects of heat. It may result directly from the transfer of thermal energy *or* indirectly when some other form of energy is converted into thermal energy. Examples of the latter are the skin burn seen in electrocution (electrical energy), burns occurring as a result of friction (physical energy), and thermal energy resulting from a chemical reaction (chemical burns). Traditionally injuries resulting from wet heat sources, such as hot water and steam, are known as *scalds*.

The annual number of individuals suffering thermal injuries in the United Kingdom (UK) is approximately 250,000, with 175,000 presenting to accident and emergency departments and 13,000 requiring hospital admission.[1] A detailed account of the epidemiology of thermal injuries is given by Lawrence[2] but in summary:

> Burns and scalds tend to be seen at the extremes of age[3–5] and have highest incidence in those less than two years old.[6] Twenty percent of burns occur in children aged up to four years and of these 70% are scalds. Sixty percent of burns occur in patients aged 15 to 64 mostly as a result of contact with flames. About 10% occur in people aged over 65.[1] As with most forms of trauma, burns are commoner in males than females[7] and are often associated with intoxication by alcohol.[8]

Burns on living persons often have considerable medicolegal significance, especially in children when the possibility of nonaccidental injury may be raised. In many jurisdictions if burns may have caused or contributed to death, a medicolegal postmortem examination will result.

The aims of this chapter are to describe the medicolegal assessment of burns, including electrical burns, discuss the mechanisms by which they cause death, and describe the postmortem effects of heat on the human body.

General Assessment of Burns

Clinically, burns are assessed primarily according to the percentage of the body surface involved and the depth of the burns. The former is principally of use in predicting the likelihood of systemic complications and death (see later). The latter

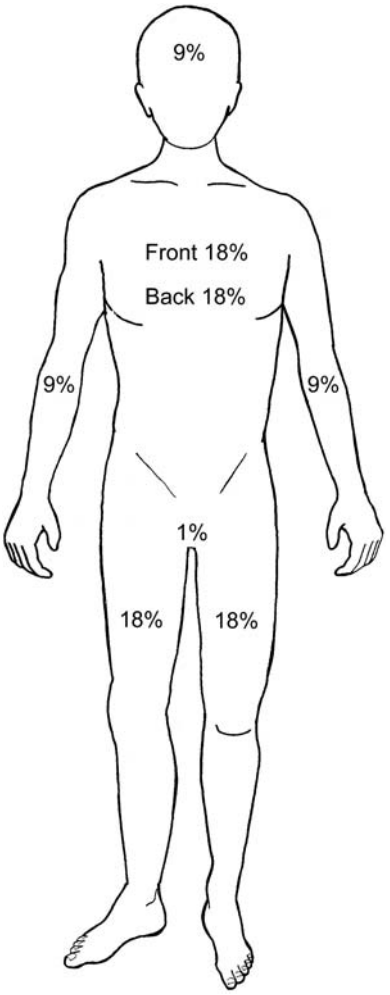

Fig. 9.1. Estimating the surface area of the body burned (rule of nines).

determines how the wound will heal and whether skin grafting likely will be necessary. The extent of burns in adults is determined by the rule of nines[9,10] (Figure 9.1). In infants the head accounts for 18% and each lower limb 13.5%.[11]

Burns are categorized by depth into first, second, and third degree. First- and second-degree burns are both partial thickness, whereas third-degree burns involve complete destruction of all skin appendages. The most superficial type of heat-related damage, erythema, usually resolves in a few days. Even extensive first-degree burns usually heal in 10 to 14 days with no scarring. They are accompanied by blistering and are very painful. Second-degree burns heal more slowly, are relatively painless, and heal with scarring if not treated. Third-degree burns are painless, heal by granulation tissue, and scar if left untreated.[12] Occasionally, most often in black-skinned races, burns are complicated by keloid scar formation characterized by tumor-like growth extending beyond the original injury.

Medicolegal Assessment of Burns

Introduction

The principal medicolegal issue in the assessment of an injury resulting from heat usually is how the injury was caused and particularly was the injury caused as a result of a deliberate act or by accident? It is important to take the individual injury in the context of all the information available about the patient.

Most burns and scalds are accidental. The history in such cases often reveals significant risk factors for accidental thermal injuries. Thus, most elderly scald and burn victims have some form of psychomotor impairment.[5,13] Epileptics are particularly prone to suffering burns following a fit.[7,14] A history of alcohol intoxication may be obtained.[7]

A very small proportion of burns are deliberately *self*-inflicted. Approximately 2% or 3% of burns unit admissions are the result of self-immolation.[15]

An important minority of burns and scalds are deliberately inflicted by another person, usually in the context of child or elder abuse. An estimated 3% to 10% of burns in children are the result of nonaccidental injury.[16] They are particularly common in children younger than 3 years. Approximately 10% or 20% of nonaccidental injuries in children involve burns or scalds.[18–20]

Even when not deliberately inflicted, a large proportion (as many as one third) of "accidental" burns in children are associated with neglect on the part of the carers.[21] Such burns and scalds may result in criminal charges against the carer.[18]

In the case of an inflicted injury such as in child abuse, the history may well be inconsistent with the injury.[22,23] The history may change and the medical presentation may be delayed. The child simply may not be capable of the activity that is said to have resulted in the injury. Repeated episodes of burning, certain patterns of burning (such as well-defined contact burns and lack of splashes in scalding), and burns associated with bruises or fractures are all suspicious.[21,22,24] Thermal abuse is seen more often in boys than in girls. Most often inflicted burns are the result of hot liquids, less often hot objects, and least often flames.[17,23]

Factors that Determine the Appearances of a Burn

The characteristics of an individual thermal injury depend upon the factors listed in Table 9.1.

In practice the first three considerations are generally of theoretical rather than practical interest. On occasion, usually in relation to either hot water or a hot object,

Table 9.1. Factors on which the characteristics of an individual thermal injury depend.

1. Part of the body involved
2. Temperature to which the tissue is raised
3. Time for which the heat is applied
4. Nature of the heat source
   A. Radiant heat
   B. Wet heat
   C. Flames
   D. Contact with a hot object

the length of time exposed to the heat may be very relevant to the distinction between accidental and inflicted injury. However, from a practical viewpoint, usually the nature of the heat source and the circumstances of the incident determine the pattern and distribution of injuries seen.

Part of the Body Involved, Temperature, and Time of Application

The susceptibility of skin to burning depends principally on skin thickness. Skin is thickest and most resistant to the effects of heat over the palms and soles and thinnest and most vulnerable on the flexor surfaces of the arms and forearms, with the epidermis ranging from 30 to 85 μm in thickness and the dermis from 500 to 2250 μm.[25]

The minimum temperature that can cause a burn is said to be 44°C, but only if the heat is applied for 6 hours or more. In contrast, an object heated to 70°C causes a burn in less than 1 second. For each degree rise in skin temperature from 44°C to 51°C, the time taken to produce a burn is halved.[26]

In relation to water, the relationship between time exposed and temperature of the water to produce (1) discomfort, (2) partial-thickness burns, and (3) full-thickness burns is discussed by Lawrence and Bull.[2] The range of preferred bath temperature is 36°C to 42.5°C (average 40.5°C) and shower temperature 38.5°C to 41°C (average 40°C).[2] In a considerable proportion of homes the hot water is above 60°C,[27] a temperature that produces instant discomfort and a scald after 5 seconds. A high proportion of homes where scalds from tap water have occurred have unsafe hot water tap temperatures.[28]

Nature of the Heat Source

The pattern and distribution of burns depends on the type of the heat source. The four principal types of heat source are radiant heat, wet heat, flames, and hot objects.

Radiant Heat

Radiant heat damage is most commonly seen in the form of "sunburn." Less commonly, it is seen as erythema abigne, usually in elderly people on the fronts of their legs as a result of sitting in front of a fire. The pattern of burn produced by radiant heat depends on the position of the body in relation to the heat source and the presence of clothing or other intervening objects.

Wet Heat

Traditionally, thermal injuries from "dry" heat sources and scalds are distinguished by the presence of charring of the tissues and destruction of hair in "dry" injuries and their absence in scalds. Charring and hair destruction result from oxidation that occurs only if the temperature is high enough and oxygen is available. Thus the absence of charring by no means indicates wet heat. Many dry burns do not show charring.

Scalds usually are accidental and may be suffered in baths and showers where they usually involve the lower part of the body.[5] However, most accidental scalds occur when a toddler overturns a cup or other container of hot liquid,[4,14,20] resulting

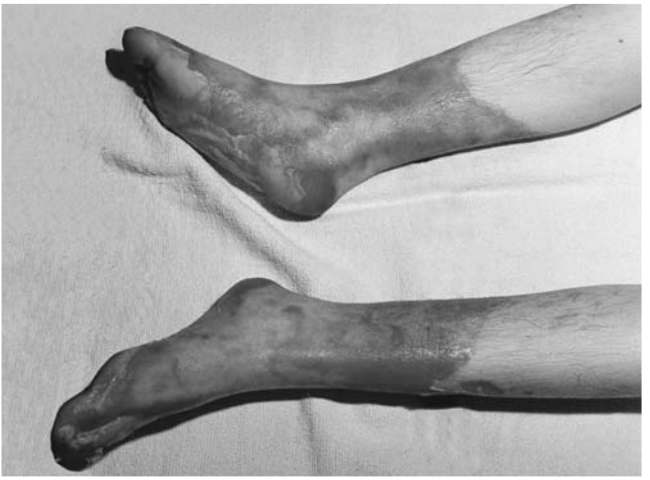

Fig. 9.2. Scalds with tide marks resulting from immersion of the feet into a hot bath.

in a poorly defined splash pattern of scalds to the face, upper chest, and arms.[20] Such scalds are multiple and noncontiguous, and running of fluid under the influence of gravity is seen.[29] Sparing of the flexures *or* skin creases may indicate the position of the body during the scalding process.[29] Splash burns may of course be inflicted rather than accidental. A child reaching up and overturning a saucepan typically sustains scalds to the submental region and axillae but will not do so if the arms are by the sides, that is, if the fluid was poured by another person.[18,24]

Inflicted scalds in children usually occur to the buttocks, perineum, or limbs because of deliberate immersion in hot water.[20,23] The resulting scald is typically deeper than those resulting from splashing and has a well-demarcated edge or "tide mark" (Figure 9.2).[17,24,29,30] Occasionally immersion-type scalds have a central area of spared skin where the child's body has been pushed against the side or bottom of the container containing the hot liquid.[29]

The presence of clothing alters the pattern of scalds produced and generally makes scalding worse by holding the hot fluid in contact with the skin[4] but may be protective if the clothing delays contact between the skin and the fluid.

Flames

Flames cause burning of hair and skin. The pattern of burns on the body may suggest a specific set of circumstances, as in elderly people whose clothing catches fire while they are cooking. In such circumstances, the burns usually occur to the front of the body rather than the back, with burns to the hands resulting from attempts to beat out the flames, to the under surface of the chin, and to the upper chest and face.[31] The most diagnostic pattern from clothing fires is burns affecting the dominant arm and adjacent parts of the body.[32]

Flash fires are particularly likely to cause burns to the head and neck, as are flames in general. A pattern of burns to the face and hands[33,34] or to the thighs,

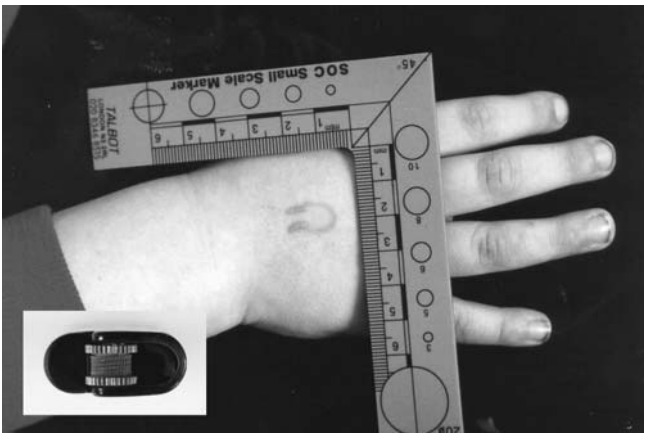

Fig. 9.3. Contact burn and the cigarette lighter that caused it.

forearms, face, and neck has been reported as a result of a flash fire during solvent abuse.[35]

With self-immolation using an accelerant the burns frequently are very extensive, with sparing of skin folds at the axillae and perineum.[31] They tend to be most pronounced over the front of the body.[15]

Hot Objects

Accidental burns from hot objects usually occur on the hands.[36] Contact burns in child abuse are often on the buttocks or perineum from the child being placed on a hot surface.[17,19] The burn often shows a pattern corresponding to the hot object causing the burn, with a well-delineated edge.[20,29] For example, the typical cigarette burn seen in child abuse is small and circular.[24] Contact burns resulting from abuse are generally well defined and even in depth (Figure 9.3), whereas accidental burns usually show more intense burning on one side.[29] Hot wax dripping from a candle or poured from a tea light will, under normal circumstances, not cause a burn.

Internal Burns

Although the vast majority of burn injuries are to the skin, a small number affect "internal" structures. These burns require separate consideration. Internal thermal injuries are most commonly seen in the mouth and less often in the esophagus and upper airways. They usually are the result of ingestion or inhalation of hot food or fluids,[37–40] with microwave-heated foods particularly deceptive, [41] but they can be the result of inhalation of hot gases.

Hot gases usually are inhaled during house fires, and internal injury is often associated with facial burns.[42] The injury produced is characterized by

erythema and ulceration of the lining of the airway, and the histologic appearances are of nonspecific tracheobronchitis.[42,43] Dry heat burns are generally not seen beyond the upper airways, as smoke cools rapidly when breathed in.[44] Indeed, thermal injury of the tracheobronchial tree is difficult to replicate experimentally other than by using steam, as dry heat generally does not penetrate beyond the larynx.[45] Steam has approximately 4000 times the heat-carrying capacity of dry air,[45,46] and thermal injury may extend much lower than with dry heat.[45,47] However, experiments in dogs showed that any thermal exposure sufficient to damage the lungs was more than enough to cause a rapidly fatal obstructive edema of the glottis.[45]

Death from Burns

Introduction

Approximately 300 individuals in the United Kingdom die of effects of burns and scalds each year.[1] The mortality of burns depends principally on the percentage of body surface area involved[48] in both adults and children.[6] The area of full-thickness loss may be an important variable.[49] The characteristics of the individual, particularly old age and the presence of significant natural disease, are also relevant. Children younger than age 4 are reported by some authors to have a higher risk of death than older children independent of burn size.[6]

According to Ryan et al.,[50] burn mortality can be predicted using three principal risk factors: (1) age older than 60 years, (2) total body surface area burned greater than 40%, and (3) presence of inhalational injury.[50] The presence of none, one, two, or three of these factors predicts mortalities of 0.3%, 3%, 30%, and 90% respectively. Others have found death less predictable.[51]

Mechanisms of Death

The mechanisms by which burns cause death are listed in Table 9.2. Acutely, death usually results from burn shock. Over subsequent days and weeks, the main cause of death is infection. Other causes of death are as seen in other forms of trauma. Occasional late deaths occur from malignant transformation in a scar. I consider these categories in turn.

Table 9.2. Mechanisms by which burns cause death.

- Burn shock
- Infection
  - Pneumonia
  - Septicemia
  - Toxic shock syndrome
- Pulmonary embolism
- Gastric ulceration
- Acute renal failure
- Scar-related malignancy

Burn shock

This term *burn shock* describes the rapidly developing hypovolemic circulatory failure seen in the first 72 hours after burn injury.[52] The physiologic changes leading to burn shock are complex. Skin burning is followed by hypovolemia, low cardiac output, hypoproteinemia, hyponatremia, and a rising hematocrit. Burn shock is the result of hypovolemia and the effects of cytokines and other inflammatory mediators. Hypovolemia in turn is the result of a combination of massive interstitial edema, intracellular edema resulting from generalized impairment of cell function, and evaporation from the burn site. The normal adult skin loses less than 40 ml of water each hour, but with extensive burns the loss can increase to 300 ml/hour.[12] Interstitial edema is the result of vasodilatation, increased microvascular permeability and increased extravascular osmotic activity around the burned tissue. The edema usually is maximal within 1 to 3 hours.[12]

There is a profound reduction in cardiac output within minutes of injury, mostly as a result of hypovolemia,[52] but cardiac output does not return to normal until 12 to 24 hours after the burn, even with prompt and effective fluid resuscitation. The situation is not explained simply by fluid loss[53] but also reflects the effects of cytokines and other inflammatory mediators.

In the 1960s burn shock was the leading cause of in-hospital death of burn victims. It is largely because of more effective treatment of burn shock that the total body surface area (TBSA) burns expected to cause death in 50% of young, otherwise healthy individuals has risen from 30% in the 1960s to 70% in the 1990s.[52] Burn shock remains the second most common cause of in-hospital death after infectious complications.[52] The immediate cause of death in such cases often is multiorgan failure.[50]

Infectious Complications

Currently the principal cause of in-hospital death in burn victims is infection.[52] The most common individual infectious cause of death is pneumonia.[54] The infection usually is airborne and less often hematogenous from wound infection.

Septicemia is another common cause of death and has a high mortality in burns patients.[55] It usually is secondary to infection of the burn site[55] but may be secondary to other sources of infection, such as pneumonia and intravascular devices.[48]

Skin burning causes general immunosuppression,[56] and the denatured protein in burn injured tissue provides a good substrate for microbial growth. The relative avascularity as a consequence of thermal thrombosis further promotes infection.[54] Not surprisingly, the risk of burn infection is proportional to the area burned.[54]

Full-thickness skin burns usually are colonized by bacteria within a few days,[56] with sparse Gram-positive organisms in the first week and dense Gram-negative organisms thereafter.[57] Most episodes of septicemia occur between 6 and 10 days after the burn.[55]

Wound infection by specific organisms such as *Streptococcus pyogenes* or *Pseudomonas aeruginosa* or heavy colonization of a burn wound predispose to invasive sepsis with organisms invading living tissue adjacent to the wound.[56] Fungi have

emerged as the most common cause of invasive burn wound infection.[57] Septicemia and high mortality are associated with invasion of viable tissue by microorganisms rather than colonization of the dead tissue.[54]

Toxic shock syndrome may result from infections by *Staphylococcus aureus* phage type 29/52.[58]

Other Early Complications

As in other trauma victims, death may be the result of general complications of trauma, such as pulmonary embolism or hemorrhage from gastric "stress" ulcers.[31,59,60] Death also may result from renal failure as a consequence of either hypovolemia[59] or septicemia.[44] The pathologic change in renal failure after burning is acute tubular necrosis.[61] Rhabdomyolysis as a result of constriction of skeletal muscle by overlying burnt tissue contributes to renal failure in some cases.[62]

Late Complications

Untreated or nonhealing wounds may lead to chronic ulceration, which carries a risk of malignant transformation, so-called Marjolin ulcer. Most burn scar-related malignancies are squamous cell carcinomas, but some other epithelial malignancies and occasional sarcomas have been reported.[63]

Pregnancy Loss

Spontaneous abortion is the probable consequence of extensive burns in the first trimester and premature labor is likely in the third trimester.[48]

Postmortem Heat Artifacts

The postmortem examination of a fire-related death is frequently complicated by the effects of continuing exposure to heat after death, leading to postmortem artifacts including destruction of soft tissue, bones, and internal organs, tissue shrinkage, body rigidity, anal dilatation, the pugilistic position, skin splitting, and heat hematomas. The main issue of medicolegal significance is simply distinguishing real antemortem changes from postmortem artifact. Occasionally the length of time the body was exposed to fire may be important, but because fires are so variable it is rarely possible to give more than a very rough estimate.[64] Such information as is available has been obtained by studying the effects of heat on the dead body in a crematorium and is summarized in Table 9.3.[65–67] The temperature reached in a crematorium (680°C) is similar to that in a house fire but is maintained at this level for a considerable period of time. The temperature reached in a room fire after flashover is on the order of 770°C to 780°C.[65]

Dehydration and tissue shrinkage indicate that the body length may be shortened by several inches and weight loss may exceed 60%.[11] Denaturation of muscle proteins by heat leads to rigidity of the body, which should not be confused with rigor mortis.[11] In a fire victim the anus is occasionally dilated as a result of shrinkage of

Table 9.3. Effects of heat on the dead body in a crematorium.

Minutes	Effect
10	Arms badly charred
10–15	Pugilistic position
15	Face and arm bones visible
20	Ribs and skull visible, vault fractures
30	Abdominal and chest cavities breached
35	Thigh bones visible
45–60	Base of skull exposed
45–150	Converted to ashes and bone fragments

General artifacts

perianal tissues because of heat.[11] Swelling, wrinkling, and vesicular detachment of the skin on the palms of the hands and the soles of the feet, as seen in prolonged exposure to a moist environment, has been described.[68]

Occasionally a body is found with the majority of the trunk reduced to ashes but the head and legs well preserved. This occurs when an item of clothing acts as a wick and body tissues, especially the fat, provide the fuel that maintains the fire, which may burn for several hours. This situation has been reproduced experimentally using animal carcases.[69]

Severe burning delays decomposition, whereas simply heating up a body accelerates decomposition.[11] Hair exposed to heat becomes frizzy and brittle and assumes a red to brown color.[64] The tongue may protrude from the mouth, and petechial hemorrhages may occasionally be found in the lids and conjunctivae.[64]

The Pugilistic Position

The *pugilistic position* refers to the fact that, after a fire, the body often is found with hands raised in front of the face. This finding is supposed to raise the suspicion that immediately prior to death the individual was involved in a fist fight. In fact it is merely the effect of shrinkage of muscles because of heat, with the larger biceps overriding the smaller triceps. Other muscles can be similarly effected, and bodies cremated on funeral pyres are said on occasion to sit up because of the effects of heat.[11]

Postmortem versus Antemortem Burns

Traditionally the presence of a red rim to a burn has been regarded as evidence of vital reaction. However, a red flare is frequently seen around postmortem burns, so this distinction cannot be considered reliable.[11,70,72] Blisters usually are part of antemortem burns but can also form after death, where they tend to be pale, yellow, and lack a red base.[11] The content of protein and chlorides is said to differ in the fluids of antemortem versus postmortem burns.[72]

Histologic examination for evidence of inflammatory reaction can be performed and may be helpful. Cutaneous erythema is characterized by dilated capillaries, occasional necrotic epidermal cells, condensation of nuclear chromatin, swelling of epidermal cell nuclei, and edema of the subepidermal connective tissue.[43] First-

degree burns show epidermal necrosis, subepidermal blister formation, and perivascular inflammatory cells.[73] Coagulative necrosis of the dermis is seen with deeper burns. Adjacent intact epidermis shows elongation of cells and cell nuclei. After 6 to 8 hours obvious leukocyte infiltration is seen,[70] but in some cases there may be a delay of more than 16 hours.[73] Thus, the absence of tissue reaction does not mean that the burn is postmortem.

Skin Splitting

Skin splitting (Figure 9.4) occurs as the tissues dry out and shrink[72] and usually is most obvious over the extensor surfaces and over the head. The splits occur only where the tissues have been severely affected by the heat and usually are obviously postmortem. The absence of related hemorrhage in relation to postmortem splits helps distinguish them from antemortem lacerations.[71,74]

Fractures

Heat causes dehydration of the bone collagen, leading to shrinkage, distortion, cracking, fragmentation, and destruction of bone. Potentially this may simulate blunt injury but not sharp force injury.[75] Heat-induced fractures and traumatic fractures may be difficult or impossible to distinguish, but scanning electron microscopy is said to be useful.[75] The relationship to severely heat damaged areas of tissue usually is obvious, but fractures, particularly of the skull, may be worrying. As heat destroys the scalp, the vault may gradually fracture and disintegrate or may explode as a result of boiling of the cranial contents.[66] Fractures at the base of the skull are said not to occur as a consequence of exposure to heat.[66,76]

Bones may fracture from physical forces applied after death, as in a collapsing building, and may be broken during recovery of the body.[75]

Fig. 9.4. Severe heat-related skin splitting.

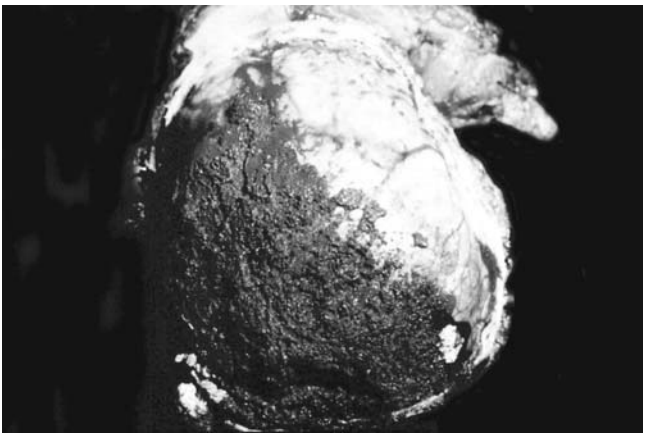

Fig. 9.5. Heat-related extradural hematoma.

Heat Hematoma

When heat is applied to the cranium, blood boils out of the spaces within the skull and the venous sinuses and may collect in a thin layer between the dura mater and the skull. This is the so-called *heat hematoma.* The blood is spongy in consistency, brown in color, thin, and bilateral (Figure 9.5),[72] in contrast to the localized, unilateral, thick, usually temporal collection of dark hematoma seen in a true extradural hematoma. Subdural hematoma does not occur as a heat artifact.[11,77]

Heat hematoma may or may not be associated with a heat-related skull fracture.[78] The dura may split under the effects of fierce local heat with herniation of the underlying brain.[79] The brain itself usually is shrunken, firm, and yellow to light brown in color.[11] Apparently artifactual hemorrhages have been reported in the brain,[80] as well as tears in the cerebral white matter[81] in individuals who were believed to be dead at the start of the fire.

Burns Caused by Electricity

Introduction

Very-high-voltage electricity such as lightning may cause thermal injury simply by passing close to the body (see later). Occasionally burns result from objects such as clothing set on fire by the electric current, but in order to cause burn injury electrical current usually has to pass from one point in the body to another. In such circumstances, injury may be seen anywhere along the route taken by the current and thus can involve any tissue in the body. However, most commonly damage occurs to the skin at one or both ends of the route taken by the current through the body, as normally the skin has the greatest electrical resistance and thus is the site where the majority of thermal energy is generated.

Factors Determining the Damage Caused by Electricity

The principal factor determining the severity of skin and soft tissue injury is the amount of thermal energy generated as the current passes. This is proportional to the square of the current, the resistance of the skin, and the time of exposure (Joule's Law). Thus the current is the most important factor in determining the degree of local damage. However, electrical supplies are traditionally classified by voltage, with electricity classified as high voltage above 1000 volts. The injuries seen in high-voltage electrocution usually are more severe than with lower voltage,[82,83] as expected from Ohm's law, which states that current equals voltage divided by resistance.

The resistance of the skin varies greatly according to the thickness of the keratin covering the epidermis and the dampness of the skin. The average resistance of skin is between 500 and 10,000 ohms,[84] but the soles of the feet and the pads of the hands may offer 1,000,000 ohms resistance when dry. When wet this resistance may be reduced by a factor of 1000.[85] Jaffé[86] states that sweating can reduce skin resistance from 30,000 to 2500 ohms.

The longer the current passes through the skin, the more thermal energy is produced and the greater the degree of tissue damage. Even very-low-voltage electricity will produce a burn if the current is maintained for long enough.[82]

Appearances of Electrical Burns

The appearances of an electrical burn depend upon the amount of thermal energy produced, the area of skin involved, the nature of the electrocuting object, and whether or not contact occurred with the object. Frequently with domestic electricity, even in fatal electrocution, there is no mark on the skin. When present the injury may be extremely small or in a hidden location, but most often the burn is seen on the hands (Figure 9.6). The presence of an electrical burn does not necessarily mean that death was the result of electrocution, as indistinguishable marks can be produced postmortem.[84]

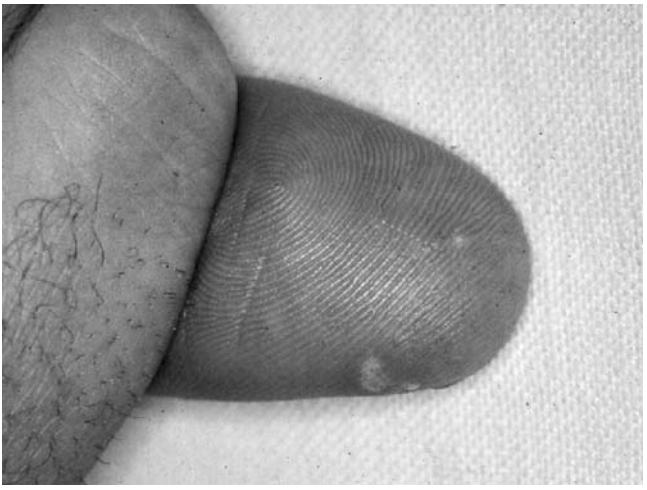

Fig. 9.6. Tiny superficial burns resulting from electrocution.

The typical mark of low-voltage electrocution is a shallow crater with blistering, sometimes a break in the skin, and sometimes a faint rim of hyperaemia.[84] Sometimes the burn is so superficial that it consists of a tiny area of coagulated keratin (Figure 9.6). A patterned burn may result, reflecting the shape of the causative object, for example, a linear mark because of contact with a wire. When there is contact between the conductor and the skin, metal ions may be transferred from the conductor onto the skin, leading to obvious discoloration.

When contact is prolonged, the skin becomes more obviously burnt and brown. If contact continues after death, charring and extensive soft tissue destruction can occur, even at domestic voltage.[84] With high-voltage electrocution the degree and extent of burning are greater, potentially with extensive deep tissue destruction sometimes involving bones, including the skull.[87]

At high voltage, contact with the electrified object is not necessary, as current may pass through the air, a phenomenon known as *arcing*. In dry air, 1000 volts will jump several millimeters and 100 kilovolts will jump approximately 35 cm.[87] The result of arcing is conversion of electric energy into thermal energy in the air. At high but not very high voltage, the resulting heat melts the outer keratin of the skin, producing a hard brownish nodule. At very high voltage, the temperature generated may be as high as 3000 or 4000°C,[83] and the resulting injury is more severe "flash" burning, often in the form of multiple discrete lesions.[11,88] Major surface burns are seen as a result of arcing in about half of the victims of high-voltage electrocution.[89]

Histologically, skin marks from electrocution are nonspecific. The burnt tissue may be eosinophilic, and the cells of the epidermis are often elongated with nuclei of the lower layers orientated horizontally.[90] A honeycomb of small spaces is seen in the epidermis, and subepidermal blister formation is seen. Identical appearances can be seen in thermal burns. Experimental injuries in pigs have shown some minor differences between heat and electrical injury.[91] Special stains for iron and copper frequently show metal deposition at the site of electric contact burns and may be useful in establishing the diagnosis.[92]

Medicolegal Assessment of Electric Burns

The skin usually shows evidence of burning at the points where the current entered and/or left the body, but injuries may not be externally obvious, for example, in the mouth or to the genitals. Contact marks in the mouth are common in young children. Burns are most commonly found on the hands in low-voltage electrocution (Figure 9.6), but only in a small proportion of high-voltage cases.[93] Suicides typically wrap or tape wires to their bodies.[84] Burns are more likely if the area of contact is small and if the voltage is high or the contact prolonged.[84,94]

Almost all high-voltage fatalities show skin burns and just over half of low-voltage fatalities do,[82,95] but fatal electrocution can occur with no evidence of skin or other injury. Electrocution occurring while immersed in water, particularly in swimming pools, is particularly likely to leave no external mark, and indeed the immediate cause of death may be drowning rather than electrocution.[84,96] Bonte et al.[97] reported "electrical marks" in eight of 48 fatal electrocutions occurring in the bath. Ten cases showed restriction of the postmortem hypostasis to the submerged areas, a finding not seen in bath deaths not involving electricity.[97]

Other external findings in fatal electrocution are nonspecific. Petechial hemorrhages on the eyelids are seen sometimes in both low- and high-voltage fatalities.[89]

Often, particularly in high-voltage electrocution, there is evidence of mechanical trauma,[89] for example, from a fall.

Lightning-Related Burns

Most lightening strikes occur outdoors, but individuals who are indoors using electrical equipment may rarely be affected.[11,84] Lightning has high voltage and current but very brief duration (less than one ten thousandth of a second).[11] Examination of the scene usually shows evidence of the lightning strike.[87] Lightning causes damage to the body as a result of (1) electrical current passing through the body on the way to the ground, with burns occurring as air nearby is heated by passing lightning and sometimes clothing is set on fire or (2) the explosive effect of the air heated up to 20,000°C in a fraction of a second. About one third to half of strikes are fatal. Clothing may be torn or destroyed,[84] eardrums ruptured, and the victim thrown some distance as a result of the explosive effect of the strike, potentially sustaining injuries. Typically the victim shows extensive burning, sometimes in the form of branching or "feathered" burns, punctate full-thickness burns, linear burns often in the folds and creases of the skin, or nondescript lesions.[84,98] Burns may occur in the pattern of metallic objects such as belt buckles. However, characteristic pattern skin burns are said to be seen in only a minority of lightning strike victims.[40,82] There is often soft tissue damage, including lacerations and bruising, usually of the head. Fractures may occur. The internal findings usually are nonspecific, as seen in other cases of electrocution, but sometimes include destruction of internal organs.

Chemical Burns

Chemical burns usually occur as a result of industrial accidents but may occur with household products. They tend to be deep and are generally said to be worse with alkalines than with acids. Cement is a common cause of alkaline burns.[16]

Summary

Burn injuries present challenging problems to the forensic physician and pathologist. The principal issue usually is causation, particularly when a distinction is to be made between an accident and a deliberate act. The principal additional issue with burn-related fatalities is establishing the cause of death, which usually is either "burn shock" or infection. Forensic pathologists need to be familiar with heat-related postmortem artifacts and their distinction from antemortem changes. It is important that electrical burns be looked for and recognized, especially in the absence of a good history suggesting electrocution.

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