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Effect of Cage Bedding on Temperature Regulation and Metabolism of Group-housed Female Mice

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Mice are generally housed in groups in cages lined with an absorbent bedding material at ambient temperature (T_a) of 20 to 24°C, which is comfortable for humans, but cool for mice. Little is known about the effects of bedding on thermoregulation of group-housed mice. To determine whether bedding material affects thermoregulatory stability, core temperature (T_c) and motor activity (MA) were monitored by use of radiotelemetry in female CD-1 mice housed in groups of four in a standard plastic cage at T_a of 23.5°C. Ten groups were tested using three types of bedding material: a deep layer of heat-treated wood shavings (DWS) that allowed mice to burrow, a thin layer of wood shavings (TWS) just covering the bottom of the cage floor, or a layer of beta chips (BC). Mice could not burrow in the TWS or BC. The T_c and MA were affected by bedding type and time of day. Mice housed with DWS maintained a significantly higher T_c (ΔT_c = 1.0°C) during the day, compared with that in mice housed with TWS and BC. During the night, T_c and MA were high in all groups and there was no effect of bedding type on T_c or MA. Effect of bedding on metabolic rate (MR) was estimated by measuring oxygen consumption for six hours in groups of four mice at T_a of 23.5°C. The T_c was significantly reduced in mice housed on the TWS and BC, but MR was unaffected by bedding type. There was a trend for higher MR in mice on BC. Compared with use of other bedding materials, housing mice on DWS and comparable materials provides an environment to burrow, thus reducing heat loss. The effects of bedding material on temperature regulation may affect rodent health and well being. Moreover, bedding will affect variability in toxicologic and pharmacologic studies whenever an endpoint is dependent on body temperature.

Ambient temperature is one of many critical environmental factors involved in housing and experimentation of laboratory rodents. The ambient temperature for rearing laboratory rodents is critical not only to the health and well being of the rodent but also to the comfort of the animal care personnel. In the *Guide for the Care and Use of Laboratory Animals*, the recommended dry bulb temperature for mouse, rat, hamster, gerbil, and guinea pig is 18 to 26°C (10). This topic of optimal environmental temperature for housing rodents has been discussed and reviewed in a variety of forums (2, 9, 12). Environmental temperature is also a critical factor in the thermoregulatory response to drugs and toxicants. It thus follows that any biological endpoint that is dependent on body temperature will likely be affected by the choice of bedding material and ambient temperature.

In the glossary of thermal physiology (8), the thermoneutral zone is defined as the range of ambient temperatures at which temperature regulation is achieved without changes in metabolic heat production. A survey of studies in laboratory mice has indicated that these animals have a thermoneutral zone of approximately 26 to 34°C (3). Differences in strain and technique may account for this wide range in the estimate of the thermoneutral zone. However, most would conclude that the lower end of the thermoneutral zone of individual mice (i.e., the lower critical temperature) is approximately 30°C. On the other hand, mice are generally housed in animal facilities at cool ambient temperatures of 20 to 24°C. Under these conditions, one would expect the mice to be cold stressed and their metabolic rate would be increased above the basal rate. A temperature zone for optimal growth, development, reproduction, and other processes also can be defined that does not necessarily match the metabolic thermoneutral zone (12). Each parameter has its own optimal temperature zone. The ideal temperature zone is estimated to be 20 to 26°C for group-housed JCL-ICR mice maintained on cedar bedding (12). Recently, it was reported that the thermoneutral zone could be defined in terms of skin temperature rather than the metabolic rate (11). It remains to be seen whether this approach could be used to study the impact of bedding material on thermoregulation.

Most information on the thermoneutral zone of mice and other rodents has been assessed in individual animals housed on a wire-screen or solid floor with no bedding material. Acrylic cages and bedding provide insulation and reduce metabolic requirements in rodents housed at standard room temperatures (5). Moreover, mice are generally housed in groups of at least five and are kept in cages with insulative bedding material covered with a Micro-Isolator™ (Lab Products, Seaford, Del.) filter lid. Under these conditions, it is assumed that the mice are in a state of thermal comfort (i.e., not cold stressed) because they are able to huddle and/or bury into wood shaving bedding. However, this has not been documented experimentally. Some bedding, such as pine shavings, is thought to provide more insulation because rodents can burrow or nest while other materials, such as chipped wood and manufactured paper bedding, are not amenable for

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burrowing behavior. Filter lids of static Micro-Isolators™ (Lab Products) limit air flow and probably reduce heat loss and metabolic requirements for thermoregulation (8).

There are very few studies of the thermoregulatory and metabolic response of group-housed mice and other rodents. This information is vital to the researchers and animal care personnel that desire a thermally comfortable environment for mice and other rodents in facilities that are also comfortable for personnel. Radio telemetry allows undisturbed monitoring of core temperature of rodents maintained in a variety of housing situations. To this end, a series of experiments was performed to assess the thermoregulatory and metabolic response of group-housed mice that were maintained on a variety of bedding materials commonly used in animal husbandry practices.

Materials and Methods

Animals. Female mice of the CD-1 strain were obtained from Charles River Laboratories (Raleigh, N.C.) at 45 days of age, and were housed in groups of four in standard plastic cages (length, 46 cm; width, 24 cm; depth, 15 cm) with heat-treated, pine wood shaving bedding material that was changed out weekly. The animals were given food (Prolab RMH, Brentwood, Mo.) in slotted hanging feeders and water ad libitum and were maintained at ambient temperature of 22 to 24°C, 50% relative humidity, and a 12:12-h light:dark cycle. Serologic profiling was performed in sentinel mice to ensure that mice were free of viral and bacterial infections. Monthly serologic analysis included screens for Sendai virus, mouse hepatitis virus, *Mycoplasma pulmonis*, cilia-associated respiratory bacillus, and parvovirus; quarterly screening included minute virus of mice, pneumonia virus of mice, epizootic diarrhea of infant mice, Sendai virus, mouse hepatitis virus, *Mycoplasma pulmonis*, cilia-associated respiratory bacillus, and parvovirus. The humane care and use of the mice was ensured by approval of all protocols by the Institutional Animal Care and Use Committee.

Surgery. Radiotransmitters were implanted in the mice to monitor core temperature and motor activity. This procedure was performed at least one week prior to an experiment in one randomly selected mouse among the group of four in each cage. Using aseptic techniques, the mice were anesthetized with sodium pentobarbital (80 mg/kg of body weight, i.p.; 50 mg/ml stock solution diluted in an equal amount of saline for injection). A small incision was made in the abdominal wall, and a transmitter (Model TA10TA-F20, Data Sciences, St. Paul, Minn.) was inserted into the abdominal cavity. The abdominal wall was closed with 4-0 suture, and the skin was closed with a wound clip. The mouse was returned to the group and allowed at least one week of recovery prior to testing.

Protocol. Two cohorts of mice were studied. Core temperature and motor activity were measured at five-minute intervals in one mouse while housed with the other three animals in clean plastic cages (identical to that for housing described previously) with a wire screen top to hold food and a water bottle. The entire top of the cage was covered with a Micro-Isolator™ (Lab Products) filter cover. The signal from the radiotransmitter was detected by a receiver board placed beneath the cage (7). Mean body weight, when tested in the environmental chamber, was 32.1 and 30.4 g for the first and second cohorts, respectively.

At 11 a.m., four cages of mice were placed in an environmental chamber maintained at an ambient temperature of 23.5°C. Ambient temperature was measured with thermocouples accurate to ± 0.1°C placed within the cage and positioned as close to the mice as possible. The bedding material in the cage was one of three types: 225 g of beta chips; 100 g of pine wood shavings (same as used for housing animals), resulting in a thin layer of bedding material that was just sufficient to cover the floor of the cage; or approximately 460 g of wood shaving bedding, resulting in a deep layer (approx. seven to 10 cm of depth) of wood shavings in the cage. When housed with the beta chips and shallow wood shaving bedding, the mice were unable to burrow into the bedding. The deep layer of bedding was chosen so that mice could burrow regardless of their location in the cage. The layer of beta chips approximates that used in many mouse cage environments. The thin and thick layer of wood shavings represent the extreme range of bedding material thickness that might be found in a mouse cage, depending on discretion of animal care personnel, research situation, and other factors. In this study, it was desirable to have a sufficiently deep layer of shavings, into which the mice would have to burrow, or a sufficiently shallow layer, into which they could not burrow, but could build a small nest. Fresh bedding was used at the start of every test.

Metabolic rate. The metabolic rate and body temperature of groups of four mice housed in cages of different bedding material was assessed by measuring the oxygen consumption while they were housed in a calorimeter (Model SEC-A 1202, Thermonetics, San Diego, Calif.). The calorimeter is a water-perfused device that provides a stable thermal environment and is an ideal method for measuring the heat loss of an organism. Moreover, by measuring the oxygen content of the air passing through the calorimeter, the oxygen consumption of an animal can also be measured. Measurement of dry heat loss in the mice in the calorimeter was intended; however, the accuracy of the measurement of heat loss is compromised if evaporative water loss is not taken into consideration. Since evaporation from the mice and bedding could not be controlled, it was later decided that the measurements of dry heat loss may not be reliable. Hence, the heat loss data are not reported. The same mice used in the temperature regulation studies described previously also were used in the calorimeter experiments. Both cohorts were tested in the environmental chamber and the calorimeter. However, while testing the first cohort, it was decided to collect the calorimeter data followed by the temperature data in the environmental chamber. For the second cohort, the metabolic data were collected after the environmental chamber experiments.

The internal dimensions of the calorimeter was $30.5 \times 30.5 \times 30.5$ cm, which make it considerable smaller than the standard cage used in the temperature experiments. Hence, a smaller plastic cage (length, 28 cm; width, 18 cm; depth, 13 cm) was used to house mice in the calorimeter. The cage was filled with a thick layer of wood shavings (150 g), a thin layer of wood shavings (25 g), or a layer of beta chips (125 g). The depth of the bedding in the small cage was proportional to that in the larger cages described previously so that the mice could similarly burrow into the deep wood shaving bedding in the calorimeter as they could in the environmental chamber. A wire mesh lid was placed over the top of the plastic cage and telemetry receiver wands were placed in close proximity to detect the telemetry signal from one of the group of four mice in the calorimeter. Mice were deprived of food and water during the six-hour test period in the calorimeter.

Ambient temperature was maintained at 23.5°C in the calorimeter and was measured continuously by use of a thermocouple. The mice were placed in a clean cage, which was then placed in the calorimeter for six hours. Core temperature, activity, heat loss, and oxygen consumption were recorded at one-minute intervals. A mass flow controller was used to meter dry air at a constant flow rate (2.73 L/min; STP) into the calorimeter. A fraction of the air leaving the chamber was dried and passed through an oxygen analyzer (Model S3A, Applied Electrochemistry, Sunnyvale, Calif.) to measure the percentage of oxygen. Metabolic rate of the rat was estimated by measuring the oxygen consumption. The change in percentage of oxygen before and after passing through the calorimeter was multiplied by the flow rate of air into the chamber. The accuracy of the oxygen consumption measurement was calibrated by burning a small alcohol lamp containing 100% ethanol inside the calorimeter. The change in weight of the lamp was used to determine the expected rate of oxygen consumption, assuming complete and steady combustion of the ethanol. The measurement of the percentage change in oxygen consumption was converted to milliliters of oxygen per minute on the basis of the calibrated standard of the alcohol lamp. Percentage of oxygen, dry heat loss, air flow rate, and calorimeter temperature were monitored at one-minute intervals by use of a data acquisition system (Dianachart PC acquisition; Model PCA-14, Rockaway, N.J.). The interior of the calorimeter was not illuminated during the measurements.

Statistics. The core temperature and motor activity data collected from mice housed in the environmental chamber were averaged into one-hour bins for statistical analysis. The telemetry data collected during the day of placement in the chamber (noon to 6 p.m.), night (6 p.m. to 6 a.m.), and next day (6 a.m. to 10 a.m.) were analyzed separately, using repeated measures, two-way analysis of variance (ANOVA) and incorporating bedding type and time as variables (GB-Stat, Silver Spring, Md.). The data collected from mice housed in the calorimeter were analyzed by averaging the data over specific time intervals during testing, then subjecting them to ANOVA. Significant treatment effects of the ANOVA were followed by use of a Tukey's protected *t*-test.

Results

Core temperature. The time course of core temperature of the group-housed mice was significantly affected by the type of bedding material (Fig. 1). Core temperature on all three treatment groups was high when they were first placed in the cages. After several hours, significant effects of bedding type on core temperature were apparent. During the light phase, core temperature of mice housed on beta chips and shallow wood shavings decreased to approximately 36.5°C, whereas that of mice housed in the deep shavings remained at 37.5°C. As the nocturnal phase approached, core temperature of mice on beta chips and shallow shavings increased significantly from their diurnal values, compared with that of mice in the deep shavings (Fig. 1). Core temperature of all three bedding groups was similar throughout the nocturnal phase. At the start of the next light phase, core temperature of mice on beta chips and shallow shavings decreased by 1°C, relative to that of mice on deep shavings. Frequency distribution of the 24 h of core temperature data illustrates the effects of bedding type on the variability of temperature regulation (Fig. 2). Mice housed on the deep

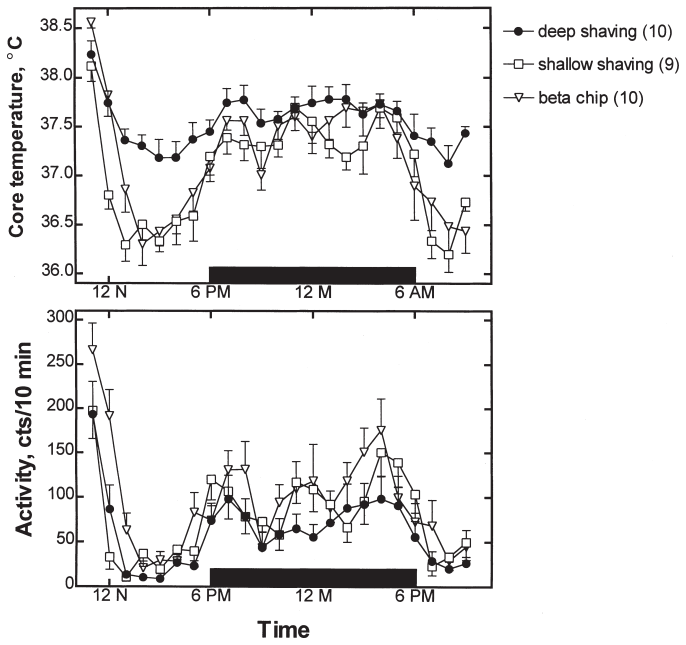

Figure 1. Time course of core temperature and motor activity of group-housed mice maintained in cages lined with beta chips, a shallow layer of wood shavings, or a deep layer of wood shavings. Groups of mice were placed in the chamber at 11 a.m. Repeated measures analysis of variance (ANOVA) was used for the following comparisons: core temperature, day 1, bedding treatment, $F(2,27) = 9.1$, $P = 0.0009$; bedding treatment-time, $F(12,162) = 5.6$, $P < 0.0001$; night, bedding treatment, not significant (NS), bedding treatment-time, NS; day 2, bedding treatment, $F(2,27) = 9.1$, $P = 0.0009$; bedding treatment-time, NS; motor activity, day 1, bedding treatment, $F(2,27) = 7.7$, $P = 0.002$; bedding treatment-time, $F(12,162) = 3.5$, $P < 0.0001$; night, bedding treatment, NS; bedding treatment-time, NS; day 2, bedding treatment, NS; bedding treatment-time, NS. Numbers in parentheses indicate number of groups of mice.

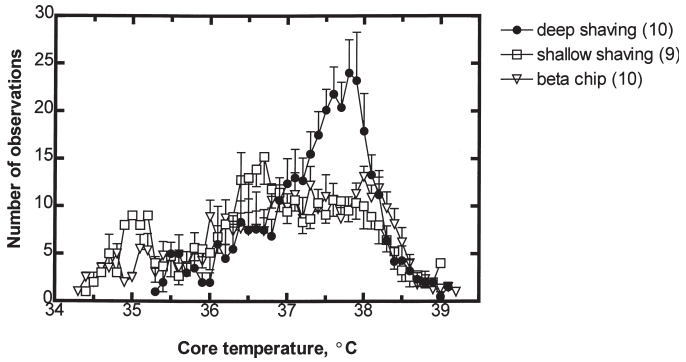

Figure 2. Frequency histograms of core temperature of mice maintained in the various types of cages (data presented in Fig. 1).

shavings had a well defined distribution of core temperature, with a mode value of 37.8°C. The distribution of core temperature of mice house on the beta chips and shallow wood shavings was broader, with no discernable peak, as was associated with the deep wood shavings.

Motor activity. The activity of mice in all three treatment groups was transiently increased when animals were first placed in the environmental chamber (Fig. 1). During this period, motor activity of mice on beta chips was significantly higher than that of mice on the other bedding types. Throughout the night and next day, motor activity was unaffected by bedding type.

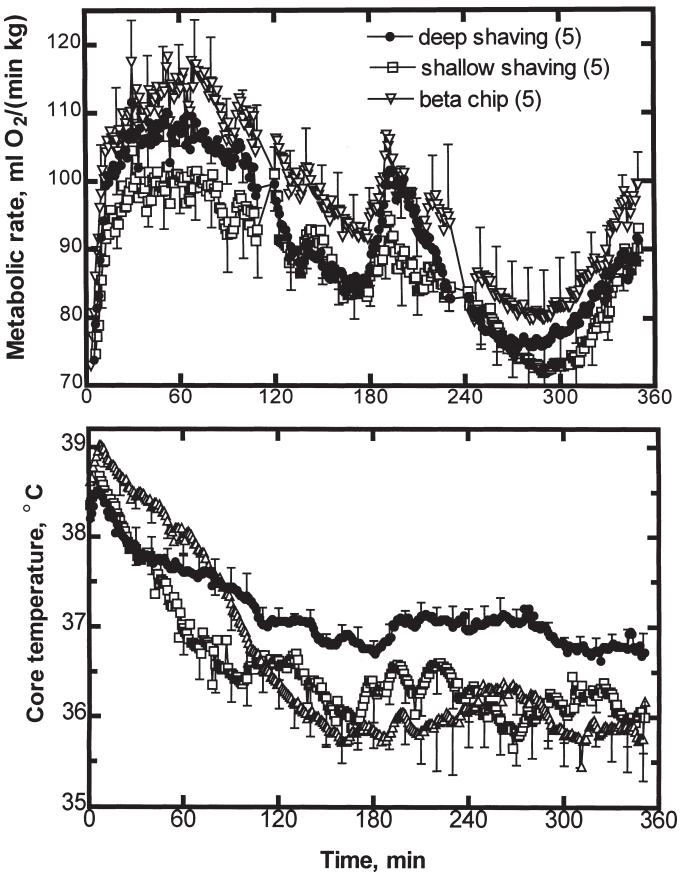

Figure 3. Time-course of oxygen consumption and core temperature of mice housed in the calorimeter while maintained in cages lined with beta chips, a shallow layer of wood shavings, or deep layer of wood shavings. For sake of clarity, standard error bars are plotted every 10 min.

Calorimeter. When mice were first placed in the calorimeter, metabolic rate and core temperature were high, reflecting the increase in activity as the animals were placed in a novel environment (Fig. 3). Core temperature and metabolism decreased gradually over the six-hour testing period. There was a trend for mice housed on the beta chips to have a higher metabolic rate during the first three hours in the chamber, but this difference was not significant. After approximately four hours in the calorimeter, metabolic rate was similar among groups on the three bedding types, but core temperature was significantly higher in mice housed on the deep wood shavings, compared with the shallow shavings and beta chip groups.

Discussion

The results of this study illustrate the importance of choice the on effect of bedding material on the basal thermoregulatory patterns of group-housed mice. A bedding of deep shavings was associated with warmer core temperatures during the daytime, but not during the night. The difference in core temperature could not be attributed to motor activity since mice housed on deep shavings had lower motor activity during the daytime, compared with that of those housed on the shallow shavings and beta chips. Although group-housed mice can huddle to conserve body heat when placed on beta chips or shallow wood shavings, this type of thermoregulatory behavior was apparently insufficient to maintain a core temperature similar to that observed in mice housed on deep shavings. The results should lead one to consider the potential thermoregulatory lability of groups of mice housed on bedding material that does not allow for burrowing behavior. Furthermore, although individual mice were not assessed in the study reported here, the results, nonetheless, indicate that, without an option to huddle as groups of mice normally do, individual mice housed with bedding material that does not allow for burrowing would be subject to thermoregulatory instability at standard housing temperatures.

The operative ambient temperature (T_o) is defined as the "...temperature of a uniform (isothermal) 'black' enclosure in which a solid body or occupant would exchange the same amount of heat by radiation and convection as in the actual nonuniform environment" (8). The rodent cage and bedding is a nonuniform thermal environment, and a simple temperature measurement made outside of the cage will not accurately predict how a mouse will dissipate heat and/or its thermal comfort. Using a mouse model (i.e., an aluminum cylinder similar in dimensions to a mouse) to estimate the T_o of various types of bedding material at an air temperature of 22°C (still air conditions), it was observed that such "mouse" placed on top of wood shaving had T_o of 25.8°C, compared with T_o of 23.8°C when placed on top of a bedding of beta chips (6). When it was buried into the wood shavings, the model mouse's T_o increased to 30°C.

Acrylic or plastic cage bottoms also provide more insulation than do metal or wire-screen floors. The ability of a thermogenic drug to increase core temperature is more effective when rats are housed on an acrylic, compared with wire-screen floors (5). Taken together, a bedding of deep wood shavings affords greater insulation and the T_o of mice housed in the deep shavings is significantly warmer than that of animals housed on the beta chip or shallow shaving bedding.

A behavioral thermoregulatory study in this laboratory indicated that group housing at standard room temperature does not ensure that mice are in an ideal zone of thermal comfort (6). A group of five female mice or individual mice at an age of two months were housed in a temperature gradient with a wire screen floor while selected ambient temperature was monitored every minute. The mean selected ambient temperature over a 24-h period for individual mice and groups of five mice was 27.1 ± 0.3 and 26.8 ± 0.1°C, respectively. That is, the grouped mice were found to huddle in the temperature gradient but their selected ambient temperature was only 0.3°C below that of individual mice. It should be noted that individual and grouped mice prefer relatively warm temperatures, compared with their standard housing temperature of 22°C. Furthermore, aged mice (11 months) preferred significantly warmer temperatures (individuals = 28.6°C; five per group = 27.7°C). Since groups of mice prefer ambient temperatures slightly below that of individual animals, it is likely that the groups of mice are not in a state of thermal comfort at 22°C. This is especially relevant when there is not an option for burrowing into bedding material as would occur with housing on wire screen floors, beta chips, or similar material.

A metabolic model was constructed on the basis that metabolic rate was expected to vary depending on type of bedding material. Because the deep shavings provide greater insulation, a higher metabolic rate was predicted for mice maintained on beta chips or shallow wood shavings. However, metabolic rate was unaffected by bedding material when measured over a six-hour period. The reason for a lack of effect on metabolic rate is likely attributable to

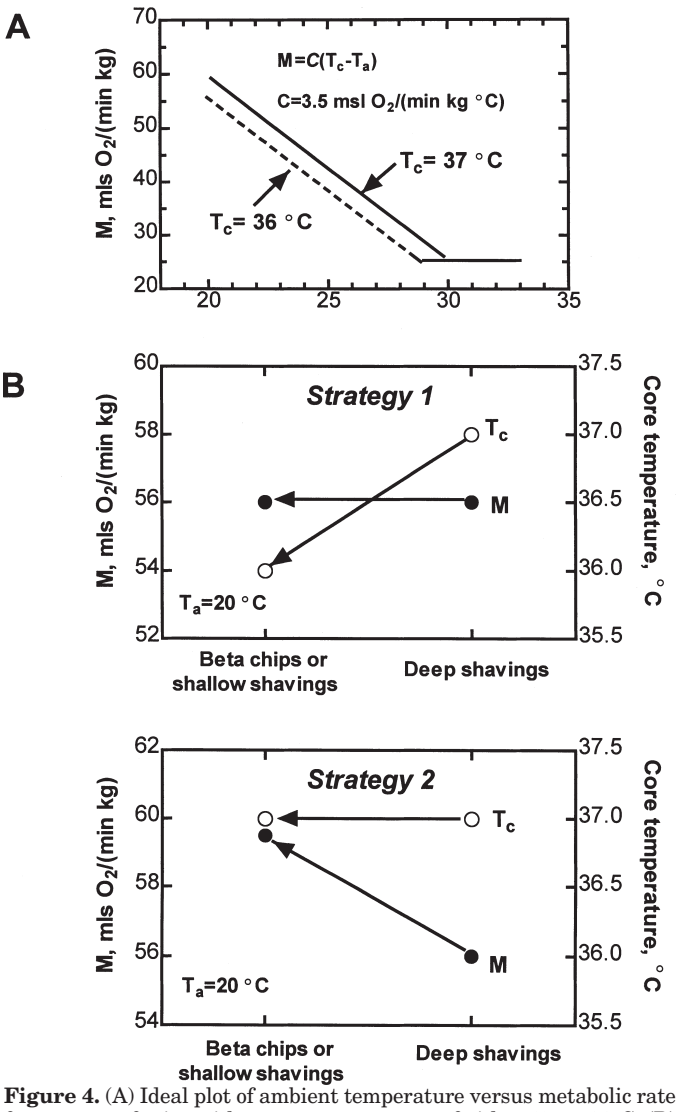

Figure 4. (A) Ideal plot of ambient temperature versus metabolic rate for a group of mice with a core temperature of either 37 or 36°C. (B) Response of metabolic rate and core temperature at an ambient temperature of 20°C when mice are moved from a cage with deep wood shavings to one with either shallow wood shavings or beta chips. This change in bedding is assumed to lower the operative ambient temperature (T_o) by 1°C. In strategy 1, core temperature of mice decreases by 1°C and metabolic rate remains unchanged. In strategy 2, core temperature is unchanged and metabolic rate must increase to maintain a balance between heat gain and heat loss. *See* text for details.

the effect of bedding material on body temperature.

Two of the most important factors that affect metabolic rate are ambient and body temperature. When housed at ambient temperatures below thermoneutrality, metabolic rate of a homeotherm (i.e., birds and mammals) can be expressed in terms of an equation derived from the principles of Newton's law of cooling (1, 3):

$$M = C(T_c - T_a)$$

where M = metabolic rate (ml of O_2/min/kg), C = whole-body thermal conductance (ml of O_2/min/kg/°C); T_c = core temperature and T_a = ambient (or operative) temperature.

Whole-body thermal conductance is the absolute value of the slope of the line predicting metabolic rate as a function of ambient temperature below thermoneutrality. The C value is generally constant and minimal at ambient temperatures below thermoneutrality; C increases markedly with heat stress and also increases from day to night (1, 3). The value of C essentially represents the metabolic cost of thermoregulation in animals exposed to temperatures below their thermoneutral zone. In other words, the higher the value of C, the greater the metabolic energy that must be expended per degree decrease in ambient temperature to maintain a constant core temperature.

The aforementioned relationship is useful for explaining how bedding material and core temperature affect metabolic rate (Fig. 4A). In this scenario, M is plotted as a function of T_a, with core temperature set at either 36 or 37°C and a constant value of C. Lowering core temperature from 37C° to 36°C shifts the lower critical temperature to the left and reduces the metabolic requirements to thermoregulate at any T_a below the lower critical temperature. Assuming a T_a of 20°C and that the T_o for deep wood shavings is 1°C higher than the T_o for beta chips and shallow wood shavings, metabolic rate may change depending on how core temperature is regulated (Fig. 4B). When the T_o decreases by moving mice from deep shavings to shallow shavings or beta chips, M will remain unchanged if core temperature is reduced (strategy 1). If T_o decreases while core temperature remains constant, then metabolic rate must increase to maintain a balance between heat production and heat loss (strategy 2). Apparently, strategy 1 is followed by mice during the daytime, as based on results of the metabolism experiment (*cf.* Fig. 3). Metabolic rate of mice on the beta chip and shallow wood shavings was essentially unchanged, but their core temperature was significantly reduced, compared with that of mice housed on deep wood shaving bedding. In the 24-h study (*cf.* Fig. 1), mice that could not burrow in the beta chips and shallow bedding material allowed body temperature to decrease by 0.9°C. On the other hand, at night when activity is high, core temperature was similar for all bedding materials. Under these conditions, mice on the beta chips and shallow wood shavings lean toward strategy 1 and should have an increased metabolic rate to maintain the same core temperature as animals housed in the deep wood shaving bedding.

The data from this study were collected from the female CD-1 mouse, a common strain of laboratory mouse. The revolution in transgenic technology and other genetic techniques has led to the development of a plethora of mouse phenotypes that are likely to have altered thermoregulatory characteristics. Since body temperature can have such a profound effect on all physiologic processes, it is important to understand how bedding material and other factors affect the thermoregulatory and metabolic responses of laboratory rodents, including the unique transgenic animals that, compared with the CD-1 mouse, are likely to have altered sensitivity to environmental challenges. Age, body weight, and number of animals housed per cage would certainly be critical factors to consider. Moreover, in pharmacologic and toxicologic studies where an endpoint is dependent on core temperature, one would expect that the type of bedding will have a profound effect on the efficacy of a drug or toxicant.

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Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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