Development of homeothermy
in infant C3H mice

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The ability of C3H mice to maintain normal nest-level body temperatures when removed from the nest and isolated at room temperature (24°C) was examined between 2 and 30 days of age. To account for the increasing body size and fur growth which might serve to decrease the rates of heat loss in the developing mouse, colonic temperatures were recorded for isolated live and dead mice matched for age, body weight, and pelage, over a 1-h exposure to 24°C. The results point to there being three stages of thermoregulatory development: 2-5, 8-15, and 18-30 days of age, with the pup being unable to maintain normal body temperature when isolated from the nest until 18 days of age. Increases in body size, weight, and growth of fur had little effect on heat loss through 18 days of age.

It is well known that the newborn of certain altricial mammals, including the common house mouse (*Mus musculus*) and the Norway rat (*Rattus norvegicus*), lack adult-like thermoregulatory capabilities and have been regarded as essentially poikilothermic (see, e.g., Adolph, 1957; Fairfield, 1948; Lagerspetz, 1962; Pichotka, 1971). When removed from the nest and isolated at an ambient temperature (T_a) lower than nest-temperature (T_n), the colonic temperature (T_{co}) of newborn pups drops quickly to levels only slightly above T_a. When in the nest, however, young mice and rats are able to maintain their body temperature (T_b) within a relatively narrow range of about 33°-37°C, even though T_a may be much lower, depending upon a combination of factors, including heat production, infant huddling, insulation quality of the nest, and maternal behaviors (see Leon, 1986, for a review). During the first 3 weeks of life, pups develop an increasing ability to maintain nest-level T_{co} when isolated at increasingly lower T_as (see, e.g., Conklin & Heggeness, 1971; Fairfield, 1948; Poczopko, 1961). The transition from a poikilothermic to a homeothermic state is not simple, and it clearly reflects the increasing ability of the infant to maintain a thermal balance between heat production and heat loss, using either physiological or behavioral means.

In adult mice and rats, exposure to a T_a below the zone of thermal neutrality results in a sequence of behavioral and/or physiological responses in order to increase T_b. The initial attempt is usually behavioral, consisting simply of moving to an area of higher T_a. If unsuccessful, adults may attempt next to conserve heat loss by means of general vasoconstriction of peripheral blood vessels and/or piloerection of fur. With further decreases in adult T_b, oxygen (O_2) consumption increases and there is an increase in heat production by both shivering and non-shivering mechanisms (see Bligh, 1973, for a review).

In newborn mouse and rat pups, these mechanisms of thermoregulation are either absent or limited (for general reviews, see Alexander, 1975; Hull, 1973; Schmidt, Kaul, & Heldmaier, 1987). For example, house mice (Ogilvie & Stinson, 1966) and Norway rats (Kleitman & Satinoff, 1982) are able to move to a higher T_a as early as 1 day of age. However, their limited locomotor abilities make this form of behavioral thermoregulation very inefficient, so that it can require up to 2 h in very young pups. If T_a is very low, the pup may not be able to reach an area of higher temperature, resulting in further heat loss, with subsequent hypothermic immobility, additional heat loss, and, eventually, death. Although vascular response to lowered T_a has been suggested as early as 4 days of age (Poczopko, 1961), most researchers have found little evidence of this ability until 12-14 days of age in the rat (e.g., Conklin et al., 1971; Taylor, 1960). Furthermore, because most mice and rats are born without fur, they lack both passive (insulation provided by fur) and active (piloerection) means of reducing the rate of heat loss that are available to the adult. Pups develop a full coat of fur between 7 and 14 days of age; however, piloerection has been reported not to be evident earlier than 14 days of age (Taylor, 1960), and the presence of fur seems to have little insulation value until 14-18 days of age in the young rat (Hahn, 1956). Notwithstanding major increases in body insulation and pelage, mice show little change in preferred T_a (T_{pref}) of 34°-37°C from 5 to 16 days of age, after which T_{pref} gradually decreases until the adult T_{pref} of 31.8°C is reached around 84 days of age (Ogilvie et al., 1966). In a more recent report (Goodrich & Wilk, 1981), T_{pref} of Swiss mice 3-14 days of age was found to be only 29°-31°C, which is similar to the T_n maintained by the parent mice.

With regard to mechanisms for increasing T_b, it is clear that nonshivering thermogenesis precedes shivering in the newborn mammal's response to cold. Brown adipose tissue, the principal effector organ for nonshivering heat pro-

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duction in many newborn mammals (see Hull, 1973, for a comprehensive review), has been reported to be at maximum levels in mice at birth, with subsequent decreases over the first 7 days of life (Tarkkonen & Julka, 1968). In contrast, shivering has not been detected until 7 days of age in the infant mouse (Arjamaa & Lagerspetz, 1979) and 11-12 days of age in the rat (Gulick, 1926; Taylor, 1960). Although infant mice (Chew & Spencer, 1967) and rats (Taylor, 1960; Thompson & Moore, 1968) may exhibit a marked increase in O_2 consumption in response to lowered T_a as early as birth, the young pups cannot respond to lowered T_a as quickly as adults, nor are they capable of sustaining high levels of O_2 consumption for long durations.

There appears to be a general consensus that mice and rats pass through several stages or phases of temperature regulatory capabilities following birth. The first phase, marked by the ability to increase O_2 consumption and heat production in response to decreased T_a, lasts through 5-7 days of age. From about 5-13 days of age, there is a marked increase in heat production capability, whether measured directly by calorimetry (see, e.g., Walker, 1967) or indirectly by O_2 consumption (see, e.g., Fitzgerald, 1953; Lagerspetz, 1962; Thompson et al., 1968), followed by the gradual increase to adult levels over the next 2 months. Although it is clear that thermoregulatory capabilities improve during this period, some researchers (e.g., Conklin & Heggeness, 1971) have argued that this improvement is due primarily to the increasing capability to produce body heat, whereas others (e.g., Spiers & Adair, 1986) have attributed it primarily to the increasing ability to prevent heat loss.

The purpose of the present study was to provide additional data regarding the thermoregulatory capabilities in the developing mouse pup, particularly the relative contributions of heat production and heat conservation over the first 3 weeks of age. In the present study, the relative abilities of pups to prevent loss of T_{co} when exposed to a T_a lower than T_n were compared in live and dead littermates from 2 to 30 days of age. The littermate pups were of identical age and were matched for body weight within each age, so it is likely that they were also similar in terms of fur growth and body insulation. Thus, it was hypothesized that T_{co} differences between live and dead mice should be due to the abilities of the live mice to produce body heat and/or to actively conserve heat loss through vasoconstriction or by piloerection of the fur.

METHOD

Subjects

The subjects were 96 C3H mice (*Mus musculus*), with 48 of each gender, born and reared in 30.4×18×12 cm polyethylene cages with wire-grid tops, wood-chips on the floors, and ad-lib access to food and water. Separate groups of 6 males and 6 females were assigned to one of eight age groups according to a modified split-litter design. The ages were 2, 5, 8, 10, 12, 15, 18, and 30 days of age. The pups remained with their mothers and littermates until testing; the colony room was on a 12:12-h light:dark cycle beginning at 0800 h, and its temperature was maintained at 24°±2°C.

Apparatus

The experiment was conducted inside a Forma Scientific walk-in environmental chamber maintained at 24°±1°C and 50% humidity. A 38.1×24.1×43.2 cm chamber, maintained at 35°±2°C by thermostatically controlled heating elements and blower-circulated air, was kept inside the environmental chamber. Smaller 12×10×10 cm compartments, with wire-mesh tops, were placed inside the 35°C chamber to house individual mice during the study.

T_{co}s were recorded by an Ellab Universal Thermocouple thermometer (Type TE3; differential sensitivity ±0.1°C). A type RM6 thermometer probe (1.2 mm in diameter) was used for mice 15 days of age and older, while an ISC probe (0.3 mm in diameter, with a silicone-covered tip to prevent colonic damage), was used for mice 12 days of age and younger. An Akro-Mils cabinet (XT-315) housed the mice inside the environmental chamber between T_{co} recordings. Each drawer of the cabinet was divided in half, providing approximately 12×8.6×7.3 cm for each mouse, and the floor was covered with a paper towel. The T_a within each drawer closed with no mouse present was 24°±0.1°C.

Procedure

At the appropriate age, each mouse was removed from the rearing cage and weighed to the nearest 0.1 g. Two male and 2 female mice from each litter were then matched within gender for body weight and assigned to one of two groups such that the mean body weight was as equal as possible for each group. Each of these groups consisted of 3 males and 3 females. The mice in the first group were then placed individually into small jars for a 10-min period and sacrificed by CO_2 exposure (hereafter referred to as the *dead* group). The mice in the second group were placed individually into similar jars for a 10-min period without CO_2 (hereafter referred to as the *live* group). Following this procedure, the mice were removed from the jars and placed into holding cages within the 35°C chamber for a 1-h period to ensure a constant T_a for all mice prior to exposure to a lower T_a, and to maintain or return the T_b of the dead mice to about 35°C, the T_b normally maintained by the pups in the nest with the presence of the mother.

Both the live and the dead mice were removed from the 35°C chamber after 1 h, T_{co}s were recorded, and the mice were then placed individually into the drawers of the cabinet maintained at 24°C. Colonic temperatures were recorded again after 5, 15, 30, and 60 min in the drawers. In this manner, each pair of live and dead pups of each gender within each age group was as identical as possible: they were littermates of identical age, near identical body weight, body mass, and pelage. Thus, any differences in T_b found between the live and dead groups within each age following exposure to 24°C T_a could not be attributed to differential passive defense of heat loss due to differences in body weight or mass, body insulation, or the amount of fur growth. Instead, any differences between the groups had to be due to differences in either the active defense of heat loss, such as vasoconstriction or piloerection, or the ability to produce body heat.

RESULTS AND DISCUSSION

Figure 1 presents the mean T_{co}s of live and dead mice for each age group as a function of the amount of time exposed to 24°C. It is quite clear that the decrement in T_{co}s over the 1-h session for live mice is inversely related to age, with both the rate and extent of decline decreasing with age until 18 days of age, after which there is little change through 30 days of age. It is quite likely that both the 18- and the 30-day-old live mice were made slightly hyperthermic by exposure to 35°C, and that their drops in T_{co}s during the first 5 min reflect a return to normal T_{co} rather than deficiencies in either heat production or heat conservation ability.

Two-day-old mice in the present study were hairless and had a mean body weight of only 1.6 g, in contrast to the full coat of fur and mean body weight of 7.9 g for
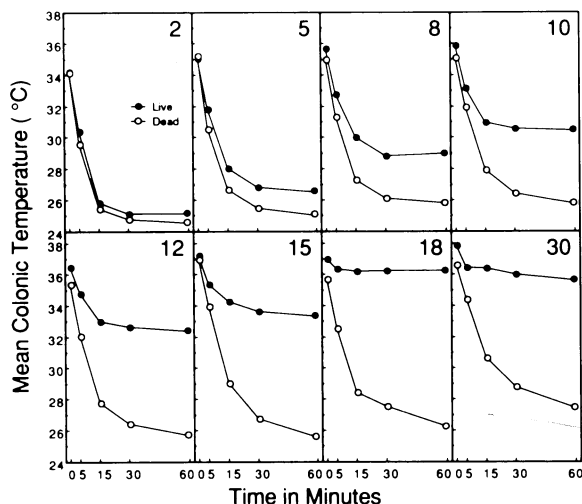
Figure 1. Mean colonic temperatures for live and dead mice as a function of age and time of exposure to 24°C.

18-day-old mice. Yet examination of the data for dead mice indicates that although older mice tended to decrease T_{co}s at a slower rate than did younger mice, temperatures at the end of the session were only slightly higher for the older and heavier mice, while absolute temperature decrements were about equal at the two ages. For example, the 18-day-old dead mice exhibited a mean T_{co} decrease of 9.5°C; the much smaller and furless 2-day-old mice exhibited a decrease of 9.6°C. From these data, it appears that factors of increased pelage and body mass alone cannot account for the steady increase in T_{co}s with increasing age for the live mice by the end of the 1-h exposure.

These data were analyzed in a four-way analysis of variance, with one repeated measure. The results indicated highly reliable main effects of age [$F(7,64) = 113.70$, $p < .0001$], of live versus dead mice [$F(1,64) = 567.38$, $p < .0001$], and of time of exposure [$F(4,256) = 1,807.01, p < .0001$]. All two-way interactions involving the factors of age, live/dead status, and time of exposure, as well as the three-way interaction depicted in Figure 1 [$F(28,256) = 10.82$], were significant (all Fs > 13.07, all ps $< .0001$). Multiple comparison tests conducted within the three-way interaction showed that, with the exception of the 18-day-old live mice, all age groups of live and dead mice exhibited reliable decreases in temperature from the first to last measurement (all Fs > 29.36, all ps $< .0001$). The 30-day-old live mice showed a reliable decrease in T_{co} over the first 5 min [$F(1,256) = 11.74, p < .001$]; the slight decreases over the remaining 55 min were not significant, suggesting that the pups were slightly hyperthermic upon removal from the 35°C chamber.

Comparisons between live and dead mice at each interval of exposure within each age revealed that 2-day-old mice did not differ appreciably at any interval [all Fs($1,256$) < 2.18], suggesting almost complete poikilothermy. However, although their initial temperatures were equivalent at the 0 interval [all Fs($1,256$) < 2.09],

5-, 8-, 10-, and 15-day-old live mice had higher colonic temperatures than did their dead age-mates at every interval over the 60-min session [all Fs($1,256$) > 5.17, all ps $< .025$]. At 12-, 18-, and 30-days of age, live mice had higher T_{co}s at every measurement (all ps $< .05$), including the initial one.

Figure 2 depicts the mean T_{co} differences (live minus dead) as a function of age after 5–60 min of exposure to 24°C T_a. Examination of these data indicates a rather consistent temperature difference from 5–60 min for both 2- and 5-day-old mice. Together with the data presented in Figure 1, these changes in T_{co} suggest that the 2-day-old mouse produces very little body heat, whereas the 5-day-old mouse produces some heat over the first 5 min, but little thereafter as its T_{co} falls at the same rate and extent as the dead mice for the remainder of the session. In 8-day-olds, T_{co} does not fall as rapidly in live mice over the first 15 min, but there is little difference over the rest of the session, indicating that 8-day-olds may be capable of not only producing more body heat, but doing so for longer durations than can 5-day-olds. The 10- and 12-day-old live mice demonstrated a similar ability to remain increasingly warmer than their dead controls over the entire session. A similar pattern is noted at 15 days and older, except that these age groups exhibit an increasing difference in T_{co} from their dead controls, particularly over the first 5–15 min. This finding suggests that mice at these ages are capable of responding to a decline in T_a more rapidly than are younger groups, and further, that they are capable of producing greater amounts of body heat for longer periods of time.

Overall, the results of this study are in general agreement with previous research suggesting that there are several phases or stages of temperature regulatory capabilities following birth. In the present study, there appear to have been three distinct stages: 2–5, 8–15, and 18–30 days of age. From 2 to 5 days of age, there is near poikilothermy, although some limited heat production may be present in
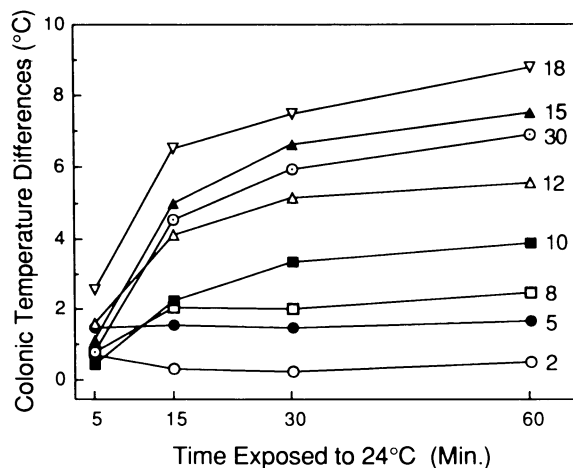

Figure 2. Mean colonic temperature differences between live and dead mice as a function of age and time of exposure to 24°C.

5-day-olds. From 8 to 15 days of age, there appears to be an increase in the ability to produce body heat, whereas the 18-day-old and older groups seem to be able to produce more body heat and/or to employ heat conservation means not available to younger mice. These data suggest a gradually increasing ability to increase body temperature which cannot be accounted for by a decrease in rate of heat loss with increasing body weight and fur growth. In fact, the increasing growth of fur from 8 to 18 days of age appeared to have virtually no insulating effect on passive heat loss, for T_{co}s of the dead mice were similar at each time interval regardless of age, in agreement with a previous report on the rat (Hahn, 1956). Thus, when several means of heat conservation (e.g., increased body mass and/or weight, pelage, or skin insulation) are controlled, the ability of the pup to maintain T_{co} when challenged by a lower T_a appears to show gradual rather than sudden improvement from 5 to 18 days of age, after which there is no further improvement through at least 30 days of age.

The results of this study indicate that young C3H mice, when exposed to a T_a of 24°C, are unable to maintain normal nest-level T_{co}s until 18 days of age, at which age they appear to be as capable as 30-day-old mice. However, it is not known whether the 18-day-old mouse would be able to prevent loss of T_{co} as well as the 30-day-old if exposed to T_as much lower than 24°C. The finding that the young mouse is unable to maintain nest-level T_{co} when removed from the nest until the 3rd week of age should be of interest to the increasing numbers of behavioral researchers (e.g., Kail & Spear, 1984; Shair, Barr, & Hofer, 1991) who examine the varying capabilities of neonatal animals in situations that require the young pup to be isolated from the nest. A number of studies have shown that a decrease in T_{co} for even a brief period during infancy may result in behavioral changes lasting into adulthood (e.g., Caldwell & Kesner, 1966; Hutchings, 1963, 1965, 1967; Schaefer, 1963; Schaefer, Weingarten, & Towne, 1962), so it would be surprising if such exposures did not also have short-term consequences.

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