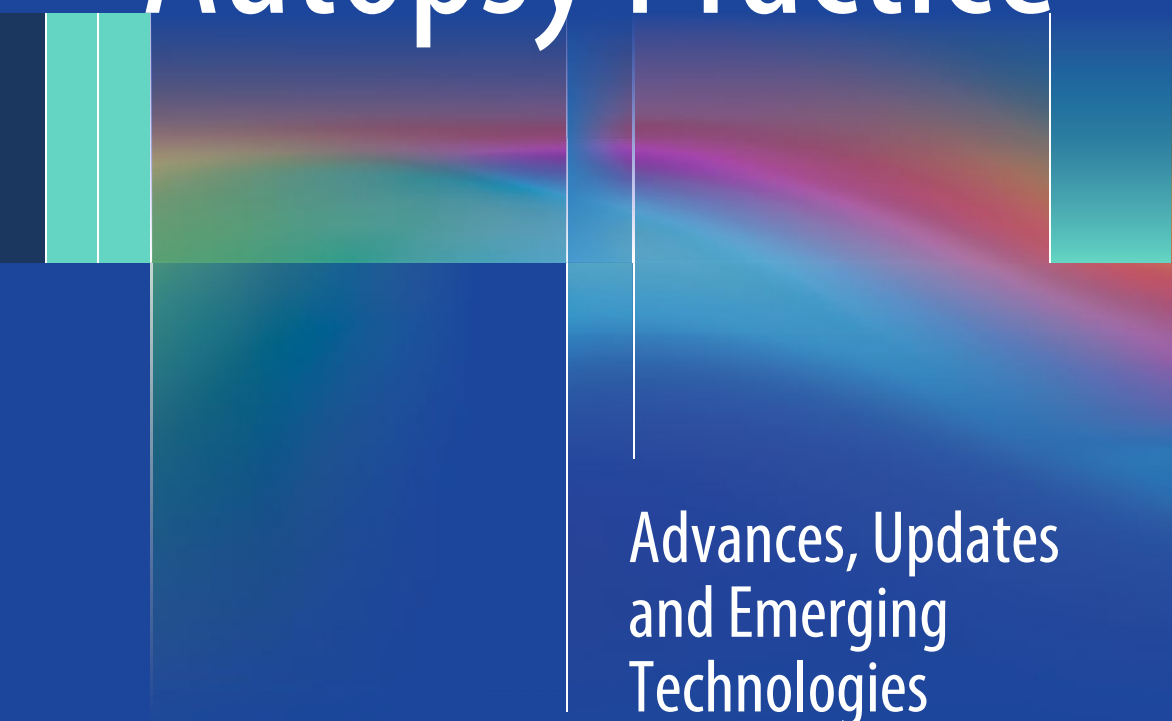
Guy N. Rutty
Editor

Essentials of Autopsy Practice

Advances, Updates and Emerging Technologies



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Preface

As the world continues to debate the role of cross-sectional imaging in autopsy practice, either as an adjunct or a replacement, what is becoming clear is that the autopsy will still exist into the foreseeable future. Despite the decline in academic forensic pathology, there remains a steady flow of emerging techniques and advances in practice, often due to translation from clinical medicine or other areas of science into autopsy investigations. With these advances and the emerging role of cross-sectional imaging, autopsy practice is developing a more multidisciplinary approach to the investigations, becoming more evidence based, with increase in quality and training to ensure that the next generation not only applies the most appropriate technique to the investigation at hand but can provide as much information in relation to the four fundamental questions of who, where, when, and by what means did the person come by their death. Practitioners not normally associated with autopsy practice, for example, radiologists, interventional cardiologists, and engineers, are now being drawn into this area of practice. The *Essentials* series has always been designed to keep all involved in the investigation of death abreast of changes within this field. As is the now established format of this series, I have identified subject matter and authors from across the world and brought them together to consider a broad range of topical subjects where changes in practice have recently occurred to assist trainees and consultants, generalists and specialists, and the new multidisciplinary team alike in keeping up to date with practice. In this edition, to embrace the multidisciplinary approach, I have deviated from the previous series by introducing chapters concerning advances in the field of forensic science, not just medicine, or approaches where different science and medicine disciplines work together, for example, pathology and engineering, pathology and radiology, or pathology and entomology. I hope, as with other editions of this series, that *Essentials* continues to provide an educational and practice resource for all involved in the investigation of death.

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Chapter 1
Postmortem Computed Tomography (PMCT) Scanning with Angiography (PMCTA): A Description of Three Distinct Methods

Bruno Morgan, Namiko Sakamoto, Seiji Shiotani, and Silke Grabherr

Introduction

Postmortem computed tomography (PMCT) is rapidly becoming a standard investigation in many mortuaries around the world. Arguments remain as to the relative merits of PMCT in comparison to traditional invasive autopsy, but there are few who would argue that PMCT has no role in the investigation of death, particularly for unnatural death.

For an imaging system to be clinically useful, it has to identify different tissues, pathologies, or objects as different; this can be referred to as "contrast resolution." This discrimination must also be in time and space (temporal and spatial resolution). The speed of scanning and spatial resolution (to identify small pathologies such as subtle bone fractures) is important, but contrast resolution is the key; if pathology cannot be distinguished from normal tissue, then there is little to gain. For basic applications this is not a problem as radiographic contrast for soft tissues is based mainly on tissue density (or more precisely electron density) and is easily

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sufficient to spot metallic foreign bodies, discern bone from soft tissues, soft tissues from fat, and all structures from air. Therefore, standard radiography and fluoroscopy are sufficient to identify metallic foreign bodies and bone fractures, and indeed these were the mainstay of postmortem imaging for many years until the development of CT imaging in the 1970s [1]. CT scanners offer the ability to reconstruct single slices of information from a body, rather than to accept a radiograph showing a summation of the whole body part. This ability to omit the overlying structures leads to a significant increase in contrast resolution, although the underlying mechanism of contrast is the same. This slice-by-slice approach also allows a better spatial awareness of abnormalities in three dimensions.

PMCT was first reported in 1983 [2], and in 1994 it was proposed that CT could prove a possible replacement to the autopsy in trauma cases [3]. This led to similar proposals for magnetic resonance imaging (MRI) [4] and for children [5]. MRI has been increasingly used for forensic imaging both in the living [6] and in the postmortem setting [7] and, for most body parts, demonstrates better contrast resolution than CT. A major clinical weakness of MRI—its sensitivity to cardiac or respiratory motion—is not a problem in postmortem (PM) use. However, MRI does have other key weaknesses that hold it back as a routine PM imaging tool: the cost is high, the equipment is much more technically demanding to operate, there are safety issues relating to the strong magnetic fields required, scan times are longer, and MRI has lower "spatial" resolution than CT, losing the fine detail required for bone trauma and lung imaging.

Recently PMCT has started to advance more rapidly for two key reasons: Firstly there has been a global increase in use and availability of scanners, causing subsequent decrease in their relative costs, and secondly the advent of multi-slice CT (MSCT). MSCT is not critical for the performance of PMCT, and interestingly one of the major advantages of MSCT in clinical practice is speed, which for PMCT just makes whole body scanning more convenient. However, MSCT does considerably improve the ability to reconstruct the axially acquired CT data into any plane desired (multi-planar reconstruction, MPR) and to create three-dimensional (3D) reconstructions, which can substantially improve the recognition and demonstration of pathology, particularly trauma. There have also been advances in the engineering of CT scanners that have allowed them to be easier to operate and to scan large volumes at high resolution without overheating. Only 25 years ago scanning a whole body in 2 mm increments would take well over an hour. This was reduced to about 10 min in the late 1990s with spiral CT (at low power) but can now be achieved in less than a minute with increments of 0.5 mm.

Due to these factors the world woke up to this possibility. Driven initially by the work of the Virtopsy® group in Switzerland [8, 9] and the eventual introduction of dedicated CT scanners into mortuaries, such as in Scandinavia and the Victorian Institute of Forensic Medicine, Australia, in 2005, the interest, experience, and research evidence base has grown. Publications are now increasing from all around the world with cooperative groups being formed such as the International Society of Forensic Radiology and Imaging (ISFRI) [10] and Technical Working Group Postmortem Angiography Methods (TWGPAM) [11]. Most recently international standards of nomenclature for publications and research have been put forward [12].

This article concerns a key and crucial development for PMCT. Although PMCT shows definite advantages over standard radiography, it still struggles to detect more subtle disease [13–15]. In clinical practice, CT is used as an adjunct to the investigation and management of the patient rather than a single diagnostic tool, and also PMCT is more difficult to interpret than clinical CT, as changes occur rapidly during and after death that can obscure existing pathology. These include edema occurring in many tissues and subsequently the appearance of gas [9]. Even in clinical CT, pathology may only cause subtle changes in tissue appearance, and it is for this reason that routine body and brain clinical CT scanning is normally "enhanced" using contrast agents that can be imbibed, injected, administered by enema, or persuaded down fistula tracks. These contrast agents alter the attenuation of an X-ray beam to make tissues appear different, relating to the distribution of the agent.

A Brief History of Angiography

The study of blood vessels and lumens of other tubes by specific opacification predates radiology and goes back to the beginning of the sixteenth century, when scientists such as Leonardo da Vinci and Jakobus Berengius studied the human body. In order to investigate the interior of hollow anatomic structures, they produced wax casts of the heart chambers and the cerebral ventricles by using maggots to remove the surrounding tissues after wax injection [16, 17]. During the seventeenth and eighteenth centuries, direct vascular injections were performed by pioneers such as de Graaf, Ruysch, Lower, and Virchow [16, 17]. The resulting vascular casts were sufficient for detailed study of the anatomy of the vascular system.

Radiopaque contrast agents were therefore developed very quickly after the discovery of X-rays in 1895. In 1896 in Vienna, Haschеck and Lindenthal demonstrated an angiogram on an amputated hand using injection of Teichmann's mixture (a mixture of lime, mercury, and petroleum) [18], and such angiographies were common by 1899 [16]. Although these early contrast agents were toxic, this did not hold back their use in cadaver studies. The use of postmortem angiography techniques to investigate the vascular supply of organs, especially the heart, became then a real boom, particularly during the first half of the twentieth century. At this time, numerous methods and injection materials existed, and all kinds of organs had been investigated in order to understand their vascular anatomy. However, by the end of the twentieth century, the use of postmortem angiography had nearly vanished, without any visible reason. Only some rare articles appeared, describing the investigation of specific parts of the vascular system such as esophageal veins [19], coronary arteries [20], intracranial arteries [21], and spinal arteries [22].

As early as 1927, Moniz performed the first cerebral angiograms in living patients, using sodium iodide under sedation, and many feel it was this, rather than the now discredited prefrontal leucotomy, that should have earned him his Nobel prize in 1949 [23]. From the 1930s to 1950s, water-soluble iodine agents, based on pyridine and then benzene rings, were developed that were reasonably well

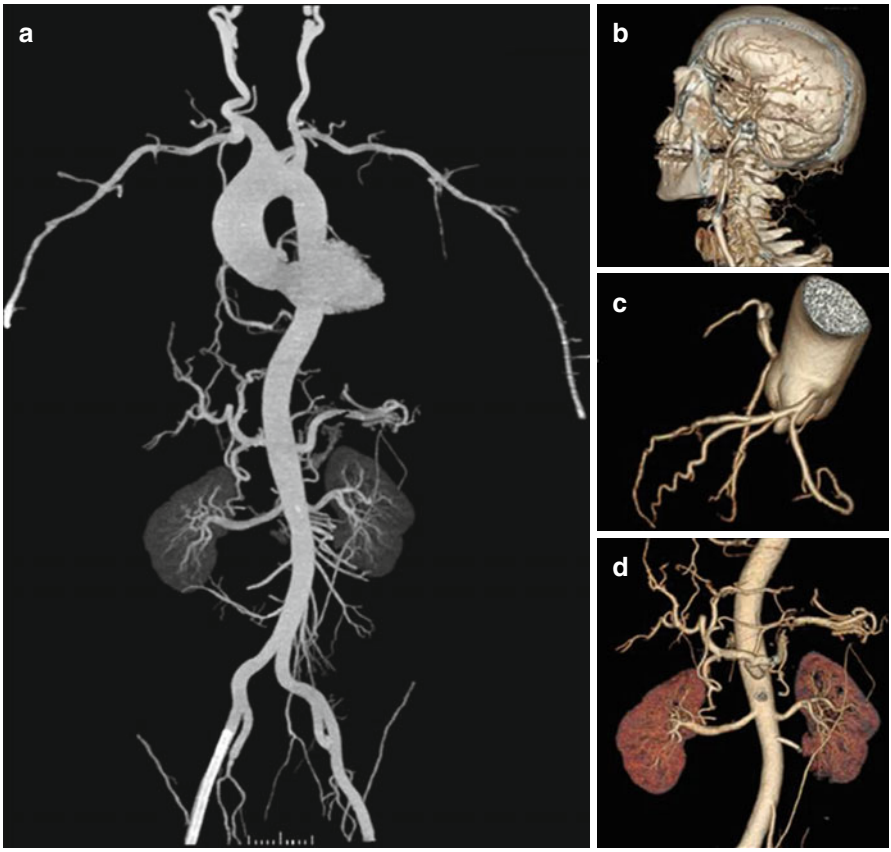

Fig. 1.1 Arterial phase of whole body PMCTA using oily contrast agent with maximum intensity projection (MIP) showing the arteries of the thorax and abdomen (**a**) and 3D volume rendering reconstructions of the cervical and intracerebral arteries (**b**), coronary arteries (**c**), and the major abdominal vessels and both kidneys (**d**) (Images courtesy S. Grabherr, Lausanne, Sz)

tolerated and could be injected into the venous system and excreted by the kidneys. These allowed both angiographic studies [24, 25] and also assessment of organ enhancement, such as intravenous urograms (IVUs) to study both kidney anatomy and function [26]. These were relatively high osmolar agents, but low osmolar compounds were developed and latterly introduced in the 1990s.

Therefore, in postmortem practice most approaches had been direct, using ex vivo organs such as for the heart [27], because enhancing the vascular system for a PMCT scan is more challenging due to the lack of an intact circulation. In clinical practice the contrast is injected, and the circulation then takes it all around the body. Radiologists are used to directly injecting contrast media into specific arteries and veins using intra-arterial or intravenous catheters, but many would be surprised that widespread arterial or venous contrast opacification could be achieved in a cadaver (Fig. 1.1). However, this would be of no surprise to embalmers going back centuries!

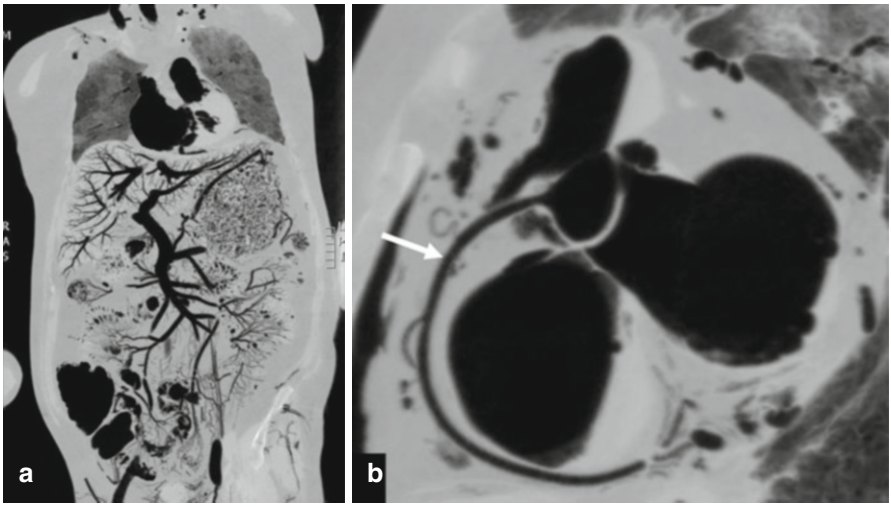

Fig. 1.2 The use of air as a negative contrast agent for PMCTA, demonstrating a "whole body" approach via catheters in the femoral vessels (**a**) and a targeted coronary approach (**b**) showing a patent right coronary artery (*arrow*) (Images courtesy of Morgan B & Rutty G, University of Leicester)

This chapter is the story of how arterial and venous contrast studies have been developed and how PMCT has been developed to PMCTA.

Contrast Agents

Any substance that alters the attenuation of an X-ray beam can be considered a contrast agent. We traditionally consider these to be agents that increase attenuation, appearing white (positive) on traditional CT images. These agents generally use substances that have a high atomic number, which increase attenuation of X-rays due to their density and the photoelectric effect. Therefore, relatively low concentrations of iodine solution or barium can have dramatic effects on the image.

The various types of contrast agent used in postmortem work have been summarized [17] and include corpuscular preparations such as barium sulfate, where the particles are suspended in water [28] or gelatin/agar [29]; lipophilic agents dissolved in oily liquids generally using iodine; casting techniques, possibly using a silicone rubber and lead oxide [30]; and the water-soluble iodinated chelates generally used in clinical imaging practice. However, an agent does not have to attenuate X-rays (positive contrast agent) in this manner to be useful. Clinically agents such as air or fat that lower the attenuation and appear black on traditional CT images are also used to improve contrast in a "negative" manner (Fig. 1.2).

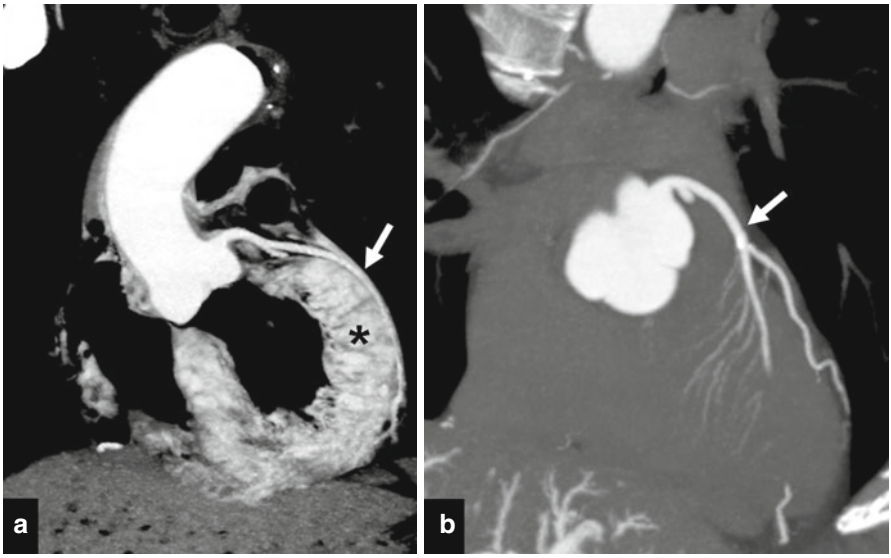

Fig. 1.3 A comparison of normal coronary artery and left ventricle studies by water-soluble contrast agent (**a**) and oily contrast agent (**b**). An opacified patent left anterior descending artery is shown in both cases (*arrow*). However, using water-soluble contrast agent leads to rapid leak of the contrast into the extracellular extravascular space (*). This is a key aspect of clinical contrast-enhanced imaging but may cause edema in the tissues if a lot of contrast is used, such as in whole body techniques, which may affect histology. Also the rapid dispersion diminishes vascular contrast requiring rapid imaging after injection (Images courtesy of Morgan B & Rutty G, University of Leicester)

While casting techniques are mostly only applicable on single organs, methods were developed to render visible the whole vascular system of a body, such as for investigating the vessels of human embryos and fetuses [19] and newborns [31]. Most methods used a simple manual injection via a syringe to introduce the contrast agent into the vascular system. Many recommendations were provided on how to perform the perfusion of single organs [17], but there were few in the last century regarding the perfusion of whole bodies. An exception is the article written by Stoeter and Voigt [19], which describes a radiologically controlled, discontinuous injection of the contrast agent, with angiography performed in the intervals.

All of these agents have their advantages and pitfalls. The key issues, whether using a corpuscular agent in suspension, an oily agent, or water-soluble agent, are related to their molecular size, viscosity, density, and osmolality, which all dictate how they disperse in the body (pharmacokinetics) (Fig. 1.3). In addition some agents such as the barium suspensions have properties that allow them to coat the walls of vessels, providing exquisite wall detail especially if the lumen is subsequently filled with a negative agent such as air. These factors will be discussed further for the techniques in the following sections.

Whole Body Infusion Angiographic Techniques in Switzerland

In 2005 Jackowski et al. from the Institute of Forensic Medicine, University of Bern, Switzerland, reported preliminary results of a new technique of minimally invasive whole body PMCTA using meglumine-ioxithalamate as a contrast agent and a roller pump, which enabled stable conditions for the injection of the contrast agent [32]. This water-soluble contrast agent solution provided excellent vessel visualization but caused tissue edema and artifacts in histological investigations when injected using their protocols, which rendered its application in medicolegal cases difficult. This was probably due to the rapid extravasation of these agents into the extravascular extracellular space and their high osmolality. In order to overcome the extravasation into the surrounding tissue, and therefore the massive edema caused, polyethylene glycol was added as a solvent [33].

At the same time the group in Bern and then Lausanne, Switzerland, started to devise a system that could deliver a postmortem circulation to resemble in vivo conditions and allow the perfusion of the body [34]. A first feasibility study, performed on an animal model, showed the success of the concept, with the use of diesel oil as a perfusate and a roller pump as perfusion device [35]. A postmortem circulation was established in adult dogs and cats. The oily contrast agent Lipiodol® Ultra-Fluide (Guerbet LLC, Bloomington, IN) was then injected during which perfusion imaging was performed at defined intervals giving a dynamic arterial, "parenchymal," and venous phase. Oily perfusates will remain intravascular, avoiding extravasation into the surrounding tissue causing edema [36, 37], and the images in the "parenchymal" dynamic phase will be different to those achieved using standard water-soluble contrast agents as the contrast does not enter the capillaries or leak into the tissue parenchyma. To adapt the technique to a human model, two essential changes were made: The perfusion device was changed from a roller pump to a modified heart-lung machine, and diesel oil was replaced by the odorless oil paraffinum perliquidum. The resulting technique was called "two-step postmortem angiography" [34], as it consisted in the establishment of a postmortem perfusion in a first step and the injection of contrast agent with simultaneous image acquisition as a second step. The obtained images displayed the vascular anatomy in detail, up to the level of arterioles. Vascular lesions such as chronic occlusion and traumatic vessel rupture could be detected [34, 38]. The major problem of the technique was the appearance of a discharge of the perfusate into the stomach and the intestine. This finding was not surprising given the combination of bacterial decomposition and autolytic activities that occurs in the gastrointestinal tract, which may lead to an early increase in vascular permeability in this region [35]. This weakness was also criticized by Ross et al. [39] who compared the application of an oily approach to the one proposed by Jackowski et al. [32]. The advantages of using oily liquids have been described [35]. Microscopic studies show that the oil blocks the capillaries due to fatty embolism, which are especially vulnerable to postmortem increases in permeability. The same principle is used in chemoembolization for cancer treatment by using chemotherapy in an oily medium, which is arrested in the small tortuous

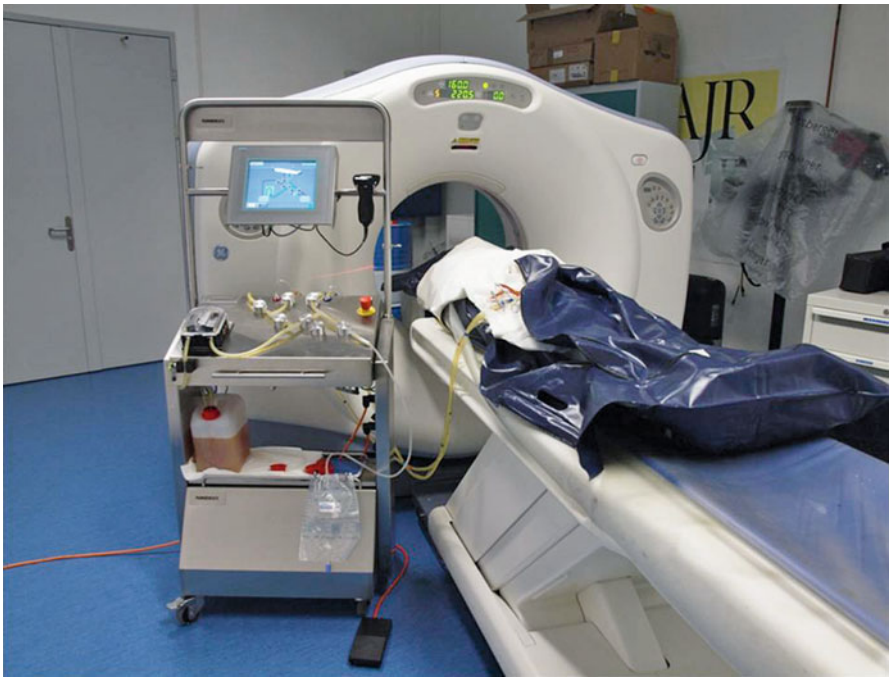

Fig. 1.4 Setup for whole body PMCTA showing the investigated body on the CT table; connected via the cannulas and perfusion tubes to a Virtangio® perfusion device (Images courtesy S. Grabherr, Lausanne, Sz)

neo-angiogenic vessels of a tumor thereby allowing higher exposure of the tumor to chemotherapy with less systemic exposure [40]. In the postmortem setting, the oil passes to the venous system through arteriovenous shunts. The level of this micro-embolization depends on the viscosity of the oily perfusion, which can be varied depending on what characteristics are required.

In the University Centre of Legal Medicine in Lausanne, Switzerland, a research group was created with the aim to develop these contrast agents and perfusates for PMCTA and to establish an easily applicable standardized protocol to achieve complete filling of the vascular system and decrease artifacts to improve diagnostic quality. In 2011, the group published their first study of 45 human cases using different perfusion protocols [41] calling their technique MPMCTA (multiphase postmortem computed tomography angiography). They used a perfusion device (Virtangio®, Fumedica AG, Sz) using a single-use set containing tube sets and cannulas inspired by the modified heart-lung machine, first used by Grabherr et al. in 2006 (Fig. 1.4) [35] and later by Ross et al. in 2008 [39]. A new oily contrast agent mixture (Angiofil®, Fumedica AG, Sz) was specifically developed by the research group for postmortem investigations [17, 38]. Chemically, the contrast agent Angiofil® is a mixture of esters (mainly ethyl esters) of polyiodinated fatty acids. It is yellowish, nearly odorless, and stable under normal conditions (room

temperature). As opposed to diesel oil [35] or paraffinum perliquidum [34], the contrast agent Angiofil® is dissolved in the more viscous paraffin oil (paraffinum liquidum). By changing the viscosity of the perfusate, extravasation into the gastrointestinal tract has virtually disappeared. By diluting Angiofil® with a solvent such as decane, its viscosity can be decreased so as to enter the capillaries to enable microangiography [42].

The study suggests the use of high perfusion volumes as well as the recording of at least three angiographic phases and a native CT scan. The standard protocol of MPMCTA consists therefore in the performance of one native CT scan followed by the cannulation of the femoral vessels of one side of the body. During the cannulation process, blood samples are collected for toxicological and biochemical analysis. Once these samples are done, contrast agent mixture composed of 6 % of Angiofil® and paraffin oil (paraffinum liquidum) is infused. The contrast (1,200 ml) is injected into the femoral artery at a flow rate of 800 ml/min prior to "arterial phase" image acquisition; 1,800 ml is then injected (800 ml/min) prior to "venous phase" image acquisition. Then a further 500 ml of contrast is injected at a flow rate of 200 ml/min to perform the "dynamic phase" image acquisition.

By using the technique of MPMCTA, the whole vascular system of the head, thorax, and abdomen is visualized (Fig. 1.1). The only exceptions comprise parts of the cerebral sinus and vessels, which may be occluded by large postmortem clots. In contrast to the earlier techniques using oily liquids, this method did overcome the problem of discharge of the perfusate into the stomach and the intestine, which was only slight, even in cases with a long time gap between angiography and autopsy. Further visualization of all vessels was possible without changing the position of the body, as proposed by Ross et al. [39]. Using three distinct phases of contrast is helpful to avoid misinterpretations, which may occur if only arterial and venous phases are performed [41]. This is similar to other dynamic diagnostic imaging tests, such as intravenous urograms and barium studies, where an abnormality should be consistent to be interpreted as a real finding.

The use of MPMCTA has increased in the last few years and has already been introduced as a routine investigation for medicolegal purposes in addition to conventional autopsy in many centers. Different studies have been performed investigating the advantages and inconveniences of MPMCTA compared to autopsy [43], technique-related artifacts [44], its performance compared to clinical in vivo CT angiography [45], its influence of biochemical markers [46], as well as its use for investigating different medicolegal case groups such as cases of sudden cardiac death [47] and coronary thrombosis [48] and cases of fatal outcome after surgical interventions [49].

The introduction of MPMCTA into medicolegal death investigation improves the accuracy of the postmortem exam compared to nonenhanced (native) PMCT alone and in some situations may even improve on conventional autopsy. For example, MPMCTA is more likely to detect a source of hemorrhage than conventional autopsy, even when not detected by premortem clinical CT angiography (Fig. 1.5). For these cases, the combination of conventional autopsy and MPMCTA has already been proposed as being a new gold standard.

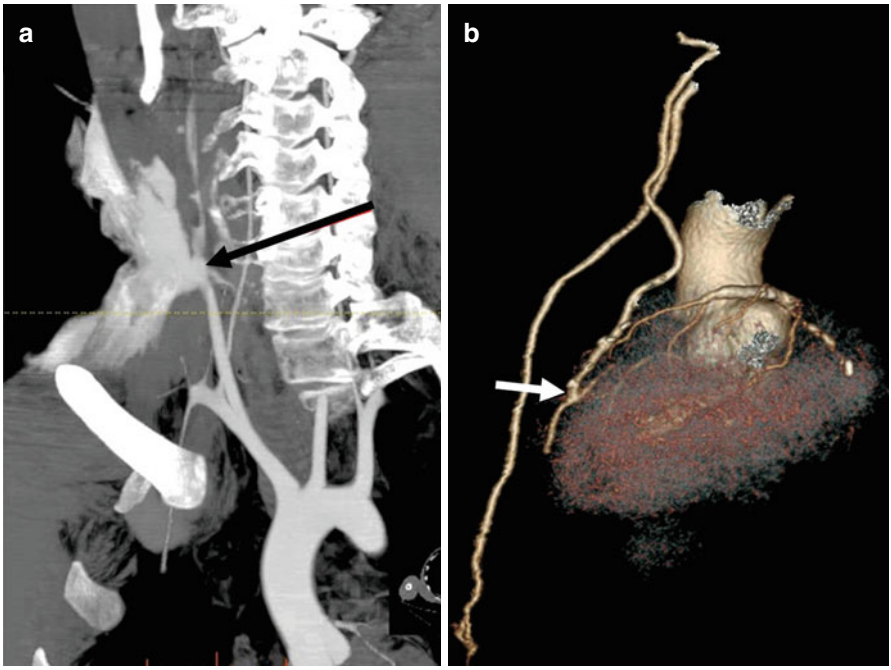

Fig. 1.5 Adult suicide using a knife. (**a**) An MIP image of arterial phase whole body PMCTA using oily contrast agent showing massive leakage of contrast agent from the right common coronary artery (*arrow*) and (**b**) 3D volume rendered image demonstrating an intact coronary bypass between the left internal mammary artery and the left inter anterior descending artery (*arrow*) (Images courtesy S. Grabherr, Lausanne, Sz)

PMCTA Using Cardiopulmonary Resuscitation (CPR) to Establish Circulation in Japan

In Japan, due to a very low rate of conventional autopsy and the highest distribution of CT scanners per population in the world, postmortem computed tomography (PMCT) is performed in more than 20,000 cases yearly [50]. The majority of these cases involve screening for unusual causes of death in patients brought into emergency rooms (ERs) in a state of cardiopulmonary arrest, where resuscitation was unsuccessful.

For PMCT, the Japanese experience is similar to other reported studies, showing that PMCT alone can give a cause of death in 30 % of cases of nontraumatic death, and is especially useful in hemorrhagic cases, including cerebral hemorrhage, aortic dissection, and aortic aneurysm rupture [51, 52]. However, as previously stated, ischemic heart disease is a factor in more than half of sudden and unexpected deaths in Japan, which is difficult to detect with PMCT. Therefore, a diagnosis of ischemic heart disease is made after review of clinical history, examination findings such as ECG, and the presence of pulmonary edema, an indirect PMCT finding suggesting

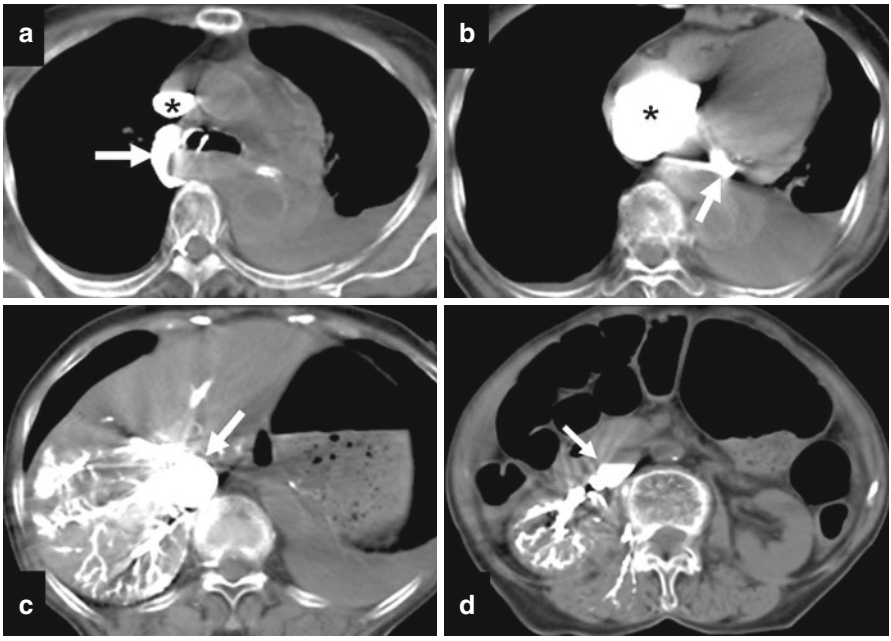

Fig. 1.6 PMCTA without using the CPR method in an 80-year-old female after a traffic accident. The contrast media infused via an intravenous catheter moved from the right brachiocephalic vein to the superior vena cava and right atrium (*), and refluxes into the azygous vein (**a**) and coronary sinus (**b**) (*arrows*), IVC and abdominopelvic venous system (**c**, **d**). Without CPR venously injected contrast media do not pass through the cardiopulmonary circulation (Reprinted with permission from Ezawa and Shiotani [72])

acute left heart failure [53]. It is also difficult to diagnose pulmonary thromboembolism with non-contrast PMCT.

The whole body and targeted PMCT angiography (PMCTA) techniques described in this chapter are customarily difficult to do in Japan where surgical management on a newly deceased patient is traditionally shunned. Instead, quite a few Japanese hospitals conduct PMCTA using a less invasive and relatively simple technique that can be simply done in a CT examination room in ordinary hospitals [54, 55]. The administration of contrast agent from the peripheral venous route alone does not provide diagnostically useful angiograms (Fig. 1.6), but PMCTA is possible in combination with chest compressions. Chest compression during cardiopulmonary resuscitation (CPR) increases blood pressure to a certain degree and generates cardiac output of approximately one-fourth compared with the normal state [56]. This phenomenon makes possible PMCTA with chest compression (CPR method). The contrast media is normally injected into an arm vein such as the cubital vein in the antecubital fossa; it enters the right atrium and moves into the right ventricle, pulmonary artery, pulmonary vein, left atrium, left ventricle, and aorta and then into the arterial tree including the proximal portions of the cerebral, coronary, celiac, and superior mesenteric arteries (Figs. 1.7 and 1.8). The PMCTA

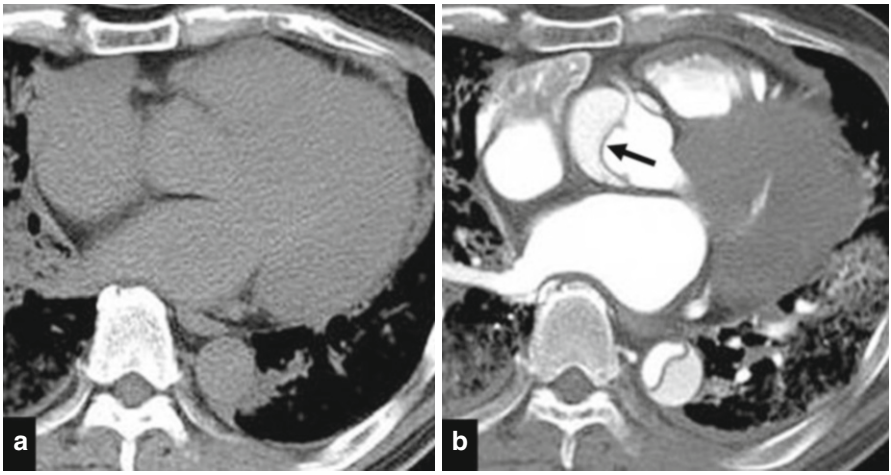

Fig. 1.7 PMCT (**a**) and PMCTA using the CPR method (**b**) in a 60-year-old male showing an intimal flap in the ascending aorta (*arrow*) due to aortic dissection (Images courtesy of Sakamoto N & Shiotani S, Tokyo, Japan)

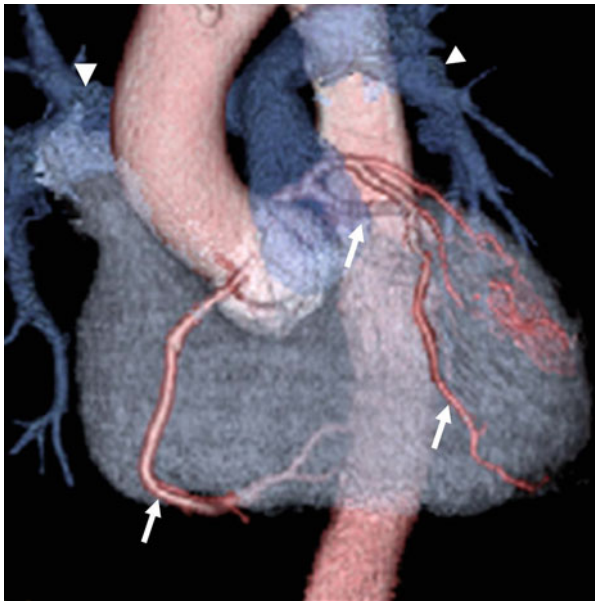

Fig. 1.8 PMCTA using the CPR method with 3D image reformats showing coronary (*arrows*) and pulmonary arteries (*arrowheads*) (Image courtesy of Sakamoto N & Shiotani S, Tokyo, Japan)

scan can therefore be performed immediately after death. This is helpful as in the Japanese experience, there is little extravasation of the contrast media within the 3-h period after death, but this increases thereafter, due to increasing postmortem vascular permeability.

The cases shown in Figs. 1.7 and 1.8 are patients arriving at the ER of Tokyo Medical Center in a state of cardiopulmonary arrest (CPA) with failed resuscitation.

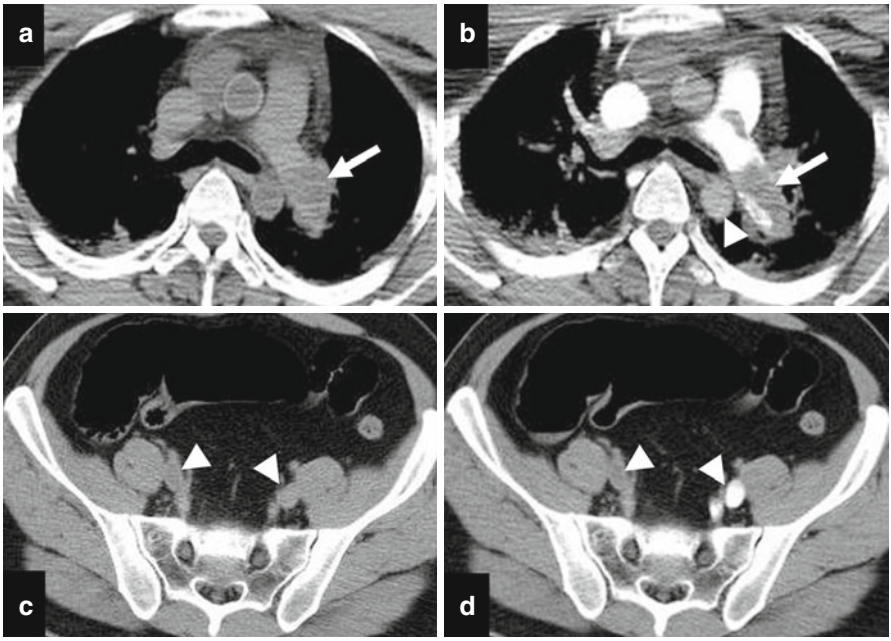

Fig. 1.9 PMCT (**a**, **c**) and PMCTA using CPR method (**b**, **d**) in a case of pulmonary thromboembolism in a male in his 40s showing an embolus in the pulmonary artery (*arrow*) and no contrast in the aorta. Pelvic images (**c**, **d**) show failure of filling of the right common iliac vein despite filling on the left (*arrowheads*). Despite this demonstration, thromboembolism remains a difficult problem for all PMCTA techniques due to variable presence of postmortem clot (Images courtesy of Sakamoto N & Shiotani S, Tokyo, Japan)

After confirmation of death, consent to perform non-contrast PMCT and PMCTA was obtained from the family of each subject. An automatic injector is used with the intravenous catheter already in place after cardiopulmonary resuscitation. Standard clinical contrast media are used at similar strengths and volumes to normal clinical CT practice, e.g., 2 ml/kg, up to 150 ml, at a rate of 1–2 ml/s. While injecting the contrast media, chest compressions are done for 2 min at a rate of 100 times/min (a total of 200 times). If on scanning passage of contrast is deemed insufficient, 200 or fewer additional chest compressions are done. However, if the total number of chest compressions exceeds 400, image contrast is degraded due to the widespread contrast media. Invasiveness to the cadaver is minimal, as only about 2 min of chest compression is done after death, compared with approximately 30 min of chest compression during the failed resuscitation management in the emergency room. In cases where chest compression cannot be effectively done, such as a multiple rib fracture case, contrast enhancement is insufficient due to lack of effective perfusion of the media in the body.

This CPR method of PMCTA allows diagnosis of arterial and occasional venous based pathology. Fig. 1.9 shows a case of filling defects in the pulmonary artery and right common iliac vein with failure of passage on contrast agent into the aorta

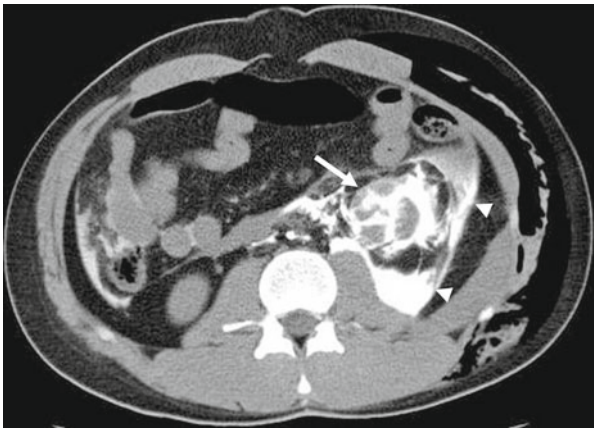

Fig. 1.10 PMCTA using CPR method in an 30-year-old male after a traffic accident showing left kidney trauma (*large arrow*) and leak into the perirenal space and retroperitoneum (*arrowheads*)

because thromboemboli hinder its passage. Using this technique it is assumed that these filling defects are not due to postmortem clot as the scans are performed early and a large quantity of tissue plasminogen activator is secreted from the vascular endothelium during sudden death, thereby increasing blood fluidity.

In cases where blood loss volume is large due to hemorrhage, CPR does not establish effective circulation and contrast enhancement of the arterial route is insufficient and reflux to the veins increases [57]. This can be useful in trauma cases as it may show organ injuries as extravasation of the contrast media (Fig. 1.10).

Targeted Coronary Angiography Techniques in the UK

There is a requirement that a medical cause should be given for all deaths in England and Wales [58]. The medical professional attending the final illness often does this, but may not be able to in cases of sudden unsuspected death. Therefore, about 45 % of cases are referred to Her Majesty's (HM) Coroner, who will often request a post-mortem investigation, normally by autopsy, to establish the cause of death, even when obviously from natural causes. A considerable number of these deaths will be due to cardiac and particularly coronary vascular disease.

Therefore, without the ability to diagnose coronary artery disease (CAD), the most common cause of non-suspicious sudden death in our population, PMCT may only be able to make a diagnosis confidently in 28–41 % of cases [13], such as for catastrophic hemorrhage. However, by combining PMCT results with detailed knowledge of the circumstances of death, the diagnosis is likely to be correct in up to 60 % [13, 14]. In clinical practice, cardiac MDCT including contrast-enhanced CT coronary angiography is fast emerging as a powerful diagnostic tool for the assessment of coronary disease in both acute and chronic cases [59–61].

If minimally invasive autopsy using PMCT is to be implemented for routine coronial autopsies, the numbers of cadavers to be examined will run into thousands

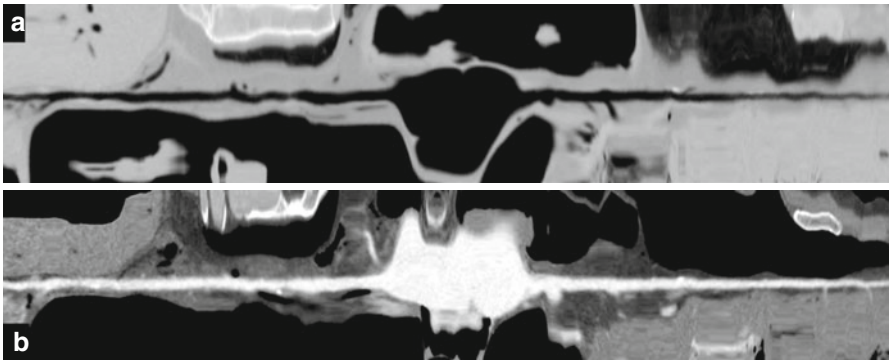

Fig. 1.11 Targeted coronary PMCTA with air (**a**) and water-soluble contrast agent (**b**) showing mildly diseased right and left coronary arteries reconstructed using straightened curved MPR (cMPR) (Images courtesy of Morgan B & Rutty G, University of Leicester, UK)

and complex approaches, such as whole body multiphase PMCTA techniques as described previously, may be impractical. Independently two centers in the UK decided to augment PMCT by direct contrast injection into the ascending aorta and therefore the coronary arteries. In both cases the objective was to provide a straightforward method of investigating for coronary artery disease that was quick, cost-effective and easy to perform [62, 63]. Both techniques describe cut down into the left carotid artery and direct insertion of a catheter of the type used for bladder catheterization. The catheter balloon is then inflated in the ascending aorta so contrast can be injected into the aortic root. Backflow occurs into the left ventricle but is resisted by the aortic valve and the compliance of the ventricle muscle and mitral valve. This allows filling of the coronary arteries.

Both techniques use standard clinical water-soluble contrast agents dissolved in water rendering them hypo-osmolar. They have not observed the same problems with widespread tissue edema affecting post procedure histological analysis [64], but the quantity of agent used is less than for whole body techniques and the concentration of iodine (mgI/ml) used is also less.

The techniques differ in their approach. The technique developed in Oxford uses only positive contrast media and makes efforts to avoid air within the vessels [63]. The system developed in Leicester, however, deliberately uses both air and contrast media (Figs. 1.2b, 1.3a, and 1.11). Another difference of the two techniques, and a potential advantage of this approach, is the use of standard clinical CT power contrast injectors to image the coronary vessels dynamically during contrast injection, which has the potential to mimic physiological pressure and provide a more meaningful assessment of vessel stenosis [64, 65]. The technique involves accessing the left carotid artery in the mortuary by cut down using a modified 14Ch Foley urinary catheter inserted by the study technician [62]. In the CT scanner the position of the Foley catheter in the ascending aorta is confirmed and the (large) balloon is inflated. A standard clinical pump injector is then used to inject 300 ml air at 6 ml/s, two times supine and then once in the right lateral decubitus position. Contrast

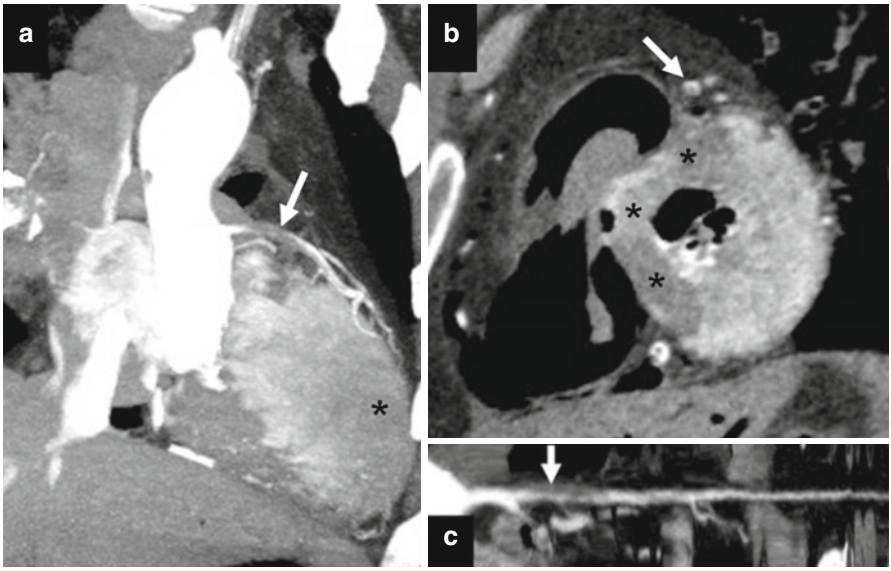

Fig. 1.12 Targeted coronary PMCTA using water-soluble contrast agent: (**a**, **c**) shows a mixed plaque occluding the left anterior descending artery (*arrows*) and (**b**) an associated perfusion deficit in the anterior septal myocardial wall (*). This sign is helpful but not specific to infarction as perfusion deficits may be seen in normal myocardium (Images courtesy of Morgan B & Rutty G, University of Leicester, UK)

(Urografin® 150 mg/ml; Bayer Healthcare, positive contrast) diluted 1:10 is then injected, first in the right lateral decubitus position and then supine. Imaging is performed to run concurrent with the end of the injection so images are acquired while the arteries are under pressure [65].

Studies comparing these techniques with autopsy show that they perform well in identifying significant ischemic heart disease (Fig. 1.12) [63, 66], and they also correlate well with detailed microscopic and histological investigations of the vessels [64]. This is in agreement with whole body approaches [47]. In fact by investigating the vessels under pressure, this technique may outperform pathology, where heavily calcified vessels may appear stenotic to the pathologist due to the trauma of sectioning the vessel for pathology assessment (Fig. 1.13) [64]. A further advantage of the localized targeted approach is that there is no significant effect on biochemical and toxicological analysis performed post procedure [67].

One issue relating to all PMCTA techniques is that in clinical cardiac angiography and CT cardiac angiography, qualitative assessment of stenosis does not necessarily correlate with clinical significance [68, 69]. It is therefore debatable that critical coronary artery stenosis alone, diagnosed by PMCTA, can be used to diagnose cause of death. However, there is evidence that severe coronary artery disease does predict for coronary occlusion [70] and even at autopsy it can be very difficult to be certain of the cause of sudden death, unless there is a clear occlusive thrombus. For this reason the cause of death is more commonly attributed to "ischemic heart

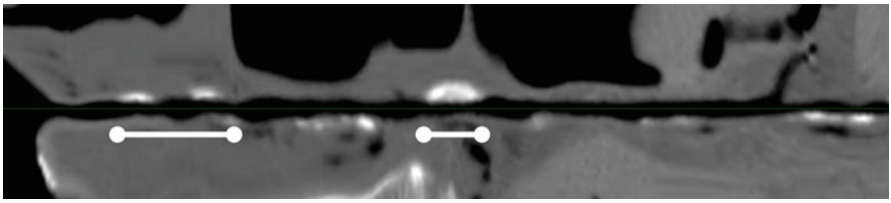

Fig. 1.13 Targeted coronary PMCTA with air showing a diseased coronary artery reconstructed using straightened curved MPR (cMPR). The areas denoted by a white line were reported as critical (>75 %) stenosis on pathological examination but are more patent after distension by air (Images courtesy of Morgan B & Rutty G, University of Leicester, UK)

disease" on the "*balance of probabilities*" rather than specifically to cardiac occlusion, if no other cause of death is ascertained [58]. In fact, in sudden death from cardiac occlusion, myocardial infarction cannot be diagnosed with absolute certainty at autopsy if death is caused by immediate causes such as arrhythmia, and similarly the presence of thrombus is not proof of "cause and effect" as thrombus has been seen in cases of noncardiac death [71].

For any investigation if the initial test gives no answer, then further testing would be performed. However, an important caveat in the use of PMCT and PMCTA, and even for autopsy, is that if two potentially lethal pathologies are present and only one is demonstrated, such as a critically stenosed coronary artery, then this may be given as the single cause of death "*on the balance of probabilities*," and the real cause of death may be missed. For PMCTA fatal pulmonary thromboembolism could be missed and therefore misdiagnosed in the presence of severe unrelated coronary artery disease. However, PMCTA, in combination with the correct clinical situation, a thorough external examination and toxicology if necessary, is unlikely to miss unnatural death.

Conclusion

These three methods provide distinct ways of providing vascular and, to a lesser extent, parenchymal contrast enhancement information to postmortem CT. All methods could be adopted in any unit providing forensic services, even when using hospital clinical scanners. The choice of method will rest on several factors, including cost, local experience, time available on the scanner, and local cultural and religious preferences. Ideally, however, the choice will also rest on using the most suitable method for the individual case. As can be seen from the development of these methods, modifications are possible to the choice of contrast media that would change the information achieved and therefore would allow each method to be further refined for the individual case. The authors have no doubt that the use of these contrast-enhanced methods will further strengthen the role of PMCT in forensic investigation.

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Chapter 2
Death by Hanging

Anny Sauvageau

Introduction

Strangulation is defined as a form of asphyxia by closure of the blood vessels and/or air passages of the neck as a result of external pressure on the neck. All authors recognize at least two types of strangulation: ligature strangulation and manual strangulation [1]. Until recently, the place of hanging within this classification was, however, more problematic. Three different types of classification were encountered: hanging as a type of strangulation, hanging as a subtype of ligature strangulation, and hanging and strangulation as different unrelated entities [1].

In an effort to standardize the classification of asphyxia, a unified system of classification was constructed in 2010, by drawing upon mainstream definitions from the literature [1]. The comparison of all the previous classifications and definitions in the forensic literature is out of the scope of the present chapter. This is presented in detail by Sauvageau and Boghossian elsewhere [1]. The following year, an international consultation on the proposed new classification of asphyxia was conducted. This new classification of asphyxia, called the "INFOR (International Network for Forensic Research) classification," is presented in Fig. 2.1 and Table 2.1. The INFOR is a research group founded in 2010 by Dr. Duarte Nuno Vieira and Dr. Anny Sauvageau. The INFOR classification has not been published yet but has been presented at the International Association of Forensic Sciences in Madeira in 2011 and in 2012 at the meetings of the American Academy of Forensic Sciences and of the National Association of Medical Examiners. According to this internationally supported classification, all asphyxias by external pressure on the neck are regrouped under the term "strangulation." Three types of strangulation are recognized, depending of the source of this pressure: "hanging" (pressure on the neck is applied by a constricting band tightened by the gravitational weight of the body or part of

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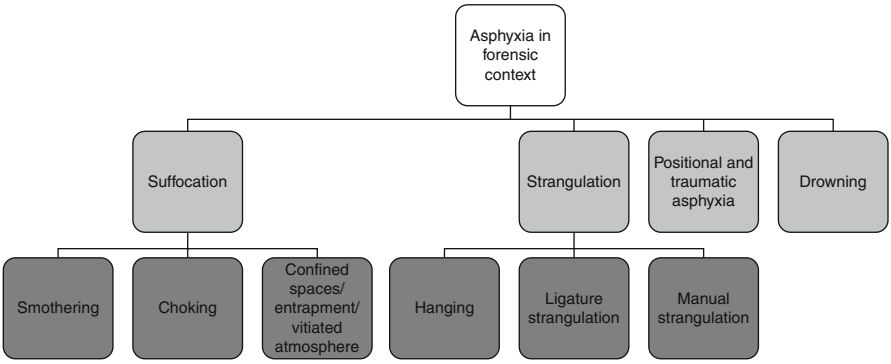

Fig. 2.1 INFOR classification of asphyxia

the body), "ligature strangulation" (pressure on the neck is applied by a constricting band tightened by a force other than the body weight), and "manual strangulation" (pressure by hands, forearms, or other limbs). Additional terms such as "throttling," "garrotting," or "mugging" are encountered from time to time in the forensic literature. Throttling refers to strangulation, usually by hand or more rarely by ligature. Garroting is a former type of Spanish judicial execution with tightening of a noose around the neck by twisting a rod within the ligature; by extension, it is sometimes use as a synonym of ligature strangulation. Mugging originally referred to strangulation by an arm crooked around the victim's neck from the rear; the meaning has been extended over the years to encompass any kind of robbery with violence. These various expressions originated from specific contexts, with a progressive widening of their meaning over time. It is recommended to avoid these terms in classifying asphyxial death cases.

In the new INFOR classification, it is recommended that all asphyxial deaths caused by external pressure on the neck structures be labeled "strangulations." For example, a drunken individual who passes out with his neck resting on the transverse bar of a chair should not be ruled out as positional asphyxia but should be ruled out as strangulation. Furthermore, when the strangulation cannot be classified as any of the three types (manual, ligature, hanging), it must be considered a "strangulation not otherwise specified." In the case of a hanging with a fall from height (in which cases death is usually caused by fracture dislocation of the upper cervical vertebrae), there is no clear agreement at this time if it should be considered to belong to the classification of asphyxia or not. Further consultation is needed on this point.

Pathophysiology of Hanging: A Historical Review

Most of the contemporary understanding of the pathophysiology of hanging and strangulation is still based on old European studies from the end of the nineteenth century and beginning of the twentieth century. A brief summary of this historical

Table 2.1 Definitions of terms in the INFOR classification

Term	Definition
Suffocation	A broad term encompassing different types of asphyxia such as vitiated atmosphere and smothering, associated with deprivation of oxygen
Smothering	Asphyxia by obstruction of the air passages above the epiglottis, including the nose, mouth, and pharynx[a]
Choking	Asphyxia by obstruction of the air passages below the epiglottis[a]
Confined spaces/entrapment/vitiated atmosphere	Asphyxia in an inadequate atmosphere by reduction of oxygen, displacement of oxygen by other gases or by gases causing chemical interference with the oxygen uptake and utilization
Strangulation	Asphyxia by closure of the blood vessels and/or air passages of the neck as a result of external pressure on the neck
Hanging	A form of strangulation in which the pressure on the neck is applied by a constricting band tightened by the gravitational weight of the body or part of the body
Ligature strangulation	A form of strangulation in which the pressure on the neck is applied by a constricting band tightened by a force other than the body weight
Manual strangulation	A form of strangulation caused by an external pressure on the structures of the neck by hands, forearms, or other limbs
Positional or postural asphyxia	A type of asphyxia where the position of an individual compromises the ability to breathe
Traumatic asphyxia	A type of asphyxia caused by external chest compression by a heavy object
Drowning	Asphyxia by immersion in a liquid

[a]The epiglottis has been accepted as the anatomical landmark between smothering and choking by only a low majority in the international survey. Further consultation work will be performed by INFOR on these two definitions

context will be presented here; but for an in-depth analysis of the historical evolution of pathophysiological hypotheses, it is recommended to consult Clement et al. [2].

In France in 1870, Tardieu concluded that the mechanism of death in hanging was respiratory asphyxia [3]. He was convinced that the asphyxia was caused by an obstruction of the trachea and not by closure of the blood vessels, based on his observation that intimal tears of the carotid arteries and congestion of the brain were uncommon autopsy findings. In the following decade, Hoffman, an Austrian author, shared this view that the asphyxia was by obstruction of the airways [4]. However, he also observed he was unable to infuse the neck vessels of hanging victims and concluded that the external pressure of the neck ligature was also creating vascular impairment. In Germany in 1886, Langreuter conducted hanging experimentations by tying a rope around the neck of cadavers who had died of natural causes [5]. He reported observing the epiglottis and the base of the tongue being pushed back against the posterior pharyngeal wall, obstructing the airways.

Based on the experimentation of Langreuter, Dixon supported the hypothesis of airways obstruction as the mechanism of death in hanging [6]. To further corroborate this view, this English author also reported the case of one of his colleague's, Ecker: The examination of the frozen body of a hanged man had revealed similar anatomic evidence of obstruction of the airways at autopsy. Dixon was convinced that the main mechanism was a respiratory asphyxia, but he thought the occlusion of the vessels might also play some minor role. To support the role of a vascular occlusion, he reported the work of Reineboth in 1895. Reineboth had conducted experimentation with tracheotomized rabbits and observed that even if the noose was tightened above the level of the tracheotomy opening, the rabbits still died. There was confusion in the scientific world at this time since this rabbit model was contradicted by two dog models. This contradiction would not be surprising nowadays as we now know dog models of hanging are not adequate for studying human hanging [7]: In dogs, the internal carotids are relatively less well developed, and the vertebral arteries contribute to the majority of the cerebral circulation; furthermore, dogs have highly developed anastomoses from the external carotids contributing to collateral blood supply to the brain.

Around the same time, Dixon was promoting airway obstruction as the mechanism of death; two English authors were establishing a distinction on this regard [8]. For Guy and Ferrier, complete hangings were associated with airway obstruction, but they believed that occlusion of the blood flow to the brain played a larger role in partial hangings.

Most of the contemporary views of the pathophysiology of hanging come from Brouadel and Lacassagne, who conducted multiple experimentations at the end of the nineteenth and beginning of the twentieth centuries, respectively [9, 10]. Brouadel demonstrated by experimentation of cadavers that the jugular vein is occluded by a pressure of 2 kg, the carotid artery by a pressure of 5 kg, the airways by a pressure of 15 kg, and the vertebral artery by a pressure of 30 kg. Based on animal tracheotomy and hanging experimentation, Brouadel confirmed the important role played by the occlusion of blood vessels in the pathophysiology of hanging. As for Lacassagne, he confirmed the pressure of 15 kg to occlude the trachea but shared a view closer to the one of Guy and Ferrier: Complete hangings were causing mainly respiratory asphyxia, whereas incomplete hangings were mainly associated with a compression of the blood vessels of the neck. For most authors of this era, it was thought that death by complete hanging would be significantly quicker than death by incomplete hanging, an assumption that would persist in the forensic world until the next millennium.

Despite the important work of Brouadel and Lacassagne, the role of respiratory versus circulatory asphyxia remained a controversial topic, with Derome favoring the vascular etiology based on his tracheotomized rabbit model [11], whereas Balthazar [12], Smith [13], and Webster [14] continued to maintain that the major element of the mechanism of death in hanging was that of respiratory asphyxia.

The possibility of a different mechanism of death was explored by several authors, cardiac inhibition by stimulation of the pericarotid nerves. This hypothesis was believed possible in some cases by Hoffman, Brouadel, Lacassagne, Balthazar, Smith, Webster, and Derome. The hypothesis was, however, rejected by Tardieu, Dixon, and Guy and Ferrier. For this second group of authors, experimentation and observation clearly revealed persistence of cardiac activity after the total cessation

of body movement and respiration, therefore infirming the hypothesis of a cardiac inhibition by vagal stimulation.

The agonal sequence documented within the forensic literature was generally vague or omitted. Brouadel presented the most detailed opinion on the time to die by hanging. According to him, it would take 5–10 min in a respiratory asphyxia (complete hanging), 12–20 min in a vascular asphyxia (incomplete hanging), and 15–20 min in a death by cardiac inhibition.

The Working Group on Human Asphyxia

Despite great advances in the forensic sciences in the last few decades, our understanding of the pathophysiology of hanging, as summarized previously, is still largely based on historical writings and experimentation from the end of the nineteenth and beginning of the twentieth centuries [2]. Since that time, there were very limited advances in our knowledge of the pathophysiology of hanging until the recent studies of filmed hangings by the Working Group on Human Asphyxia.

The Working Group on Human Asphyxia was founded in 2006 to systematically review and compare video recordings of lethal human hanging. Dr. Sauvageau founded the Working Group on Human Asphyxia in 2006, with an invitation to all forensic experts to join the group by sharing a filmed asphyxia. Dr. R. Laharpe (Switzerland) and V. J. Geberth (USA) were the first two contributors to join, followed by Dr. D. King (Canada), Dr. G. Dowling (Canada), Dr. S. Kelly (USA), Dr. C. Ambrosi (USA), and Dr. M. Benecke (Germany). The contribution of others is currently under review. In 2009, the scope of the group was extended to include nonlethal filmed human hanging and other types of filmed human asphyxia. Despite the efforts and advances of the group, it is recognized that work still needs to be undertaken before the forensic pathology world has a complete comprehensive understanding of the pathophysiology of hanging and other types of strangulation. This research group's work has, however, advanced significantly the body of knowledge available with this field of practice in a relatively limited amount of time. Apart from the work of the Working Group on Human Asphyxia, two papers on lethal filmed hangings also exist in the forensic literature: Risse and Weiler [15] and Yamasaki et al. [16]. These papers are, however, isolated case reports of one case each. The advantage of the studies by the Working Group on Human Asphyxia is the amount of cases reviewed by this group. Without the Working Group on Human Asphyxia, the agonal sequence in hanging would still remain unclear, and data would have remained anecdotic.

The Agonal Sequence

The agonal sequence that usually occurs in hanging is now well documented (Table 2.2) [17]. Considering the time of the initial application of the force to the neck to be time 0, the victim will lose consciousness on average in 10 ± 3 s.

Table 2.2 The agonal sequence in strangulation (based on a review of 14 cases of filmed hanging)

	Average time
Loss of consciousness	10 s ± 3 s
Convulsions	14 s ± 3 s
Decerebrate rigidity	19 s ± 5 s
Start of deep rhythmic abdominal respiratory movements	19 s ± 5 s
Decorticate rigidity	38 s ± 15 s
Loss of muscle tone	1 min 17 s ± 25 s
End of deep rhythmic abdominal respiratory movements	1 min 51 s ± 30 s
Last muscle movement	4 min 12 s ± 2 min 29 s

Fig. 2.2 This still from a taped autoerotic death shows the victim in *decerebration rigidity* (Reprinted with permission from Geberth [32])

Generalized tonicoclonic convulsions follow at 14 ± 3 s. A complex pattern of decorticate rigidity and decerebrate rigidity is then observed. Decerebrate rigidity (Fig. 2.2) is a postural attitude characterized by a full extension of the upper and lower limbs, with extension of the hips and knees, adduction of the legs, internal rotation of the shoulders, extension of the elbows, hyperpronation of the distal parts of the upper limbs with finger extension at the metacarpophalangeal joints, and flexion at the interphalangeal joints (marked extensor rigidity of all extremities). In decorticate rigidity (Fig. 2.3), there is an identical marked extensor rigidity of the legs but combined with rigidity of the flexors of the arms, with the arms

Fig. 2.3 This still from a taped autoerotic death shows the victim in *decortication rigidity* (Reprinted with permission from Geberth [32])

flexed and bent on the chest, the hands clenched into fists. Decerebrate rigidity usually happens first, at 19 ± 5 s, and there are from one to three occurrences of decorticate rigidity, the first one being observed on average at 38 ± 15 s. In some cases, however, the first phase of decorticate rigidity precedes decerebrate rigidity. Synchronously with the decerebrate rigidity, at approximately 19 ± 5 s, very deep abdominal respiratory movements are observed and heard. The victim is heard breathing loudly and regularly while the abdomen is seen contracting rhythmically, creating a rocking of the midsection. At 1 min 17 s ± 25 s, the muscle tone is lost and the body becomes flaccid and limp. The deep rhythmic abdominal respiratory movements cease at 1 min 51 s ± 30 s. Isolated muscle movements are observed from time to time, with the last observable movement happening at 4 min 12 s ± 2 min 29 s.

The usual pattern of decerebrate rigidity preceding decorticate rigidity deserves a further comment. Decerebrate rigidity indicates lesions at the midbrain level, the midbrain being vascularized by tributaries of the vertebral arteries. Decorticate rigidity on the other hand is indicative of impairment to the cerebral cortex, the premotor areas of the cerebral cortex being vascularized by tributaries from the carotid. Considering that the carotid arteries and jugular veins are more prone to occlusion in most hanging than the deeply located vertebral arteries, it would have been logical to assume that decerebrate rigidity should be the first to appear. This is, however, not the case. Further research is needed to understand this observation.

Interference of the Vestibular System in the Postural Attitudes

This agonal sequence is observed with striking similarities in almost all filmed hangings documented so far. The only exception to this sequence was the unusual case of a hanging without decerebrate and decorticate rigidities [18]. A 52-year-old man hanged himself by completely suspending himself from a ring in the ceiling. When he stepped off the stool, the movement of the body stepping off the stool created a rotary movement around the ceiling's ring, and the body started to revolve around the ring. Apart from the rolling around the ceiling's ring, the body stayed motionless for the duration of the movie, and no decerebrate or decorticate rigidity was observed. It was suggested that the lack of usual motor responses in this particular case might have been caused by the vestibular stimulation of the rotary movement. It is well known that the decerebrate and decorticate postural reflexes are triggered by three types of afferent sensory information: muscle proprioceptors (sense changes of length or tension in muscles), vestibular receptors (detect head motion), and visual inputs (sense movement in the visual field). The vestibular system plays a particularly important role in the postural attitude of decerebrate and decorticate rigidities. In animal models, cutting the vestibular nerves or destroying the labyrinths abolishes the input from the vestibular system and reduces decerebrate rigidity. Stimulating the anterior lobe of the cerebellum also reduces decerebrate rigidity. In this exceptional case of hanging without decerebrate and decorticate rigidity, the revolving movement of the body is hypothesized to have interfered with the development of the postural attitude.

The Consequences of the Agonal Sequences on the External Examination

The development of decerebrate and decorticate rigidities are accompanied by relatively sudden wide range movements of the limbs. In enclosed environments such as staircases or closets, the arms are seen and heard banging on walls [19]. Bruises and abrasions have been described as a normal constituent of death by hangings. The usual pattern of bruises in hanging (Fig. 2.4) is the posterior aspect of the upper limbs and the anterior aspect of the lower limbs [19]. The presence of bruises on the anterior part of the upper limbs or the posterior part of the lower limbs is not considered usual injuries accompanying the agonal sequence itself, and further violence by an aggressor should be investigated. There are several other external and internal findings potentially associated with hanging. These are to be found in every forensic textbook and will not be discussed here.

Time to Death

The time to die by hanging is often said to be approximately 3–5 min. There is, however, no forensic study to sustain this estimate of 5 min [20]. This number appears to be based on three types of studies: a series of near-hanging victims in

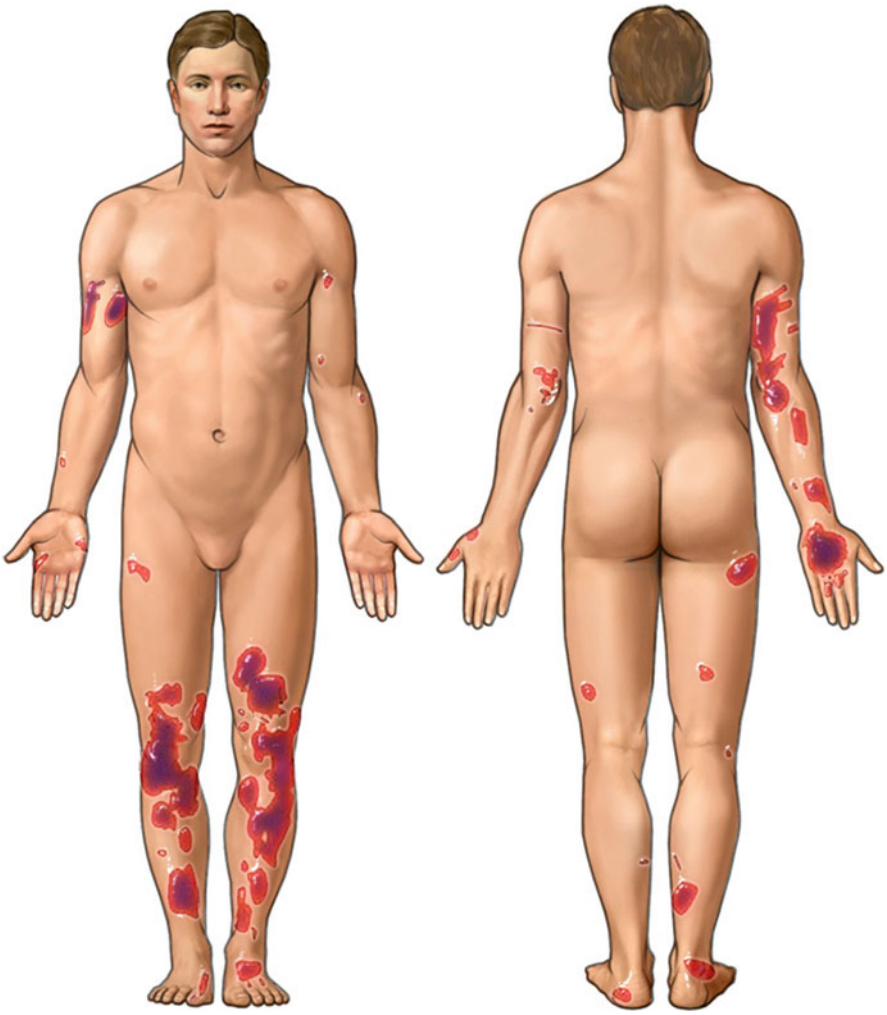

Fig. 2.4 Usual pattern of bruises in hanging (Reprinted with permission from Sauvageau and Geberth [33]. Illustration Courtesy of Medical Legal Art "Illustration Copyright 2010 Medical Legal Art www.doereport.com")

emergency medicine, studies of carotid endarterectomy, and physiopathological studies of brain ischemia. A summary of the studies that led forensic experts to think death was in 4–5 min, and why these studies are not valid to sustain such and assertion will be presented here. A complete review can be found in Sauvageau et al. [20].

There are a few studies that evaluated the outcomes of unsuccessful attempts at hanging (so-called "near-hanging" victims). Of these studies, four correlate the duration of the hanging with the outcome [21–24]. The studies on near-hanging victims are doomed by a strong flaw: The duration of hanging is based on the patient's recollection or on the time the patient was last seen. These studies are thus not precise enough to be valid to estimate the time to die by hanging.

Carotid endarterectomy studies have also been used to support the time to death in hanging. These studies are numerous and clearly demonstrate that unilateral carotid clamping can be tolerated without irreversible damage for more than 10 min in most patients [25–28]. However, very limited information about hanging can be drawn from these studies. The studies of carotid endarterectomies are not a good source of data for hanging since the carotid clamping is unilateral and not combined with obstruction of the venous return.

The fact that neurons undergo irreversible damage in 3–5 min of ischemia whereas myocardial cells can survive for 20–30 min is well known to all pathologists [29]. Less known is that the timeline to neuron death is based on animal studies: There is no study to document a threshold of 3–5 min of ischemia to cause irreversible brain damage in human beings [20]. Studies of brain ischemia have provided thresholds for ischemic brain damage in rodents, pigs, cats, dogs, and nonhuman primates, but there is no study on the threshold of ischemia to cause irreversible brain damage in humans.

As it would be unethical to undertake live human experimentation to further consider this important pathological question, despite the time to die by hanging been often said to be 3–5 min, there are no convincing scientific studies to validate this assertion [20]. There is no scientifically valid answer at this time on the time required to die by hanging and strangulation. There is, however, some partial answer on the time to irreversibility that came once again from the study of filmed hangings by the Working Group on Human Asphyxia.

Time to Irreversibility

The majority of filmed interrupted hangings or ligature strangulations are revealing victims losing consciousness and presenting convulsions with disruption of the strangulation process in its very early stage followed by full recovery of the victim. These short nonlethal filmed strangulations are unfortunately not very informative as to the time to irreversibility. Two nonlethal filmed hangings, however, are very informative.

The first one is the case of a 35-year-old autoerotic practitioner that hanged himself from a shower rod by using a pair of pajama pants as a ligature [20]. The man is seen going through the first three phases of the agonal sequence (loss of consciousness, convulsions, and decerebrate rigidity). The pants were not tied strongly to the shower rod and the man fell to the ground, interrupting the hanging. He then quickly regained consciousness and presented a full recovery without any noticeable symptoms. This first film demonstrated that the agonal sequence is reversible at least up to the decerebrate rigidity.

The second interesting nonlethal filmed hanging is a sadomasochistic film showing a young volunteer woman stepping off a chair to a complete suspension hanging. This unpublished case has been reviewed by the Working Group on Human Asphyxia, with the special collaboration of Mark Benecke and Lydia Ewelin Benecke. The

Table 2.3 The point of irreversibility in the agonal sequence

	Average time	Reversible or not
Loss of consciousness	10 s ± 3 s	Yes
Convulsions	14 s ± 3 s	Yes
Decerebrate rigidity	19 s ± 5 s	Yes
Start of deep rhythmic abdominal respiratory movements	19 s ± 5 s	Yes
Decorticate rigidity	38 s ± 15 s	Yes
Loss of muscle tone	1 min 17 s ± 25 s	?
End of deep rhythmic abdominal respiratory movements	1 min 51 s ± 30 s	?
Last muscle movement	4 min 12 s ± 2 min 29 s	?

woman is seen going through the following agonal sequence: loss of consciousness (15 s), convulsions (16 s), decorticate rigidity (17 s), and deep rhythmic abdominal respiratory movements (18 s). A witness in the room finally realized that something was wrong and pulled the rope down. The hanging is interrupted at 44 s. Decerebrate rigidity is observed at 45 s and a second phase of decorticate rigidity at 50 s. The body relaxes to a neutral position at 1 min 16 s, and she regains consciousness, without any sequela, and gets up at 1 min 30 s. This second case demonstrates that the agonal sequence of hanging is reversible up to the point of decorticate rigidity.

The point of irreversibility in hanging and strangulation remains unknown at this time (Table 2.3): Is it the loss of muscle tone (1 min ± 25 s), the end of the deep rhythmic abdominal respiratory movements (1 min 51 s ± 30 s), or the last isolated muscular movements (4 min 12 s ± 2 min 29 s)? At this point in time, the only honest and scientifically valid answer to how long it takes to suffer irreversible damage in hanging and other forms of strangulation remains that we unfortunately do not know. Series of nonlethal filmed hangings have demonstrated, however, that death by hanging is not a rapid type of death and that neck constriction up to decorticate rigidity have been tolerated with uncomplicated recoveries.

Impact of Various Factors on the Agonal Sequence

When comparing the time required to die in a hanging with complete suspension versus a hanging with incomplete suspension, it would seem logical to assume death would be faster in complete suspension, considering the weight on the ligature and the degree of occlusion of the structures of the neck is greater in the complete form. Studies of filmed hangings do not support this assumption [17, 30]: The agonal sequence does not seem to differ significantly between complete and incomplete suspension nor between different positions of incomplete suspension (e.g., upright or kneeling).

Expert witnesses sometimes state in court that a victim intoxicated by ethanol will die more quickly from strangulation than a sober victim [17]. There is no

Table 2.4 Timing of the agonal responses: comparison of autoerotic hangings versus non-autoerotic hangings

	Autoerotic hangings	Non-autoerotic hangings
Loss of consciousness	11 ± 3 s	10 ± 3 s
Convulsions	13 ± 3 s	15 ± 4 s
Decerebrate rigidity	20 ± 6 s	18 ± 2 s
Start of deep rhythmic abdominal respiratory movements	17 ± 3 s[a]	25 ± 6 s[a]
Decorticate rigidity	40 ± 16 s	32 ± 16 s
Loss of muscle tone	2 min 5 s ± 23 s[a]	1 min 31 s ± 9 s[a]
End of deep rhythmic abdominal respiratory movements	1 min 56 s ± 31 s	1 min 38 s ± 32 s
Last muscle movement	4 min 23 s ± 2 min 59 s	3 min 42 s ± 39 s

[a]Comparisons between both groups using Mann–Whitney U were significant at $P < 0.05$

animal or human study to support this assumption. In the series of filmed hangings, one victim was known to be intoxicated with ethanol, and this intoxication did not accelerate the timing of the agonal responses. Further studies are needed to consider the influence of alcohol and/or drugs upon this sequence.

Similarly, there are no studies to support any discussion of the effect of any drugs, legal or illegal, on the time to die by strangulation. Any discussions on the possible effect of drugs would be pure speculation.

In the case of autoerotic practitioners, it could have been argued that they might develop over time, a degree of ischemic habituation, with a deceleration of the timing of the sequence. On the other hand, it would seem logical that since they often play with the asphyxia for a longer period before the final accidental lethal hanging, the sequence would be accelerated. A comparison of the agonal responses in autoerotic versus non-autoerotic hangings reveals that overall, the timing of the responses to hanging are not significantly different between both groups (Table 2.4). The timing of only two agonal responses differs significantly between both groups: In autoerotic practitioners, the start of the deep abdominal respiratory movements is significantly accelerated, and the loss of muscle tone is significantly delayed.

Mechanisms of Death

Deaths by hanging and strangulation have traditionally been attributed to three possible mechanisms: closure of the blood vessels of the neck, compression of the air passages, and vagal stimulation by pressure of the baroreceptors in the carotid sinuses and the carotid body. This traditional explanation of the mechanisms of death is largely based on historical writings and experimentation from the end of the nineteenth and beginning of the twentieth centuries [2].

The studies of filmed hangings by the Working Group on Human Asphyxia have challenged two of these mechanisms. First, the respiratory movements in filmed hangings are not just visualized but are also clearly audible. This observation challenges the complete compression of the air passages as the mechanism of death. It should be mentioned, however, that the rhythmical contraction of the diaphragm with abdominal and thoracic wall movement does not prove that the air is actually entering or exiting the lungs. As for the hearing of breath sounds, some may argue that this does not eliminate the possibility of significant partial airway obstruction. For example, choking deaths are encountered with partial airway obstruction by food or foreign body. Therefore, the studies of the Working Group on Human Asphyxia strongly support that tracheal obstruction is not complete in hangings, but it would be premature to totally exclude some implication of partial airway obstruction in the mechanism of death.

The second mechanism of death challenged by the studies of the Working Group on Human Asphyxia is the cardioinhibitory reflex by pressure on the baroreceptors in the carotid sinuses and the carotid body [17, 30]. If this cardioinhibitory reflex was an important mechanism of death in hanging, rapid almost instantaneous death should have been observed. The Working Group on Human Asphyxia has currently reviewed more than 30 cases of filmed lethal and nonlethal hangings. However, not a single rapid death was observed in all the lethal and nonlethal filmed strangulations. On the contrary, all lethal and nonlethal films analyzed so far have presented a strikingly similar pattern of agonal sequences. Further research is needed, but at this point in time, there is no clear scientific evidence to support rapid death by cardioinhibitory reflex in hanging, whereas there are multiple cases to support a slower death by another mechanism.

Despite there being no final answer to the question as to the mechanism of death in strangulation, the scientific evidence seems to point towards the obstruction of the blood vessels as the main mechanism of death.

Amount of Pressure Required for Occlusion of the Neck Structures

The amount of pressure required for causing the occlusion of the neck structures is found in most textbooks: 2 kg (4.5 lb) for the jugular veins, 5 kg (11 lb) for the carotid arteries, 15 kg (33 lb) for the trachea, and 30 kg (66 lb) for the vertebral arteries. These numbers come from old cadaveric studies, mainly by the French authors Brouardel and Lacassagne [2, 9, 10]. Though these numbers are generally accepted and often presented in court, the study of filmed hangings casts doubts on this theory.

To illustrate the problem with the theory of the amount of pressure on the neck structures, let us consider the first case of the Working Group on Human Asphyxia's series as an example. In that case, a 67.3 kg man is observed hanging with both feet on the ground. According to the proportion of the body weight applied to the hanging ligature in incomplete hanging as documented by Khokhlov [31] (Table 2.5), the pressure applied on the ligature in that case would be 66 % of the body weight, so

Table 2.5 Proportion of the body weight applied to the hanging ligature in incomplete hanging

Position of the incomplete hanging	Proportion of the body weight applied to the ligature (%)
Standing toes touching the ground	98
Standing feet flat on the ground	66
Kneeling, buttocks down	74
Kneeling, buttocks up	64
Sitting, back suspended upright	18
Sitting, back suspended backward	32
Lying down, face down	18
Lying down, face up	10

Adapted from Khokhlov [31]

approximately 44 kg. This pressure should be sufficient to completely occlude not only the jugular veins and the carotid arteries but the trachea as well. Nevertheless, this case is one of the movies with great sound quality and clear audible respiration.

The explanation to this problem might be that the cadaveric studies were overlooking an important factor: the angle of the pressure on the structures of the neck. Though the original French texts [9, 10] are not descriptive enough compared to the modern standard of describing *materials and methods*, it seems that the weight required to occlude the structures of the neck had been studied with pressure vectors applied perpendicularly to the structures. In typical hangings, the pressure vectors are more diagonal, with a greater angle to the neck structures. This would have the effect of lessening the true amount of pressure applied on the neck. Another factor may have been underestimated in the old French studies: the anatomical level of the ligature on the neck (i.e., at the level of the hyoid bone, the tracheal cartilage, the cricoid cartilage, or below), which could impact the amount of pressure required.

Thus, the observation of filmed hangings has shed doubts on the classic theory of the amount of pressure to occlude the structures of the neck. It is not recommended to use these numbers without commenting that the scientific validity of these numbers is unsure. Modern studies should be conducted to evaluate the amount of pressure required to occlude the neck structures, taking into account the angle of the pressure vector and the anatomical level of the applied pressure.

Conclusion

For centuries, forensic pathology was practiced as an art more than a science. Unfortunately, a part of our current body of knowledge still comes from this era. The knowledge of forensic pathology needs to be scrutinized with the modern view of an evidence-based approach. Several theories and concepts we are taking for granted will not survive this closer examination. The recent re-questioning of the old traditional concepts of the pathophysiology of death by strangulation is a good example of the work that we need to accomplish as a field.

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Chapter 3
Marine Toxins

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Introduction

Intoxication from marine organisms is an increasing problem worldwide. There is a strong possibility that global climate change, which alters marine temperatures and salinity, has been accompanied by redistribution of marine species around the world. This would have effects not only in distant but also in home waters around the UK. Why is this of concern? There are many examples of previously unrecognized marine-related toxicological events causing serious harm to human health and even deaths.

One is the incident at Minamata, Japan's worst case of industrial pollution, which occurred over approximately 36 years until 1968. Between 1932 and 1968 a chemicals factory released methyl mercury into Minamata Bay. From around 1950 local cats were noted to be behaving erratically, having convulsions, and dying, with some even falling into the sea and drowning. Birds began to fall out of the sky, and fish die-offs were noted in increasing numbers in the bay [1].

By the middle of the 1950s, symptoms had begun to appear in humans; these included loss of fine motor control, stumbling while walking, or developing violent tremors. By 1956 so many cases had been noted that on May 1 the local hospital director reported "an epidemic of an unknown disease of the central nervous system," which was presumed to be infectious. Casualties experienced social stigma. By October more than 40 cases had been reported and 14 fatalities identified. Apparently healthy mothers were found to deliver babies with unusual neuromuscular and other anomalies who were ultimately found to have been poisoned in utero.

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It was not until 1959 that organic mercury was identified as the cause. The highest concentrations of mercury were in the sludge at the wastewater discharge channel from the factory, decreasing gradually out to sea—indeed the contamination was so severe that it was economically viable to mine the sludge at the wastewater channel. From this source it was identified that large quantities of mercury were present in fish and shellfish caught in the bay. In total more than 900 people were reported to have died of "Minamata disease," and it has been estimated that up to two million people suffered health problems as a consequence of ingesting contaminated seafood.

In January 2013, governments worldwide agreed to a global, legally binding treaty to prevent mercury emissions and releases, addressing mercury contamination of the environment. The "Minamata Convention," as it is called, provides controls and reductions of mercury use across a number of products, processes, and industries using mercury ranging from thermometers and lightbulbs to coal and other mining and cement making. It will be open for signature at a special meeting of the United Nations (UN) in Japan in October 2013.

It is thus important that unusual, new, or changing distributions and patterns of disease and mortality are flagged up and appropriately investigated in a timely manner. In this chapter, we describe some of the most noteworthy toxic poisoning syndromes that may affect consumers associated with eating fish contaminated by algal toxins and other contaminants. We also present a short overview of those envenomating sea creatures that may be encountered in UK waters, alongside a description of the effects of some of the most toxic of marine fauna worldwide. This chapter will end with a short discussion on the impact that climate change may have on the burden of marine toxin disease in the UK.

Ingestion of Seafood

While there is increasing public awareness of the dangers of eating bacterially contaminated seafood, awareness of the important public health problem of inherent and accumulated algal toxins in seafood has received less attention.

Many fish and shellfish, including much of the shellfish that we ingest, depend on microscopic phytoplankton to feed. Approximately 60–80 of the 5,000 species of marine phytoplankton produce potent marine toxins [2]. The phytoplankton that produce toxins are distributed throughout the world, with certain toxins concentrated in particular parts of the world. These toxins accumulate in the tissues of shellfish and other fish that filter feed on the plankton. These fish or shellfish are in turn ingested by animals further up the food chain before ingestion by humans. This process of increasing concentration up the food chain is known as marine bioaccumulation or bioconcentration.

Fig. 3.1 Satellite image of an algal bloom off the Devon and Cornwall coast in 2004 (*Source*: NASA)

Under appropriate environmental conditions, phytoplankton can proliferate with extraordinary rapidity leading to "blooms" of algae, which change the water color to red, brown, or green, giving rise to the phenomena known as "red tide." Indeed, one of the earliest possible references to an algal bloom comes in the book of *Exodus*: "… all the waters that were in the river turned to blood, fishes died, Egyptians could not drink the water of the river" [3]. These blooms may stretch for many miles in length (see Fig. 3.1 for example), and it has been reported that the toxin may be so concentrated in seawater in these circumstances that it can be aerosolized by surf and cause a transient syndrome of inhalational intoxication in humans, irritating the respiratory tract [4]. Red tides are increasing in frequency. The mechanism for this is not entirely clear but has been linked to both climate change and transport of shipping ballast [5].

Bioaccumulation is not unique to shellfish; gastropods, crustaceans, and fish can all accumulate toxins from feeding and thus pose a threat to human health. In some circumstances, careful preparation of fish and shellfish may eliminate the most heavily contaminated parts [6].

The Impact of Climate Change on Frequency of Algal Blooms in UK Waters (Adapted from UK CCRA 2012)

It is clear that climate change may lead to increases in algal blooms, some of which may be harmful to either biodiversity or humans if the food chain is affected. Increases are projected along the north coast of Cornwall, the Firth of Clyde, the Moray Firth, and Northeast England. However, in a scenario of medium emission levels, marked increases are projected off the south coast of Sussex and in places along the South Devon coast by the 2080s.

Increased water temperatures would place additional pressures on freshwater ecosystems. Warmer temperatures can act to displace cold-water species to cooler regions. If migration is not possible, then extinction may occur. A higher water temperature holds a lower volume of oxygen leading to eutrophication (a process where water bodies receive excess nutrients that stimulate waterborne organism growth); thus algal blooms incidences could increase. Blooms act to starve the ecosystem of oxygen and so water quality would deteriorate through a fall in oxygen levels, release of sediment-bound phosphorus, and altered mixing patterns. Migration routes, species composition, and nutrient cycling could all be expected to change [7].

Toxicity Associated with Shellfish

While seafood is less commonly associated with outbreaks of food poisoning than other food sources [6], there are a number of specific potential human health hazards from toxins that accumulate in fish and shellfish. Intoxications from seafood are likely to be underreported due to under-recognition. Throughout the world, toxins produced by algae are held responsible for approximately 60,000 human intoxications yearly [8].

A number of different toxidromes result from ingestion of these algal toxins, which are summarized at Table 3.1. The toxic syndromes that may occur from seafood harvested with the UK and Europe are paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), and amnesic shellfish poisoning (ASP), although an effective shellfish monitoring system acts as a sentinel and has prevented serious impact on human health. This has, however, led to long-term closures of fisheries with severe economic consequences [2].

UK residents may be exposed to other syndromes from consuming contaminated imported food or while travelling abroad. Poisoning from seafood ingestion has been examined in some considerable detail with a large volume of literature and textbooks published. This section covers the following more notable occurrences with case studies:

Table 3.1 Mechanisms of marine envenomation, organisms of interest, and UK presence

Toxic syndrome	Geographic distribution	Toxin and source	Symptoms	Mechanism of action	Time to onset	Food source
Paralytic shellfish poisoning	North Sea, Japan, northeast/northwest USA, southern Chile	Saxitoxin produced by dinoflagellates	Facial and perioral paresthesia, headache, dizziness, muscular weakness, ataxia, nausea, vomiting, respiratory suppression, death	Sodium channel blockade	30 min–4 h	Shellfish and other fish
Diarrhetic shellfish poisoning	Europe, Japan, Africa	Okadaic acid produced by dinoflagellates	Diarrhea, nausea and vomiting, abdominal cramps, fever	Protein phosphatase inhibition	30 min–12 h	Shellfish
Amnesic shellfish poisoning	Eastern Canada, northeast and northwest USA	Domoic acid from diatoms	Nausea, vomiting, diarrhea, headache, visual disturbances, weakness, cranial nerve palsies, autonomic nerve dysfunction, amnesia, pain, seizures, coma, death	Glutamate receptor stimulation	GI symptoms within 24 h, neurological symptoms within 48 h	Shellfish
Neurotoxic shellfish poisoning	Western Florida, Caribbean	Brevetoxin from dinoflagellates	Paresthesia, abdominal pain, dizziness, diplopia, diarrhea, gait disturbance, reversed temperature perception, headache, muscular pain, respiratory distress	Opens sodium channels	3–6 h	Shellfish and other fish
Ciguatera	All tropical areas	Ciguatoxin, maitotoxin, and others from dinoflagellates	Facial and perioral paresthesias, headache, reversed temperature perception, arthralgia, rash, cyanosis, insomnia, myalgia, extremity pain, loss of hair and nails, motor disturbance, coma, and death	Ciguatoxin opens sodium channels, maitotoxin opens calcium channels	3–30 h, can be recurrent or induced later	Large predatory reef fish (including barracuda and snapper)

(continued)

Table 3.1 (continued)

Toxic syndrome	Geographic distribution	Toxin and source	Symptoms	Mechanism of action	Time to onset	Food source
Scombroid	Worldwide, associated with poor storage of fresh fish	Histamine, possibly others	Bright pruritic facial and truncal rash, flushing, sweating, perioral tingling, headache, facial and tongue swelling, wheeze, nausea, vomiting, diarrhea, tachycardia, cardiovascular collapse	Histamine actions	Minutes to hours	Tuna, mackerel, mahimahi among others
Tetrodotoxin	Eastern Asia, particularly China and Japan	Tetrodotoxin produced by bacteria; occurs with saxitoxin from dinoflagellates	Numbness of face and extremities, sensation of impending doom, ascending paralysis, respiratory failure, cardiovascular collapse, death	Sodium channel blockade	30 min–3 h	Puffer fish and other mixed species

- Paralytic shellfish poisoning (PSP)
- Diarrhetic shellfish poisoning (DSP)
- Amnesic shellfish poisoning (ASP)
- Ciguatera
- Tetrodotoxin
- Scombrotoxicity

Paralytic Shellfish Poisoning (PSP)

Definition and Epidemiology

PSP is the most common and widespread of the toxic syndromes associated with seafood. This is a severe disease of rapid onset that may be life threatening or fatal. The toxidrome produced is exclusively neurological and is caused by saxitoxin and other closely related marine toxins produced by dinoflagellates [4].

Mode of Action and Symptomology

Saxitoxin has a mode of action that blocks voltage-gated sodium channels in nerve and muscle cell membranes, blocking nerve transmission [4]. For symptoms and onset time, see Table 3.1. Death results from respiratory muscle paralysis; ingestion of a large dose may cause respiratory arrest and death within 2 h of ingestion. An outbreak in Guatemala affected 187 people, hospitalizing 70 % and killing 26 [9]. There is much published research and data on the nature of saxitoxin and its mode of action, as it has been considered as a potential chemical warfare agent.

Diagnosis and Treatment

There is no known antidote to saxitoxin and thus supportive treatment (where necessary to include airway, ventilatory, and circulatory support) is the main treatment strategy. A definitive diagnostic test is available that requires mouse bioassay of the suspected food or liquid. Since this is often unavailable, the diagnosis is usually made on clinical features along with a positive history of eating shellfish in the previous 24 h. It is of note that puffer fish, the classic vehicle of ingestion of tetrodotoxin (see later), can also cause saxitoxin poisoning [10].

Amnesic Shellfish Poisoning (ASP)

Definition and Epidemiology

Amnesic shellfish poisoning, in contrast to paralytic or diarrhetic shellfish poisoning, which are caused by toxins accumulated from dinoflagellate species, is caused by a

toxin released by a diatom. The toxin in question, domoic acid, is released by *Pseudo-nitzschia multiseries* and was the first shellfish toxin to be attributed to a diatom [11].

The first described outbreak of amnesic shellfish poisoning was in 1987 in Canada; 107 people became unwell after eating contaminated blue mussels [12]. The victims were reported to have suffered neurotoxic and gastrointestinal effects but also an acute loss of memory. Until this outbreak it had been thought that algal toxins were only produced by dinoflagellate species and diatoms had not been considered as a possible source. Domoic acid has since been found in a variety of bivalve molluscs (scallops, clams, oysters, etc.) as well as crabs and lobsters. Those aged under 40 years generally reported diarrhea while the more severe neurological effects including seizures were reported by those aged over 65 years or with other chronic diseases such as chronic renal failure. Three patients died in this group. At postmortem, severe damage to the hippocampus and other parts of the brain was found. A dose–response relationship is thought to exist; in the Canadian outbreak, those who ingested 15–20 mg were unaffected. Ingestion of levels in excess of 135 mg caused serious illness [12].

Mode of Action

Domoic acid is a glutamate receptor agonist and disrupts normal function of the central nervous system. The glutamate receptor conducts sodium ion channels; domoic acid acts to open the sodium channels, leading to sodium influx and depolarization. This leads to continuous neuronal stimulation and a rise in intracellular calcium and subsequent swelling and lysis of cells within the hippocampus [5].

Symptoms

The onset of symptoms is slower than that seen in paralytic or diarrhetic shellfish poisoning, with onset within approximately 24 h. For more detailed symptomology, see Table 3.1.

Treatment

There is no antidote available for domoic acid or amnesic shellfish poisoning. Treatment is supportive and includes careful monitoring of vital signs and fluid and electrolyte balance. Airway management as well as management of seizures may be required in severe poisoning.

Diarrhetic Shellfish Poisoning (DSP)

DSP is a toxic syndrome with rapid onset and purely gastrointestinal symptoms, which are typically severe but self-limiting. It is caused by okadaic acid, which inhibits eukaryotic protein phosphatases. Sodium release by intestinal mucosal cells

is thought to cause the eponymous diarrhea [4]. Outbreaks have been described in the UK [13]. Symptoms generally start within 30 min of exposure and lead to diarrhea and abdominal pain that last for 3–4 days. Okadaic acid is a potent tumor-growth promoter [14] and immunosuppressant, but there is little data on the effect of acute or chronic exposure in humans [4].

Toxicity Associated with Eating Contaminated Fish

Ciguatera Fish Poisoning

Definition and Epidemiology

While there have been very few cases of ciguatera fish poisoning in the UK, it can produce serious poisoning with long-term neurological effects. It has been estimated to affect between 25,000 [15] and 50,000 [16] people globally every year; however, accurate epidemiology is difficult to assess since reporting is a requirement in only a few countries. Cases in the UK have arisen from imported contaminated fish [2].

The term "ciguatera" refers to food poisoning caused by ingestion of toxic coral reef fish [17]. Two groups of compounds are implicated in the poisoning seen: ciguatoxin and maitotoxin. Both are produced by the same dinoflagellate species.

Herbivorous tropical reef fish ingest *Gambierdiscus toxicus* dinoflagellates. The toxin builds up in larger predatory fish, having accumulated while moving up the food chain, including sea bass, snappers, surgeonfish, moray eel, and most commonly barracuda. For this reason, the sale of barracuda for human consumption is banned in parts of Florida [6].

Schlaich et al. [18] describe an outbreak of ciguatera poisoning on a cargo ship, which was docked at the port of Hamburg, following consumption of bigeye and grouper fish species caught while in the Caribbean. All 14 of the sailors who consumed the fish developed gastrointestinal or neurological symptoms to varying extents; an experimental assay confirmed the presence of ciguatoxin in the fish. The attack rate was high, with 14 of the 15 crew unwell, which interrupted marine operations due to both the number of crew affected and the chronicity of symptoms. It is important to be aware that ciguatera fish poisoning is a risk for seafarers who travel through tropical or subtropical areas; they represent an occupational group at risk of this potentially fatal fish poisoning due to unsafe food sources [18].

Mode of Action

Ciguatoxin activates voltage-dependent sodium channels on the cell membrane following ingestion of the contaminated fish. A wide range of signs and symptoms develop (see Table 3.1). The most characteristic symptom of ciguatera is the reversal of thermal sensation, termed "dry ice syndrome."

Tetrodotoxin Poisoning

Definition/Epidemiology

Tetrodotoxin poisoning is a serious medical emergency that can cause death through respiratory paralysis and respiratory failure. The importance of advanced and sometimes protracted advanced life support cannot be underestimated.

Tetrodotoxin is frequently involved in fatal food poisoning. Until relatively recently it was thought that puffer fish (a member of the Tetradontidae family) was the sole source of the toxin. Tetrodotoxin has now been isolated from crabs, the blue-ringed octopus, a goby, molluscs, flatworms, and even a terrestrial amphibian leading to the discovery that the primary source of the toxin is a symbiotic bacterium [19], which produces the toxin as a product of metabolism.

Tetrodotoxin poisoning is very well known due to its association with puffer fish. Puffer fish, or fugu, is a traditional Japanese delicacy and may be consumed raw as sashimi or in a variety of other preparations including fugu soup. There are more than 120 species of puffer fish, which are widely distributed throughout the tropics. Only select species of puffer fish are used and Japanese chefs must be certified before being able to prepare and serve fugu. The toxin itself is located in visceral tissues such as the liver, gonads, and sometimes the skin. Muscle tissue is nontoxic or only weakly toxic [20, 21]. Anecdotal reports suggest that some diners seek to place a small amount of the toxin-containing liver on the meat they digest in order to induce the sensation of perioral tingling. However, tetrodotoxin is exceptionally potent; in one case report, a woman died 45 min after consuming the liver of a puffer fish [22].

Since 2002, mixed tetrodotoxin and saxitoxin toxicity in puffer fish has been described [23], particularly in the waters around Florida. In January 2002, the poison control center in Tampa reported a man who had been hospitalized with numbness and tingling of the hands with diarrhea and vomiting after consuming puffer fish caught during a recreational fishing trip on Florida's central east coast [10]. The puffer fish was found to contain large amounts of saxitoxin and only trace amounts of tetrodotoxin; further 28 poisonings were reported in the same area over a 2-year period. Much of the Japanese fugu industry is predicated on the fact that tetrodotoxin is concentrated in tissues other than the muscle and thus, with careful preparation, muscle tissue will be safe to eat even in the most toxic of animals. Saxitoxin is found at high concentrations in the muscle tissue of the puffer fish in Floridian waters and not just the viscera, meaning that no standard method of preparation would be able to make the fish safe for consumption. As in shellfish, saxitoxin occurs in puffer fish due to accumulation through the food chain following ingestion of dinoflagellate species. As discussed previously, a change in the global distribution of these toxigenic algal species has the potential to cause contamination of this already potentially hazardous fish product.

Mode of Action

Tetrodotoxin is readily absorbed from the gastrointestinal tract. It alters the initial increase in sodium permeability of nervous cell membranes resulting in nerve block. It is able to block both sensory and motor nerves and is thus a powerful tool in research. Tetrodotoxin has been considered as a hypotensive agent and potent respiratory inhibitor and has been used clinically as a pain-relieving agent in cases of patients suffering from neurogenic form of Hansen's disease (leprosy) [17]. Tetrodotoxin and a number of its derivatives have been examined for local anesthetic action.

Scombroid Fish Poisoning

There are sources of toxicity from ingestion of fish that are not due to algal contamination and are not due to inherent toxicity; thus they do not easily fall into previous sections. The most noteworthy is scombroid fish poisoning (SFP), which is due to bacterial breakdown of naturally occurring chemicals in harvested fish to a harmful toxin.

Definition/Epidemiology

Scombroid fish poisoning (SFP) is a chemical intoxication from eating contaminated tuna, mackerel, herring, or other members of the Scombroidea family of fish [24, 25]. SFP occurs with relatively high frequency in the UK, with 71 general outbreaks and incidents reported to the HPA's Foodborne and Non-Foodborne Gastrointestinal Outbreak Surveillance System (eFOSS) between 1992 and 2009, affecting 336 people [26]. SFP outbreaks most frequently occur in the warmer summer months after consumption of fish that has been improperly stored, handled, or prepared [24, 25].

Mode of Action

Scombroid fish are naturally high in histidine, which is converted to histamine by bacteria if storage conditions are inadequate to control bacterial growth. Histamine is heat stable and survives subsequent processing, including canning. Consumption of fish with elevated levels of histamine results in the illness. Bacterial spoilage and production of histamine may occur at any stage in the food chain (i.e., from landing the fish, at the processing plant or in the distribution system, or in catering premises or homes), and adequate temperature control is key in preventing bacterial growth and histamine formation [26].

Symptoms

The symptoms of SFP are related to histamine consumption and include a spreading rash across the face, neck, and chest; nausea and vomiting; diarrhea; abdominal pain; headaches; dizziness; and palpitations. For more detail, see Table 3.1.

Treatment

Most mild cases respond adequately to prompt antihistamine treatment, although circulatory collapse, shock and acute pulmonary edema have been described [5], which necessitate standard management in line with European Resuscitation Council guidelines [27].

Toxicity Associated with Sea Immersion

Subtropical species are occurring with increasing frequency in European waters [28]. In recent summers, including 2012, there have been outbreaks of people affected by weever fish stings while bathing from UK beaches, and there have been increasingly numerous sightings of Portuguese men-of-war as far north as the Scottish coasts, a species not usually associated with these waters.

There are a number of mechanisms by which individuals may be envenomated by marine animals. These are detailed in Table 3.2, along with some examples of interest of those animals that may envenomate by each method. Further information on certain of the species is provided in this text; this is a very large area for which much published work exists.

Stings

Venomous Fish

More than 200 species of fish are venomous including stingray, scorpion fish, zebra fish, stonefish, toadfish, catfish, surgeonfish, and ratfish as well as several species of shark; however, these fish are generally found in shallow water, reefs, kelp beds, or inshore in tropical waters of the South Pacific. It is the weever fish that are of greatest relevance in the UK.

Summer 2010 saw a number of reports of weever fish (*Echiichthys vipera*) poisonings around the UK, with more than 30 cases of envenomation occurring in 1 week. These fish are classically associated with the warm waters between Morocco and the Atlantic coasts of France and Spain but increasingly are found on the shorelines of the south coast of England and Wales during the warmer summer months.

Table 3.2 Symptoms, global distribution, and speed of onset of ingested toxic syndromes

Mechanisms of marine envenomation, organisms of interest, and UK presence		
Mechanism	Major organisms of interest	Currently a risk in the UK?
Stings – from specialized apparatus that puncture skin and introduce venom	Weever fish	Yes
	Sea urchin	
	Crown-of-thorns starfish	
	Stingray	
	Stonefish	
	Cone shells	
Bite	Blue-ringed octopus	No
	Sea snakes	No
Nematocysts	Box jellyfish	Yes
	Other jellyfish	Yes
	Portuguese man-of-war	Yes

Adapted from Ellenhorn et al. [14]

Details of the precise numbers of envenomations by weever fish are difficult to obtain as many go unreported [29].

Echiichthys vipera is about 15 cm long and hides in sand, leaving only its spikes exposed. These puncture the skin (usually on the foot, since the most common cause of envenomation is standing on the fish) and venom enters the body. This causes excruciating pain, which can last for many hours or even days.

Complications of weever envenomation including localized gangrene and necrosis have been reported, but fatalities are rare. Treatment consists of immersing the affected area in the hottest possible water that can be tolerated, particularly over 40 ° Celsius if possible, since all fish venoms are proteins and are characterized by instability due to changes in temperature. Weever venom is no exception. Pain can be treated conventionally with analgesics, although stings can easily be prevented by wearing protective footwear [14].

Nematocysts

Nematocysts are spring-loaded venomous glands, which respond to stimuli by sudden eversion that penetrates prey and delivers a dose of venom through a connecting tube. Stimuli can be touch, or other chemical or mechanical stimulation, and are a vital mechanism in trapping and paralyzing prey for feeding. For examples of organisms that employ this method of envenomation, see Table 3.2.

There are a number of characteristics unique to nematocysts, which on some animals may extend on tentacles for up to 30 m. They can remain functional even after amputation from the host organism and thus must be removed carefully from the skin of a victim to prevent further envenomation of victim or responders. Furthermore, they may become embedded in the skin without discharging, which can then be stimulated by attempted removal.

Fig. 3.2 The mauve stinger jellyfish (*Source*: Hans Hillewaert http://commons.wikimedia.org/wiki/File:Pelagia_noctiluca_(Sardinia).jpg)

Jellyfish and Siphonophores

In Scotland in summer 2011, the Torness nuclear power station was forced to shut down both reactors due to huge numbers of jellyfish obstructing cooling systems [30], while in 2012 Spain saw an invasion of the jellyfish *Pelagia noctiluca*, commonly known as the mauve stinger due to its coloration (see Fig. 3.2). More than 1,000 bathers reported being stung in 1 week off the Costa Blanca [31]. While these stings are painful and distressing, they are rarely dangerous. Treatment consists of the careful removing or scraping of any remaining jellyfish tissue from the skin, followed by the application of vinegar. In exceptional cases, antivenom may be required along with supportive and symptomatic medical treatment.

Often mistakenly considered a jellyfish, the Portuguese man-of-war *Physalia physalis* (Fig. 3.3) is not a single creature but actually a "siphonophore" or floating colony of hydrozoa. In 2009, warnings to bathers were issued by authorities in Pembrokeshire, Wales, after a number of sightings, while dozens of *Physalia physalis* were reported along long stretches of the Irish coast from 2009 to 2010.

Stings from *Physalia physalis* cause a characteristic series of red welts on the skin, which can persist for several days. Pain subsides after an hour or so but severe allergic reactions have been reported after the venom spreads to lymph nodes [29]. Deaths are possible. In one case report, a woman emerged from the ocean with a

Fig. 3.3 *Physalia Physalis* – the Portuguese man-of-war (*Source*: United States Department of Commerce, National Oceanic and Atmospheric Administration (NOAA) http://commons.wikimedia.org/wiki/File:Portuguese_Man-O-War_(Physalia_physalis).jpg)

Portuguese man-of-war wrapped around both of her arms. She became comatose, suffered considerable myocardial ischemia, and died after a number of days in intensive care [32].

Immediate treatment is to avoid further contact and remove remaining tentacles, which may still be attached to the victim. The affected area should be washed with seawater; freshwater worsens symptoms. As with venomous fish, hot water helps to denature the venom. Vinegar is not of use on the management of Portuguese man-of-war poisoning [29].

Bites

Octopus

While several species of octopus exist in British waters, none are poisonous to man. All octopuses contain venom of some sort, which contributes to their method of attacking and catching prey.

Fig. 3.4 Blue-ringed octopus (Source: Jens Petersen http://commons.wikimedia.org/wiki/File:Hapalochlaena_lunulata2.JPG)

However, there is just one octopus (or cephalopod) species that is potentially harmful to man: the blue-ringed octopus (or octopi since there are, in fact, four different species) (Fig. 3.4). This deserves special mention here as it is widely recognized as one of the most venomous species in the world—on land or sea. It is a small animal, approximately 20 cm across at most, which lives among the coral reefs of the South Pacific and Indian Oceans. While the bite from such a small animal is slight and resembles little more than a slight laceration, with or without drawing blood, the saliva of the octopus contains tetrodotoxin, which is best known as the toxin contained in puffer fish (*vide infra*). Tetrodotoxin is a potent sodium channel blocker and is one of the most toxic chemicals known to man, rapidly causing death through respiratory muscle paralysis. The toxin is found in a number of seemingly unrelated animals including sea stars, snails, harlequin frogs, and a flatworm. It has been discovered that the salivary glands of these animals are heavily colonized with tetrodotoxin-producing bacteria that have a symbiotic relationship with their host.

Discussion

The study of marine toxins is an emerging area of knowledge since many have only been identified in the last 25 years. However, as exemplified by the Minamata incident, contamination of food sources in a manner not previously known to be toxic

can be of insidious onset and not be reflected in human health for many years. Minamata disease has been in the spotlight again recently following the UN agreement of the "Minamata Convention on Mercury" in January 2013, which introduces controls to phase out mercury in many products. This historical example highlights the importance of drawing attention to unexplained changes in disease patterns and demonstrates the involvement of toxicological contamination that may not be immediately evident. Indeed, at Minamata, the symptoms observed were presumed for many months to be infectious in origin.

Current surveillance in the UK and Europe suggests that there is a low incidence of human disease associated with marine toxin ingestion and envenomation. However, these illnesses are likely to be greatly underreported and the species involved are often not identified.

The apparently low incidence of disease can, in part, be attributed to monitoring programs in place. These programs apply to both the fishing industries and those industries that have waste products to dispose of. Legislation requires that shellfish entering the human food chain are monitored to control the risk of shellfish poisoning. The Food Standards Agency (FSA) is responsible for the monitoring of marine toxins within the UK by collecting and analyzing samples of shellfish and water from around the harvesting regions. Toxin threshold values have been produced and maximum acceptable limits set. If the levels of toxins in the samples exceed these limits, the FSA communicate to the local authority for the relevant infected beds, who can close the beds to harvesting until daily tests have returned negative results for 2 consecutive weeks [2].

The statutory laboratory testing methods for shellfish toxins vary throughout the UK. The Centre for Environment, Fisheries and Aquaculture Science (CEFAS) monitors PSP and DSP in England, Wales, and Scotland and ASP in England and Wales. In Northern Ireland, the Department for Agriculture and Rural Development (DARD) has conducted the testing program. Discovery of a range of novel DSP toxins, among other concerns, has led to investigation of new methods to be able to effectively monitor marine toxin levels in seafood in the future [2].

With the changing hydrological conditions and the general idea that toxic algal species are increasing in their geographical location and frequency, it is important to determine the possible future threat in the UK. Indeed, in 1999 the presence of Pacific diatom algae *Neodenticula seminae* was detected in the North Atlantic for what is thought to be the first time in 800,000 years, potentially providing the first evidence of trans-Arctic migration from the Pacific in modern times. The introduction of Pacific species to the North Atlantic not only brings such organisms much closer to our fisheries and bathing waters but raises the possibility of hybridization with the marine flora and fauna native to the UK [33].

One of the key indicators of the impacts of climate change on biological populations is the observation of changes in the annual life cycle of marine species. Changes in sea temperature can lead to large, difficult-to-observe changes in the marine food web, which will ultimately affect the food available to fish, birds, and large marine mammals. Work in the North Sea has demonstrated that plankton species there are very sensitive to regional climate warming, which can easily lead to a mismatch between any one species and its source of food. This will have important consequences

for the health of all species within the ecosystem, including man. These issues are described in an indicator-based report of the European Environment Agency [28]. Rising sea temperatures are already providing conditions conducive to an increase in viruses, bacteria, and harmful algae in the seas around the UK. The risk of health problems caused by marine and freshwater pathogens is thus projected to increase [7].

The problem of toxic algal blooms and their impact on our domestic food supplies and fish stocks featured in the UK Climate Change Risk Assessment (CCRA) government report, which was laid before Parliament on 25 January 2012. Increases in regional sea temperatures have triggered a major northward expansion of warmer water plankton in the Northeast Atlantic and a northward retreat of colder water plankton [7]. This northerly movement is about 10 ° in latitude (1,100 km) over 40 years, which has accelerated since 2000 [28].

As a species, we too are increasingly exploring ever more distant parts of the world for tourism, with leisure and professional trips to the far South Pacific now a realistic prospect for many UK travellers.

Conclusion

This chapter has presented an overview of both the most common and most serious toxidromes and effects from ingestion of, or envenomation by, marine animals. The objective has been to give the reader an introduction to the science, epidemiology, and effects of marine toxins as a foundation for further reading as required. We have drawn attention to the effects that climate change and global redistribution of species could potentially have on the distribution of disease from marine toxins and highlight current issues in both testing and surveillance of disease. While changes in sea temperature, salinity, and marine redistribution are unlikely to bring such species as the blue-ringed octopus to UK home waters in the near future, increased distant foreign travel and global trade in fish stocks means that the health effects of marine toxins are an emerging issue of which both health professionals and the general public should remain aware of.

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Chapter 4
The Dismembered Body

Guy N. Rutty and Sarah V. Hainsworth

Introduction

Postmortem dismemberment is relatively rare within the United Kingdom. Since 2003, 28 cases have been recorded in England and Wales by the Serious Organized Crime Agency's (formerly National Police Improvement Agency) Missing Persons Bureau (personal communication 2013). This averages two to three cases per year. Such cases often pose challenges for the investigating police force, as often members of the police investigative team, and even the pathologist, may not have encountered such a case previously.

Dismemberment associated with homicide is commonly performed for one of three reasons: first, to make the transportation of the remains from the site of the death to a place of disposal more straightforward; second, to make an attempt to make it more difficult to identify the victim; and third, there is a total disrespect for life or abhorrence of the victim [1–4]. Dismemberment, or more specifically decapitation, can also occur accidentally: for example, in cases where decapitation results from impact with vehicles or trains or by suicide (e.g., in death by hanging where the victim jumps from a first-floor window; or ties one end of a rope to a tree, the other to their neck, and then drives away from the scene). Removal of a limb from a person who subsequently dies can occur in an attempt to rescue a person, for example, when trapped under a train where the removal of a limb may be required as part of prehospital emergency treatment [5–8].

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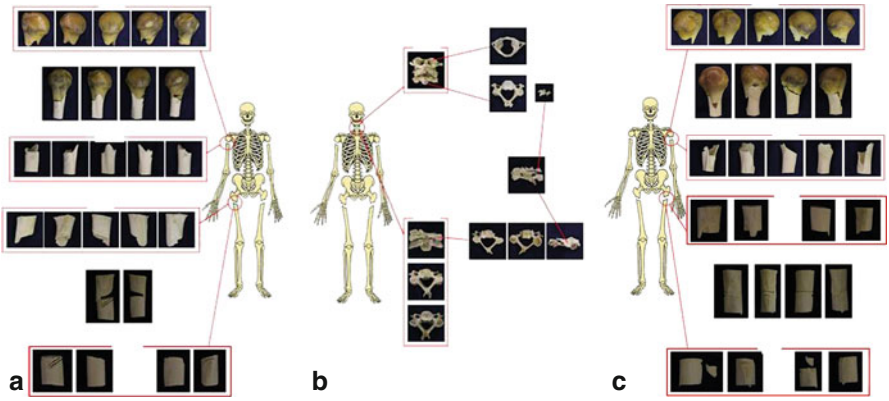

Fig. 4.1 Composite image showing the typical six body part dismemberment. The bones at each site have been cleaned, examined, and then finally (after all examinations) fit matched

This chapter presents a personal approach to the investigation of a dismembered body associated with a homicide: hence often in the text there is the use of the word "we" (i.e., the authors' experience). Firstly it presents an overview from a pathological viewpoint of an approach from scene to mortuary in considering the four standard questions of who the person was, where, when, and by what means they came by their death. The second part of the chapter then considers from an engineering viewpoint the instruments that may have been used to dismember the body and which methods can be used to identify the said instrument. We touch upon but do not detail the police, scene of crime, or forensic science laboratory investigation as this is beyond the scope of this chapter.

Anatomical Sites of Dismemberment

From the authors' experience, it is unusual for the body's limbs to be removed through the joints. Pathologists will know from experience that to remove an arm or a leg through the shoulder or hip joints is not easy. If such a case is encountered, then it is reasonable to consider that the perpetrator has a degree of knowledge of anatomy and/or butchery skills.

The sites of dismemberment that the authors normally encounter—to the point that if a body part is discovered, you can predict to the police how many further parts are yet to be discovered—are the head and neck removed at the approximate level of the fourth or fifth cervical vertebrae, both arms removed through the proximal third of the humerus, and both legs removed through the upper part of the shaft of the femur leaving the torso and pelvis as a single piece. Thus it is the authors' experience that the body is normally dismembered into six pieces (Fig. 4.1).

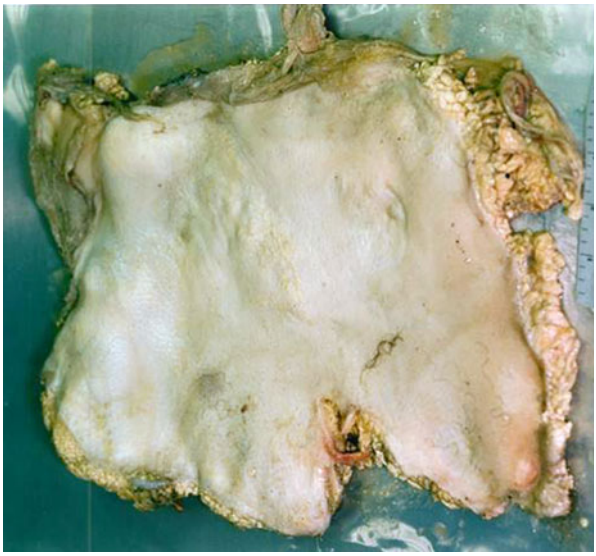

Fig. 4.2 A section of skin removed during the process of dismemberment

That is not to say that a larger number of pieces may not be generated. We have encountered cases where the chest and abdomen have been opened and the cavity contents eviscerated, as well as a case where the skin, subcutaneous tissue, and muscle were removed in square or oblong segments of repetitive sizes. Skin with tattoos, digits, genitalia, and breasts may all be removed in an attempt to hinder identification—although to go to this length is unusual in the authors' experience (Fig. 4.2).

Scene of Dismemberment

If dismemberment is undertaken indoors, an element of planning will be necessary. Which room in the house to use; what instrument(s) to effect the dismemberment? If they do not have the correct tools, a trip to the local do-it-yourself or hardware store may be required, in which case they may be captured on closed-circuit television (CCTV) on the way to, in, or from the store. Credit card records may reveal purchase of equipment.

The bathroom is a room that has been used on several occasions we have encountered, although other rooms in properties are encountered as well as outside scenes. We have even encountered a case undertaken in the back of a van. The body may be placed into or over the bath. In doing so the neck, for example, or a limb can be placed into such a position to facilitate dismemberment by sawing. However, if they have not dismembered a body previously, they will find that sawing through the skin and muscle without cutting away the tissue to expose the bone is difficult. Thus the first attempt to remove a limb may result in multiple saw marks to the skin where an

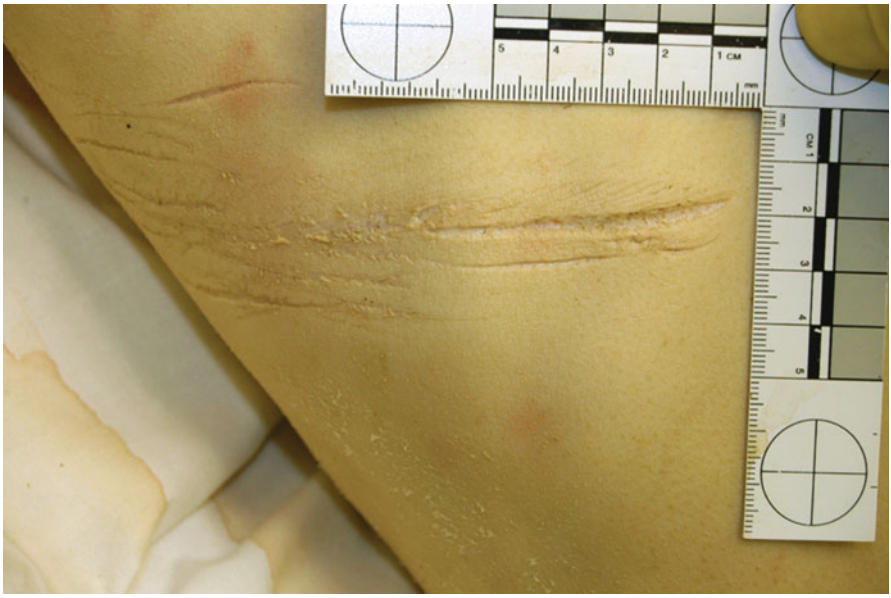

Fig. 4.3 Saw marks to skin caused during the initial attempt to dismember the victim

attempt has been made to saw through the skin without cutting it with a knife first (Fig. 4.3). These marks are of evidential value when trying to identify the instrument used and so care must be taken to preserve any such marks, and photograph them, at right angles, with a suitable scale. The limb may move on the edge of the bath, resulting in so-called false-start kerfs (see later), and as they saw through the limb, tool marks may be left on the edge of the bath or similar edge.

Several saws may be used due to the wrong choice of instrument, blunt blades, or blades breaking. Broken blades may on occasion be left inside the remains. As the perpetrator dismembers the body, the body will leak blood and thus the site of dismemberment will become contaminated by blood. The dismemberment instrument will become contaminated by bodily fluids and tissue that can be recovered at a later date, assuming the saw is recovered. Fluid and tissue can be washed down sinks or flushed down toilets. These sites should be checked for such evidence. One can have the victim's hair in their own property sink traps, but to find fat, muscle, or organ tissue at this site is an important finding. Such material should be sought by the crime scene investigators, who should consider sending it to a pathologist, not a forensic scientist, to be prepared in a similar manner to a cytology preparation. Forensic science laboratories may not be used to such preparation techniques, and thus a strategy of whom is to examine what, and in what manner, should be developed early prior to the investigation of any potential site of dismemberment.

The discussion of the scene examination is beyond the scope of this chapter. However, body fluids may seep through floorboards or between tiles. Often an attempt is made to clean up such fluid, but appropriate scene blood detection

systems can be applied to identify dilute blood, which can then be sampled for DNA analysis. On occasion this is anticipated, and plastic sheeting may have been placed down prior to dismemberment; however, this then needs to be disposed of. The sheeting may not only have the victim's blood and tissue on it but may also bear tool marks from the instrument used during the dismemberment.

Disposal

Disposing of a body is, at times, not as easy as one might think. Having now dismembered the body into six pieces, the perpetrator will need to dispose of the body parts, assuming that the dismemberment is not undertaken at the scene of disposal or that they have not undertaken the dismemberment for purpose three, mentioned previously, in which case they may simply leave the parts intentionally where they are for others to find.

It is not unusual to wrap the body parts inside something, usually plastic bags, to facilitate moving the parts from the place of dismemberment to the disposal site. The reason to do this may be twofold. First, bags and wrappings will limit leakage of blood from the remains onto seats or carpets in vehicles. However, a sealed bag may, in fact, potentially capture such fluid for subsequent retrieval and analysis. Second, in the case of limbs, limbs may be folded in half at the elbow or knee joints, placed into black domestic waste bags, which in turn may be taped closed to aid carrying (Fig. 4.4a, b). The authors have encountered a case when this was done, and the limbs then placed into a bag so the perpetrator could make several trips by bicycle to the final deposition site of the remains. On recovery, the pathologist should take extreme care in the handling and removal of any wrappings because perpetrator evidence such as hairs, fibers, and DNA may be present within the bags or on the tape. Bags arising from a sequential roll can subsequently be linked through the manufacturer's perforations. The bags may show brand names to assist with identifying where they may have come from, which in turn may lead to CCTV images of the perpetrator. They may also contain other items along with the body parts that the perpetrator has intentionally placed inside the bag. An example we encountered of this was a homicide over the Christmas period where Christmas decorations were placed in the bag with the decapitated head.

Disposal Site

The disposal site(s) of the remains may be meticulously planned or can be spontaneous or undertaken in panic. When planning has occurred, a record of the disposal site(s) may be discovered during the investigation. If such a plan exists, this will assist the investigating police force to narrow down the search parameters.

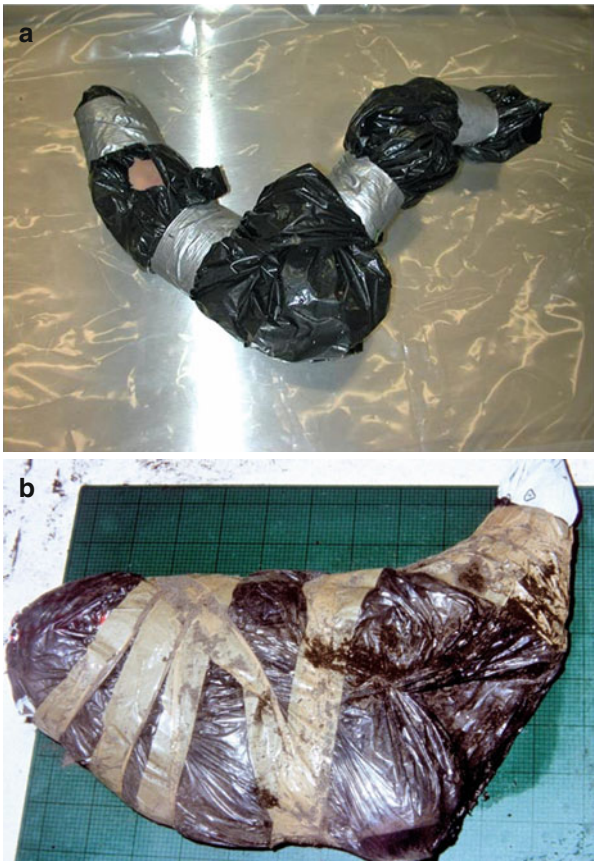

Fig. 4.4 (**a**) A limb wrapped in black domestic waste bags sealed with silver-colored adhesive tape. (**b**) A lower limb is folded in half at the knee, wrapped in domestic waste bags, and sealed with adhesive tape

We have encountered a case where the remains were placed into a domestic refuse bin, which proved effective as the refuse was taken for incineration without further inspection. We have encountered remains that have been buried in the back garden of the scene property, sometimes with an attempt to burn them first. Other burial sites may occur in habited or rural locations. Once we encountered a dismembered body buried in the soil of a number of graves within a cemetery. Water—for example, canals, land drains, rivers, and reservoirs—has been encountered as a disposal site more often, in our experience, than burials. The perpetrator may drive over a large distance depositing remains from bridges or banks into water. Although these may sink, sometimes they may wash up on the shore or be thrown such that the remains land on water vegetation, never entering the water. This can lead to the remains being found by bird watchers, dog walkers, or anglers who then alert the police to the find (Fig. 4.5).

The recovery of remains from water will require the use of specialist police underwater search teams and may involve the use of equipment such as side-scan sonar. Purpose-designed water recovery body bags may aid the recovery process.

Fig. 4.5 A dismembered partial limb is discovered in water by a member of the public

Often, if the remains are spread over a large geographic area, there may be a considerable time period before the discovery of the first and last remains, assuming all are recovered. During this period, water-disposed remains can be subject to predatory action or damaged by the action of weirs, lock-gate sluices, and boat propellers. An attempt to distinguish between antemortem trauma and such postmortem damage should be undertaken at the subsequent postmortem examination, although this may prove problematic at times.

On discovery of the first remains, it is always advisable for the pathologist to visit the scene. First, it must be confirmed that the remains are human, i.e., not animal, a prosthesis, or a model. The finding of a limb—for example, an arm or a leg—does not mean that the original owner of the limb is dead. Only when you have a vital organ/anatomical area such as the head or torso do you have a dismemberment. The pathologist can confirm this at the scene and participate in the discussion concerning the best strategy for body part recovery and transport to the most appropriate mortuary for examination. A sample of water should be recovered at the scene of water disposals and a number of solid samples from on top, under, and distant to the body part for surface or buried remains.

Examination of a Dismembered Body Part

For the purpose of this next section, the authors will present an approach to the examination of a dismembered leg, assuming that a single leg has been discovered and presented for examination. By taking this approach it will be demonstrated how

much information in relation to who, where, when, and how can be provided to the investigation police from a single limb. The same approach is applicable to any body part and will be repeated as each new find is discovered until all body parts have been found and examined.

Radiology

Prior to the autopsy examination, the limb should first be subject to a radiological examination. This would normally be undertaken by use of computed tomography (CT). This can be undertaken with the limb within a sealed limb bag. The involvement of a forensic radiologist and an anthropologist (and forensic odontologist if examining a head) with experience in the use of postmortem computed tomography (PMCT) is advisable. The analysis does not have to be undertaken at the site of examination and the use of remote radiology can prove beneficial [9, 10]. The following can be obtained at this stage from the PMCT examination:

1. An estimation of age, gender, and stature [11].
2. An examination for natural bone disease, for example, tumors, cysts, or evidence of arthritis or abnormal joint wear due to a gait abnormality.
3. An examination for previous trauma, both soft tissue and bone. Historical projectiles such as air-rifle pellets or shotgun shot may be identified.
4. An examination for antemortem trauma. The exclusion of trauma is also of use to determine, for example, the absence of a classic fracture of a pedestrian road traffic collision.
5. An examination for the site of dismemberment including the potential means of dismemberment.

Examples of these findings are illustrated in Fig. 4.6. These images become of evidential value and can be used at a subsequent trail to illustrate the findings to a jury. PMCT can thus start to build up a picture of who the missing person was, how they came by their death, and how they have been disposed of.

Trace Evidence

As stated previously, any bag/wrapping should be opened with care following an agreed forensic recovery strategy. The inside of bags and tape can have DNA and/or fingerprints from the perpetrator on them.

The limb should be placed onto a sterile or at least clean surface. It should be photographed on both sides as well as from the proximal and foot ends. Photography of any identified trace evidence, natural disease, tattoos, trauma, or postmortem change should all be undertaken during the examination with the use of an

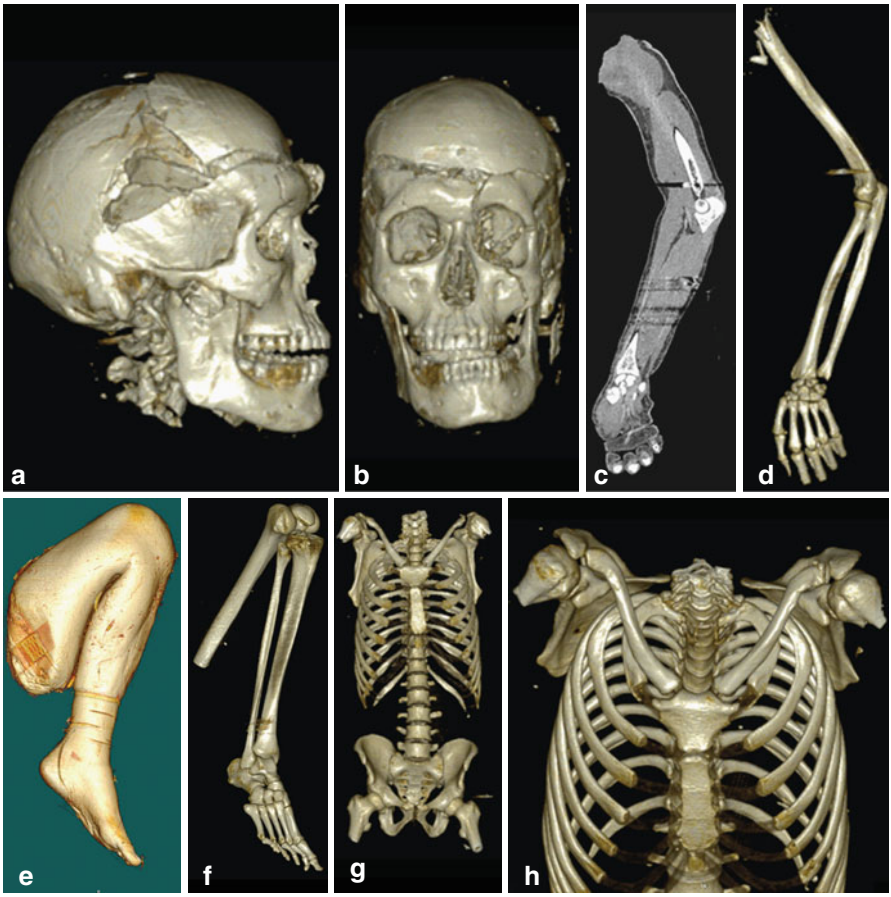

Fig. 4.6 PMCT used to provide two-dimensional (2D) and three-dimensional (3D) images of dismembered body parts for the purpose of identification and trauma assessment. (**a**, **b**) 3D bone reconstructed right and front views of a dismembered head. (**c**, **d**) A left arm showing the position of an old air-gun pellet in the arm. (**e**, **f**) A left leg showing 3D surface view and 3D bone view, the latter of which can be used for age and stature estimation. (**g**, **h**) 3D bone image of a dismembered torso with close-up of site of dismemberment through the neck and both arms

appropriate scale. Care should be taken to take photographs at right angles to the subject of interest.

The ankle areas should be examined for evidence of bindings/restraint. The whole limb should be inspected to look for evidence of the limb lying in a corrosive substance that may give an indication of where the limb has been prior to final disposal. The entire surface of the limb can be swabbed for offender DNA using dry and wet swabs to increase DNA yield. The entire surface can then be tapped for fiber lifts. Care should be taken to examine the cut end of the limb and any saw marks in the skin for paint or debris that could have arisen during the process of dismemberment.

Crime-lite

The use of alternative lighting techniques can aid the examination at this stage. We have found the use of a mortuary-sited Crime-lite such as the Crime-lite® ML2 (Foster and Freeman, Worcestershire, UK)[1] which can assist with the identification of potential sexual, ballistic, and fiber stains and trace evidence as well as the enhancement of patterned skin injuries.

Clothing and Jewelry

If clothing remains, this can be removed and packaged appropriately. Usually clothing has been removed from legs, although the authors have encountered socks and footwear still remaining. The size, material, and manufacturer label details should all be recorded and photographed as in a conventional so-called DVI approach, as at this stage you have an unidentified missing person who may be of international origin. The use of the Interpol Disaster Victim Identification pink forms may facilitate this process.[2] Jewelry may still be present to the ankles (chains) or toes (rings).

Who

The process of identification started with the radiological examination and is now continued. The gender, ethnicity, and age can be considered from the external examination, although caution should be expressed as errors can arise at this stage by dogmatic statements based on the color of the skin, the distribution of limb hair, or epiphysis fusion in the developing human or those of mixed ethnic origin. Stature can be estimated by the removal of an intact long bone, although PMCT can also be used to achieve this [11].

The feet including the nails should be examined for congenital, natural (e.g., the presence of pustular psoriasis), and acquired disease/pathology. The foot length should be measured, prints taken, and the involvement of a forensic podiatrist considered. The nails can be used for isotopic analysis for identification purposes [12].

Blood, if present, muscle, or bone (the latter usually from the mid-shaft of the femur) should be sampled for DNA identification. A method for taking samples from the femur for DNA examination has been reported by Maat [13]. Care should be taken to use sterile instruments when exposing the bone and a sterile saw blade for sampling the bone.

[1] http://www.fosterfreeman.com/index.php last visited March 2013.

[2] http://www.interpol.int/INTERPOL-expertise/Forensics/DVI-Pages/Disaster-victim-recovery-form.

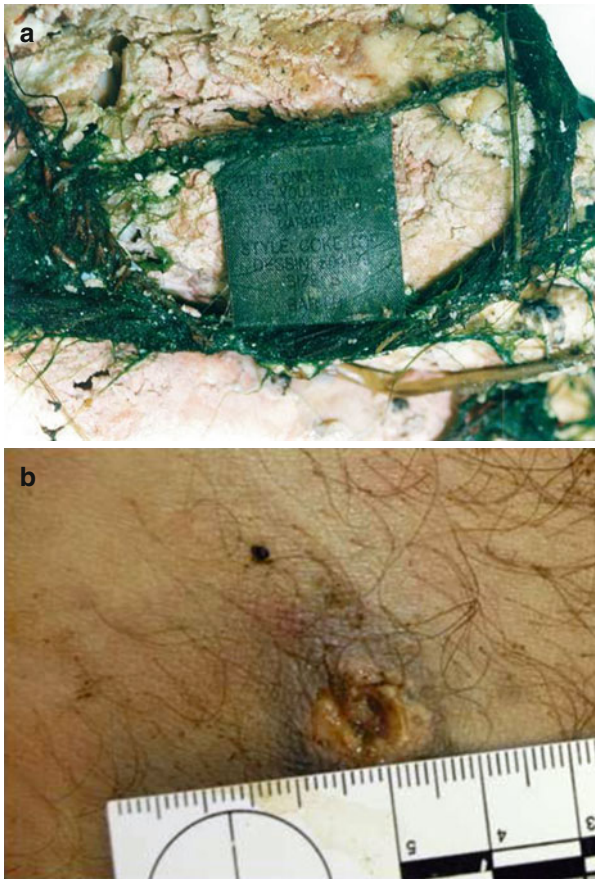

Fig. 4.7 (**a**) A label on residual clothing on the body part. (**b**) A sinus associated with chronic intravenous drug abuse

General Examination

As with an external examination of a whole body, the pathologist should seek out the presence of the following. This will continue to build up a picture of who the person was and also provide information as to potential reasons why they came by their death. Examples of the information gathered at this stage are shown in Fig. 4.7a, b:

1. Congenital or dysmorphic features
2. Body modifications such as surgical operations or tattoos
3. Presence of prosthesis, which may bare a unique number that can be checked within medical notes or from manufacturer's records
4. Presence of natural disease to the skin, soft tissues, vessels, joints, and bones
5. Historical scars including surgical, injury, and self-inflicted
6. Evidence of subcutaneous or intravenous drug abuse
7. Antemortem injury including blunt and sharp trauma, burning, projectiles, and evidence of torture
8. Postmortem changes, including the action of predators

When

As always the estimation of the time since death is difficult, especially if all one has is a leg. However, if maggots are present, these should be sampled in the conventional way and submitted to a forensic entomologist who may be able to assist with this particular question. The general state of decomposition should be recorded. Mid-shaft femur and toenails should be collected and submitted for isotopic analysis. The use of short-half-life radioactive isotopes as well as bone mineral composition can be used to build up a picture of when the person may have died and the geographical area they may have lived in during life [14, 15].

Laboratory Examinations

As well as the trace evidence, DNA and time since death samples, samples can be obtained for toxicology examinations (blood, muscle, bone) and histology of any wounds for wound dating [16] along with other considerations such as the histological examination of muscle to consider whether or not the body had been frozen prior to disposal [17].

Bone End Removal and Preparation

Up to this point, while examining the leg, one has concentrated on the recovery of trace evidence, considering the questions of who, where, when, and how they came by their death. At all times care must be taken not to damage the cut end of the limb because the bone end contains vital evidence as to how the person was dismembered.

When ready the pathologist should carefully examine the cut skin, soft tissue, muscle, and bone of the dismembered body part. The presence of any injury to the skin should be recorded. There may be, as previously explained, a saw mark where an attempt was made to saw through the skin. The nature, number, and direction of the injuries should be noted along with the position on the limb—for example, to the front, side, or back—as this will inform the investigating team as to the way the body was presented to the saw during the dismemberment act. Trace debris may be present within these marks.

The cut end of the bone should be photographed with care without removing any tissue from the bone. As much of the skin and muscle should then be removed without cutting or scraping muscle from the bone itself as this will add tool marks to the bone. This will allow the cut end to be freed from the mass of the thigh muscle. Some muscle will inevitably remain on the sample at this stage. Going approximately 5 cm back from the cut end of the bone, to ensure no false-start kerf marks or breakaway spurs are damaged, one can now dissect down to the femur and expose

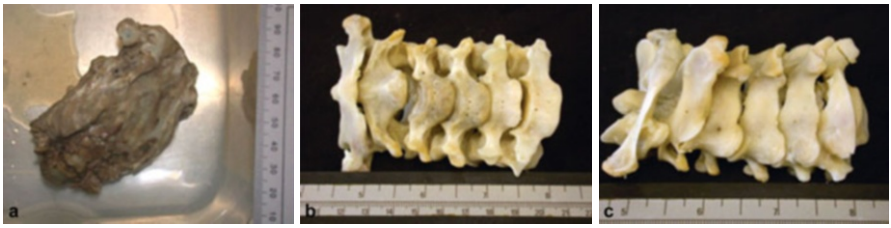

Fig. 4.8 (**a**) The cervical spine from a dismembered body is cleaned (**b**, **c**)

the bone. The bone is then cut in half to produce a sample with the evidential cut end. This specimen *must not* be fixed in formalin fixative as this will cause difficulties at the next preparation stage of the bone. The bone end should be taken to a suitable laboratory for cleaning and for forensic engineering examination (Fig. 4.8).

There are several reported means of cleaning bone specimens from manual cleaning to water maceration, hot-water maceration, enzymatic maceration, and insect consumption [18]. Whichever method is chosen by the pathologist, they should ensure that it does not affect the subsequent tool mark investigation. We use the method described by Mairs et al. [18], which uses domestic washing detergents to remove the residual flesh from the bone. We have found this to be effective in tissue removal but to allow detailed tool mark analysis. Thus the bone should be cleaned in preparation for tool mark examination.

Analysis of Tool Marks in Dismemberment Cases: Techniques and Tools

A variety of implements have been recorded as being used in dismemberment cases. The most common types of tools used are knives, saws, and axes, but other implements such as garden loppers have also been used to dismember bodies. In order to differentiate between the types of tool marks left on bone by the different implements, it is essential to have an understanding of the types of tools that may be used and the marks that they leave and the techniques that can be used to analyze the marks. This section will introduce the different tools that may be used in dismemberment cases and also the different imaging techniques that we use for analyzing tool marks including photography, stereo microscopy, scanning electron microscopy, and X-ray micro-computed tomography.

Tools and Tool Marks

Tool marks are the marks left in a softer material when a harder material is used to cut or strike it. Two types of tool mark are possible: imprints left by the indentation

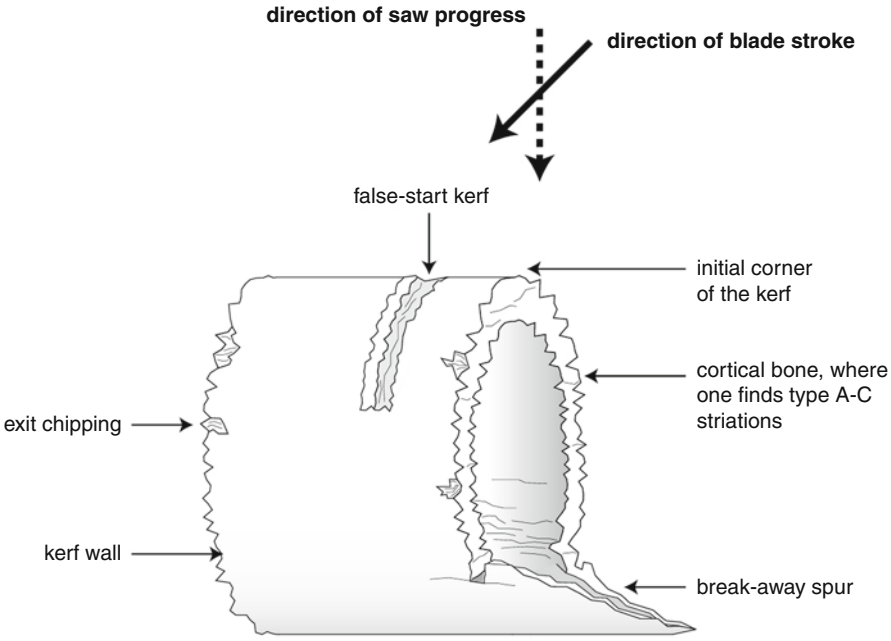

Fig. 4.9 The features of marks made by a saw on bone (Reprinted with permission from Saville et al. [20])

of the tool into the softer surface or striations that are left by the edge of the tool by either a sawing or cutting action. For saws, the nature of the mark that is left depends on the size, shape, width, and set of teeth and the sawing action of the user [19]. The analysis of saw marks is complex because the saw has multiple cutting teeth and is repeatedly moved to generate the witness mark. The slit mark made by a saw is referred to as the *kerf* (Fig. 4.9). Kerf walls and floors contain important information about the saw that was used. For knives, if used with a cutting as opposed to sawing action, the tool marks that are left are often v-shaped slits, with vertical striations on the slit wall in the case of serrated or scalloped blades. For saws, striations are normally oriented horizontally to the direction of saw movement. Tools generally leave a "negative" imprint of the tool itself in the material, and as such the imprints can be used to help determine the size and shape of the tool's tip or imperfections on the blade. Striations are parallel lines that are caused by a tool's blade either by cutting or sawing the material. For sawing, the marks are parallel to the blade's length. For cutting, the orientation of the marks depends on the direction of cutting.

The goal of tool mark analysis in forensic medicine is to compare the marks left on bone or cartilage with the suspected tool or instrument under conditions as close as possible to the conditions under which the original tool marks were made [20–23].

Tool mark experts try to match the mark found on the bone or cartilage to weapons that are thought to have made the mark. This can be done by making new marks

from the suspect weapon and comparing the dimensions and markings with the ones that were characterized from the bone or cartilage [22, 24, 25]. Traditionally, the impressions or striations are compared with a 3D stereo comparison microscope, which allows both samples to be examined at the same time, and regions where the marks correspond can be determined from carefully matching the positions of the samples. If a good match is found over a sufficient area then the marks are deemed to have been made by the same instrument. This technique is used for matching marks from saws, screwdrivers, chisels, knives, hammers, etc.

For marks on wood, metal, and polymers, tool marks are often clear and well retained. Bone is also a relatively hard, stiff material and this will also retain good marks. Cartilage is relatively soft and low in stiffness and the marks retained can be less clear. Both cartilage and bone have to be defleshed before tool mark analysis can be performed and after this the samples are dried. Tool mark analysis on bone and cartilage is more challenging because of these factors.

When investigating tool marks, it is helpful to note the material that the tool marks appear in and record the dimensions of the tool mark including length, width, thickness, depth of mark, angle, and/or diameter. The overall geometric shape of the mark is useful and features such as whether or not it consists of straight, curved, round, square, oval, or triangular marks. Any trace evidence from paint or other materials should also be recorded. Tool marks can be subdivided by a number of classifications including scrape (e.g., knife or saw mark), impression, puncture, or a combination of these. Any marks that are found can be compared to marks made by the suspected implements. The comparison marks can be prepared by looking at the marks made in a similar substrate (i.e., pig femur might be used for marks in bone) or a soft annealed metal sheet made from, e.g., copper or aluminum. Sometimes, jewelers wax can also be used.

Tools with a Sawing Action: Saws

Handsaws are generally made from rolled strips of metal with teeth cut into them. Saws are usually categorized by type (e.g., tenon saw, hacksaw, hardpoint saw) and by the number of teeth. The number of teeth is measured in points per inch or teeth per inch. Most saws have a "set," which means that the teeth usually bend to alternate sides. The set widens the kerf width and helps prevent the blade from jamming or binding. The set patterns can either be alternate (most common), where the teeth are alternately bent in opposite lateral directions, or raker, where the raker is a specialized tooth that occurs every fifth to seventh tooth to rake materials or imperfections from the kerf floor. The rakers themselves are not set, but the teeth between them are a wavy set where groups of teeth are alternately bent to the sides. Wavy-set saws have small teeth, which makes bending individual teeth difficult. The shape of the sawtooth is usually classified as either rip- or crosscut. Rip-cut teeth are flat chiseling teeth usually found on wood saws and designed to cut along the grain of the wood; generally rip-cut teeth are larger than crosscut teeth. Crosscut teeth are

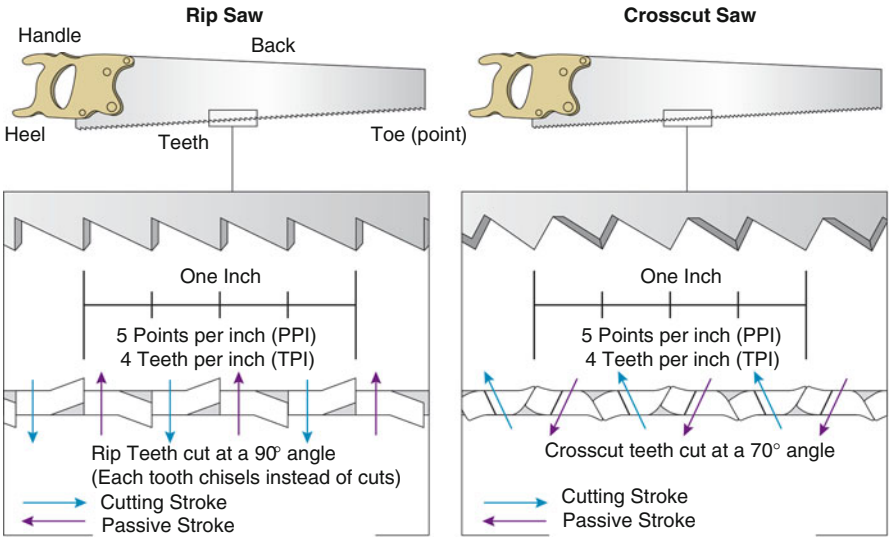

Fig. 4.10 The typical set of teeth for rip- and crosscut saws

filed at opposing angles, typically 70°. The tooth terminates in a point and the teeth cut material rather than chisel it. In modern do-it-yourself stores, saws are often designated as hardpoint. Hardpoint refers to the fact that the saw has been made from a steel that allows for the teeth to be hardened, which improves the wear resistance of the blade. There is a compromise to be made between hardness and toughness as the hardest saw teeth may be brittle. Hardpoint saws have a crosscut tooth pattern. The typical set of teeth saws is shown in Fig. 4.10. Figure 4.11 shows a range of common saws used in dismemberment cases and the teeth geometries and set.

There are a number of indicators on a sawn bone that may help with determining whether or not the saw was in good condition:

1. Sharp teeth leave a clean cut; missing or blunt teeth, or other damage to the blade, will leave a rougher kerf wall [26].
2. If the blade is bent, the saw will bind or jam as it is cutting. Deep false-start kerfs can indicate that the saw blade was bent. Bent blades are difficult to saw with and this can lead to more tiring conditions for the person sawing. There may be more hesitation marks in the cut.
3. If the teeth are not evenly set (i.e., some teeth protrude more than others), then the teeth drag and the cut is usually rough. If all teeth on one side project farther than on the other, then the blade twists during use and this can be identified by a kerf wall that changes orientation with depth of cut.
4. Good saw blades taper, which helps stop the blade binding and gives a clean cut.
5. Most blades have a crown, i.e., a slight outward curve at the blade center. The crown increases the pressure on the teeth in the center during cutting. Typically, sawyers do not use the entire length of the blade; at most two-thirds of the length

Fig. 4.11 A typical (**a**) hardpoint, (**b**) tenon, and (**c**) hacksaw showing the geometry (*middle column*) and set of the saw teeth (*end column*), respectively. For the hardpoint and tenon saws, the saw teeth are set in a crosscut geometry. The hacksaw has a wavy-set teeth setting

is used. This means that the effect of missing or bent teeth at the center of the blade has a bigger effect on the tool mark than those at the outer edge.

The kerf width can be helpful in determining the type of saw used [20], but is not a unique measure as some very different saws can give kerf measurements that are similar. It is important to look at kerf widths under the microscope, as this can sometimes show that a kerf width comprises of several passes of the blade. Kerf widths do not directly relate to saw blade thickness; as has been described earlier, most saw blades taper and are wider at the teeth to prevent the blade jamming during sawing. In order to see whether the kerf width matches a suspected implement, cuts can be made into defleshed pig femur and compared with the mark found on the dismembered part.

For handsaws, examination of kerf walls by environmental scanning electron microscopy (ESEM) has identified three distinct types of striations that have been previously designated as type A, B, and C striations [20]. Figure 4.12a, b shows an ESEM micrograph of the three types of striations that can be seen. Type A striations are large bands that appear on the kerf wall bordered by deep furrows that arise as the saw is pulled backwards on the passive pull stroke when the teeth are aligned. The striations are between 1 and 4 mm in size and represent the amount of material cut on each stroke. Type B striations are smaller striations occurring within a type A striation and represent the material removed by an individual tooth. Type C striations occur within the type B striations and are created by imperfections from the leading edge of a particular sawtooth. These striations allow discrimination between saws as they are not influenced by either the pressure or speed of the saw blade. Type C striations are seen in small bands, which can be smaller than 1 μm.

Circular saws, power saws, and chain saws leave a range of different marks compared to handsaws. Circular saws tend to leave tool marks that follow a circular

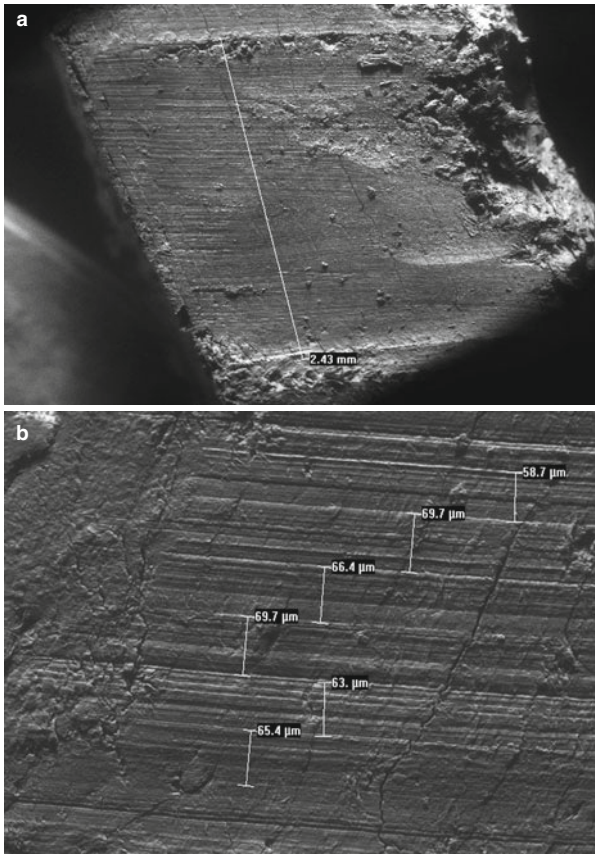

Fig. 4.12 An environmental scanning microscope image of the different type of striations that can be seen from a saw cut on bone. (**a**) Type A striations. (**b**) Type B and Type C striations within type B striations

pattern on the kerf wall. The kerfs of circular saw cuts tend to be relatively smooth. The width of the cut is determined by the widest part of the blade and depends on whether or not the teeth on the saw blade are tipped with, for example, cermet cutting points. Power saws that saw with a reciprocating action leave straight kerfs that run parallel to the direction of the blade movement. Chain saws tend to leave a circular pattern on the kerf wall but with very wide false-start kerfs if they can be found. There is also considerable bone loss and the geometry of the bones that remain does not necessarily fit—there is a substantial gap upwards of 5 mm depending on the blade and chain width. The wall of the kerf is smooth but there may be entrance and exit chipping. The kerf widths of power tools are generally larger than those of hand tools [26].

Tools with a Cutting/Slicing Action: Knives

Walker and Long [27] made a systematic study of tool marks on bone from knives and axes on fresh metapodials from cattle. They studied the pressure and angle of application, motion used, and length of blades to determine how steel knives, axes,

and bifacially flaked chert tools left tool marks. They found that the analysis of cross sections of the tool marks allowed them to relate tools to butchering marks on wolf bones. The width of the kerf was found to increase with additional load as the knife pressed deeper into the bone. Knives and axes left v-shaped cross sections but the chert tools left rougher cross sections. Lewis [28] made a study looking at distinguishing sword marks from those left by knives. The hind limbs of cows were struck with a broad sword, machete, katan, samburu, scimitar, or knife. The swords produced marks that were clearly different from knives. The machete and scimitar produced a conchoidal flaking and shards of bone, whereas knives produced smooth kerf walls. Sword marks produced features associated with both sharp force and blunt force trauma, i.e., a sharp smooth wall where the edge of the knife enters the bone and other features such as lateral cracking, chipping, and breaking of the bone. The sharpness of the edge of the blade was important in determining how much sharp force trauma versus blunt force trauma is seen. Shaw et al. [29] studied the relationship between the v-shaped angle left on bones against the knife geometry and elasticity of the bone. Both need to be taken into account when determining whether or not a particular knife left a mark. Additionally, vertical striations can often be found on the inner surfaces of the v-shaped grooves and these can be matched to the tool profile in the same way as saw marks in some instances.

Tools with an Impact: Axes/Cleavers/Hatchets

An axe is a tool whose modern use is intended to shape, carve, split, and cut wood, although historically axes have been used as weapons and they are used in a modern forensic context in cases of dismemberment. The axe head, which is wedge shaped, is attached to a shaft. Axes come in different sizes and shapes, from small hand axes used with one hand through to longer axes where a two-handed swinging action is more common. Axes have a shallow wedge angle, but a similar tool known as a splitting maul that is used for splitting large logs has a deeper angle to drive a split into the log. The wedge angle concentrates the pressure applied via the axe at the blade and the handle of the axe works as a lever; thus, the longer the handle, the greater the applied force assuming that the grip is at the end of the handle away from the head. Most axes have steel heads with handles (or hafts) that are made from either wood (hickory or ash are common) or fiberglass. Hatchets are a specialist type of axe with a wedge on one side and a hammer on the other. Other types of axes are available as gardening tools; these tend to be a variation on the ancient billhook. A typical example of an axe and a billhook are shown in Fig. 4.13a, b.

Humphrey and Hutchinson [30] looked at the characteristics of hacking trauma on partially fleshed pig bones caused by machetes, cleavers, and axes. The axe wounds were clearly recognizable with chattering, crushing, and fracture. Lynn and Fairgrieve [31] also examined the trauma produced on pig bones by axe and hatchet but where the bones had not been previously defleshed. They found a number of different types of fractures including curve transverse and spiral fractures in defleshed femora and longitudinal fractures in fleshed fibula.

Fig. 4.13 (**a**) A typical billhook with a fiberglass handle. (**b**) A typical hand axe with a fiberglass handle

Fig. 4.14 (**a**) A typical surface produced by an axe on a bone surface. Note the large area of break-off spur to the left-hand side where the bone has fractured. A v-shaped notch can also be seen where the axe has cleaved into the bone. (**b**) The straight parallel-sided cuts into a pig femur (near the knuckle) where the parallel-edged billhook has cleaved into the bone

The typical damage imparted on bone by an axe and billhook is shown in Fig. 4.14a, b, respectively. The bones show examples of fracture and rough, hewn fracture surfaces. The momentum imparted to the blades when swung results in a high energy at impact and fracture and splitting of the bone surfaces. For Fig. 4.14b, it is possible to see the v-shaped profile of the billhook on the bone.

Tools with a Scissor Action: Garden Loppers/Bolt Cutters

Tools such as garden loppers can be used in dismemberment cases. Figure 4.15 shows a typical pair of ratchet garden loppers. These loppers are geared and have long lever handles, which means that the force exerted on the blade is substantial. The cut surface of a bone cut by loppers is very straight sided (see Fig. 4.16) and the cut surface is flat and relatively featureless. There is some evidence that the cortical

Fig. 4.15 A pair of garden loppers with extendable arms

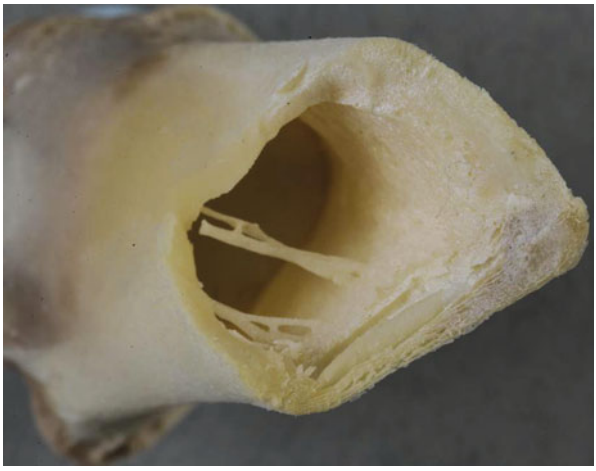

Fig. 4.16 A pig femur that was cut by the garden loppers in Fig. 4.15 showing a flat and relatively featureless tool mark. There is some splitting of the cortical bone evident towards the right-hand side of the bone

bone has split as the bone has been cut through. Flat featureless cuts such as these can often be related to cuts made with a scissor type of action. A feature of these tool marks is that cutting may occur from one or both sides, depending on whether or not both surfaces have cutting edges or whether one side of the tool has a grip surface. If a grip is present, there may be evidence of this on one side of the bone; alternatively, the side with the grip surface may show signs of crushing. These features can be helpful in guiding the search for a suitable tool or identifying a specific tool if similar tool marks can be made on a suitable bone such as a pig femur.

Analyzing Tool Marks: Protocols

The initial examination of any tool mark on skin/bone or cartilage is by eye or with a low-power hand lens. The eyes are good at observing colors that may be important if, for example, there is any debris from the tool used for the dismemberment.

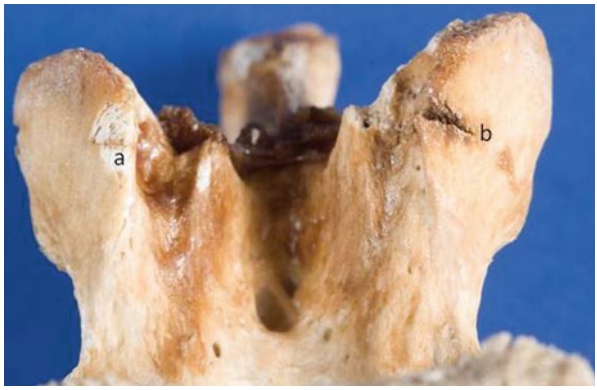

Fig. 4.17 Location of the two marks (**a**, **b**) shown on an L2 vertebra. The marks are oriented at different angles and therefore these marks were made with two separate cuts of a blade

In particular, saws may leave debris from paint on the blade (hacksaw blades are particularly prone to this) and loppers or axes may similarly leave traces of paint. This trace evidence needs to be recovered. The first tool in any analysis should always be what is observed by eye. Additionally, while it may be tempting to put cut ends of bone back together, for example, to demonstrate that they match, actual contact between the surfaces should be avoided as it can damage the marks that have been left. Cartilage or bones for examination may need to be excised to look at a higher magnification or using different analysis tools. When transporting any bones, the surfaces of interest should be kept out of contact with other surfaces as, again, this can damage the surface. Before examination of tool marks on bone, the bone may need to be defleshed using a suitable method, with care to avoid overzealous preparation of the bone [18], which can lead to bone softening and, in extreme cases, loss of bone.

One of the disadvantages of the examination by eye is that while observations can be recorded in a descriptive sense, it does not give a permanent scientific record of the examination, and therefore the initial view is often recorded using macrophotography. Macrophotography of bones can often be useful for identifying the initial location of marks. Figure 4.17 shows the typical detail that can be observed using macrophotography. The figure shows an L2 vertebra from a body that was dismembered with a knife. The macrophotography is helpful for determining the number of cuts and the orientation of the cut marks. For example, by observing the alignment of the cuts on the articular processes of the vertebra, it is immediately apparent that the marks must have been made by two distinct cutting processes.

In order to take high-quality macrographs, the illumination should be carefully arranged so that shadows are minimized. A good-quality digital SLR camera with a macrophotograph lens is required, and a tripod or camera stand helps to allow images to be obtained without flash. A ruler or other feature can be used for recording scale; although sometimes where an image has considerable depth of field, it can be difficult to arrange for the ruler and feature of interest to be in focus at the same time.

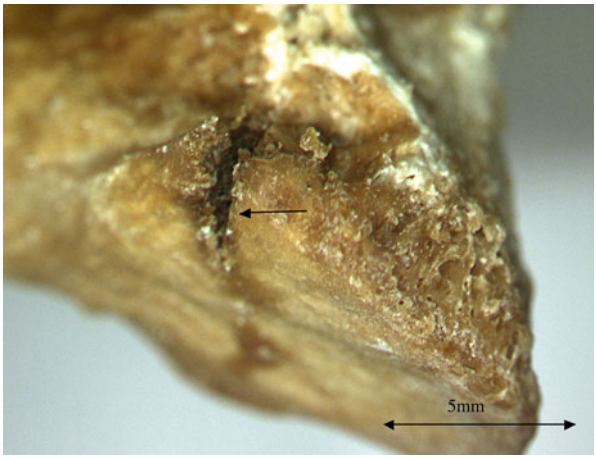

Fig. 4.18 A cut mark (*arrowed*) in the right inferior articular facet of an L2 vertebra. Note the V nature of the cut. This identifies the cut as having been made by a blade with a V profile rather than by a saw with a parallel blade. Saw marks are usually characterized by square-shaped kerf floors

Stereo Microscopy

It is the parallax between views of an object (i.e., the lateral difference in position) from your two eyes that allows you to see objects in three dimensions. In optical microscopy, to achieve a stereo image, a stereo microscope uses two eyepieces taking light from two different optical paths to achieve the same effect. Another feature of stereo microscopes—as opposed to traditional reflected or transmitted light microscopes that are used for viewing, for example, histological sections—is that they allow a large working distance between the bottom of the lens and the sample. They also have the facility to zoom in magnification, as compared to the steps in magnification as you switch between objective lenses in a standard reflected light microscope. The ultimate magnification of stereo microscopes is limited by the numerical aperture (NA) of the lens. Unfortunately, there is a trade-off between optimizing optical lenses for depth of focus and resolution, and this limits the maximum magnification that it is possible to use with a stereo microscope. The magnification of the image is the eyepiece magnification multiplied by the objective lens magnification or the camera magnification multiplied by the objective lens magnification if you are observing the image on a PC. As with reflected light microscopy, it is important to include the scale on any image. When you observe the sample through the binoculars, you will see a good perception of depth. When you take a single image through the camera, you will lose this depth perception as you lose the parallax. Samples need to be rigidly clamped when recording the image to ensure images are as sharp as possible. If the 3D information related to the cut shape is required, other techniques such as optical profilometry can also be helpful. Figure 4.18 shows a stereo microscope of a cut mark in bone and clearly shows the v-shaped profile indicating that a knife was used to make the mark.

Scanning Electron Microscopy and Environmental Scanning Electron Microscopy

When an electron beam strikes a sample, the electrons interact with the material to produce either additional electrons or X-rays. These signals are emitted from the surface and detected. Different signals can be used to gain different information about the sample. Secondary electrons are the most commonly used signal; these give topographic information (i.e., information on the surface geometry). Backscattered electrons give atomic number information; the brighter the image, the higher the atomic number phase or element from which the electrons come. One of the big advantages of using an electron microscope is that electrons give images that have excellent depth of focus, and because they effectively have a small wavelength, they give excellent resolution, and thus, the scanning electron microscope gives advantages over traditional comparison microscopes [32, 33]. As a practical guide, it may be possible to work at magnifications up to 200,000× and resolve particles that are approximately 40 nm in diameter. In order to allow electrons to travel sufficiently far, electron microscopes require a vacuum environment and conducting specimens so that the electrons that interact with the specimen disperse and do not lead to imaging artifacts from charge buildup. Over the last 20 years, environmental or low-pressure scanning electron microscopes have become available. These microscopes have a high vacuum in the electron gun, which is separated from the chamber where the specimen sits. The specimen can be at a relatively low vacuum, and imaging gases such as water, air, and nitrogen are used. These gases help to neutralize charge buildup on the specimen and mean that insulating samples, such as bone or cartilage, can be imaged without having to conductively coat the specimen. This is essential for forensic applications where it is desired to not change the specimen in any way. Scanning electron microscopes also have large chambers and, as an example, specimens up to the size of a house brick may be accommodated in large-chamber microscopes (although not all areas may be accessible to imaging). Scanning electron microscopes are particularly helpful in imaging striations in bone, particularly the fine striations left on saw marks. Figure 4.19 shows a montage of scanning electron microscope images taken of a saw mark on a nylon 6,6 surface. There are several inset boxes showing cuts made on different samples with the same saw but different sawyers. It can clearly be seen that there is an excellent correlation between the different marks and thus this technique is essential for performing matches of this nature.

Micro-Computed X-Ray Tomography

Computed X-ray tomography has routinely been used for medical applications since the early 1970s. In computed X-ray tomography, an X-ray source is located on one side of a body and the detector is located 180° around from the source.

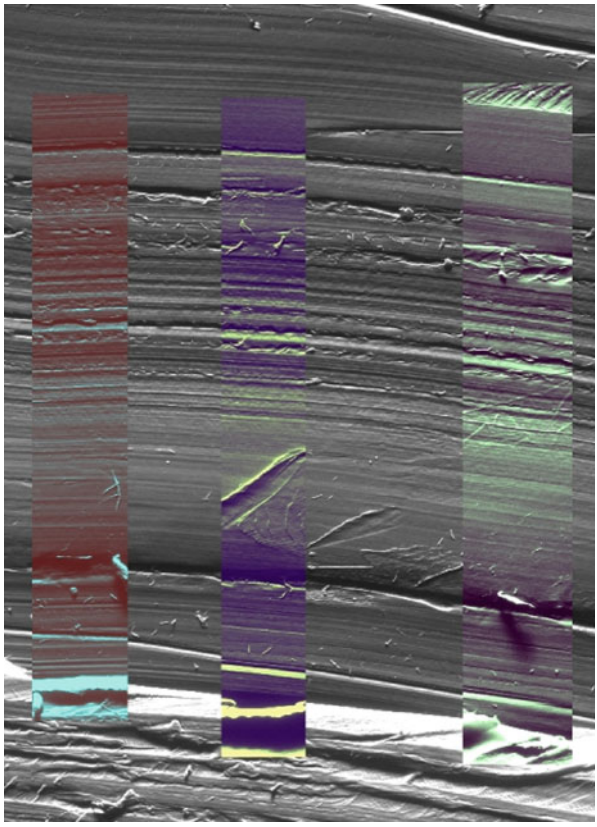

Fig. 4.19 A montage of scanning electron microscope images of a cut on a nylon 6,6 surface. The three inset boxes are of cuts made on different samples with the same saw. It can be seen that it is possible to almost exactly match the striations from the different cuts, illustrating that the striations are showing marks from the individual teeth on the saw blade (Reprinted with permission from Saville et al. [20])

The detector and source are rotated around the stationary body and X-ray radiographs are taken at a number of positions. The radiographs are then reconstructed using sophisticated software to produce a 3D image of the body.

In other fields such as engineering, micro-computed X-ray tomography has recently become available. This allows higher-resolution imaging and greater detail of tool marks on bone to be retrieved. Recently, we have demonstrated the use of micro-CT in forensic applications [9] including the analysis of tool marks on bone. Figure 4.20 shows the difference between CT and micro-CT in terms of the rotation of components. The blue arrows show the rotating parts in each case.

Figure 4.21a, b shows a typical image of tool marks in bone that was taken with a Nikon Metrology XTH 225 micro-CT scanner, with a PaxScan detector. Data was reconstructed using Nikon Metrology's proprietary software and all rendering and subsequent analysis was performed in VGStudio Max 2.1. For examining bones and cartilage, typically X-ray emitter conditions of between 95 and 115 kV with a current range of 115–142 mA and a filter of 0.5 mm thick copper are used.

One of the key questions about X-ray tomography is, "What is the resolution?" The resolution depends on the size of the pixel (picture element) matrix employed

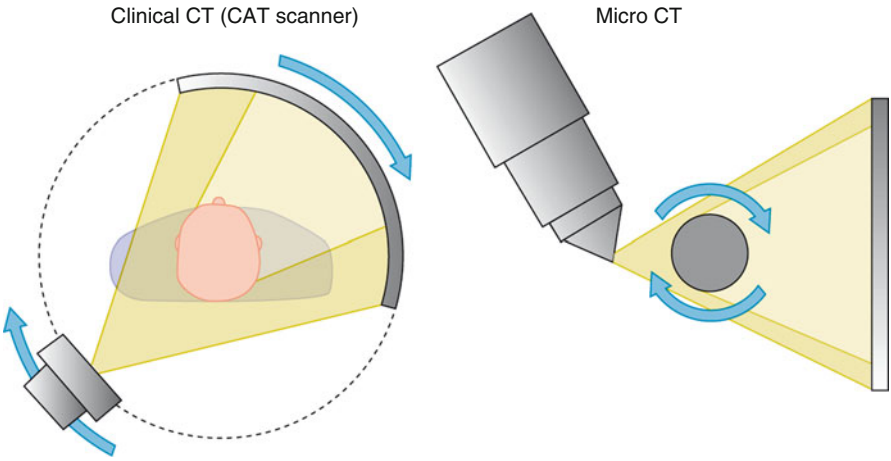

Fig. 4.20 A schematic diagram showing the difference between clinical CT, where the X-ray source and detector rotate around the body, and micro-CT where the sample rotates and the X-ray source and detector remain in fixed positions

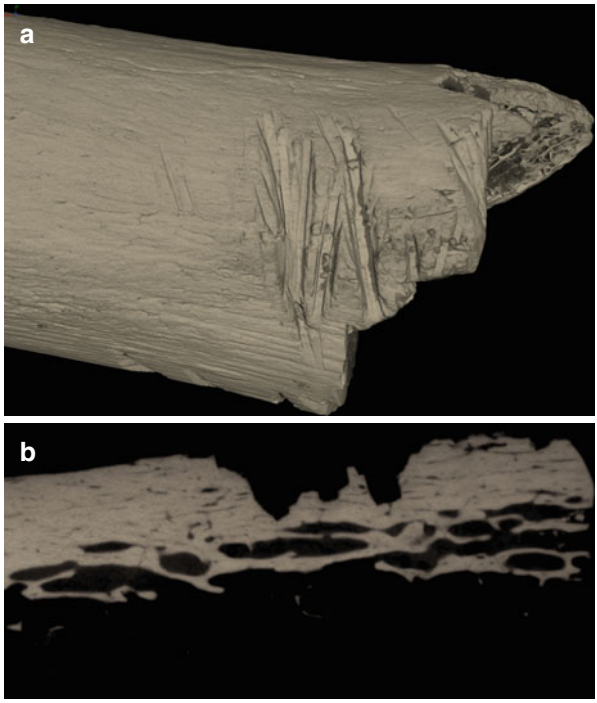

Fig. 4.21 X-ray CT images of saw tool marks on a femur showing the resolution that can be obtained. (**a**) Shows the top view with multiple false-start kerfs in different directions. (**b**) Shows the cross section through the kerfs in the line shown in (a)

and the subsequent spacing of volume elements (voxels). The spatial resolution is also influenced by several other factors, including the inherent resolution of the X-ray detector, focal spot size, geometric magnification, stability of the rotation mechanism, and the filtering algorithm utilized for CT reconstruction.

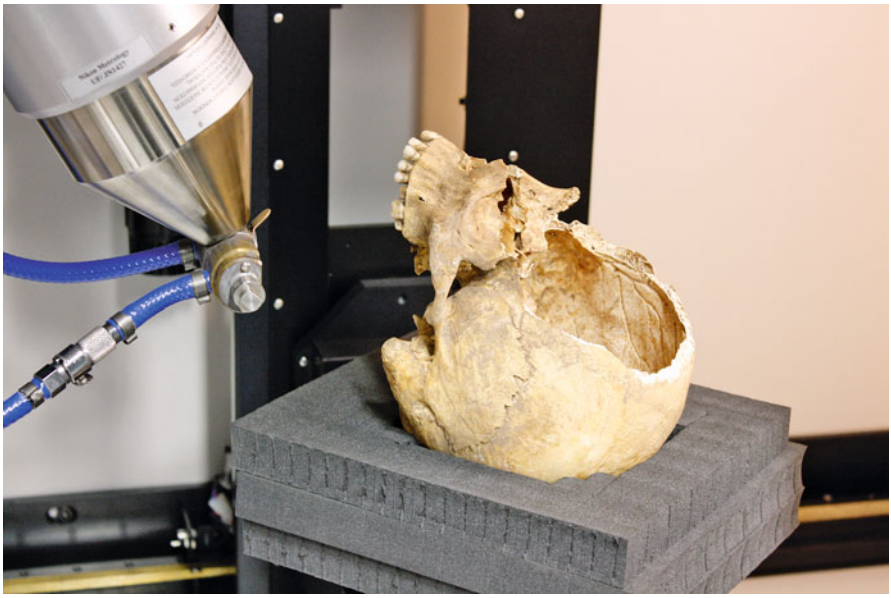

Fig. 4.22 Image of the X-ray micro-CT showing a large sample of a skull close to the X-ray source

In practice, for micro-CT (and clinical CT), the geometric magnification is important. The X-ray emitter and the camera for detecting the X-rays are a fixed distance apart, and therefore the geometric magnification depends on how close the sample is placed to the emitter (source of X-rays) or detector. The closer the object is to the emitter, the larger the projected image captured by the detector. The sample must be able to be rotated in micro-CT to 3D images to be generated from taking multiple X-ray slices as the sample is rotated in the X-ray beam, and this gives a practical limitation on how close the object can be to the emitter. The combination of the geometric magnification and distance from source to detector gives the improved resolution in micro-CT as compared to conventional CT. Figure 4.22 shows the typical size of sample that can be accommodated and imaged in a micro-CT scanner.

A further factor that controls the resolution is the spatial resolution. This is controlled by how much the parts of the object being scanned move between the different radiographic slices. The part of the object that is at the greatest distance from the center of rotation moves the most. The smaller the movement between slices, the better the resolution. Finally, the number of frames taken is averaged to improve the signal-to-noise ratio. To obtain very high-quality images, long scan times may be necessary; so, for example, for high-resolution imaging, each bone may be scanned for between 6 and 8 h, although lower-resolution images can be obtained in shorter times.

One of the advantages of micro-CT as opposed to the other imaging tools that can be used to look at marks on bones or cartilage is that by virtue of the image being reconstructed from many slices, each slice is in focus and thus there are none

of the issues of parts of the sample being out of focus that happen with the other techniques. Another of the real advantages of micro-CT is that there is no specular reflection from the surface as can sometimes be an issue with stereo microscope images of bone. Also, the images can be presented in a form that is accessible to juries.

Conclusion

A range of investigative tools can be employed to fully understand the tool marks left on bone and cartilage in dismemberment cases. A full and thorough investigation will involve examination by eye followed by micro-computed X-ray tomography, stereo microscopy, and, finally, scanning electron microscopy. The marks should be compared to marks made in other samples with the suspected tools to determine whether or not a match can be made. Tool mark analysis is a critical part of the investigation in any case involving dismemberment.

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Chapter 5
Medicolegal Autopsies and Pharmacogenetics

Antti Sajantila

Introduction

A medicolegal cause of death (CoD) investigation uses a multidisciplinary approach and expertise from forensic pathology, imaging, toxicology, biochemistry, and genetics. An effective use of all of these disciplines is crucial for individual citizen's safety and legal protection. Apart from the safety aspect and individual cases, medicolegal autopsies produce unbiased and reliable data to investigate unexpected natural and injury or toxic deaths. When performed in a reasonable frequency, medicolegal autopsies also archive unique datasets for studies to characterize lifestyle, for example, substance use, which may affect morbidity and mortality at population level. Thus, large medicolegal CoD databases can reveal trends or patterns that can be used by policymakers to implement preventive actions and to make evidence-based health or legislative initiatives.

With new technology, such as next generation sequencing [1, 2], toxicological analysis without reference standards [3] and from alternative matrices [4, 5], and bioinformatics [6, 7], the amount of information available from various forensic sample types is increasing. The new avenues in human identification include characterization of unidentified bodies by (1) search for the geographical origin of the individual [8], (2) assessment of the relative age of the sample donor (remains) [9, 10], and (3) external visible traits of the sample donor such as eye and hair color [11, 12]. The latest technical progresses in human identification indicate that multiple DNA datasets can be analyzed in a single tube [13] from samples collected from a crime scene.

The same genomic and toxicological advances are of great interest for medicolegal CoD investigation. Thus, previously unavailable information of disease conditions and

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toxicological effects can be obtained from molecular autopsies [14–17], toxicology and drug interaction studies [18], and postmortem pharmacogenetic studies [19–22].

This chapter describes the basic concepts of pharmacogenomics, and how genetics and toxicology can aid forensic pathology, and together form an essential part of the CoD and manner of death (MoD) investigation.

Pharmacogenetics and Pharmacogenomics

Definitions

Due to the great scientific interest and potential clinical importance of the genetic basis for drug response, the United States Department of Health and Human Services and Food and Drug Administration (FDA) has defined *pharmacogenomics* (PGx) as "the study of variations of DNA and RNA characteristics as related to drug response" and *pharmacogenetics* (PGt) as "a subset of pharmacogenomics (PGx) defined as the study of variations in DNA sequence as related to drug response" in their release "The Definitions for Genomic Biomarkers, Pharmacogenomics, Pharmacogenetics, Genomic Data and Sample Coding Categories" [23]. In other words, PGt/PGx deal with the underlying inheritable factors in interindividual differences in the response to prescription drugs. With emerging technologies it is, for the first time, possible to offer a holistic molecular-level explanation to the clinically known notion of "responders and nonresponders." PGt/PGx has already revolutionized biomedicine and pharmacology. Today, clinical pharmacology combined with genetic knowledge form together with pharmacokinetics (PK) and pharmacodynamics (PD) the basis of pharmacology. The ultimate goal of PGt/PGx is to fulfill the promise of a most effective and safe drug treatment tailored for each individual patient and to minimize the unexpected adverse effects of prescribed drugs.

Early Observations

Since the time of Pythagoras in 510 BCE, it has been known that some individuals may have adverse effects when ingesting certain substances, such as the ingestion of fava beans [24]. The idea of the use of genetic testing to ensure effective drug treatment was born in the first half of the twentieth century. In 1902, Archibald Garrod [25] anticipated that genetic tests may be useful in optimizing patient's drug treatment. However, the concept of pharmacogenetics was maturated based on the laboratory studies to solve patients' problems (cases) in the 1950s. In 1952, Hockwald et al. [26] described that African-American patients using the antimalarial drug primaquine developed intravascular hemolysis. In 1956, it was shown that the cause for this undesired response was a deficiency of glucose-6-phosphate dehydrogenase [27]. Around the same time it was recognized that the response to the tuberculosis drug isoniazid

Table 5.1 Some milestones in the development of concepts and crucial data in PGt/PGx described in this chapter

Year	Milestone	Author(s)	References
510 BCE	Description of the danger of fava beans	Pythagoras	[24]
1902	"Inborn errors of metabolism" Anticipation of genetic tests for drug treatment	Garrod	[25]
1952	Primaquine causes intravascular hemolysis in African-American patient, but not in Caucasians	Hockwald et al.	[26]
1954	Interindividual difference in response to isoniazid used for tuberculosis treatment	Hughes et al.	[28]
1956	Glucose-6-phosphate dehydrogenase deficiency is the reason for intravascular hemolysis during primaquine treatment	Carson et al.	[27]
1956	Interindividual difference in response to succinyl cholinesterase is due to deficiency in	Lehrman and Ryan	[30]
1957	pseudocholinesterase	Kalo and Staron	[31]
1957	Summary of findings relating heritability and differences in drug metabolism	Motulsky	[32]
1959	"Pharmacogenetics" term used for the first time	Vogel	[33]
1960	Slow and rapid acetylators for isoniazid described	Evans et al.	[29]
1977	Polymorphic hydroxylation of debrisoquine described	Mahgoub et al.	[37]
1979	Pharmacogenetic defective in oxidation of sparteine	Eichelbaum et al.	[38]
1988	CYP2D6 gene cloned and characterized in DNA level described	Gonzales et al.	[39]
2000	First case in postmortem pharmacogenetics described	Sallee et al.	[87]
2000	Pharmacogenetics and pharmacogenomics form an important basis for clinical medicine, forensic medicine and academic research		

showed interindividual differences and that patients could be classified as rapid or slow acetylators [28, 29]. After studies by Lehman and Ryan [30], and Kalow and Staron [31], who independently showed that prolonged neuromuscular paralysis after succinylcholine administration varied due to a deficiency in the metabolizing enzyme pseudocholinesterase in some patients, the concept of a genetic basis for these phenomena was summarized by Motulsky in 1957 [32]. In 1959, Vogel [33] then coined the term "pharmacogenetics," which has evolved from the idea of monogenic traits to pharmacogenomics with genome-wide perspective [34–36].

In the 1970s, the molecular mechanisms of the aforementioned phenomenon began to be uncovered. Two groups independently observed disadvantageous and unexpected patient effects of the drugs debrisoquine and sparteine [37, 38]. It was shown that both drugs are metabolized by a cytochrome P450 (CYP) monooxygenase. Finally, in 1988 Gonzalez et al. [39] cloned and characterized CYP2D6 as the first polymorphic gene affecting the drug response. Some of the above described and other milestones of the development of PGt/PGx with references are listed in Table 5.1.

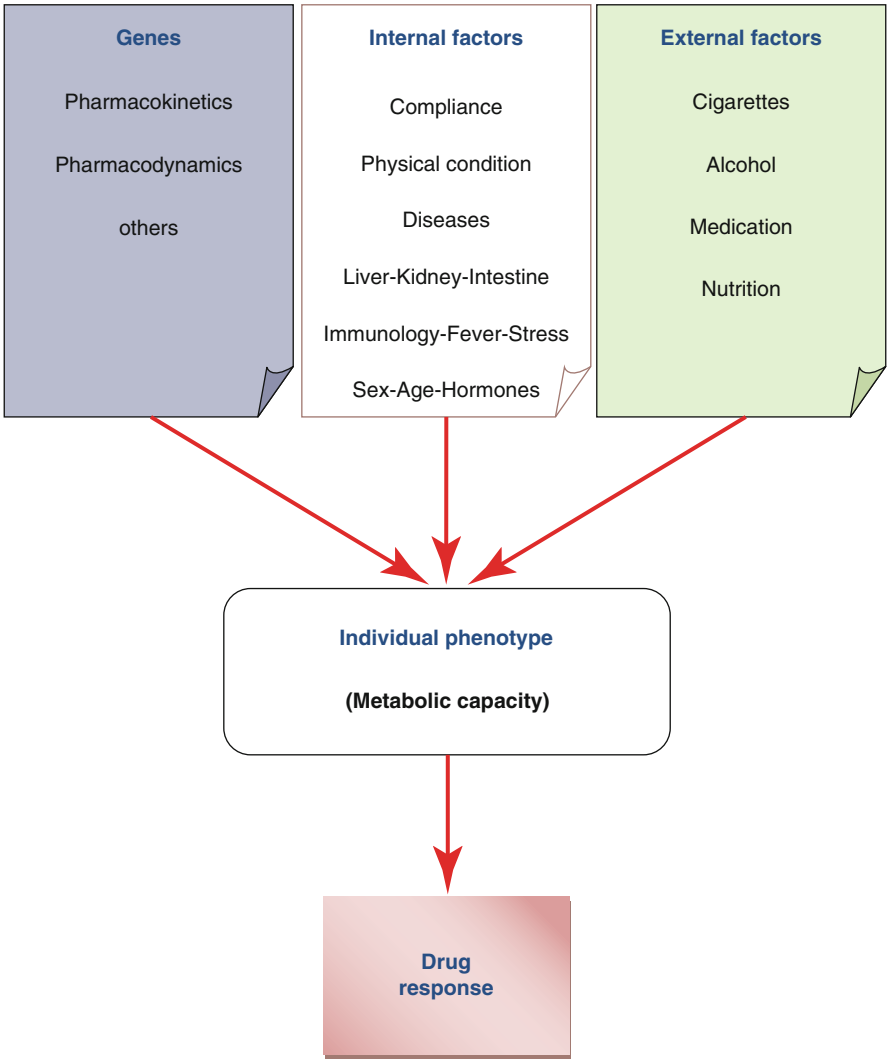

Fig. 5.1 The individual drug response is a combination of factors that have genetic, internal, and external compounds

Current Understanding of Genetic Basis for Drug Response

The response to a therapeutic drug is a puzzle of multiple factors to be classified to genetic factors, external or environmental, and internal or patient-specific factors (Fig. 5.1). They can all temporarily or permanently change an individual's response to drugs. The internal factors include patients' compliance, developmental stage (age and sex), physiological factors (e.g., mental or physical stress), and hormonal, seasonal, or circadian changes. Specific diseases—diabetes, immunological defects,

infections, fever, or gastrointestinal diseases—can also have an effect on drug response. The external factors, consisting of personal environmental history, lifestyle, concomitant use of alcohol(s) or drug(s), and exposure to environmental toxins or certain food products, can also change the response to therapeutic drugs. The genetic variation relevant to PGt/PGx has an effect on either the PK or PD of drugs. In addition, genetic systems with other mechanisms, such as human leukocyte antigen (*HLA*), human epidermal growth factor receptor two (*HER2*), Kirsten rat sarcoma two viral oncogene homolog (*KRAS*), and some ion channel modifying genes (e.g., long QT syndrome), may warrant for individual dosing with prescribed drugs.

The genes affecting the PK of drugs have an effect on either the adsorption, distribution, metabolism or excretion (ADME), and they form the largest and best known group of pharmacogenetic factors till now. The most common and largely studied is the cytochrome P450 (CYP) family enzymes (such as CYP2D6, CYP2C9, CYP2C19) [35, 36]. These enzymes are part of the xenobiotic metabolism system protecting us against harm caused by foreign compounds ingested or administrated to our body, for example, through nutrition or drug therapy [24]. The xenobiotics are cleaned from our body by modification (phase I reaction), conjugation (phase II reaction), and excretion (phase III reaction), which detoxify and remove foreign substances from cells with the aid of a variety of enzymes. The phase I reaction enzymes modify the functional groups of the foreign substances, and the subsequent phase II reaction enzymes conjugate these modified functional groups with, for example, acetate, sulfate, glutathione, and glucuronic acid. The phase II reaction is catalyzed by transferases (e.g., glutathione S-transferases, N-acetyltransferases, and thiopurine methyltransferase). Phase III involves excretion of the foreign substance conjugates and their metabolites from cells. In this last phase, for example, the multidrug resistance protein (MPR) family can catalyze the ATP-dependent transporters and remove conjugated products to the extracellular matrix to be excreted from the body. Another clinically relevant transporter is solute carrier organic anion transporter family, member 1B1 (*SLCO1B1*) gene, related to statin-induced myopathy, which also acts in phase III reactions (Fig. 5.2).

The genes involved in PD are responsible for the molecular structure or receptor targeted by the medication, or they influence the signalling or metabolic pathway involved in the disease. Examples of PD genes are catechol-O-methyltransferase (COMT), dopamine transporter, and receptor two (DAT1 and DRD2, respectively) genes and monoamine oxidase B (MAOB) gene

An interesting interplay of genes affecting both PK and PD of a drug is the case of warfarin therapy. Patients with CYP2C9*2 and CYP2C9*3 alleles show reduced warfarin metabolism, require a lower daily dosage, and have a greater risk for bleeding with a standard dosage [40]. It has been proposed that gene variants involved in PD and thus altering drug target pathways, may affect drug efficacy in a pronounced manner. Indeed, in warfarin therapy vitamin K epoxide reductase (VKOR) is the target of warfarin, and polymorphisms in the VKOR complex subunit one gene (*VKORC1*) affect the warfarin dosage required for therapeutic anticoagulation. The proportion of this PD-driven *VKORC1* variation is much greater than interindividual variation caused by PK-driven CYP2C9.

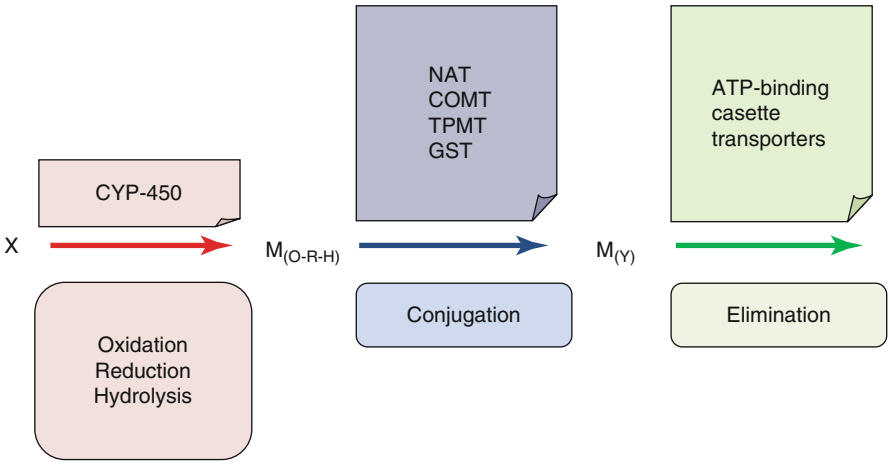

Fig. 5.2 Xenobiotic metabolism. Phase I (*red arrow*), phase II (*blue arrow*), phase III (*green arrow*). $M_{(O\text{-}R\text{-}H)}$ is usually a minor reactive metabolite of the oxidation, reduction, or hydrolysis in the phase I reaction mediated by the *CYP-450* enzymes. Well-known enzymes of the phase II conjugation reaction are *N-acetyltransferase* (*NAT*), *catechol-O-methyltransferase* (*COMT*), *thiopurine methyltransferase* (*TPMT*), *and glutathione-S-transferase* (*GST*). The metabolism is continued in the phase III, where, for example, the ATP-binding cassette transporters can transport a variety of conjugated substances out of the cells to be excreted from the body. The smooth endoplasmic reticulum of the liver is the main site of drug metabolism. Other organs for drug metabolism are the epithelial cells of the gastrointestinal tract, lungs, kidneys, and skin

Pharmacogenomics and Unexpected, Adverse Effects of Drugs

Undesired effects of drug treatment are known as *adverse drug reactions* (ADRs). The World Health Organization (WHO) factsheet No. 293 defines ADRs as "an unintended response to a drug occurring at a conventional dose and used for disease in prophylaxis, diagnosis, therapy or for modification of physiological functions" [41]. Classic examples of ADRs are the disfigured infants whose mothers used thalidomide for morning sickness during pregnancy, thromboembolism related to oral contraceptives, and muscle ache and degeneration during statin use. The WHO definition of ADRs does not include therapeutic failures, noncompliance and intentional or accidental poisonings. An adverse drug event (ADE) is a term used in ADRs or events caused by medication errors [42]

Pirmohamed et al. [43] classified ADRs into type A and type B reactions, and Edwards and Aronson [44] additionally to type C–F reactions. Classification of ADRs facilitates comparison of ADRs associated with different drugs and systematic collection of the data into large databases (e.g., WHO-Uppsala Monitoring Centre [45]).

The capacity of the therapeutic effect of a drug in a clinical trial (efficacy) is the basis for a drug's transition to medical practice and an indicator of a drug's effectiveness in patient use. Documentation of ADRs is difficult and depends on the type of ADRs (mild, severe, fatal) and reporting institution (general

practice, general hospital, university hospital). Studies on pharmacovigilance and pharmacoepidemiology (International Society of Pharmacoepidemiology [46]) are effective ways of detecting ARDs [47].

In a meta-analysis Lazarou et al. [48] found that ADRs are associated with ~100,.000 deaths/year in US hospital in-patients. Based on their study, Lazarou et al. estimated an overall incidence of 0.32 % for fatal ADRs in US hospital in-patients. In concordance, a prospective Scandinavian study [49] with 13.992 patients, concluded that the incidence of lethal ADR was estimated to be 0.95 %, and Pirmohamed et al. [50] observed 0.15–2.3 % ADR lethality for 18,820 hospital in-patients.

From the concepts and data presented above, it is clear that PGt/PGx is of great medicolegal interest [20, 21, 51] and may have potential legal consequences [51, 52]. Indeed, as an analog to personalized medicine, a necessity for the development of personalized justice has recently been expressed referring to the use of postmortem PGt/PGxin medicolegal CoD investigation [53].

Medicolegal Investigation of Death

Forensic Pathology

In many countries where a public system for CoD investigation exists, a suspected unnatural death falls into a police-led investigation including full medicolegal autopsy in order to establish the CoD and MoD. According to the WHO, the definition of the underlying CoD is the (1) disease or trauma that initiated the train of morbid events leading directly to death or (2) the circumstances of the accident of violence that produced the fatal injury [54]. The MoD is the circumstances that led to the underlying CoD and is classified as natural, occupational, accidental, suicide, homicide, or war. In some countries, medical treatment is considered as an additional class of MoD. In addition, use of the intermediate CoD and immediate CoD are used in CoD certificates, when appropriate. Underlying CoD and MoD are the archived parameters for national and international comparative statistics [55].

An "autopsy negative" case [56] refers to a situation where an autopsy is not sufficient to determine the CoD (and MoD) after autopsy and standard auxiliary tests, for example, histology, toxicology, and biochemistry. Various forms of sudden unexpected death (SUD) are typical examples of autopsy negative cases. WHO definition for a SUD is "a death, which occurs within 24 h after the beginning of the symptoms of the disease or condition that lead to the death" [54]. Most SUDs are classified as sudden cardiac deaths (SCDs) [57–59], sudden death in epilepsy (SUDEP) [60], or sudden infant death syndrome (SIDS) [61]. Autopsy negative cases are concentrated in young age groups, but the prevalence of these cases in the age group of 1–35 years varies depending on the study, the institution, the population and the age groups, and the intensity (possibility) of auxiliary tests from 3 to 53 % [16, 58].

Forensic Toxicology

Forensic toxicology is thematically divided to human performance toxicology, doping control, workplace testing, and postmortem toxicology (i.e., death investigation toxicology). Postmortem toxicology derives from the toxicological study of the ADRs and chemicals on humans. The objective of postmortem toxicology is to analyze prescription drugs, narcotics, poisons, or other toxic agents in dead individuals for medicolegal purposes and to interpret the results for CoD investigation. Postmortem toxicology employs methods used in clinical medicine and academic laboratories (clinical pharmacology, analytical chemistry, etc.), but postmortem research has also led to the new analytical methods and instrumentation [62]. In addition, understanding of postmortem effects on drugs [63] and the interpretation of the results in the medicolegal context are important areas of postmortem toxicology research [64].

The interpretation of postmortem toxicology results is complicated by various factors, for example, patient's compliance, adherence, external factors, diseases, genes, and behavioral habits. Also borrowing and sharing of medication [65], co-use of prescription drugs with over-the-counter drugs, abuse of prescribed drugs [66] and enhancement of the effect of illicit drugs with prescription drugs [67] can be behind unusual findings. Postmortem toxicology findings are crucial for establishment of CoD and MoD in cases of intoxication, particularly in the evaluation of suicides by alcohol, drugs, or their combination [68]. In the course of medicolegal investigation, postmortem drug screening is also important in assessment of clinical maltreatment and medical negligence [69].

Polypharmacy is a common phenomenon in modern society and this largely affects the forensic toxicology findings and interpretation. Therefore, data of drug-drug interactions from clinical data, such as the **S**wedish, **F**innish, **IN**teraction **X**-referencing database, (SFINX) [70], are of utmost important to be translated to the forensic toxicology case-work interpretation [71] and can be mirrored against the data obtained from large forensic toxicology databases [72]. Relevant new insights can be revealed from forensic data, which can be used as one key foundation for drug safety, for example, by analysis of fatal toxicity indices [73, 74].

Postmortem Pharmacogenetics: Concepts and Preliminary Data

When CoD and MoD of a death cannot be unequivocally established by traditional autopsy, the newly developed molecular approaches may help to solve some of these autopsy negative cases. For the application of genetic tests in the CoD investigation, a term "molecular autopsy" has been coined [75]. The objective for the molecular autopsy is to reduce the number of undetermined CoDs (and MoDs) and to better understand the mechanisms underlying such deaths.

One such area stems from the principles from clinical PGt/PGx translated to the postmortem investigations. Indeed, some eye-opening cases have been published (see later). This field is still immature and has been termed as postmortem pharmacogenetics [21, 76], medicolegal (pharmaco)genetics [21], or toxicogenetics

[77]. The terminology refers to the aspects of use of PGt or PGx in postmortem, medicolegal or forensic context, and the genetic susceptibility to fatal adverse reactions.

The cost-effective use of PGt/PGx in medicolegal investigation of death requires integrating research in forensic pathology, toxicology, and genetics [20, 21, 53]. The information to be collected in a medicolegal autopsy for systematic postmortem PGt/PGx studies include (1) medical history and other background information, (2) detailed knowledge on the pathophysiological conditions or diseases, (3) concentrations of all drugs and their relevant metabolites, and (4) genotype data of the gene pathways related to the drugs found. Few studies have already been carried out in these lines for some drugs [19, 22, 78–81]. All the data collected in this manner should be considered along with the fact that an individual's genetic constitution, and external and internal factors (Fig. 5.1) are acknowledged.

Earlier, the phenotype from an individual was obtained by urine analysis on drug-by-drug basis, and thus calculating the "metabolic ratio" (metabolic capacity or phenotype) for the particular drug [82]. Currently, the phenotype is predicted from the genotype(s), which is a significant tool for personalized drug therapy. Indeed, with the new analytical methods and rapid genotype production, some guidelines for deducing phenotype from the genotype in clinical PGt/PGx have already been suggested [83, 84], but generally, the probability of prediction of the phenotype from the genotype still remains a challenge [85]. Again, similarly to the clinical work, a study objective for PGt/PGx in medicolegal autopsies is predictive interpretation of the results using genotype data mirrored in drug-drug metabolite ratios [78, 79, 86]. So far, studies using deduction of the metabolism phenotype from genotype have been based on the assumption of dominance; i.e., most efficient variant in the genome determines the phenotype.

A well-studied example is the *CYP2D6* gene with four phenotypic classes, which can be inferred from genotype data:

1. Individuals who lack the functional enzyme are poor metabolizers (PMs).
2. Carriers of two decreased-function variants or a combination of one decreased-function variant and one nonfunctional variant are intermediate metabolizers (IMs).
3. Those who have at least one fully functional variant are extensive metabolizers (EMs).
4. Carriers of active gene duplication or another mutation increasing the enzyme activity in conjunction with a functional variant are ultrarapid metabolizers (UMs) (Fig. 5.3).

It is clear that many postmortem studies are needed for the understanding of the genotype-phenotype relationship CoD and MoD investigation, but using the CYP2D6 as a model gene, it has been shown that, at least in the case of some drugs, the common finding in forensic toxicology, the multidrug use, does not hamper genotype-phenotype interpretation [78].

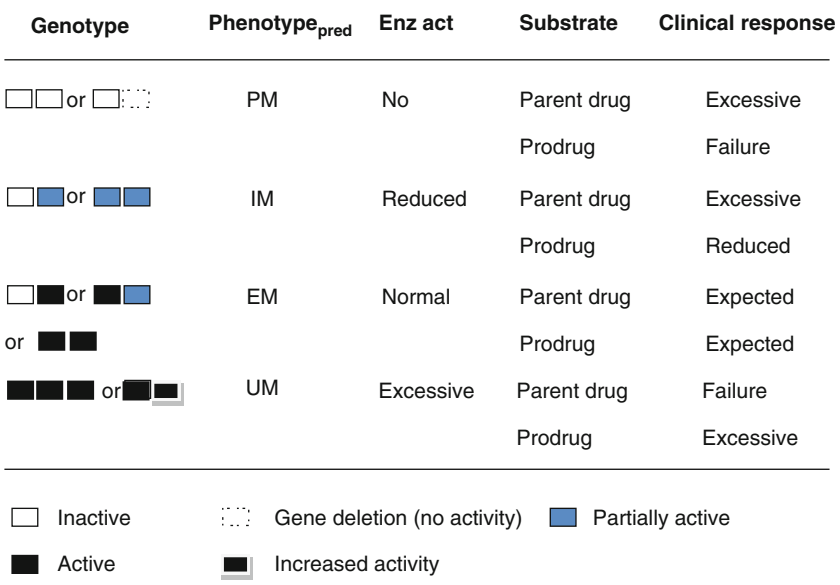

Fig. 5.3 Genotypes, predicted phenotype (Phenotype$_{pred}$) classification, enzyme activity (Enz act), substrate, and potential clinical response

Illustrative Medicolegal CoD Investigations Cases Using PGt/PGx Approach

Pioneering Cases and Studies

Ín the following, some selected cases from the literature are summarized, showing practical use of postmortem PGt/PGx. The examples are methodological developments, proof of principle type cases of unsuccessful psychiatric treatment or pain management, and suicides.

Sallee et al. [87] were the first to show that a PM genotype, related to poor drug metabolism, led to a fatal drug intoxication of a 9-year-old boy with fluoxetine treatment prescribed for behavioral problems; he died of fluoxetine intoxication. An unexpectedly high concentration of fluoxetine and norfluoxetine (major active metabolite) was found in postmortem analysis and warranted PGt analysis. The child had a completely defective *CYP2D6* gene resulting in a poor ability to metabolize CYP2D6 substrates, such as fluoxetine. With the new PGt-based evidence, the investigation of the adoptive parents was terminated.

After the case reported by Sallee et al. [87], CYP genotyping has been used to aid interpretation of postmortem toxicology results in oxycodone- [88], methadone- [89], and fentanyl-related deaths [90]. Also UM phenotypes have been associated with severe or fatal ADRs, as the enzyme can catalyze the conversion of a prodrug into an active compound. Such cases have been reported involving CYP2D6 and

Table 5.2 Drugs and PGt markers reported in medicolegal case work and literature

Drug	Drug class	ACT-code	Gene	References
Fluoxetine	Antidepressant (SSRI)	N06AB	CYP2D6	[87]
Oxycodone	Analgesic (opioid)	N02AA	CYP2D6	[88]
Methadone	Dependence (withdrawal)	N07BC	CYP2D6	[89]
Fentanyl	Anesthetic/Analgesic	N01AH/N02AB	CYP3A	[90]
Codeine	Analgesic (opioid)	N02AB	CYP2D6	[51, 91]
Doxepin	Antidepressant (nsMRI)	N06AA	CYP2D6 CYP2C19	[86]
Amitriptyline	Antidepressant (nsMRI)	N06AA	CYP2D6 CYP2C19	[79]
Venlafaxine	Antidepressant	N06AX	CYP2D6	[71]
Digoxin	Cardiac glycoside	C01AA	ABCB1	[22]

SSRI selective serotonin reuptake inhibitor, *nsMRI* nonselective monoamine reuptake inhibitor

codeine [51, 91]. Koren et al. [51] reported an eye-opening case, describing a neonate, who died at the age of 13 days. Postmortem toxicology revealed morphine intoxication. The mother had been given codeine for episiotomy pain. Codeine is demethylated to morphine via catalyzation by CYP2D6. In the course of the CoD investigation, it was found that the mother carried a *CYP2D6* gene duplication associated with increased metabolism of codeine to morphine, thus explaining the high concentration of morphine in the neonate, who acquired the morphine from the mother's milk.

These original and pioneering cases are illustrative for the usefulness of PGt/PGx testing when carefully applied in medicolegal context. Particularly, the cases prescribed by Sallee et al. [87] and Koren et al. [51] show examples, that those cases could have been interpreted as an infanticide/poisoning or medical misconducts/negligences; while after the PGt testing, the CoD was still intoxication; the MoD was demonstrated to be accidental in both cases. The original cases with their drugs and PGt markers are listed in Table 5.2.

More systematic case control studies were later carried out [19, 78–81] showing that (1) *CYP* genotyping is feasible in a systematically collected postmortem sample material from cadavers in various physical conditions and (2) genotypes correlated well with the observed phenotype, i.e., parent drug to metabolite ratio.

An important line of systematic research in postmortem PGt/PGx is the study of antidepressants and suicide. Depression is an important risk factor for suicides and therefore is a common finding in medical history in suicide cases. Tricyclic antidepressants (TCAs) have been the basis of antidepression therapy for more than four decades. Amitriptyline, which is a traditional TCA, has high efficacy and low cost and is therefore widely used [92]. The drawback of amitriptyline is that it has a narrow therapeutic range and high toxicity at increased concentrations, which makes it a common finding in forensic toxicology [93]. The main CYPs involved in amitriptyline metabolism are CYP2C19, causing its demethylation to the active compound nortriptyline, and CYP2D6 mediating the hydroxylation reactions of both amitriptyline and nortriptyline [94]. The study by Koski et al. [64] indicated that there is

concordance of amitriptyline-metabolite ratios with *CYP2C19* and *CYP2D6* genotypes even in the presence of typical confounding factors (diseases and polypharmacy) in the postmortem investigation.

The CYP2D6 genotyping has been used interestingly to analyze cases with MoD classified as suicide. From a previous study [95], it was known that the frequency of CYP2D6 gene duplication occurs more frequently in patients with persistent mood disorders and antidepressant treatment had no response. In an interesting study Zackrisson et al. [96] showed that the occurrence of *CYP2D6* gene duplications are increased among suicide cases indicating ultrarapid metabolism. Ahlner et al. [97] analyzed two medicolegal autopsy groups—(violent) suicides and natural deaths—and found that the CYP2D6 gene with more than two functional alleles occurred more than 10-fold greater in the suicide group, when compared with the group of natural deaths. The underlying mechanism is still unclear, but hypotheses of insufficient drug efficacy due to the UM phenotype or CYP2D6 related personality characteristics have been speculated.

Conclusions

The principles and cases described in this chapter demonstrate that adding PGt/PGx methodology as part of the medicolegal genetic arsenal can have tremendous medicolegal impact. The capability to exploit the genetic basis for contributing to the determination of CoD and MoD should be pursued vigorously and may open new avenues for the medicolegal practitioner to collaborate in basic research. Similarly to the patients in the clinic, deceased individuals clearly form a population within which the individuals may differ in their pharmacogenetically relevant genes.

It should be noted, however, that some of the studies, which can be performed in medicolegal settings using appropriate ethical approvals, are not impossible in the clinical setting. Therefore, a PGt/PGx approach will also contribute substantially to more comprehensive endpoint data for drug safety and translation of this knowledge to the clinics.

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Chapter 6
Forensic Entomology: A Synopsis, Guide, and Update

Ian Robert Dadour and Beryl Morris

Introduction

A death occurs. If it is a result of foul play followed by a time interval prior to discovery, and there has been an association of insects with the corpse, then there is a good chance that an entomologist will be required to help determine when the crime occurred. The discipline is called *forensic entomology* and in more recent times has become the gold standard for estimating the time since death [1]. Within this chapter, the reader will appreciate that there is much more that insects can offer when they become involved with a crime scene. Apart from their value in determining an accurate estimate of the chronological interval of death, forensic entomologists are able to extract drugs and gunshot residues (GSR) (entomotoxicology) from larvae [2] and make determinations on species identification as well as host substrate using DNA from where the larvae were collected [3]. So what is forensic entomology? The following chapter will describe its history; it will define the actual science and detail its many applications as a tool to help solve crime. In addition, the following chapter should serve as a guide for pathologists and other scientists investigating a corpse as to the location of insect material and how to sample and preserve collected specimens.

Forensic entomology is comparable to many of the other sciences that now have an affiliation with forensics. Forensics is a Greek word meaning "in the forum" and,

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in its more contemporary guise, it is any science or skill set that is used to solve a crime that typically concludes in a court of law.

Forensic entomology has been a useful tool for crime scene investigators for the best part of a century in the Western world. There have been numerous case histories now where entomology has played a crucial role in helping to work out the details of a crime or unattended death. However, three cautionary details apply to forensic entomology [4]:

1. Insects are animals, and it is not always possible to rely on animals to do what is expected of them, even when conditions seem favorable.
2. All care must be taken in the collection of entomological evidence; this should be gathered only by individuals who have knowledge and experience and/or accreditation as a practitioner in this activity.
3. Entomological evidence that is likely to appear before a court should be analyzed by individuals who have demonstrable experience with the aspects and assumptions of entomology that are applied to medicolegal investigations.

As previously stated, the term "forensic entomology" is generally used to describe the study of insects and other arthropods associated with criminal events [5]. In practice, however, most crime scene examiners are not entomologists, so any non-backboned animal associated with a legal investigation has in the past been sent to a forensic entomologist for analysis. Such animals may include snails, millipedes, spiders and mites, land shrimps, and flatworms, which can be passed on by the entomologist to an expert associated with that animal group. This range of invertebrate fauna is further expanded when a corpse is located in a water body and a variety of crustaceans may then be presented for examination. Unfortunately, very few species of animals, apart from a small number of insects and mites [6], have been studied sufficiently to have any reliable use in forensic entomology [4].

What Is Forensic Entomology?

The field of forensic entomology can be divided as follows [7, 8]:

- Urban entomology (e.g., civil actions relating to insect- and human-built structures, as may occur with termites and buildings) [9, 10]
- Stored product entomology (e.g., civil actions related to insect infestations of commodities such as food) [11, 12]
- Medicolegal entomology (e.g., criminal cases involving the estimate of time since death for decomposing remains of humans or animals) [13]

The last of these categories, medicolegal entomology, includes determining where and when the human death occurred, cases involving possible sudden or suspicious death, and criminal misuse of insects [14].

Uses for entomology in forensic situations seem to be broadening continually [15, 16]. This breadth is evidenced by applications in specific legal cases related to detection of:

- Toxins, drugs, and GSR [17–22]
- Injuries after death [23]
- Movement of vehicles and transport of remains [24]
- Movement of people through bites or infestations [25]
- Neglect of children and the elderly [26–29]
- Food contamination by insects [30, 31]
- Veterinary and wildlife forensics [32–35]

Estimating minimum time since death is still one of the most fundamental questions following a death, and the application of the developmental rates of insects associated with a corpse has become most essential in calculating the postmortem interval (PMI) in legal situations [1]. There are a number of other ways of establishing minimum time elapsed since death [36], including histological, chemical, and bacteriological methods, but such traditional techniques reputedly lose precision if more than a day has elapsed since death [37]. This has now been extended using modern molecular biology techniques, whereby the parameters responsible for changes in DNA yield have been researched as a possible measure [38].

There has always been a need for a broader range of methods that can be applied to determining PMI, and this is where entomology can be of use. Indeed, the case should be argued for pathologists, entomologists, and anthropologists to be considered as part of a continuum, working together for improved information on time since death [1]. Forensic pathology is about the process of autolysis, entomology with the early decay stages, and anthropology with the later stages. As such, the pathologist first calls on the entomologist when insects are present and then the anthropologist to assist with time since death assessments.

Determining minimum time since death is generally based on two considerations:

- The time it takes for insects attracted by carrion to arrive on a body
- The temperature-dependent rate at which carrion-eating insect species develop through their life cycles

With respect to time taken for carrion-eating insects to arrive on a body, there are many environmental- and species-specific factors that will determine which insects will utilize a corpse. However, a predicted order of corpse utilization typically occurs. Therefore, the first arrivals will be those insects attracted by and feed only on a fresh corpse [39–41]. In contrast, other types of insects will not be attracted by a corpse until it is in one of the later stages of decay. So, the presence of an insect known to be attracted only by a dry, decayed corpse indicates that the corpse has been dead for some time and has already passed through fresh, bloat, and wet decay stages. Insects involved in feeding on decaying flesh include blowflies, beetles, wasps, and moths, and, by the time the last species arrives, the earliest arrivals are

generally no longer present. Within the first hour, blowflies are generally the first insects to arrive at the site of decomposing flesh and are, to date, the only insect group to provide reliable estimates of PMI [42]. There are many species of blowflies, each with its own habits. Therefore, in entomological applications to forensic situations, it is critical that the species is correctly identified. Most forensic entomologists can identify genus and species, but typically the judiciary requests that actual identifications are conducted morphologically and/or genetically by a qualified taxonomist (Dadour 2011, personal communication).

The second consideration concerns the rate of development of insects associated with carrion. This requires knowledge of how climate, topography, vegetation, and other environmental factors will influence how quickly an insect grows from egg to adult (oviparous) (or in some species larvae only are born: ovoviviparous) on a corpse. Temperature (the most important) and rainfall (humidity) are two critical weather factors to consider. It is therefore necessary to find out the recent weather patterns for the area in which a body is found to estimate how overall insect activity in the area and rate of growth of those insects found feeding on a corpse may have been influenced by such factors [43].

History of Forensic Entomology

Sung Tz'u (1235) translated in 1981 [44] was the first known account of entomology being involved in a legal matter. The original details describe an incident involving a Chinese peasant who died of wounds inflicted by a sickle. The investigator assembled the farmers in the village and had them place their sickles on the ground. All were placed in the sun with flies being attracted to one sickle. As a consequence, the owner confessed guilt.

However, forensic entomology stayed unrecognized until the work of several European pioneers whose case studies were published in the last half of the nineteenth century. Dr. Bergeret d'Arbois is credited as being the first Westerner to apply "entomology" to a forensic situation. Bergeret identified some arthropods from a child's body found in 1850 behind a mantelpiece [14] and as a consequence of their identity and biology suspicion lay with previous rather than current short-term occupants of the house.

Megnin [45] is attributed as establishing entomology as a useful forensic tool when he published a treatise on the fauna of cadavers and their legal applications based on 15 years of work at the Paris morgue. Megnin [46] was the first to suggest that an exposed corpse would undergo a series of predictable changes during decomposition and that these stages would be visited by a succession of specific arthropods. By the end of the nineteenth century, the use of entomology in forensic cases was also well established in North America [47, 48].

Literature during the early part of the twentieth century is depleted on the topic of forensic entomology case work and research. In the UK, the first case utilizing forensic entomology occurred in late 1935 [49]. Police recovered about 70 pieces of

butchered human remains belonging to two females from a Scottish ravine that the media of the day dubbed "The Devil's Beef Tub." In this case, the forensic pathologists relied heavily on maggots to provide an estimate of the time of death.

The pioneers of regularly using forensic entomological evidence in European courts during the last century were Leclercq and Nuorteva. Publications of their research and involvement in police cases over a number of years were principally responsible for the growing interest by police in Western countries in gathering insect specimens from crime scenes. This resulted in a resurgence of interest in forensic entomology [50–56].

An Overview of Succession

Two types of succession typify the decomposition of a cadaver. The first succession pattern is the actual decomposition of the body, and it is generally categorized as gross morphological changes [1]. This section will not dwell on these changes as overviews of these patterns have been documented extensively [57, 58]. However, from a forensic entomology perspective, probably the most informative sequence is the four stages described by Reed [59] as they are not subjected or altered by climatic change. A fifth and final stage termed "skeletal" was added by Goff [60]. These stages include:

1. Fresh stage: begins with death; continues until early stages of bloating.
2. Bloat stage: begins during early stages of bloating; loss of hair begins; ends when bloating ceases.
3. Decay stage: begins when bloating ceases; hair loss is conspicuous; skin is usually broken in one or more places; soil within 30 cm of carcass is pulverized by burrowing activity of insects; ends when most of the carcass remnants are relatively dry.
4. Dry stage: begins when only small amounts of decay tissue remain; the limits of the stage are difficult to define due to lack of pronounced events marking the beginning and end, and diversity in appearance of similar-aged carcasses and fauna living on them; considerable moisture due to rain, dew, or underlying soil and litter may be present; small amounts of semisolid putrefying material is occasionally present on the ground under solid remnants; ends when no carrion fauna remains.
5. Skeletal stage: characterized by skeletal remains and hair and an absence of carrion fauna. During this stage some useful forensic information can be derived from the soil composition under the corpse [6, 60–62].

The second pattern of succession is based upon the behavior and biology of (mostly) immature insects occurring and developing on a cadaver following death. This locality-specific but predictable succession is then correlated with the temperature-dependent developmental data for the immature insects found on the cadaver in order to estimate the PMI [4].

The use of successional data in the estimation of PMI assumes that following death, an orderly and predictable succession of insect species occurs on a cadaver. In a terrestrial environment, insects are generally the first organisms to locate a body following death. The carrion community is comprised of four categories. The first category includes insects classified as necrophages that feed from the body itself and are most useful in estimating the PMI. The second category consists of predators and/or parasites that may feed on other species that have already colonized the body, and as more research is being conducted especially on the parasites, this category is also becoming a useful tool for estimating the PMI [63–65]. The third category is far less useful for estimating the PMI and includes omnivorous species such as beetles, which occur later in the decomposition process. The last category is the adventive or incidental species, which simply visit by chance and generally have little forensic relevance [18]. Insect evidence no matter what category it falls into should only be discounted by a forensic entomologist.

As the body progresses through the stages of decomposition, from fresh dead to bloat, decay, dry, and skeletal stages, the resource changes chemically [66], and as a consequence the odors emitted by the corpse also change [64, 65, 67]. The odors vary in attractiveness to different insects, and as the body decomposes and various resources are depleted, new insect types will colonize, being more suited to the current decompositional stage [68]. These insect taxa reflect the physical and chemical changes in the body and are therefore predictable and useful in estimation of PMI.

Flies (Diptera) and beetles (Coleoptera) are the insects most frequently collected from corpses [69], and although both groups are important, the flies are the focus of most forensic invertebrate research and applications. The blowflies (Diptera: Calliphoridae) are usually the first insects to arrive following death. Female flies will deposit eggs or live larvae around orifices or wound sites on the corpse, and larvae will secrete enzymes and bacteria, facilitating consumption of the soft tissues of the corpse. Larvae will feed through three stages of growth (instars) each punctuated by the molting of their size-restricting cuticle, enabling further growth. At the cessation of feeding, larvae will pupate in soil, clothing, or beneath surrounding objects if inside a dwelling, and following a period of metamorphosis, the adult fly emerges. The empty pupal casings may persist around the corpse for many years and even longer in soil.

The arrival of blowflies, and subsequently their larvae, is coincided or followed quickly by the arrival of the flesh flies (Diptera: Sarcophagidae), other carrion flies (Diptera: Muscidae), and predaceous beetle species such as rove beetles (Coleoptera: Staphylinidae), carrion beetles (Silphidae), clown beetles (Histeridae), skin beetles (Dermestidae), and checkered beetles (Cleridae). A variety of other fly families may be found in association with the body, and hide beetles (Trogidae) and larvae of some of the aforementioned beetle groups may feed on carrion itself, often on remains of hair, skin, and clothing in late decomposition [24].

As mentioned previously, when the body is decomposing in a terrestrial environment, the substrate beneath it is also altered. This initiates a series of changes in vegetation and soil fauna, beginning a succession of arthropods affected by the

decomposing corpse above. This is a most important aspect of sampling and collecting for the entomologist or proxy, as the environment directly underneath the corpse may conceal arthropods of forensic importance. Generally, this sampling can only occur after the corpse is removed from the scene and taken to the mortuary (see later section: General Methods for Collecting and Preserving Insect Material).

Bornemissza [61] observed the greatest effect of the decomposing corpse on the soil beneath to occur during the black putrefaction and butyric fermentation stages. Fluid seepage contributes to development of a crust of hair, plant matter, and the uppermost soil layer beneath the body. During fermentation, the decomposition fluids released from the body, along with the waste products excreted by the insects feeding on the body, combine to kill the plants beneath the body and alter the soil fauna, altering the microenvironment [6, 70].

During the decay stage, the soil beneath the carrion may become disturbed to a depth of approximately 3 cm by the action of arthropods, particularly dipteran larvae, burrowing [59]. Decomposition fluids and associated arthropods are reported to affect the soil to a depth of 14 cm, with most effect in the upper soil layers. The area directly beneath the body, the "carrion zone," serves as a decompositional zone occupied by carrion dwellers, distinct from a surrounding area of approximately 10 cm, which provides an "intermediate zone" of both carrion and regular soil-dwelling invertebrates [61]. Generally, 10–20 cm away from the body, the soil fauna is typical of general litter-dwelling fauna, but perhaps the size of these zones may be dependent on the size of the carrion, as Bornemissza's work was based on guinea pigs, and human decomposition may produce greater amounts of fluid.

Nomenclature and Insect Life History

The biomass of insects is huge with an estimated (extrapolated) species richness ranging from 3 to 80 million species. Five major insect orders stand out for high species richness: Coleoptera (beetles), Diptera (flies, mosquitoes), Hymenoptera (wasps, ants), Lepidoptera (butterflies and moths), and Hemiptera (true bugs) [71]. The first three orders have adapted directly or indirectly to using carrion as a resource.

The vast majority of the insects lack a common name. A scientific name once applied to a species is recognized throughout the world, although there may be the occasional species being described twice.

Insects owe their success to several adaptations [72]. They mostly produce large numbers of eggs to help compensate for predation or fewer live larvae to escape predation. Wings allow insects to travel far for food or in search of mates and to escape their enemies. A further advantage is the ability of insects to feed on an amazing variety of food materials. Most plant species are utilized by some insect for food and/or shelter. Many insects prey on other insects, a number depend on blood derived from mammals or birds, some feed on the waste products of larger animals

or on decaying plants and animals, and there are others that feed only on dried animal remains including hair and feathers. Insects as poikilotherms can reduce their metabolic activity when conditions are unfavorable (i.e., extreme temperatures, water, or food shortages) and can colonize areas of limited food and water supply (i.e., deserts) and as small organisms can adjust to temperature rather quickly to make maximum use of favorable thermal conditions [71].

The external anatomy of most insects includes four wings. However, true flies have only one pair (the forewings, the second pair modified into stabilizers), while beetles use only the hind pair in flight with the forewings serving as a protective covering. Some insects, generally more primitive forms, never developed flight, while some did have wings but due to the niches they now occupy, their wings have been lost.

Another insect attribute is their external skeleton, which is segmented which in turn facilitates movement. However, the tough external skeleton impedes insect growth. The skeleton is constrained as to how much it can grow. As a consequence, insects must molt their exoskeletons. Immediately after molting, they increase in size, but with the hardening of the new skeleton, growth is once again constrained until the next molt. To offset the problem of constantly molting, some insects have developed a soft-bodied larval stage (grub, caterpillar, or maggot) reducing the number of required molts. Amongst the Diptera, the larvae (or maggots) generally undergo 3 molts; the final one forms the pupa and during this period—termed "metamorphosis"—the adult insect forms [73].

Like the Diptera, the more advanced insect groups' (moths, beetles, flies, wasps, and fleas) development passes through four stages: egg, larva (maggot, grub, caterpillar), pupa (chrysalis, cocoon), and winged adult. Pupation is a period of rest between the feeding and growing larval stage and the final transformation (metamorphosis) into an adult. Those families of flies that have a maggot-like larval stage have simplified the process of pupation whereby the last larval skin hardens and darkens and forms a puparium. In certain blowfly species, the period taken from egg to adult may be as short as 10 days, but as stated previously this time is greatly influenced by climate, especially temperature and humidity [71].

The majority of the other insect groups have no pupal stage, and the young (nymphs) usually resemble the adults except that they lack wings. For example, in grasshoppers, which may molt eight times, the wings grow a little with each molt and appear fully developed only after the last molt. The most primitive insect orders are wingless, and their young closely resemble the adult forms.

In adult flies, the most important external stimuli are those concerned with odors [64, 65], light [71], moisture, and wind direction [74, 75]. Receptors in the antennae detect odor. For blowflies, the most powerful reaction shown by all species is their attraction to the specific odors of decomposition, more especially odors of carrion or decaying animal matter, of the feces of human beings and other animals, and the odors given off under certain conditions by the skin and wool of living sheep. Very importantly, different species of flies react differently to successive stages in the process of decomposition.

General Methods for Collecting and Preserving Insect Material

Numerous accounts of how to collect at a crime scene or a decomposing animal have been documented and published [16, 76, 77]. The following is a précis on much of the current literature and the authors' experience.

At the Scene

In many cases when insect material is observed at homicides, suspicious deaths, and suicides, then the medical examiner, coroner, pathologist, or trained forensic field officer will call an entomologist. In some cases, although not ideal, any one of these attending persons may do the collections on behalf of an entomologist. At homicides when the entomologist attends, that person is generally integrated into the forensic team dealing with the case. Typically, the entomologist arrives with a kit and dons a disposable and protective type coverall, which includes booties, gloves, and a head covering. At this point the entomologist can, under the direction of forensic officers, assess the evidence and acquire the necessary tools to collect the evidence. The kit should contain tools such as a net and/or sticky paper for the collection of adult fly insect; forceps, spoons, and paintbrushes for collecting immature and some adult insects; collecting jars that are capable of being ventilated for storing insect samples; pens and pencils for writing labels; thermometers (standard but more appropriate infrared) for checking temperatures of large visible masses of fly larvae; and some form of refrigeration—either a cooler containing ice or freezer blocks or a fridge in which to place collected specimens. Prior to delivering specimens to an entomologist, never place insect material directly into a freezer for killing or storage of immature insects.

What to Collect

Although experience is required to sample from a corpse at a scene, it is reasonably straightforward if the person collecting has some basic skills and understanding about what to collect. One major assessment within the environment where the body is situated is if any other decomposing material is closely associated with the corpse. The original observation of the visible insect material on the corpse should include the accessible areas where immature insects are likely to be found. The main areas are the head region, due to the large number of orifices present, and also the hairline; the armpits; between fingers and toes; back of knees or front of elbow if both are bent; where the body and substrate meet; orifices in the groin region; any wound; and the in situ area under the corpse following its removal to the mortuary. It is important to search and sample beneath the corpse as well, so an entomologist or

their proxy will be required to collect samples when the corpse is first located and when it is removed. In any one of these locations, a fly larval masses may be present, and the temperature of this mass should be recorded. Studies have shown that these larval masses produce elevated temperatures, which influences the developmental time and consequently affects the PMI [78]. Always place samples into ventilated containers, and do not place live fly and beetle larvae together. Following removal of the corpse, a weather station (generally a miniature Stevenson Screen containing a data logger) should be placed as near as possible to the scene. The weather station should remain in place for 7–10 days.

Preservation

Although a number of different methods are available to preserve collected immature insects, the best procedure following sampling as suggested above is to place all samples into a cooler or refrigerator. All samples can then be conveyed to the entomology laboratory to be processed and preserved. The preferred process in the laboratory is to place the insect material firstly into hot water (approximately 80 °C). Hot water prevents autolysis in the larva by destroying proteins in the gut, which would otherwise darken the specimens during storage, and it reduces the elasticity of the cuticle [79]. Following the hot water treatment, all immatures (eggs, larvae, and pupae) can be placed directly into 70–90 % ethyl alcohol with one exception and that is pupae that must be pierced to allow infusion of the preservative into the pupal case [80]. Sometimes cooling and/or refrigeration is not available, and the preservation processes will need to be conducted at the scene. Processing at the scene can involve a number of steps depending on whether hot water and preservatives are available. In essence, some alcohol will need to be sought, which may include a white spirit such as vodka. If such a liquid is used, this needs to be swapped out into 70–90 % ethyl alcohol at the earliest opportunity. There are many solutions not suited for preserving insect immatures, including methyl alcohol and formalin [79]. During autopsy most preservation issues do not arise as refrigeration is available, and specimens collected can be stored appropriately pending being sent to an entomologist.

However, some other issues can arise at the mortuary, and this is because sampling of the insect evidence on many occasions occurs only during the autopsy. As a consequence, the time period between corpse discovery and the autopsy may range from a few hours to several days, but generally no more than a week. During most of this time, the corpse and the insects feeding on it are stored in a cool room in the mortuary (with temperatures approximating 4 °C). The purpose of refrigeration is to slow down the decomposition of the corpse and the activity and the development of the necrophagous insects (poikilotherms) on or in the corpse. In some cases prior to autopsy, the corpse may be shifted from the cool room to the laboratory to have other procedures (e.g., fingerprinting, inspection of clothing) conducted. These periodic transfers in and out of cooling may affect the development rate of insects reliant on the corpse, which will affect the PMI.

Although there are several studies that have been conducted to determine development of dipterans when subjected to low temperature and its potential effect on the estimation of PMI, no information is available on the effects of moving a corpse in and out of the cool room environment. In addition, all these studies have been focused on larva and pupa, not on the effect of cooling of fly eggs. The results of the effects of cooling on all other life-history stages have demonstrated significant increases in development time [81–87].

Contemporary Research in Forensic Entomology

There is still much to learn about forensically useful insects. Besides their seasonal occurrence in various geographical regions and their rates of development in the many situations of forensic interest, research worldwide continues to explore how this material can be best utilized and increase the credibility of forensic evidence.

Entomotoxicology (Drugs and Gunshot Residue)

It is becoming more commonplace that insects associated with a corpse can be used for toxicological analyses [2, 88]. Forensic toxicology is usually associated with poisons and other illicit substances or their metabolic subproducts found during a death investigation. This combined with the pathology, biology, and pharmacology evidence will typically determine the circumstances of death [2].

Goff and Lord [89] reported that the use of illicit drugs has increased in recent years on a global scale. Many victims having used drugs that cause death may remain undiscovered for different periods of time. Mainstream toxicological technologies require samples of the highest quality and best conditions for analyses. However, such samples become more difficult to extract, especially when the body is highly decomposed. Other factors that may prohibit proper sampling include mummification, a lack of body fluids such as blood or urine, the presence of high levels of alcohol or carbon monoxide, and in some instances due to the religious beliefs of the person that forbid sampling directly from the body [90].

Over the last 35 years, insects have become an alternative source of samples for toxicological analyses. In addition, many toxic substances can modify the development of arthropods [89] and in doing so alter the determination of the postmortem interval. This relatively new science has been coined *entomotoxicology*, with the first reports showing that adult house flies accumulated metals such as copper, zinc, and iron [91]. Following this, Beyer et al. [17] wrote a technical note based on a highly decomposed corpse discovered after 14 days in a wooded area. The fly larvae collected confirmed that the person had consumed phenobarbital. Later, Nuorteva and Nuorteva [92] extracted mercury from a number of different species of fly larvae feeding on fish tissue. More recently, Roeterdink et al. [21] extracted metals

such as antimony, barium, and lead associated with gunshot residues [93]. During the last 25 years, extractions have been made from fly larvae of pesticides and drugs. The following is a compilation of these extractions from fly larvae, which is by no means complete but includes malathion [20, 94], parathion [95], and drugs and narcotics such as alimemazine, bromazepam, clomipramine, levomepromazine, oxazepam, triazolam [96, 97], cocaine [98, 99], amitriptyline, propoxyphene and acetaminophen [100], opiates [101–103], temazepam, trazodone and trimipramine [104], salicylates, paracetamol, amphetamines and barbiturates [105, 106], and derivatives from amphetamines [107]. Furthermore, when a body is highly decomposed, beetle larvae may also be present, but currently limited information is available on drug detection from these larvae [103, 108].

Sampling

The principle to sampling toxicological specimens is that all the apparatus used to collect and store the sample must be clean and preferably autoclaved. Insects, like tissue and fluids, must be collected in vials free of preservatives or any external contamination. Parafilm can be put in a lid of a glass vial to avoid contamination of the sample by rubber seals and metal lids.

Prior to analysis, all samples should be kept refrigerated, and a part of each analyzed specimen should be retained, stored in the refrigerator, and be kept as a reference sample. The toxicological analysis should be conducted as soon as possible to guarantee the integrity of samples.

In the laboratory, live larvae should be sacrificed by freezing prior to analysis. Following death they must be rinsed in distilled water and pupae/puparia rinsed with methanol prior to extraction to avoid contaminants. Prior to toxicological analysis, the substances to be analyzed must be taken into account [109]. It is important to emphasize that all material used for analysis must be stored in clean glass containers to avoid contamination of phthalate present in plastic vials.

Hydrocarbons: A New Tool

A thin epicuticular layer of wax covers the cuticle of all insects. This wax is a compound containing alcohols, hydrocarbons, fatty acids, waxes, acylglycerides, phospholipids, and glycolipids [110]. The purpose of this waxy layer is to prevent desiccation and penetration of microorganisms [71]. In the majority of the insects, the wax layer is dominated by hydrocarbons [111]. Cuticular hydrocarbons are found in all life stages of insects and are biologically stable. Their biosynthesis is genetically based and modulated by factors such as reproductive status [112], developmental stage [113], diet [114], or temperature [115, 116].

Currently, research on cuticular hydrocarbons has identified that the profiles of these compounds found on larvae and pupae change over time [117–119]. If these

changes occur as part of the development of necrophagous insects, then these hydrocarbons could be a very useful tool in estimating the age of a larvae or pupae and hence could enhance the accuracy of the PMI.

There are two techniques used to collect cuticular hydrocarbons from the cuticle of the insect: via liquid extraction or solid phase micro-extraction (SPME) [110]. It has now been established that many different hydrocarbons exist on the cuticle of insects and that each insect generally has a very distinctive hydrocarbon profile. This profile is fast becoming a valuable tool in identifying species—a research area referred to as chemotaxonomy and now used extensively in plant taxonomy [120, 121].

This latest technique in forensic entomology has the potential to firstly identify the species of immature necrophagous insects. As will be discussed later, DNA has become extremely useful in determining species identification; however, this process is time consuming and not very sensitive when using either spent eggs or pupae. Like DNA, hydrocarbons are species specific and can be compared with a database of profiles of known species; however, species identification can be completed in a few hours [122]. There is also recent evidence that insects could have varying cuticular hydrocarbon profiles, depending on what geographical region where they occur [123, 124]. Once profiles for different regions are established, then hydrocarbon profile could play a role in identifying if a body has been relocated. This would be achieved by comparing the profile of the hydrocarbons on insects associated with the corpse to those of the local insects found in a certain region.

The second area in which cuticular hydrocarbons can be a valuable tool in aiding forensic entomology and alluded to earlier is in establishing the postmortem interval (PMI). It has been demonstrated that a significant change over time of the hydrocarbon profile on the larvae and pupae of the blowfly *Chrysomya rufifacies* has been observed [117]. Furthermore, Roux et al. [119] provided evidence (with a precision of 1 day) for the importance of hydrocarbons as an alternative method for evaluating the postmortem interval demonstrating changes in hydrocarbon profiles in three calliphorids of forensic interest: *Calliphora vomitoria*, *C. vicina*, and *Protophormia terraenovae*.

The Value of DNA in Forensic Entomology

In forensic entomology case work, there is a growing need to use molecular techniques [125]. This comprises numerous aspects such as identification, host detection, including victim suspect associations, and postmortem interval. The first two aspects have become quite important, whereas research on DNA degradation as a measure for the postmortem interval has not yet become a mainstream technique.

Identification

Research into molecular techniques has become more common as a research tool, providing more equivocal identification of immature stages of flies [126–129].

Globally, an increasing number of publications now emphasize the molecular identification of forensically important blowflies: Canada [130], France [131], USA [132], UK [133], Australia [126, 129], and Harvey et al. [126] for comparison of southern African and Australian species. However, in case work many of these techniques are used only as support for more traditional morphological identifications.

Molecular techniques have many advantages over morphological techniques in that species can potentially be readily identified at all life-history stages. For example, a reliable genetic identification of forensically important flies can be performed from empty puparia and/or their fragments, although DNA degradation can deeply compromise the genetic analysis of older fly puparia [134]. "On-site" identification of first-stage maggots may also become a reality through development of rapid immunodiagnostic assays [135].

To develop suitable diagnostic tests for use in forensic entomology, species-specific molecular markers—regions of DNA used for identification purposes—need to be identified for interspecific distinction. DNA sequencing produces data of high information content and allows both intra- and interspecific comparison. In particular, sequencing of the mitochondrial region encoding the cytochrome oxidase I (COI) gene has proved useful in evolutionary studies, population genetics, and systematics due to the relatively high degree of variation in the region [131]. Generally, mitochondrial DNA (mtDNA) has a higher mutation rate than nuclear DNA and, therefore, an increased chance of generating species-specific markers. In addition, mtDNA may be isolated more easily than nuclear DNA [136]. This is clearly advantageous to forensic studies where specimens may be incomplete or in poor condition.

Ten or so years ago, it would have been thought that DNA analysis would replace any need to rear through fly larvae to adults for identification and possibly would assist in questions of how much time may have been spent in a particular developmental stage. However, in forensic entomology, application of molecular biology techniques and knowledge are still in their infancy. Not only does one need unique stretches of DNA sequence common to all members of the taxon in question (or at least subsets of such a group), but also that such DNA stretches should be distinct from all other taxa [137]. To reach a point of reliable, robust identification from molecular techniques, considerably more geographic sampling and pooling of data from various researchers, is needed.

Host Determination

As stated previously, identification is still the main focus, but DNA analysis of the gut contents of certain arthropods, including maggots, has been used successfully in establishing victim/suspect associations [132, 138], and determining whether the larvae collected from a corpse assumes that these larvae have developed entirely on this resource [138]. Generally, other signs of decomposition, on and around the corpse, will justify this assumption [139]. Situations where host confirmation is necessary include the discovery of a corpse in an area where food scraps are present,

or a corpse located in a bushland area where alternate animal carcasses may provide suitable development sites for fly larvae.

Estimating the PMI based on the oldest larvae at a scene may therefore be flawed; as such larvae may have developed on a substrate external to the corpse (see previous discussion of sampling techniques). It may be necessary for the forensic entomologist to confirm the food source on which larvae completed their development. In case work, the PMI may be disputed as a consequence of an alternative feeding resource. As a result, the material present in the digestive system of the larvae needs to be identified.

Recently, molecular biological techniques have been used to identify the sources of blood meals consumed by adult hematophagous insects such as mosquitoes [140] and crab lice [141]. Human DNA has also been isolated from beetles that had fed on human skeletalized remains [142].

Critical in host detection is the quality and quantity of host DNA present in the alimentary canal of the insect. Importantly, the isolation method used to obtain host DNA from the larvae should not result in further degradation of the DNA and should provide a sufficient quantity of DNA, which can be detected and used in subsequent molecular procedures [141].

Sampling

Collecting insects for DNA examination requires the application of different preservation techniques than those required for insects to be used in morphological studies [143]. Appropriate techniques include freeze drying or preserving in 95 % ethanol. While forensic entomologists with appropriate molecular genetic training can undertake the study of insect DNA, it is also likely that the process of identification may be undertaken by a non-entomologist—a molecular technologist using relevant standard operating procedures in the forensic laboratory. Similar to choosing a forensic entomologist for PMI analyses, take the same care when choosing a molecular biologist that he or she has an awareness of the expected analytical standards and nature of legal systems.

Temperature Effects and Maggot Masses

Each fly species has its own unique developmental profile, even those that are genetically close, as demonstrated by Nelson et al. [144] in a study of sibling species of *Chrysomya*. There are numerous studies on rearing flies associated with cadavers/carcasses at constant temperatures [84–86, 145, 146]. However, these records do not cover all species known to be forensically important. Furthermore, there is an obvious gap in information on development rates of these species at many temperatures, including populations of the same species in different geographic localities; some species have yet to be studied, and thermal limits and optimum temperatures are practically unknown for each of the life stages.

There is a lack of uniformity in investigations that have measured the effect of temperature on larval development. Some have used instar change to measure development, while others have used larval length or weight, and a few studies use a combination of instar and either length or weight [147, 148]. Most of these studies have not recognized that instar change is a reflection of maturation and indicates the "real" age of larvae, whereas length and weight are measures only of somatic growth [149].

Dadour [150] demonstrated the effect of temperature and density on *C. dubia*. The experiment was set up to measure the effect of density and temperature (fluctuating [30 °C/19 °C] versus constant [24 °C]) on larval development in *C. dubia*. Densities of 100, 500, 1,000, 2,000, 3,000, 4,000, and 5,000 were selected based on observations of larval masses on carcasses in the field. The parameters measured were larval instar changes and larval length. In general, the effects of temperature and density on the rate of larval instar change were not pronounced. At 20 h of development, temperature had a significant effect on larval instar. The proportion of larvae attaining second instar at the fluctuating temperature regime was greater than at the mean constant temperature, for larvae developing at densities of 100, 1,000, 2,000, 3,000, and 5,000.

Larval density had a significant effect on larval instar between 38 and 42 h of development. At these sampling times, 90 % of the larvae reared at densities of 3,000, 4,000, and 5,000 had reached third instar at both constant and fluctuating temperature regimes. For all other densities at both temperature regimes, less than 20 % of larvae had matured to third instar. Larvae developing at a density of 4,000 and at a constant 24 °C required the least amount of time to reach third instar. At a fluctuating temperature regime, larvae reared at a density of 3,000 had the shortest developmental time to reach third instar.

Overall, there was no significant difference in the rate of larval development of *C. dubia* at a fluctuating temperature of 30 °C and 19 °C when compared with the mean constant temperature of 24 °C. This suggests that both temperatures of the fluctuating regime lie within the threshold and optimum temperature for the larval development of *C. dubia*. The only significant difference in proportion of larvae at each larval instar, between constant and fluctuating temperature regimes, was found at 20 h of growth, probably as a result of the initial exposure of larvae to 30 °C at the fluctuating temperature. Exposure to 30 °C in the first 12 h of growth is therefore likely to have initially increased the rate of larval development at the fluctuating temperature regime compared to the mean constant temperature of 24 °C. This initial increase was not sustained as development proceeded.

A significant difference occurred in the length of larvae developing at different temperature regimes and densities. However, there was variation in the length of newly deposited first instar larvae, which implies that there is natural variation in the length of larvae of the same age deposited by adult female *C. dubia*.

The variation in larval length between treatments indicated that larvae did not have to reach a certain size before instar change took place. Therefore, the time of instar change was a response to the time of exposure to the environmental conditions and not to larval length. The small amount of variation in larval length within

treatments indicates that length is a good measure of larval age only when larval density and environmental temperature are known. The greatest variation in larval length was observed around the time of instar change, when larvae had shed the cuticle of the previous instar. This had also been observed in the larval development of *C. vicina* and *C. vomitoria* [151]. Therefore, the age of larvae of the same instar can be determined using larval length. This is particularly useful for determining the age of third instar larvae where an increase in larval size by six to seven times is observed [152].

Further evidence of the effect of temperatures comes from studies in Western Australia inside vehicles. In one set of experiments, Voss et al. [153] showed higher temperatures, rates of decomposition, and insect succession between exposed pig carcasses on the soil surface and those enclosed within a vehicle following carbon monoxide poisoning. Another study, this time of temperatures in the cabin of parked vehicles [154], showed the importance of vehicle color, amount of glass, and if the window was open 2.5 cm or 5 cm on the internal temperature of the vehicle and also produced a model for temperatures in vehicles.

Morris [155] showed the degree to which temperatures where maggots are developing in pigs, goats, rabbits, and sheep can differ from ambient temperatures recorded at the site of the decomposing carcasses. Field experiments with pigs showed that when the pig was not infested by insects at all, the temperature within the pig carcass quickly reflects ambient. Alternatively, when a sheep and a pig were infested with maggots, the temperatures within the abdomen of both animals rise above ambient.

The most distinctive feature of blowfly larval feeding aggregations is heat production, that is, the capacity to generate heat within the aggregations, which can exceed ambient temperatures by 30 °C or more [31, 156, 157]. Although larval mass heat effects are accepted, the relationship between larval mass effect and larval development time remains difficult to assess [13, 158].

From these results, one could presume that the temperatures are almost impossible to estimate in forensic situations where we do not have access to the past history of the corpse or the local weather patterns. However, Morris [155] observed (and recorded on time-lapse film) in field experiments the way in which the maggots move their feeding site in response to temperature stimuli. This behavior was backed up by results of laboratory experiments on responses to temperatures [31]. In particular, maggots move away from extreme temperatures in the carcass or food medium and toward the optimum for their development. Many forensic entomologists use ambient temperatures as their base for estimating a time since death based on insect fauna. The observations and experiments by Morris suggest that in many situations, such as corpses found in summer with high numbers of maggots present (maggot mass), it would be more appropriate to use the optimum temperature for estimates of the temperatures driving development for the maggot species involved, at least during the feeding stage of the third instar, unless evidence exists of mass mortalities of maggots.

Other researchers have come close to suggesting the importance of maggot behavior in controlling the temperature at which the maggot mass is developing.

Dallwitz [159] stated that, for life stages capable of movement, the extent of development occurring at high measured temperatures may be of slight importance, since the insect may frequently be able to select a microenvironment where temperatures are nearer to optimal.

Deonier [156] postulated that the heat generated by blowfly larvae in carcasses enables the species to survive periods when weather conditions are unfavorable to adult activity. Waterhouse [160] took up these thoughts and observed that many larvae are killed by high temperatures generated in a carcass by the maggot activity, and the temperatures and mortality vary directly with the degree of overcrowding. Waterhouse [160] also found that when temperatures near 52 °C were reached, larvae, both fully grown and immature, left the carcass. Many perished and the primary species were the first to leave. Morris [31] also found this when carcass temperatures were 22 °C above ambient, *L. cuprina*, *C. augur*, and *Ch. rufifacies* all spilled out of the carcass. Morris [31] suggested that the demise of numbers of maggots is useful in decreasing the overall temperature of the maggot mass and may be part of a behavioral adaptive advantage of some primary flies.

Waterhouse's study [160] also found that when a carcass becomes a seething mass of *Chrysomya* spp. larvae, immature primary larvae would leave it in large numbers. It coincided with the time the carcass temperatures rose to their peak. Waterhouse was not able to establish whether the *Chrysomya* larvae caused the primary flies to depart because of the high temperatures generated by the *Chrysomya* larvae.

Fuller [161] may have been observing the same temperature phenomenon when carrying out experiments on interspecific competition. She, however, concluded that *Lucilia* larvae were "definitely repelled by those of *Chrysomya* [sic]." Even when they were well established and there was an abundance of food, *Lucilia* larvae became disturbed and some left the carcass when *Chrysomya* larvae were added.

Influences of clothing on the insect colonization of corpses have had little attention until recently in 2009 and in 2011. Both studies were preliminary in that only small numbers of replicates were used; two seasons (autumn and summer) were used in the Kelly at al. [162] study, and one season (autumn) over 2 years was used in the Voss et al. [163] study. Interestingly the comparisons made between the clothed pigs dressed in T-shirts and shorts in both studies, one in Africa and the other in Australia, largely agree with each other in that there was no evidence of a delay in Calliphoridae arrival or oviposition. However, *Lucilia sericata* in the Australian study oviposited 24 h earlier on clothed carcasses compared to unclothed carcasses, and this trend was consistent, within years, between all replicates [163]. No such delays were evident in the African study [162], which introduces a potential for error in the estimation of time since death when PMI is based on the arrival and developmental time of this primary colonizing species. Furthermore, within years, the duration of the wet decay stage was approximately 6 days longer for clothed than unclothed carcasses. In both studies maggot masses were present on all carcasses during wet decay, and internal carcass temperatures were consistently above ambient temperature. It seems clothing potentially protects larvae from environmental conditions and facilitates greater movement across the carcass surface contributing to the greater visibility and distribution of maggot masses.

Conclusion

As Jean Fabre [164] so eloquently quoted about the humble maggot:

"At the surface of the soil, exposed to the air, the hideous invasion is possible; aye, it is the invariable rule. For the melting down and remoulding of matter, man is no better, corpse for corpse, than the lowest of the brutes. Then the Fly exercises her rights and deals with us as she does with any ordinary animal refuse. Nature treats us with magnificent indifference in her great regenerating factory: placed in her crucibles, animals and men, beggars and kings are one and all alike. There you have true equality, the only equality in this world of ours: equality in the presence of the maggot."

This chapter by no means completes all the information that can be extracted from insect material. However, it highlights the "bread and butter" aspect of forensic entomology, which is to provide first and foremost a postmortem interval. Now we know that the decomposition process is much more than this and that the insect material present on a corpse can unlock other secrets to do with the way someone died.

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Chapter 7
Advances in the Use of Latent Finger Marks

John W. Bond

Introduction

This chapter considers recent advances in our ability to enhance and visualize finger marks deposited on surfaces that are likely to be encountered by the pathologist at a crime scene or postmortem examination. These surfaces include fabric, leather, skin, and metal, some of which have traditionally been known to be problematic when enhancing finger mark deposits. Many of the techniques described here are developments of existing technology, while others are entirely new methods of enhancing and visualizing finger mark deposits. This chapter has been organized by sample type (fabric, skin, etc.) with the intention of making the contents more readily accessible for the pathologist to retrieve quickly the latest techniques applicable to different surfaces. Finally, a more general overview of other recent advances in finger mark visualization is given, with particular emphasis on noninvasive and intelligent finger mark recovery techniques.

The Use of Finger Marks to Solve Crime

The use of finger marks deposited at the scene of a crime as a means of identifying an offender and hence solving the crime was first suggested in the nineteenth century [1, 2]. Subsequent work by Francis Galton, Edward Henry, and others well over a hundred years ago led to the establishment of finger mark identification as a recognized means of identifying offenders (see, e.g., Berry and Stoney [3] for a review

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of the history of fingerprinting). Today, finger marks continue to play an integral part in the investigation of a wide range of criminal offenses from volume crime, such as burglary and vehicle crime, to serious and major crime. In the UK, evidence from finger mark identifications solves one-and-a-half times more residential burglary offenses than DNA identifications and over twice as many thefts of motor vehicle offenses [4].

When a person's finger (or palm) comes into contact with a surface, secretions of sweat are deposited onto the surface leaving an impression of the papillary ridge pattern of the finger. This is referred to as a *latent finger mark*. Outside the UK, finger marks deposited in this way may be referred to as *fingerprints*. In the UK, the use of the term fingerprints is generally restricted to finger impressions taken from individuals for comparison with finger marks recovered from the scene of a crime. Identification of a finger mark to an individual is based on the accepted facts that [5, 6]:

1. The patterns of papillary ridges (known as characteristics) differ between fingers on the same individual and between individuals.
2. The patterns do not vary topographically during a person's lifetime.

Identification of an individual by means of their fingerprints thus requires that the pattern of ridges (characteristics) left behind at the scene of a crime matches exactly the characteristics from the individual. Recently, Kuchen and Newell [7] developed a computer model for the creation of the ridge characteristics based on a buckling instability of the basal cell layer of the fetal epidermis. The accepted uniqueness of an individual's fingerprint ridge characteristics has led to the widespread use of the term *fingerprint* to mean any telltale signature, not necessarily in the field of forensic science [8].

Three glands—the eccrine, sebaceous, and apocrine—contribute to sweat secretion [9]. Both the hands and soles of the feet contain only eccrine glands, while sebaceous glands are generally localized to regions of the body containing hair follicles, face, and scalp. Apocrine glands are restricted to regions of the body such as armpits and genital areas [9]. Therefore, eccrine and sebaceous secretions are normally the only contributors to finger mark sweat deposits.

Eccrine sweat comprises mostly water (>98 %) together with inorganic secretions (such as alkali metal salts), amino acids, and sugars. Sebaceous sweat comprises mainly fatty acids, waxes, and squalene. While eccrine secretion is directly onto the skin surface, sebaceous secretion is via the sebaceous duct [9, 10]. Table 7.1 shows the main constituents of these glandular secretions [11].

Latent finger marks deposited at crime scenes are, generally, not easily visible to the naked eye as they are translucent. Therefore, over the years, many physical and chemical techniques have been developed to enhance finger marks to make them visible and, hence, more easily identifiable [11, 12]. Today, a range of such techniques exists, and these are employed routinely, depending on factors such as the age of the finger mark, its history (i.e., has it been wetted) and, very importantly, the surface on which the finger mark was deposited (see, e.g., a recent review by Becue et al. [13] on the enhancement of finger marks using either chemical or nanoparticle stains).

Table 7.1 The principle constituents of human glandular secretions

		Constituents	
Source	Location	Inorganic	Organic
Eccrine glands	All over the body, but the only type of glands on the palms of the hands and the soles of the feet	Chloride	Amino acids
		Metal ions (Na^+, K^+, Ca^{++})	Proteins
		Sulfate	Urea
		Phosphate	Uric acid
		Bicarbonate	Lactic acid
		Ammonia	Sugars
		Water (>98 %)	Creatinine
			Choline
Apocrine glands	In the groin and the armpits; associated with hair follicles around the genitals and mammary glands	Iron	Carbohydrates
		Water (>98 %)	Sterols
Sebaceous glands	All over the body, except on the palms of the hands and the soles of the feet; highest concentration is on the forehead and on the back; associated with hair roots	_	Glycerides (30–40 %)
			Fatty acids (15–25 %)
			Wax esters (20–25 %)
			Squalene (10–12 %)
			Sterol esters (2–3 %)
			Sterols (1–3 %)

The following sections each deal with a specific surface on which finger mark deposits might be encountered, with a final section giving a general overview of other, recent, advances in finger mark visualization.

Fabric

The recovery of sweat-deposited finger marks on fabrics is known to be extremely problematic with no recognized process in the UK to enhance their visualization [12]. Until recently, radioactive sulfur dioxide was suggested as offering the best possibility of finger mark development, although success rates were known to be, at best, low [12]. Recently, Fraser et al. [14] explored the potential for vacuum metal deposition (VMD) to enhance such finger marks on fabric. VMD is a well-known, highly sensitive technique for the enhancement of sweat finger marks on smooth, nonporous surfaces such as plastics (i.e., polythene carrier bags) and is known to be particularly successful if the finger mark is aged or the plastic has been wetted, circumstances in which other techniques produce less successful results [12]. VMD, originally introduced as a technique for depositing metal coatings onto glass to form mirrors [15], is a process in which thin layers of metals (typically gold followed by zinc) are sequentially thermally evaporated onto the surface of the sample at a

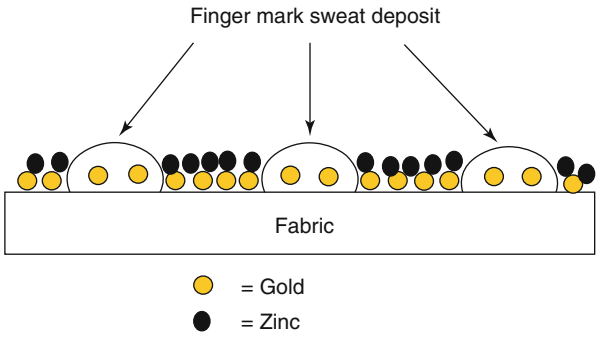

Fig. 7.1 Schematic representation of vacuum metal deposition of gold followed by zinc, showing zinc deposited on gold that is absent on the finger mark sweat deposit

pressure of ~3×10^{-4} mbar [14]. Evaporated gold atoms cluster together on the surface of the plastic, forming agglomerates that can penetrate into the surface of the finger mark sweat. The evaporated zinc then preferentially adheres to the gold, and, as some of the gold has penetrated the finger mark sweat, less zinc is deposited onto the finger mark. Thus, a contrast in thickness of zinc can be observed between the substrate and the finger mark, rendering the finger mark visible (Fig. 7.1).

Fraser et al. considered white samples of cotton, nylon, polyester, and polycotton. The weave of all of these garments was not less than three threads per millimeter, which is the suggested minimum weave for the use of radioactive sulfur dioxide [12]. Normal (i.e., not artificially prepared) finger mark sweat deposits were taken from 15 donors who, in turn, grabbed a sample of fabric with a hand for 10 s. After deposition, the samples were kept for time periods ranging from 1 to 28 days before VMD treatment. Results showed that greater ridge detail was revealed on the shinier, tighter weave, materials like nylon. The duller, more porous fabrics like cotton showed only "empty" finger marks (i.e., no ridge detail present) or only grab marks. Generally, the less aged the finger mark before VMD treatment, the more ridge detail was developed. Fraser et al. noted that samples where an empty finger mark was revealed might prove useful for more targeted swabbing for cellular DNA material, thus making the recovery of DNA evidence on such samples less speculative.

Although not using fabric as a substrate, I-Heng et al. [16] have carried out a simplified VMD process to develop finger marks by thermally evaporating zinc oxide, which they found condensed preferentially onto finger mark sweat deposited on a polyethylene terephthalate (PET) substrate. I-Heng et al. found this to be a more effective VMD process than the conventional gold/zinc evaporation, particularly when the finger marks were aged.

Thus, the success of Fraser et al. in enhancing finger marks on fabrics with gold/zinc VMD and the success of I-Heng et al. in finding alternatives to gold/zinc evaporation open the possibility of other metal combinations being considered.

When considering the enhancement of marks deposited on fabrics in a contaminant, Farrugia et al. [17–19] have investigated the enhancement of marks contaminated with blood. Although the work of Farrugia et al. relates to the enhancement of footwear marks rather than finger marks, it is possible that such techniques might be adapted for use with finger mark deposits in blood on fabrics, and therefore this

work is discussed here. In three related papers, Farrugia et al. considered the chemical enhancement of footwear impressions in blood using protein stains [17], peroxidase reagents (leuco crystal violet, leuco malachite green, fluorescein, and luminol) [18], and amino acid staining (ninhydrin, 1,8-diazafluorenone-9-one (DFO), and 1,2-indanedione) [19]. The fabrics tested were white, black, and patterned cotton; white and black polyester and nylon; blue denim; and brown bovine leather. Results showed that most protein stains successfully enhanced marks on light-colored fabrics, enhancement on dark-colored fabrics requiring the use of fluorescent protein stains. The same results were obtained for the peroxidase reagents. For the amino acid stains, ninhydrin produced enhancement of marks on the light-colored fabrics, and the other two amino acid stains failed to produce any enhancement on any of the fabrics. In a separate paper, Farrugia et al. [20] considered the enhancement of marks in blood on fabrics by first of all "lifting" or recovering the marks for subsequent chemical treatment in the laboratory. An alginate (more usually employed to take a dental impression) was used to recover the marks, and acid black or leuco crystal violet selected as a protein stain and peroxidase reagent, respectively. With white and black cotton and nylon and denim fabrics, both chemicals produced good results, although the authors cautioned against the routine use of leuco crystal violet because of health and safety concerns.

Farrugia et al. [21] have also considered the enhancement of marks deposited on fabric with urine as a contaminant. Again using white, black, and patterned cotton; white and black polyester and nylon; blue denim; and brown bovine leather as test fabrics, Farrugia et al. found that, despite urine from different individuals exhibiting different fluorescent properties, strong fluorescence of urine-contaminated footwear marks was observed at 476 nm with an excitation bandwidth of 350–469 nm. Chemical enhancement showed that ninhydrin, DFO, 1,2-indanedione, and 4-dimethylaminocinnamaldehyde (DMAC) all produced some enhancement, even 2 months after deposition, with DFO and lighter, more porous, fabrics (cotton and patterned cotton) giving the best results.

With soil as a contaminant, Farrugia et al. [22] found that 2,2′-dipyridyl gave good results on all light-colored fabrics tested, particularly synthetic fibers such as polyester and nylon/Lycra, with a variety of soil samples tested from different areas of Scotland.

Skin

Like fabrics, skin is thought to be one of the most difficult surfaces from which to recover finger mark sweat impressions [11]. Attempts to enhance finger marks on skin have included examination with light sources (visible and UV), direct lifting with adhesive tape, and fuming with cyanoacrylate (superglue) [23]. Recently, Trapecar and Balazic [23] have considered finger mark recovery from skin on both living subjects and cadavers. They investigated magnetic, Swedish Black (soot), and Silver Special powders and, in addition, both cyanoacrylate fuming and

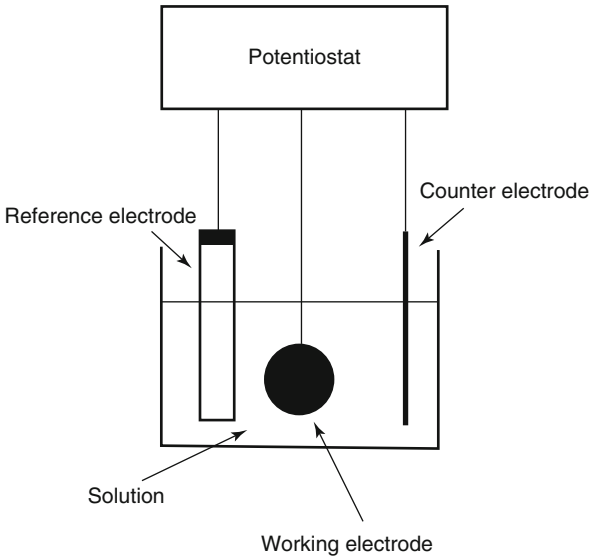

Fig. 7.2 Schematic representation of the electrochemical cell used by Bersellini et al. [26]

ruthenium tetroxide (RTX) on cadavers. Enhancement was carried out within 2 h of deposition, and the cadavers had been deceased for 20–30 h and had been stored for at least 12 h at 6 °C. Identifiable finger marks were enhanced by magnetic black and Swedish Black powders on both living subjects and cadavers and with RTX on cadavers. In addition, some ridge detail (although not producing identifiable finger marks) was observed with cyanoacrylate fuming on cadavers. The quality of the finger marks was not influenced by the initial contact time during donation or whether the subject was alive or dead. Thus, as was noted for fabrics, identification of areas of finger contact on skin might prove useful for more targeted swabbing for cellular DNA material.

More recently, Trapecar [24] has extended this work and shown that silicone (a casting material, applied to the enhanced finger mark after mixing with a hardener) and white fingerprint gelatin (a thick, low-adhesive gelatin layer with a carrier of linen rubber) were the best vehicles to recover the enhanced finger mark from the skin.

Drahansky et al. [25] have considered how diseases of the skin may adversely affect the enhancement and recovery finger marks and have commented on how such diseases might also affect the use of fingerprint scanners.

Metals

Bersellini et al. [26] demonstrated finger mark visualization on metallic surfaces using an electropolymerization process. Bersellini et al. observed that the sebaceous component of a finger mark deposit acted as an electrical insulator to the surface of a metal substrate. By setting up an electrochemical cell with a monomer solution of pyrrole in sulfuric acid (Fig. 7.2), Bersellini et al. produced

polymerization of the pyrrole on a metal substrate except in regions covered by a sebaceous-rich finger mark. The end result was thus a negative impression of the finger mark. Subsequently, this work has been developed by Beresford and Hillman [27] who electrochemically enhanced finger mark deposits on stainless steel by deposition of an electrochromic polymer (polyaniline). By varying the applied potential, the polymer's optical characteristics could be continuously and reversibly adjusted to optimize the visual contrast of the finger mark deposit against the polymer background. Beresford et al. [28] have shown how another electrochromic polymer—poly (3,4-ethylenedioxythiophene) (PEDOT), a derivative of the polythiophene family—when compared with three traditional finger mark enhancement methods (dusting with dry powder, wet powder, and cyanoacrylate fuming), produced superior results for finger mark deposits on stainless steel when the samples had either been heated or washed with a soap solution.

The discoloration of a metal surface through exposure to oxidizing agents is well known, as is the wearing away of metal through corrosion [29]. The ability of some metal elements and alloys to resist corrosion and the principles of dissimilar metal corrosion form the basis of metal corrosion theory, which is used in practice to prevent unwanted corrosion. Metal corrosion by sweat has been investigated extensively by dermatologists, and a link was established many years ago between palmar hyperhidrosis, the salt content of sweat, and metal corrosion [30–32].

When latent finger marks are deposited on metal surfaces, recent research has examined finger mark visualization techniques that exploit the chemical reaction that can occur between the metal surface and the finger mark residue. This reaction, effectively a corrosion of the metal surface, results in a change to both the chemical and physical characteristics of the metal surface. The use of such techniques could be considered noninvasive as they require no physical or chemical development of the finger mark prior to visualization.

Williams et al. [33–35] have demonstrated finger mark visualization on metals using a scanning Kelvin microprobe. A Kelvin probe exploits a discovery made by Lord Kelvin in the nineteenth century that two dissimilar metals in close electrical contact will each display a net charge, which is equal and opposite (i.e., one positive and the other negative). This charge is associated with electron flow between the two metals, when connected electrically, to equalize their Fermi levels. The Fermi level is derived from Fermi-Dirac statistics and represents the highest occupied energy level in a solid, that is, the Fermi energy or the energy of that quantum state [36]. From this, the minimum energy (usually measured in electron volts) needed to remove an electron from the solid to a point immediately outside the solid surface (i.e., the energy needed to move an electron from the Fermi level energy) is defined as the work function of the solid (φ [phi]). By measuring the electrical potential required to offset the charge between two dissimilar metals connected electrically, and knowing the work function of one of them, the other work function can be derived. This is shown schematically in Fig. 7.3.

Williams et al. used a scanning Kelvin microprobe to measure the potential difference (offset potential) arising between a wire probe and a metal surface containing a finger mark deposit due to differences in their respective work functions.

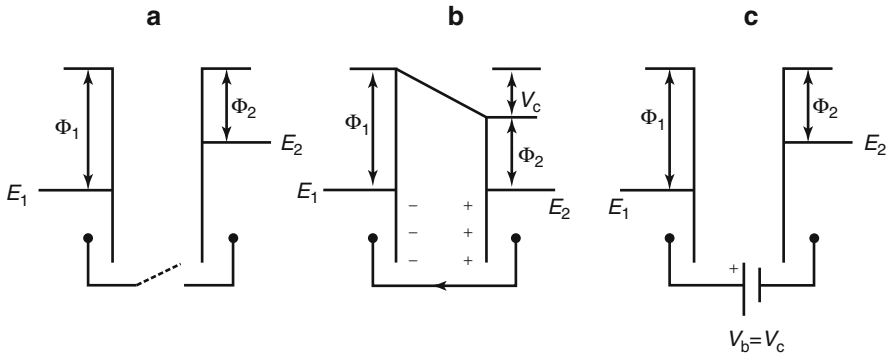

Fig. 7.3 Fermi level and work function for two dissimilar metals in which E and φ (phi) represent the Fermi energy and work function of metals 1 and 2, respectively. (**a**) No contact, (**b**) with electrical contact, and (**c**) with offset potential v_b which is set equal to the work function difference v_c

The magnitude of this potential difference is affected by the finger mark deposit and finger mark corrosion of the metal surface. By measuring this variation in potential, the finger mark was visualized in terms of potential difference. The usefulness of this technique was demonstrated by visualizing finger marks deposited beneath layers of soot or paint and also on brass cartridge cases where finger marks were deposited post-firing.

Williams et al. noted a serendipitous finding that rubbing the finger mark deposit with a dry cloth several days after deposition had little effect on their measurements. This led to the consideration of how easily common metals (such as brass and copper) might be corroded by finger mark deposits [37]. It was found that heating planar brass disks on which a finger mark had been deposited produced durable corroded images of the finger mark on the metal that were resistant to cleaning [37].

A practical use of this effect was demonstrated by heating brass disks with finger mark deposits in a controlled fire, approximately 24 h after deposition [37]. At its peak, the fire reached a temperature of 390 °C and comprised paraffin-soaked cloth, wood, and plastic. After the fire had cooled, the disks were washed in warm water containing a few drops of a commercial detergent (to remove soot and debris) and revealed finger mark corrosion visible beneath the fire debris.

Finger mark corrosion on a wider range of metallic elements and alloys has also been explored and explained in terms of the thermodynamics of the chemical reaction between the metal and the finger mark deposit [38].

Based on the aforementioned findings, novel finger mark visualization techniques have been developed to visualize finger marks deposited on brass surfaces after the deposit itself has been removed by washing or wiping.

Most easily, visualization has been achieved by considering the reflection of visible light from brass that has been subject to surface oxidation in air at elevated temperatures. Wightman and O'Connor [39] investigated the visualization of finger marks deposited on planar brass, aluminum, and stainless steel disks that were heated subsequently to temperatures between 200 and 900 ° C. They confirmed

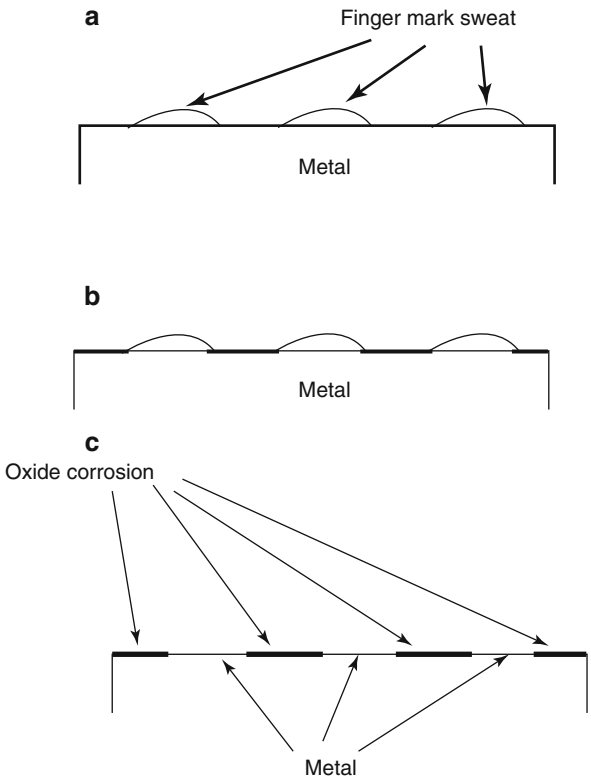

Fig. 7.4 Schematic representation of differential oxidation as described by Wightman and O'Connor [39] showing (**a**) finger mark sweat deposits on a metal surface, (**b**) oxidation to areas not covered by the sweat, and (**c**) the ridge lines showing as metal with oxidized areas in between

results from earlier work related to finger mark visualization on heated metals [37] with, frequently, no additional enhancement being necessary in order to visualize the finger mark ridge characteristics. They postulated that visualization occurred due to differential oxidation, that is, the finger mark sweat acted as a barrier to oxidation on those parts of the metal surface covered by the sweat (Fig. 7.4). Wightman and O'Connor believed that visualization was enhanced by interference colors resulting from a thin oxide film on the metal surface not covered by the sweat. Enhancement through optical interference has also been observed for finger marks deposited on brass disks in Iraq [40], where the increased air temperature (relative to the UK) resulted in a zinc oxide layer that produced optical interference as the viewing angle of the disk was adjusted in natural daylight. The thickness (t) of this zinc oxide film was found to be 70 nm $\leq t \leq$80 nm, which is consistent with values reported for zinc oxide formed during the electrochemical corrosion of brass in an aqueous saline solution [41].

Optical interference occurs in thin films (such as an oxide deposit on metal) when, for a given wavelength, the optical path difference between rays reflected and refracted at the air-thin film interface differ by an integral number of wavelengths, as shown in Fig. 7.5. This is because both the reflected and refracted rays undergo a phase shift of π (pi) radians at an optically denser medium

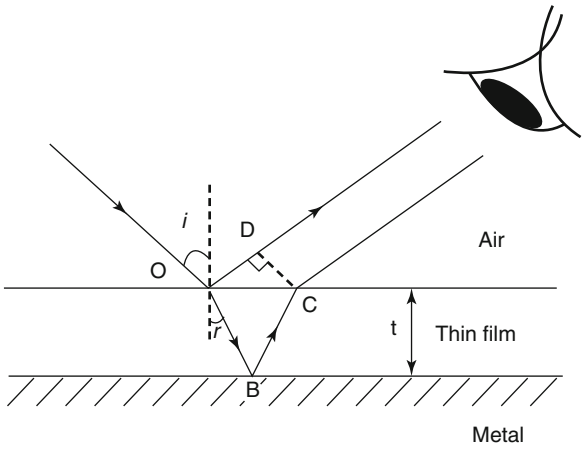

Fig. 7.5 Optical interference in a thin film caused by an optical path difference between rays reflected (*OD*) and refracted (*OBC*) at the air-thin film interface. The angles *i* and *r* represent the angles of reflection and refraction, respectively, and t is the thickness of the thin film

(the air/oxide interface for the reflected ray and the oxide/brass interface for the refracted ray). From Fig. 7.5, it can be deduced that constructive interference occurs when [42]:

$$2nt\cos(r) = m\lambda \tag{7.1}$$

where n is the refractive index of the thin oxide film of thickness t, r the angle of refraction, m an integer, and λ (lambda) the wavelength of incident light. From an extended light source such as natural daylight, a thin film would produce a change in color with viewing angle. An extension of this as a means to visualize finger mark corrosion has been to selectively digitally color map visible light reflected from the surface of brass using proprietary computer software [43]. Enhancement by digital color mapping was found to be optimal when the brass was heated to ~250 ° C, with areas of oxidation having a mirrorlike appearance.

Visualization has also been achieved in a more sophisticated fashion by employing a technique that requires the application of a potential to the brass (>1 kv) followed by the introduction of a conducting carbon powder (grain size ~10 μ[mu]m) [37]. The introduction of the conducting powder is facilitated by using Cascade Developer, which comprises ~400 μ(mu)m spherical beads that are coated with the conducting powder. By rolling the spherical beads back and forth across the charged brass surface, the conducting powder adheres preferentially to the areas of corrosion on the metal thus enabling the finger mark to be visualized.

The preferential adherence of the conducting powder to the corroded parts of the brass was explained by further experimentation that showed corroded parts of the brass had a potential lower than that applied to the bulk, typically up to 12 V for an applied potential of 1,400 V [44]. These experiments revealed that the junction between the bulk and corroded brass can exhibit the characteristics of a rectifying metal–semiconductor contact (known as a Schottky barrier) with the corroded brass exhibiting the properties of a p-type semiconductor, this most likely being oxides of copper [44].

As a further practical application of this technique, finger marks deposited on brass cartridge cases pre-firing could be visualized post-firing (and after the cartridge cases had been cleaned) [37]. Attempts to visualize finger marks on both fired and unfired cartridge cases have attracted much research in recent years, not least because of the problems it has presented in terms of heat damage to the finger mark deposit caused during the firing process [45–48]. This visualization technique has now been employed successfully to examine brass shell casings recovered from homicide investigations where conventional cyanoacrylate fuming has been tried unsuccessfully [49].

Most recently, the use of these techniques to visualize finger mark corrosion has been extended to copper pipe bombs, made from 1 mm thick copper pipe and detonated with a low explosive power powder [50]. A visual examination for finger mark corrosion revealed ridge details on several fragments that were enhanced by selective digital mapping of reflected visible light as described above.

Other Recent Advances

Finally, other recent advances in finger mark enhancement and visualization are considered where the technique is either noninvasive or intelligent. Noninvasive techniques, in which there is no physical contact or chemical reaction with the deposit or substrate, are favored as they do not in any way compromise the sample for subsequent analysis by other techniques. Intelligent fingerprinting includes techniques that not only enable visualization of the finger mark but also have the potential to provide additional information about the donor from the components present in the deposit, be they exogenous or endogenous.

Noninvasive Visualization

Noninvasive finger mark visualization, without physical contact or chemical reaction with the deposit, offers the potential to use this in conjunction with other finger mark visualization techniques and, in addition, to recover other forensic evidence types, such as DNA.

Worley et al. [51] considered a new method of finger mark visualization on polypropylene using micro-X-ray fluorescence. A beam of X-rays incident on a finger mark deposit gives rise to X-ray emission in which the wavelengths of the emitted X-rays are characteristic of the elements present in the finger mark. Thus, an eccrine-rich finger mark deposit would be expected to emit X-rays at wavelengths characteristic of the ionic salts present as shown in Table 7.1. This physical relationship was discovered by Henry Moseley in the early years of the twentieth century and is known as Moseley's Law. Through experimentation, Moseley determined that the square root of the frequency of the emitted X-rays was proportional to the

atomic number of the emitting element [52]. Imaging X-ray emissions characteristic of a number of elements, Worley et al. visualized finger mark deposits with a resolution of a few micrometers. In addition to sweat finger marks, Worley et al. used a variety of artificial sources of relevant elements contained in such things as sunscreen, hand lotion, and a banana skin.

Worley et al. conceded that a prior knowledge of the general location of a finger mark on a substrate is necessary before employing this technique, and, to some extent, this would be true also of the work of Bersellini et al. [26] and Beresford and Hillman [27] as scaling up the electrochemical cell to cater for a large substrate might be problematic. In this respect, neither of these techniques could be described as "speculative." That is, the ability of a technique to cover a large area of substrate in a relatively short time interval looking speculatively for finger marks. Clearly, speculative techniques offer advantages for those engaged in this work. However, Worley et al. did cite benefits of micro-X-ray fluorescence such as the detection of contaminants in the deposit (i.e., explosive material), no requirement for visible contrast with the substrate, and it being a noninvasive technique. With regard to this latter point, while Worley et al. did not consider whether the X-ray bombardment might adversely affect other evidence types present (such as DNA), the development of noninvasive techniques has gained in momentum in recent years.

Dubey et al. [53] reported recently the use of optical coherence tomography (OCT) to visualize finger mark deposits, a technique more usually associated with biomedical tissue imaging. OCT uses an interferometer, a device used to measure short distances or changes in distance by the production of optical interference fringes, which was proposed originally by Albert Michelson in the nineteenth century [54]. Together with a super luminescent diode broadband light source, Dubey et al.'s OCT employed an interferometer to detect only coherent (as opposed to scattered) reflected light, providing an image of the finger mark deposit. More recently, Dubey et al. [55] have extended this work to produce both a phase and amplitude map of latent finger mark deposits that provided tomographic as well as topographic images.

A growing method of noninvasive visualization is reflected in the work of Crane et al. [56], who employed Fourier transform infrared microspectroscopy (FTIR). This technique is a development from infrared spectroscopy and uses, in addition, Fourier filtering of the detected infrared signal to remove substrate images with periodic patterns, such as might be encountered on bank notes. Thus, background patterns can be effectively "removed" from the image, improving the contrast between the finger mark deposit and the substrate background. The infrared spectroscopy part of the process utilizes molecular absorptions in the mid-range infrared part of the spectrum (wavelength between ~25 and ~2 μ[mu]m) at specific wavelengths that are characteristic of the molecular structure under observation. The molecules rotate or vibrate at these specific wavelengths, which correspond to discrete energy levels in the molecule known as vibrational modes. For finger mark deposits, Crane et al. measured the infrared transmittance corresponding to the asymmetric O-C-C stretch ester vibrational mode.

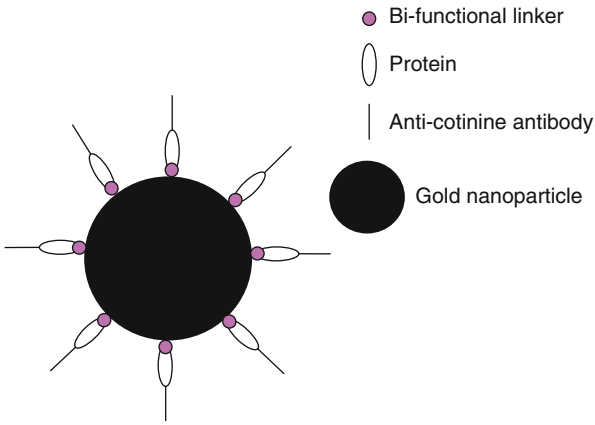

Fig. 7.6 Schematic representation of antibody–nanoparticle conjugates [60]

Crane et al. also revealed the presence of associative evidence within a finger mark deposit in the form of a material fiber that was spectroscopically analyzed along with the finger mark. Crane et al. asserted that an invasive finger mark visualization technique may have interfered with the spectral image obtained from the fiber. Like Worley et al. [51], Crane et al. conceded that FTIR works best when one knows where to look for the finger marks.

Tahtouh et al. [57] used FTIR to visualize finger mark deposits that had been subject to cyanoacrylate fuming, FTIR being an alternative to using a dye. They found best contrast between the finger mark and the substrate by imaging the C=O stretch vibrational mode of cyanoacrylate. Tahtouh et al. did not compare their results with those that would be obtained through more conventional dye and light source excitation of fumed cyanoacrylate. Therefore, any advantages of FTIR imaging of cyanoacrylate-fumed finger marks are not clear, particularly as the non-invasive benefit of FTIR is lost due to the finger marks being cyanoacrylate fumed. Tahtouh et al. did, however, make reference to the potential use of FTIR to detect contaminants (as suggested by Worley et al. for micro-X-ray fluorescence) and the use of Raman spectroscopy for the detection of drugs of abuse in latent finger marks [58, 59].

Intelligent Fingerprinting

Leggett et al. [60] have described the detection of drug metabolites in eccrine sweat secretions, deposited as a latent finger mark. Specifically, they detected the presence of cotinine, a metabolite of nicotine, from individuals who smoked tobacco products. Leggett et al. fabricated gold nanoparticles (16 nm in diameter) functionalized with multiple anti-cotinine antibodies to enhance the reaction between the antibody and the cotinine antigen within the finger mark deposit. Figure 7.6 shows a

schematic representation of the antibody–nanoparticle conjugates. The anti-cotinine nanoparticle conjugates were pipetted onto latent finger marks and incubated. After the introduction of a fluorescent agent, the finger marks were again incubated and then fluorescence images taken.

Leggett et al. concluded that, in addition to identifying metabolites of drugs, their metabolite-enhanced image of the finger mark deposit was sufficient to identify the finger mark donor. They noted that the ultimate sensitivity of this detection technique had still to be determined but the potential functionalization of nanoparticles with other antibodies might enable more general illicit drug screening or medical diagnoses.

More recently, Szynkowska et al. [61] have shown how time-of-flight secondary ion mass spectrometry (ToF-SIMS) can be used to visualize contaminants in finger mark deposits on metal substrates. ToF-SIMS has become one of the most sensitive analytical techniques in surface science [61]. Fingers were contaminated with either $Ni(NO_3)_2.[6H_2O]$, gunpowder residues, gunshot primer residues, As_2O_3, methamphetamine, or methylenedioxymethamphetamine (MDMA; ecstasy). In addition to the identification of these trace amounts of exogenous substances, which do not naturally occur in finger mark sweat, the presence of these contaminants enabled visualization of the finger mark ridge lines.

Identification of both exogenous and endogenous material deposited as a finger mark has been exploited by Bradshaw et al. [62] to deconvolute overlaid finger marks using Matrix-Assisted Laser Desorption Ionization Mass Spectrometry Imaging (MALDI MSI). Using spectra obtained in positive ion mode in the mass range between m/z 50 and 1,000, Bradshaw et al. were able to separate overlapping finger marks using ion signals that were characteristic of each finger mark from either endogenous or exogenous deposits. Finger marks were deposited onto aluminum either endogenously or exogenously, the latter by the donor handling a condom or by drinking coffee 30 min prior to deposition. A final set of finger marks was taken by depositing endogenous material onto stainless steel kitchen knives. Finger marks were separated by imaging both endogenous and exogenous material, including both substances ingested and secreted through sweat, as well as finger marks containing minute amounts of sebaceous material.

Lambrechts et al. [63] considered components present in sebaceous finger mark sweat that would fluoresce when excited by a range of wavelengths from UV (350–380 nm) to the green part of the visible spectrum. Donors deposited finger marks onto thin layer chromatography (TLC) plates, and emission and excitation spectra were obtained with a luminescence spectrometer. TLC was then used to identify the components in the finger mark deposits. The results indicated that the chlorophyll decomposition product, pheophorbide A, is present in some finger marks. Lambrechts et al. hypothesized that it might be possible to retrieve information about a donor's diet from the observed autofluorescence of the finger mark deposit.

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