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CASE REPORT

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Heat stroke in an incubator: an immunohistochemical study in a fatal case

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Abstract The authors report the unique case of an 8-day-old infant succumbing to heat stroke caused by an abnormal increase of the environmental temperature in an incubator. At postmortem examination, second-degree burns were detected, and macroscopic and microscopic findings were typical for a heat-related death. An immunohistochemical study was performed. At the same time, a detailed examination of the incubator was conducted, revealing a malfunctioning of the temperature and relative humidity control system. We suggest that the diagnosis of heat stroke has to be confirmed on the basis of a detailed postmortem examination and a complete immunohistochemical investigation of heat shock proteins, molecules produced acutely in response to heat stress.

Keywords Heat stroke · Incubator · Heat shock protein · Hyperthermia

Introduction

Infant and early childhood death caused by environmental hyperthermia (fatal heat stroke) is a rare event sometimes reported in the international literature: generally, death occurs in motor vehicles or in beds [1]. Infants and young children left unattended in motor vehicles are at risk of heat stroke and death because intravehicular temperatures can increase quickly to lethal levels, especially when the vehicles are parked in direct sunlight [2, 3, 4, 5]. Hyperthermic illness and death in bed is often the result of a combination of several factors (well wrapped infant, exertional self-overheating in bed) [6, 7, 8, 9, 10]. This report describes the death of an 8-day-old infant lying in a disfunctioning incubator causing a fatal increase of environmental temperature.

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Case history

R.T. born in the 36th week of pregnancy was found unresponsive at 6:00 a.m by nurses of the hospital, lying in an incubator; spontaneous respiration was already absent, pupils were dilated, there was no light reflex, skin was dry and with burns, and the trunk was hot. Cardiopulmonary resuscitation was unsuccessful. In the history the infant had been healthy and well nourished, no anatomical abnormalities were reported and his last nourishment was at 00:30 a.m. He presented typical aspects of second-degree burns (Fig. 1) at external examination. A postmortem examination was ordered by the public prosecutor's office and carried out a few days after death. A technical report on incubator functioning was also conducted.

Postmortem findings

The body was 45 cm in length and 1,728 g in weight, the skin was diffusely dehydrated and presented also erythema on the right side of the body. On the right side of the body (mandibular ramus, chest, shoulder, axilla, arm, hand, leg) and on the left wrist, second-degree burns of various size and form, were also observed.

A complete autopsy was performed. Petechial haemorrhages were detected over pericardial, epicardial and pleural surfaces, trachea and bronchi appeared apparently free from gastric content, lungs were congested and oedematous. In the stomach 20 ml of thick yellowish material was found. The examination of other organs was unremarkable.

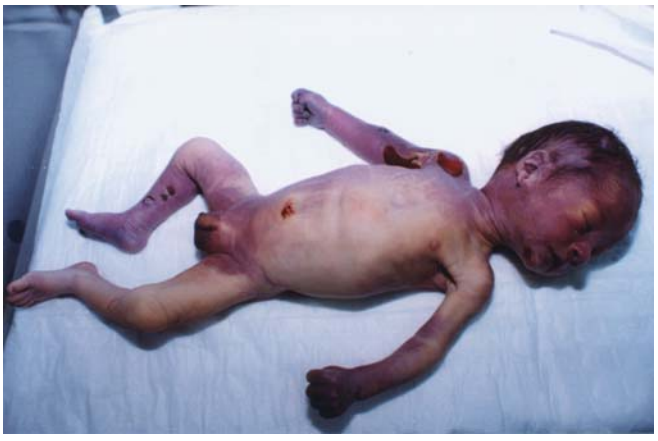

Fig. 1 Macroscopic view of the body of the infant showing second-degree burns

Histological studies

A routine microscopic histopathological study was performed by using formalin-fixed paraffin-embedded tissue sectioned at 4 μm and stained with haematoxylin-eosin and Weigert elastic. Immunohistochemical investigation of lungs, skin, trachea and heart samples were performed utilising monoclonal antibodies (NovoCastra, UK) anti-heat shock protein (HSP 90, 70, 27) and anti-CD 15 (Dako, Netherlands); polyclonal anti α-lactalbumin antibodies (Dako, Netherlands) were also utilised on lung samples. We used 4 μm thick paraffin sections mounted on slides covered with 3, aminopropyl-triethoxysilane (Fluka, Switzerland). A pretreatment was necessary to facilitate antigen retrieval and to increase membrane permeability to antibodies The primary antibody was applied in a 1:50 dilution for HSP 90 and CD 15, in a 1:100 dilution for HSP 70, a 1:20 dilution for HSP 27, a 1:400 dilution for α-lactalbumin and incubated for 120 min at 20°C (HSP 90, HSP 27, CD 15, α-lactalbumin) and overnight at 20°C (HSP 70). The positive reaction was visualised by 3,3 diaminobenzidine (DAB) peroxidase, according to standard methods. The sections were counterstained with Mayer's haematoxylin, dehydrated, covered and observed under a Leica optical microscope.

Histological findings

The histological findings of the trachea revealed disappearance of epithelium and nuclear elongation in the residual epithelial fragments. In the lungs interstitial and intra-alveolar oedema and haemorrhages, acute stasis and areas of acute emphysema were found; in bronchial lumen sloughed-off epithelial cells and moderate eosinophilic amorphous material were detected. Skin sections of burned areas showed a detachment of the upper epidermal areas mainly extending through the basal cell layers with eosinophilic amorphous material and few polymorphonuclear neutrophilic leukocytes in the vesicle fundus. The deeper parts of stratum papillare and underlying upper layers of the chorium were characterised by marked hyperaemia, oedema and to a lesser extent inflammatory cell infiltrates. In other areas the dermo-epidermal detachment was more evident and inside the microvesicles eosinophilic amorphous material, erythrocytes and some inflammatory cells were present (Fig. 2). The presence of polymorphonuclear neutrophilic leukocytes was confirmed by the positive reaction for CD 15. All these findings suggested typical second-degree cutaneous burns.

The cutaneous and tracheal heat injury was confirmed by the positive results to the immunohistochemical reaction for HSP 90, 70 and 27 (Fig. 3). The immunohistochemical staining in lungs samples for α-lactalbumin, a human milk protein, was negative.

The examination of other organs was unremarkable except for brain oedema and generalised haemostasis.

Incubator examination

The incubator was a Vickers Mod 59 built in the first half of the 1970s; technical examination revealed that the incubator did not conform to in force CEI norms because the metallic components close to the lying board exceeded the maximum temperature of 40°C, established from CEI norms, and also because a thermal switch, signalling excess of temperature over 38°C, was missing. Technical assessments showed that the environmental temperature

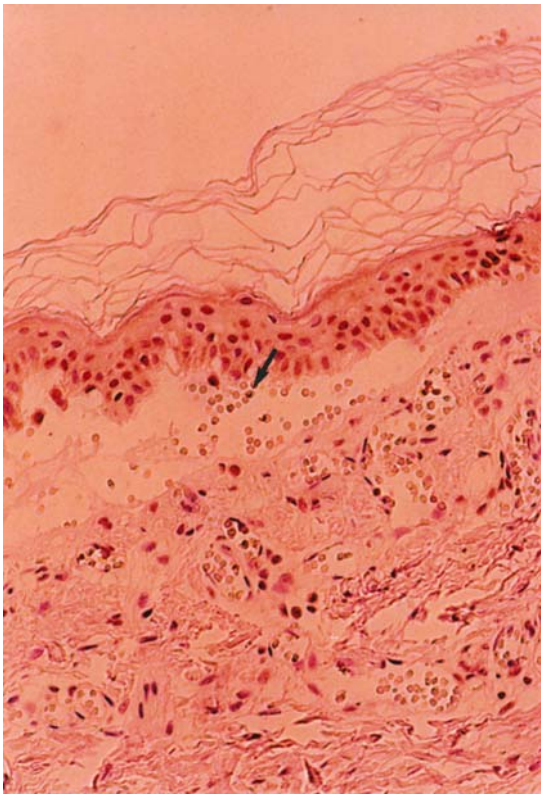

Fig. 2 Cutis with marked hyperaemia, oedema and inflammatory cell infiltrates. Eosinophilic amorphous material, erythrocytes, and few polymorphonuclear neutrophilic leukocytes in the vesicle fundus (*arrow*) (H&E ×20)

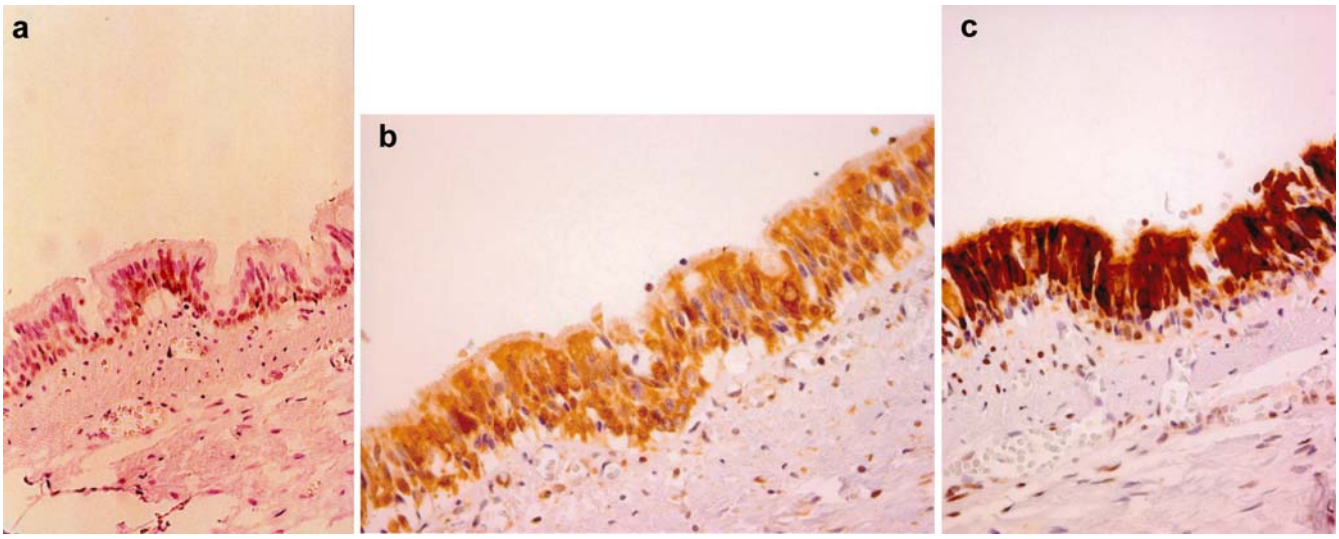

Fig. 3a-c Heat-related changes in epithelium of the trachea, **a** HSP 27 (×40), **b** HSP 70 (×60), and **c** HSP 90 (×80)

set at the beginning was not held constant, showing changes of more than 12°C in a period of 2 h [11]. In the incubator relative humidity was not held constant changing in a range of 30–90%, due to the control system malfunction and fixed on the minimum value of relative humidity and also because of the water level, which was under the minimum standard necessary for the correct functioning of the incubator.

It was also demonstrated that maintenance of the electric circuit had not been performed for at least 3 years, the temperature control system (triac) had been badly maintained and some of its components were strongly oxidised. The incubator was also dirty and a lot of dust, organic material and algae were detected in the water tank for the air humidity.

It was also established that the maximum value of air temperature was 46°C, and the steel components under the lying board next to the infant achieved a temperature of 55.6°C; an alarm control system signalling increasing of temperature was absent.

Discussion

On the basis of macroscopic, microscopic findings, and technical assessment, heat-stress induced pulmonary oedema was recorded as the cause of death.

Our case represents a unique case of heat-related death in infancy due to the malfunctioning of the incubator in which the infant was lying. Technical assessment of the incubator demonstrated a careless maintenance and a malfunctioning of the temperature and relative humidity control systems, so the infant was exposed to an environmental temperature of about 46–55.6°C for at least 5 h, because the alarm system was absent.

Heat stress is a life-threatening failure of the body's thermoregulatory system. Sweating ceases, which drastically reduces the evaporative cooling mechanism. There is a typically physical collapse and hyperthermia, with core body temperatures reaching and often exceeding 41°C [7, 12].

The National Association of Medical Examiner Ad Hoc Committee on the Definition of Heat-Related Fatalities recommends the following definition of "heat-related death": a death in which exposure to high ambient temperature either caused the death or significantly contributed to it [13]. The diagnosis of heat-related death has to be based on a history of exposure to high ambient temperature and the reasonable exclusion of other causes of hyperthermia [14]. It is important to emphasise that humidity is a major component of heat stress, sometimes even more important than air temperature [15]. The diagnosis may be established from the circumstances surrounding the death, investigative reports concerning environmental temperature, and/or measured antemortem body temperature at the time of collapse. In cases where the antemortem body temperature cannot be established but the environmental temperature at the time of collapse was high, an appropriate heat-related diagnosis should be listed as the cause of death or a significant contributing condition [14]. Autopsy findings are not specific and they depend on the duration of survival after hyperthermic exposure: intrathoracic, cutaneous and conjunctival petechiae, pulmonary and cerebral oedema, disseminated intravascular coagulation (DIC) [1]. A few cases of heat-related deaths in infancy are reported in literature, generally involving children found left in closed automobiles; experimental studies demonstrated that with all windows closed, the temperature rose from an ambient level of 36°C to a maximum of 67°C within 15 min and remained high until the doors were opened [2, 3, 4, 5, 16]. More often, heat-related death in infancy is connected to well wrapped children found lifeless in bed [10, 17] or to an excessive temperature of the electric coverlet [8]. Children do not adapt to extremes of temperature as effectively as adults when exposed to high climatic heat stress and the adaptation of adolescents falls somewhere in between. In fact, children have a greater surface area to

body mass ratio than adults, which causes a greater heat gain from the environment on a hot day and a greater heat loss to the environment on a cold day; children produce more metabolic heat per mass unit than adults during physical activities that include walking or running. The sweating capacity is also considerably lower in children than in adults, which reduces the ability of children to dissipate body heat by evaporation [15].

As the body temperature rises to temperatures above 41°C, there is generalised vasodilatation, increased cardiac output, and redistribution of blood away from the splanchnic organs to the skin in order to maximise heat loss, cardiac dilatation, depression of respiratory activity and a generalised process of cellular damage; this often results in circulatory collapse, heart failure and distributive shock.

In this case an immunohistochemical study has been performed researching on tissue heat shock proteins (HSP 70, 27, 90) which are a group of proteins that are rapidly induced in response to physiological stress, including hyperthermia [18], infections [19, 20], tumors [21, 22], ischemic stimuli [23, 24] and exposure to toxicants [10]; experimental studies revealed HSP 70 expression in the early post-burn stages [25]. HSPs fulfill a range of functions, including cytoprotection and the intracellular assembly, folding, and translocation of oligomeric proteins, and represent a rapid response to altered redox states [23, 26, 27]. In addition to acting as cellular chaperones, HSPs mediate cytoprotection by associating with and hindering the action of key apoptotic proteins, and by facilitating the degradation of misfolded intracellular proteins by the ubiquitin/proteasome system, so-called protein triage [23]. How HSPs might impact patient's response to heat stroke and the temporal changes in HSPs expression are questions that currently have few answers [28, 29].

Our case suggests the possibility of heat-related death in an absolutely unsuspected environment. The diagnosis of heat stroke has to be confirmed on the basis of a detailed postmortem examination and a complete immunohistochemical study based on heat shock proteins, molecules produced acutely in response to heat stress.

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