

HEART FUNCTION, CIRCULATION AND RESPIRATION IN EMBRYO AND HATCHING

Early Development of Thermoregulatory Competence in Chickens: Responses of Heart Rate and Oxygen Uptake to Altered Ambient Temperatures

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ABSTRACT

Avian embryos need a heat supply from the external environment to develop, because heat loss from an egg overwhelms heat production of an embryo and accordingly they cannot maintain body temperature constant during incubation. In the domestic fowl, a minute homeothermic competence appears during the end of incubation with subsequent large augmentation soon after hatching. However, hatchling's metabolic rate still produces less heat than that required to offset heat loss. This stage prior to reaching "full-blown" homeothermy is designated as "power-limited". In this power-limited stage of thermoregulation, development of thermoregulatory competence in response to altered ambient temperature (T_a) involves the rapid maturation of heart rate (HR) regulation. The first part of this report reviews briefly: (1) early development of homeothermic thermal and metabolic responses to altered T_a in chick embryos and hatchlings, and (2) HR responses to altered T_a in newly hatched chicks during the power-limited stage of thermoregulation, which depend on the state of development of thermoregulatory competence. The last part concerns our recent preliminary experiment and hypothesis with regard to the early development of thermoregulatory competence in hatchlings developing in the power-limited stage. The preliminary experiment shows possible influence of brooding temperatures (*i.e.* preferred brooding T_a of 35°C and low brooding T_a of 24–27°C) to the development of thermoregulatory competence in terms of HR responses to altered T_a . We hypothesise that homeothermic-metabolic response to altered T_a develops in parallel with the homeothermic HR response and additionally the brooding temperature will influence the maturation of homeothermic-metabolic response so that hatchlings maintained at low T_a will develop the homeothermic-metabolic response earlier than those at the preferred brooding T_a . The result shows that homeothermic-metabolic response obtained in the white leghorn chickens does not develop in parallel with the homeothermic HR response obtained previously in the broiler chickens. In addition, maturation of homeothermic-metabolic response is observed on just hatched chicks maintained at both the preferred brooding T_a and low T_a . The failure of proving the hypothesis suggests other possibilities of which investigation will improve our knowledge of thermoregulation in avian embryos and hatchlings.

Keywords: thermoregulatory competence, heart rate response, metabolic response, embryo, hatchling, altered ambient temperature, preferred brooding temperature, cooling exposure, warming exposure, chick, duck, emu

1. INTRODUCTION

Avian embryos developing inside an eggshell need a heat supply from the environments, *i.e.* parent birds or

an incubator. When they are exposed to low ambient temperature (T_a), the embryos cannot maintain body temperature (T_b) because of their small heat production compared with a possible large decrease in T_a caused

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by isolation of heat supply and so avian embryos are effectively poikilotherms. In the domestic fowl (*Gallus gallus*), thermal conductance of the egg is estimated to be about $70\text{--}75\text{ mW}^\circ\text{C}^{-1}$ during incubation, while embryos generate heat at most of about $130\text{--}140\text{ mW}$ prior to pipping and about 160 mW during external pipping (EP) at an incubation temperature (T_a) of 38°C (Tazawa *et al.*, 1988a, 1988b, 2001). Accordingly, these metabolic heats raise the egg temperature about 2°C or a bit more above T_a during the last stages of incubation (Tazawa and Rahn, 1987). However, if the eggs are exposed to an environment 10°C cooler than T_a , the rate of heat loss during cooling exceeds the embryos' maximum rate of heat production by about 5–6-fold. In fact, if the embryos are exposed to air 10°C cooler than the egg's centre they have to generate heat at a rate of about 800 mW to keep the egg temperature steady (Turner, 1986). Eventually, chick embryos cannot maintain constant body temperature when they are exposed to low T_a and thus they are poikilothermic. However, the transition from poikilothermy to homeothermy begins to occur during the late stages of incubation. When chicken eggs were cooled gradually for a prolonged period to low T_a to make the imbalance between heat loss from the eggs and heat production of the embryos as small as possible, 18-day-old pre-pipped embryos showed a weak metabolic response to cooling with a subsequent augmented response in externally pipped embryos (gradual cooling test, Tazawa *et al.*, 1988b). When the drop of T_a was small, *e.g.* 6°C , a feeble compensatory metabolic response was also confirmed in the late pre-pipped embryos. Namely, during prolonged cooling with the small drop in T_a , they had a temperature coefficient (Q_{10}) of oxygen uptake (\dot{M}_{O_2}) lower than 2 (the Arrhenius value; Tazawa *et al.*, 1989; Kuroda *et al.*, 1990). A transient homeothermic-metabolic response to cooling greater than 3°C was also demonstrated in late pre-pipped and pipped chick embryos (Freeman, 1964).

While the thermoregulatory competence begins to emerge during the last stages of embryonic development in chickens, it is rapidly augmented soon after hatching (Romijn and Lokhorst, 1955; Freeman, 1964, 1967, 1971; Wekstein and Zolman, 1967; Tazawa and Rahn, 1987), or after a predicted time of hatching irrespective of emergence from the eggshell (Tazawa and Rahn, 1987; Tazawa *et al.*, 1988b). However, the hatchling's metabolic rate still produces less heat than that required to offset heat loss. In models of the

development of thermoregulation, illustrating the transition from poikilothermy to homeothermy in precocial and altricial birds (Tazawa *et al.*, 1988b; Whittow and Tazawa, 1991), this stage prior to reaching "full-blown" homeothermy where hatchlings are capable of fully defending their body temperature against cooling has been designated as "power limited". In this stage, the hatchling's capacity to generate heat is limited. In chickens, development of thermoregulatory competence during the power-limited stage still progresses after hatching (Tazawa and Rahn, 1987). Previously, it was shown that the development of thermoregulatory competence in response to altered T_a during the first week of post-hatch involved the rapid maturation of heart rate (HR) regulation. HR responses to altered T_a in newly hatched chicks during the power-limited stage of thermoregulation depended on the state of development of thermoregulatory competence (Khandoker *et al.*, 2004). Another preliminary experiment indicated that brooding temperature of hatchlings influenced the development of thermoregulatory competence in reference to HR responses to altered T_a . Based on our previous experience with the thermoregulatory responses of HR, we hypothesise that metabolic responses to altered T_a in chick hatchlings during the power-limited stage also depend on the state of development of thermoregulatory competence and brooding temperature. The present report reviews briefly the HR responses to altered T_a in chick hatchlings and the possible influences of brooding temperature on the HR responses. In addition to the review, we examine whether or not metabolic responses to altered T_a also depend on the state of development of thermoregulatory competence and brooding temperature.

2. DEVELOPMENT OF THERMOREGULATORY COMPETENCE IN EMBRYOS AND HATCHLINGS

Many studies on the thermogenesis in chick embryos and hatchlings show that during embryonic development chickens are substantially poikilothermic and after hatching rapidly develop the competence to maintain body temperature (Romijn and Lokhorst, 1955; Freeman, 1964, 1967, 1971; Wekstein and Zolman, 1967; Tazawa and Rahn, 1987). Development of thermoregulatory competence can be shown by determining egg temperature (T_{egg}) or body temperature (T_b) of animals that are exposed to low T_a .

The equilibrium temperature of eggs and hatchlings at T_a of 38°C and quasi-equilibrium temperature at T_a of 28°C in chickens are shown in Figure 1 (Tazawa and Rahn, 1987). A quasi-equilibrium state of embryo's temperature is defined as a change in T_{egg} of less than 0.1°C h^{-1} and has been reached 5-hour after exposure to low T_a . The equilibrium temperature of the embryos increases steadily to reach a value of about 2°C above T_a of 38°C just before hatching due to increasing metabolism during development while maintaining a constant thermal conductance of the egg. After hatching, T_b increases further to reach a plateau about 3°C above T_a of 38°C on day 3–4. After exposure to T_a of 28°C for the 5-hour period, T_{egg} reaches the quasi-equilibrium temperature that also gradually increases with embryonic development to reach a value of about 1°C above T_a just before pipping and hatching. In consequence, the large, prolonged drop in T_a swamps the nascent thermoregulatory response of T_{egg} to T_a . However, upon hatching, the quasi-equilibrium temperature 5-hour after exposure to the lower T_a is remarkably higher than 28°C and reaches levels approaching 38°C by day 3. This augmented T_b confirms the previous studies on the rapid development of thermoregulatory competence soon after hatching (Romijn and Lokhorst, 1955;

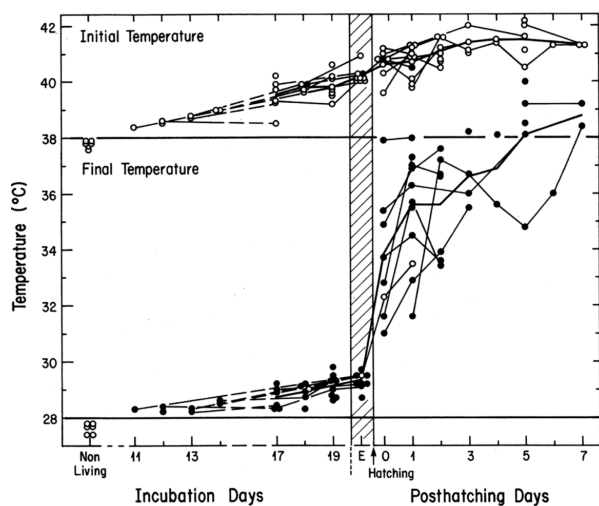


Fig. 1 The temperature of eggs and hatchlings before and after 5-hour exposure to ambient temperature of 28°C. The shaded area shows external pipping period and symbol E indicates that the embryos pip externally the eggshell. Initial temperature corresponds to the equilibrium temperature of the eggs and hatchlings at 38°C before exposure to 28°C, and final temperature corresponds to the temperature of eggs and hatchlings that reaches after 5-hour exposure to 28°C; that is, quasi-equilibrium temperature. Solid lines connect the results from the same individuals. Reproduced with permission from Tazawa and Rahn (1987).

Freeman, 1964, 1967, 1971; Wekstein and Zolman, 1967). Additionally, the closed circles in the initial temperature and open circles in the final temperature show the equilibrium temperature and quasi-equilibrium temperature of a single embryo that fails to hatch at predicted time of hatching. Although it stays within the eggshell on day 22 (equivalent to day 0 post-hatch) and day 23 (day 1 post-hatch), the quasi-equilibrium temperature is remarkably above that at the EP period. This result indicates that emergence from the eggshell at a time of predicted hatching is not necessary for an increase in metabolic rate in response to cooling.

The ratio of oxygen uptake (\dot{M}_{O_2}) after 5 hours at a T_a of 24°C to that at 37°C in chick embryos and hatchlings is shown in Figure 2 (Tazawa and Rahn, 1987). The ratio is remarkably constant at about 40% throughout the last half of incubation until hatching. The Q_{10} of \dot{M}_{O_2} is about 2, indicating a simple physiochemical process without an attempt of thermoregulation by the embryos. However, immediately upon hatching, the ratio increases to 70–95%, supporting rapid development of thermoregulatory competence. The two ringed circles in Figure 2 show two eggs that pipped, but the embryos failed to emerge from the eggshell on day 22 of incubation as in the case of the embryo shown in Figure 1. Their \dot{M}_{O_2} is appreciably higher than that during the previous day, indicating again that emergence from the eggshell is not necessary for augmented metabolic response to

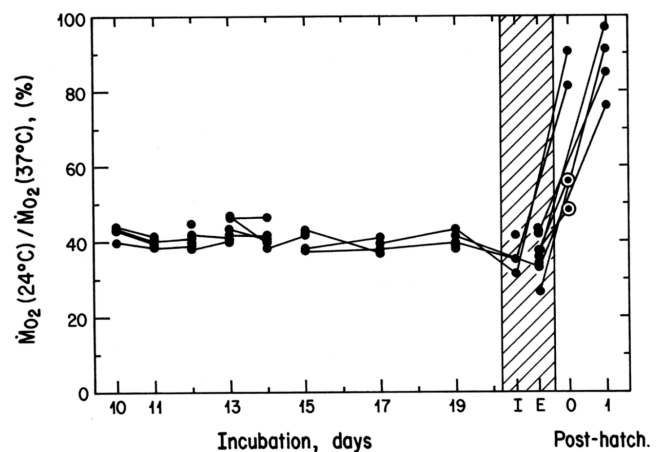


Fig. 2 The ratio of oxygen uptake at quasi-equilibrium temperature of 24°C after 5-hour to that at equilibrium temperature of 37°C in chick embryos and hatchlings. The shaded area corresponds to pipping period and symbols I and E indicate internally pipped and externally pipped embryos. Reproduced with permission from Tazawa and Rahn (1987).

cooling and rapid development of thermoregulatory competence occurs in unhatched embryos that reach a maturity to hatch. The similar thermoregulatory response of an unhatched embryo is shown by changes in its HR in response to cooling (Tazawa *et al.*, 2001).

3. HEART RATE RESPONSES TO ALTERED T_a IN NEWLY HATCHED CHICKS

While the thermoregulatory competence improves immediately upon hatching, it involves the rapid maturation of HR regulation in birds (Tazawa *et al.*, 2002; Tamura *et al.*, 2003; Khandoker *et al.*, 2004). The HR response to altered T_a in newly hatched chicks depends on the state of development of thermoregulation. The maturation of the thermoregulatory HR response to altered T_a has been investigated on the broiler hatchlings in Muroran, Japan (Khandoker *et al.*, 2004). Figure 3 shows representative responses of instantaneous heart rate (IHR) and skin temperature (T_s) to altered T_a in newly hatched chicks. The hatchlings were kept in a brooding cage placed under an electric lamp maintaining an environmental temperature at about 24–27°C until the experiment. After setting non-invasively three flexible ECG electrodes and thermistor probe on the skin of the chest wall and abdomen, the chicks were placed in a programmable incubator maintained initially at 25°C. The chicks were kept at the temperature of 24–27°C, so the measurement of IHR and T_s was began soon after placing them in the incubator. After 1-hour measurement at 25°C, T_a was changed to 35°C for the next hour and then returned to 25°C for an hour. On day 0, *i.e.* the period of 24 hours after hatching, measurements were made during the first 8-hours after hatching (group 0–7 hours), the second 8-hours (group 8–15 hours), and the following two 4-hour periods (group 16–19 hours and group 20–23 hours).

A chick in group 0–7 hours (Figure 3, panel A) shows a flat and narrow HR baseline with spontaneous HR decelerations during the first 1-hour exposure to 25°C. Upon exposure to increased T_a (35°C), HR baseline increases in an exponential fashion in parallel with increase in T_s , and it decreases as in T_s decreases during the final cooling period. Accordingly, this chick responds to the warming and cooling bout with a thermoconformity pattern of HR baseline. As the age of hatchlings advances, T_s at T_a of 25°C increases and

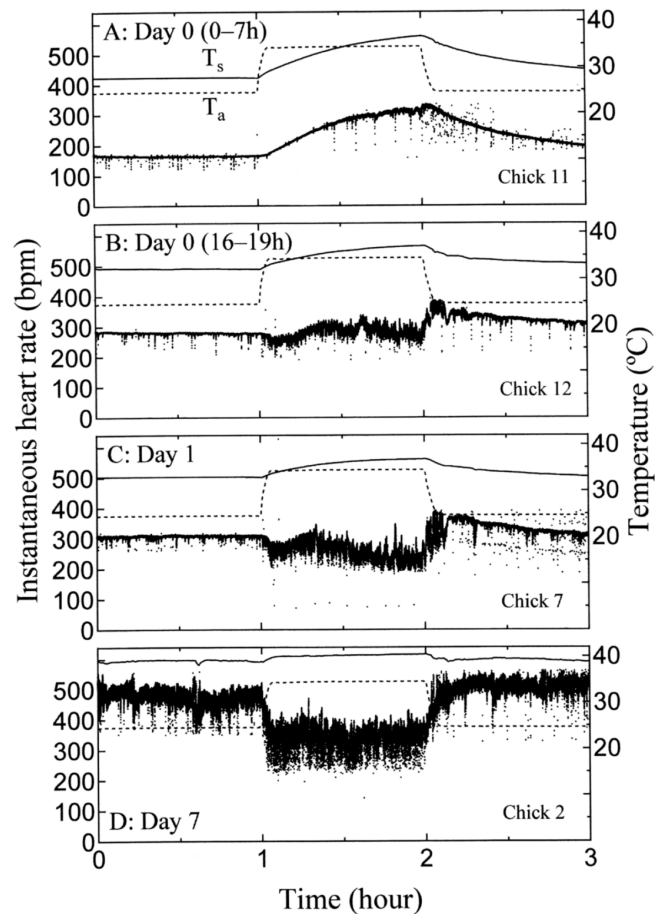


Fig. 3 Responses of instantaneous heart rate (IHR) and skin temperature (T_s) to altered ambient temperature (T_a) in four chick hatchlings with different ages. T_a shown by dotted line is changed from 25°C for the first 1-hour period to 35°C for the next 1-hour period and finally to 25°C for the last 1-hour period. T_s shown by solid line responds to the step change in T_a with an exponential fashion. The magnitude of changes in T_s becomes small as hatchlings advance, indicating development of thermoregulatory competence with age. The HR baseline is flat and narrow at 25°C in 0-day-old chicks and becomes wide with age as is shown in 7-day-old chicks. The changes in HR baseline in response to warming are different even between two 0-day-old hatchlings and turns from thermo-conformity response (0–7 hours hatchling) to thermoregulatory response (7-day-old hatchling) with various responding patterns in chicks with intermediate ages. Additionally, the HR baseline at 35°C becomes wide with age, indicating that HR fluctuations increased by exposure to warm environment of 35°C are further increasing with age. Modified from Khandoker *et al.*, 2004.

the elevation in T_s during warming at 35°C becomes small, indicating development of thermoregulatory competence. A chick in group 16–19 hours (Figure 3, panel B), of which thermoregulatory competence is more developed, responds to warming with wide range HR baseline that remains at a similar level to the

preceding 1-hour period. In addition, HR baseline increases transiently against cooling during the last 1-hour period. The HR baseline of a 1-day-old chick (Figure 3, panel C) decreases in response to warming and increases against cooling. The thermoregulatory patterns of HR response during warming and cooling bouts are more evident in a 7-day-old chick of which T_s changes minutely (Figure 3, panel D). These are the representative patterns of the responses of HR and T_s to warming and cooling bouts according to chick age, which suggests that the developmental stage (age) has a significant effect on the HR response to altered T_a and thermoregulatory competence.

The results of the responses of T_s and HR to warming and cooling bouts measured for 114 hatchlings ranging in age from day 0 to day 7 are

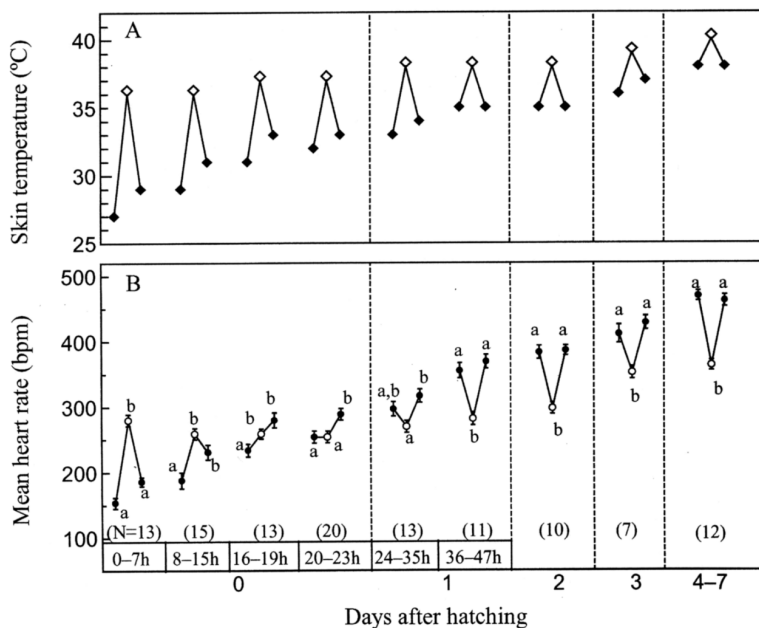


Fig. 4 Changes in skin temperature (T_s) and heart rate (HR) at the first 25°C (closed symbols), the middle 35°C (open symbols) and the last 25°C (closed symbols) during the post-hatch week. The hatchlings on the day of hatching (day 0) are divided into four groups and those on day 1, two groups. Twelve hatchlings on days 4–7 are pooled into one group. T_s at 35°C increases with age and the difference between T_s at 25°C and T_s at 35°C (ΔT_s) decreases with age, indicating that thermoregulatory competence is increasing with the lapse of time, particularly during the day of hatching. Mean HR is decreasing from about 280 bpm to 250 bpm during the day of hatching with subsequent increase to about 350 bpm on days 4–7. Meanwhile, HR at 25°C increases from about 150 bpm during the first 8-hour period on day 0 to 480 bpm on days 4–7.

Accordingly, HR at 25°C which is far below that at 35°C during the early period of day 0 turns to exceed it during the late day 0 and the early day 1. While the development of thermoregulatory competence is shown by decrease in ΔT_s , HR is regulated from thermo-conformity response to thermoregulatory response. Reproduced with permission from Khandoker *et al.*, 2004.

summarised in Figure 4 (Khandoker *et al.*, 2004). For T_s and HR responses, the mean value of the last 10-minute period of individual 1-hour exposure periods was obtained and all the mean values for a given developmental stage were averaged. As the chicks age and develop, the T_s increases when exposed to the warm T_a (35°C) and the magnitude in the change of T_s between warming and cooling exposures decreases, indicating that thermoregulatory competence is progressing with age. Meanwhile, HR increases on warming and decreases on cooling in the two youngest groups (0–7 hours and 8–15 hours on day 0), showing the thermoconformity pattern in HR response. During the last 8-hour period of day 0, the thermoconformity response of HR to warming turns to be insignificant change and the insignificant increase of HR during cooling turns to be significant. Therefore, HR increases significantly against the decrease in T_a , showing a thermoregulatory response. The insignificant change in HR during warming and the significant increase in HR during cooling are maintained during the first half of day 1 with subsequent thermoregulatory response during the rest of day 1. Chicks at 2 days of age and older show a thermoregulatory pattern of HR response to altered T_a .

The development of thermoregulatory competence differs among the birds. The difference is evident between the altricial and precocial birds (*cf.* Whittow and Tazawa, 1991). Among the precocial species, it is shown that early thermoregulatory competence progresses in aquatic domestic ducks (*Anas platyrhynchos* var. *domestica*) more than in terrestrial chickens (Kuroda *et al.*, 1990). The size of body also influences the development of thermoregulatory competence. In general, the larger the hatchling is, the smaller the increase in mass-specific heat production required to maintain body temperature during cooling exposure. The development of thermoregulatory competence involves the maturation of HR regulation, and so HR responses to altered T_a should be different between precocial species. Measurements of HR responses to cooling in large ratite emu hatchlings show the thermoregulatory response on the day of hatching and suggest that ability of thermo-

regulation progresses considerably before hatching compared with chickens (Tamura *et al.*, 2003).

Accordingly, we provisionally examined ducks and emus for HR responses to altered T_a (unpublished data). In the duck, IHR baseline tends to decrease with cooling when the plumage is still wet soon after hatching, but a few hours later when the plumage dries HR baseline increases in response to mild cooling. During the external pipping period, some duck embryos respond to mild cooling with an increase in HR baseline, while other embryos decrease HR baseline with or without initial increase. The early thermoregulatory response of HR seems to be more advanced in ducks than in chickens. In the emu a marked increase in HR baseline in response to mild cooling is observed in externally pipped embryos. Even internally pipped (IP) embryos tend to increase HR baseline during an early period of mild cooling. This thermoregulatory HR response in ratite IP embryos corresponds to that in chick hatchlings on the day of hatching (day 0) and that in duck embryos during the external pipping period.

4. POSSIBLE INFLUENCE OF BROODING TEMPERATURE TO THERMOREGULATORY RESPONSES OF HEART RATE

When the changes in T_a induce no response of carbonic acid discharged by respiratory exchange in animals, this condition is termed the “neutral condition” (Pembrey, 1895; Freeman, 1964) and the zone of thermal neutrality for newly hatched chicks is narrow around 35°C (Freeman, 1963). The thermoneutral temperature is now defined as the temperature that produces the lowest heat production of the animals and a preferred ambient temperature for hatchlings is situated near the thermoneutral temperature (Nickelmann and Tzschentke, 2002). Meanwhile, for studies on ontogeny of thermoregulation, hatchlings are kept at T_a of 25°C with the possibility of moving to a warmer place (Nickelmann and Tzschentke, 2002). In our previous experiment investigating into the maturation of thermoregulatory HR response to altered T_a , the hatchlings were kept at T_a of 24–27°C. The preferred brooding T_a is much higher (*circa* 35°C), so a low brooding T_a may induce a thermal stress to the hatchlings. Accordingly, it is feasible that the low brooding T_a prior to experiments

influences the development of thermoregulatory HR responses to altered T_a .

In a preliminary experiment, influence of brooding temperature on HR responses to altered T_a was briefly investigated on broiler chicks in Muroran. Chick hatchlings were divided into two groups soon after hatching. Hatchlings in one group were kept in the incubator maintained at 38°C (group A) and those in group B were moved into a cage placed in a laboratory room and warmed by an electric lamp to 24–27°C. Individual chicks were tagged to identify the time of hatching. ECG electrodes and a thermistor probe were attached on a hatchling in the same manner as in the previous experiment (Khandoker *et al.*, 2004). The hatchling was put in the programmable incubator of which temperature was set at 35°C. After an hour, the measurements of IHR and T_s were made for the first hour at 35°C, then a 2-hour period at 25°C, a third hour at 35°C and finally an hour at 25°C. Two hatchlings from groups A and B were selected so that ages were similar and eight hatchlings were examined for the HR responses to the series of cooling, warming and cooling bouts.

Figures 5 and 6 present the responses of IHR and T_s in four hatchlings kept at 38°C and 24–27°C environments prior to the experiment, respectively. The ages of hatchlings were 22-hour (panel A in both Figures 5 and 6), 30 and 32-hour (panel B), 38 and 39-hour (panel C) and 47 and 40-hour (panel D). The solid lines indicate changes in T_s in response to altered T_a . The mean of T_s at 35°C during the first 1-hour exposure was 38.7°C (SD = 0.7) and 38.6°C (SD = 0.3) for chicks of groups A and B, respectively. In both groups, T_s during the 2-hour cooling period decreased in an exponential fashion to the values of final 10-minute period that tended to increase with ages. The mean of T_s during the final 10-minute period at 25°C was –7.5°C (SD = 1.0) and –5.3°C (SD = 2.8) lower than T_s at 35°C for chicks of groups A and B, respectively. Although statistical analysis was not carried out due to the small number of experimental animals, these values, as well as patterns of T_s changes during the first 2-hour cooling, indicate that thermoregulatory competence improved in advanced hatchlings of both groups, but the largest improvement occurred in group B. Meanwhile, HR baseline tended to increase upon cooling that began at the first hour of recording, but decreased with the lapse of time towards the end of the 2-hour cooling period, particularly in group A (Figure 5). The subsequent

warming at 35°C was accompanied by increases in HR baseline even in advanced chicks in group A (e.g. 38-hour and 47-hour chicks), showing a thermoconformity response of HR to warming. After 1-hour of warming, HR baseline transiently increased upon cooling with a subsequent gradual decrease as during the first cooling. As a result, although the chicks maintained at 38°C responded to cooling with transient thermoregulatory increases in HR baseline at the beginning of cooling, their HR changes during warming were a thermoconformity response (Figure 5).

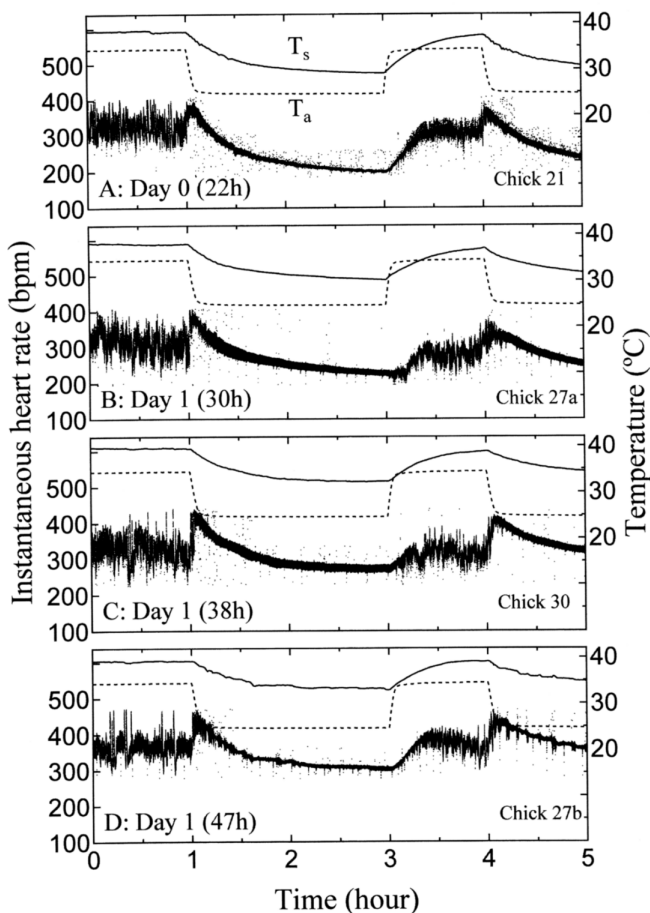


Fig. 5 Responses of skin temperature (T_s) and instantaneous heart rate (IHR) to changes in ambient temperature (T_a) during 5-hour period in four newly hatched chicks that were kept in 38°C environment after hatching. T_s and T_a are shown by solid and dotted lines, respectively. The mean values of T_s and IHR during the last 10-minute period in the first 35°C environment, the next 25°C environment and the second 35°C environment are 38.2°C, 29.2°C and 37.2°C and 319 bpm, 200 bpm and 311 bpm for the 22-hour chick, 37.9°C, 30.2°C and 36.7°C and 307 bpm, 227 bpm and 291 bpm for the 30-hour chick, 39.5°C, 32.5°C and 38.7°C and 313 bpm, 271 bpm and 312 bpm for the 38-hour chick, and 39.2°C, 33.1°C and 39.0°C and 362 bpm, 303 bpm and 355 bpm for the 47-hour chick, respectively.

On the other hand, advanced chicks maintained at 24–27°C changed HR baseline with a thermoregulatory pattern in response to altered T_a (Figure 6). A 22-hour chick (panel A) showed the thermoconformity pattern for the HR baseline. A 32-hour chick (Figure 6, panel B) increased HR baseline transiently in response to cooling which began at 1-hour and 4-hour of the recording and decreased HR baseline transiently during warming with subsequent transient increases and again decreased towards the end of warming. While a 39-hour old chick (Figure 6, panel C) maintained almost constant HR baseline in response to warming, a 40-hour old chick (Figure 6, panel D) down-regulated HR baseline during warming, showing a thermoregulatory response. Although the experimental data were obtained from only four hatchlings in both groups and lacked statistical analysis, Figures 5 and 6 indicate possible influence of brooding temperature on the development of thermoregulatory competence and the HR regulation in relation to the development of thermoregulatory competence in chick hatchlings.

5. METABOLIC RESPONSES TO ALTERED T_a IN CHICK HATCHLINGS

5.1. Hypothesis

Based on the previous study on the homeothermic response of HR to altered T_a (Khandoker *et al.*, 2004) and the preliminary experiment on the possible influence of brooding temperature on the development of thermoregulatory competence in relation to HR responses, we hypothesised that homeothermic-metabolic response to altered T_a develops in parallel with the homeothermic response of HR in chick hatchlings. In addition, the brooding temperature will influence the maturation of homeothermic-metabolic response so that hatchlings maintained at low T_a will develop the homeothermic-metabolic response earlier than those at the preferred brooding T_a (35°C).

5.2. Materials and methods

White Leghorn chicken eggs were brought from Texas A & M University to the University of North Texas and were incubated for 20 days in a Lyon forced-draught incubator maintained at a temperature of 37.5°C and a relative humidity of about 60%. Eggs were turned automatically every 4h. On day 20, all eggs were

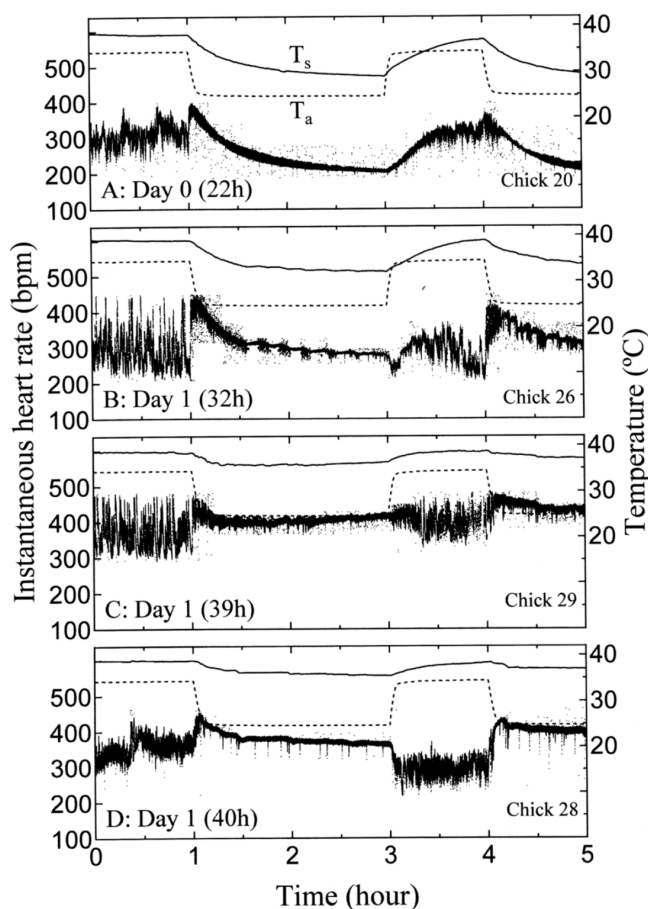


Fig. 6 Responses of skin temperature (T_s) and instantaneous heart rate (IHR) to changes in ambient temperature (T_a) during 5-hour period in four newly hatched chicks that were kept in 24–27°C environment after hatching. T_s and T_a are shown by solid and dotted lines, respectively. The mean values of T_s and IHR during the last 10-minute period in the first 35°C environment, the next 25°C environment and the second 35°C environment are 38.1°C, 29.0°C and 36.8°C and 313 bpm, 206 bpm and 315 bpm for the 22-hour chick, 38.9°C, 32.1°C and 38.8°C and 287 bpm, 279 bpm and 254 bpm for the 32-hour chick, 38.4°C, 36.3°C and 38.6°C and 365 bpm, 415 bpm and 413 bpm for the 39-hour chick, and 38.8°C, 35.5°C and 38.2°C and 361 bpm, 364 bpm and 305 bpm for the 40-hour chick, respectively.

transferred to a hatching tray in the incubator and allowed to hatch. The hatching tray was divided by crossing rectangular cardboards 10 cm wide into square sections with a side about 8 cm long so that each section could contain a single egg. A digital camera took a photo of the eggs in the hatching tray every 30 minutes through the day and night to determine the time of hatching. Upon hatching, individual chicks were identified by numbers indicating date and time of hatching written on a tag attached to the leg and stayed there for about an hour to allow for down to dry. They were then trans-

ferred alternately to an incubator maintained at 35°C (group A) or a brooding cage placed under an electric lamp maintaining temperature at 24–27°C (group B). Water and food were given in the incubator and the brooding cage. The day of hatching was designated as day 0.

Metabolic rate was determined as oxygen uptake (\dot{M}_{O_2}) by a hatchling using flow through respirometry previously described for chicken eggs (Dzialowski *et al.*, 2002). Hatchlings were placed in individual PVC respirometers (with an approximate volume of one litre) and submerged into a water bath. A gas mixture (20.94% O_2/N_2) was pumped from a cylinder through the individual respirometers. The flow rate of the gas mixture ventilating the respirometers was regulated with fine needle valves and was measured at the inflow side of the respirometers for the individual chicks. The gas mixture passed through copper coils submerged in the water bath prior to entering the respirometers to allow it to equilibrate the temperature of the water bath. The outflow gas from the respirometers passed through drierite and soda lime to remove water vapour and CO_2 respectively before the percentage of O_2 was measured using a Sable Systems O_2 analyser and recorded with a data acquisition system (Sable Systems). \dot{M}_{O_2} was calculated using the equation of Hill (1972) and expressed as $mlO_2 h^{-1}/g^{-1}$ at standard temperature, pressure and dry (STPD).

The body temperature (T_b) of individual chicks and T_a inside the respirometers were measured to 0.1°C by fine gauge thermocouples. For cloacal temperature, the thermocouple wire was wound through three small holes in the centre of a small plastic disk about 1 cm in diameter so that 1–1.5 cm length of wire came out of the plastic disk. The end of wire was inserted into the cloaca and the plastic disk was glued to the feathers with superglue. After the experiment, the plastic disk was removed from the feathers by melting superglue with acetone.

The responses of \dot{M}_{O_2} and T_b to a cooling and warming bout were measured on 0, 1, 2 and 3 day-old hatchlings in both groups. The single measurement for a hatchling was made over a 4-hour period. The hatchling of known age had the thermocouple placed in the cloaca and was placed in the respirometer. A lid of the respirometer had a small hole through which two thermocouple wires were passed in advance. One thermocouple measured T_a in the respirometer and another had a female connector to connect a male connector from the thermocouple in the cloaca. After

the lid was closed hermetically, the hole was also hermetically sealed with clay. Initially, the respirometer with the hatchling was submerged in a water bath maintained at about 35–36°C for 2-hour period. Then, the water in the bath was replaced at once with cool water (*circa* 24–25°C) that was maintained in it for the next hour. Lastly, the water was warmed back to 35–36°C for the final hour by replacing the bath at once with warm water. During these cooling and warming bouts, the averaged values of \dot{M}_{O_2} , T_b and T_a during the last 10-minute period prior to water change were determined and used as the value of individual parameters at the altered T_a . After the experiment, the hatchling was taken out of the respirometer and the thermocouple was removed from the cloaca. The hatchling was weighed to 0.1 g and returned to the original incubator or cage. The data are shown as means and standard deviations.

The effects of the thermal environment and age on \dot{M}_{O_2} and T_b were tested with multivariate repeated-measures ANOVA. To examine the significance of differences between group means after an ANOVA was significant, Tukey's *post hoc* test was made.

5.3. Results

Four-hour exposure experiments were made on 58 hatchlings in group, A which were kept at 35°C, and 43 hatchlings in group B, which were kept at 24–27°C. The results on day 0 were anticipated to show various patterns of \dot{M}_{O_2} response to altered T_a depending on the time of measurement, and so the chicks were supposed to be divided into four groups according to the age. However, the anticipated various patterns for \dot{M}_{O_2} response were not observed and accordingly the results on day 0 were divided into only two groups; group 0–11 hours and group 12–23 hours. Masses of hatchlings in the two groups are shown in Table 1. In both temperatures, masses of 1-day hatchlings were significantly lower than those of group 0–11 hours and 2-day hatchlings. Between groups A and B, masses at individual ages were not significantly different.

The mean values of T_b and \dot{M}_{O_2} for 0-day chicks classified into two age groups, 1-day and advanced chicks on days 1, 2 and 3 are shown in Figure 7. T_b s at the initial preferred T_a (35°C) on individual post-hatch days (*i.e.* developmental pattern) were not significantly different between hatchlings maintained at either the warm or cool environment. The developmental

Table 1 Mean masses with SD of chicks in groups A and B

Chick age	Group A chick mass (g)	Group B chick mass (g)
0–11 hours	39.6 ± 3.7	41.4 ± 4.7
12–23 hours	36.4 ± 4.2	37.2 ± 4.2
Day 1	35.7 ± 3.6	34.6 ± 2.8
Day 2	40.4 ± 3.6	38.9 ± 5.0
Day 3	41.5 ± 7.7	35.7 ± 7.6

patterns of T_b during cooling were not significantly different between the two brooding temperature groups. Meanwhile, developmental changes in T_b at both T_a 's of 35°C and 24–27°C occurred on day 2, *i.e.* T_b s on days 2 and 3 were significantly higher than those on days 0 and 1. The results of statistical analysis on \dot{M}_{O_2} were the same as those for T_b . The developmental patterns of \dot{M}_{O_2} at the preferred T_a and low T_a were not significantly different between groups A and B with significant increases in \dot{M}_{O_2} on day 2.

5.4. Discussion

5.4.1. Lack of transitional responses of \dot{M}_{O_2} to altered T_a during the day of hatching

In contrast to the hypothesis based on the previous study on HR responses to altered T_a in chick hatchlings, responses of \dot{M}_{O_2} to altered T_a did not show various patterns on day 0 in either group A or B. \dot{M}_{O_2} was up-regulated during cooling and down-regulated during warming, showing the thermoregulatory response even in the newly hatched (0–11 hour) chicks from both groups.

\dot{M}_{O_2} is a function of HR and O_2 pulse which is determined by a product of the stroke volume and arterio-venous O_2 content difference and so we hypothesised that \dot{M}_{O_2} would respond to altered T_a as did HR on the assumption that the O_2 pulse during warming and cooling bouts would not change significantly so as not to influence \dot{M}_{O_2} . However, we failed to obtain the metabolic responses to altered T_a that would change in parallel with HR responses on the day of hatching. HR responses to altered T_a on day 0 changed from the thermoconformity pattern to the thermoregulatory pattern (Khandoker *et al.*, 2004), while metabolic responses to altered T_a are thermoregulatory pattern throughout all the experimental days. If both are the case, the increase in \dot{M}_{O_2} in response to cooling during the first half of day 0 is accompanied with the decrease in HR and instead a

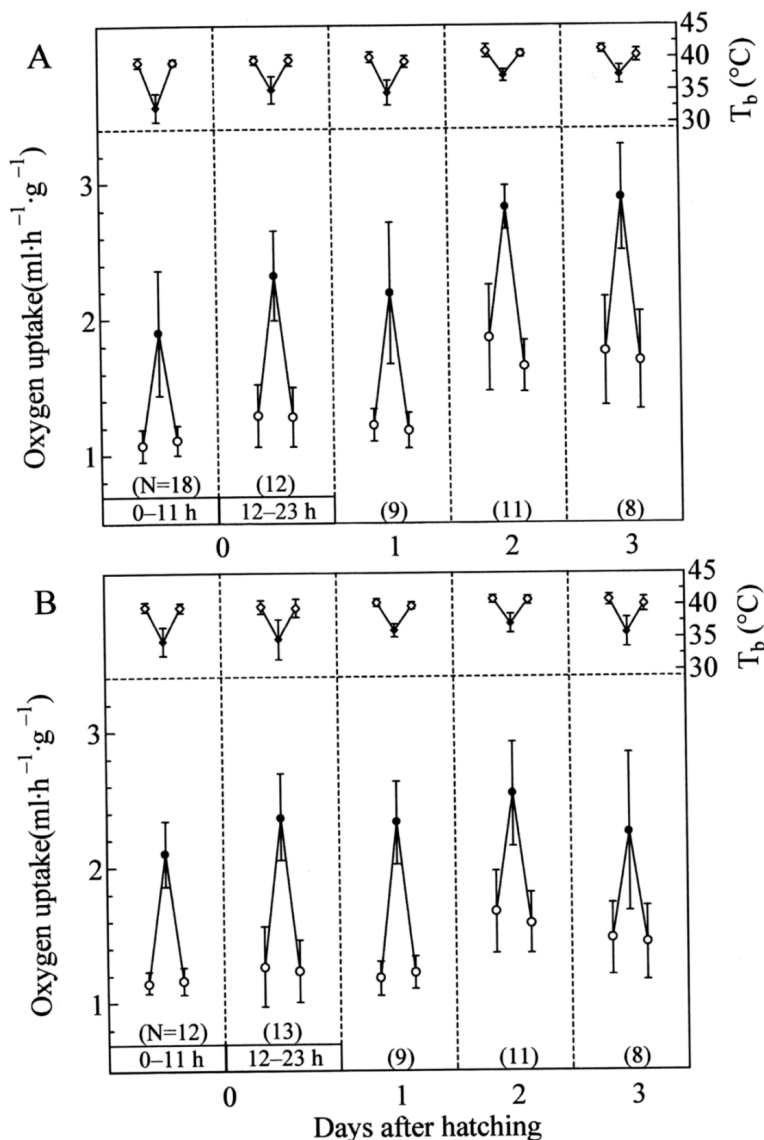


Fig. 7 The changes in body temperature (T_b) and oxygen uptake (\dot{M}_{O_2}) during cooling and warming bouts in developing chick hatchlings kept at T_a of (A) 35°C and (B) 24–27°C. \dot{M}_{O_2} changes inversely against the changes in T_b in response to altered ambient temperature, showing thermoregulatory metabolic response soon after hatching.

large increase in O_2 pulse. During the last half of day 1 and afterwards, the increase in \dot{M}_{O_2} in response to cooling is accompanied by an increase in HR and accordingly a small increase or decrease in O_2 pulse. Thus, the O_2 pulse is predicted to have large changes in response to warming and cooling bouts during the first half of day 0 with subsequent small change at advanced ages. However, it is questionable that the stroke volume and/or the arterio-venous O_2 content difference of just hatched chicks can change largely in response to altered T_a .

Another possibility to explain the inconsistency between the metabolic responses and HR responses is due to the different breeds used in the two experiments, *i.e.* white leghorn eggs in Texas and broiler eggs in Muroran. Investigations into the development of tonic vagal control of HR in chick embryos have produced controversial results. While cholinergic chronotropic control began to occur around the end of the second week of incubation in broiler chickens (Höchel *et al.*, 1998; Chiba *et al.*, 2003), white leghorns lacked this control during the entire period of embryonic development (Crossley and Altimiras, 2000). Bantam chick embryos also showed obvious cholinergic tone and it was suggested that different breeds might develop different levels of cholinergic chronotropic control at different times (Crossley *et al.*, 2003). The early development of thermoregulatory competence may also differ between different breeds of chickens.

5.4.2. No significant effects of brooding T_a on development of homeothermic metabolic response

Metabolic responses to altered T_a were not significantly different between both groups of hatchlings maintained at a high temperature (35°C) and at a low temperature (24–27°C). The thermoneutral temperature (35°C) may be a preferred T_a for the newly hatched chicks in terms of the changes in IHR fluctuation. In the low temperature environment (25°C), HR baseline of newly hatched chicks is narrow, indicating lack of HR fluctuation. The HR baseline becomes wide when hatchlings are exposed to warm temperature of 35°C and also in advanced hatchlings which are provided with more developed competence of thermoregulation even in the low temperature environment. Thus, it is assumed that HR fluctuations occur in chick hatchlings that live in thermal environment favourable to their thermoregulatory competence. A low T_a in contrast to the preferred T_a results in thermal stress depressing HR fluctuations. It seems that the thermal stress, *i.e.* exposure to low T_a or cold conditioning, does not necessarily induce adverse effects on physiological functions of hatchlings. In

broiler chickens, short repetitive cold exposure at an early age (cold conditioning) improves their thermotolerance in later life (Shinder *et al.*, 2002). We hypothesised that a mild thermal stress would improve development of thermoregulatory competence in newly hatched chicks. The present results fail to prove the hypothesis, but at least indicates that the thermal stress does not induce adversely development of homeothermic metabolic response in newly hatched chicks. Another mild thermal stress, *e.g.* exposure to T_a less than 10°C below the preferred T_a , may prove the hypothesis. The influence of brooding temperature on HR responses to altered T_a remains to be investigated again in broiler chickens and because of the possibility of different results due to different breeds, it should also be investigated in white leghorn chickens.

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