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Telemetric Evaluation of Body Temperature and Physical Activity as Predictors of Mortality in a Murine Model of Staphylococcal Enterotoxic Shock

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Background and Purpose: **Hypothermia and death are used as experimental markers in murine models of staphylococcal enterotoxic shock. This study determined whether body temperature and physical activity, monitored telemetrically, could predict impending death and provide an earlier, more humane experimental endpoint.**

Methods: **The study consisted of two iterations (experiments 1 and 2) to determine reproducibility of the model. Each experiment consisted of 24 BALB/c mice surgically implanted with intra-abdominal telemetry transmitters and then injected intraperitoneally with sublethal or lethal doses of staphylococcal enterotoxin B (SEB) and/or lipopolysaccharide (LPS). Core body temperature and physical activity were continuously monitored in all mice for 10 days before, and 5 days after, injections. Additionally, in experiment 2, subcutaneous temperatures were compared with core body temperatures obtained by telemetry.**

Results: **Body temperature and physical activity were reduced in mice after administration of SEB and LPS, or LPS alone, but not SEB only. There was a significant ($P < 0.05$) correlation between mortality and body temperature ($P = 0.0077$), but not physical activity ($P = 0.97$).**

Conclusion: **Body temperature proved to be an early indicator of mortality in this murine model of staphylococcal enterotoxic shock.**

Staphylococcal enterotoxins (SE), produced by *Staphylococcus aureus*, are proteins classified serologically and structurally into nine groups designated as SEA–SEJ (1–3). The SE are superantigens that bind to the class-II major histocompatibility complex and subsequently stimulate T cell proliferation (1). These toxins induce fever, hypotension, and enhance endotoxic shock via marked increases of pro-inflammatory cytokine levels in the blood (1, 4, 5). In humans, the SE are associated with food poisoning, toxic shock, pseudomembranous enterocolitis, and autoimmune diseases, such as rheumatoid arthritis and Kawasaki disease (1, 6–12).

Animal models for studying the in vivo effects of SE have been developed in kittens (1), non-human primates (1, 13), rabbits (14), and goats (15). Historically, nonhuman primates have been the species of choice for investigating SE intoxication because they develop clinical signs of disease and pathologic changes similar to those in humans affected with SEB-induced food poisoning and toxic shock (12, 16, 17). Clinical signs in primates typically include initial hyperthermia, followed by vomiting and diarrhea, although severe cases can progress to hypothermia, shock, and death (12, 16, 17).

More recently, murine models have been developed that successfully characterize SEB intoxication (18, 19). In mice, SEB can cause rapid weight loss, hypoglycemia, thymus atrophy, arthritis, and immune suppression (20–24), which are dose-, model-, and strain-dependent effects. At high SEB doses, or in manipulated models, hypothermia, lethal toxic shock, and death can also occur (20–24). The effects of SE in mice are potentiated by D-galactosamine (25), actinomycin D (18), or lipopolysaccharide (LPS) (19). Mice are also sensitized by depletion of endogenous glucocorticoids via adrenalectomy (26) or infection with a nonlethal influenza virus (27). Alternatively, transgenic mice expressing human T-cell receptor (β chain) (28) or severe combined immunodeficient mice transplanted with human fetal liver and thymus cells are used without further manipulation (29).

Investigators continue to formulate vaccines (30–32) and therapeutics (33, 34) to protect against the SE. To facilitate that effort, a murine model of staphylococcal enterotoxic shock was established at this institute to explore similar medical strategies (19). Death has typically been the endpoint in these studies, which is also true for many other toxicologic and vaccine development protocols, although use of the moribund state is becoming more frequent. However, defining and recognizing moribund animals often involves subjective assessment requiring observer interpretation, thereby introducing variability. Similar to comatose humans, moribund animals may not experience pain. Therefore, moribundity may be of questionable benefit in reducing animal pain and distress when used as the endpoint. As a result, investigators are attempting to find objective indices that predict impending death preferably before an animal reaches the moribund state.

Recently, hypothermia was determined to be a quick, reliable, and objective indicator of LPS-potentiated SEB intoxication in mice (35), inviting further evaluation as an alternative experimental endpoint in lieu of death. As part of an institutional commitment to minimize pain and distress in laboratory animals by defining alternative non-death endpoints, this project

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involved use of radiotelemetry, a refinement over a previous temperature-based SE mouse study (35), to monitor body temperature and physical activity in a murine model of staphylococcal enterotoxic shock.

Materials and Methods

Animals: Male BALB/cAnNCr mice (9 to 10 weeks old and > 25 g) were purchased from the National Cancer Institute Animal Production Program (Frederick, MD). Following a 2-week acclimation period, mice were individually housed after implantation of telemetry transmitters. Mice were free of antibodies to the following pathogens: *Mycoplasma pulmonis*; ectromelia virus; cytomegalovirus; GD7, hepatitis, lymphocytic choriomeningitis and, minute viruses; parvovirus; pneumonia and polyoma viruses; reovirus 3; rotavirus; and Sendai virus.

The study room was maintained at 21.2 ± 2°C with a relative humidity between 30 and 70%, a 12/12-hour light/dark cycle, and no less than 12 air changes/h. Mice were housed in solid-bottom, polycarbonate Micro-Isolator™ cages (Lab Products, Inc., Seaford, DE) with paper chip bedding (Alpha-Dri™; Shepherd Specialty Papers, Inc., Kalamazoo, MI) and given food (Harlan Teklad diet No. 7022, NIH-07) and water ad libitum. Nestlets® (Ancare Corp, North Bellmore, NY) were provided for enrichment and to encourage activity. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee and performed in an AAALAC International-accredited facility in accordance with recommendations in the *Guide for the Care and Use of Laboratory Animals* (36).

Experimental design: The LPS-potentiated murine model (19) was used for this study of staphylococcal enterotoxic shock. To confirm reproducibility of the model, two iterations (experiment 1 [n = 24] and experiment 2 [n = 24]) were performed 8 weeks apart, using the same study room and telemetry equipment. Intra-abdominal telemetry transmitters were surgically implanted into the peritoneal cavity. Thereafter, mice were observed twice daily at approximately 8 AM and 4 PM. Baseline temperature and activity data collection was begun 10 days after implantation of the transmitters. Twenty days after implantation, mice were given SEB and/or LPS and were observed for 5 days. Dead mice were removed immediately. Five days after SEB administration, all remaining mice were euthanized by intraperitoneal (IP) injection (0.2 ml) of Euthasol® (Delmarva Laboratories, Inc., Midlothian, VA). Intra-abdominal transmitters only were implanted into mice of experiment 1, whereas intra-abdominal transmitters were implanted into the mice of experiment 2 followed 13 days later by subcutaneous (SC) implantation of temperature transponders.

To examine the effects of SEB and/or LPS on temperature and activity, mice for each experiment were randomly assigned to the following four dosage groups: 10 μg of SEB (SEB only), 10 μg of SEB plus 80 μg of LPS (high SEB), 0.5 μg of SEB plus 80 μg of LPS (low SEB), and 80 μg of LPS (LPS only) (Table 1). The SEB was administered at time 0 and followed 4 hours later by LPS. All injections (0.2 ml/mouse) were given IP, and all dilutions were made in phosphate-buffered saline (PBS [pH 7.4]). The low and high SEB doses were approximately 1.5 and 30 times the median lethal dose (LD_{50}), respectively (34).

Surgical implantation of transmitters: Veterinary technicians, unaware of the study design and dosage groups, randomly selected each recipient from among the group-housed mice. Anesthesia was induced by an IP injection (0.25 ml) of an anesthetic cocktail consisting of ketamine HCl (6.06 mg/ml), acepromazine (0.06 mg/ml), and xylazine (0.67 mg/ml). A Data Sciences International (DSI) PhysioTel® transmitter (model TA10TA-F20), weighing 3.8 g and occupying 1.75 cm^3, was aseptically implanted into the peritoneal cavity of each mouse after a 1-cm midline incision caudal to the xiphoid process. The abdominal wall and skin were closed with 6-0 Vicryl (Ethicon, Inc., Somerville, NJ), and topical adhesive (Nexaband S/C®, Veterinary Products Laboratory, Phoenix, AZ) was applied sparingly over the apposed skin edges. Each mouse received 2 ml of warmed lactated Ringers solution SC prior to placing in a clean, freshly bedded, cage that was located on a recirculating water blanket. Once ambulatory, mice were returned to the study room and, for 5 days after surgery, were closely examined twice a day for evidence of pain or incisional complications.

Table 1. Experimental groups and mortality data

Experimental group	SEB Dose[a] (μg/mouse)	LPS Dose[b] (μg/mouse)	No. Dead/total EXP 1	EXP 2
High SEB	10	80	8/8	9/10
Low SEB	0.5	80	4/8	5/8
LPS only	0[c]	80	0/4	0/4
SEB only	10	0[c]	0/4	0/2

All injections (0.2 ml) were given IP, using PBS as a diluent.
[a] SEB or PBS injections were given at time 0 h.
[b] LPS or PBS injections were given 4 h later.
[c] Mice each received 0.2 ml of PBS.
EXP = experiment.

Telemetry system: The DSI monitoring system (Figure 1) consisted of a silicone-coated transmitter, receiver (model RLA 1020, DSI), data exchange matrix (BCM-100, DSI), and data acquisition system with accompanying software (Dataquest A.R.T. Version 1.1, DSI). The transmitter temperature range (-5 to 43°C) had initial accuracy and resolution of 0.01°C, and maximal drift of 0.05°C over the warranted 6-month battery life. Activity data measured relative movement only, which is dependent on orientation and distance between the transmitter and receiver.

Telemetric data collection: The data acquisition system was programmed to sample body temperature and physical activity every 15 minutes, and to calculate hourly moving averages for each mouse. Data collection began 10 days after surgery and continued until death or euthanasia at 5 days post-toxin administration.

Subcutaneous temperature monitoring: An adjunct method was used in experiment 2 to verify the core body temperatures recorded by the DSI telemetry implants. Biomedic Data Systems (Maywood, NJ) Implantable Programmable Temperature Transponders™ (IPTT-100) were injected SC into mice (n = 24) on the dorsum between the shoulder blades, using a Biomedics injector needle assembly (2.2-mm-diameter needle), 13 days after surgical implantation of the DSI transmitters and 7 days prior to toxin administration. The Biomedics Notebook™ System DAS-5002 was used to record temperatures twice a day, starting at time 0 until death or euthanasia.

LPS and SEB: Purified SEB (Toxin Technology, Sarasota, FL) and *Escherichia coli* LPS O55:B5 (Difco Laboratories, Detroit, MI) were reconstituted in sterile, pyrogen-free PBS and stored at -50°C. The endotoxin values of SEB, as measured by use of a *Limulus* amebocyte lysate assay (BioWhittaker, Walkersville, MD), were < 1 ng of endotoxin/mg of protein.

Statistical analysis: Temperature and activity data were

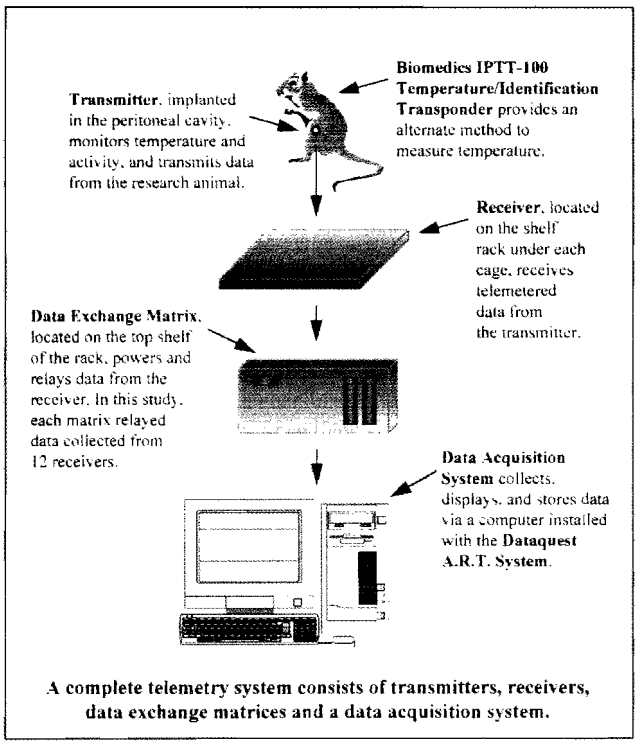

Figure 1. Schematic diagram of the telemetry system.

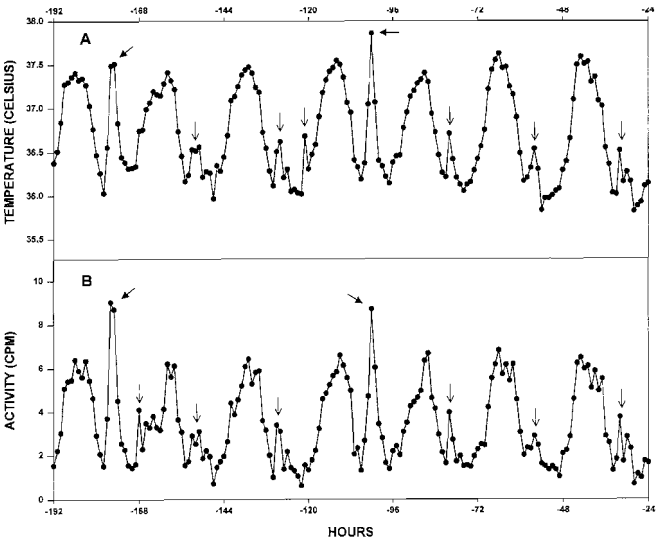

Figure 2. Normal pre-experimentation diurnal rhythms in core body temperature and physical activity for mice of experiment 1 (n = 24). Mice (n = 24) used in experiment 2 had comparable patterns. Points represent average temperature and physical activity readings. Activity was measured in counts per minute (cpm). Time -192 h represents 4 PM 8 days prior to time 0. Increases in temperature and physical activity at -176 h and again at -104 h are due to cage changing (solid arrows) which occurred at approximately 8 AM every Tuesday and Friday. The smaller increases in both parameters at approximately -152 h, -128 h, -80 h, and -32 h are due to daily checks (open arrows) performed in the morning, which involved visual observation of each animal through the shoebox cage.

evaluated as survivors and non-survivors for each experiment, rather than comparing the four dosage groups. Reproducibility was tested by determining differences in intercepts and slopes between the two experiments, using logistic regression analysis (SAS version 6.12, SAS Institute, Cary, NC). The odds of death associated with minimum temperature, minimum activity, and/or time from SEB injection also were calculated, using logistic regression analysis. By using the information generated from this analysis, effective temperatures (ET) were calculated by solving the logistic regression equation for probability of death (P = %), using statistical estimates of A (intercept) and B (slope) ($\log_e$ [P/1-P] = A + B[T]; T = minimum temperature ETP [ETP represents the effective temperature resulting in a probability of P% of dying]). The ET represents an estimated body temperature in the population that results in a probability of P% of death. All tests were performed at the 95% confidence level (P = 0.05). The paired student's t-test was used to compare intra-abdominal and SC temperature readings obtained in experiment 2. Additionally, mean (± SEM) temperature was calculated for the two measurement methods.

Results

All mice had similar diurnal rhythms of body temperature and physical activity prior to toxin administration (Figure 2). The highest average temperature (37.5 ± 0.4°C) and activity (6.6 ± 2.7 counts per minute [cpm]) readings were recorded at 1 AM, whereas the lowest temperature (35.9 ± 0.4°C) and activity (0.7 ± 0.9 cpm) measurements were obtained at 1 PM. Spikes in temperature and activity were readily apparent during routine husbandry procedures, such as cage changing and daily clinical rounds.

Physical activity data did not readily distinguish between survivors and non-survivors (Figure 3). Logistic regression analysis did not detect an association between minimum activity and the odds of death (P = 0.97). Activity levels decreased to an average of < 1 cpm within 6 hours after administration of SEB plus LPS, or LPS alone, whereas activity in mice receiving SEB only remained unchanged. Activity levels of survivors gradually increased until normal diurnal patterns were regained at approximately 102 hours post-SEB , whereas activity of non-survivors remained low until death.

Mortality data related to minimum temperature are summarized in Table 1. Minimum temperatures and time course for survivors and non-survivors are summarized in Table 2 and depicted graphically in Figure 4. Logistic regression analysis indicated a significant association between death and minimum temperature (P = 0.0077), but not the actual time of minimum temperature (P = 0.74). All surviving mice began to exhibit normal diurnal temperature fluctuations at approximately 102 hour post-SEB. Comparison of intra-abdominal versus SC temperature measurement methods were not significantly dissimilar (P = 0.06), with a mean difference of 0.2 ± 0.1°C. Wide variations in body temperature were observed among survivors in the low SEB and LPS only dosage groups, ranging from 21.1 to 33.4°C, whereas non-survivor minimum temperatures ranged from 20.7 to 24.8°C. Such wide interindividual variation among surviving mice, as well as minimum temperature overlap between survivors and non-survivors, were unexpected findings. To our knowledge, the degree of hypothermia observed among surviving mice of this study has not been reported previously.

From experimental data, the statistical temperature response obtained by use of the logistic regression model provided the following estimates of ET: ET_{99} = 19.1°C, ET_{90} = 21.4°C, ET_{75} = 22.4°C, ET_{50} = 23.4°C, ET_{10} = 25.5°C, and ET_{01} =

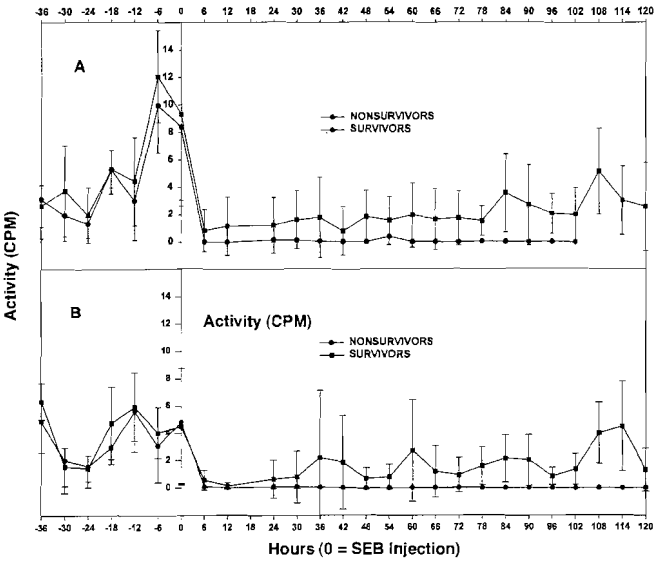

Figure 3. Physical activity expressed as cpm **(A)** Experiment 1 results with survivors (n = 12) and non-survivors (n = 12). The spike noted at -6 h was due to cage changing procedures. **(B)** Experiment 2 results with survivors (n = 10) and non-survivors (n = 14). Points represent the mean ± SD for each group. In each experiment, one non-surviving animal experienced a prolonged period of inactivity (represented by the point with no SD bars on the non-survivor line).

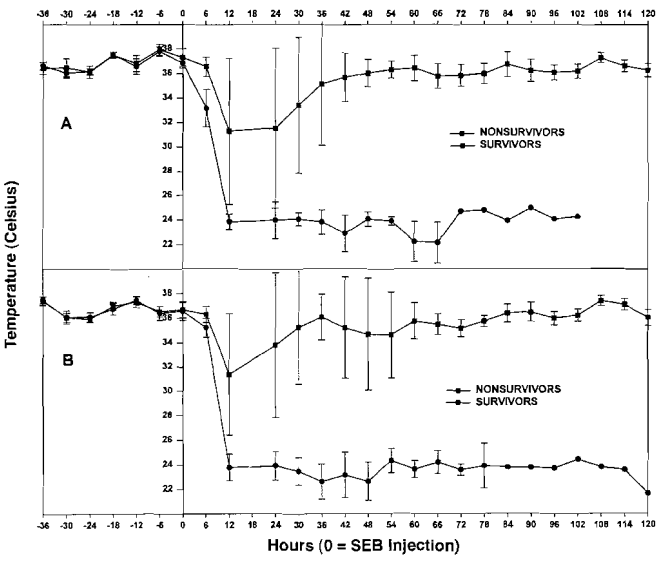

Figure 4. Body temperature **(A)** experiment 1, **(B)** experiment 2. Points represent the mean ± SD for each group. In each experiment, one non-surviving animal experienced a prolonged period of hypothermia (represented by the point with no SD bars on the non-survivor line).

27.7°C. Thus, when 23.4°C is reached in this model, mice have an equal chance of dying or surviving, whereas at 22.4°C, they have a 75% chance of dying and a 25% chance of surviving. However, unlike the statistical estimates, 86% (25/29) of all mice whose temperature decreased below 23.4°C (ET_{50}) died, and 96% (25/26) of mice injected with SEB and LPS died when their temperature decreased below 23.4°C.

Reproducibility of the two experiments was confirmed by no slope or intercept differences at the 95% confidence level. This model was reproducible with no significant difference in mortality and minimum core body temperature ($P = 0.99$) or physical activity ($P = 0.56$) between the two experiments.

Discussion

Reduced body temperature has been recognized as an indicator of endotoxin-induced morbidity and mortality in laboratory animals since 1949 (37). Recent research has effectively used body temperature to monitor the effects of various agents on mice and to define appropriate experimental endpoints before death (38–44). For example, a body temperature ≤ 32°C in mice infected with respiratory influenza virus is highly predictive of mortality (40). Likewise, ≤ 34°C accurately predicts terminal illness in bacterial virulence studies with *Pseudomonas aeruginosa*, *S. aureus*, and *S. epidermidis* (42). Similarly, body temperatures < 28°C signal impending death in influenza virus-infected mice (39). These examples clearly demonstrate that "lethal" hypothermia is a relative term and must be defined for each experimental paradigm.

Hypothermia appears to be a promising alternative endpoint to death in this murine model of staphylococcal enterotoxic shock. However, results of this preliminary study did not reveal a definitive fatal hypothermia temperature (FHT), previously defined as the body temperature at which mice will inevitably die (40). When body temperatures reached ≤23.4°C (ET_{50}), 86% of all mice died and 96% of mice injected with SEB and LPS died. The actual percentages observed are higher than the estimated 50% mortality, and are probably explained by the small sample size and the extremely steep slope of the logistic regression temperature response. Although it appears that the FHT for this model would likely fall between the ET_{50} (23.4°C) and ET_{90} (21.4°C), additional work is necessary to further delineate a clearer FHT.

These experiments also established that mice do not develop an initial hyperthermic response like that observed in primates and rabbits exposed to SEA or SEB (12, 14, 16, 17). Since previous SE studies had not proven or disproven this fact, this unique murine physiologic reaction to SE remained an unknown until now. Indeed, hypothermia appears to be a common response to many infectious and toxic insults in rats and mice (37, 38, 40, 45–47). Whether reduced body temperature in mice confers protective resistance appears to depend on the specific agent, as evidenced by the work of several authors who have reported variable effects on survival from thermal gradients provided to virally infected mice (48, 49).

Hypothermia, measured via transponder technology, was recently discovered to be a quick and reliable indicator of SE intoxication in BALB/c and C57BL/6 mice (35). Within 12 hours, 10 or 0.5 μg of SEB plus 80 μg of LPS induced a hypothermic response and hourly SC temperatures averaged 29.7 ± 0.7°C and 30.5 ± 1.4°C, respectively. In this radiotelemetric study, which involved identical doses of SEB and LPS, more marked reductions in body temperature were recorded.

One likely explanation for the differing hypothermic values could be the animal manipulations involved when measuring temperatures. Subcutaneous readings, obtained every hour for 12 hours after SEB administration, required physical handling of each mouse due to group housing (35). For purposes of this telemetric study, human-animal contact was not necessary to obtain core body temperature after toxin injections. However, in

Table 2. Minimum temperatures and time for survivors and non-survivors

Experimental group		Experiment 1 Minimum temperatures (°C)	Experiment 1 Time[b] (h)	Experiment 2 Minimum temperatures (°C)	Experiment 2 Time[b] (h)
High SEB	N[a]	21.1, 21.1, 21.3, 21.3, 22.3, 22.4, 23.2, 23.3	≤ 36	20.7, 21.0, 21.0, 21.1, 21.1, 21.3, 22.2, 22.5, 24.8	≤ 48
	S	None	NA	34.5	≤ 36
Low SEB	N	21.2, 21.2, 21.2, 23.1	48–102	21.0, 21.1, 21.3, 21.5, 21.6	48–120
	S	23.5, 23.6, 30.8, 32.8	24–96	21.1, 32.6, 32.6	10–40
LPS only	S	23.3, 23.6, 24.3, 31.5	≤ 36	21.5, 23.1, 26.3, 33.4	≤ 36
SEB only[c]	S	NA[d]	NA[d]	NA[d]	NA[d]

[a] N = Non-survivor; S = Survivor.
[b] Time period (hours) after SEB of lethality (N) or minimum temperature (S).
[c] Mice that received SEB only did not have decreased body temperatures outside of the normal diurnal patterns.
[d] NA = Nonapplicable.
See Table 1 for key.

experiment 2, lifting of cage tops was required to obtain subcutaneous temperatures, but avoided direct animal manipulation. Because repeated handling of mice induces marked increases in body temperature (44), hourly handling of the mice may have contributed to increased body temperature and could partially explain the "higher" hypothermic readings in the transponder study (35).

Another variable that may have played a role in the resulting temperatures is the actual time of SEB administration (7 AM in the Stiles, et al. [35] study versus 4 PM in our study). Observed physiologic effects can vary with the time of agent administration (50). Identical concentrations of SEB and LPS were given to the same mouse strain in both studies, but the resulting hypothermia differed by approximately 8°C.

Lastly, differences in the temperatures recorded by telemetry and subcutaneous transponders may involve housing conditions. Mice in the earlier study (35) were group-housed and noticeably huddled together (51), whereas animals in this investigation were housed individually. Previous research demonstrates that group-housed mice have significant increases in temperature, presumably due to increased activity and aggregation (44). Likewise, huddling is an effective behavioral means of conserving body heat observed in many animal species. As a result, group housing may also have contributed to the "higher" hypothermic temperatures in the previous staphylococcal enterotoxic shock study (35). Thus, temperature variations observed between this telemetry study and the previous transponder-based experiment (35) may be explained by a combination of factors, including animal manipulations, time of toxin administration, and housing conditions.

Many investigators continue to use rectal or infrared thermometry, manual techniques that require animal handling and restraint. Negative effects of human interaction on animal physiology are well known (44, 52), and generally introduce measurement error and variability into the data. Infrared thermometry commonly produces values with moderately high inter-measurement variability (38), whereas repeated insertion of rectal temperature probes can cause mucosal tears, leading to septicemia and death in mice (53). The phenomenon of human-induced hyperthermia and hyperactivity in response to cage cleaning, handling, and restraint has been reported in telemetrically monitored mice (44). Certainly, the effects of cage changing and general husbandry on murine temperature and physical activity were well illustrated in our study. Telemetric monitoring of body temperature avoids human interaction and, thus, represents a substantial refinement alternative over conventional techniques.

As with any technique, telemetry also possesses some disadvantages, including the requirement for a major surgical procedure and 10-day recovery period, the possibility of implantation-associated infections, and the initial high cost of equipment. Additionally, due to cost and space restrictions, limited numbers of animals may be monitored at any one time. On the other hand, important advantages exist with this technology (44). Most notably, telemetry provides an accurate representation of the physiologic effects of various agents because measurements are made remotely without human interaction. Collection and analysis of data are more efficient and less time consuming, since the computer operating system can sample data at programmed intervals and subsequently make various calculations. In addition, a more complete representation of the time course profile of the physiologic response can be obtained as a result of frequent data sampling. Significantly, the larger quantity and higher quality of the data acquired with telemetry results in fewer animals required per experimental group. Overall, telemetry offers an efficient and precise method to obtain large amounts of quality data, which may outweigh the disadvantages.

Radiotelemetry provided useful, previously unreported information about the degree of hypothermia that a mouse can tolerate, as well as the thermoregulatory response of mice to SEB and LPS. The study also indicated that body temperature, not physical activity, is significantly correlated with mortality, though additional studies need to be completed to clearly define a FHT.

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