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ORIGINAL ARTICLE

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Albumin as a marker of plasma transudation in experimental skin lesions

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Abstract Liquid chromatography measurements of albumin levels were obtained for experimental incision wounds, excoriations, and heat and freezing injuries of different ages. Hemoglobin levels in tissue specimens were measured and an equivalent amount of blood-related albumin was subtracted from the analysis results. In specimens taken immediately after death, the mean albumin level as compared to control skin was increased by about 2-fold in freezing injuries aged 60 min. In all other lesions, the same increase was observed even after 30 min. The mean albumin level was about 3-fold as compared with the control skin in excoriations aged 30 min, heat and freezing injuries aged 4 h, and incision wounds aged 12 h. An approximately 5-fold increase was seen in heat and freezing injuries aged 1 and 2 weeks. A marked decrease occurred in mean albumin levels in all lesions aged 4 weeks. An increase in albumin in wounds and excoriations was demonstrable also in specimens taken 3 days postmortem. Postmortem hypostasis resulted in a 1.1 to 1.4-fold increase in mean albumin levels in wounds and excoriations inflicted 1 min postmortem.

Keywords Albumin · Transudation · Plasma · Wound · Excoriation · Heat and freezing injuries

Introduction

In forensic medicine the estimation of age and vitality of lesions is problematic. Since Walcher's [1, 2] observations on inflammatory cells and hemosiderin in lesions, it has been a favourite topic for research. By using different methods numerous studies on inflammatory cells, enzymes, histamine, serotonin, different proteins and more recently on inflammatory mediators, chemokines, adhesion molecules, growth factors etc. [3, 4, 5, 6, 7, 8, 9, 10, 11] have been performed.

In the present paper observations on plasma transudation [12] have been applied for forensic medical purposes. To investigate the pathology of the inflammatory reaction, several experimental studies have been performed in which the leakage of the dye-albumin complex or that of radioactive albumin has been followed over time after different types of injury [13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29]. In forensic medical practice, measuring tracer-marked proteins in lesions is not possible, and injured tissues often contain variable amounts of blood proteins because of hemorrhages. In this work, the albumin content of tissue was measured in specimens of experimental incision wounds, excoriations, and heat and freezing injuries. The amount of albumin directly related to the presence of blood in the tissue was estimated and subtracted from the analysis results.

Materials and methods

Male Sprague-Dawley rats with an average weight of 500 g were used. Under ether narcosis, 5 cm-long incision wounds perforating the skin were made on the right side of the dorsal skin. After different periods of survival the rats were killed by neck dislocation under ether narcosis, and an approximately 2 mm-thick zone from the edges of the incision wounds was taken immediately after death. The observations were made until the wounds were 15 days old, after which determination of the exact edges of the healing wounds was problematic.

Excoriations were made on the right side of the dorsal skin under ether narcosis using coarse sandpaper and by holding the skin in a fixed position with forceps during the procedure. In these excoriations the epidermis and the superficial part of the dermis were removed. The excoriated area of the skin was on average about 3 cm^2. The entire excoriated skin layer was taken as a specimen immediately after death and different periods of survival.

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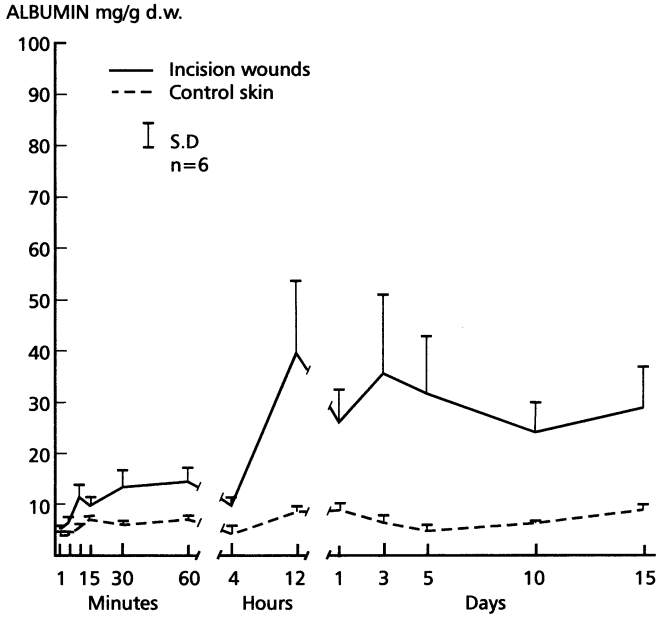

Fig. 1 Albumin mg/g dry weight in incision wounds and in control skin. Means and standard deviations (SD) are given. The mean values for the incision wounds aged from 5 min to 15 days are significantly higher than the means in control skin ($p<0.01$ or $p<0.001$)

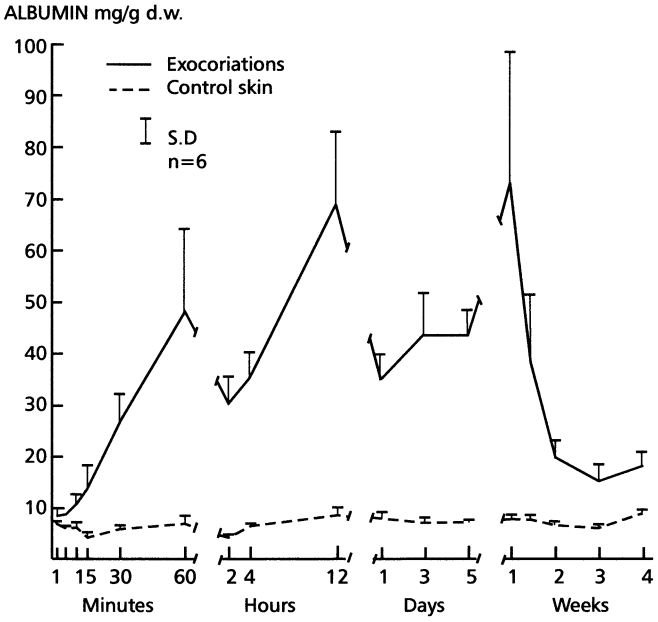

Fig. 2 Albumin mg/g dry weight in excoriations and in control skin. Means and standard deviations (SD) are given. The mean values for the excoriations aged from 5 min to 4 weeks are significantly higher than the means in control skin ($p<0.01$ or $p<0.001$)

Specimens around some incision wounds and excoriations were also taken 3 days postmortem after storage at temperatures of 4°C and 22°C.

Some incision wounds and excoriations were also inflicted on the dorsal skin of rats at 1 min postmortem. The time of death was regarded as the moment when circulation had been stopped in thoracotomy by ligature around the large vessels entering and leaving the heart (the same method was used to define the time of death in all experiments where the survival was 30 min or less). Rats were then kept dorsal side down to bring about hypostasis in wounds and excoriations. Specimens were collected at 1 h postmortem at 4°C and at 3 days postmortem at 4°C or 22°C.

Heat injury was inflicted by pressing the open end of a plastic tube containing boiling water against shaved skin for 60 s. This resulted in a circular heat injury to an area of about 3 cm^2 on the right dorsal skin of the rats. After different periods of survival, the entire injured area was taken as a specimen immediately upon death.

Freezing injury was inflicted by pressing the closed end of a metal tube containing liquid nitrogen against shaved skin for 5 min. The entire skin layer in a 3 cm^2 area on the right dorsal side was frozen. After different periods of survival the injured skin region was excised as a specimen immediately after death.

Heat and freezing injuries were also inflicted on the dorsal skin of rats at 1 min postmortem. The rats were then kept dorsal side down to result in hypostasis or dorsal side up for no hypostasis. Specimens were collected at 4 h postmortem at +22°C and at 3 days postmortem at +4°C or +22°C.

In all series of experiments, control specimens were taken from the contralateral side of the dorsal skin. All specimens were stored at −70°C.

The specimens for albumin analysis were shredded with scissors in 0.05 M Tris-HCL buffer pH 8.6 added at 10 ml per gram wet weight and then thoroughly homogenized. The homogenate was centrifuged at 13,000 rpm for 15 min. Supernatants were separated using a needle and syringe, and before analysis, the supernatants were filtered through 0.45 µm microfilters. Albumin was analyzed from the filtered supernatants by high performance liquid chromatography (Perkin Elmer) with an anion-exchange column "Mono Q" (Pharmacia), and by using 0.05 M Tris-HCL buffer (pH 8.6) and a linear gradient from 0 to 0.5 M sodium chloride over 10 min [30]. Rat albumin (Sigma) was used as a standard.

The haemoglobin content in the specimens was estimated by hemin analysis [31]. The preparation of the specimens for this analysis was as described earlier [32] except that centrifugation at 13,000 rpm for 15 min was used. The relationship between haemoglobin and albumin content in the blood of 6 live rats was determined. This was then used in estimations of albumin content which was directly related to blood content in the specimens, and the amount of albumin so obtained was subtracted from the analysis results.

Albumin was expressed as mg/g dry weight of tissue, and for this purpose a sufficient piece of specimen with known wet weight was dried in an oven at 120°C for at least 24 h and weighed. In the figures and tables, the mean at each time point is based on the results obtained from 6 experimental animals ($n=6$). Altogether 510 experimental animals were used in these experiments. The "Principles

Table 1 Albumin mg/g dry weight in incision wounds and excoriations of different ages

Age of lesions		1 min	15 min	30 min
Incision wounds	M	11.2	20.7	21.0
	SD	1.0	4.4	2.7
Control skin	M	10.0	7.0	9.5
	SD	2.5	1.8	0.6
Excoriations	M	11.1	28.3	37.2
	SD	1.3	2.4	3.9
Control skin	M	6.7	7.2	7.4
	SD	1.0	0.6	3.5

Specimens taken at 3 days postmortem at a temperature of 4°C.
M Means and *SD* standard deviations are given (n=6).
The mean values for wounds and excoriations aged 15 and 30 min are significantly higher than those for control skin (p<0.001).

Table 2 Albumin mg/g dry weight in incision wounds and excoriations of different ages

Age of lesions		1 min	30 min	60 min
Incision wounds	M	12.3	11.9	24.3
	SD	0.8	2.5	5.5
Control skin	M	9.6	6.0	6.8
	CD	1.2	1.3	1.0
Excoriations	M	12.6	39.2	49.3
	SD	1.6	9.6	6.7
Control skin	M	13.8	10.4	11.1
	SD	2.5	1.1	1.0

Specimens taken at 3 days post-mortem at a temperature of 22°C.
M Means and *SD* standard deviations are given (n=6).
The mean values for wounds and excoriations aged 30 and 60 min are significantly higher than those for control skin (p<0.001).

of laboratory animal care" (NIH publication No. 85–23, revised 1985) were followed, as well as specific national laws were applicable. This study was approved by the local ethics committee for animal experimentation. Statistical analysis was performed using Student's *t*-test.

Results

When specimens from the wounds and excoriations were taken immediately after death, the mean level of blood-unrelated albumin was about 2-fold in the edges of incision wounds aged 30 min–4 h as compared with control skin. In wounds aged 12 h–15 days the corresponding figure was 3 to 4-fold. In wounds aged 1 min to 15 min the mean albumin level was only about 1.2 to 1.5-fold as compared with control skin (Fig. 1).

In excoriations, the mean albumin level was 4-fold or greater in wounds aged 30 min–10 days as compared with control skin. After 2–4 weeks, the albumin level decreased being only about 2-fold as compared to control skin. In excoriations aged 1 min–10 min only a slight increase from 1.2 to 1.8-fold as compared to controls was observed (Fig. 2).

When samples from the wounds and excoriations were taken at 3 days postmortem at 4°C or 22°C and the dorsal side of the experimental animals faced upwards, the increase in blood-unrelated albumin during survival of 1 min, 30 min and 60 min was similar to that of specimens taken immediately after death (Tables 1 and 2).

The effect of postmortem hypostasis was tested in wounds and excoriations inflicted at 1 min postmortem in experimental animals that had been placed dorsal side down for 1 h at 4°C or for 3 days at 4°C or 22°C. In all groups, for both incision wounds and excoriations, the mean value of blood-unrelated albumin increased slightly from 1.1 to 1.4-fold as compared to control skin, indicating that some effusion of plasma does occur in lesions postmortem and especially during hypostasis. The differences between means were, however, not statistically significant as compared to control skin (p>0.01, Table 3).

In heat injuries for the specimens taken immediately after death, the mean albumin level was about 2-fold in injuries aged 30 min, 3-fold in injuries aged 1–24 h, and 5-fold in injuries aged 1–2 weeks as compared to control skin. After 4 weeks the albumin had decreased to levels similar to those in control skin (Fig. 3). In the heat injuries inflicted at 1 min postmortem, the albumin level was not significantly increased as compared to the control skin, and neither hypostasis nor different storage temperatures changed this result (Table 4).

In freezing injuries, the albumin level was about 2-fold in injuries aged 60 min, 3-fold in injuries aged 4 h–3 days, and 5-fold in injuries aged 1–2 weeks. After 4 weeks albumin levels had decreased to levels similar to those of the control skin (Fig. 4). In the freezing injuries inflicted at 1 min postmortem, a slight statistically insignificant increase of up to 1.2-fold was observed in the specimens stored for 4 h at room temperature (Table 5).

Table 3 Effect of postmortem hypostasis on albumin (mg/g dry weight) in lesions inflicted at 1 min postmortem

Time and temperature of hypostasis		1 h 4°C	3 Days 4°C	3 Days 22°C
Incision wounds	M	9.9	11.5	6.5
	SD	1.2	1.6	1.8
Control skin	M	8.8	10.0	6.1
	SD	0.8	1.0	0.9
Excoriations	M	12.8	9.6	10.9
	SD	2.5	3.5	0.7
Control skin	M	9.6	6.8	8.9
	SD	0.9	1.1	1.6

M Means and *SD* standard deviations are given (n=6).
The mean values for the postmortem wounds and excoriations are not significantly different from those for control skin (p>0.01).

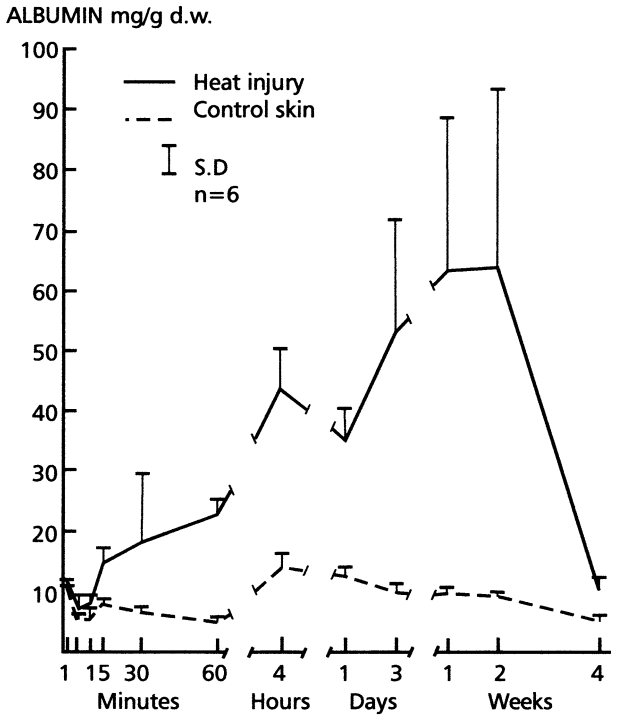

Fig. 3 Albumin mg/g dry weight in heat injuries and in control skin. Means and standard deviations (SD) are given. The mean values for the heat injuries aged 60 min to 2 weeks are significantly higher than those for control skin ($p<0.01$ or $p<0.001$)

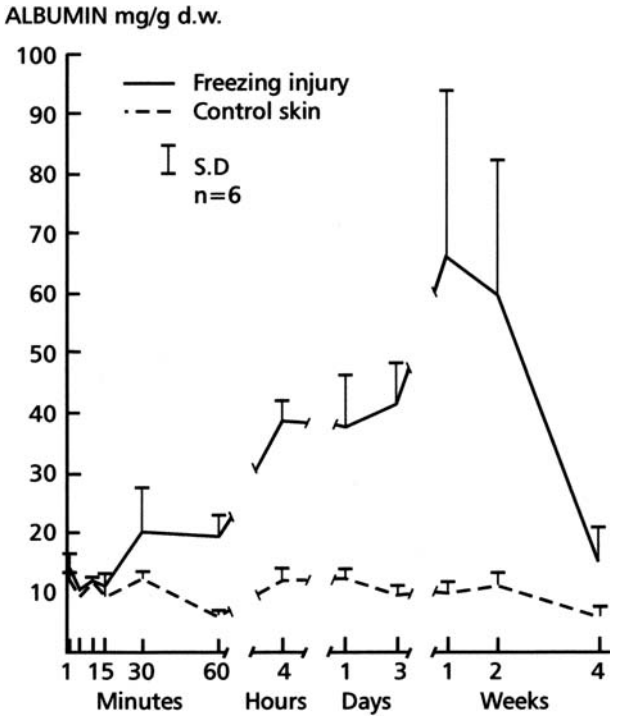

Fig. 4 Albumin mg/g dry weight in freezing injuries and in control skin. Means and standard deviations (SD) are given. The mean values for the injuries aged 60 min to 2 weeks are significantly higher than those for control skin ($p<0.01$ or $p<0.001$)

Table 4 Albumin mg/g dry weight in heat injuries inflicted at 1 min postmortem

Time and temperature of storage		4 h +22°C	3 Days +4°C	3 Days +22°C
Without hypostasis				
Heat injury	M	13.4	11.4	14.6*
	SD	3.1	2.3	1.7
Control skin	M	16.8	11.0	19.5*
	SD	2.0	2.1	1.3
With hypostasis				
Heat injury	M	9.7	15.4	11.3
	SD	2.3	4.4	2.4
Control skin	M	13.9	19.7	12.9
	SD	2.5	2.6	2.4

Specimens were taken after storage of 4 h or storage of 3 days at temperatures of +4°C or +22°C with or without hypostasis.
M Means and *SD* standard deviations are given.
Mean values for heat injuries are not significantly different ($p>0.01$) from those for control skin, *except in one pair of values.

Table 5 Albumin mg/g dry weight in freezing injuries inflicted at 1 min postmortem

Time and temperature of storage		4 h +22°C	3 Days +4°C	3 Days +22°C
Without hypostasis				
Freezing injury	M	14.6	13.8	11.9
	SD	2.2	1.6	1.6
Control skin	M	12.4	14.5	12.9
	SD	3.0	2.9	2.5
With hypostasis				
Freezing injury	M	14.2	13.4	13.4
	SD	2.6	1.7	2.1
Control skin	M	12.7	14.6	13.3
	SD	1.1	2.5	4.1

Specimens were taken after storage of 4 h or storage of 3 days at temperatures of +4°C or +22°C with or without hypostasis.
M Means and *SD* standard deviations are given ($n=6$).
Mean values for freezing injuries are not significantly different from those for control skin ($p>0.01$).

In control skin albumin levels, marked differences were observed, ranging from about 5–20 mg/g dry weight.

Discussion

The liquid chromatography method used for albumin determination was originally developed for human plasma proteins by Tomono et al. [30]. They showed by using immunoelectrophoresis that in their method some other plasma proteins namely α-2HS glycoprotein, α-2 macro-

globulin, β-lipoprotein, C3-component and fibrinogen partially coincided with the peak of albumin. Because our purpose was to use albumin as a marker protein for plasma transudation only and not to measure the absolute amounts of albumin, no efforts were made to estimate portions of other proteins coinciding with the albumin peak. Albumin was however a good marker protein since its peak in liquid chromatography was clearly different from the massive peak of haemoglobin which contaminated all lesion specimens. The concentration of albumin in plasma is also much higher than that of the other proteins mentioned above.

It is well known that all vital lesions contain variable amounts of blood proteins such as albumin due to haemorrhages. For this reason, it was necessary to estimate the amount of albumin directly related to the presence of blood outside or inside vessels. This was done by measuring the concentration of haemoglobin in lesions and by using the average ratio of haemoglobin and albumin in the blood of the rats. The relationship in the whole blood obtained, about 0.1 g albumin per gram haemoglobin, is in concordance with reports in earlier literature [33, 34]. The amounts of albumin directly related to the presence of blood in the specimens mostly varied by less than 10% from the analysis result. The highest value noted was 38%. The value obtained for "blood-unrelated albumin" does not, however, exactly represent the transudation of plasma proteins because the methods used are unable to differentiate between the albumin in the interstitial space of tissue and that inside the lymphatic vessels.

In measurements of the haemoglobin content of specimens, hemin analysis was used relating the amount of hemin to the change in absorbance [31]. Thus most of the harmful effects of turbidity were eliminated in the extracts.

Control skin contained a certain amount of albumin which varied markedly between experimental animals. According to the literature, of all tissues, skin and muscle contain the most extravascular albumin [35].

The increase of blood-unrelated albumin was about 2-fold in incision wounds and about 3-fold in excoriations after 30 min. These results are consistent with earlier reports, in which a rapid albumin effusion in different types of lesions has been demonstrated [13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29]. Also according to the literature, in the effusion an immediate transient response occurs within 10 min, which is then followed by a delayed prolonged response hours after the initial stimulus [15, 16]. Our results showed a rather small increase in the albumin level in the incision wounds during the first 4 h, followed by a higher increase after 12 h. In the excoriations, the increase of albumin was more rapid and greater, the higher level being attained in 60 min. The excoriations likely contained more damaged tissue than the incision wounds, which were produced by a sharp knife, probably resulting in more intensive release of factors inducing vascular permeability. In addition to histamine and serotonin, such factors are known to include prostaglandins PGI 2 and PGE 2, leucotrienes, platelet-activating factor, C5a complement component, bradykinin, fibrinopeptides,and fibrin-split products, which are released from tissue cells, endothelium of vessels, and during the coagulation process [6, 12, 36, 37, 38, 39].

Although most earlier studies have concentrated on a relatively short period after infliction of lesions, some works have demonstrated that increased transudation of albumin could be present in certain lesions even after 30 days [21]. According to the literature there is a turnover (transudation and removal via lymphatics) of serum proteins in vital lesions. In 1-day-old skin lesions produced by sulphur mustard, serum proteins were turned over once in about 8 h, and in 3 and 6-day-old lesions once in about 35 h [24]. Turnover of plasma proteins might explain the rather large variation in albumin levels of lesions studied.

Heat injuries were produced by exposing rat skin to boiling water for 60 s. The burned area was approximately 3 cm^2 being below 5% of the body surface area of the rats used. "Full thickness" (third-degree) skin injury has been described to occur with only a 10 s exposure to boiling water [40]. According to the literature, 100°C steam over 20 s resulted in a 4 mm-deep zone of necrobiosis in the skin [41].

Experimental frostbite was induced by a metal chamber containing liquid nitrogen. The chamber was kept in contact with the skin for 5 min. After this the skin was observed to be frozen all the way through, resulting in a frostbite area of about 3 cm^2. Frostbite induced by liquid nitrogen over a 5 -min period on a rabbit ear was previously shown to produce third or fourth-degree injuries [42].

The increase of albumin in injured tissue was similar in both heat and freezing injuries. The start of the increase was, however, a little slower in the latter. One reason for this might be the time needed for thawing of tissue in freezing injuries. An increase of albumin in burned tissue has been observed in many earlier experimental works [25, 26, 27, 28]. For example in heat injuries brought on by a 10 s application of boiling water and resulting in 10% of the rats body surface area being burned, a 2.8-fold increase in albumin content was reported after 60 min, as measured by radial immunodiffusion [25]. That increase was nearly the same as the one observed in this study in heat injuries after 60 min. Interestingly, however, in the other study, had the burned area been 40% of the body surface area, then the albumin increase after 60 min would have only been 1.3-fold compared with the control value. The authors explained the difference by more severe hypovolaemia in the 40% group, resulting in a more pronounced fall in capillary hydrostatic pressure [25]. The same phenomenon has also been noted in several studies [43, 44]. In an earlier study examining freezing injuries induced by a temperature of −30°C over 30 min in the rat hindlimb, tissue water had increased from 2.1 to 4.5 ml/g d.w. in 30 min after the start of thawing. Simultaneously, albumin clearance measured by radioactive albumins was reported to rise from 0.02 to 2.4 ml/g d.w. The albumin content of the tissue had not been measured in this study [45].

In the present work, the highest albumin levels in burned or frozen skin were observed during the period from 3 days to 2 weeks. The albumin content had returned to control levels after 4 weeks. In heat injuries induced by applying 65–95°C water for 10 s, after about 3 weeks the blood-lymph barrier was observed to have normal permeability, the wound to have healed, and no significant edema to have occurred [15]. The minimum healing time after sustaining a severe dermal heat injury has been reported to be several weeks [46].

In heat injuries two phases of increased vascular permeability have been described [47]. The early phase appears during the first hour and the late phase approximately 4 h post-burn. The early phase develops as a result of complement activation together with anaphylatoxin release and mast cell secretion of histamine. This leads to increased xanthine oxidase activity and production of oxygen radicals. The early phase has been shown to be neurophil-independent [48, 49]. In the late phase, inhibition of the adhesion molecules E-selectin and L-selectin and ICAM-1 reduces vascular leakage. The same happens with neutrophil depletion by antibodies. This phase is complement-independent, but the proinflammatory cytokines IL-6, IL 1, and TNF-α have been established to play an important role, resulting in increased vascular injury and neutrophil influx. Neutrophil-generated reactive oxygen species are assumed to be responsible for the local tissue damage. About 60% of the injury is estimated to be explained by the abovementioned mechanisms while 40% is thought to be directly heat-related [50]. Also frostbite injuries are believed to be mediated more by free radicals than by a direct cold effect [51, 52]. Moreover in freezing injuries, the vascular changes are probably connected to injury of the nerves in cutaneous tissue [53].

Diffusion of plasma proteins is known to take place in tissues postmortem [54, 55, 56]. In the present experiments, the increase of albumin was, however, still demonstrable in the vital incision wounds and excoriations from which the specimens were taken at 3 days postmortem at temperatures of 4°C and 22°C. Diffusion and hypostasis, however, seemed to result in an artificial, statistically insignificant increase of albumin levels in postmortem lesions. While conclusions drawn from rat experiments are not usable in human autopsy practice, we could nonetheless expect that in the human body, postmortem diffusion and hypostasis might have a much greater influence due to the much higher vertical gradient and amount of plasma than in the rat. We could speculate that only when the lesions are located in non-hypostatic areas, might the increase of albumin be of any use for estimation of age and vitality of lesions. In addition the results of this paper are based on model experiments that represent a certain type and size of injury. The degree of the injury and its relationship to the body surface area have been shown to be important factors in modifying results of at least heat injuries [25, 43, 44, 46]. This could presumably also apply to other type of injuries. In freezing injuries, it should be noted that thawing during the lifetime is a prerequisite for reactions to occur in the frozen tissue. However, in forensic medical practice, many frostbite injuries, although incurred antemortem, may not thaw until postmortem.

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