Inflammatory, hemostatic, and clinical changes in a baboon experimental model for heatstroke

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Departments of 1Comparative Medicine, 2Pathology and Laboratory Medicine, 4Biostatistics Epidemiology and Scientific Computing, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia; 3Institut National de la Santé et de la Recherche Médicale U479, Faculté Xavier Bichat, Paris, and 3Hôpital Louis Mourier, AP-HP, Colombes, France

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Bouchama, A., G. Roberts, F. Al Mohanna, R. El-Sayed, B. Lach, S. Chollet-Martin, V. Ollivier, R. Al Baradei, A. Loualich, S. Nakeeb, A. Eldali, and D. de Prost. Inflammatory, hemostatic, and clinical changes in a baboon experimental model for heatstroke. J Appl Physiol 98: 697–705, 2005. First published October 15, 2004; doi:10.1152/japplphysiol.00461.2004.—The mortality and neurological morbidity in heatstroke have been attributed to the host’s inflammatory and hemostatic responses to heat stress, suggesting that immunomodulation may improve outcome. We postulated that an experimental baboon model of heatstroke will reproduce human responses and clinical outcome to allow testing of new therapeutic strategies. Eight anesthetized juvenile baboons (Papio hamadryas) were subjected to heat stress in an incubator maintained at 44–47°C until rectal temperature attained 42.5°C (moderate heatstroke; n = 4) or systolic arterial pressure fell to <90 mmHg (severe heatstroke; n = 4) and were allowed to recover at room temperature. Four sham-heated animals served as a control group. Rectal temperature at the end of heat stress was 42.5 ± 0.0 and 43.3 ± 0.1°C, respectively. All heat-stressed animals had systemic inflammation and activated coagulation, indicated by increased plasma IL-6, prothrombin time, activated partial thromboplastin time, and D-dimer levels, and decreased platelet count. Biochemical markers and/or histology evidenced cellular injury/dysfunction: plasma levels of thrombomodulin, creatinine, creatine kinase, lactic dehydrogenase, and alanine aminotransferase were increased, and varying degrees of tissue damage were present in liver, brain, and gut. No baboon with severe heatstroke survived. Neurological morbidity but no mortality was observed in baboons with moderate heatstroke. Non-survivors displayed significantly greater coagulopathy, inflammatory activity, and tissue injury than survivors. Sham-heated animals had an uneventful course. Heat stress elicited distinct patterns of inflammatory and hemostatic responses associated with outcome. The baboon model of heatstroke appears suitable for testing whether immunomodulation of the host’s responses can improve outcome.

Heat stress; hyperthermia; interleukin-6; inflammation; coagulation; multiorgan system dysfunction

Heatstroke is a life-threatening illness characterized by a rapidly increasing core body temperature (Tc) (>40°C) and central nervous system abnormalities such as delirium, convulsions, and/or coma after exposure to a high ambient temperature (classical or nonexertional heatstroke) or strenuous exercise (exertional heatstroke) (8, 25). Heatstroke can progress to multiple organ dysfunction/injury syndrome (MODS) and death, despite adequate lowering of the victim’s body temperature and intensive care (1, 8, 15, 25). Up to 30% of survivors may sustain permanent neurological damage (8, 15). The high mortality and neurological morbidity in heatstroke, despite cooling and supportive treatment, are largely due to the fact that the mechanisms of MODS are not well understood and that no specific treatment is available (8).

Studies in humans and animals suggest that the inflammatory and hemostatic responses of the host to heat stress contribute to the multiple tissue and organ injury in those who survive the initial deleterious effects of hyperthermia (1, 4–13, 15, 23, 27–31, 34–36, 40). Hemorrhagic diathesis is invariably present in victims of fatal heatstroke, and autopsy findings include hemorrhage and necrosis with widespread microthrombi in lungs, brain, kidneys, heart, liver, and gut (1, 13, 15, 31, 34, 36, 40). High levels of pro- and anti-inflammatory cytokines are detected in humans and in animal models, and they correlate with the organ failure and fatal outcome (5, 8, 9, 12, 23, 27–30). Normalizing the body temperature by cooling does not prevent inflammation, activation of coagulation, and progression to MODS (5–9, 23). These findings suggest that disseminated intravascular coagulation and excessive activation of inflammation may be major pathological mechanisms.

Recent findings in small-animal models of heatstroke suggest that immunomodulation of the host response may alter the clinical course of heatstroke and thereby improve outcome (27–29). In rats and rabbits, heatstroke induces production of TNF-α and IL-1 in the central nervous system and systemically, and this is associated with severe neuronal injury and high mortality. The administration of an IL-1 receptor antagonist or corticosteroids before the onset of heatstroke attenuates neurological injury and improves survival (27–29). However, extrapolation of data from small laboratory animals cannot predict reliably the human responses because of interspecies differences. In addition, the assessment of clinically relevant outcomes such as the cognitive and neurological disturbances that are major complications of heatstroke is not easy because of anatomic and physiological differences from humans (8, 15, 25, 42, 43). Moreover, emerging evidence from studies on sepsis suggests that important interactions occur in vivo between the inflammatory and coagulation pathways and treatment that inhibits both reduces mortality (2, 14, 17, 32, 38, 39).

For these reasons, the experimental nonhuman primate model of heatstroke appears better suited to the examination of...
Animals

MATERIALS AND METHODS

Table 1. Thermal responses in baboons subjected to heat stress

<table>
<thead>
<tr>
<th>Heat Response</th>
<th>Sham-Heated Control</th>
<th>Moderate Heatstroke</th>
<th>Severe Heatstroke</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>4.5±0.2</td>
<td>4.2±0.4</td>
<td>4.2±0.3</td>
</tr>
<tr>
<td>Incubator temperature, °C</td>
<td>27.7±0.5</td>
<td>44.2±0.9</td>
<td>44.0±0.4</td>
</tr>
<tr>
<td>Incubator humidity, %</td>
<td>36.3±1.1</td>
<td>35.4±1.7</td>
<td>36.1±1.9</td>
</tr>
<tr>
<td>Duration of heat exposure, min</td>
<td>267±52</td>
<td>303±61</td>
<td>361±52</td>
</tr>
<tr>
<td>Tc, maximum, °C</td>
<td>36.5±0.3</td>
<td>42.5±0.0</td>
<td>43.3±0.1*</td>
</tr>
<tr>
<td>Heat load, °C/min</td>
<td>0</td>
<td>249±43.6</td>
<td>316±35.6</td>
</tr>
<tr>
<td>Heating rate, °C/min</td>
<td>0</td>
<td>0.019±0.003</td>
<td>0.026±0.005</td>
</tr>
<tr>
<td>Time at &gt;40.4°C, min</td>
<td>0</td>
<td>155±47</td>
<td>180±45</td>
</tr>
<tr>
<td>Cooling rate, °C/min</td>
<td>0.056±0.005</td>
<td>0.048±0.007</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Tc, core temperature. Sham-heated control animals are shown for indication. Statistical comparisons were made by Student’s t-test between moderate and severe heatstroke groups. *Difference between groups was significant (P < 0.05).

stroke) or hypotension occurred (systolic arterial pressure <90 mmHg), which was taken as the onset of severe heatstroke, as in other reported studies (19, 20, 28, 29). Sham-heated baboons were handled in an identical manner but without heat stress and served as a control group.

After an overnight fast with water ad libitum, each baboon was immobilized by an intramuscular injection of 15 mg/kg ketamine hydrochloride, intubated, and given a continuous intravenous infusion of ketamine (20–25 mg·kg⁻¹·h⁻¹) and diazepam (0.4–0.8 mg/kg intravenously every 2 h) with a concomitant infusion of dextrose normal saline at 5 ml·kg⁻¹·h⁻¹, to maintain anesthesia and vascular stability. A percutaneous angiocatheter was inserted into the cephalic vein, and indwelling venous and arterial catheters were placed aseptically via femoral cutdown, for administration of drugs and fluids, continuous monitoring of blood pressure, and blood sampling.

Methods

Heat stress protocol. After stabilization, the study animals were placed in a prewarmed neonatal incubator (Isolette infant incubator; Air-shield, Hatboro, PA) maintained at 44–47°C and relative humidity of 33–39%. Tc was monitored by a reusable, pediatric rectal thermistor probe calibrated for 0 –70°C with an accuracy of ±0.15°C (Yellow Springs Instruments, Yellow Springs, OH). The probe was positioned 7–8 cm above the anal sphincter. For the severe heatstroke group, exposure was terminated when systolic arterial pressure fell to <90 mmHg, and this occurred when Tc rose above 43°C. For the moderate heatstroke group, exposure was terminated when Tc reached 42.5°C, regardless of the systolic arterial pressure.

The animals were removed from the incubator and allowed to cool passively at an ambient temperature of 26–29°C. Normal saline was given as needed to maintain a mean arterial pressure (MAP) >60 mmHg. All animals in the study group were maintained under anesthesia and monitored until the last sample (time T + 3 h) for the first day of the study was taken, normalization of the Tc, and stabilization of the vital signs, or death. Anesthesia was then discontinued; vascular access and orotracheal tube were removed. Survivors were observed and assessed for evidence of bleeding or neurological changes for an additional 72 h. Baboons surviving for 72 h were considered perma-

Table 2. Vital sign changes in control and heat-stressed study groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>T –8</th>
<th>T +0</th>
<th>T +1</th>
<th>T +2</th>
<th>T +3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP, * mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>119±12</td>
<td>120±11</td>
<td>117±7</td>
<td>120±8</td>
<td>140±10</td>
</tr>
<tr>
<td>Moderate heatstroke</td>
<td>95±6</td>
<td>150±13</td>
<td>118±2</td>
<td>110±5</td>
<td>102±9</td>
</tr>
<tr>
<td>Severe heatstroke</td>
<td>102±6</td>
<td>82±8</td>
<td>119±24</td>
<td>116±11</td>
<td>108±9</td>
</tr>
<tr>
<td>DAP, * mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>70±12</td>
<td>62±6</td>
<td>55±8</td>
<td>58±9</td>
<td>75±5</td>
</tr>
<tr>
<td>Moderate heatstroke</td>
<td>45±4</td>
<td>87±8</td>
<td>69±5</td>
<td>56±3</td>
<td>54±4</td>
</tr>
<tr>
<td>Severe heatstroke</td>
<td>60±11</td>
<td>40±2</td>
<td>44±7</td>
<td>36±3</td>
<td>35±2</td>
</tr>
<tr>
<td>RR, * breaths/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>26±3</td>
<td>25±2</td>
<td>25±2</td>
<td>24±2</td>
<td>26±2</td>
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<tr>
<td>Moderate heatstroke</td>
<td>28±3</td>
<td>48±5</td>
<td>42±4</td>
<td>43±6</td>
<td>38±3</td>
</tr>
<tr>
<td>Severe heatstroke</td>
<td>29±4</td>
<td>40±3</td>
<td>43±3</td>
<td>46±4</td>
<td>59±4</td>
</tr>
<tr>
<td>SpO₂, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>99±1</td>
<td>98±1</td>
<td>97±2</td>
<td>98±2</td>
<td>92±5</td>
</tr>
<tr>
<td>Moderate heatstroke</td>
<td>93±6</td>
<td>99±1</td>
<td>100±0</td>
<td>100±0</td>
<td>100±0</td>
</tr>
<tr>
<td>Severe heatstroke</td>
<td>98±2</td>
<td>97±3</td>
<td>99±2</td>
<td>100±0</td>
<td>100±0</td>
</tr>
</tbody>
</table>

Values are means ± SE changes in systolic arterial pressure (SAP), diastolic arterial pressure (DAP), respiratory rate (RR), and pulse oximetry saturation (SpO₂) at baseline (time T = 8 h), onset of heatstroke (T = 0 h), and cooling (T +1, T +2, and T +3 h). NA, not available. *Difference between groups by ANOVA for repeated measurements was significant (P < 0.05). No significant difference in SAP, DAP, and RR was evident when severe was compared with moderate heatstroke.

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nent survivors and were subsequently killed with pentobarbital sodium (100 mg iv) for necropsy. The animals in the control group were sham-heated in the same neonatal incubator preset at a temperature of 27.7 ± 0.3°C and humidity of 36 ± 3% for a time comparable with that for the study groups.

Monitoring and blood and tissue sampling. Rectal temperature, heart rate, arterial pressure, and oxygen saturation measured by pulse oximetry were monitored continuously with a bedside monitor (Marquette 7010, Milwaukee, WI). Urine output was monitored hourly via a Foley catheter inserted aseptically. Blood samples were collected at baseline before commencement of heat exposure (T = 8 h), at the end of heat exposure (T = 0 h), and 1 (T + 1), 2 (T + 2), 3 (T + 3), 12 (T + 12), and 36 (T + 36) h. Blood samples at 12 and 36 h were drawn by percutaneous arterial femoral puncture after anesthesia with ketamine 20 mg/kg im. Tissue samples were taken from deceased baboons immediately and from survivors after euthanasia.

Biochemical measurements and tissue preparation. Properly calibrated and controlled automated devices were used to determine complete blood counts (CellDyn 4000, Abbott Diagnostics, Santa Clara, CA), liver and renal profiles (Hitachi 912, Mannheim-Boehringer, Mannheim, Germany), and coagulation profiles (BCS, Dade-Behring, Miami, FL).

Interleukin-6 (IL-6) and thrombomodulin were assayed in plasma by using specific ELISA (Quantikine, R&D Systems, Minneapolis, MN, and Diagnostica Stago, Asnières, France, respectively), according to the manufacturers’ instructions. The detection limits were 3 pg/ml and 6.87 ng/ml, respectively.

Light microscopy analysis of brain, liver, and gut was performed on tissue slices fixed in neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin.

End points. Three end points were studied. They were 1) inflammatory and hemostatic responses to heat stress: white blood cell count (WBC), plasma IL-6 concentrations, prothrombin time (PT), activated partial thromboplastin time (aPTT), D-dimer, fibrinogen, and platelet count; 2) cell and tissue injury: plasma creatinine and thrombomodulin concentrations, alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and creatine kinase (CK) activities were used as markers of renal dysfunction and endothelial, hepatic, and muscular cell injury, respectively, and histopathology of brain, liver, and gut; and 3) outcome: mortality, or neurological morbidity in survivors.

Thermal calculations. Heat stress was quantified by determination of heat load, a product of magnitude of Tc above 40.4°C and duration of hyperthermia, as described elsewhere (22, 24). Tc was recorded at 15-min intervals and heat load (°C-min) was calculated as Σ time interval (min) [Tc (°C) above 40.4°C – 40.4°C]. Heating rate (°C/min) was calculated as [maximum Tc (°C, Tc max) attained during heat
exposure. \( T_c \) (°C) recorded before heat exposure] / time (min) to attain \( T_{c, max} \). Cooling rate (°C/min) was calculated as [\( T_{c, max} \) (°C) – 40.4°C] / time (min) for passive cooling to \( T_c \) of 40.4°C.

### Statistical Analysis

Statistical analysis was performed using a computer program package (SAS Institute, Cary, NC). Excel software was used for the line graphs. Comparisons between groups during the course of the observation period were performed by repeated-measures ANOVA. ANOVA was used to determine significance of difference in means between groups at given times. Linear regression was applied to determine correlation coefficients. Differences were considered significant at \( P < 0.05 \), and the data are expressed as means ± SE.

### RESULTS

**Thermal and Cardiorespiratory Responses to Heat Stress and Passive Cooling**

Table 1 shows that the intensity of heat stress assessed by the \( T_{c, max} \), time above \( T_c \) of 40.4°C, and the heat load was more marked in severe than in moderate heatstroke. Heat stress induced tachycardia, elevation of blood pressure, and tachypnea with no significant changes in oxygen saturation (Table 2). There was a significant correlation between \( T_c \) and systolic arterial pressure (r = 0.64 and \( r = 0.79 \), \( P < 0.0001 \)), heart rate (r = 0.90 and \( r = 0.85 \), \( P < 0.0001 \)), and respiratory rate (r = 0.30, \( P < 0.01 \) and \( r = 0.42 \), \( P < 0.001 \)) during heat exposure in severe and moderate heatstroke, respectively.

During cooling, the decrease in \( T_c \) was similar for all heat-stressed animals, suggesting that cardiovascular collapse and tissue injury had not affected the ability of the animals to eliminate heat (Fig. 1 and Table 1). Systolic and diastolic blood pressure, MAP, and heart and respiratory rates (during cooling) were significantly different between the three groups (Fig. 1 and Table 2); however, no significant difference was evident when severe heatstroke was compared with moderate heatstroke. Animals in both groups required normal saline to maintain MAP > 60 mmHg. Although the animals in the severe heatstroke group received a significantly larger volume of fluid than those with moderate heatstroke (416 ± 66 vs. 281 ± 31 ml; \( P < 0.05 \)), the increase in MAP was only temporary at \( T + 1 \) h, after which it declined and remained significantly lower (\( P = 0.02 \)) (Fig. 1). This was associated with a lower urine output (72 ± 40 vs. 272 ± 38 ml). The low MAP was essentially due to a low diastolic arterial pressure, suggesting that the shock state is distributive (Table 2). Sham heating induced mild increase in heart rate and blood pressure without the pattern of progressive rise observed in the study groups (Fig. 1 and Table 2).

**Inflammatory and Hemostatic Responses to Moderate and Severe Heatstroke**

IL-6 and leukocytes. Figure 2A shows that circulating IL-6 was not detected in any animal at baseline (\( T = 8 \) h). Plasma IL-6 concentrations were increased at the onset of heatstroke (\( T + 0 \) h) in all heat-stressed animals compared with sham-heated control group (\( P < 0.0001 \)). A large difference between the plasma concentrations of IL-6 in animals with severe vs.

**Fig. 3. Hemostatic response patterns in control and heat-stressed study groups.** Values represent means ± SE in prothrombin time (PT), activated partial thromboplastin time (aPTT), D-dimer, fibrinogen, and platelet count. The difference between the 3 groups was significant by repeated-measurements ANOVA for PT, aPTT, D-dimer, and platelets. A significant difference in PT and D-dimer (but not for aPTT and platelets) was evident when moderate heatstroke was compared with control (\( P < 0.001 \) and \( P < 0.01 \), respectively). NS, not significant.
moderate heatstroke was observed during cooling ($T + 1$, $T + 2$, and $T + 3$ h) ($P < 0.001$) (Fig. 2A). The plasma IL-6 concentrations were sustained in moderate heatstroke, whereas a steep increase was noted in severe heatstroke. The peak IL-6 levels coincided with the severity of the clinical manifestations, tissue injury, and death.

Figure 2B shows that the leukocyte response patterns were significantly different among the three groups from the onset of heatstroke ($T + 0$ h) through the cooling ($T + 1$, $T + 2$, and $T + 3$ h) and recovery period ($T + 12$, $T + 36$ h) ($P < 0.0001$). A significant leukopenia occurred at the onset of severe heatstroke, reaching a nadir of $2.4 \pm 2.6 \times 10^9$/l at $T + 1$ h because of marked neutropenia ($0.7 \pm 0.9 \times 10^9$/l), before returning to normal. Baboons with moderate heatstroke developed significant leukocytosis, which peaked at $T + 3$ h and remained elevated at $T + 12$ h. The control baboons displayed a mild leukocytosis during the experiment.

Coagulation. Figure 3 shows that, before heat exposure, the animals in the three groups had normal global coagulation tests, namely PT, aPTT, D-dimer, platelets, and fibrinogen. Heat stress induced activation of coagulation as indicated by significantly increased PT, aPTT, and D-dimer and decreased platelet count. The time course and intensity of the coagulation activation diverged widely between severe and moderate heatstroke. The hemostatic variables remained mildly deranged in moderate heatstroke but were markedly disturbed in severe heatstroke. The rapid decline in platelets count ($124 \pm 70 \times 10^9$/l) and fibrinogen ($0.7 \pm 0.5$ g/l) in $<3$ h suggests that the hemostatic response in severe heatstroke was insufficiently compensated (39).

Cellular Injury, Organ Dysfunction, and Outcome in Moderate and Severe Heatstroke

**Metabolic changes.** Figure 4 and Table 3 show that heat stress resulted in metabolic alteration, cellular injury, and organ dysfunction, differing in magnitude and time course according to its severity. Animals with severe heatstroke displayed an oliguric renal failure with an increase in blood urea and plasma creatinine levels, hypobicarbonatremia, hyperchloremia, and mild to moderate changes in blood sugar, sodium, and potassium levels (Table 3). Blood sugar should be interpreted with caution because the animals were receiving dextrose with their normal saline, as a preventive measure after severe hypoglycemia was observed in the first baboon. Moreover, the variables measured were not adjusted to blood volume, and these could have been affected by the large difference in fluid balance between the study groups. Although blood volume was not measured, the lack of statistically significant difference in hematocrit levels when animals with severe or
significant difference in hematocrit level was evident when severe was com-

bicarbonate (HCO₃⁻, mmol/l), and glucose (mmol/l). *Difference between

K⁺). sinusoidal dilatation (solid arrow) engorged with blood cells (dashed arrows) that extends across the CV wall (arrowhead) and accompanied by accumulation

either lying free (solid arrow) or adhering to the endothelium (dashed arrows) in moderate heatstroke (Fig. 5). Early Purkinje cells necrosis (solid arrows) and
edema of lamina propria (solid arrow) in severe heatstroke (Fig. 5); desquamation of surface epithelium (arrowheads) and markedly dilated capillaries with engorgement by red blood cells

moderate heatstroke results in more extensive tissue injury than moderate

ical markers and/or histopathology suggest that severe heat-

there are two response patterns distinguished by magnitude and time

course and their relation to outcome. Moderate heatstroke

resulted in mild to moderate inflammatory and hemostatic

responses, which were self-limited and subsided after 36 h with

recovery of the animals. Severe heatstroke led to an excessive

response that appeared to be out of control and culminated in

the demise of the animals. These observations support the

hypothesis of the physiological/pathophysiological role of in-

flammation and hemostasis in heatstroke similar to trauma and

sepsis (2, 8, 18, 32, 38, 39).

Studies in patients with heatstroke show marked elevation of pro-

and anti-inflammatory cytokine levels, acute-phase pro-

tiens, leukocytosis, and activation of endothelial cells as sug-

gested by increased circulating markers such as von Wille-

brand factor antigen, intercellular adhesion molecules, and

moderate heatstroke are compared (Table 3) suggests that any

effect on measured variables is likely to be minimal.

The animals with moderate heatstroke showed a similar

pattern of metabolic alteration but at much lower magnitude

and with the exception of sustained hypokalemia.

Cellular and organ injury/dysfunction. Changes in biochemical

markers and/or histopathology suggest that severe heat-

stroke results in more extensive tissue injury than moderate

heatstroke (Figs. 4 and 5). Animals with severe heatstroke

exhibited a marked increase in plasma thrombomodulin levels

and activities of LDH and ALT, suggesting endothelial and

liver injury. The CK activity was also elevated but without

reaching statistical significance. Histological examination

showed that the injury to the liver was multifocal, disrupting

the hepatocellular architecture, and included sinusoidal con-

gestion with intrasinusoidal and central vein accumulation of

erthrocytes and neutrophils (Fig. 5). The injury to the jejunum

was located in the villi, with tissue loss and desquamation

and exposure of the lamina propria where there was edema together

with capillary dilatation and congestion by erythrocytes.

Changes in the liver and jejunum were minimal in moderate

heatstroke (Fig. 5). The central nervous system (CNS) showed

cytoplasmic eosinophilia and nuclear pyknosis in the scattered

neurons of the hippocampus and pallidum, as well as in the
cerebellar Purkinje cells; this was more pronounced and wide-

spread in severe than in moderate heatstroke (Fig. 5). No

significant abnormalities were noted in the control animals.

Outcome. All animals with severe heatstroke died. The

median time of death was 189 min (range: 145–265 min) from

the onset of heatstroke. There were no fatalities in moderate

heatstroke; however, three of four survivors had lethargy and

weakness in all limbs, which subsided after 72 h.

DISCUSSION

The aim of this study was to characterize the inflammatory

and hemostatic responses to heatstroke, their time course
during cooling and rehydration, and their relation to cellular

injury, organ dysfunction, and death. For this purpose, anes-

thetized baboons were subjected to environmental heat to a
degree similar to that of hot climates (44–47°C), until their Tc

attained 42.5°C (moderate heat stroke) or to the onset of severe

heatstroke marked by hypotension, as described in experi-

mental rodent and monkey models (19, 20, 28, 29). The baboons,

like the monkeys, became hypotensive at Tc above 43°C and

exhibited a high mortality rate (19, 20). Anesthetized sham-

heated baboons handled in an identical manner but without

heat stress were used for comparison.

This study establishes clearly that systemic inflammation

and activation of coagulation represent important components

of the host response to heat stress. It also shows that there are

two response patterns distinguished by magnitude and time

course and their relation to outcome. Moderate heatstroke

resulted in mild to moderate inflammatory and hemostatic

responses, which were self-limited and subsided after 36 h with

recovery of the animals. Severe heatstroke led to an excessive

response that appeared to be out of control and culminated in

the demise of the animals. These observations support the

hypothesis of the physiological/pathophysiological role of in-

flammation and hemostasis in heatstroke similar to trauma and

sepsis (2, 8, 18, 32, 38, 39).

Table 3. Blood electrolytes, hematocrit, and glucose levels in control and heat-stressed study groups

<table>
<thead>
<tr>
<th></th>
<th>T = 8</th>
<th>T = 0</th>
<th>T = 3</th>
<th>T = 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea*</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Moderate heatstroke</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Severe heatstroke</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
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<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
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<td>10.0</td>
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</tbody>
</table>

Values are mean ± SE changes in blood urea (urea, mmol/l), hematocrit (%), potassium (K⁺, mmol/l), sodium (Na⁺, mmol/l), chloride (Cl⁻, mmol/l), bicarbonate (HCO₃⁻, mmol/l), and glucose (mmol/l). *Difference between groups by ANOVA-repeated measurements was significant (P < 0.05). †No significant difference in hematocrit level was evident when severe was compared with moderate heatstroke.
soluble thrombomodulin (4, 5, 7–9, 12, 23, 30, 35). The present study shows for the first time that similar changes can be replicated in the experimental baboon model for heatstroke. We found an early systemic inflammatory response before the occurrence of hypotension, as indicated by increased production of IL-6, a key cytokine that modulates local and systemic acute inflammatory response, which encompasses hepatic production of acute-phase proteins, leukocytosis or leukopenia, and endothelial cell activation or injury assessed by increased thrombomodulin levels (8, 18, 41). As in humans, the inflammatory response was sustained during the course of moderate heatstroke and was exacerbated when heatstroke was more severe (5, 8, 9, 23). The animals with severe heatstroke exhibited a marked increase in plasma IL-6 levels that continued to rise during resuscitation and cooling, peaking just before their demise. This is strikingly similar to the kinetics of circulating IL-6 in humans with near-fatal heatstroke, in whom cooling attenuates but does not suppress the inflammation and in which the highest plasma IL-6 levels correlate with poor outcome (5, 8, 9, 23).

Previous studies in monkeys and rodents suggest that endotoxin, originating from the gastrointestinal tract, fuels the inflammatory response (19, 20, 22, 26). In heat-stressed monkeys, endotoxin enters the circulation at a Tc as low as 40°C, and its level increases with the rise in Tc, reaching maximum above 43°C (19, 20). A series of elegant work in rodents attributed the leakage of endotoxin to gut and liver being damaged by heat and ischemia (22, 26). Endotoxin, a major cell wall component of gram-negative bacteria, is a potent agonist for the release of cytokines and thus could have contributed to the exacerbation of the systemic inflammation observed in baboons with severe heatstroke. Although endotoxin was not measured in this study, the pathological examination revealed widespread injury to liver and gut in severe heatstroke, thus lending support to this mechanism.

Coagulation and fibrinolysis are frequently activated during heatstroke and may progress to disseminated intravascular coagulation (DIC) (1, 6, 13, 15, 31, 34, 36, 40). The occurrence of DIC in heatstroke has been associated with poor outcome (1, 6, 15, 25, 34). This study confirms that heat stress induces activation of coagulation that can progress to full-blown DIC as a function of the severity of heatstroke. The time course, namely the rapid worsening of DIC, despite cooling in baboons with severe heatstroke, closely resembles that described in near-fatal heatstroke patients (1, 6, 15, 34). There is no specific therapy for the coagulopathy of heatstroke, essentially because the defective physiological mechanisms of the coagulation pathways are not well known (1, 6, 8, 15, 25, 34). This study shows that baboons can be used for identifying the pathogenic mechanisms of the coagulation disturbances in heatstroke and for testing novel therapies directed to these pathways (2, 32, 38, 39).

The endothelial cell represents one of the main targets for the actions of coagulation proteins and cytokines (7, 8, 16, 17, 35, 39). Endothelial cells express thrombomodulin, a transmembrane glycoprotein that inhibits the procoagulant activity of thrombin (3, 16). The thrombin-thrombomodulin complex accelerates the activation of protein C, which in turn neutralizes factors Va and VIIIa, resulting in anticoagulation (16). Inflammatory cytokines downregulate thrombomodulin gene expression and internalize thrombomodulin, thus reducing its availability at the endothelial surface (3, 16). Paradoxically, these mediators are associated with increased circulating levels that have been attributed to the cleavage of thrombomodulin from the cell surface and liberation in the circulation after endothelial injury (3). Hence increased circulating thrombomodulin is regarded as a marker of the degree of endothelial injury (3). In this study, inflammation and coagulation are activated simultaneously; they progress in parallel and are associated with outcome. Nonsurviving animals exhibited greater inflammatory activity, coagulopathy, and endothelial injury than surviving animals, suggesting, as evidenced in sepsis, that their cross talk may have contributed to endothelial injury, organ failure, and death (2, 14, 16, 17, 32, 38, 39). This observation enhances the importance of the link between inflammation and coagulation to the definition of new therapeutic approaches (2, 14, 17, 38, 39).

This study demonstrates that experimental heatstroke in baboons can mirror the full spectrum of human heatstroke. Both groups of moderate and severe heatstroke fulfilled the clinical triad used for the diagnosis of classic human heatstroke, namely, hyperthermia, CNS alteration, and a history of exposure to a high ambient temperature (8, 25). Although the CNS alterations could not be assessed in the baboons immediately before and during cooling because of the effects of anesthesia, the neurological alterations observed in the survivors at 24 h suggest that CNS injury was probably masked by the anesthesia. This is supported by histological evidence of cortical neuronal and cerebellar Purkinje cell death. Purkinje cell shrinkage and disappearance is a characteristic feature of human heatstroke at necropsy. This feature is contributory to the progressive cerebellar atrophy with debilitating and permanent sequelae for survivors (8, 15, 25, 42, 43). The baboon model duplicates this specific injury and hence affords the possibility to unravel its mechanisms.

Many of the clinical and laboratory features of classic heatstroke, such as a distributive shock, normal or low potassium levels, and hyperglycemia, are also manifest in this model (8, 25, 33, 37). However, there are a few differences between heatstroke in baboons and in humans. The baboons are under the influence of anesthesia, which is known to interfere with both thermoregulatory and cardiovascular responses. Also, the observed leukopenia and metabolic alterations, such as severe hypoglycemia and hyperchloremia, are uncommon in humans with classic heatstroke, although in this study the latter may have been partly iatrogenic because of the large amount of normal saline given for resuscitation (8, 25, 37). The shock state observed in human heatstroke is usually responsive to cooling and volume expansion, but this was not the case in the baboons (8, 25). Finally, to avoid adding another confounding factor, vasopressive drugs were not used for resuscitation. As a result, the baboons remained hypoperfused, and ischemic injury may have superimposed and masked more specific heat injury.

In conclusion, although the number of animals in the study groups was small, the baboons displayed a uniform response and reacted similarly to humans with moderate to fatal heatstroke, in terms of inflammatory and hemostatic responses. Moreover, cellular injury, neurological morbidity, and mortality reproduced closely the biological and clinical manifestations in human disease. The baboon could therefore be a suitable model for future study on the inflammatory and coagulation pathways in heatstroke and for testing whether thera-
heuristic intervention targeting these pathways can alter the clinical course and improve outcome.

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