SPONTANEOUSLY ARISING DISEASE: REVIEW ARTICLE

Some Challenges in Forensic Veterinary Pathology: A Review

R. Munro*† and H. M. C. Munro†

*Royal Veterinary College, London and †Royal (Dick) School of Veterinary Studies, Edinburgh, UK

Summary

Forensic veterinary pathology is a diverse discipline that is in an early phase of its development. Common challenges include estimation of the age of skin wounds and bruises, the diagnosis of drowning and estimation of the time since death. However, many details of the pathological findings related to these various aspects await validation. The ‘multispecies’ nature of veterinary pathology, combined with the preponderance of published observations originating from animal experimentation, rather than casework, poses two challenges. Firstly, extrapolation of results between species may jeopardize the reliability (and credibility) of the forensic opinion. Secondly, experimental studies may not truly reflect the spectrum of changes seen in actual cases (e.g. extent of injuries, infection, age and health of victim). With regard to drowning, diagnosis based on post-mortem findings remains problematical. Methods for estimation of the time since death (also known as the post-mortem interval) continue to be a major focus of study, with fresh avenues such as post-mortem diagnostic imaging offering interesting possibilities.

Keywords: bruising; drowning; forensic veterinary pathology; time since death

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Correspondence to: R. Munro (e-mail: Ranald.Munro@ed.ac.uk).

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Introduction

The work of the forensic medical pathologist and the forensic veterinary pathologist is essentially similar, but there is a major difference. The work of the former focuses on one species only, while that of the latter encompasses multiple species of great variety, ranging as it does through cases involving companion animals (including exotic species), farmed livestock and wildlife. The complexity of multispecies forensic pathology renders a review of all aspects, in all species, unwieldy and confusing. For this reason, the present review concentrates on four selected aspects of forensic veterinary pathology that, in the present state of knowledge, are sources of difficulty. These areas are (1) estimation of the age of skin wounds, (2) estimation of the age of bruising, (3) the diagnosis of drowning and (4) estimation of the time since death.

Veterinary pathologists are often encouraged to state when a skin wound was inflicted or to give opinions on the age of bruises. Similarly, it is often of primary interest to investigators to establish the time of death as accurately as possible and there is an expectation that the pathologist can provide the answer. Also included in the challenges facing the veterinary pathologist is the troublesome subject of drowning, which has been the focus of extensive research on both human corpses and experimental drowning of various other species. Courts rely on veterinary pathologists to unravel the necropsy evidence with a view to establishing whether an animal, recovered from water, was alive at the time of immersion. Additionally, the veterinary pathologist will from time to time be required to examine a cadaver where there is concern that the animal may have been drowned in a bath or other receptacle. Such cases may be further complicated if the body has been moved to another location.

What is the Meaning of ‘Forensic’?

Definitions are important in the avoidance of misunderstandings and this is particularly relevant when words or phrases are used both in ‘everyday language’ and in medical terminology. ‘Forensic’ is just such an example, since its original meaning of ‘relating to the law’ has broadened over recent years to implying ‘a detailed investigation and collection of evidence regardless of whether or not there is a specific legal case or enquiry pending’ (Cooper and Cooper, 2007b). For the purpose of this review, the word ‘forensic’ is used in the context of ‘relating to the law’. Further confusion can arise through differing interpretations of the terms ‘forensic medicine’, ‘forensic pathology’ and ‘forensic science’.

‘Forensic medicine’ (sometimes also called ‘legal medicine’) refers to the application of medical and veterinary knowledge to the elucidation of evidence for the courts. To some, forensic medicine and forensic pathology are recognized as being separate, but closely allied to one another; hence the departmental title ‘Pathology and Forensic Medicine’ that is found in many veterinary institutes worldwide. ‘Forensic science’, however, is generally understood to encompass factors such as scenes of crime examination, ballistics tests, DNA analysis, toxicology, etc. Nevertheless, it should be recognized that some workers may include pathology under the term ‘forensic science’.

Reports from forensic veterinary pathologists may be requested in both criminal and civil cases. Civil cases are concerned with the settlement of private differences between members of a community and are distinct from cases where a criminal charge is involved. The standard of proof necessary in civil cases is often less exacting than that required in criminal cases, with the decision depending on the ‘balance of probabilities’ rather than a need ‘to satisfy the court beyond reasonable doubt’ that a crime has been committed (Ross and Chalmers, 2009). Nevertheless, the task of the forensic pathologist remains the same: to provide meticulous records, care and attention to detail, clear reporting and recognition that the report is produced to aid the court to arrive at a just decision (Munro and Munro, 2008b). Given the nature of civil proceedings, which frequently are settled out of court and therefore may not come to the attention of the general public, very little has been published, in English language peer-reviewed journals, on the details of veterinary evidence in civil cases. Thus, this review is biased towards criminal offences.

Brief History

In the medical field, the development of forensic medicine (using the term in its broadest sense) has been well documented (Fisher and Platt, 1993; Wecht, 2005). At the forefront was Italy, and by the second half of the 16th century medical legal autopsies were being conducted in a number of European countries.
The first formal academic lectures in forensic pathology were held by Johann Michaelis and Johannes Bohn at the University of Leipzig in the mid to late 17th century (Smith, 1954 cited by Finkbeiner et al., 2009). Towards the end of the 18th century, three Chairs in forensic medicine were created in Paris, Montpellier and Strasbourg, but it was not until 1807 that the first Chair of Legal Medicine in the English speaking world was established in Edinburgh, Scotland. Shortly after, the first Professor of Medical Jurisprudence was created by the College of Physicians and Surgeons of New York City in 1813 (Camps, 1976).

In contrast, the development of forensic veterinary medicine has not been formally documented. However, in recent years the publication of a number of English language textbooks and journal articles highlights the increasing interest in this area (Stroud, 1995; Cooper, 1998; PAW, 2005; Sinclair et al., 2006a; Cooper and Cooper, 2007a, 2008; Merck, 2007a; Munro and Munro, 2008a, 2011; Newbery and Munro, 2011). Wildlife forensic investigation continues to generate attention throughout the world and two recent textbooks covering this complex area (Huffman and Wallace, 2012; Cooper and Cooper, 2012) are a welcome addition to the literature.

**Estimation of the Age of Skin Wounds**

Ohshima (2000) was of the view that in forensic pathology ‘wound examination is the most important matter and that it requires much experience of forensic practice’. Other pathologists may place the emphasis elsewhere, but in many cases it can be of considerable evidential interest to determine whether a wound occurred before or after death. This is not always an easy or simple distinction since the lack of reaction around the wound margin does not necessarily indicate that a wound occurred post mortem. However, a well-developed response around the wound margin is a clear guide to ante-mortem injury. Even so, care should be taken when assessing microscopical changes. For example, it has been recognized for many years that small numbers of leucocytes at wound margins do not necessarily signify ante-mortem injury (Raekallio, 1980). Indeed, Saukko and Knight (2004d) caution that leucocytic infiltra-
tion can occur in wounds in people ‘even several hours after death’.

If the wound is adjudged to be ante-mortem in character, the period that has elapsed from the time of injury to death of the animal (or presentation for veterinary examination) may be of significance. Ideally, forensic veterinary pathologists should have a thorough understanding of the pathophysiology of wound healing in the species of animals under investigation. However, there may be no such current knowledge.

Wound healing is complex, but detailed knowledge of the process has expanded greatly since the 1980s (Swaim and Krahwinkel, 2006). Much of the understanding of the stages of wound healing, based on microscopy, has been gleaned from experimental studies in a range of animals including mice (Hiss et al., 1988; Birch et al., 2005), pigs (Sullivan et al., 2001) and guinea pigs (Cox et al., 1989). Therein lies a problem, because experimental studies may not truly reflect the spectrum of changes related to the size, type and anatomical location of the wound, or take account of the extent of infection in the wound, or the age, species or health of the victim. The complications affecting wound healing are considered by Demetriou and Stein (2011).

Animal models are frequently used in researching human conditions. The results of these experiments are then extrapolated despite substantial differences existing between the reactions of the ‘model’ and human beings. For this reason, Saukko and Knight (2004c) consider the use of results from animal experiments as an unreliable basis for opinions in the context of human forensic pathology. An example concerns the dating of human wounds using rats as the experimental model. Conclusions based on such extrapolated data should be viewed with some caution since the major process involved in wound closure in rats is contraction, whereas epithelialization is the primary mechanism in people (Gottrup et al., 2000). The obverse of that must also be true, where the dating of wound closure in rats based on the published rate of epithelialization of human wounds would be unsound. This has wide implications for forensic veterinary pathology, for it casts doubt on the reliance paid, in many veterinary publications, on extrapolation of results from studies on people to a range of animal species. As noted above, and by other researchers (Chvapil et al., 1979), direct inter-species comparisons suffer from the same uncertainties.

Hosgood (2006) provides a detailed review of the stages of wound healing. The healing process is traditionally divided into three phases: inflammatory, proliferative and maturation (Ohshima, 2000). However, these phases are not defined precisely in time and all overlap to some extent (Baum and Arpey, 2005). Early blood clotting and the development of provisional extracellular matrix are followed by scab formation, inflammation and debridement (including migration of leucocytes into the injury and a shift from neutrophil predomination in the early inflammatory period to macrophages in the older lesions), angiogenesis, fibroplasia,
epithelialization, contraction and remodelling/maturation. These observations provide the pathologist with guidelines that may facilitate the development of evidence-based estimates of the time that has elapsed since injury. Investigators interested in the detection of vasoactive compounds or mediators of wound healing may find the original papers cited by Hosgood (2006) helpful. Additionally, the excellent overview by Kondo (2007) of collagens, cytokines and growth factors, as markers for wound viability and age, points the direction for future research.

The old veterinary saying ‘cats are not small dogs’ is particularly apposite in forensic work. Wound healing in cats generally occurs more slowly than in dogs. Granulation tissue, for example, appears at approximately the same time in both species, but Bohling et al. (2004) found that the subsequent rate of formation was more rapid in dogs, resulting in the median filling time of wounds in dogs to be 8 days compared with 20 days in cats. Epithelialization, contraction and time to total healing also showed specific differences, with each of these processes being slower in cats compared with dogs (Bohling and Henderson 2006).

Similarly, ‘ponies are not small horses’ when it comes to wound healing. In ponies, the early inflammatory response is more intense than in the horse, leading to uncomplicated healing. Studies by Wilmink et al. (1999a) and Wilmink and van Weeren (2005) showed second intention healing in ponies to be significantly quicker compared with horses, with the rate of wound contraction being better. These authors point out that granulation tissue in horses remains irregular and purulent for longer. At the histological level, neutrophil populations in second intention in ponies are high during the first 3 weeks then decrease rapidly. In horses, they are slower to rise, but persist for longer. Additionally, there is strict organization of myofibroblasts in ponies compared with horses, particularly in metatarsal wounds (Wilmink et al., 1999b). It is also worthy of note that metatarsal wounds in horses are unrepresentative of other cutaneous wounds and may increase in size during the first 2 weeks post injury (Wilmink et al., 1999a).

Responses to cutaneous wounding differ in horses and cattle, with horses showing a more rapid development of granulation tissue, while growth and differentiation of connective tissue in cattle 10 days post injury is more pronounced than in horses (Dinev and Dzhurov, 1987). Shehata et al. (1992) conducted an interesting investigation into the healing of the wounds in the pinnae of cattle and buffalo ears following the application of plastic ear tags. Species differences in the rate of healing were highlighted. The histopathological observations at various stages are shown in Table 1.

The plethora of papers covering numerous aspects of wound healing in people and laboratory rodents may tempt the well-meaning, but unwary, to use this information during the compilation of forensic veterinary reports. However, it would be wise to bear in mind the words of Lucius Accius (170–86 BC) ‘vigilandum est semper; multae insidiae sunt bonis’ (‘always be on your guard; there are many snares for the good’).

### Estimation of the Age of Bruises

Vanezis (2001) defined a bruise as ‘...a collection of blood, visible to the naked eye as an area of discolouration, which has extravasated into the surrounding tissues after vascular disruption, principally as a result of trauma or occasionally spontaneously, as a result of a disease process’. A broader definition by May and Hamdy (1966) that bruising is ‘tissue injury, without laceration whereby cells and blood vessels are crushed with a resulting release of cellular fluids and blood into the injured area’ supports the view that bruising may involve degeneration and

<table>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<td>Healing of wounds in cattle and buffalo ears following application of ear tags</td>
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</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–3 days</td>
<td>Haemorrhage, oedema, necrosis, neutrophilic infiltration</td>
</tr>
<tr>
<td>7 days</td>
<td>Appearance of healing in the form of vascular connective tissue</td>
</tr>
<tr>
<td>2 weeks</td>
<td>Regeneration of epithelium begins</td>
</tr>
<tr>
<td>3 weeks</td>
<td>Healing of auricular cartilage observed in both cattle and buffalo and epithelialization in cattle complete</td>
</tr>
<tr>
<td>4 weeks</td>
<td>Complete healing of the dermis and epidermis in cattle but epithelialization in buffalo incomplete</td>
</tr>
<tr>
<td>6 weeks</td>
<td>Epithelialization complete in buffalo</td>
</tr>
<tr>
<td>8–26 weeks</td>
<td>Scar tissue avascular and contracted accompanied by absence of hair follicles and both sweat and sebaceous glands</td>
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inflammation of muscle and adipose tissue (Thornton and Jolly, 1986).

Trauma is often the primary focus in a forensic investigation. More specifically, assessment of when trauma occurred vis-a-vis the time of death can be environmentally crucial. The pressure to define the time that has elapsed since injury has generated considerable research into methods for dating of bruises in a range of species including guinea pigs, rats, rabbits, poultry, calves, adult cattle and lambs (Hamdy et al., 1957; May and Hamdy, 1966; McCausland and Dougherty, 1978; Thornton and Jolly, 1986). There are, however, no objective reports on the development and healing of bruises in, for example, dogs, cats or horses.

Inter-species variability in the detail of the timing of the appearance of markers such as haemosiderin can be considerable (Vanezis, 2001). Consequently, despite the confidence expressed by Thornton and Jolly (1986) that ‘experiments using animals will remain the most practicable means of developing and refining wound ageing techniques for eventual forensic application’, considerable caution should be exercised in the extrapolation of data from one species to another.

Economic loss related to bruising of livestock going to slaughter is recognized as a worldwide problem (Anon, 1954; McManus and Grieve, 1964; Marshall, 1977; McNally and Warriss, 1996; Gallo et al., 2005; Andrade et al., 2009; Huertas, 2009; Alende, 2010). The potential financial benefit of eliminating these unnecessary costs to the industry has driven efforts to identify the causes of bruising and the times when it occurs (McCausland and Millar, 1982). Central to these investigations is the development of reliable ways of dating bruises (Hamdy et al., 1957).

Various approaches have been attempted to determine the length of time that has elapsed between the event causing the injury and the post-mortem examination of the bruise. These investigations have considerable relevance to forensic pathology when improper use of sticks or poor livestock handling are the focus of welfare prosecutions.

Gross Examination of Bruises

Gross examination of bruises may permit estimation of an approximate age based on the criteria set out by Gracey et al. (1999). These authors consider that at 0–10 h post injury, bruises are red and haemorrhagic. By 24 h the colour has darkened and the bruise becomes watery in consistency between 24 and 38 h. Bruises over 3 days old appear rusty—orange and have a ‘soapy’ feel.

Earlier experimental work by McCausland and Dougherty (1978) found that lesions inflicted at slaughter showed reddening of subcutaneous and muscle tissue. Examination of the injuries 8 and 24 h after they were induced showed no significant difference in the appearance of the lesions at these two times. However, compared with earlier lesions, the reddened area was wider and deeper, with a small quantity of clear fluid detectable in the muscle fascia and subcutaneous tissues of both calves and lambs. ‘Pockets of un-clotted blood’ affected the reddened areas in some lambs. By 48 h, some differences were noted between lambs and calves. Generally, the lesions in lambs were similar to those seen during the 8–24 h post-injury period, although the fluid collections tended to be yellow or green. In calves, at 48 h, the affected muscle was yellow—red and the lesions were drier and smaller.

Tramline bruising caused by sticks or poles is often referred to as ‘stick marks’ (McNally and Warriss, 1996) and are described by Wilson (2005) as ‘two straight red weals with a clear area in between’. Such lesions are commonly noted in pigs, but can also affect cattle (McNally and Warriss, 1996). Similar lesions are well described in medical forensic texts (Saukko and Knight, 2004b). The parallel bruises result from stretching and rupture of small blood vessels at the margins of the line of compression corresponding to the strike of the stick.

Microscopical Examination of Bruises

McCausland and Dougherty (1978) studied the histological changes in bruises induced experimentally in calves and lambs. Based on the extent of haemorrhage, fibrin formation, relative numbers of neutrophils and macrophages and muscle fibre damage, they proposed a scheme for ageing bruises up to 2 days old. Observations in this study were made at 0, 8, 24 and 48 h and reference should be made to the original paper for the details of the changes described. Little difference was found between the reactions in these two species and the authors further believe that the results are equally applicable to mature animals.

To overcome some of the inherent variability associated with observer error and interpretation of histopathological changes, Thornton and Jolly (1986) investigated ageing of ovine bruises over 0–72 h by application of a Bayesian probability model. The confidence values suggested that the Bayesian probability model could be applied successfully to age bruises into two broad categories only: 1–20 h and 24–72 h (c.f. the more optimistic view of McCausland and Dougherty, 1978). However, Thornton and Jolly
(1986) do point out that accuracy was increased when more than one tissue sample was examined and that for forensic purposes multiple sampling would, presumably, be routine.

Other Tests of Bruises

Many attempts have been made to estimate the age of bruises using biochemical and histochemical methods (see McCausland and Dougherty, 1978; Vanezis, 2001). Cattle and rabbits were the subjects of experiments to assess the usefulness of chemical means in the determination of the age of bruises (Hamdy et al., 1957). Bilirubin, formed during the degradation of haemoglobin, was not detectable (using a 10–20 min exposure to Fouché’s reagent at room temperature) until 50–60 h post injury. Bruises 60–72 h old developed a very light blue colour at 60 h and were blue by 72 h. Three to 5-day-old bruises were distinguished by a diffuse dark green at the periphery with a brown centre. Old bruises (5–8 days) showed little or no blue colour.

Electrical conductivity tests on bruised tissues were also undertaken by Hamdy et al. (1957), but appear to be of limited practical value. In addition to the requirement to have un-bruised control samples from symmetrical areas, it was found that the size of the bruise and the quantity of fat present in the tissue affected the conductivity.

Bruising in Poultry

Bruising in chickens may be visible within seconds following trauma (Hamdy et al., 1961b). Table 2 sets out (as modified by May and Hamdy, 1966) the gross morphological colour changes observed over 120 h post injury following experimental bruising of chickens (Hamdy et al., 1961a).

It is important to note that these descriptions apply to live birds, but after death all bruises revert to dark red–purple and lose the yellow and green pigments (Hamdy et al., 1961b). Nevertheless, Bremner and Johnston (1996) consider these signs are suitable for use in ‘fresh carcases’ (although these authors do not define a ‘fresh carcase’), but they agree that the yellow bruising fades as the carcasse ages.

The influence of environmental temperature on the colour of bruises in chickens is a consequence of haemoglobin being degraded to biliverdin in birds kept at 30°C and to bilirubin in poultry reared at 21°C or below.

Previous work by Kaiser and Smith (1958) showed that breed and bodily condition affected the appearance and detection of bruises. Hamdy et al. (1961b) noted that bruises in young birds (4–6 weeks of age) tend to heal faster than in older birds.

Table 3 shows the ages of poultry bruises, based on the colour reactions using Fouché’s reagent (Hamdy et al., 1961a). These experiments adopted the methodology developed by Hamdy et al. (1957) for bruises in cattle and rabbits.

**Table 2**

<table>
<thead>
<tr>
<th>Age of bruise</th>
<th>External appearance in 21°C environment</th>
<th>External appearance in 30°C environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal tissue</td>
<td>No colour</td>
<td></td>
</tr>
<tr>
<td>0–13 h</td>
<td>Pink, within minutes turning brown</td>
<td></td>
</tr>
<tr>
<td>14–24 h</td>
<td>Diffused light blue along with pink and brown</td>
<td></td>
</tr>
<tr>
<td>24–36 h</td>
<td>Diffuse light green, especially at the periphery, with a brown colour in the centre of the bruise</td>
<td></td>
</tr>
<tr>
<td>2–3 days</td>
<td>Diffuse dark green along with brown centre</td>
<td></td>
</tr>
<tr>
<td>3–4 days</td>
<td>Dark green spots or crystals embedded in the bruised area, with no brown colouration</td>
<td></td>
</tr>
<tr>
<td>5 days</td>
<td>Sometimes slight blue colour</td>
<td></td>
</tr>
</tbody>
</table>

(Adapted from Hamdy et al., 1961a)
Experiments on Drowning

Numerous experiments involving the drowning of cats, dogs, guinea pigs, mice, rabbits and rats have been conducted. The purpose of these experiments, based on the assumption that the process of drowning in ‘animal models’ in fresh and salt water would be the same as that in people, was to unravel the pathophysiology of drowning in people (Swann et al., 1947). Early experiments (Brouardel and Vibert, 1880; Yamakami, 1922, 1923; Martin, 1932; Karpovich, 1933; Banting et al., 1938; Lougheed et al., 1939; Jetter and Moritz, 1943; Kyllstra, 1962) were undeniably inhumane and unethical by modern standards, involving, as they did, conscious animals. In addition, the scientific methodologies were seriously flawed by (1) asphyxiation of control animals by clamping the trachea or by strangulation and (2) clamping the oesophagus to prevent swallowing of water. These procedures rendered the results invalid in the context of ‘natural drowning’.

In 1963, Fuller reviewed freshwater drowning in people and pointed out that the course of human drowning does not follow the course predicted by Swann and colleagues’ experiments (1947) on dogs. Nevertheless, this did not prevent further experimental studies using lightly anaesthetized dogs in the coming years (Farthmann and Davidson, 1965; Modell et al., 1966; Giammona and Modell, 1967). Eventually, in 2005, Lunetta and Modell stated ‘It is generally agreed that although pathophysiological differences between drowning in freshwater or saltwater may be observed in experimental models, these have little or no clinical significance for human drowning’. These authors also noted the recurring experimental design fault, whereby the quantities of water inhaled during experimental drowning of dogs are greatly in excess of those that have been calculated to be aspirated in 85% of human drownings.

This background raises serious moral and ethical questions regarding the use of data generated by cruel or scientifically flawed studies. In this regard, the excellent appraisals of the ethics of citing results of Nazi medical experiments (Moe, 1984; Talia, 2002) provide balanced guidance to the dilemma. From a human drowning perspective, the results of the experimental drowning studies in animals lack validity and applicability and as such there seems no scientific or moral justification for their citation. As far as forensic veterinary pathology is concerned, and without condoning these distasteful experiments, which make for distressing reading, it may be possible to salvage some good from them. As a consequence of improving ethics on the use of experimental animals, these experiments will not be repeated, but they remain a source of pathology data on drowning, particularly in dogs. Table 4 summarizes a number of gross observations recorded in the various reports (cited above) on experimental drowning.

Lung Weights of Drowned Dogs

Hyde et al. (1989) reported that, using computed tomography (CT) and re-breathable gases, the lungs of healthy mongrel dogs weighed 19 ± 5 g/kg body weight (BW). In comparison, Giammona and Modell (1967) found the lung weights of their lightly anaesthetized drowned mongrel dogs to be 34.9 ± 5.3 g/kg BW, 35.4 ± 3.6 g/kg BW and 38.7 ± 4.7 g/kg BW, depending on whether the dogs were drowned with distilled water, seawater or chlorinated distilled water, respectively. Despite the increased weights of lungs from drowned dogs, it should be noted that Lougheed et al. (1939) and Swann et al. (1947) found that most lungs from experimentally drowned dogs floated in water.

In all of this, it should be borne in mind that the ratio of normal lung weight/body weight will vary substantially according to breed, age and bodily condition of the dog (e.g. a young, fit greyhound or collie, vis-a-vis a fat, middle-aged Labrador).

Microscopical Examination of the Lungs from Drowned Animals

Microscopical changes in the lung after drowning may include alveolar overdistension, attenuation of alveolar septae, narrowing of alveolar capillaries, alveolar rupture and intra-alveolar haemorrhage and flooding by pale eosinophilic fluid (Munro and Munro, 2008c). However, Reidbord (1980) cautioned that in most cases of human drowning ‘the pathologist is faced with a variety of non-specific histologic changes that preclude a definitive diagnosis’. As a consequence, no single histopathological change, or combination of changes, is considered...
Large quantities of froth and fluid flow from the nostrils.

Clear or pink frothy foam is present in the trachea and major airways in 80% of dogs drowned in freshwater. More froth is noted in seawater drowning than in freshwater drowning.

The entire bronchial system is usually filled with a frothy fluid.

The dependent parts of lung lobes appear atelectatic and haemorrhagic.

Congested or haemorrhagic areas may be scattered elsewhere on the lung surfaces.

Crepitus can occur.

Various authors (Lougheed et al., 1939; Swann et al., 1947; Modell et al., 1966) agree that the gross appearance of the lungs in freshwater and seawater drowning is similar.

The right side of the heart and the great veins are usually engorged, while the chambers of the left heart may be virtually empty.

Large quantities of water may be present in the stomach when artificial respiration or vomiting have not already affected its removal.

Table 4
Gross changes in experimental drowning

- Large quantities of froth and fluid flow from the nostrils.
- Clear or pink frothy foam is present in the trachea and major airways in 80% of dogs drowned in freshwater. More froth is noted in seawater drowning than in freshwater drowning.
- The entire bronchial system is usually filled with a frothy fluid.
- The dependent parts of lung lobes appear atelectatic and haemorrhagic.
- Congested or haemorrhagic areas may be scattered elsewhere on the lung surfaces.
- Crepitus can occur.
- Various authors (Lougheed et al., 1939; Swann et al., 1947; Modell et al., 1966) agree that the gross appearance of the lungs in freshwater and seawater drowning is similar.
- The right side of the heart and the great veins are usually engorged, while the chambers of the left heart may be virtually empty.
- Large quantities of water may be present in the stomach when artificial respiration or vomiting have not already affected its removal.

pathognomonic for drowning in people. This may also be the case for other species.

The ultrastructural changes described in rat lung following experimental intratracheal perfusion of either fresh or salt water showed clear differences. In freshwater drowning there was severe disruption of all cellular organelles with marked endothelial vesiculation. Salt water caused relatively little ultrastructural alteration, resembling the changes seen in acute hypoxia (Reidbord and Spilz, 1966; Reidbord, 1966, 1967).

The Diatom Test

Diatoms are unicellular algae which are found in almost all aquatic and damp terrestrial habitats (Horton et al. 2006). Although literally hundreds of peer-reviewed papers have been published on the potential of diatom testing for the diagnosis of drowning, its reliability remains controversial (Bortolotti et al., 2004; Lunetta and Modell, 2005; Horton et al., 2006). Problems confounding the reliability of this test include (1) contamination of the samples at the time of necropsy examination or during processing of tissues, (2) passive penetration into bodies following immersion, (3) absence of diatoms in some cases of human drowning and (4) the occurrence of falsepositives, whereby diatoms are identified in bodies that have had no contact with water.

Nevertheless, Merck (2007b), citing literature referring entirely to human cases, considers that suitably controlled diatom testing is a valid means of confirming drowning as the cause of death in veterinary cases.

The rather sketchy account of an investigation by Giri and Tripathi (1994) on immersion of anaesthetized and dead dogs in water, suggests that examination of liver, spleen, brain and bone marrow for diatoms might be helpful in distinguishing between submersion before or after death. However, the lack of information on the time interval between immersion and necropsy examination rather undermines the application of their findings. This report also confirms that low numbers of diatoms can be recovered from lungs, heart and kidney in dogs that died from causes other than drowning and which had no contact with water.

Recent investigations involving immersion, in freshwater, of piglets that had died from natural causes (Giancamillo et al., 2010) confirmed the existence of ante-mortem contamination by diatoms and that post-mortem contamination also occurs. In contrast, Bortolotti et al. (2011) found no diatoms in the lungs or the bone marrow of the sternum of people who had died from causes other than drowning.

Because many species of diatoms are habitatspecific, considerable efforts have been made to use diatoms to identify the location where a person may have drowned. This research led to the development of the ‘diatom-based quantitative reconstruction technique’ (Horton et al., 2006) but, to the best of the authors’ knowledge, this methodology has not been employed in veterinary forensic pathology.

At the present time, it seems prudent to proceed on the basis that ‘diatom testing cannot be accepted, in a court of law, as a definitive method for establishing death by drowning’ (Lunetta and Modell, 2005).

Estimation of Time since Death

Establishment of the time since death is a daily task in human forensic casework (Henssge and Madea, 2007). Routine murder enquiries usually attempt to discover whether particular individuals were in the
area at the time and had the opportunity to commit the offence, or if they can provide an alibi. Estimation of the post-mortem interval (i.e. the period between death and medical examination) may assist investigators in narrowing this ‘window of opportunity’, thereby eliminating specific events and suspects. Because of the importance of this task, much time and energy have been invested in researching an almost bewildering variety of methods for determining the time since death.

Great caution must be taken when providing an estimate of the post-mortem interval. Knight (1988) states ‘The margin of error remains large and unpredictable, even in controlled research conditions, let alone for the more variable environment of an actual scene of death’. The lack of accuracy and reliability of most methods was also of concern to Henssge et al. (2006b). However, over the last 30 years some improvements have been made, but Swift (2010) concludes that ‘It remains debatable whether there is any single, reliable and accurate means of estimating the time since death during the early post-mortem interval’. In a veterinary context, the picture is particularly complex since requests from prosecutors, police or defence agents for time of death estimates are driven by diverse objectives depending on the category and species of animal involved.

In companion animals, for example, investigations of suspected non-accidental injury (i.e. deliberate injury) are in many ways similar to human assault cases, where placing the accused in the location at the estimated time of the crime can be of importance. However, in contrast to human forensic casework, it may (depending on the jurisdiction involved) be a legal requirement, central to a prosecution, to determine whether suffering occurred in the period between assault and death. It follows that in such cases establishment of the time of death is important.

Wildlife crime investigations are less concerned with issues of suffering because they are usually focused on regulatory matters (e.g. ‘out of season’ shooting of game animals, poaching and breaches of statutory time limits). Many countries have enacted laws to provide protection of wildlife through limiting hunting to specific periods of the year and by requiring trappers to visit their traps and snares at least once in any 12 or 24 h period. Estimation of the post-mortem interval can be helpful during investigation of breaches of these regulations. Establishment of the approximate time of death of protected species (e.g. badgers) can serve the same purpose of determining alibi and opportunity, just as for human and companion animal deaths.

With regard to farm livestock, the desire to establish the time of death may be related to questions on (1) duration of neglect, (2) failure to dispose of carcasses within statutory time limits, (3) uncertainties over death during transport, (4) suspected fraudulent insurance claims and (5) time of onset of serious husbandry problems (e.g. interruption of water supply to poultry houses).

Methods for Estimating Time since Death

There are two basic approaches to estimation of time of death: (1) measurement of change that takes place at a known rate (e.g. rigor mortis, cooling of the body and putrefaction), and (2) comparison of the occurrence of events known to have taken place at a specific time with the time of death (e.g. extent of digestion of last meal).

The sources of evidence that are relied on when attempting to estimate the time of death in people include those present in the body (corporeal), those present in the vicinity of the body (environmental and associated evidence) and anamnestic evidence based on the deceased’s ordinary habits, movements and day-to-day activities (Pounder, 1995). The first two categories are important in animal cases. However, it could be postulated that evidence based on ‘day-to-day activities’ could also be helpful in certain instances of a forensic veterinary investigation — for example a case of suspected illegal shooting of ducks that have a regular pattern of flights to and from particular ponds or feeding sites.

Techniques and procedures used to estimate the post-mortem interval in mammals and birds have been outlined by Edge (1984), Erlandsson (2003), Cooper and Cooper (2007c), Merck (2007c), Munro and Munro (2008d), Okene (2010) and Sinclair et al. (2006b). The specific issues related to determination of the post-mortem interval in reptiles and amphibians are highlighted and discussed by Cooper (2012). In human forensic casework, Henssge and Madea (2007) believe that ‘most methods for estimation of the time since death are of only academic interest’. These authors are of the opinion that all methods of estimation of time since death must demonstrate quantitative measurement and include a mathematical description. They also suggest that any proposed method should be accompanied by proof of precision of the method on independent material, and that quantification of those factors that influence the method needs to be provided. In general, field studies are necessary to demonstrate the practicality of any method. Similar strictures apply to methods adopted in veterinary forensic investigations.

Techniques for estimation of time of death are listed in Table 5. However, many of these are, currently, of
limited practical value in legal cases because lack of validation of the methods renders the results open to challenge. For example, the use of electrical excitability of muscle to determine the time of death in the early post-mortem period may be questioned on the basis that the reaction of the muscle is affected by the manner of death (Madea and Henssge, 1990; Elmas et al., 2007). Similarly, although studies on post-mortem degeneration of DNA extracted from porcine muscle suggest that this technique may be useful during the first 72 h after death (Watson, 2010), validation of this method awaits detailed investigation of the impact of environmental variables such as ultraviolet radiation, heat, high humidity and fungal/bacterial contamination.

There is an accepted body of knowledge and experience on rigor mortis and decomposition that allows experienced veterinary pathologists to estimate the post-mortem period within approximate blocks of time such as <24 h, 1–3 days, 3–7 days, 7–21 days, weeks, months or years (Munro and Munro, 2008d). These changes are well described in standard veterinary pathology texts (e.g. Maxie, 2007) and are not considered further in this review. Nevertheless, experimental studies (Turner and Wiltshire, 1999; Wilson et al., 2007; Schotsmans et al., 2012) designed to simulate ‘clandestine burials’ revealed interesting insights into the effects of soil type, hydrated lime and quicklime on the process of decay of pig cadavers. These findings might have relevance to some veterinary investigations.

It is also worth noting that in the field of human forensic pathology, experienced pathologists frequently underestimate the time since death (James and Knight, 1965) and it is probable that similar errors are commonplace in veterinary pathology. This is a source of concern when a Court seeks veterinary guidance in relation to the time of death.

Forensic entomology can be of considerable value in veterinary cases (Anderson and Huitson, 2004). Although identification of the types and stages of maggots and beetles is outside the competence of most veterinary pathologists, knowledge of the correct procedures for the collection of entomological evidence is a necessary skill. Byrd et al. (2010) provide up-to-date guidance on appropriate methods.

Some research conducted on temperature-based methods, post-mortem chemistry, histopathology, electron microscopical changes and post-mortem radiology is, however, of relevance to forensic veterinary pathology. These methods may provide the means of refining the rather crude estimates based on evidence of decomposition or open fresh avenues for further investigation and independent validation.

**Temperature-Based Methods.** A carefully controlled study of beagle dogs weighing between 8.5 and 15 kg demonstrated the potential usefulness of rectal probes for estimation of time of death over the initial 10 h after death (Erlandsson and Munro, 2007). The data collected in this study suggested that the post-mortem interval can be estimated in 2 h bands or periods. For example, the rectal temperature at 60 min post mortem was clearly distinguishable from that taken 3 h post mortem. Similarly, the body temperature 5 h post mortem was distinct from that recorded 7 h post mortem etc. After 10 h the rate of drop of rectal temperatures of individual dogs showed greater variability, resulting in overlap and less clear separation. Nevertheless, under the conditions prevailing in this study (ambient temperature of 10.9–16.8°C), by 17–17.5 h post mortem the rectal temperature of each dog was <19°C. Body temperatures approximated ambient temperature 24–48 h post mortem. Contrary to the findings for human cadavers (Knight, 1988; Henssge, 2002), Erlandsson and Munro (2007) found no evidence of a plateau in the rectal temperature curve in the early part of the post-mortem period.

Two studies from Malaysia provide data on the cooling of dogs at ambient temperatures of 24–36°C. Abdulazeez and Noordin (2010) inserted stirring thermometers into liver and rectum while Okene (2010) recorded external ear canal, rectal and hepatic cooling using probes and thermocouples. These authors are of the opinion that the exponential and linear equations developed during their studies may prove useful for the estimation of the time of death in the initial 7 h post mortem. Although the exponential model may provide a slightly more accurate estimate, the linear model appeared mathematically easier to use in the field. Abdulazeez and Noordin (2010) found cooling under tropical conditions was less consistent than in temperate climates. When body temperature was close to ambient temperature, the cooling curve showed peaks and short plateaux
that lasted for an average of 70 min. Under these tropical conditions, body temperature reached ambient temperature in 26 ± 8 h.

The literature on estimation of time of death of people makes frequent reference to a lag phase, or plateau, in the cooling curve derived from rectal temperature measurement in the early period after death. However, the studies by Baccino et al. (1996) demonstrated no lag phase if the temperature measurements were taken from the external ear canal. Erlandsson (2003), Abdulazeez and Noordin (2010) and Okene (2010) failed to demonstrate a plateau in dogs, irrespective of the organ where the temperature readings were taken. This is of some importance as it highlights the caution and attention to detail necessary when extrapolating between species. Further consideration needs to be given to potential differences arising through the use of differing methodologies and equipment.

Merck (2007c) notes the ‘rule of thumb’ cooling rate of 1.5°F/h that is sometimes used in estimating the time of death of people and that it is recommended to add 1–2 h to the post-mortem interval in cases where a plateau may have occurred. She comments that ‘it makes sense to apply this rule to animal cases as well’. Sinclair et al. (2006b), citing Rauch (2003), also comment on the temperature plateau and the 1.5°F loss per hour in rectal temperature. However, the studies by Erlandsson (2003), Abdulazeez and Noordin (2010) and Okene (2010) suggest there is no scientific evidence to support the extrapolation, to canine cadavers, of guidelines formulated for human cadavers. The same caveat may well apply to other species.

Establishment of reliable evidence to support prosecutions for out-of-season killing of deer was the primary aim of a number of studies conducted in North America. The widespread practice of ‘field dressing’ culled deer, whereby the abdominal cavity is opened and the gastrointestinal tract removed soon after the deer has been shot, renders rectal temperature measurement impossible or meaningless in these animals. Consequently, thigh and/or nasopharyngeal temperatures in culled North American deer were recorded by Neubrech (1960), Gill and O’Meara (1965), Pex et al. (1983), Woolf et al. (1983), Kienzler et al. (1984), Cox et al. (1994) and Hadley et al. (1999). In addition to the thigh and nasopharyngeal temperatures, these investigators recorded ambient temperature, deer weight and the approximate time that elapsed between shooting and sampling. These studies allowed the construction of tables, temperature charts (Kienzler et al., 1984) and a field manual (Kienzler and Fuller, 1983) to assist rangers and law enforcement agencies to estimate time since death, taking into account BW and prevailing ambient temperature. Although affected by uncertainties (e.g. variations in body temperature at time of death), this approach was endorsed 20 years ago as being of practical value (Oates, 1992). The development of computer software programmes by Kienzler (1983) and Cox et al. (1994) made this task easier.

Experiments on the cooling rates of pig cadavers were conducted by Kaliszanz et al. (2005). These studies aimed to assess the practical value of the ‘two-exponential’ model for time of death estimation, in comparison with the single exponential model. Although these experiments were undertaken to assist in human forensic investigations, they provide useful insights into the estimation of the time since death in pigs.

Pin probes (connected to two channel thermometers) inserted into the eyeball, orbital tissues, ‘rump’ muscle and rectum measured the decrease in body temperature, beginning 75 min after death. The single exponential model applied to eyeball cooling allowed very precise estimation of the time of death up to 13 h post mortem. Thereafter, better time-of-death estimations were obtained from muscle or rectal probes. Limited numbers of pigs were used in these experiments. Consequently, it is desirable that other research groups validate this method.

Post-mortem Chemistry. The search for reliable chemical markers that correlate strongly with time since death continues to be a popular area of research in human forensic medicine. In the past many of these studies focused on vitreous humour (Madea et al., 1989) because it is generally less affected by autolysis and is isolated from the influences of the large organs in the chest and abdomen (Saukko and Knight, 2004a). Following the experiences in human studies, attempts were made to correlate potassium levels in vitreous humour and glucose in aqueous humour with the time since death in deer. Pex et al. (1983) demonstrated the fall in glucose in aqueous humour in black-tailed deer to be a useful marker during the first 8 h post mortem. Rising potassium levels in vitreous humour of mule deer were considered to have a logarithmic, rather than a linear (arithmetical), relationship with the lengthening post-mortem interval (Johnson et al., 1980). However, Woolf and Gremillion-Smith (1983) advised caution because this technique is based on intraocular post-mortem autolysis, which can vary widely.

A variable rate of autolysis is not the sole drawback of this type of marker. Lack of knowledge of the ‘normal’ ante-mortem levels of chemicals in the vitreous or aqueous humour undermines confidence in the
relevance of the findings. Similarly, the effects of disease or nutritional status on the distribution and concentration of these chemicals remains to be studied. Consequently, in a veterinary context, these uncertainties result in chemical markers in vitreous and aqueous humour being viewed as providing supporting evidence, rather than the primary means, of estimating time since death for deer.

The situation in dogs is slightly further advanced with regard to vitreous humour. Schoning and Strafuss (1980) examined 60 adult mongrel dogs and helped to establish baseline values for ante-mortem and post-mortem levels of sodium, chloride, potassium, urea nitrogen, glucose and creatinine. However, these tests do not appear to have been adopted as standard methods of estimation of the post-mortem interval in domestic dogs.

Research has also been conducted into the use of protein markers for estimation of the time since death. Analysis for cardiac troponin I in cattle (Sabucedo and Furton, 2003), although semi-quantitative in nature and temperature dependent up to 37°C, shows potential, but awaits validation. Nagaraj et al. (2005) studied changes in skeletal muscle in goat carcasses stored at 5°C for 3–20 days, with the primary purpose of understanding the processes involved in the tenderization of meat. Proteolytic breakdown products appeared after 6 days storage and the detection of a 55 kDa polypeptide was a consistent feature of muscle samples from 12-day-old carcasses. Again, these findings are interesting, but validation will, however, be necessary if they are to form the basis of alternative methods for estimation of the time since death, for legal proceedings.

**Histopathology and Electron Microscopy.** In addition to collecting temperature data, Erlandsson (2003) noted post-mortem gross and microscopical changes in beagle dogs. Subsequently, Erlandsson and Munro (2007) drew up a table of differential findings at various time points (<1 day, 3 days, 7 days and 23 days) after death. They found heart, liver, lungs, pancreas, thonsils, thyroid and urinary bladder to be the most useful organs for highlighting histological changes at different times in these animals. However, the usual caveats apply to extrapolation of these data to other breeds of dog or different species. Nevertheless, this study raises interesting possibilities for the use of histopathology and immunohistochemistry in refining the estimation of time since death. Similarly, the investigation of time-dependent changes in post-mortem testis histopathology in the rat (Bryant and Boekelheide, 2007) provides valuable background information on autolytic changes at specific times. Expansion of this research to encompass other species and different environmental circumstances would inevitably establish a sounder basis for exploring this approach to estimation of the post-mortem interval.

Although somewhat more esoteric, but nevertheless valuable, is the work of Pallot et al. (1992) who showed that autolytic changes in the carotid body may be misinterpreted if account is not taken of the delay between death and fixation of the tissue sample. The ‘beyond reasonable doubt’ principle used in criminal proceedings applies equally to veterinary pathology as it does to other types of evidence. Therefore, elimination of doubt over the significance of particular histological observations could be of crucial importance.

The study by Nagaraj et al. (2005) outlines changes to Z-disks in goat skeletal muscle observed by transmission electron microscopy (TEM). Six days after death there was little alteration of these structures, but degradation of the Z-disks was noted to be ‘considerable’ by 12 days. Munoz et al. (1999) employed TEM to relate autolytic changes in canine myocardial cells to the post-mortem period. Morphometrics characterized (1) decreased numbers of mitochondria, (2) increased mitochondrial volume and (3) increased surface areas of both outer and inner mitochondrial membranes, as the post-mortem interval extended from 0 to 240 min.

**Post-mortem Radiology.** The concept of the ‘virtual autopsy’ (also known as ‘virtopsy’) using CT and magnetic resonance imaging (MRI) is a developing sub-speciality of post-mortem radiology (O’Donnell and Woodford, 2008). In human forensic pathology, CT scanning of the deceased is increasingly being adopted as a routine procedure before conventional post-mortem examination. However, significant differences between clinical radiology and post-mortem imaging are potential pitfalls (Flach et al., 2010).

There is a considerable shortage of information on the use of advanced imaging techniques in veterinary post-mortem examination. Exceptions include the essentially practical experimental studies on the comparative sensitivity of conventional necropsy examination and CT scanning in detection of bone fractures in piglets (Cattaneo et al., 2006). More fundamental, and less likely to become a mainstream procedure, is the use of proton magnetic resonance spectroscopy to detect new metabolites of autolysis and putrefaction in pig and sheep brains (Cecil et al., 1998; Ith et al., 2002; Banaschak et al., 2005).

Heng et al. (2008), using conventional radiography, described post-mortem changes in the abdomen of 41 cats that had been humanely destroyed and kept at 4°C for up to 12 h before radiography. Of these cats, 11 (27%) had intravascular gas, with the liver being the most common site. Intravascular gas was also detected in the aorta, femoral artery, coeliac
and cranial mesenteric arteries and the caudal superficial epigastric artery. Interestingly, only two cats showed distension of the small intestines, while a solitary cadaver was affected by gas dissecting the tissues in the wall of the large intestine (pneumatosis coli).

Heng et al. (2009b) subsequently studied the post-mortem radiographic appearance of the abdominal organs of dogs. However, these studies were conducted on canine cadavers kept at ambient temperatures of 22–33°C. Consequently, the results are not directly comparable to the feline study. The high ambient temperatures resulted in rapid decomposition and prevented the study extending beyond 24 h after death. Increased gas in the gastrointestinal tract was observed as early as 8 h. By 16 h, gas was observed in the liver, caudal vena cava and peritoneal cavity in all cadavers. Abdominal distension was a feature in five of the six dogs at this time. The authors suggest that, in ambient temperature of 22–33°C, detection of gas in the abdominal cavity indicates that death occurred at least 8–16 h before radiography. Okene (2010), who also conducted his experiments in ambient temperatures of 22–33°C, found that the intestines, liver and hepatic vein were the radiological markers of choice for estimation of time of death. The observations made in this latter study included radiographs taken 6 h after death, reducing by 2 h the time selected by Heng et al. (2009b) as the start point.

Serial thoracic radiographs detected post-mortem gas accumulation in the pleural cavity in one dog after 8 h and in all dogs by 16 h (Heng et al., 2009a). Okene (2010) found that 90% of dogs in his study showed varying degrees of gas accumulation in the pleural cavity 12 h after humane destruction. However, he cautioned that compression of thoracic organs by abdominal distension may limit accurate interpretation of the development of pneumothorax.

In the study by Heng et al. (2009a), gas in the cardiovascular system was detected first in the right ventricle, coronary vessels, main pulmonary artery and caudal vena cava. This contrasts with Okene’s investigations (2010) where the right atrium and cranial vena cava had evidence of gas 6 h after death. This study also recorded a time dependent increase in accumulation of gas in the cranial vena cava from 12–24 h. There being no trauma or surgical interventions in the dogs before humane destruction, in either study, it can be concluded that all the gas in the cardiovascular system was a consequence of putrefaction.

Clearly, more research is needed to determine the value of post-mortem radiology in relation to estimation of time of death in veterinary cases. However, there is another aspect to this research. Gas accumulation occurred in the scapulohumeral joints of dogs in the absence of joint disease or a history of stress to the joints. It appears that this gas results from post-mortem decomposition and is not a manifestation of local bone ischaemia or other degenerative changes (‘vacuum phenomenon’) (Heng et al., 2009a). This finding reinforces the importance of being aware of post-mortem artefacts that may lead to misinterpretation.

**Conclusion**

This review focuses on four aspects of forensic veterinary pathology. Although each presents particular diagnostic challenges, there are difficulties that are common to all. These include lack of detail of the timing of changes, the danger of extrapolation of findings from one species to another, limitations of experimental models to reflect the spectrum of changes seen in actual cases, and the constant requirement for the pathologist to provide a balanced, factual interpretation of post-mortem findings. Great reliance is placed on forensic pathologists to guide investigations into alleged offences. Investigators wish for clear, unequivocal answers. Currently, however, much remains to be discovered about the development and resolution of lesions in different species; therein lie exciting possibilities for the expansion and refinement of the forensic veterinary pathology knowledge base.

**Conflict of Interest Statement**

The authors have not declared any conflict of interest that may arise from being named as an author on the manuscript.

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