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Original works

Vital reactions to frostbite of the ear and paw skin in guinea pigs exposed to the cold

Jorma Hirvonen

Department of Forensic Medicine, University of Oulu, Kajaanintie 52D, SF-90220 Oulu, Finland

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Summary. Vital reactions to frostbite in the paw and ear skin of guinea pigs were studied in order to find an animal model for frostbite in cases of accidental hypothermia. One group of animals was rendered hypothermic (rectal temperature, 30°C) by exposure to an ambient temperature of −20°C, and samples were taken from the frozen skin. A second group was rendered hypothermic and rewarmed in warm air at 45°C, and samples were taken from the thawed skin. The only vital reaction in the first group (freezing time, 4–5 h) was mild initial inflammation, which was expressed in granulocyte adhesion to the vessel wall and the migration of a few cells into the dermis. The inflammatory reaction was more distinct in the second group (freezing and thawing together 5–7 h), with a large number of granulocytes being present in the dermis. Oedema and hyperaemia were also present in the frostbitten tissue after thawing, but no signs of necrosis developed. The alkaline-phosphatase reaction demonstrated the presence of granulocytes more clearly than H & E or Masson trichrome staining. Vital reactions were more advanced in the ear skin. It is concluded that vital reactions are very scarce in cases of frostbite, even after several hours' exposure, unless the tissue is allowed to thaw.

Key words: Vital reactions, frostbite – Hypothermia – Frostbite, vital reactions

Zusammenfassung. Vitale Reaktionen bei Pfoten- und Ohrenhauterfrierungen wurden an Meerschweinchen erforscht, um ein Tiermodell für Erfrierungen bei akzidenteller Hypothermie zu definieren. Eine Gruppe von Tieren wurde bei −20°C unterkühlt (T_{rec} 30°C) und die Proben wurden der gefrorenen Haut entnommen. Die andere Gruppe wurde unterkühlt (T_{rec} 30°C), und erwärmt in warmer Luft (+45°C), und die Proben wurden der aufgetauten Haut entnommen. In der ersten Gruppe (4–5 Std Erfrierungszeit) bestand die einzige vitale Reaktion in einer leichten Entzündung im

Anfangsstadium, die sich in einer Festsetzung der Granulozyten an der Gefäßwand und der Wanderung einiger weniger Zellen in die Dermis zeigte. Die entzündliche Reaktion was ausgeprägter in der zweiten Gruppe (insgesamt 5–7 Std Erfrierungs-/Auftau-Zeit) mit mehr Granulozyten in der Dermis. Es traten auch Ödem und Hyperämie in den aufgetauten Erfrierungen auf, jedoch waren Anzeichen für Nekrose nicht festzustellen. Die alkalische Phosphatase-Reaktion zeigte die Granulozythen deutlicher als H-E und das Masson Trichrom-Färbung. Die Reaktion in der Ohrenhaut war fortgeschrittener. Die Schlußfolgerung ist, daß vitale Reaktionen bei Erfrierungen auch nach einigen Stunden im Frost sehr gering sind, wenn das Gewebe nicht aufgetaut wird.

Schlüsselwörter: Erfrierungen, vitale Reaktionen – Vitale Reaktionen, Erfrierungen

Introduction

One of the most distinct and rapid vital reactions to physical injury is inflammation, which is morphologically demonstrable from various manifestations, e.g. an increase in biogenic amines and an accumulation of granulocytes in the lesion and at the site of injury repair (see Raekallio 1977).

Frostbite is a special type of lesion, since no major disruption of the tissue occurs during its first phase, the main injury being intracellular, i.e. both in the parenchyma itself and in the capillary endothelial cells. This injury is difficult to demonstrate morphologically, as preservation of the frozen tissue is excellent in this early phase before the tissue thaws and the blood circulation is restored, after which necrosis becomes visible. This usually takes 2–3 days (Carpenter et al. 1971).

In earlier experiments on local deep freezing, we found that, after thawing, a strong inflammatory reaction develops within a few hours in damaged skin and the underlying adipose tissue (Laiho and Hirvonen 1971), while another experiment showed that, vital reactions in frostbitten skin are scarce when an animal dies of cold, even though the skin may have been exposed to frost for 2–6 h (Hirvonen et al. 1982). The difference in the intensity of these reactions appears to be due to the fact that no thawing and subsequent recirculation occur when a victim dies of cold exposure. Accordingly, we set out to study vital reactions to frostbite in two types of skin in an animal model in which accidental hypothermia with or without subsequent rewarming is simulated. Such experimental models are of great practical value for the light they may shed on the difficult situation, in which a frozen body is found, and data are needed to determine the cause of death and the length of exposure. For the treatment of congelations, in which the aim is to develop more tissue-saving surgical methods, all kinds of basic research are important.

In the present study, animals were exposed to a temperature of −20°C until they had become hypothermic and were then rewarmed to normal body temperature. The progression of the early inflammatory reaction in the frozen paw and ear skin was investigated using histological and histochemical methods.

Materials and methods

Twenty-four adult guinea pigs of both sexes (weight, 610–1030 g) that had been kept in a normal laboratory colony and fed with pellets, vegetables and water were divided into three groups. Seven animals were exposed to a temperature of −20°C until their rectal temperature was 30°C, which took an average of 4–5 h, and then killed. A further 10 animals were similarly cooled until their rectal temperature T_{rec} was 30°C and were then rewarmed at 45°C to their initial body temperature of 39°C before being killed. Cooling and rewarming together took an average of 5–7 h. Seven animals without any exposure to lower temperatures served as controls.

Skin samples were taken from the upper surface of the front paws and across the ear lobes, where the skin had frozen during exposure. One sample from each area of skin was immediately immersed in liquid nitrogen for enzyme histochemistry, while another was fixed in 10% formalin for paraffin sectioning.

The following methods were employed, based on our earlier experiences:

1. Masson trichrome was used for connective tissue, and haematoxylin-eosin (A & E) was used for ordinary morphology according to the *Manual of the Armed Forces Institute of Pathology* (Luna 1968)
2. The alkaline-phosphatase (A1Pase) reaction for granulocytes was visualized using the α-naphthyl phosphate Fast Blue RR method (Barka and Andersson 1965).

The acid-phosphatase reaction was also tested, but the results were inconclusive and were therefore omitted from the final analysis.

Results

Histological and histochemical observations

Group I (seven guinea pigs exposed to −20°C until T_{rec} = 30°C)

Ear. The epidermis showed no changes using H & E and Masson stainings. Slight signs of inflammation were seen in the dermis of four samples, this taking the form of either granulocyte adhesion to the endothelium of the venules or the presence of a few granulocytes between collagen fibres. The collagen fibres of one sample were stained red after Masson staining. No thrombi or even thrombocyte aggregates were seen in this sample group, nor were any haemorrhages or oedemas observable. In frozen sections stained for A1Pase, granulocytes could be observed on the inner walls of the venules and capillaries in all seven samples, and outside the vessels in two samples. In one sample, the quantity of granulocytes was considerable (Fig. 1).

Paw. The epidermis appeared to be intact, and neither haemorrhages nor obvious oedemas could be observed. A few granulocytes were seen between the collagen fibres after H & E and Masson staining. No thrombi were observed in the vessels. A few A1Pase-positive granulocytes were visible in the dermis of frozen sections in six samples and at the venule wall in one sample (Fig. 2).

Group II (ten guinea pigs exposed to −20°C until T_{rec} = 30°
and then rewarmed to 39°C)

Ear. Inflammation was visible in all ten samples after H & E and Masson staining. Granulocytes and macrophages had already invaded the dermis. The in-

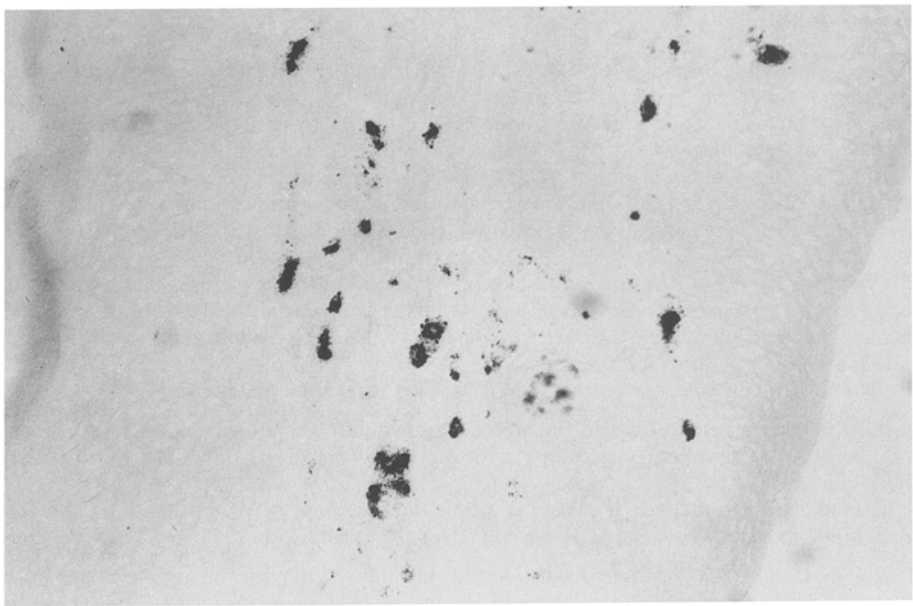

Fig. 1. A number of granulocytes are present in the venules and subepidermal tissue of the frozen ear lobe of a guinea pig exposed at −20°C until its body temperature had reached 30°C. Alkaline-phosphatase reaction. × 160

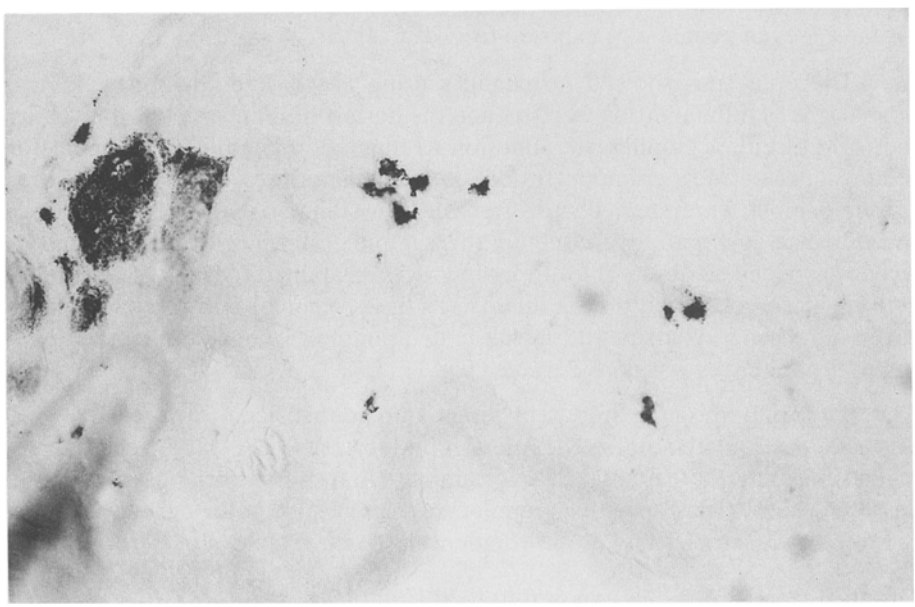

Fig. 2. A few granulocytes showing a strong alkaline-phosphatase-positive reaction are visible in the venules and capillaries of the frozen paw skin of a guinea pig cooled to a body temperature of 30°C. The inflammatory reaction was less intense at this site than in the ear. Alkaline-phosphatase reaction. × 160

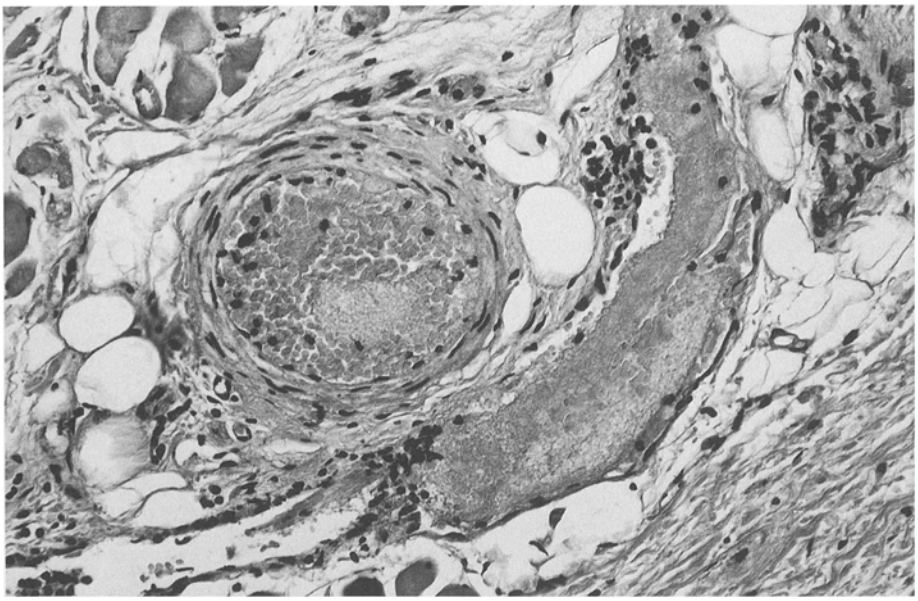

Fig. 3. Thrombocyte aggregates in an artery and adjacent vein from an ear lobe that was frozen and then thawed. Also note the granulocytes that have settled at the venule wall. H & E. × 160

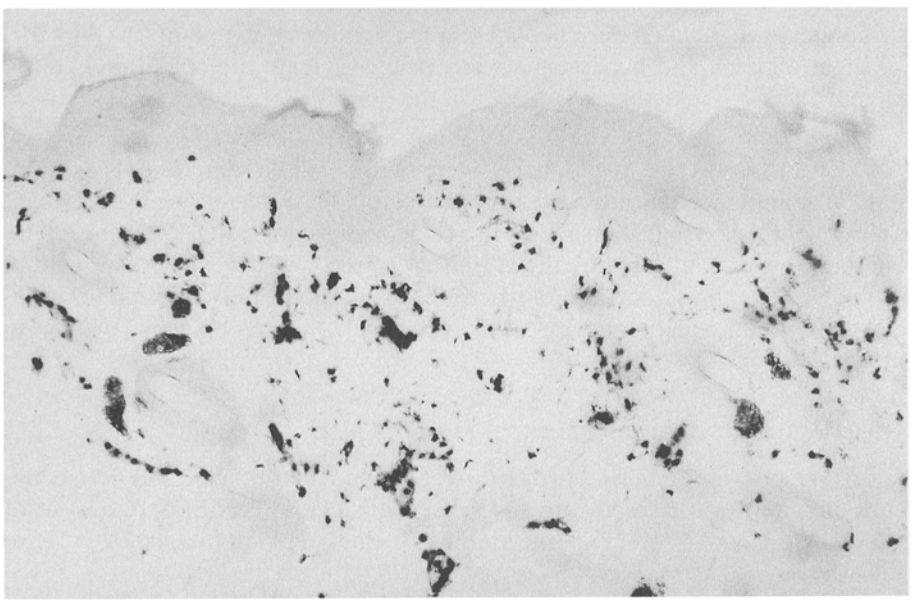

Fig. 4. A distinct inflammatory reaction is visible in this paw skin which was first frozen and then thawed as the animal was rewarmed to a body temperature of 39°C. There was a considerable difference between the intensity of the reaction in the unthawed frozen tissue (Fig. 2) and that in the frozen and thawed tissue. Alkaline-phosphatase reaction. × 60

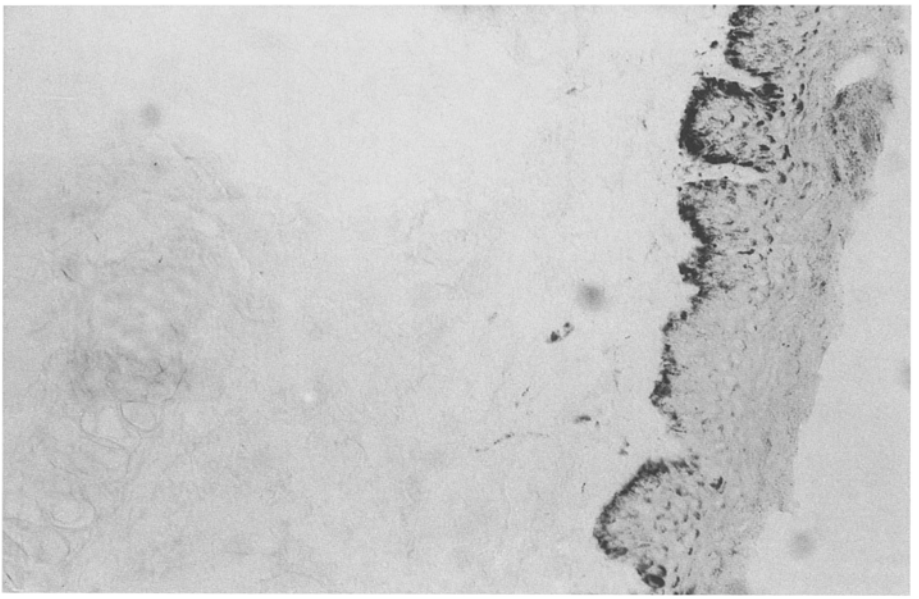

Fig. 5. Ear lobe of a control animal. No alkaline-phosphatase-positive cells are present in the dermis. × 160

flammatory cells were even more clearly visualized by the A1Pase reaction in the venules and capillaries as well as in the dermis itself. Small thrombi or thrombocyte aggregates were detected in the venules of five samples (Fig. 3).

Slight oedema and hyperaemia were visible beneath the epidermis, but no haemorrhages were detectable deeper in the dermis.

Paw. Granulocytes and macrophages were seen in all ten samples at H & E and Masson staining; collagen fibres stained red after Masson staining were present in three samples. Venous thrombi were seen in one sample. The presence of inflammation was confirmed by the A1Pase reaction, which revealed large numbers of positive granulocytes and macrophages in the venules and capillaries as well as between collagen fibres. Mild hyperaemia and oedema was present in the lower layer of the dermis (Fig. 4).

Group III (controls: seven non-exposed guinea pigs)

Ear. Masson staining showed two to three granulocytes in the dermis of two animals, but none of these features were seen in H & E sections. A couple of granulocytes were present in the dermis of one sample stained for A1Pase (Fig. 5).

Paw. A couple of granulocytes were seen in dermis samples from two animals after H & E and Masson trichrome staining, and a similar incidence of granulocytes was observed in three other animals using the A1Pase reaction.

Discussion

The present results confirm and supplement our earlier findings on vital reactions to frostbite, and they further support the notion that few morphological changes are detectable in fresh premortem frostbite cases in spite of hours of exposure to frost (Hirvonen et al. 1982). Only a slight inflammatory reaction involving granulocytes was observed in the initial phase. Indeed, this reaction could not be detected in all of the frostbite cases in which no thawing had been allowed to take place.

One reason for the slowness of the vital reaction to frostbite is probably the vasoconstriction which occurs under cold conditions, so that the precapillary arteries remain constricted unless the skin thaws. Thawing is followed by hyperaemia only in those parts of the tissue where the vessels are not completely necrotized, and leucocytes are able to gather only in the functioning venules and then invade the surrounding tissue. Another explanation for the slow inflammatory reaction observed in the present frostbite cases is that the skin was thawed relatively slowly in warm air. This has been shown to cause more damage to vessels than rapid thawing in warm water (Carpenter et al. 1971). A third explanation is that the guinea pigs were, or had been, hypothermic, a state during which the blood circulation is generally slow, particularly in the peripheral parts of the body.

Our experiments also demonstrated that vital reactions are more pronounced in the ear skin, which is a looser and more vascular tissue than the comparatively dense paw skin. Ear tissue is also exposed to the cold from both sides and becomes frozen more rapidly, while thawing also probably occurs more swiftly than in the paw skin. These factors might explain the difference in the intensity of the vital reaction at these two sites.

Microthrombi in the venules have been found to be a frequently occurring factor in the pathogenesis of frostbite. Their appearance occurs in a later phase, but in the present short-duration congelations, these features were only observed in 3 out of 17 ear skin samples, all being from guinea pigs that had been rewarmed. It can be concluded that platelet aggregates are not prone to develop in acute frostbite because of diminished circulation in the freezing phase. The thrombi tend to form after thawing, i.e. when endothelial damage and local congestion are developing.

The implication of the present results for forensic pathology is that it is necessary to look for very minor changes in the dermis of the frozen skin in order to determine the vital character of the thermal injury. An increased number of granylocytes in the venules and their adherence to the endothelium would seem to be the important differences as compared to controls.

Personal experience of accidental hypothermia cases suggests that almost no clear vital reactions are observable in the skin of the limbs, with mild hyperaemia perhaps being an exception; also, no data are currently available concerning rapid inflammatory changes in frozen skin after the rewarming of human hypothermia victims.

The degree to which minor vital changes can be observed in fresh frostbite material also depends on the histological method used. The present study

clearly showed the advantage of the A1Pase method as compared to H & E and Masson trichrome staining for demonstrating early cellular reactions to thermal injury. The activity of this enzyme is high in guinea pig granulocytes, and thus, this procedure enables the accumulation of these cells at the venule wall and, later, in the dermis to be demonstrated much more clearly than the other two methods tested. It is difficult – or even impossible – to identify the few granulocytes among the other cells in paraffin sections, but in the case of A1Pase staining, the background remains almost entirely negative, which considerably improves the discernibility of these structures. I am unaware of any other staining methods which could demonstrate possible changes in fresh frostbite material. The trichrome method used here sometimes revealed reddish discoloration of collagne fibres, but this cannot be regarded as a reliable vital reaction.

The other possibility for demonstrating early vital reactions to experimentally induced frostbite is to use biochemical assays of histamine and serotonin, as has been done in the case of mouse ear lesions (Penttilä et al. 1974). These amines are present in decreased amounts after frostbite, a change which is also demonstrable post mortem.

It is difficult to judge the timing of frostbite on the basis of vital reactions, since the course of such changes depends on many factors, e.g. the degree and speed of freezing brought about by the ambient temperature, the speed of thawing and whether thawing has taken place at all. The schedule for the inflammatory reaction observed here resembles that found in rat skin subjected to severe frostbite, in which neutrophils were observed at the border of the necrosis within 1–4 h (Cummings and Lykke 1973).

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