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The Effect of Ageing on Enzyme Histochemical Vital Reactions

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Summary. In order to study the effect of ageing on enzyme histochemical vital reactions, an experimental study was made on young (aged 2 months) and old (2 years) rats. There were no age-dependent differences between the negative vital reactions (decrease of enzyme activity in the central zone near the wound edge). There were quantitative differences between the enzymatic responses demonstrable in the periphery of wounds of young and old animals. However, the positive vital reactions, as an initial increase in enzyme activity in the peripheral wound zone, appeared in the same chronological order in both age groups. The biological time-table, based on the consecutive appearances of certain enzymes histochemically demonstrable in the wound periphery, is thus useful as one of the modern methods for the timing of wounds, even in older individuals.

Zusammenfassung. Um den Einfluß des Alterns auf die fermenthistochemisch nachweisbaren Vitalreaktionen zu untersuchen, wurde mit alten (zweijährigen) und jungen (zwei Monate alten) Ratten experimentiert. Es gab keine altersabhängigen Unterschiede zwischen den sog. negativen Vitalreaktionen (Verminderung der Enzymaktivität der inneren Wundzone) bei den beiden Altersgruppen. Quantitative Differenzen zwischen den enzymatischen Reizbeantwortungen (Zunahme der Enzymaktivität in der äußeren Wundzone) bei jungen und alten Tieren wurden nachgewiesen. Die positiven Vitalreaktionen, also die allererste Zunahme der Aktivität von den verschiedenen Enzymen in der äußeren Wundzone, erschienen in derselben chronologischen Reihenfolge bei den beiden Altersgruppen. Der biologische Zeitplan, der sich auf die nacheinanderfolgende, histochemisch erfaßbare Erscheinung von gewissen Enzymen gründet, ist also brauchbar als eine moderne Methode zur Altersbestimmung der Wunden auch im höheren Lebensalter des Untersuchungsobjekts.

Key words: Vital reactions, effect of ageing on — Wound healing, effect of ageing on — Wound enzymes — Traumatology, estimation of age of injuries.

Introduction

According to experimental and clinical studies, (Doberauer, 1962; Struck and Engelhardt, 1971) wounds in young animals and men heal faster than in older individuals. Further, conditions such as far-advanced senility, cachexia, and very severe and multiple injuries may impair the local reaction of the skin (Raekallio, 1967).

During the last 14 years, enzyme histochemical studies have been applied for the estimation of the age of injuries (Raekallio, 1960, 1961, 1965, 1970, 1973; Arima and Nagamori, 1965; Tanaka, 1966; Fatteh, 1966; Pioch, 1966; Friebel and Woohsmann, 1968; Berg, 1969, 1972; Lo Menzo, 1969; Oya, 1970; Lindner, 1971) and many others (cf. several literature references in Raekallio's book, pub-

lished 1970). Raekallio (1961, 1965) has presented a biological time-table based on the consecutive appearances of enzymes in the wound periphery. The time-table is useful in the rough timing of vital wounds (Knight, 1971; Berg, 1972; Schmidt, 1973). However, there have been no systematic studies on the possible effect of ageing on enzyme histochemical vital reactions. In order to study the effect of ageing on the enzymatic response to injury, an experimental study was made on young and old rats.

Methods

Square skin wounds (1 cm^2) were excised in an unshaved dorsal area. Groups of young (aged 2 months) and old (2 years) male rats were killed 1, 2, 3, 4, 8, 12, 24, and 48 hrs after wounding. For each time interval, 5 young and 5 old animals were used. The total material thus consisted of 90 rats of the same laboratory stock. When wounded, each animal was kept in a separate cage lest additional injuries should be caused by other rats of the group.

A flap of skin containing the wound with its surroundings (about half an inch in each direction) was removed immediately after killing the animal. One half of the tissue block was fixed overnight in 10% formalin at +4°C (refrigerator temperature) for the demonstration of esterase and adenosine triphosphatase activities and for histological examination. The other half was fresh-frozen using isopentane chilled with solid carbon dioxide. Histochemical methods for adenosine triphosphatases and for aminopeptidases were applied to the frozen specimen. The histochemical methods have been described and discussed in detail previously (Raekallio, 1961, 1964, 1965, 1970).

In order to describe changes of enzyme activity from one magnitude class to another, the intensity of staining was indicated by grades 1 to 5, grade 1 representing the enzyme activity in the intact skin of the animal. Grade 5 corresponds to the maximum activity demonstrable histochemically.

Results

Soon after injury two zones could be seen around the wound (Fig. 1). In the immediate vicinity of the wound edge, a central or superficial zone, 200 to 500 μ in depth, showed decreased enzyme activity. Surrounding this, a peripheral zone, 100 to 300 μ deep, exhibited an increase in enzyme activity.

Both in the young and old animals the activity of esterases and adenosine triphosphatases started to increase at about 1 hr, that of aminopeptidases at about 2 hrs, and the activity of acid phosphatases and alkaline phosphatases at 4 and 8 hrs, respectively. Although the enzymes appeared in the same chronological order in both of the age groups, the enzyme reaction in the young rats was manifold in intensity (cf. Figs. 1, 2, 3, and 4). This difference was also apparent when the changes of enzyme activity from one magnitude class to another were numerically indicated by grades 1 to 5. For example, 3 hr after wounding the increase in adenosine triphosphatase activity was twice as great in young rats as in the old ones (Fig. 5). Further, old animals required approximately twice as much time to attain the maximum intensity of enzyme activity, histochemically demonstrable in the wounds (Fig. 5). Analogous results were achieved by using the histochemical methods for the other hydrolytic enzymes mentioned in the "Methods". There is thus a histochemically demonstrable, age-dependent delay in the maximum intensity of the enzymatic response to injury in the wounds inflicted on old rats. Histologically also, the leucocytic infiltration in the wound periphery was slightly inferior in numerical strength in the old animals as compared to the cellular response in the young ones.

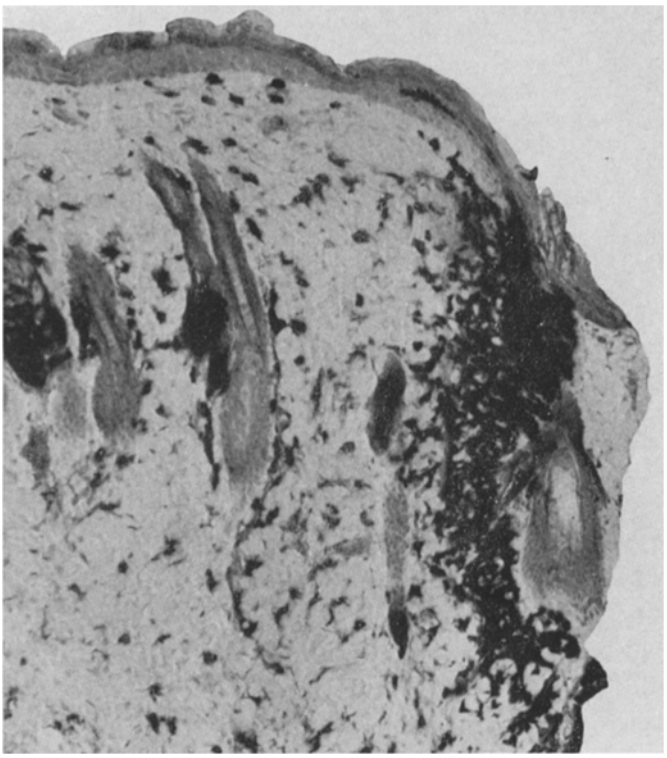

Fig. 1. Alkaline phosphatase activity in an 8-hour wound of a young rat

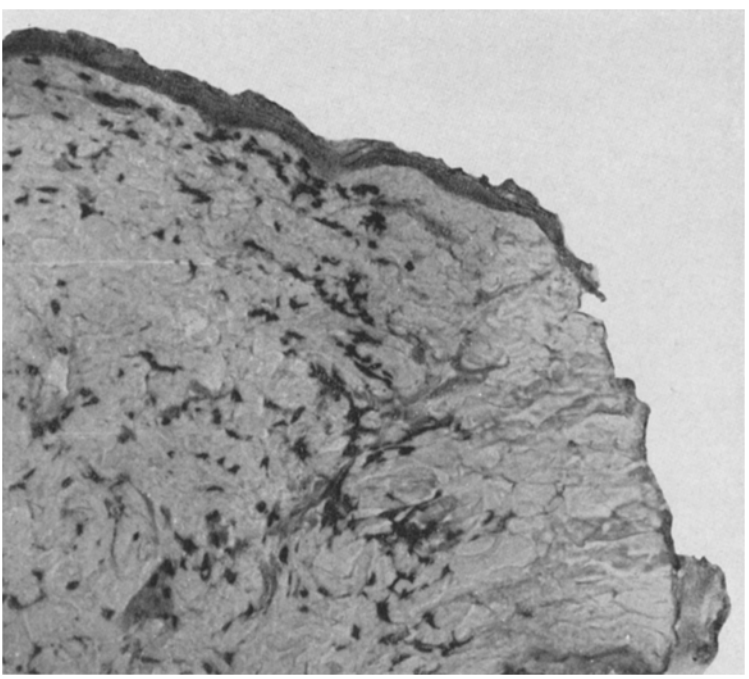

Fig. 2. Alkaline phosphatase activity in an 8-hour wound of an old rat

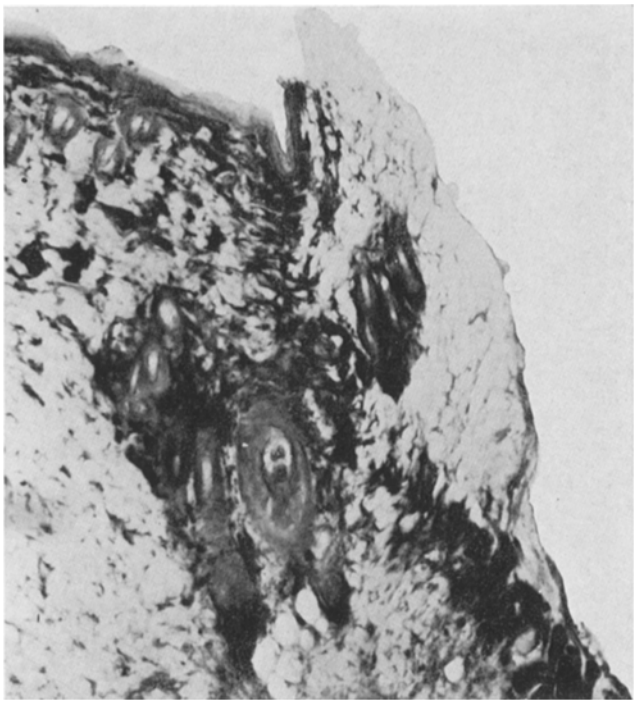

Fig. 3. Adenosine triphosphatase activity in an 1-hour wound of a young rat

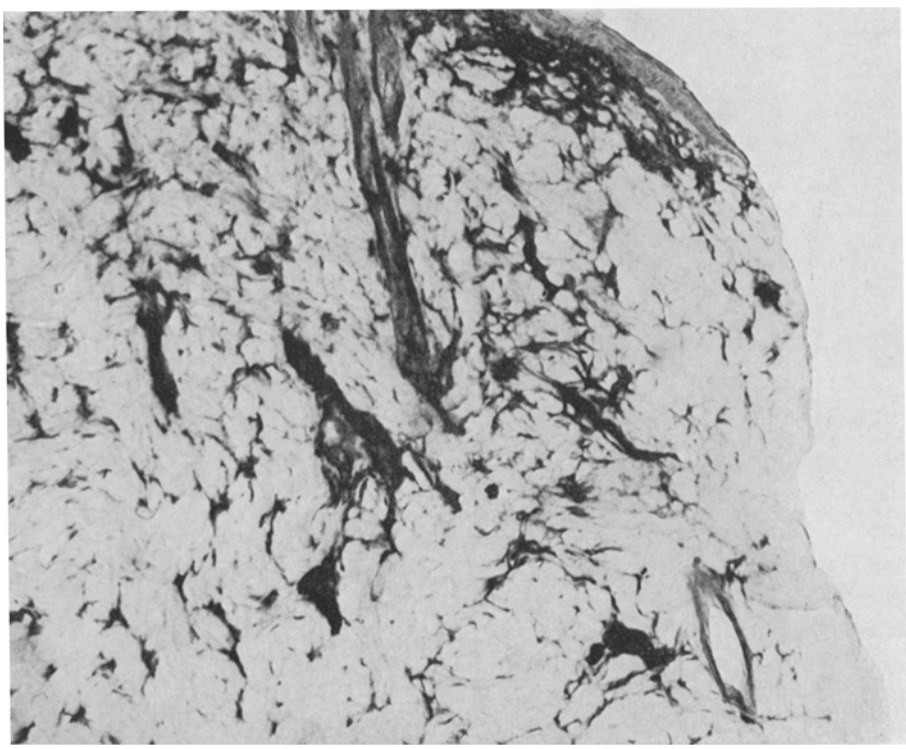

Fig. 4. Adenosine triphosphatase activity in an 1-hour wound of an old rat

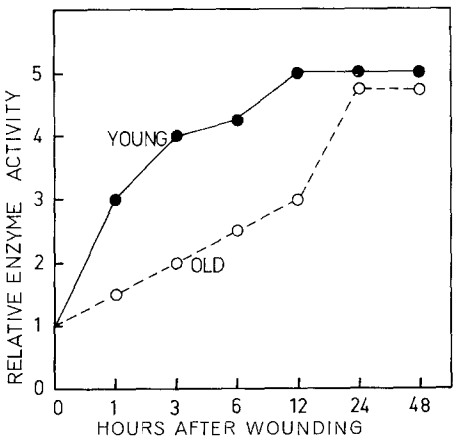

Fig. 5. Time course of the increase in adenosine triphosphatase activity in the wounds of young (——•——) and old (— —o— —) rats. Each point (• or o) represents the mean grade for 5 rats. The dispersion was never greater than ± ½ grade. In order to reduce the influence of the subjectivity of the evaluation, the staining intensity was independently graded by both of the authors

Discussion

The decrease of enzyme activity in the central (superficial) zone is an early sign of imminent necrosis (Raekallio, 1961, 1965, 1970; Berg, 1972). The regressive phenomena in the central zone are called negative vital reactions, since no such local decrease in enzyme activity is observed in the wounds inflicted after death (Raekallio, 1961, 1965, 1973). There were no demonstrable differences between the negative vital reactions of the young and old animals.

The increased enzyme activities in the peripheral wound zone are called positive vital reactions, since there are no such changes in post-mortem wounds (Raekallio, 1961, 1965, 1973). The initial increase in enzyme activity represents, among other things, an adaptive defence mechanism of the local fibroblasts and of the invading leucocytes as an enzymatic response to injury (Raekallio, 1960, 1961, 1965, 1970). The ability to adapt to environmental changes is a fundamental property of life. The molecular basis of adaptation is the synthesis and degradation of enzymes as well as the modification of their physiological functions (Adelman, 1972). Wounding is an example of the changes to which the organism has to adapt itself. This is why it is not surprising that the enzymatic response to injury is demonstrable during the very first hours after wounding.

One biochemical expression of adaptive response is the fluctuation of enzyme activities. Adelman (1972) has described the mammalian enzyme adaptation in response to some nutrional, hormonal, pharmacological, or physiological manipulations as follows: The basal level of enzyme activity, following a characteristic latent period of time ranging from minutes to weeks (cf. the biological time-table, showing the appearance of certain enzymes in the wound periphery, Raekallio, 1961, 1965), increases to a maximum level and generally is maintained at that level for a characteristic period of time following withdrawal or utilization of the inducing stimulus. Such a fluctuation in the activity of an enzyme must be

attributed to the production of a modified or distinct, new enzyme species, the modification of catalytic behaviour, the alteration of an enzyme concentration, or some combination of these (Adelman, 1972). Aminopeptidases are a representative group of enzymes accumulating in the wound periphery. In our laboratory we have biochemically investigated aminopeptidases in early wound healing (cf. several literature references in Raekallio's book, 1970; Mäkinen and Raekallio; Raekallio and Mäkinen, 1967—1969). According to these studies, the intensification of aminopeptidase activity, histochemically and biochemically demonstrable from 2 hr after injury, involves an actual increase in the amount of enzymes and not only an activation. The extra amounts of aminopeptidases in wound tissue originate to a noticeable degree in the injured tissue itself. They are not derived solely from blood plasma or from the invading leucocytes, although the enzymatically active immigrating cells participate, of course, in the increase in aminopeptidase activity. The accumulation of enzymes in the local fibroblasts involves an accelerated *de novo* synthesis of enzyme protein as an adaptive enzymatic response to injury.

According to the definition of Goldstein (1971), ageing is a progressive, unfavourable loss of adaptation. This theory is in accordance with our present results. At a given point of time during the earliest post-operative period, the increase in enzyme activity in the wounds of an old animal is much less manifest than in a young one. Further, old animals require approximately twice as much time to attain the maximum intensity of the enzyme activity. Thus, an age- to attain the maximum intensity of the enzyme activity. Thus, an age-dependent impairment of the *quantity* of the enzymatic response to injury characterizes an ageing organism as an expression of its decreased ability to adapt itself to the environmental changes caused by wounding.

From the forensic point of view, it is important that, inspite of the quantitative differences, the enzymes appear in the same *chronological* order both in the young and old animals. The same biological time-table is thus valid for both age groups. This means that the initial increase in the activity of a given enzyme, for example, adenosine triphosphatase, is histochemically demonstrable at the given point, one hr, after wounding.

It is well known that there are certain exceptions in the biological time-table when it is applied to human autopsy material. In the material (Raekallio, 1967) collected from 43 victims of traffic accidents, there were 5 cases with a slightly exceptional appearance of enzymes. Among them, there was 1 case (an 81 years old man) with a slight delay in the appearance of alkaline phosphatase activity. However, the influence of age is more important in later stages of healing than in the acute response to injury (Hegemann *et al.*, 1950; Berg, 1972). Thus the age of the victim does not have any significant effect on the chronological order of the enzyme histochemical vital reactions. The methods of enzyme histochemistry may thus be used as a guide for the estimation of the approximate age of the wounds of young and old individuals. However, it is apparent that a forensic pathologist should never time a wound on the basis of one histochemical, biochemical, or histological finding only. Now, as in the past, all autopsy findings, the examination of the body at the scene and, when available, the case history, must all be taken into account in order to reconstruct cases of violent death.

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