The laboratory rat as a model for hyperthermic syndromes in humans


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HUBBARD, R. W., W. T. MATTHEW, J. D. LINDUSKA, F. C. CURTIS, W. D. BOWERS, I. LEAV, AND M. MAGER. The laboratory rat as a model for hyperthermic syndromes in humans. Am. J. Physiol. 231(4): 1119-1123. 1976. —To assess the lethal syndromes in humans, the laboratory rat as a model for hyperthermia will clearly distinguish between survivors and fatalities, the exercise-induced core temperature at exhaustion (between 40.4 and 43°C), mortality increased within 24 h. A dose-response curve with an LD₅₀ equivalent to a core temperature of 41.5°C was calculated. Although differences in body response curve with an LD₅₀ equivalent to a core temperature of 41.5°C represented a base line above which mortalities occurred. With increasing core temperature at exhaustion (between 40.4 and 43°C), mortality increased within 24 h. A dose-response curve with an LD₅₀ equivalent to a core temperature of 41.5°C was calculated. Although differences in body response curve with an LD₅₀ equivalent to a core temperature of 41.5°C represented a base line above which mortalities occurred. With increasing core temperature at exhaustion (between 40.4 and 43°C), mortality increased within 24 h. A dose-response curve with an LD₅₀ equivalent to a core temperature of 41.5°C was calculated. Although differences in body response curve with an LD₅₀ equivalent to a core temperature of 41.5°C represented a base line above which mortalities occurred.

heatstroke; heat exhaustion, rat model; exercise to exhaustion

OF THE RECOGNIZED HEAT DISORDERS (30), the most serious is acute heatstroke which results in widespread tissue injury and death (19). Two factors have apparently retarded the study of the pathophysiology of heatstroke through controlled experimentation: 1) the high risk of mortality (17) precludes its purposeful induction in man, and 2) the widely held belief that cessation of sweating is a cardinal sign of heatstroke (15) has a priori prevented the use of nonsweating animal models. Although there is considerable evidence that a breakdown of heat-dissipating mechanisms and lack of sweating may precipitate heatstroke (2, 3, 14, 16, 30), there are also numerous reports of heatstroke accompanied by profuse sweating (7, 19, 25-27, 31). These conflicting observations have been synthesized into a more general concept that either damage or overload to a heat-dissipating mechanism can result in excessive body temperature and heatstroke (29). It was hypothesized and confirmed in studies on dogs (24) that heatstroke develops when excessive body temperature, itself, becomes a noxious agent. Elevations in rectal temperature above 43°C and heatstroke were achieved by exercising dogs at 43-45°C and by sedentary exposure to 50°C, but not by exercising the animals at 18°C.

In contrast to man, in whom heatstroke levels of body temperature have been estimated at 40.6 (17) and 41.1°C (12), none of the dogs with rectal temperatures below 43°C showed signs of heatstroke. This and a number of other factors have led us to assume that heat exhaustion and exertion-induced heatstroke death could be better modeled in untrained, adult laboratory rats exercising at or near room temperature. Heatstroke has been observed in humans (28) working on relatively cool days (26°C) and has affected untrained, unacclimatized, and overweight individuals (13, 19-21), as well as conditioned athletes (12, 27, 29). It is well known that exercising trained and untrained rats at room temperature results in hyperthermia (4, 8, 11) and that similar levels of hyperthermia induced as a result of passive heating cause rats to succumb to heat exhaustion and death (1, 6, 10, 18, 23). The purpose of this study was to develop an animal model that could be used to define the relationship between body temperature and work in producing fatal hyperthermic syndromes.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Charles River CD strain) of equivalent ages were caged individually in an environmental chamber maintained at 26°C and 49 ± 17% relative humidity (RH). The air in this chamber (13 x 11 x 6 feet) was replaced at a rate equivalent to 1.4 room volumes per hour. All rats were fed a diet of Purina laboratory chow and water ad libitum. Rats were selected when their prefast weights were between 485 and 545 g and were fasted 18-24 h before use. Animals were either run to exhaustion at one of four ambient temperatures (5, 20, 23, or 26°C) or were restrained in an appropriate-sized wire cage which was placed in a small environmental chamber set at 41.5°C ambient until their core temperatures reached 42.3°C ± 0.1°C. The motor driven treadmill was similar to the one described by Pattengale and Holloszy (22). Rats were run up a 6" incline at 11 m/min but were allowed a 2-min rest period after 20 and 40 min of work. Exhaustion was achieved under a shock-avoidance contingency. It was defined as that point at which rats could not keep pace, and when placed on their backs would not right themselves. Core (6.5 cm rectal probe) and tail skin temperatures were measured using copper/constantan thermocouples in
conjunction with a thermocouple reference oven (Acromag model 340) and a 10-channel data acquisition system (Esterline Angus model D-2020) with a teletype printer. After reaching exhaustion or a predetermined core temperature, all rats were monitored at 26°C ambient while resting in plastic cages lined with bedding. After recovery, animals were returned to their cages (26°C) and allowed water but no food for 24 h.

The LD₅₀ was estimated by the method of Reed and Muench and the standard error by the procedure of Pizzi (32). Significance testing was carried out by using the Student t test. P values >0.05 are omitted from the tables. Work done was calculated from the formula: kg m = body wt (kg) \times \text{running time (min)} \times \text{treadmill speed (m/min)} \times \text{inclination of treadmill (sin)}.

RESULTS

The effect of exhaustive exercise at different ambient temperatures on rat core temperature and subsequent survival is depicted in Fig. 1. This histogram represents the results from 123 animals run to exhaustion at ambient temperatures of 5, 20, 23, and 26°C. A number of observations should be noted: 1) rats forced to run at different ambient temperatures displayed a range of overlapping core temperatures at exhaustion, 2) all rats run at 5°C survived for 24 h, 3) with increasing core temperatures at exhaustion (0.5°C-increments above 40.0°C), the percentage of rats dying within 24 h increased (0, 10, 48, 74, and 88% mortality at 42.5°C), and 4) between core temperatures at exhaustion of 41.1 and 42.5°C, one-third of the rats survived 24 h and two-thirds died.

The data from Fig. 1, when plotted as percent mortality versus core temperature at exhaustion, generated the dose-response curve shown in Fig. 2. A core temperature of 40.4°C represented a threshold hyperthermia above which mortalities occurred in exhausted animals. This threshold temperature, therefore, became a logical base line for calculating the thermal exposure in degree-minutes. The LD₅₀ and standard error were equivalent to a core temperature at exhaustion of 41.5 ± 0.1°C. Thus, the probability of death in exhausted rats resting at 26°C appeared directly related to the core temperature at collapse.

In order to gain insight into other factors contributing to the death of these animals, the data were arranged into four categories (Table 1). These categories approximate, in general, a continuum from work in the cold and subsequent survival to no work in the heat and subsequent death. The groups were: 1) all rats that ran to exhaustion at 5°C; 2) rats that ran to exhaustion at 20, 23, or 26°C had elevated core temperatures (41.1-42.5°C) and survived; 3) rats that ran to exhaustion at 20, 23, or 26°C had elevated core temperatures (41.1-42.5°C) and died; and 4) sedentary rats heated in restraining cages at 41.5°C ambient until their core temperatures reached 42.2°C. Although rate run at 5°C ran 85% longer and did proportionately more work than those exercising at or near room temperature (20, 23, or 26°C), there were no deaths in 24 h. Furthermore, when the data from rats run at 20, 23, or 26°C were separated retrospectively into survivors and fatalities (groups 2 and 3), these groups displayed identical run times and rates of increase in core temperature. However, in spite of the same range in core temperatures examined (41.1-42.5°C), both the initial and exhaustion core temperatures were significantly higher in the group dying within 24 h (group 3). Finally, although rats restrained at 41.5°C ambient performed no measurable work and had similar rates of rise in core temperature as exhausted survivors, the sustained hyperthermia resulted in 100% mortality.

Table 2 shows the effect of exhaustive work and hyperthermia on the weight losses and cooling rates of untrained rats. Animals run at 20, 23 or 26°C and surviving 24 h (group 2) lost body weight during the run at a rate 2.6 times that of rats running at 5°C (group 1). Although group 3 (24-h fatalities) had running times identical to group 2 (Table 1), they sustained a higher rate and percentage of body weight loss. In contrast, the rate of weight loss in restrained, heated rats (group 4) was significantly less, but the longer exposure time (Table 1) resulted in equivalent body weight losses.

The extent of hyperthermia (time vs. intensity) was calculated as an area above a base-line core tempera-
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FIG. 2. Dose-response curve of percent mortality with 24 h vs. core temperature at exhaustion. All rats recovered at 26°C with water supplied ad libitum. A core temperature of 40.4°C represented a threshold heat load above which death could occur in exhausted rats within 24 h.

However, Lewis et al. (18) demonstrated conditions under which rapid dehydration of rats occurred in spite of lack of panting. The major route of water loss appeared related to the continuous licking and wetting of the body that occurs in the heat. The average weight loss for a 1-h exposure to ambient temperatures between 39 and 45°C was equivalent to 30 g/kg body wt (18). This rate of body weight loss is similar to the rate of sweat loss in unacclimatized men in the heat (12). Subsequently, Hainsworth (9) has shown that there are two components necessary for the survival of rats at high ambient temperatures: physiological secretion of saliva in response to hyperthermia and appropriate behavioral utilization of the saliva for cooling. By working the rats on a treadmill at or near room temperature or by restraining them in the heat, we hoped to achieve both dehydration and rapid hyperthermia through limiting the grooming behavior. The use of the treadmill at or near room temperature was suggested by the observation that heatstroke often occurs in highly motivated individuals such as athletes and soldiers engaged in some form of programmed work and, at times, on relatively cool (26°C) days (27, 28). By running rats in the cold, we hoped to assess any lethal cardiovascular or shocklike effects of work independent of hyperthermia in the absence of work. A 24-h survival period was suggested by the observations of Malamud et al. (19). In this classic description of 125 heatstroke fatalities in military recruits, death occurred in less than 24 h in approximately 70% of the cases. In a large percentage of these, the men were relatively unacclimatized and overweight which suggested the use of large, untrained rats (500 g). Since heavier rats have a lower surface area to mass ratio and must do more work at any given speed and incline, they should produce and store more heat.

In contrast to earlier experiments determining the tolerance time or time until death of rats continuously exposed to heat (6) or heat plus work (23), the degree of hyperthermia achieved in these experiments was a variable related to both the run time to exhaustion and the given ambient temperature. Although no rats run to exhaustion at 5°C subsequently died within 24 h (Fig. 1, Table 1), the duration of the work in some individuals raised their core temperatures to levels at which others succumbed. This observation reinforces and extends the earlier assumption (28) that heatstroke levels of hyperthermia can occur under relatively cool conditions. Furthermore, since rats run 85% longer and did proportionately more work in the cold (Table 1), these results suggest that hyperthermia may seriously complicate endurance type studies conducted at or near room temperature.

In the series reported by Malamud et al. (19), the core temperatures on admission to the hospital ranged from 36 to 44°C, and nine of those with temperatures below 41°C subsequently rose to hyperthermic levels. The striking dose-response relationship found between increasing percent mortality in exhausted rats and core temperatures between 40.4 and 43°C (Fig. 2) is consistent with theses human data (19) and the apparent threshold levels of body temperature (40.6°C) for heat-

<table>
<thead>
<tr>
<th>% MORTALITY WITHIN 24 HOURS</th>
<th>CORE TEMP AT EXHAUSTION (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>40.0</td>
</tr>
<tr>
<td>20</td>
<td>41.0</td>
</tr>
<tr>
<td>40</td>
<td>42.0</td>
</tr>
<tr>
<td>60</td>
<td>43.0</td>
</tr>
<tr>
<td>80</td>
<td>44.0</td>
</tr>
</tbody>
</table>

Table 1: Dose-response curve of percent mortality with 24 h vs. core temperature at exhaustion. All rats recovered at 26°C with water supplied ad libitum. A core temperature of 40.4°C represented a threshold heat load above which death could occur in exhausted rats within 24 h.
TABLE 1. Effect of work and core temperature on mortality of exhausted rats

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Ambient Temp, °C</th>
<th>Percent Mortality 24 h</th>
<th>Fastiging Body WT, g</th>
<th>Work Done, kg m*</th>
<th>Run Time, min</th>
<th>Initial Tr, °C</th>
<th>Δ Tr To Exh, °C/min</th>
<th>Tr Exh, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Run exhausted</td>
<td>5</td>
<td>0</td>
<td>30</td>
<td>484 + 15†</td>
<td>56 + 16</td>
<td>100 + 30</td>
<td>38.4 ± 0.6</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>2) Run exhausted (41.1-42.5°C)</td>
<td>20, 23, 26</td>
<td>0</td>
<td>22</td>
<td>488 ± 17</td>
<td>32 ± 12</td>
<td>57 ± 23‡</td>
<td>38.1 ± 0.7</td>
<td>0.07 ± 0.03‡</td>
</tr>
<tr>
<td>3) Run exhausted (41.1-42.5°C)</td>
<td>20, 23, 26</td>
<td>100</td>
<td>41</td>
<td>486 ± 15</td>
<td>31 ± 12</td>
<td>55 ± 22</td>
<td>38.4 ± 0.6‡</td>
<td>0.07 ± 0.03</td>
</tr>
<tr>
<td>4) Sedentary restrained</td>
<td>41.5</td>
<td>100</td>
<td>20</td>
<td>481 + 14</td>
<td>80 + 32§</td>
<td>38.1 + 0.9</td>
<td>0.06 ± 0.03</td>
<td>42.3 ± 0.12†</td>
</tr>
</tbody>
</table>

All rats recovered in a constant-temperature chamber maintained at 26°C. After recovery measurements were made, rats were returned to their cages and allowed water but not food ad libitum. * kg·m = body wt (g) × running time (min) × treadmill speed (m/min) × inclination of treadmill (sin). † Mean ± SD. ‡ P < .05 for Student t test between the mean ± SD and the mean immediately above it. § Time at 41.5°C.

TABLE 2. Effect of exhaustive work and hyperthermia on weight losses and cooling rates of untrained rats

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Ambient Temp, °C</th>
<th>Fastiging Body WT, %</th>
<th>Rate of Wt Loss, mg/g per h</th>
<th>Body Wt Loss, %</th>
<th>Tr Exh, °C</th>
<th>Area Above 40.4°C, deg/cm²</th>
<th>Postexh Cooling, °C/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Run exhausted</td>
<td>5</td>
<td>0</td>
<td>30</td>
<td>11.9 ± 6.9</td>
<td>1.9 ± 0.7</td>
<td>39.7 ± 0.7</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>2) Run exhausted (41.1-42.5°C)</td>
<td>20, 23, 26</td>
<td>0</td>
<td>22</td>
<td>30.0± 16.0</td>
<td>2.7± 1.3</td>
<td>41.5± 0.3</td>
<td>0.04± 0.02</td>
</tr>
<tr>
<td>3) Run exhausted (41.1-42.5°C)</td>
<td>20, 23, 26</td>
<td>100†</td>
<td>41</td>
<td>36.8± 13.6</td>
<td>3.2± 1.0</td>
<td>41.9± 0.4</td>
<td>0.04± 0.02</td>
</tr>
<tr>
<td>4) Sedentary restrained</td>
<td>41.5</td>
<td>100</td>
<td>20</td>
<td>25.3± 10.9</td>
<td>3.0± 0.9</td>
<td>42.3± 0.1</td>
<td>0.07± 0.01</td>
</tr>
<tr>
<td>5) Sedentary restrained</td>
<td>41.5</td>
<td>Sac§</td>
<td>5</td>
<td>42.3 ± 0.1</td>
<td>5</td>
<td>0.07 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD in columns 5-8 and column 10. Cooling rate was calculated during first 30 min of recovery at 26°C ambient temperature. * 51% died overnight, 49% died within 3-4 h. † 15% died overnight, 85% died within 42 ± 45 min. ‡ Sacrificed when core temperature approached 42.3°C. § P < .05 for Student t test between the mean ± SD and the mean immediately above it.

stroke estimated by Leithead and Lind (17). These results suggest a continuum of increasing risk with rising levels of hyperthermia above this core temperature.

The trend toward increasing core temperatures and death with exercise to exhaustion at increasing ambient temperatures (Fig. 1) supports the contention of Gilat et al. (7) that work and external heat are to be considered as collaborators, supplementing one another in producing heatstroke. This result raises the question: to what degree does work, per se, contribute to heatstroke death? The data in Table 1 indicate a lack of correlation between death in 24 h and either the run time to exhaustion or the amount of work done. In fact, these two factors as well as the rate of increase in core temperature were identical in the exhausted survivors and the fatalities. However, the average core temperature at exhaustion was significantly higher in the group dying within 24 h (Table 1, group 3). This appeared to be the result of superimposing an equivalent amount of work on a higher initial core temperature. To this extent, initial core temperatures were a predisposing factor to heatstroke death. This result has obvious implications for situations requiring programmed work in the heat by large numbers of individuals. The results from the restrained-heated and the cold-run rats further suggest but do not prove that the amount of work, per se, was not the cause of death. However, further research should compare the incidence of mortality between working and sedentary animals at equivalent heat loads measured in degree-minutes.

The data in Table 2 demonstrate striking differences in the rate of weight loss when nonsweating rats are run to exhaustion in the cold and at or near room temperature. The rate of weight loss in the cold probably reflects the combined contributions of urinary and respiratory water losses. This rate of weight loss was increased 2.6-fold by running at room temperature and probably reflects additional water losses through salivation. These salivary water losses, coincidental with hyperthermia, appear analogous both in rate (18) and neural control (5) to the sweat rates of unacclimatized humans (12). Similar body weight losses were obtained in the restrained rats at 41.5°C ambient. The data in Table 2 indicate two factors that clearly distinguish between survivors and fatalities. The first is the slow rate of postrun cooling in potential fatalities. This characteristic may prove useful in predicting morbidity or in assessing the benefits of prophylactic treatment. Since restrained rats, taken to a core temperature of 42.3°C and then killed by concussion, had a cooling rate equal to that of survivors, potential fatalities are not simply metabolically near death, but must be producing endogenous heat in excess of what they can dissipate effectively. The second important characteristic of potential fatalities is their greater area under the heating and recovery curves. The extent of hyperthermia in groups 2, 3, and 4 appears inversely
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proportional to survival time (footnotes, Table 2). Thus, although differences in body weight loss, core temperature at exhaustion, and cooling rate will clearly distinguish between survivors and fatalities, the severity of heat injury as inferred from survival times is best measured by the time versus intensity of hyperthermia in degree-minutes. Area analysis of rats which survived and those that died revealed that the area of the survivors was much smaller than those that died (21 ± 10 vs. 52 ± 28 in those exposed to work at room temperatures and 120 ± 55 for those exposed to external heat alone). These results are quantitatively similar to those obtained by Shapiro et al. (24) in dogs, but in the later study the base-line temperature above which the area was calculated was 43°C. In the present study, the base-line temperature was 40.4°C, which is similar to the apparent threshold heatstroke levels of body temperature (40.6°C) estimated for men by Leithead and Lind (17).

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In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences—National Research Council.

The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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