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VITAL REACTION AS ENZYMATIC RESPONSE TO INJURY

—WITH REGARD TO DISTINCTION BETWEEN ANTEMORTEM AND POSTMORTEM SKIN WOUNDS BY HISTOCHEMICAL METHODS—

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ABSTRACT

In order to discover early vital reactions the authors investigated the changes in the activity of alkaline phosphatase, acid phosphatase and esterase in antemortem and postmortem skin wounds.

Dermal connective tissues adjacent to the wound edge showed a decrease in the activity of the three enzymes from 2 hours after vital wounding. Surrounding the decreased zone, along the wound edge, the activity of the three enzymes increased simultaneously in local fibroblasts from 2 hours and in immigrating leucocytes from 4 hours after vital wounding. The authors consider that these phenomena belong to the intravital reactions, since there were no such changes in postmortem wounds.

Epidermis and hair follicles in the vicinity of the wound edge revealed an increased esterase activity as early as 30 minutes after vital wounding. Such a phenomenon was also seen even in postmortem skin wounds which were inflicted within 2 hours after death and preserved for a certain time interval. On alkaline phosphatase and acid phosphatase, increased reactions in the epidermis and hair follicles could not be observed. It seems that this increase in esterase activity corresponds to the intermediate reactions.

Accordingly, whether a wound is of vital origin or not, might not be decided solely by the earlier increase in esterase activity. Further, because of the simultaneous activation of the three enzymes, the estimation of the age of wounds should be based rather on the intensity and localization of the enzyme activities.

INTRODUCTION

The differentiation between antemortem and postmortem skin iniuries has always been one of the medicolegally significant problems. In spite of such importance, this has been based so far on several macroscopic findings and observations by histologic sections[1)2)3)].

In 1960, Raekallio[4)-12)] made an experimental study on guinea pigs and

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demonstrated histochemically an activity of several enzymes in the earliest phase of wound healing. He could reveal and localize earlier functional changes, not detectable by conventional histologic technics. He noticed two zones around vital wounds. In the vicinity of the wound edge, a central or superficial zone, 200 to 500 μ in depth, showed decreasing enzyme activity. Surrounding the central area, a 100 to 300 μ deep peripheral zone exhibited an increase in enzyme activity. Since there were no such changes in postmortem wounds, Raekallio called the former negative vital reactions and the latter positive vital reactions. On the other hand, methods of enzyme histochemistry were applied to forensic medicine, and because of the consecutive appearance of the positive vital reactions, this methods proved of great use not only in earlier diagnosis but also in more accurate estimation of the age of vital wounds. Later, some medicolegal investigators[13-18] became interested in this problem and confirmed his results.

However, cannot such vital reactions be really observed after death?; and the following idea occurred to us that somatic death might not be identical with 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; certain activities continue, and reactions of cells and tissues to stimuli can be produced by artificial means for a considerable time afterwards. For example[19], the muscles will contract to direct electrical stimulation for up to 3 hours after death when postmortem changes in the fibres herald the onset of rigor mortis. The corneal reflex and pupillary light reflex disappear at the time of death, but the instillation of atropin into the eye up to 4 hours after death, by its direct effect on the muscle of the iris, will cause some dilatation and, likewise, eserin will cause constriction up to 1 hour after death. A cornea, required for transplantation into a living patient, need not be removed from a dead body for 6 hours and satisfactory transfusions have been made of blood taken from bodies dead for 6 hours. Similarly the dermal cells and tissues still maintain the ability to function for some time after somatic death.

From this reason the authors expect that the very early reaction in wound edge could also take place even shortly after their infliction. In order to confirm our hypothesis we carried out the following experimental investigations on guinea pigs and examined the changes in the activity of alkaline phosphatase, acid phosphatase and esterase in antemortem and postomortem skin wounds.

MATERIALS AND METHODS

Experimental procedure

a) Antemortem skin wounds

Incised skin wounds, 1 cm in length and 2–3 mm in depth, were made in a shaved dorsal area of guinea pigs. Each was decapitated 0, 1/4, 1/2, 1, 2, 4, 8, 16, 24, 48, 72 and 120 hours after wounding and skin flaps, 1×1 cm, containing the wounds, were removed immediately.

b) Postmortem skin wounds

As soon as guinea pigs were decapitated, similar wounds were made, kept at room temperature and removed 0, 1/2, 1, 2, 4, 8 and 16 hours afterwards. Same experiments were performed 1/2, 1, 2, 4, 8 and 16 hours after death.

Histochemical technics

The wounds obtained were rapidly frozen with solid carbon dioxide, maintained at $-70°C$ with a mixture of acetone and dry ice. Frozen sections were cut in a cryostat at 10–14 μ and then fixed for 5 minutes at 4°C in neutral buffered 10 per cent formalin.

After the fixation alkaline phosphatase activity was stained by the method of Burstone[20], acid phosphatase activity by the method of Takamatsu[21] and esterase activity by the method of Pearse[22]. The sections were incubated at 37°C for 30 minutes, at 37°C for 2 hours and at room temperature for 5 minutes, respectively. After being washed in distilled water, the sections were mounted in glycerin jelly and examined under a microscope.

RESULTS

a) Antemortem skin wounds

In antermortem skin wounds inflicted with in 1/4 hour before death there were no recognizable changes in the activity of the three enzymes. As early as 1/2 hour after vital wounding the epidermis and hair follicles in the vicinity of the wound edge revealed an increased esterase activity. This increase in esterase activity was seen in wounds up to 8 hours old, disappearing in wounds older than 16 hours. Alkaline phosphatase and acid phosphatase showed no such changes.

In dermal connective tissues adjacent to the wound edge, a 200 to 500 μ deep central zone, a decrease in the activity of the three enzymes occurred 2 hours after wounding and persisted all through the wound healing.

Surrounding the central zone, the activity of alkaline phosphatase, acid phosphatase and esterase began to increase in fibroblasts existing in the dermal connective tissue from 2 hours after wounding. This formed a bandlike peripheral zone, 100 to 300 μ in depth, situated parallely along the wound edge and which became more intense in 4-hour and 8-hour wounds. On the other hand, from 4-hour vital wounds there immigrated leucocytes which showed a marked activity of the three enzymes. The enzyme-active leucocytes intensified the picture of the peripheral zone, disappearing in 16- to 24-hour

TABLE 1. The Changes in Alkaline Phosphatase Activity in Antemortem Skin Wonnds

Site of increased reaction	Age of wounds (hrs.)									
	1/4	1/2	1	2	4	8	16	24	48	72
Epidermis and hair follicles	−	−	−	−	−	−	−	−	−	−
Fibroblasts	−	−	−	+	+	++	+++	+++	++	+
Leucocytes	−	−	−	−	+	++	+	−	−	−

TABLE 2. The Changes in Acid Phosphatase Activity in Antemortem Skin Wounds

Site of increased reaction	Age of wounds (hrs.)									
	1/4	1/2	1	2	4	8	16	24	48	72
Epidermis and hair follicles	−	−	−	−	−	−	−	−	−	−
Fibroblasts	−	−	−	+	+	++	+++	+++	++	+
Leucocytes	−	−	−	−	+	++	+	−	−	−

TABLE 3. The Changes in Esterase Activity in Antemortem Skin Wounds

Site of increased reaction	Age of wounds (hrs.)									
	1/4	1/2	1	2	4	8	16	24	48	72
Epidermis and hair follicles	−	+	+	+	+	+	−	−	−	−
Fibroblasts	−	−	−	+	+	++	+++	+++	++	+
Leucocytes	−	−	−	−	+	++	+	−	−	−

wounds, whereas the stainability of the fibroblasts reached a maximum at that time and then decreased gradually. 72 hours after injury, the bandlike zone of the fibroblasts lost the enzyme activity, constituting a crust, and the epidermis was newly reconstructed just beneath the crust.

b) Postmortem skin wounds

In any postmortem skin wounds there were no such changes in the activity of the three enzymes as occurred in the fibroblasts or leucocytes in vital skin wounds. Unlike alkaline phosphatase and acid phosphatase, however, an increased esterase activity in the epidermis and hair follicles in the vicinity of the wound edge could be observed even in postmortem skin wounds which

TABLE 4. The Changes in Esterase Activity in Postmortem Skin Wounds (Epidermis and Hair Follicles)

		Time from wounding to fixation (hrs.)						
		0	1/2	1	2	4	8	16
Time from decapitation to wounding (hrs.)	0	−	+	+	+	+	+	+
	1/2	−	+	+	+	+	+	+
	1	−	±	+	+	+	+	+
	2	−	−	±	+	+	+	+
	4	−	−	−	−	±	±	±
	8	−	−	−	−	−	−	−

were made shortly after death and preserved for a certain time interval at room temperature.

In wounds inflicted immediately after death or in 1/2-hour postmortem wounds this afore-mentioned increase in esterase activity was recognized by preservation for more than 1/2 hour, in 1-hour postmortem wounds for more than 1 hour, in 2-hour postmortem wounds for more than 2 hours and in 4-hour postmortem wounds occasionally by preservation for more than 4 hours. In postmortem wounds injured more than 8 hours after death the increased reaction could not be recognized by histochemical methods in any case. The postmortem stainability of esterase activity was gradually intensified depending on the preserving time after the infliction of wounds.

On alkaline phosphatase and acid phosphatase there were no such postmortem changes in the epidermis and hair follicles.

DISCUSSION

Raekallio[4)-12)] was the first to show that increased enzyme activity was seen during the very first hours of wound healing. He determined various enzyme activities by histochemical methods and demonstrated in the peripheral zone an increase in alkaline phosphatase activity 8 hours, acid phosphatase activity 4 hours and esterase activity as early as 1 hour after vital injury.

Since Raekallio, there have been many studies concerning histochemically demonstrable enzymes participating in wound healing. Pioch[13)] reported comparable findings on burns. According to Fatteh[14)15)], there were no great differences between experimental and medicolegal autopsy materials, but the enzyme changes in men appeared more delayed than in guinea pigs. Friebel and Woohsmann[16)] made syringe injections into rat skin and claimed that on the basis of temporal changes of the maxima and activity differences of nine

enzymes a reliable statement regarding the time of the syringe injections became possible. But they paid no regard to the distribution of the enzyme activities.

From our results of animal experiments, however, there are no temporal differences in the increased reactions in the dermis between alkaline phosphatase, acid phosphatase and esterase. We observed increased activities of these enzymes in the local fibroblasts in the peripheral zone from 2 hours after wounding and in the immigrating leucocytes from 4 hours. Different from alkaline phosphatase and acid phosphatase, we found an increased esterase activity in the epidermis and hair follicles in the vicinity of the wound edge as early as half an hour after wounding.

Esterase is known to be the earliest detectable enzyme. According to Fatteh[14)15)], the increase in esterase activity began in men 10 minutes and in guinea pigs 30 minutes after vital wounding. Arima and Nagamori[17)] also noticed a weak reaction of esterase activity in the wounded skin of rats sacrificed 3 to 15 minutes after the cutting. According to Tanaka[18)], the activity of esterase increased in wound edge of dermis 15 minutes after infliction of wounds and in hair follicles 2 hours after. He noticed a difference in the increased reaction of the hair follicles from that of the dermis. Our studies also indicate that the increased reactions of epidermis and hair follicles differ from that of fibroblasts or leucocytes.

On the other hand, we also recognized an increased esterase activity in the epidermis and hair follicles even in postmortem skin wounds which had been inflicted within 2 hours after death and preserved for a certain time interval at room temperature. Besides, Hou-jensen[23)] demonstrated a bandlike structureless amount of nonspecific esterase in a skin lesion produced in a rat 1 hour after death.

The biological interpretation of the apperarance in skin wounds of these enzymes has been discussed in previous reports[4)5)6)8)10)15)17)18)]. However, the precise role of these enzymes in wound healing has not been clarified.

Raekallio[4)-10)] believes that the initial increase in enzyme activity in the peripheral zone represents an adaptive defense mechanism by local connective tissue cells, as a response to injury. Besides functioning as a defense barrier, the enzymes appearing in the peripheral zone may play a part in more specific regenerative processes. The immigrating leucocytes contribute to the intensification of the enzyme activity, initiated by the local fibroblasts. The decrease in enzyme activity in the central zone should be considered as an early sign of imminent necrosis. Arima and Nagamori[17)] presume that the enzymes are carried to the destroyed layer by blood, whereby the enzymes are activated and accumulate there. On Fatteh's[14)15)] supposition, the enzymes within the damaged cells spill out into the tissue spaces and more enzyme may be brought to the site of injury by the fluid exudate or by the infiltrating

leucocytes.

The reason why only esterase activity increases in the epidermis and hair follicles vitally or postmortally is not clear. Since the increase in esterase activity occurs without a blood-supply after death, it is impossible to explain by the presumption[17] that the enzymes are carried to the wound periphery by blood. If the cells are damaged and the enzymes are released into the extra cellular spaces[14)15)], the increased reaction ought to take place in alkaline phosphatase or acid phosphatase as well as in esterase. From the fact that it needs a certain time for the increase in esterase activity to appear as the intensification of the stainability after postmortem wounding, the authors suppose that this phenomenon might be due to local synthesis of esterase within the cells in response to injury, independent of the individual control. It is reasonable that the very early vital reaction may also occur within a short time after death because the dermal cells and tissues still maintain the ability to function for some time after somatic death.

Orso's[24] classified the vital reaction into the following six groups:

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. postmortem reactions, seen after cellular death, and
6. signs of decomposition.

According to this classification, the postmortem increase in the activity of esterase in the epidermis and hair follicles corresponds to the intermediate reactions. On the other hand, the antemortem increase in the activity of alkaline phosphatase, acid phosphatase and esterase in the fibroblasts or leucocytes belongs to the intravital reactions.

No matter how the reason, medicolegally, whether a wound is of vital origin or not, might not be decided solely by the earlier increase in esterase activity, because this increase in the epidermis and hair follicles is also seen even in postmortem skin wounds which were inflicted within 2 hours after death and preserved for a certain time interval. Since the activity of alkaline phosphatase, acid phosphatase and esterase begin to increase in the fibroblasts or leucocytes simultaneously, it is impossible to date vital wounds by temporal differences of the increased reactions of the dermis. The dating of wounds should be based rather on the intensity and localization of the activity of the three enzymes.

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EXPLANATION OF FIGURES

FIG. 1. Alkaline phosphatase activity in a 2-hour vital wound showing a thin band of activated fibroblasts. ×100
FIG. 2. Alkaline phosphatase activity in a 4-hour vital wound. Fibroblasts in the upper part and infiltrating leucocytes in the lower part. ×100
FIG. 3. Alkaline phosphatase activity in a 16-hour vital wound showing typical peripheral zone and central zone. ×40
FIG. 4. Acid phosphatase activity in a 4-hour vital wound. ×40
FIG. 5. Acid phosphatase activity in a 24-hour vital wound showing its maximum activity in fibroblasts. ×40
FIG. 6. Acid phosphatase activity in a 72-hour vital wound. Epidermis is newly reconstructed beneath the crust. ×40
FIG. 7. Esterase activity in a 1/2-hour vital wound showing a weak reaction in epidermis and hair follicles in the vicinity of the wound edge. ×40
FIG. 8. Esterase activity in a 2-hour vital wound. ×200
FIG. 9. Esterase activity in a 8-hour vital wound. Not only epidermis and hair follicles but also fibroblasts and leucocytes reveal an increased esterase activity. ×40
FIG. 10. Esterase activity in a 1/2-hour postmortem wound, preserved for 2 hours. Epidermis and hair follicles exhibit a weak reaction of the esterase activity. ×40
FIG. 11. Esterase activity in a 2-hour postmortem wound, preserved for 8 hours. The same findings as Fig. 10. ×40

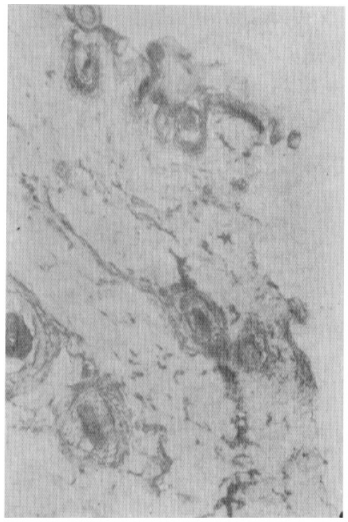
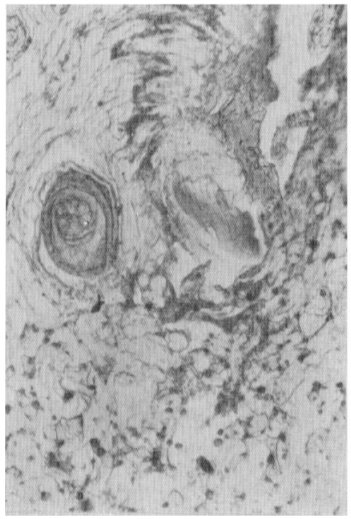


FIG. 1



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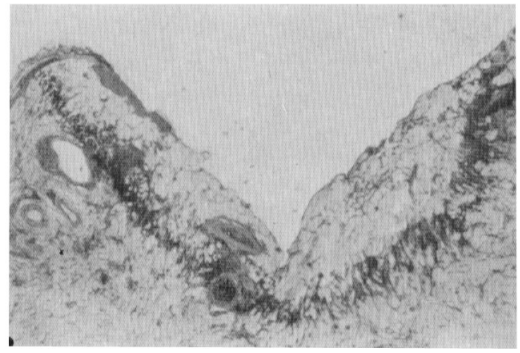

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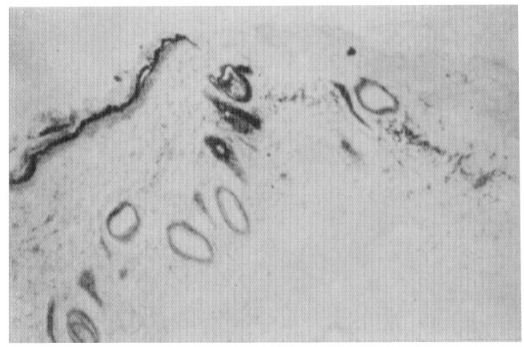
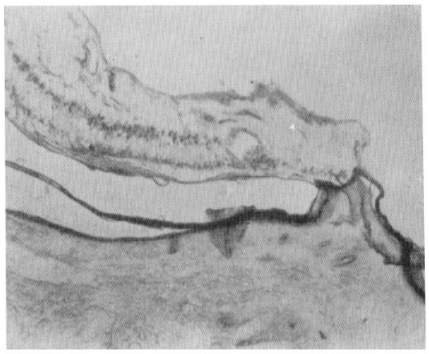


FIG. 4

FIG. 5



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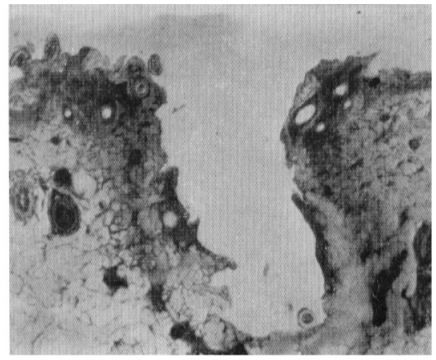

FIG. 9

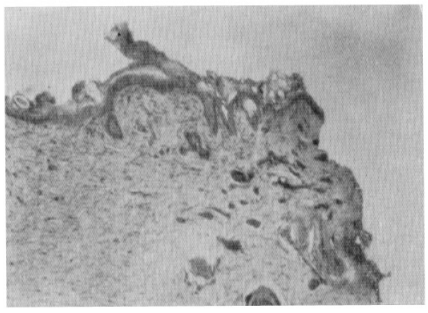

FIG. 7

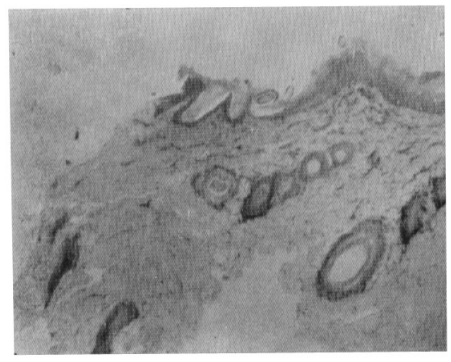

FIG. 10

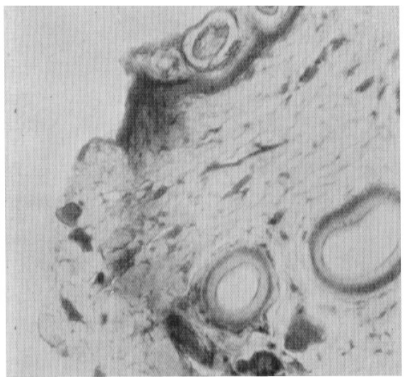

FIG. 8

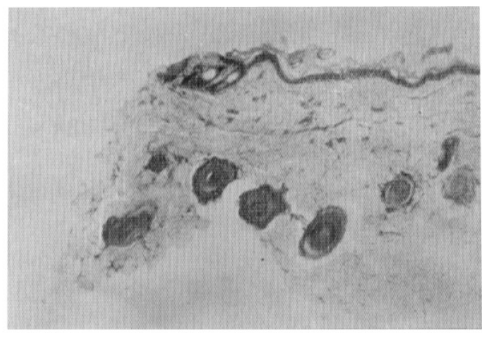

FIG. 11