Z. Rechtsmedizin 73, 83—102 (1973)

Übersichtsreferat—Review Article

Estimation of the Age of Injuries by Histochemical and Biochemical Methods

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Received June 6, 1973

Summary. The author reviews the literature dealing with the application of histological, histochemical, and biochemical methods for the estimation of the age of wounds (in the skin and other organs), hanging marks, bruises, burns, and electric injuries. According to the data presented, it is possible to distinguish biochemically between ante-mortem and post-mortem skin injuries after a survival time of as little as a few seconds or minutes. The methods of enzyme histochemistry act as a guide to the estimation of the approximate age of injuries inflicted 1—16 hrs before death. The determination of the age of injuries is more reliable when several independent methods are simultaneously used. In addition to histology, biochemical and histochemical methods are applicable even in forensic practice.

Zusammenfassung. An Hand der neueren und älteren Literatur wird ein Überblick über die histochemischen und biochemischen Methoden gegeben, die bisher für die forensische Wundalterbestimmung herangezogen worden sind. In Zusammenhang mit der kritischen Bewertung verschiedener Parameter werden auch eigene experimentelle und praktische Erfahrungen von den posttraumatischen Gewebsreaktionen mitgeteilt.

Um eine Altersbestimmung von Verletzungen vornehmen zu können, ist die genaue Kenntnis des zeitlichen Ablaufs der Reparationsvorgänge notwendig, der nur in tierexperimentellen Serienuntersuchungen zu ermitteln ist. Es ist jeweils die Prüfung notwendig, inwieweit dieser experimentell ermittelte Reparationsablauf mit jenen Reparationsvorgängen zeitlich korrelierbar ist, die durch Untersuchungen von Verletzungen des Menschen bekanntgeworden sind.

Früheste fermenthistochemische Befunde bei Hautwunden werden 30—60 min nach der Läsion beobachtet (Esterase, Adenosin-Triphosphatase). In der unmittelbaren Nähe der Wundfläche wird ein Rückgang der Fermentaktivität (negative vitale Reaktion) sichtbar. In der Wundperipherie erkennt man eine Zunahme der Enzymaktivität (positive vitale Reaktion). Keine entsprechenden Veränderungen kommen in den postmortal erzeugten Wunden vor. Außerdem kann man auf Grund von fermenthistochemischen Untersuchungen das Alter der 1—16-Std-Wunden schätzen und dadurch die immer noch erforderliche histologische Untersuchung bereichern. Ein wesentlicher Vorteil der Fermentdarstellung liegt in ihrer enormen Autolyse- und Fäulnisresistenz. Sehr wichtig ist für die forensische Altersbestimmung von Hautverletzungen der Wechsel des Zellbildes vom granulocytären bis zum histiocytären Infiltrat der Subcutis. Für die biochemische Altersbestimmung von Verletzungen ist der biochemische Nachweis einer Histamin- und Serotoninvermehrung sehr geeignet. Beginnende biochemisch nachweisbare Veränderungen werden im allgemeinen auf einen Zeitraum von etwa 1—5 min nach der Läsion gelegt.

Für die Altersbestimmung der Verletzungen von anderen Organen stehen, im Gegensatz zu den ziemlich eingehend untersuchten Hautverletzungen, verhältnismäßig wenige Untersuchungen zur Verfügung. Ein Überblick über die Befunde bei Verletzungen des Fettgewebes und der Leber und über Extraktionswunden wird gegeben. Die morphologische und histochemische Altersbestimmung von gedeckten Suffusionen geht nach gleichen Gesichtspunkten vor wie diejenige von offenen Wunden. Die baustein-histochemischen Methoden bereichern

die fermenthistochemische Untersuchung von Verbrennungen und Strommarken. Mit enzymhistochemischen Methoden wird die Abgrenzung vitaler Knochenbrüche von postmortalen Veränderungen nach einer Überlebenszeit von etwa 10 Std möglich.

Die Einführung der histochemischen und biochemischen Methodik auf dem Gebiet der Wundaltersbestimmung bedeutet einen großen forensischen Fortschritt, den man, als eine Bereicherung des histologischen Status, in der praktischen Diagnostik nicht mehr missen möchte.

Key words: Injuries, estimation of age — Vital reactions, enzyme histochemistry, biogenic amines — Traumatology, estimation of age of injuries.

1. Introduction

In cases of death by physical violence there is always one major point to be determined by the forensic pathologist: were the injuries inflicted before death (intra vitam) or post mortem? Further, it is often important to estimate the interval between trauma end death. The following examples illustrate the importance of the estimation of the age of injuries.

A jealous young man believed that he had killed his fiancée when he hit her on the head with a stone. He carried the body to a railway track in order to simulate an accident or suicide. 4 hrs later, a train arrived and caused fatal injuries. Histological and histochemical studies indicated that a laceration on the head was produced several hours before the final fatal wounds caused by the train. The girl had obviously been unconscious after the initial head injury.

A man died of a heart attack while walking along a road. The body was later run over by a car. The lack of a biochemically demonstrable increase in the histamine and serotonin contents in the injuries, as compared to a control sample of neighbouring intact skin, helped to reconstruct the course of events. Accordingly, the driver was exonerated from the charge.

It may be possible by naked-eye estimation to state that a wound is ante-mortem in origin if it shows evidence of a marked inflammatory reaction. Under average conditions, the edges of a wound are red and swollen after a lapse of about 12 hrs. This is far too long to satisfy the demands of a medicolegal examination. An ante-mortem wound is customarily identified by its profuse bleeding. However, haemorrhages, even with abundant fibrin formation, are no longer reliable vital reactions (Mueller, 1964; Laiho, 1967).

Walcher (1930, 1936), Simpson (1965), and others have, with good reason, pointed out the importance of studying the lesions microscopically in order to distinguish between ante-mortem (vital) and post-mortem injuries. According to Walcher (1930), the leucocytic reaction was the earliest histological sign of inflammation. The pavementing of the inner vessel walls by leucocytes and the beginning of their extravasation may be seen during the first hours after wounding. However, the appearance of a few leucocytes in the wound periphery does not justify the diagnosis of a vital reaction, because these cells are a regular component of the connective tissue. The point of time of the appearance of distinct leucocytic infiltration is still a matter of debate. According to different authors it varies from 4 hrs (Walcher, 1930, 1936; Raekallio, 1961; Lindner, 1962; Giacometti, 1967; Robertson and Hodge, 1972), 6 hrs (Ross and Benditt, 1961), 12 hrs (Smith, 1945), 13—18 hrs (Russell, 1951), even to 24 hrs (Allgöwer, 1956). This discrepancy

seems to be due to different opinions regarding the amount of leucocytes for which the name "leucocytic infiltration" could be applied. In subcutaneous adipose tissue the cellular reaction is earlier (30—60 min in human material) demonstrable than in the dermis (Hirvonen, 1968; Berg, 1972). In the vicinity of corneal wounds polymorphonuclear leucocytes are seen at 24 hrs (Latessa and Ross, 1964).

Histological investigations have contributed substantially to our knowledge of local vital reactions and of the estimation of the age of injuries. However, there still remains a latent period of 1 to 4 (or even more) hrs during which the distinction between ante-mortem and post-mortem injuries is not possible by histologically detectable morphological changes.

It is now known (Raekallio, 1960, 1961, 1965a, b, 1970, 1972; Pioch, 1966a; Lo Menzo, 1969; and others) that morphological alterations are preceded by functional changes and these, moreover, often correlate with the action of enzymes. Thus, it is to be expected that the demonstration of enzymes and other substances, causing many important changes, could reveal earlier reaction than could the visualization of the resulting, morphologically demonstrable changes. Enzymes and other substances, being essential in the inflammatory reaction to injury, can be demonstrated histochemically and biochemically.

2. Open Skin Injuries Caused by Mechanical Force

A wound is a disruption of the anatomical continuity of tissues caused by the application of mechanical force to the body. It is usual to distinguish between incised wounds (cuts), stab wounds (punctures), and lacerations. Abrasion is a destruction of the skin which usually involves the superficial layers of the epidermis only. As many investigators have observed, abrasions are of two types: the "graze" caused by oblique force, and the die-like impression produced by an object striking the skin more or less at right angles. Basically, the mechanism of injury is not different (Robertson and Hodge, 1972).

Orsós (1935) made an attempt to distinguish between ante-mortem and post-mortem injuries by using the staining method of Mallory. Orsós used the term "metachromasia" to describe the difference in colour seen in connective tissue fibres injured before death, as compared with those in undamaged parts of the skin. He believed that this "metachromasia" was due to dehydration and to the relative increase in the globulin content of the proteins. Orsós, as early as the year 1935, emphasized the difference between somatic and cellular death. The former is initially characterized by cessation of respiration, heart contractions and circulation, and by loss of sensibility and reflexes. With somatic death not all cells and organs cease to function immediately and simultaneously — a fact very well known and used by modern transplantation surgeons. Orsós used the following classification of reactions:

1. intravital reactions, occurring when the whole organism is still able to function,
2. agonal reactions, seen just before somatic death,
3. signs of somatic death,
4. intermediate reactions, produced by individual cells still able to function after somatic death,
5. post-mortem reactions, seen after cellular death, and
6. signs of decomposition.

The classification by Orsós (1935) is still of value. By contrast, Blum (1937) noted that a colour change, similar to the "metachromasia" of Orsós, occurred not only in wounds made during the intermediary phase but also in those cut after the dissolution of rigor mortis. Lindner and Hölzer (1962), Becker and Lindner (1965), and Lindner (1968) have shown, by using modern qualitative and quantitative methods, that the colour change is not due to any actual metachromasia. By contrast, the change is caused by a competitive staining, partly due to the blue colour with aniline blue and partly to the red staining by acid fuchsin.

Ökrös (1938) believed that certain changes of elastic fibres (retraction, aggregation etc.), characteristic of ante-mortem injuries did not occur in post-mortem wounds. Similar changes, however, were demonstrated in post-mortem lesions, too (Stössel, 1950). Lindner and his group (Lindner, 1971) have shown that the various methods used for the demonstration of elastic and collagen fibres (Ökrös, 1938; Lindner, 1959, 1962; v. Schweinitz, 1959; Lindner and Freytag, 1960; Brack *et al.*, 1962), do not make possible the distinction between intravital and intermediate reactions or between ante-mortem and post-mortem injuries.

Carbohydrates and mucosubstances have been histochemically studied in order to distinguish between ante-mortem and post-mortem wounds and to estimate the age of injuries. These substances were previously called acid mucopolysaccharides. For the majority of carbohydrate biochemists the expressions mucopolysaccharide and mucoprotein are now out of date. Pearse (1968) refers to Gottshalk (1966) who says, however, that "each author defines mucopolysaccharides and mucoproteins in his own way, resulting in a great confusion of the true meaning, if any, of these terms". For convenience, I shall use the term acid glycosaminoglycans, preferred by many experts in the field, meaning the different chondroitin sulphates, and hyaluronic acid. The bulk of skin mucosubstances is known to be composed of them.

Dunphy and Udupa (1955), Raekallio (1961), Lindner (1962), and Nevelös and Gee (1970) have shown that acid glycosaminoglycans decrease in the so-called central zone of 32-hour ante-mortem wounds. This zone is a 200—500 μ deep area in the immediate vicinity of the wound edge (Fig. 1). The decrease, evident in the central zone, may be called a negative vital reaction (Raekallio, 1961, 1965b, 1972). No acid glycosaminoglycans are histochemically demonstrable in the central wound zone 64 hrs after injury (Raekallio, 1961). According to Nevelös and Gee (1970), acid glycosaminoglycans remain present in ante-mortem hanging and strangulation marks.

There is an increase in acid glycosaminoglycans in a 100—300 μ deep peripheral wound zone, surrounding the central area (Fig. 1). This increase appears at about the same time (32 hrs) as the decrease in the central zone (Raekallio, 1961). Lindner (1971) has demonstrated a so-called true ("echt") metachromasia (cf. Lindner, 1960, 1971) in the periphery of ante-mortem wounds. This phenomenon does not correspond to an actual increase in acid glycosaminoglycans. By contrast, this change is due to the staining of the released acid glycosaminoglycans, and it becomes histochemically demonstrable 2—4 hrs after wounding (Schallock and Lindner, 1957; Lindner, 1971). A primary acidosis and disturbances in electrolyte and water distribution precede the release of acid glycosaminoglycans. Sylvén

(1941) and Dunphy and Udupa (1955) have demonstrated an increase in acid glycosaminoglycans up to the 5th day after wounding. There is no increase in these substances in post-mortem wounds (Raekallio, 1961).

The changes revealed by the histochemical demonstration of acid glucosaminoglycans require for their manifestation a relatively long time-interval between the infliction of an injury, and death. This is why their demonstration cannot be used in the distinction between ante-mortem and post-mortem injuries. By contrast, the histochemical study of acid glycosaminoglycans may be used in the estimation of the age of injuries in later stages of healing, *inter alia*, in fatal cases of the "battered-baby syndrome".

Nucleic acids have been histochemically studied for the estimation of the age of wounds and other injuries. Regenerating fibroblasts (Bunting and White, 1950) show an augmentation of ribonucleic acid (RNA), whereas the desoxyribonucleic acid (DNA) content of the nuclei does not vary as much (Johnson and McMinn, 1960; Haley and Williamson, 1962; Williamson and Guschlbauer, 1963). The highest concentrations of RNA appear 7—9 days after wounding (Williamson and Guschlbauer, 1961). According to Raekallio (1961), cytoplasmic RNA increases in the peripheral zone (Fig. 1) of wounds inflicted 32 hrs before death. The histochemical stainability further increases up to 64 hrs. This, and in addition the accumulation of Feulgen-positive nuclei of polymorphonuclear leucocytes 4—8 hrs after wounding, is called the positive vital reaction (Raekallio, 1961). In the central zone (Fig. 1), the stainability for both RNA and DNA of the connective tissue cells decreases from 16 hrs, and that of epidermal cells from 64 hrs after the ante-mortem injury (negative vital reaction, Raekallio, 1961). These vital changes are detectable for five days after death and no such zones are seen in the wounds inflicted post mortem (Buris, 1970). The histochemical demonstration of nucleic acids is thus of supplementary value in the estimation of the age of skin wounds.

During the last 13 years, histochemical enzyme studies have been applied for the estimation of the age of injuries (Raekallio, 1960; Raekallio, 1972; cf. several literature references in Raekallio's book, published 1970). The histochemical methods have been described and discussed in detail (Raekallio, 1961, 1964, 1965b, 1970). For the histological and histochemical examination, the wounds (or parts of them) are excised with their surroundings, about half an inch in each direction. One half of the tissue block is fixed overnight in neutral 10% formalin at +4°C (refrigerator temperature) for the demonstration of esterase and phosphatase

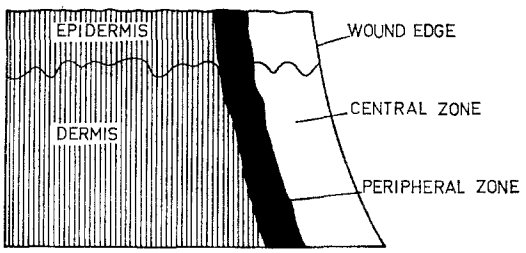

Fig. 1. Schematic diagram showing the zones of an ante-mortem skin wound

activities and for histological examination. The other half is fresh-frozen using isopentane chilled with dry ice. The histochemical methods for adenosine triphosphatases and for aminopeptidases are applied to the frozen specimen.

The central zone (Fig. 1) shows decreasing enzyme activity as an early sign of imminent necrosis. The regressive phenomena in the central zone are called negative vital reactions since no such decrease in enzyme activity is observed in the wounds inflicted after death.

The peripheral zone (Fig. 1) exhibits an increase in enzyme activity, representing, among other things, an adaptive defence mechanism of the local connective tissue cells as an enzymatic response to injury (Raekallio, 1961, 1965a, b, 1970). The increase in enzyme activity in the peripheral wound zone may be called a positive vital reaction since there are no such changes in post-mortem wounds. The activity of esterases and adenosine triphosphatase increases at about 2 hrs, and that of acid phosphatases and alkaline phosphatases at 4 and 8 hrs, respectively. The consecutive appearance of the positive vital reactions, demonstrable by the various methods of enzyme histochemistry, allows the construction of a biological time-table which may be useful in the rough estimation of the age of ante-mortem wounds (Fig. 2). These vital changes are demonstrable for 5 days after death and no increase in enzyme activity is seen in the wounds inflicted post-mortem. The histochemical demonstration of enzymes is thus of great help to a forensic pathologist when faced with the problem of determining the age of wounds.

There are, however, certain exceptions in the biological time-table when it is applied to human autopsy material. Raekallio (1967) collected material from 43 victims of traffic accidents. Most of the injuries were lacerated but there were also some incised wounds caused by glass or other sharp parts of the vehicles. The victims had survived for a known period of from some minutes to several days. The bodies were autopsied 1—2 days after wounding. In this material there were 5 cases with a slightly exceptional appearance of enzymes. For example, in 1 case (an 81 year old man) alkaline phosphatase activity was not increased in the periphery of a laceration, inflicted 10 hrs before death (from chest injury). This delay in the enzymatic response to injury may have been due to the senility of the victim. According to experimental and clinical studies (Carrel, 1910; Her-

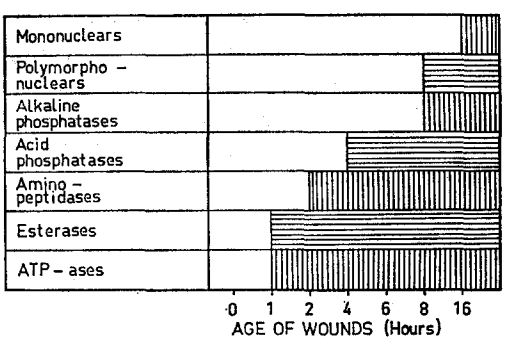

Fig. 2. Schematic diagram showing the histochemical estimation of the age of ante-mortem skin wounds

nández-Richter and Struck, 1970; Struck and Engelhardt, 1971), wounds in young animals and men heal faster than in older individuals. The influence of the age is, however, more important in later stages of healing than in the acute response to injury (Hegemann *et al.*, 1950; Berg, 1972). According to Raekallio (1967), conditions such as far-advanced senility, cachexia, and very severe and multiple injuries may impair the local reaction of the skin. Berg (1972) has shown that bleeding, cold environment, and cranial fractures do not detain the inflammatory reaction as Walcher (1936) had believed.

Dotzauer and Tamaska (1968), Wagener (1969), and Hou-Jensen (1969) have expressed sceptical views on the usefulness of the methods of enzyme histochemistry in the estimation of the age of wounds. However, studies by Arima and Nagamori (1965), Tanaka (1966), Fatteh (1966), Friebel and Woohsmann (1968), Berg (1969), Lo Menzo (1969), Pioch (1969), Oya (1970), Schroll and Sasse (1971), and Lindner (1971) have confirmed the presence of an early enzymatic response to injury in ante-mortem wounds and injuries, caused by blunt force. Further, these authors have demonstrated its absence in post-mortem injuries. Thus, the methods of enzyme histochemistry act as a guide to the estimation of the approximate age of the wounds, especially those inflicted 1—16 hrs before death. Secondly, these methods reveal vital changes around skin wounds, inflicted as little as 1 hr before death. This is about 4—8 times earlier than was possible when only histological methods were used.

The reaction to injury must be immediate. The problem is: are we able to detect the very earliest reactions in a wound and thus demonstrate its ante-mortem origin? Histamine and serotonin are known to participate in the acute inflammatory process (cf. Zweifach *et al.*, 1965; Houch and Forscher, 1968; Bertelli and Houck, 1969; Schultz, 1970; Porter and Knight, 1970; Williams and Fudenberg, 1971; Büchner, 1971; Lepow and Ward, 1972; Ramwell and Pharris, 1972; and others). These and related results suggest that endogenous histamine is responsible for the initiation of vascular changes in inflammation, but that other mechanisms are then required to maintain them. Serotonin or 5-hydroxytryptamine is another vasoactive substance which has been demonstrated in early inflammatory exudates (cf. the books mentioned before in connection with histamine). Many types of injury release serotonin parallel to histamine, especially in the earliest post-traumatic period.

Fazekas and Virágos-Kis observed in 1965 an increase in the free histamine content at the vital hanging mark. Their results were confirmed by Berg (1966) and by Raekallio and Mäkinen (1966). In order to study the histamine content of skin wounds at controlled circumstances, Raekallio and Mäkinen (1966), Berg *et al.* (1968), Merli *et al.* (1969) made experimental investigations on animals. Similarly, experimental investigations on the serotonin content have been performed (Raekallio and Mäkinen, 1969a; Merli *et al.*, 1969). Further, histamine and serotonin contents have been studied in forensic autopsy materials (Berg *et al.*, 1968; Raekallio and Mäkinen, 1970). A sample, about 2 g of the injured skin (for example, a piece of the ligature mark in cases of hanging) is removed. In addition, a control sample, equal in size, is taken from the neighbouring intact skin of the same body. This is necessary since there are great individual and regional differences in the free histamine and serotonin content of skin. Post-mortem

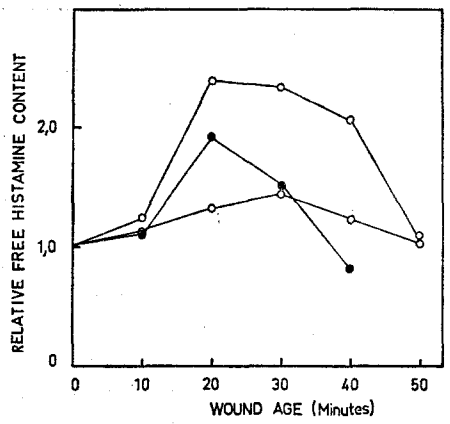

Fig. 3

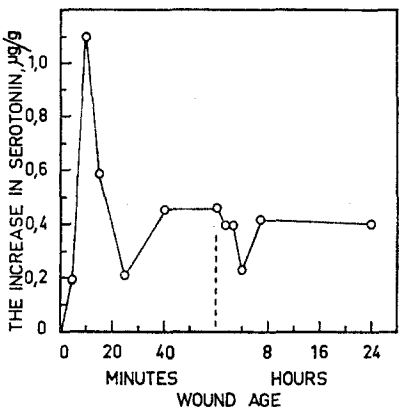

Fig. 4

Fig. 3. Acute histamine reaction in the guinea-pig. The diagram shows relative free histamine contents of the wound surroundings, as compared to the free histamine values (= 1) of a control sample of the same animal

Fig. 4. The diagram shows that the serotonin content depends on the age of ante-mortem wounds. The increase in the serotonin content in a group of animals, wounded at a given time before death, is always compared to the serotonin content (= 0 in the figure) of the post-mortem control wounds of the same mice

wounds, inflicted at the autopsy, serve as additional controls. The extraction of the free histamine from skin is performed with Tyrode's solution as described by Fazekas (1965). The free histamine of the extract is most easily measured spectro-fluorimetrically according to the method of Shore *et al.* (1959). The serotonin is extracted and measured spectrofluorimetrically according to the method of Udenfriend *et al.* (1955). For details of the methods the reader is referred, for example, to our previous paper (Raekallio and Mäkinen, 1970).

The acute increase in the free histamine content of guinea-pig skin is shown in Fig. 3. The maximal increase occurs within 20—30 min after wounding. The increase in the serotonin content is demonstrable still earlier, the maximal increase occurring within 10 min after wounding (Fig. 4). There is another, although slighter, increase in serotonin between 40 min and 2 hrs.

In addition to the amines mentioned before, even the demonstration of catecholamines (noradrenaline) by Eränkö's (1955) fluorescence method has been applied for the estimation of the age of injuries (Penttilä, 1971). In these experiments, injuries inflicted 60 min before death could be distinguished from the post-mortem wounds. The rapid (8—16 hrs) disappearance of the fluorescence after death limits the usefulness of this method in forensic practice.

On the basis of autopsy studies, a distinct increase in serotonin and free histamine content indicates the ante-mortem origin of an injury even in man (Berg, 1969; Raekallio and Mäkine, 1970; Berg and Bonte, 1971; Berg, 1972; Raekallio, 1972). According to Raekallio and Mäkinen (1970), the increase in the serotonin content of the wounded tissue must be at least twofold, and that in histamine 1,5-fold or more (as compared to a control sample of neighbouring intact skin), in order to indicate with certainty that the wound was inflicted before death.

Berg and Bonte (1971) have compared the results of biochemical serotonin and histamine determinations with each other. A great increase in the serotonin content with a simultaneous slight increase in histamine characterizes an agonal injury. In wounds inflicted 5—15 min before death, there is a relatively greater increase in histamine than in serotonin. The case is just the reverse in injuries inflicted 15—60 min or more before death. Anyhow, the biochemical serotonin and histamine determinations are most important in the estimation of the age of the injuries inflicted during the first 60 min before death and in the distinction between ante-mortem and post-mortem wounds. Injuries inflicted more than 1 hr before death may be dated enzyme histochemically and if the wounds were caused more than 4—8 hrs ante-mortem, even histologically, by using the methods for the histochemical demonstration of acid glycosaminoglycans or nucleic acids.

There is no increase in the histamine and serotonin content in post-mortem wounds. The absence of the histamine reaction does not necessarily indicate that a wound was inflicted post-mortem (as Fazekas claimed in 1971), but a positive histamine reaction, *i.e.* a distinct increase in the histamine content, does indicate the vital origin of an injury (Berg, 1969, 1972; Merli *et al.*, 1969; Raekallio and Mäkinen, 1970; Raekallio, 1972; and others). A distinct increase in the serotonin content also indicates the vitality of a wound, and the increase in serotonin seems to be more constant than that in histamine (Berg, Raekallio and their co-workers). The ante-mortem increase in the content of both of these amines is well demonstrable during the first 4—5 days after death, at least in the European conditions of climate (Raekallio and Mäkinen, 1970; Lürssen, 1972). The changes in the serotonin and histamine content may thus be used in forensic practice as the earliest trustworthy vital reactions so far known.

It is obvious that the determination of the age of wounds becomes more reliable when several independent methods are used. Therefore, in addition to biochemical estimations of histamine and serotonin, enzyme biochemistry has been applied for the estimation of the age of injuries. Raekallio and Mäkinen (1967, and later on, cf. numerous literature references in Raekallio's book, published 1970) have fractionated enzymes by several biochemical methods and shown that the increased aminopeptidases in vital wounds differ qualitatively from the corresponding enzymes in intact skin or in post-mortem wounds. Manning and Dipasquale (1967) have demonstrated 2 peaks of alkaline phosphatase activity at 12 hrs and 10 days after injury.

By using isoelectric focusing in polyacrylamide gel, Jarecki *et al.* (1970) have shown differences in esterases, and Raekallio and Mäkinen (1971) in aminopeptidases, if the injuries were inflicted 30 min or longer before death. The same method revealed no changes in the enzyme patterns of acid phosphatase activity. By contrast, the increased alkaline phosphatase activity in ante-mortem wounds differs qualitatively from the corresponding enzymes of the same animal (Mäkinen and Raekallio, 1973). The difference becomes demonstrable in the curves depicting enzymes of ante-mortem wounds inflicted 2 hrs or more before death, when alkaline phosphatases are isoelectrically focused in polyacrylamide gel (Fig. 5). The activity of histidinedecarboxylase corresponds to the histamine content in ante-mortem wounds. The determination of the enzyme is, however, less useful in the timing of wounds than the measuring of the corresponding amine (Berg

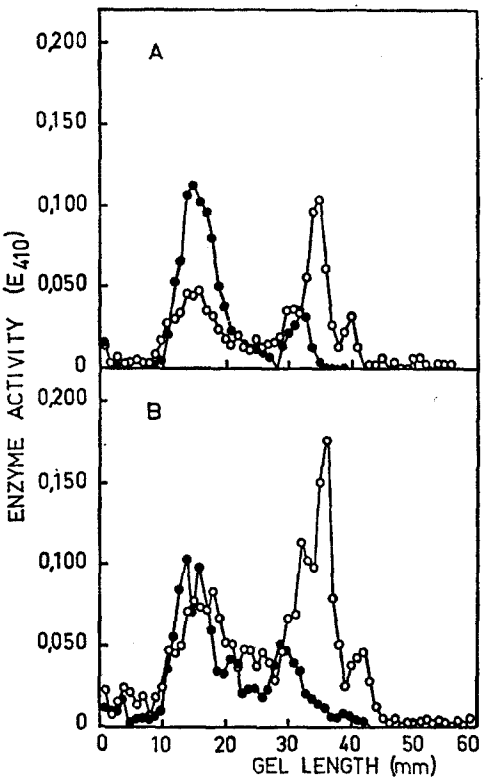

Fig. 5. Isoelectric focusing of alkaline phosphatases in a vital (O) and in a post-mortem control wound (●). A: a 2-hour wound. B: a 4-hour wound

et al., 1971; Berg, 1972). According to the present author, enzyme biochemistry is very important in experimental wound healing research. By contrast, enzyme histochemistry, and, especially, biochemistry of histamine and serotonin, are much more useful in forensic practice.

3. Wounds in Organs other than the Skin

There are some, mostly experimental, studies on the estimation of the age of wounds affecting internal organs. Karkola (1972) studied liver wounds enzyme histochemically and histologically. The severance of circulation of the liver lobe causes an ischaemic coagulation necrosis to the peripheral direction of the wound. Histologically, accumulation of blood and fibrin can be observed but the first changes in the parenchymal cells are not seen before 4 hrs. By most of the 13 enzyme histochemical methods used by Karkola, an increase or decrease in enzyme activity made the injury visible during the first 2 hrs. Phosphorylase and branching enzyme activities disappeared as early as at 30 min after wounding. With other enzymes the loss of the activity took place at 8 to 16 hrs but succinate dehydrogenase, acid phosphatase and non-specific esterase survived much longer.

The mechanically traumatized cells in the edge of wound were destroyed far more rapidly than those in the ischaemic area, this being best observed when using methods for the demonstration of oxidoreductases.

The inflammatory reaction in the periphery of the liver wounds can best be detected by demonstrating the activity of alkaline phosphatase. Migrating connective tissue cells appear as early as at 30 min to 1 hr after wounding. The activation of the fibroblasts in the peripheral wound zone is seen with most of the enzymatic reactions. Aminopeptidase is the best method for demonstrating the proliferative growth of scar tissue in the late stage of healing.

Beneke (1972) studied histologically injuries of adipose tissue, liver and kidney. According to him, the following parameters are of importance: the presence of necrosis or fatty degeneration of the damaged cells, the appearance of polymorphonuclear leucocytes, the appearance of iron pigment, the changes in the size of nuclei and mitoses in the parenchymatous and connective tissue cells in proximity of the injury, the appearance of newly formed acid glycosaminoglycans, collagen, and elastic fibers. Cellular repair is especially dependent on the extent of the injury.

Tooth extraction wounds have been studied by Todo (1968a, b), Plagmann (1970), and Huusko (1973). When studying the initial cellular response with tritiated thymidine, Todo (1968a) showed that the uptake of ^{3}H-thymidine reaches its peak at 4 days after tooth extraction. The maximum increase in labelled cells in the periosteum outside the socket was seen at 1 day after the operation. Enzyme histochemically (Todo 1968b), the granulation tissue in the fundus of the socket shows on the 5th day a moderate activity of alkaline and acid phosphatases and a low activity of oxidoreductases. The first new bone in the socket appears on the 10th day and transformation of fibroblasts into osteoblasts in the granulation tissue is associated with an increase in the activity of alkaline and acid phosphatases and oxidoreductases, demonstrable in the 10th—20th day wounds. Unfortunately, the healing process during the first 2 days was ignored in the experimental studies by Todo.

Plagmann (1970) made both experimental and patient studies on the healing of tooth extraction wounds. According to him, a typical pattern of activity is histochemically and cytochemically demonstrable for the following enzymes during the healing process: succinate dehydrogenase, lactate dehydrogenase, and acid phosphatase. Even in tooth extraction wounds, there is a decrease of enzyme activity in the immediate vicinity of the wound edge (central zone) and an increase in the wound periphery (peripheral zone). Additionally, the earliest phase of healing has been biochemically studied by Huusko (1973). By using several 2-naphthylamides as substrates, he demonstrated a decrease of aminopeptidase activity immediately after tooth extraction. At 1 hr the activity was decreased by 20—35% as compared to the control samples, depending on the substrate used. The activity reached the control level about 1 day or later after the operation.

4. Bruises

Bruises, contusions, or ecchymoses are injuries which are characterized by the effusion of blood into the tissue spaces. They are usually caused by blunt weapons

but they can be produced in other ways, e.g., by the pressure of the fingers in throttling.

Ante-mortem bruises must be differentiated from post-mortem dissection artifacts. Prinsloo and Gordon (1951) investigated this problem by performing the autopsy in 18 cases in the routine way, *i.e.* by removing the organs of the neck *en masse* by downward traction from the floor of the mouth and by examining them thereafter individually. They found extravasations of blood in the deep connective tissues in 16 of the 18 cases, although there was no evidence or suggestion of any ante-mortem cervical injury in these cases. Even by microscopical examination it was not possible to distinguish between dissection artifacts and ante-mortem bruises caused shortly before death. Therefore, these authors recommended the following dissection of the neck in medicolegal autopsies: the dissection should be immediately deep to the skin, through the platysma, the skin being held with a toothed forceps and the dissection carried out with a sharp, long-handled scalpel. If the skin is flayed from the body in this way and care is taken not to divide any of the great veins in the neck, a clear exposure of the subcutaneous tissues and the ventral surfaces of the anterior muscles of the neck is obtained. The subcutaneous dissection should be carried up to the lower border of the lower jaw, well laterally on the side of the neck and downwards to meet the reflection of the tissues at the base of the neck and the clavicle. After inspection the investing layer of deep cervical fascia is incised and reflected from the ventral surfaces of the anterior cervical muscles. After all the cervical tissues have been examined *in situ*, the pharynx, larynx, oesophagus and the surrounding tissues are brought down to the level of the clavicles and opened for further study.

On the grounds that blood usually loses its coagulability after death, a clotted haemorrhage was earlier considered to be a sign of vital reaction. Walcher (1936) assumed and Mueller (1964) showed that clotting of tissue haemorrhage may occur as late as about 6 hrs post-mortem. Likewise, the presence of fibrin in a bruise was earlier held to indicate an intravital injury. However, Walcher (1936), Piédelièvre (1938), and Mueller (1964) demonstrated fibrin in post-mortem haemorrhages. By immunohistochemical studies, Laiho (1967) showed that post-mortem fibrin cannot with certainty be distinguished from vital fibrin. Further, the absence of fibrin from a bruise does not exclude the possibility that the injury may be of ante-mortem origin.

The first infiltration of leucocytes is histologically demonstrable approximately 4 (Berg and Ebel, 1969) to 6 (Marchand, 1901) hrs after bruising. Intact erythrocytes may be seen at the site of extravasation for several weeks. This suggests that extravasated blood does not constitute a strong irritant to subcutaneous tissues (Robertson and Mansfield, 1957; Robertson and Hodge, 1972). According to Berg and Ebel (1969), hemosiderin is found regularly 90 hrs, hematoidin occasionally only 9 days after bruising. The development of granulation tissue in bruises is very rare. In patients with severely decreased vital functions frequently (but irregularly and hardly predictably) delayed tissue reactions occur (Berg and Ebel, 1969).

Acid glycosaminoglycans have been studied in order to distinguish between ante-mortem and post-mortem bruises. Robertson and Mansfield (1957) mentioned that there are changes in the metachromasia of the connective tissue after bruising.

Nevelös and Gee (1970) used several histochemical methods (Alcian blue-PAS, dialysed iron-PAS, both of them controlled by enzyme digestions) for the demonstration of acid glycosaminoglycans in bruises. According to them, there is an initial decrease of these substances, becoming apparent almost instantaneously after bruising. In haemorrhages, when at least 1 hr had elapsed between injury and death, there were no acid glycosaminoglycans demonstrable. Bruises which were several days old showed, with the increase in cellular elements of the connective tissue, the reappearance of acid glycosaminoglycans.

Recently, enzyme histochemical methods have been applied for the estimation of the age of bruises. According to the results of the animal experiments by Pioch (1969), there is an increase in esterase activity as early as about 30—45 min after the effect of a blunt force. In human material, the earliest reaction (Berg and Ebel, 1969) was an increase in adenosine triphosphatase activity about 2½ hrs after bruising. Aminopeptidase and esterase increased at 4½ and 7 hrs, respectively. Biochemically, lysosomal enzymes increased by the 2nd day after bruising and reached a maximum between the 3rd and 5th day (Brown and Hamdy, 1965).

5. Burns

Burns are produced by the application of flame, or hot liquids or gases or solid heated substances to the surface of the body.

It may be possible on naked-eye examination to state that a burn is ante-mortem in origin if it shows evidence of an advanced inflammatory reaction. For example, if a vesicle caused by a burn contains pus it may be assumed that the burn was produced before death. However, in most cases a histological examination is necessary. A distinct leucocytic infiltration is trustworthy evidence that the burn was produced ante-mortem (Unna, 1894; Ullmann, 1922, 1932; Moritz, 1947; Mueller, 1953; Malik, 1971). Most authors report that there are no unequivocal histological changes earlier than 6—8 hrs after the infliction of a burn.

Vesicles caused by burning are usually produced ante-mortem. This is even more evident if there are fibrin and leucocytes demonstrable in the vesicle (Mueller, 1953; Prokop, 1960). However, vesicles can appear shortly after death (Gonzales *et al.*, 1954).

By using histochemical methods for the demonstration of proteins, Pioch (1966a, b, 1971) was able to show a decrease of these reactions in the burned area and an increase in reactivity of the surrounding peripheral zone. These changes appeared in animal experiments as early as 45 min after burning. Enzyme histochemically, there was a decrease in esterase activity in the central zone (i.e. in the burned area), and an increase in the activity of the peripheral zone 30 min after burning. Similar results were reported by Malik (1971), as far as esterase is concerned. He demonstrated a decrease of the following enzymes in the central zone at the times given: alkaline phosphatase at 1 hr, acid phosphatase at 30 min and aminopeptidase at 1 hr. In the peripheral zone there was an increase in activity, becoming apparent at 4, 2, and 1 hrs with the same enzymes respectively. The activity of esterase increased in the peripheral zone 30 min after burning. To the best of my knowledge, no reports have been published on the application

of enzyme histochemical methods for the distinction between ante-mortem and post-mortem burns in any extensive human material.

6. Electric Injuries

Electricity is a form of energy which under certain conditions can injure the body and cause death. There are two forms of death from electrocution: 1. deaths caused through contact with electrical conductors and 2. those caused by lightning-stroke. The pathological changes in both of them are essentially similar.

The current mark is a specific lesion observed at the sites of entry and exit of the current. The skin offers most of the resistance to the passage of an electric current through the body and that part of the electrical energy is transformed into the so-called Joule's heat in the skin. Current marks cannot be differentiated from ordinary burns on histological examination (Schäffner, 1964; Somogyi *et al.*, 1964; Pioch, 1966b; Nevelös and Gee, 1970). The elongation of nuclei is not a distinctive feature of the current mark since similar changes may be seen in a frostbite, too.

By using polariscopy and fluorescence microscopy, Somogyi *et al.* (1964) demonstrated alterations of collagen structure in current marks. Histochemically, the central zone of the current mark showed no acid glycosaminoglycans. With a gradual transition towards undamaged areas these substances reappeared (Somogyi *et al.*, 1964; Nevelös, and Gee 1970). There are metallic particles in most current marks due to the contact with conductors and to the Joule's heat. Schäffner (1964) and Buris *et al.* (1967) have demonstrated histochemically copper, iron, zinc, and aluminium particles in current marks and thus distinguished them from burns. Enzyme histochemically there is a loss of lipoamide dehydrogenase (=NAD-diaphorase) activity in the central zone of the current mark. An increase in esterase activity in the peripheral zone is histochemically demonstrable, if the victim survives 30—45 min or more after the electrocution.

7. Fractures

Fractures of the bones and cartilages are rather common. They may be caused by direct violence, by indirect violence, or by muscular action.

According to Watson-Jones (1952) there are three histologically demonstrable stages in the healing of fractures. 1. The initial stage is characterized by inflammation and formation of the granulation tissue during the first few days. At 1 to 8 hrs the fracture defect is filled and surrounded by extravasated blood and inflammatory exudate. Numerous polymorphonuclear leucocytes appear in the exudate at 4 hrs. 2. Within a week small areas of new bone are laid down around blood vessels in an irregular interwoven manner. This "woven" bone is formed by periosteal, endosteal, and marrow reticulum cells. The growth of "woven" bone represents a stage of temporary repair corresponding to union by primary callus formation. 3. The trabeculae of "woven" bone gradually undergo resorption and are replaced by lamellae of bone which are laid down in parallel plates corresponding to the lines of stress in the bone. This period of consodilation into mature lamellar bone may take as long as 1 year.

Fracture repair has been studied enzyme histochemically in order to collect data for the estimation of the age of fractures. According to Raekallio and his co-workers (Raekallio and Mäkinen, 1968; Raekallio and Mäkinen, 1969b; Raekallio and Kovács, 1969; Raekallio *et al.*, 1970) the activity of phosphatases and several oxidoreductases begins to decrease in a 200—500 μ deep central zone in the immediate vicinity of the fracture line. In the same area necrosis can be histologically demonstrated 2 days after the injury. In a peripheral zone, farther away from the fracture line, the activity of the same enzymes begins to increase from the 10th hour onwards in the osteoblasts and osteogenic cells of the inner periosteal cells. Kovács and Harsányi (1971) demonstrated an increase in aminopeptidase activity in these cells 6—8 hrs after fracturing. Lactate and lipoamide dehydrogenases show the strongest, succinate and glucose-6-phosphate dehydrogenases the slightest intensification of activity. The proliferation of the periosteal cells after 16 hrs contributes to the increase in enzyme activity, remaining strong during the first 3 days after the injury. Similar phenomena can be observed in the endosteum cytochrome oxidase showing the most intense reaction there. The enzyme histochemical studies reviewed were performed on animals. To the best of my knowledge, there have been no reports published on the application of enzyme histochemical methods for the estimation of the age of fractures in any extensive human material.

To conclude a more accurate estimation of the age of different injuries and the distinction between ante-mortem and post-mortem wounds is now possible. This distinction is possible, by using biochemical methods, after a survival time of as little as a few seconds or minutes. By using scanning electron microscopy, an aggregation of fibrin, thrombocytes and erythrocytes has been demonstrated in arterial injuries as early as 3—5 sec after injury (Böhm and Tschomakov, 1972, 1973). The methods of enzyme histochemistry act as a guide to the estimation of the approximate age of the wounds and other injuries, especially those inflicted 1—16 hrs before death.

References

Allgöwer, M.: The cellular basis of wound repair. Springfield (Ill.): Charles C. Thomas 1956

Arima, T., Nagamori, H.: A histochemical study on skin wound. Kagaku Keisatsu Kenkyosho Hohoku **18**, 193—197 (1965)

Becker, K., Lindner, J.: Qualitative und quantitative immunologische Untersuchungen an der atherosklerotischen Gefäßwand. In: Emmerich, R., Perlick, E. (Hrsg.), Gefäßwand und Blutplasma. Jena 1965

Beneke, G.: Altersbestimmung von Verletzungen innerer Organe. Z. Rechtsmedizin **71**, 1—16 (1972)

Berg, S.: Beitrag zur Frage der humoralen Vitalreaktion am Ort der Gewalteinwirkung. 45. Tag. dtsch. Ges. gerichtl. soz. Med., Freiburg im Breisgau 1966

Berg, S.: Der Beweiswert der Todeszeitbestimmung (Überlebenszeit). Beitr. gerichtl. Med. **25**, 61—65 (1969)

Berg, S.: Die Altersbestimmung von Hautverletzungen. Z. Rechtsmedizin **70**, 121—135 (1972)

Berg, S., Bonte, W.: Praktische Erfahrungen mit der biochemischen Wundalterbestimmung. Beitr. gerichtl. Med. **28**, 108—114 (1971)

Berg, S., Ditt, J., Friedrich, R., Bonte, W.: Möglichkeiten der biochemischen Wundalterbestimmung. Dtsch. Z. ges. gerichtl. Med. **63**, 183—198 (1968)

Berg, S., Ditt, J., Kunze, P., Garbe, G.: Beziehungen zwischen Histamingehalt und Aktivität der Histidindecarboxylase im Bereich von Hautverletzungen. Z. Rechtsmedizin **69**, 26—40 (1971)

Berg, S., Ebel, R.: Altersbestimmung subcutaner Blutungen. Münch. med. Wschr. **111**, 1185—1190 (1969)

Bertelli, A., Houck, J. C. (Ed.): Inflammation biochemistry and drug interaction. Amsterdam: Excerpta med. Foundation 1969

Blum, H.: Zur Frage der Unterschiedlichkeit vitaler und postmortaler Gewebsveränderungen am Beispiel der Strangfurchen beim Erhängungstod. Virchows Arch. path. Anat. **299**, 754—766 (1938)

Böhm, E., Tschomakov, M.: Ein Sekundenphänomen der vitalen Reaktion, demonstriert an Stichverletzungen von Arterien. Z. Rechtsmedizin **71**, 234—242 (1972)

Böhm, E., Tschomakov, M.: Frühe Merkmale einer vitalen Reaktion — Untersuchungen an Schnittverletzungen der Rattenhaut. Z. Rechtsmedizin **72**, 111—118 (1973)

Brack, W. J., Lindner, J., Jasper, A.: Reaktionsformen von Kollagenfasern (II). Quellung, Kontraktion, Relaxation und Schrumpfung. Acta histochem. (Jena) **13**, 195—219 (1962)

Brown, W. E., Hamdy, M. K.: Lysosomal enzymes in bruised tissue. Proc. Soc. exp. Biol. (N. Y.) **119**, 778—783 (1965)

Büchner, T.: Entzündungszellen im Blut und im Gewebe. Stuttgart: Gustav Fischer Verlag 1971

Bunting, H., White, R. F.: Histochemical studies of skin wounds in normal and scorbutic guinea pigs. Arch. Path. **49**, 590—600 (1950)

Buris, L.: Histochemical examinations at vital and postmortem injuries. Acta histochem. (Jena) **38**, 65—69 (1970)

Carrell, A.: The treatment of wounds. J. Amer. med. Ass. **55**, 2148—2150 (1910)

Dotzauer, G., Tamaska, L.: Hautveränderungen an Leichen. In: Handbuch der Haut- und Geschlechtskrankheiten. Erg.-Werk Bd. I, Teil 1, S. 708—786. Berlin-Heidelberg-New York: Springer 1968

Dunphy, J. E., Udupa, K. N.: Chemical and histochemical sequences in the normal healing of wounds. New Engl. J. Med. **253**, 847—851 (1955)

Eränkö, O.: Distribution of fluorescing islets, adrenaline, and noradrenaline in the adrenal medulla of the hamster. Acta endocr. (Kbh.) **18**, 174—179 (1955)

Fatteh, A.: Histochemical distinction between antemortem and postmortem skin wounds. J. forens. Sci. **11**, 17—27 (1966)

Fazekas, I. G.: Der Gehalt verschiedener Verletzungen an freiem Histamin als Vitalreaktion. Z. Rechtsmedizin **68**, 86—94 (1971)

Fazekas, I. G., Virágos-Kis.: Der Gehalt der Erhängungsfurche an freiem Histamin als vitale Reaktion. Dtsch. Z. ges. gerichtl. Med. **56**, 250—260 (1965)

Friebel, L., Woohsmann: Die Altersbestimmung von Kanüleeinstichen mittels enzymhistochemischen Methoden. Dtsch. Z. ges. gerichtl. Med. **62**, 252—260 (1968)

Giacometti, L.: The healing of skin wounds in primates. J. invest. Derm. **48**, 133—137 (1967)

Gonzales, T. A., Vance, M., Helpern, M., Umberger, C. J.: Legal medicine, pathology, and toxicology. New York, N. Y.: Appleton-Century-Crofts, Inc. 1954

Gottschalk, A. (Ed.): Glycoproteins, their composition, structure, and function. Amsterdam: Elsevier 1966

Guschlbauer, W., Williamson, M. B.: Appearance of proteins in regenerating wound tissue. Canad. J. Biochem. **41**, 820—823 (1963)

Haley, H. B., Williamson, M. B.: Application of present knowledge of wound healing to clinical surgery. Surg. Clin. N. Amer. **42**, 15—34 (1962)

Hegemann, G., Nickell, J., Tischler, F.: Beobachtungen über den Wechsel der Doppelbrechfähigkeit und der Trypsinresistenz Kollagener Fasern beim Altern von Narbengewebe. Klin. Wschr. **28**, 362—364 (1950)

Hernández-Richter, H. J., Struck, H.: Die Wundheilung. Theoretische und praktische Grundlagen. Stuttgart: Thieme 1970

Hirvonen, J.: Histochemical studies on vital reaction and traumatic fat necrosis in the interscapular adipose tissue of adult guinea pigs. Ann. Acad. Sci. fenn. A 5, **136**, 1—96 (1968)

Houck, J. C., Forscher, B. K. (Ed.): Chemical biology of inflammation. Oxford: Pergamon Press 1968

Hou-Jensen, K.: Nogle enzym-betingede vitalreaktioner og deres retsmedicinske betydning. Aarhus: Universitetsforlaget 1969

Huusko, P.: Personal communication to the author. (1973)
Jarecki, R., Arndt, U., Schultz, C., Klein, H.: Zur Unterscheidung vitaler und postmortaler Wunden durch Bestimmung des Esterasemusters der Haut. Z. Rechtsmedizin **67**, 313—318 (1970)
Johnson, F. R., McMinn, R. M. H.: The cytology of wound healing of body surfaces in mammals. Physiol. Rev. **35**, 364—412 (1960)
Karkola, K.: Enzyme histochemical studies on wound healing in rat liver. Acta path. microbiol. scand. Section A., **Suppl. 228**, 1—56 (1972)
Kovács, M., Harsányi, L.: Über histochemisch nachweisbare vitale Reaktionen am Knochengewebe. Acta histochem. (Jena), **Suppl. 9**, 419—426 (1971)
Laiho, K.: Immunohistochemical studies on fibrin in vital and postmortem subcutaneous haemorrhages. Ann. Acad. Sci. fenn. A. 5 **128**, 1—85 (1967)
Latessa, A. J., Ross, M. H.: Electron microscope studies of nonpenetrating corneal wounds in the early stages of healing. Exp. Eye Res. **3**, 298—303 (1969)
Lepow, I. H., Ward, P. A. (Ed.): Inflammation, mechanism and control. New York, N. Y.: Academic Press 1972
Lindner, J.: Histochemische und biochemische Untersuchungen der traumatisch gestörten Beziehung zwischen Grundsubstanz und Kollagenfasern. Verh. dtsch. path. Ges. **43**, 61—69 (1959)
Lindner, J.: Histochemische und fermenthistochemische Untersuchungen am Granulationsgewebe. Symposium der Italienischen Gesellschaft für Pathologie, Mailand, Oktober 1959. Milano: Idos Verlag 1960
Lindner, J.: Die Morphologie der Wundheilung. Langenbecks Arch. klin. Chir. **301**, 39—70 (1962)
Lindner, J.: Der rheumatische Bindegewebsstoffwechsel und die pathologische Struktur der Bindegewebe bei rheumatischer Arthritis. Verh. dtsch. Ges. inn. Med. **74**, 1315—1349 (1968)
Lindner, J.: Vitale Reaktionen. Acta histochem. (Jena) Supp. **9**, 435—467 (1971)
Lindner, J., Freytag, G.: Reaktionsformen von Kollagenfasern (I). Acta histochem. (Jena) **9**, 231—246 (1960)
Lindner, J., Hölzer, K. H.: Quantitative immunologische Eiweißbestimmung in nativen Gewebsschnitten. Verh. dtsch. path. Ges. **46**, 188—194 (1962)
Lo-Menzo, G.: Lesioni vitali e post-mortali. Catania: Edigraf 1969
Lürssen, B.: Das postmortale Verhalten der Gehalte an freiem Histamin und 5-Hydroxytryptamin in der Leichenhaut. Med. Diss., Göttingen 1972
Mäkinen, P.-L., Raekallio, J.: Biochemical distinction between ante-mortem and post-mortem skin wounds by isoelectric focusing in polyacrylamide gel. II. Experimental investigation on phosphatases. Zacchia **48**. In press 1973
Malik, M. O. A.: Enzyme changes in the early phase of healing skin burns in guinea-pigs. Brit. J. exp. Path. **52**, 345—352 (1971)
Manning, J. P., Dipasquale, G.: The effect of vitamine A and hydrocortisone on the normal alkaline phosphatase response to skin wounding in rats. J. invest. Derm. **49**, 225—229 (1967)
Marchand, F.: Der Prozeß der Wundheilung. Stuttgart: Enke 1901
Merli, L. D., Gualdi, G., Ronchi, G. U.: La diagnosi chronologica delle ferite cutanee. Zacchia **44**, 380—391 (1969)
Moritz, A. R.: Studies on thermal injury. Pathology and pathogenesis of cutaneous burns. Experimental study. Amer. J. Path. **23**, 915—941 (1947)
Mueller, B.: Gerichtliche Medizin. Berlin-Göttingen-Heidelberg: Springer 1953
Mueller, B.: Zur Frage der Unterscheidung von vitalen bzw. agonalen und postmortalen Blutungen. Acta Med. leg. soc. (Liège) **17**, 43—46 (1964)
Nevelös, A. B., Gee, D. J.: Vital reaction in the epithelial connective tissue ground substance. Med. Sci. Law **10**, 175—177 (1970)
Oya, M.: Histochemical demonstration of some hydrolytic enzymes in skin wounds and its application to forensic medicine. Jap. J. leg. Med. **24**, 55—67 (1970)
Ökrös, S.: Gerichtlich-medizinische Bedeutung des elastischen Fasersystems der Haut. Dtsch. Z. ges. gerichtl. Med. **29**, 485—495 (1938)

Orsós, F.: Die vitalen Reaktionen und ihre gerichtsmedizinische Bedeutung. Beitr. path. Anat. **95**, 163—237 (1935)

Pearse, A. G. E.: Histochemistry, theoretical and applied, third edition, Vol. I. London: J. & A. Churchill Ltd. 1968

Penttilä, A.: Catecholaminfluoreszenz als Zeichen der vitalen Reaktion an Schnittflecken des Samenstranges der Ratte. Beitr. gerichtl. Med. **29**, 104—112 (1972)

Piédelièvre, R.: Les ecchymoses en médecine légale: remarques sur le coagulation du sang. Proc. 1e congrès internat. Méd. lég. Méd. soc., p. 584 (1938)

Pioch, W.: Die histochemische Untersuchung thermischer Hautschäden und ihre Bedeutung für die forensische Praxis. Lübeck: Max Schmidt-Römhild 1966(a)

Pioch, W.: Histologisch-histochemische Untersuchungen zur Identifizierung von Strommarken. Dtsch. Z. ges. gerichtl. Med. **57**, 165—169, 1966(b)

Pioch, W.: Epidermale Esterase-Aktivität als Beweis der vitalen Einwirkung von stumpfer Gewalt. Beitr. gerichtl. Med. **25**, 136—145 (1969)

Pioch, W.: Die Bewertung histochemischer Befunde nach thermischen und stumpfen Hautverletzungen unter forensischem Aspect. Acta histochem. (Jena) Suppl. **9**, 405—410 (1971)

Plagmann, H.-C.: Untersuchungen über die Epithelisierungsvorgänge bei Extraktionswunden am Menschen und im Tierexperiment. Med. Diss. Kiel 1970

Porter, R., Knight, J. (Ed.): Energy metabolism in trauma. London: J. & A. Churchill 1970

Prinsloo, I., Gordon, I.: Post-mortem dissection artefacts of the neck and their differentiation from ante-mortem bruises. S. Afr. med. J. **25**, 358—369 (1951)

Prokop, O.: Lehrbuch der gerichtlichen Medizin. Berlin: VEB Verlag Volk und Gesundheit 1960

Raekallio, J.: Enzymes histochemically demonstrable in the earliest phase of wound healing. Nature (Lond.) **188**, 234—235 (1960)

Raekallio, J.: Histochemical studies on vital and post-mortem skin wounds: Experimental investigation on medicolegally significant vital reactions in an early phase of wound healing. Ann. Med. exp. Fenn. Suppl. **6** 39, 1—105 (1961)

Raekallio, J.: Histochemical distinction between antemortem and postmortem skin wounds. J. forens. Sci. **9**, 107—118 (1964)

Raekallio, J.: Histochemical demonstration of enzymatic response to injury in experimental skin wounds. Exp. molec. Path. **4**, 303—310, 1965(a)

Raekallio, J.: Die Altersbestimmung mechanisch bedingter Hautwunden mit enzymhistochemischen Methoden. Lübeck: Max Schmidt-Römhild 1965(b)

Raekallio, J.: Application of histochemical methods to the study of traffic accidents. Acta Med. leg. soc. (Liège) **20**, 171—178 (1967)

Raekallio, J.: Enzyme histochemistry of wound healing. Stuttgart: Fischer 1970

Raekallio, J.: Determination of the age of wounds by histochemical and biochemical methods. Forensic Sci. **1**, 3—16 (1972)

Raekallio, J., Kovács, M.: Adenosine triphosphatase activity in the initial phase of fracture healing. Acta morph. Acad. Sci. hung. **17**, 41—46 (1969)

Raekallio, J., Kovács, M., Mäkinen, P.-L.: The appearance of oxidoreductases in healing fractures. Acta path. microbiol. scand. Section A. **78**, 658—664 (1970)

Raekallio, J., Mäkinen, P.-L.: Histamine content as vital reaction I. Experimental investigation. Zacchia **41**, 273—284 (1966)

Raekallio, J., Mäkinen, P.-L.: Aminopeptidases in serum and skin of rats during early wound healing. Ann. Med. exp. Fenn. **45**, 224—229 (1967)

Raekallio, J., Mäkinen, P.-L.: The appearance of esterases in healing fractures. Acta path. microbiol. scand. **73**, 304 (1968)

Raekallio, J., Mäkinen, P.-L.: Serotonin content as vital reaction I. Experimental investigation. Zacchia **44**, 587—594 (1969a)

Raekallio, J., Mäkinen, P.-L.: Alkaline and acid phosphatase activity in the initial phase of fracture healing. Acta path. microbiol. scand. **75**, 415—422 (1969b)

Raekallio, J., Mäkinen, P.-L.: Serotonin and histamine contents as vital reactions II. Autopsy studies. Zacchia **45**, 403—414 (1970)

Raekallio, J., Mäkinen, P.-L.: Biochemical distinction between antemortem and post-mortem skin wounds by isoelectric focusing in polyacrylamide gel. I. Experimental investigation on arylaminopeptidases. Zacchia **46**, 281—293 (1971)

Ramwell, P. W., Pharriss, B. B. (Ed.): Prostaglandins in cellular biology. New York, N. Y.: Plenum Press 1972

Robertson, I., Hodge, P. R.: Histopathology of healing abrasions. Forensic Sci. **1**, 17—25 (1972)

Robertson, I., Mansfield, R. A.: Ante-mortem and post-mortem bruises. J. forens. Med. **4**, 2—10 (1957)

Ross, R., Benditt, E. P.: Wound healing and collagen formation. I. Sequential changes in components of guinea pig wounds observed in the electron microscope. J. biophys. biochem. Cytol. **11**, 677—700 (1961)

Russell, W. O.: Estimation of the age of wounds and disease processes. Ann. west. Med. Surg. **5**, 950—953 (1951)

Schäffner, M.: Untersuchungen über Histologie und Metallisation nach elektrischen Einwirkungen auf die Haut. Dtsch. Z. ges. gerichtl. Med. **56**, 269—280 (1965)

Schallock, G., Lindner, J.: Beitrag zur Frage der Entmischungszustände in den Grundsubstanzen des Bindegewebes. Medizinische **1**, 12—20 (1957)

Schroll, R., Sasse, D.: Histochemische Untersuchungen zum Energie- und Pentosephosphatstoffwechsel bei der epithelialen Wundheilung. Histochemie **26**, 349—361 (1971)

Schultz, J.: Biochemistry of the phagocytic process. Amsterdam: North Holland 1970

Shore, P. A., Burkhalter, A., Cohn, V. H., Jr.: A method for the fluorometric assay of histamine in tissues. J. Pharmacol. exp. Ther. **127**, 182—196 (1959)

Simpson, K.: Taylor's principles and practice of medical jurisprudence, 12th edition, London: J. & A. Churchill Ltd. 1965

Smith, S.: Forensic Medicine, a text-book for students and practitioners. London: J. & A. Churchill Ltd. 1945

Somogyi, E., Rózsa, G., Sótonyi, P.: Histochemische und fluoreszenzoptische Untersuchungen der Strommarke. Acta morph. Acad. Sci. hung. **13**, 311—315 (1964)

Stössel, H.-G.: Die Frage der vitalen und postmortalen Reaktionen des elastischen Fasersystems in der Haut, geprüft an Hand von Stichverletzungen. Med. Diss., Heidelberg 1950

Struck, H., Engelhardt, G. H.: Altersabhängigkeit der Wundheilung. Z. Geront. **4**, 356—368 (1971)

v. Schweinitz, H. A.: Mechanische und chemische Schädigungen der elastischen Fasern. Verh. dtsch. path. Ges. **43**, 69—75 (1959)

Sylvén, B.: Über das Vorkommen von hochmolekularen Esterschwefelsäuren im Granulationsgewebe und bei der Epithelregeneration. Acta chir. scand. **86**, Suppl. 66 (1941)

Tanaka, M.: The distinction between antemortem skin wounds by esterase activity. Jap. J. leg. Med. **20**, 231—239 (1966)

Todo, H.: Healing mechanism of tooth extraction wounds in rats. I. Initial cellular response to tooth extraction in rats studied with ^{3}H-thymidine. Arch. oral Biol. **13**, 1421—1427 (1968a)

Todo, H.: Healing mechanism of tooth extraction wounds in rats. II. Histochemical observations on hydrolytic and oxidative enzymes in tooth extraction wounds in the rat. Arch. oral Biol. **13**, 1429—1443 (1968b)

Udenfriend, S., Weissbach, H., Clark, C. T.: The estimation of 5-hydroxytryptamine (serotonin) in biological tissues. J. biol. Chem. **215**, 337—344 (1955)

Ullmann, K.: Über die durch Hitzeeinwirkungen hervorgerufenen gewerblichen Hautschädigungen. In: Oppenheim, Rille, Ullmann: Die Schädigungen der Haut durch Beruf und gewerbliche Arbeit. Bd. 1, 97—159. Leipzig 1922

Ullmann, K.: Thermische Schädigungen. In: Jadassohn, J.: Hdb. d. Haut- und Geschlechtskrankheiten. Bd. IV, Teil 1, 171—437. Berlin 1932

Unna, P. G.: Die Histopathologie der Hautkrankheiten, pp. 81—97. Berlin 1894

Wagener, T. D.: Die enzymhistochemische Altersbestimmung mechanischer Hautwunden in der forensischen Praxis. Med. Diss., Göttingen 1969

Walcher, K.: Über vitale Reaktionen. Dtsch. Z. ges. gerichtl. Med. **15**, 16—57 (1930)

Walcher, K.: Die vitale Reaktion bei der Beurteilung des gewaltsamen Todes. Dtsch. Z. ges. gerichtl. Med. **26**, 193—211 (1936)

Watson-Jones, R.: Fractures and Joint Injuries. Fourth Edition, Edinburgh: E. & S. Livingstone 1952

Williams, R. C., Fudenberg, H. H. (Ed.): Phagocytic mechanisms in health and disease. Stuttgart: Georg Thieme Publishers 1972

Williamson, M. B., Guschlbauer, W.: Changes in the concentration of ribonucleic acid during wound tissue regeneration. Nature (Lond.) **192**, 454—455 (1961)

Zweifach, B. W., Grant, L., McCluskey, R. T.: The inflammatory process. New York, N. Y.: Academic Press 1965

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