Microscopic Diagnosis In Forensic Pathology

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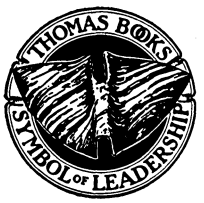

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Preface

MOST TEXTBOOKS of legal medicine devote little attention, if any, to the microscopic features of forensic pathology. To our knowledge, this is the first book on microscopic forensic medicine in the English literature. It is our conviction that this volume will provide sound and helpful guidance to forensic pathologists in a variety of circumstances, including early aging of injuries, routine and special enzymatic studies, the study of metallization of electrical burns by electron microscopy, and many other relevant histological categories of microscopic analysis.

We have gathered as contributors a distinguished panel of forensic scientists, who offer an invaluable distillate of their vast experience. We have attempted to cover the most common (although by no means all) forensic situations in which a microscopic examination is indicated. It was not possible, of course, to deal with every clinical pathological entity. For example, we chose not to cover sudden infant death syndrome, a pathological entity which is determined more by exclusion, and has no characteristic or pathognomonic micropathological findings. The micropathology of poisoning, in our opinion, deserves a separate treatise, and is therefore only incidentally treated within some of the chapters, such as the one dealing with the microscopic profile of drug toxicity.

It is our hope that ultimately this book will provide much needed stimulus to forensic pathologists; give proper emphasis to the role of microscopic examinations in medical-legal investigative work; and catalyze new efforts in the research and teaching of microscopic forensic pathology.

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Microscopic Diagnosis In Forensic Pathology

Chapter 1

Histological Estimation of the Age of Injuries

J. RAEKALLIO, M.D.

Introduction

WHEN EXAMINING VICTIMS of violence, no forensic pathologist is fulfilling his role when he confines his report merely to a numbered list of the injuries found and to their description. Even the cause of death is sometimes less important than the reconstruction of events. This is often possible by careful examination of the wounds and other injuries. As a classic example, a person may, after death, be run over by a car. If the examiner does not recognize that the injuries were caused after death, innocent people may be arrested or even found guilty. On the other hand, defective observations may lead to the acquittal of guilty persons. For example, the body of a murdered person may be put on a railroad track in order to simulate a suicide or an accident. Thus, justice in these matters often rests upon the accuracy in observation and interpretation of the injuries found. The estimation of the age of injuries and the distinction between vital (antemortem) and postmortem wounds are of prime importance.

The examination of the naked-eye appearance of wounds in corpses may be of some help when deciding whether the injuries are of vital origin. In this case, the extravasated blood is imbibed by the damaged tissues. The wound edges gape and become swollen after a lapse of about 12 hours. A small wound may show scab formation after approximately 24 hours, and when a wound has become infected, pus may be seen after about 36 hours. Epithelium begins to grow at the edges of a wound after a period of about 24 hours and epithelialization of small clean wounds may be complete in 4-5 days (Gordon, Turner, and Price 1953). It may thus be possible by naked-eye examination to state that a wound is antemortem in origin, especially if it shows evidence of a marked inflammatory reaction. This is not always the case and a lapse of about 12 hours is far too long to satisfy the demands of a forensic examination.

OPEN SKIN INJURIES CAUSED BY MECHANICAL FORCE

Because of the uncertain and variable results of naked-eye examination of wounds, it is important to study the injuries microscopically. This is

advisable both in order to distinguish between antemortem and postmortem wounds and to time wounds inflicted before death for the reconstruction of events.

A wound is a disruption of the anatomical continuity of tissues caused by the application of a mechanical force to the body. It is usual to distinguish between incised wounds (cuts), stab wounds (punctures), and lacerations. Incised wounds are caused by sharp weapons or objects such as knives, jagged portions of metal, or pieces of broken glass. Stab wounds are caused by long narrow instruments with blunt or pointed ends. Lacerations are wounds in which the tissues are torn as a result of the application of blunt force to the body. An abrasion is a destruction of the skin that usually involves the superficial layers of the epidermis only.

The histological estimation of the age of injuries is based on the morphology of the various stages of *wound healing.* The series of events in response to the initial injury generally follow in a definite order. Ross (1968) divided tissue repair into three periods: (1) the inflammatory phase, which lasts 1-3 days after the injury, (2) the proliferative phase, lasting to day 10-14, (3) the reorganization or remodeling phase of healing, which is of varying length, lasting at least several months. None of the phases is distinctly separate, since each blends into the next.

THE INFLAMMATORY PHASE of wound healing is characterized by (a) the vascular, (b) the hemostatic, and (c) the cellular response to the injury. In the *vascular response,* the sequence of events depends on the severity rather than the nature of the injurious agent. The vascular response develops in three distinct phases. The first is vasoconstriction, beginning within a few seconds and lasting for a few minutes. The second is an early vasodilatation, accompanied by increased permeability to plasma protein, which attains a maximum response in about 10 minutes. The third, delayed phase requires several hours and culminates in the infiltration of the tissue with leukocytes and is accompanied by stasis and local hemorrhage. These phases are due to the release of vasoactive substances (Spector and Willoughby, 1965). In practice, the infiltration of the injured tissue by leukocytes is the most important histological characteristic of the delayed vascular response.

The *hemostatic response* means the spontaneous arrest of bleeding. Agglutinated platelets arrest hemorrhage by rapidly sealing all cut vessels larger than capillaries. The capillaries are sealed by a red cell fibrin clot. Platelet aggregation occurs at first without fibrin formation, but *fibrin* is laid down later with the appearance of a more massive blood clot where a fibrin network enmeshes randomly distributed platelets, erythrocytes, and leukocytes. Lendrum et al. (1962) have described the method of Martius scarlet blue, which seems to be the most useful single method for the demonstration of fibrin. Fibrin stains yellow with the Martius scarlet blue

technique for the first 16 or so hours after production, and thereafter stains bright red (Pullar, 1973). The method of Martius scarlet blue is given in the chapter appendix.

The *cellular response* begins with the aggregation and margination of leukocytes on the vascular wall within half an hour of wounding. This in practice is difficult to detect with any certainty in human material from the usual cases of forensic interest (Pullar, 1973). The early cellular response is dominated by polymorphonuclear leukocytes. At the onset of a lesion both polymorphonuclear and mononuclear cells emigrate together, but there is a predominance of polymorphonuclear (mostly neutrophilic) cells, the ratio being 5:1. Later (at 16-24 hours) the polymorphonuclear cells move away from the site of inflammation, but the mononuclear cells remain, the ratio falling to 0.4:1 (Spector, Lykke, and Willoughby, 1967).

The point of *the appearance of a distinct leukocytic infiltration* is still a matter of debate. According to different authors, it varies from 4 to 24 hours. In guinea pigs, some polymorphonuclear leukocytes migrate out of the vessels 4 hours after wounding. In the vicinity of an 8-hour excision, *polymorphs* are more numerous, but they do not yet constitute a well-defined zone around the lesion. At 16 hours, it is possible to distinguish histologically two zones (Figs. 1-1 and 1-2). In the vicinity of the wound edge, a *central zone* of up to 500 microns in depth is characterized by *degenerative* changes. Karyolysis and karyorrhexis progress in the connective tissue cells, the epidermal cells maintaining their staining properties better (Raekallio, 1961). At some 32 hours, necrosis is apparent in the connective tissue, the nuclei being barely stainable. Surrounding the central zone, a 100 to 200 micron deep *peripheral zone* exhibits, in addition to activated fibroblasts, mostly neutrophilic *polymorphonuclear* cells (also termed *neutrophilic granulocytes* by many authors). Their number increases to the maximum during day 1, remains at that level until day 2-3, and then decreases. This increase and decrease in granulocytes coincides with an equally prominent rise and fall in the amount of fibrin (Ross, 1968). The granulocytes are followed into the peripheral wound zone by large mononuclear cells or *macrophages* at 16 to 24 hours, reaching their maximum concentration within approximately 48 hours. *Lymphocytes* are found in large numbers at a somewhat later stage, reaching their maximum concentration about day 6 after the injury (Ross, 1968).

The regressive phenomena in the *central* wound zone are called *negative vital reactions,* since no such local degenerative changes are observed in wounds inflicted after death. Similarly, the inflammation and other progressive phenomena in the *peripheral* wound zone may be called *positive vital reactions* (Raekallio, 1961).

At some 32 hours, necrosis is apparent in the central zone, the nuclei being barely stainable. In the peripheral zone, macrophages are princi-

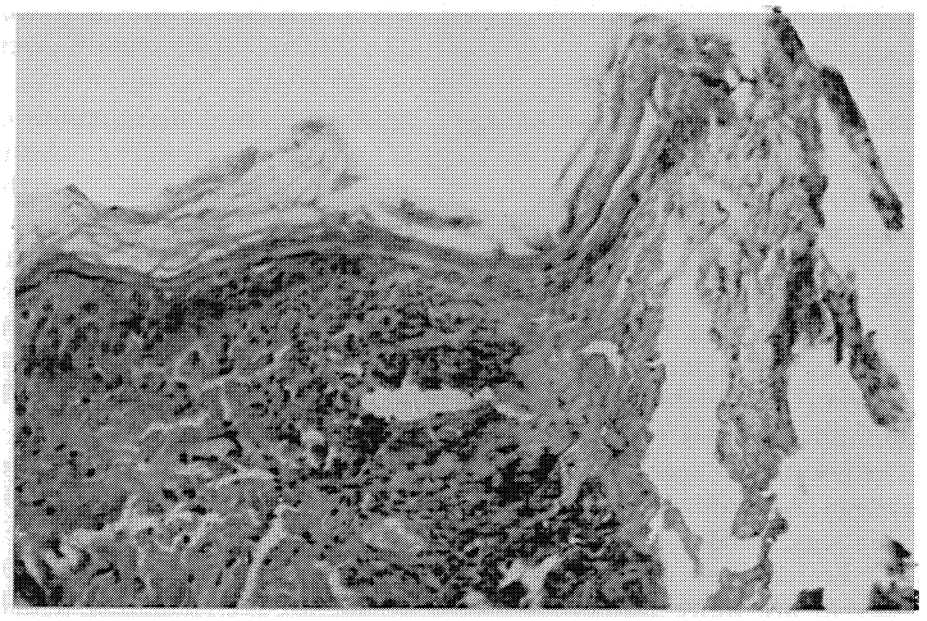

Figure 1-1. Histological view of a 16-hour antemortem wound showing the peripheral zone with migrated cells, and the developing necrosis in the central zone (on the right in the picture).

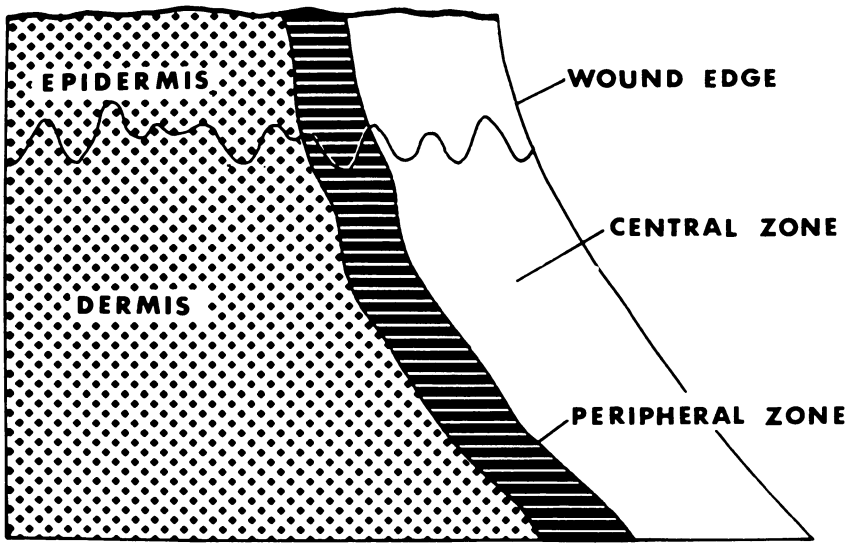

Figure 1-2. Schematic diagram showing the zones on an antemortem skin wound.

pally responsible for the increase in the number of cells in the dermis. Only occasional mitotic figures are seen within the connective tissue. The epithelial cells of the peripheral zone are enlarged. A few isolated figures are seen in the stratum basale (Raekallio, 1961).

At 64 hours, there is advanced necrosis in the central zone. In the peripheral zone, macrophages and activated fibroblasts dominate. Some mitoses are also seen in the dermis, but mitotic figures are more numerous in the lower layers of the thickened epidermis (Raekallio, 1961).

In *human wounds,* the inflammation proceeds slower than in laboratory animals. In forensic autopsy materials, some neutrophilic granulocytes are seen perivascularly at 4 hours. A definite peripheral wound zone, consisting of infiltrating granulocytes and activated fibroblasts, is recognizable at 8-10 hours (Raekallio, 1965, 1966, 1970; Robertson and Hodge, 1972; Pullar 1973). There are, however, authenticated cases in which a distinct leukocytic infiltration has taken still longer to become apparent (Pullar, 1973).

Among the inflammatory cells, the *neutrophils* are essential in the control of infection at the wound site. Further, they are capable of engulfing and digesting nonmicrobial material (Schilling, 1968). Large numbers of degenerated neutrophils can be seen during the first 24 hours. The lysis of neutrophils, with resultant release of their stores of hydrolytic enzymes, represents an important function of this cell (*cf.* Chapter 2).

Many names have been given to the large mononuclear cell that plays so important a part in the later stages of acute inflammation. The word *macrophage* means "large eater" and suitably designates a large phagocytic cell. It can ingest bacteria and also debris that results from the breakdown of cells or their products. Macrophages are termed histiocytes or clasmatocytes by some authors.

Morphologically, there are small (diameter of 7 to 8 microns) and large (around 12 microns) *lymphocytes.* As is well known, the rounded nucleus of a lymphocyte is its most prominent feature. There is very little cytoplasm around the nucleus. The essential function of the small lymphocyte is to mediate the immune response (Schilling, 1968). Together with its close relative, the *plasma cell,* the lymphocyte constitutes the so-called "small round cell infiltration" that dominates the picture in the late stages of acute inflammation. The plasma cell is larger than the lymphocyte, with a more abundant cytoplasm and an eccentric, spherical nucleus. The latter contains coarse, angular, densely staining flakes of chromatin that sometimes are arranged like the spokes of a wheel. The plasma cells are a very important source of circulating antibodies.

During acute inflammation, *mast cells* degenerate and liberate histamine, heparin, and hydrolytic enzymes into their surroundings. Mast cell histamine may participate as an edema-producing substance in the early

phase of inflammation (*cf.* vascular response). Mast cell granules, which are the chief distinguishing feature of this cell, do not show up in H and E sections. In toluidine blue or methylene blue preparations, the mast cells appear under low power as large dark oval cells. Under oil immersion the cytoplasmic granules, about 0.5 micron in diameter, are often so densely packed that no details of the dark-stained mast cells may be seen. Mast cells are absent from one-day-old wounds. This is followed by a reappearance and degranulation of these cells between day 3-7 postoperative (Miller and Whitting, 1965).

The *origin* of the new mononuclear cells in the healing wound is still a matter of debate. Some authors assume that these cells are derived from preexisting fibroblasts, others regard them as migrating from the blood.

THE PROLIFERATIVE PHASE of wound healing is characterized by epithelial and connective tissue repair. The earliest detectable change in the *epidermis* is a thickening of the epidermis remaining at the wound margin. This thickening appears to result from an increase in the volume of the epidermal cells adjacent to the wound (Odland and Ross, 1968). In clean, incised skin wounds of experimental animals, the epidermis grows down into the dermis within 24 hours (Gillman and Penn, 1956). The cut edge of the epidermis apposed to the clot shows distinctive cytoplasmic processes that project toward the clot. In small superficial wounds, migration usually appears to proceed symmetrically from both sides towards the center of the wound. Thus, in profile the regenerating epidermis has the appearance of a pair of wedges directed towards each other at the middle of the wound (Odland and Ross, 1968). In some instances, migration from one side of the wound commences in advance of that from the other side so that, at the time of sampling, there appears to be migration from one side only of the wound.

The epithelial growth in human wounds commences in the interval between 24 and 48 hours. The advancing epidermis appears to follow a plane defined by a fibrin net, which in turn is enclosed by serous exudate containing inflammatory cells. This plane lies deep to the wound crust. Epithelization of small wounds and abrasions is invariably complete 3 days, and often 2 days, after the injury.

After wound closure the newly regenerated epidermis becomes highly stratified and thicker than the normal surrounding epidermis. Epidermal thickness decreases to nearly normal by day 5-7 after the injury (Odland and Ross, 1968).

Healing by primary intention occurs when epithelial cells bridge across the wound under the dry superficial clot in a clean incision. With healing by secondary intention, when the defect is too large for epithelial cells to grow across, the cells of granulation tissue gradually invade and replace the dry fibrin covering. In this case the wound closure relies on epitheliza-