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Maturation of the homeothermic response of heart rate to altered ambient temperature in developing chick hatchlings (*Gallus gallus domesticus*)

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Khandoker, A. H., K. Fukazawa, E. M. Dzialowski, W. W. Burggren, and H. Tazawa. Maturation of the homeothermic response of heart rate to altered ambient temperature in developing chick hatchlings (*Gallus gallus domesticus*). *Am J Physiol Regul Integr Comp Physiol* 286: R129–R137, 2004. First published October 2, 2003; 10.1152/ajpregu.00316.2003.—On the basis of evidence showing that instantaneous heart rate (IHR) of chick hatchlings responds to exposure to altered ambient temperature (Ta; Tazawa H, Moriya K, Tamura A, and Akiyama R. *Comp Biochem Physiol A* 131A: 797–803, 2002), we elucidate here the developmental timeline for the homeothermic response of HR in newly hatched chicks (*days 0–7*) maintained at room temperature (~24–27°C). Hatchlings were exposed to Ta of 25, 35, and 25°C for 1-h periods, respectively, and IHR was measured together with skin temperature (Ts) during this warming and cooling bout. Early 0-day-old (0 day) chicks responded to warming and cooling exposures with various changes in HR baseline. In newly hatched chicks (0–7 h old), HR baseline was elevated during warming (Δ126 beats/min, $n = 13$) and declined during cooling ($-\Delta$94 beats/min). With progress of development on *day 0*, the elevation of HR baseline during warming decreased and advanced 0-day chicks tended to decrease HR baseline during warming rather than increase HR. The more developed 1- to 7-day-old chicks exhibited the expected homeothermic decrease in HR during warming. The diurnal variations of HR responses during warming and cooling on the first day of post-egg life indicate that pronounced development of thermoregulatory competence occurs during the day of hatching (*day 0*). The response of IHR fluctuations to altered Ta was observed in the form of low- and high-frequency oscillations. High-frequency oscillations corresponding to respiratory sinus arrhythmia developed as the hatchlings aged. There was a significant increase in the number of chicks exhibiting both low- and high-frequency oscillations that depended on age and the development of thermoregulatory competence of hatchlings.

heart rate variability; instantaneous heart rate; skin temperature; thermal stress

DEVELOPMENT OF THERMOREGULATORY competence in birds has been studied in both altricial and precocial species before and after hatching, and models and mechanisms were suggested for the development and adaptation of avian thermoregulation (5, 9, 17–19, 29, 30). While development of thermoregulation in altricial species is an event after hatching, precocial chickens and ducks are provided with incipient thermogenesis during the later stages of incubation with subsequent rapid increase after hatching (7, 8, 12, 13, 17, 20, 23–25). In these ontogenetic studies on thermoregulation, the metabolic and thermal responses of embryos and hatchlings to altered ambient temperature (Ta) were investigated. If the changes in Ta had no effect on metabolic rate of animals, this Ta was termed the thermally neutral temperature (7). For newly hatched chicks, the thermoneutral temperature was ~35°C, which might be the preferred Ta of chicks. Ta lower than the thermoneutral temperature may impose a cold stress on chick hatchlings.

There is evidence that instantaneous heart rate (IHR) and HR baseline in late embryos and hatchlings of chicken and emu respond to cooling and warming (21, 26, 27). In newly hatched chicks, three types of IHR fluctuations have been reported: types I, II, and III (14). Type II HR fluctuations exhibit oscillations with a mean peak frequency of 0.07 Hz, which we designated as low-frequency (LF) oscillation of HR (13). A previous experiment elucidated that type II LF oscillation was induced or increased in oscillatory frequency when hatchlings were exposed to low Ta and inversely the exposure to high Ta eliminated type II HR oscillation (27). Similarly, type I high-frequency (HF) oscillations related to respiratory sinus arrhythmia (RSA) were observed during warming and cooling in these hatchlings (27). These changes in HR fluctuations were also accompanied by changes in HR baseline. In another experiment with a 22-day embryo that pipped externally the eggshell, but failed to hatch, HR baseline was elevated and began to oscillate in response to cooling (26). The maturity and thermoregulatory response of this embryo might have been equivalent to a hatchling.

These studies suggest that the development of thermoregulatory competence in response to altered Ta involves the rapid maturation of HR regulation. We hypothesize that HR responses to altered Ta in newly hatched chicks depends on the state of development of nascent thermoregulatory competence, which will increase with age. In the present experiment, we determine the changes that occur in HR baseline and IHR in response to warming and cooling in newly hatched chicks to elucidate the timeline for the development of HR regulation with relation to the thermoregulatory competence of the hatchling.

MATERIALS AND METHODS

Incubation and hatchlings. Fertile eggs of broiler chickens (*Gallus gallus domesticus*) were obtained from a local hatchery in Japan. Eggs were placed in horizontal position in a forced-draught incubator and rotated automatically 90° every 3 h. Temperature of the incubator was maintained at 38°C with relative humidity of ~60%. On *day 20* of incubation, all eggs were transferred from a suspended tray to individual cages put on a floor of the incubator and allowed to hatch.

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Every 30 min a digital photo of the eggs was taken through the day and night to determine the time of hatching. On hatching, individual chicks were identified by serial numbers indicating time of hatching written on a tag attached to the leg and stayed there for ~1 h to allow for down to dry. Then they were transferred to a brooding cage placed under an electric lamp maintaining temperature of the vicinity at ~24–27°C until experiment. All the chicks were held in this cage put in a laboratory under the natural light condition before experimentation began and provided with water and food ad libitum. The day of hatching was designated as *day 0*.

Measurement of IHR. IHR was determined in real time from an ECG with the aid of a computer as described previously (14, 15). A chick to be examined was taken at random from the brooding cage, and three flexible Ag/AgCl gel ECG electrodes were attached noninvasively to the chest wall and ventral abdomen (14, 15). Two chicks were put into a grounded wire cage ($13 \times 24 \times 16$ cm^3, measuring cage), and the electrode wires were fixed above the back with adhesive tape so that they could move in the measuring cage. The wires were fixed to the measuring cage and connected to an amplifier with electrically shielded wires. The amplified and band-passed ECG signals were sampled at a frequency of 12,000 Hz by a 16-bit analog-to-digital converter (sound card). The sampled ECG signals were displayed on the computer monitor, and a threshold of the voltage was set on the monitor so that it could cross individual R waves above background noises. IHR (in beats/min) was calculated from a time interval between two adjacent R waves that crossed the threshold line. Each value of IHR was plotted in a graph on the monitor for provisional display and simultaneously saved in a computer file.

Measurement of temperature. The temperature of the skin (Ts) was measured with a thermistor probe 1 mm in diameter that was placed between the skin and one of the ECG electrodes fixed on the chest wall under the wing. The thermistor wire was fixed above the chick's back together with ECG electrode wires. Another thermistor probe was fixed in the vicinity of top of the measuring cage and measured Ta. Ts and Ta were recorded every 20 s in the computer file with an accuracy of 0.1°C. The present experiment measured Ts as a surrogate of body temperature (Tb), because it was convenient to set the thermistor probe at the same site as the ECG electrode. In addition, attaching the probe on the skin should lessen strain to hatchlings compared with insertion of the probe into the body (e.g., cloaca). Therefore, Ts was used for referential information to homeothermic responses of HR in the present study.

Procedure of exposure. Measurements were made for 114 chicks ranging in age from 0 to 7 days. Individuals were examined once and weighed before installation of ECG electrodes and thermistor probe. The measuring cage containing the chick that was installed with electrodes and probe was placed in a programmable incubator with temperature set at 25°C for the first 1 h, then at 35°C for the next 1 h and at 25°C for the last 1 h. During the warming and cooling bout lasting for a 3-h period, IHR and Ts were continuously measured together with Ta. Four hatchlings were simultaneously examined in the programmable incubator. No water and food were given during the measurement.

Statistical and power spectrum analysis. Effects of the thermal environment and age on HR baseline and Ts were tested with multivariate repeated-measures ANOVA. To test for differences between group means after an ANOVA was significant, we used Tukey's post hoc test.

Power spectrum analysis of IHR was made by fast Fourier transform to examine the fluctuations in IHR as described previously (14). The presence of any peaks in the IHR power spectrum corresponding to main oscillatory frequency was noted, and the number of hatchlings exhibiting the peaks was tallied. To test for developmental trends in the presence or absence of HF and LF oscillations, we used a Cochran-Armitage trend test. This statistical test examined the development of HF and LF peaks in the power spectrum by comparing the influence of hatchling age on the change in occurrence of the number of individuals exhibiting an HF or LF peak in the power spectrum. The developmental trends were examined during the initial cooling and the first warming exposures. *P* values obtained from the Cochran-Armitage trend test were corrected using Bonferroni procedures. Effects of the thermal environment and age on the specific peak frequency in the HF and LF oscillations were tested using a multivariate repeated-measures ANOVA. Only those hatchlings that exhibited HF or LF oscillations at all three testing temperatures were included in the analysis. Tukey's post hoc test was used to examine differences between treatments within a developmental age. All statistics were carried out using SAS 8.2. The level of significance for all tests was $P < 0.05$.

RESULTS

Horal classification of hatchlings. Three-hour exposure experiments were made on 114 hatchlings ranging in age from *day 0* to *day 7* posthatch (Table 1). Because the results on *day 0* showed various patterns of IHR responses to warming depending on the time of measurement, they were divided into four groups according to the age when the measurements were begun. On *day 0*, measurements on chicks were made during the first 8 h after hatching (*group 0–7 h*), the second 8 h (*group 8–15 h*), and the following two 4-h periods (*group 16–19 h* and *group 20–23 h*). The *day 1* posthatching chicks were also broken into two groups: *group 24–35 h* and *group 36–47 h*. Masses of hatchlings tended to increase on development from *day 0* to *day 7* (Table 1).

Continuous recordings of responses. Representative patterns of Ts and IHR responses to altered Ta in four hatchlings belonging to the four horal groups on *day 0* are shown in Fig. 1. Ts baseline during the first 25°C exposure was almost constant, and the mean value of the last 10-min period was defined as the reference Ts (referred to as Ts_{ref}). In response to step-wise increases in Ta from 25 to 35°C (i.e., warming) and decreases from 35 to 25°C (cooling), Ts of the four hatchlings changed in a fashion of the first order exponential response. However, Ts_{ref} and the magnitude of change in Ts (i.e., the difference between Ts_{ref} and Ts at the end of warming, referred to as ΔTs) were different between the four individuals. For

Table 1. *Measurement time (day and night)*

	N_T	Daytime N_D 0600 to 1800	Nighttime N_N 1800 to 0600	Hatchling Mass, g
Day 0				
0–7 h	13	7	6	49.0±4.1
8–15 h	15	7	8	46.7±4.0
16–19 h	13	2	11	47.2±2.4
20–23 h	20	7	13	48.1±2.9
Day 1				
24–35 h	13	3	10	47.6±1.7
36–47 h	11	1	10	49.1±2.9
Day 2	10	7	3	48.8±0.7
Day 3	7	4	3	51.3±2.7
Day 4	3	3	0	52.5±2.7
Day 5	3	3	0	52.1±3.0
Day 6	4	2	2	62.0±4.2
Day 7	2	2	0	69.0±6.7
Total	114	48	66	48.8±0.7

Values are mean ± SD. N_T is the total number of animals measured at each stage of development. N_D and N_N represent the number of animals measured during daytime and nighttime, respectively.

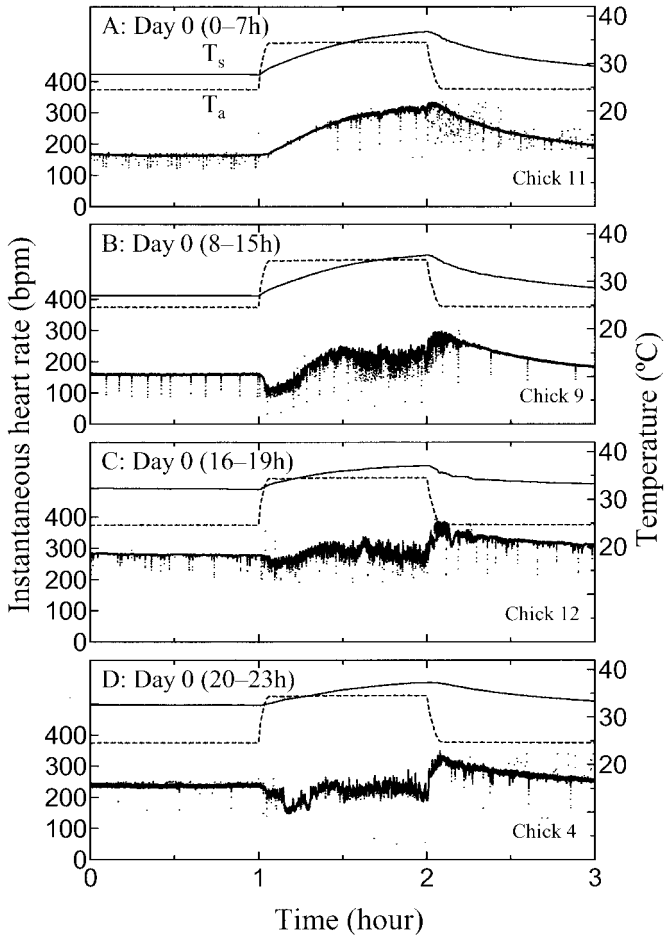

Fig. 1. Responses of instantaneous heart rate (IHR, shown by individual points) and skin temperature (Ts, solid line) to altered ambient temperature (Ta, dashed line) in 4 chicks classified into 4 groups on the day of hatching (*day 0*). *A*: chick in *group 0–7 h* (body mass 48.1 g). *B*: chick in *group 8–15 h* (47.0 g). *C*: chick in *group 16–19 h* (45.0 g). *D*: chick in *group 20–23 h* (51.5 g). bpm, Beats/min.

comparison, Ts at the end of warming was designated by the mean value during the last 10-min period of warming (referred to as Ts_{35}). Ts at the end of cooling was also determined by the mean value during the last 10-min period of cooling (Ts_{25}). Whereas Ts_{ref} and ΔTs of the *group 0–7 h* chick (Fig. 1*A*) were 27.8°C and +8.7°C, respectively, those for the *group 20–23 h* chick (Fig. 1*D*) changed to 32.5°C and +4.7°C. Then, during the second exposure to 25°C, Ts_{25} of the *group 0–7 h* chick decreased to 29.7°C, whereas that of the *group 20–23 h* chick remained high (33.5°C). Ts_{25} of both the hatchlings did not return to Ts_{ref}. The difference between T_{35} and T_{25} that was also designated as the magnitude of Ts change (ΔTs) was −6.8°C and −3.7°C for the two chicks, respectively.

HR baseline was narrow and sporadically decelerated in the environment of low temperature (25°C) in these four 0-day chicks. On exposure to 35°C environment, the *group 0–7 h* chick elevated HR baseline in parallel with the change in Ts accompanied with small increases in HR fluctuation, whereas other advanced chicks changed HR baseline in various fashions accompanied with augmentation of HR fluctuations. For HR responses, the mean value of the last 10-min period of the first 25°C exposure was defined as the reference MHR (referred to as MHR_{ref}). MHR_{35}, MHR_{25}, and ΔMHR were determined by the mean values during the last 10-min period of warming and cooling and the difference between MHR_{ref} and MHR_{35} or that between MHR_{35} and MHR_{25}. MHR_{35} tended to return to MHR_{ref} with elapse of time on *day 0*, and accordingly ΔMHR during warming turned from +146 beats/min in the *group 0–7 h* chick to −11 beats/min in the *group 20–23 h* chick.

Typical responses of IHR and Ts to altered Ta in four hatchlings on *days 1*, *2*, *3*, and *7* posthatching are presented in Fig. 2. Ts_{ref} increased with age from 33.0°C in the 1-day chick (Fig. 2*A*) to 39.0°C in the 7-day chick (Fig. 2*D*). Ts_{35} also increased from 36.8°C in the 1-day chick to 40.4°C in the 7-day chick, but ΔTs decreased from 3.8°C in the 1-day chick to 1.4°C in the 7-day chick. MHR_{ref} increased from 307 beats/min in the 1-day chick to 473 beats/min in the 7-day chick, and HR baseline widened with age. On exposure to 35°C, HR baseline declined and became wider, which was augmented with age. The decline of MHR during warming, i.e., ΔMHR, was −70 beats/min, −115 beats/min, −124 beats/min, and −126 beats/min for the 1-day, 2-day, 3-day, and 7-day chicks, respectively.

Mean values of Ts and HR change with development. The developmental stage (age) of the chick had a significant effect

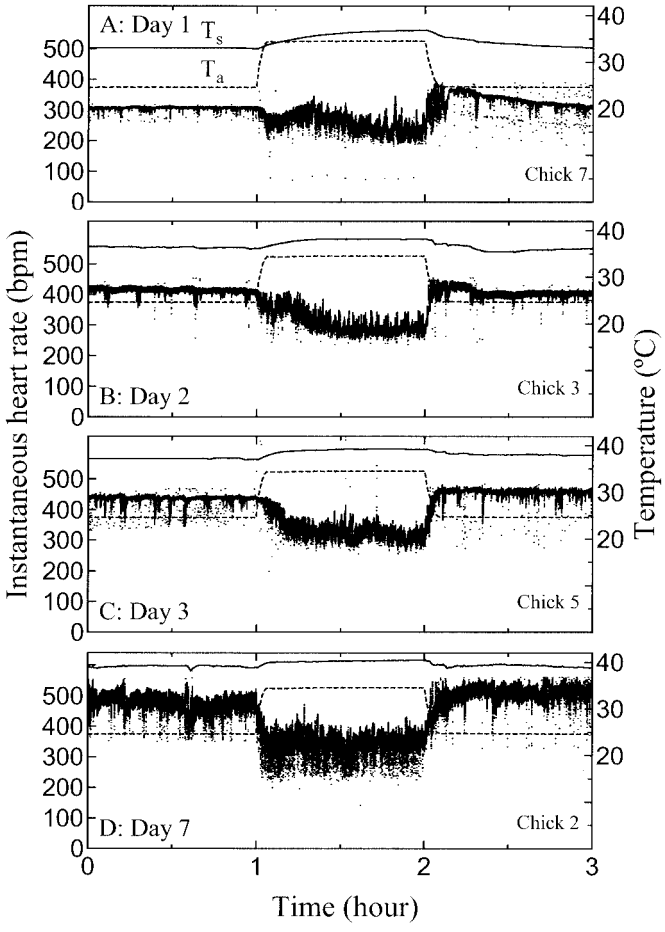

Fig. 2. Responses of IHR and Ts to altered Ta in chicks on *day 1* (*A*; 50.1 g), *day 2* (*B*; 48.2 g), *day 3* (*C*; 55.3g), and *day 7* (*D*; 62.3 g). *Chick 7* on *day 1* was classified in *group 24–35 h*.

on the MHR and Ts response to warming and cooling. Figure 3 presents mean values of Ts and MHR for 0-day chicks classified into four age groups, 1-day chicks classified into two age groups and other advanced chicks. There was a significant interaction between Ta and age on the HR of the developing chick ($P < 0.001$). HR increased significantly on warming in the two youngest groups of chicks, *groups 0–7 h* and *8–15 h* ($P < 0.05$). On return to cooling, HR returned to baseline levels in the youngest group, but remained elevated in *group 8–15 h*. In the *group 16–19 h* chicks, HR significantly increased during the first warming period ($P = 0.03$) and remained elevated on return to the cool environment ($P = 0.999$). In chicks 2 days and older, HR was elevated in the cool environment and decreased significantly in the warm environment ($P < 0.001$).

Ta had a significant effect on Ts for all age groups ($P < 0.001$; Fig. 3*A*). Ts increased significantly during warming at all age groups ($P < 0.05$). However, the magnitude of ΔTs in the cool and the warm environment decreased as the chicks aged and developed.

To further investigate the response of HR to changes in temperature, Fig. 4 shows the change in MHR (ΔMHR) plotted against the corresponding change in Ts (ΔTs) during the initial warming and the following cooling in individual chicks beginning on *day 0* through *day 7* posthatch. The slope of this relationship is defined as the "apparent gain" of ΔMHR against ΔTs, presenting approximate values of the gain. Initially in 0–7 h *day 0* chicks, an increase in MHR is positively correlated with an increase in Ts with gain of +15 beats·min^{-1}·°C^{-1}. As the chick develops, the gain decreases to +8, 0, and −4 beats·min^{-1}·°C^{-1} for *groups 8–15 h*, *16–19 h*, and *20–23 h* on *day 0*, respectively. For *days 1*, *2*, *3*, and *4–7* chicks, the apparent gain was negative and −14, −32, −29, and −68 beats·min^{-1}·°C^{-1}, respectively.

IHR fluctuation and oscillation. Figure 5 presents representative time-expanded recordings of IHR at the altered Ta in three hatchlings of different ages. In a newly hatched chick of *group 0–7 h* on *day 0* (Fig. 5*A*; *chick 11* of Fig. 1), HR baseline was flat during the first low Ta (25°C, Fig. 5*A*, *top*) and widened during warming at 35°C. As indicated by the inset (Fig. 5*A*, *middle*), the wide baseline was caused by HR oscillation with frequency of 1.1 Hz. This HR oscillation disappeared during the subsequent cooling (Fig. 5*A*, *bottom*). In another slightly older 0-day chick (Fig. 5*B*; *chick 12* of *group 16–19 h* in Fig. 1), HR baseline was also flat with sporadic decelerations during cooling (Fig. 5*B*, *top* and *bottom*), and it oscillated with a low frequency of 0.06 Hz during warming (Fig. 5*B*, *middle*). In an advanced 2-day hatchling (Fig. 5*C*; *chick 3* in Fig. 2), HR baseline elevated and oscillated with LF of 0.07 Hz at 25°C, accompanied with HF oscillation of 1.9 Hz (Fig. 5*C*, *top*). On warming, the LF oscillation disappeared, whereas the HF oscillation remained with the decrease in frequency (1.0 Hz; Fig. 5*C*, *middle*). When the hatchling was cooled again, HR baseline elevated and oscillated again with a low frequency of 0.07 Hz, accompanied with an HF oscillation of 1.9 Hz (Fig. 5*C*, *bottom*).

IHR fluctuations and oscillations were present in all ages of chicks, but the occurrence and frequency of the spectral peaks differed in response to thermal stress and age (Tables 2 and 3). Mean spectral peaks of HF oscillations corresponding to type I oscillations ranging from 1.33 to 1.85 Hz were observed in all age groups during the initial measurement period except the youngest hatchlings (Table 2). At all ages where spectral peaks were observed, the major HF peak decreased significantly

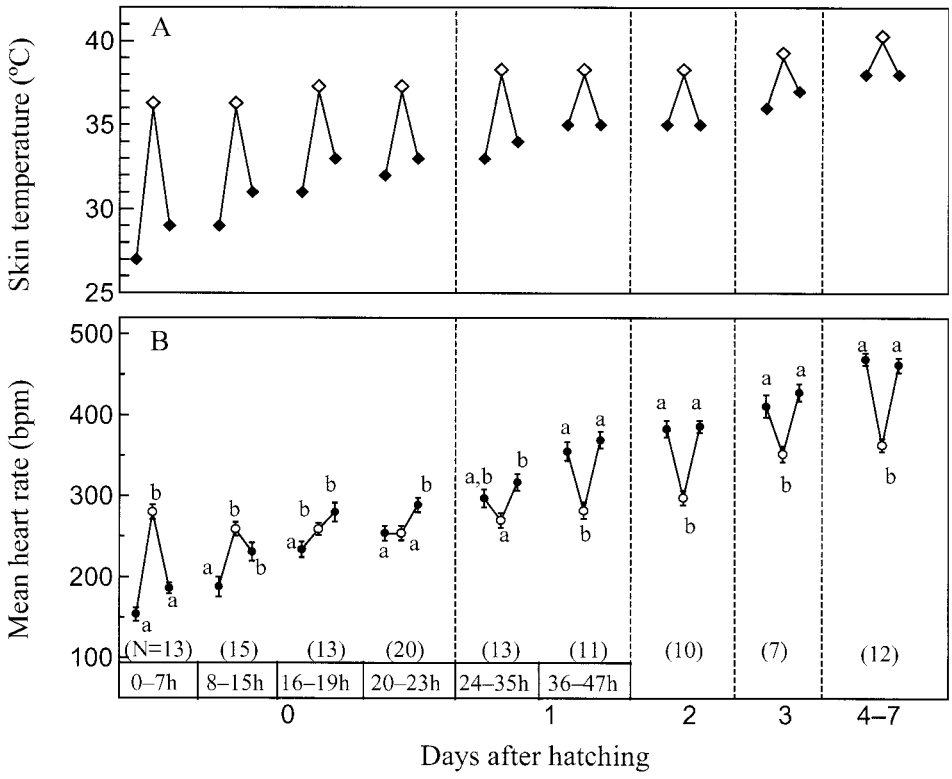

Fig. 3. Mean and SE of Ts (*A*) and mean HR (MHR; *B*) during the last 10-min period of exposures to the first 25°C (Ts_{ref} and MHR_{ref}) and 35°C (Ts_{35} and MHR_{35}) and the second 25°C (Ts_{25} and MHR_{25}) determined for 0-day chicks classified into 4 horal groups (0–7 h, 8–15 h, 16–19 h, and 20–23 h), 1-day chicks in 2 groups (24–35 h and 36–47 h), and other advanced chicks. *A*: mean Ts of each age group connected by solid lines, with ◆ on *left* indicating Ts_{ref}, ◇ in *center* showing Ts_{35}, and ◆ on *right* showing Ts_{25}. SE bars are obscured by the symbols. For all ages, Ts during cooling was significantly lower than during warming. *B*: MHR changes during warming and cooling with solid lines connecting MHR of each age group. ● on *left* show initial mean HR before warming (MHR_{ref}), ○ in *center* show mean HR after warming (MHR_{35}), and ● on *right* show mean HR after cooling (MHR_{25}). For each stage of development, data points with different letters are significantly different from each other ($P < 0.05$). Numerical figures in parentheses show number of chicks measured in each age group. Chicks examined on *days 4*, *5*, *6*, and *7* are pooled into the 1 group.

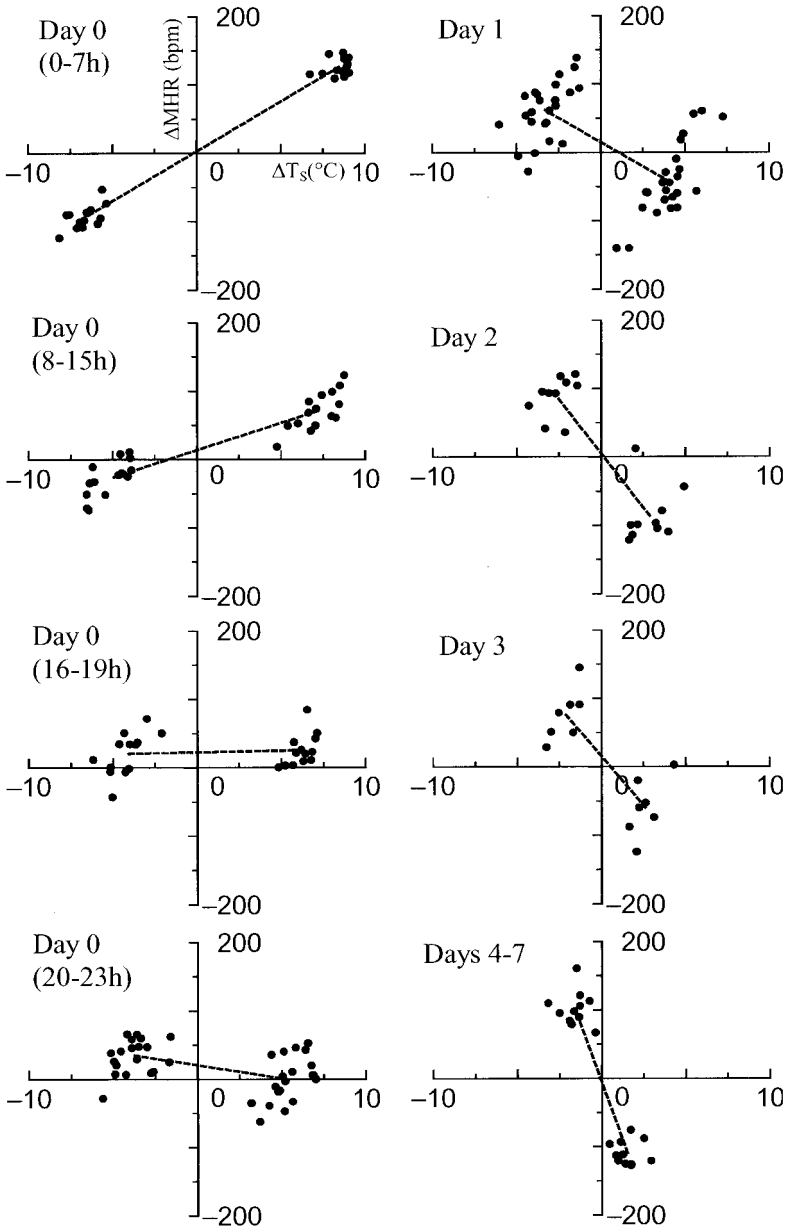

Fig. 4. Relationship between ΔTs and ΔMHR during warming (*right* half of the coordinate) and cooling (*left* half of the coordinate). Dashed line connects the mean values of ΔTs and ΔMHR between warming and cooling, and the slope was defined as apparent gain of ΔMHR against ΔTs.

during the warming period compared with either cooling periods (Table 2; $P < 0.01$ for all cases). The number of hatchlings exhibiting an HF oscillation increased significantly with age (initial hour $P < 0.001$; subsequent warming $P = 0.004$). Initially in the *group 0–7 h* hatchlings, none of the animals had an HF oscillation during the cooling periods. By *days 4–7*, the HR of all hatchlings had an HF peak (Table 2).

Type II HR oscillations in the LF range were less prominent than the HF oscillations (Table 3). As with the HF oscillations, a significant developmental trend in the number of animals that exhibited LF oscillations was revealed by the Cochran-Armitage trend test during both the initial cooling period ($P < 0.001$) and the subsequent warming period ($P < 0.0001$). During the initial measurement before warming, LF oscillations were not detected until the hatchlings reached the second half of *day 1* posthatch. By *days 4–7*, all of the hatchlings exhibited LF oscillation that was significantly lower during warming than cooling ($P < 0.01$).

DISCUSSION

Development of thermoregulatory competence. A measurement of Tb is needed to elucidate the development of HR regulation as it pertains to the thermoregulatory competence in developing chick hatchlings. The typical reference deep Tb in birds is the colonic temperature. However, here we measured Ts as a proxy for Tb. The main drawback with measuring Ts in place of Tb is that Ts is more dependent on Ta than Tb. However, we anticipated that Ts would provide a competent measure of the thermoregulatory ability of the hatchling by placing the thermistor probe underneath the ECG electrode, preventing the probe from direct contact with the air. Even if the area of the skin underneath the electrode were not getting

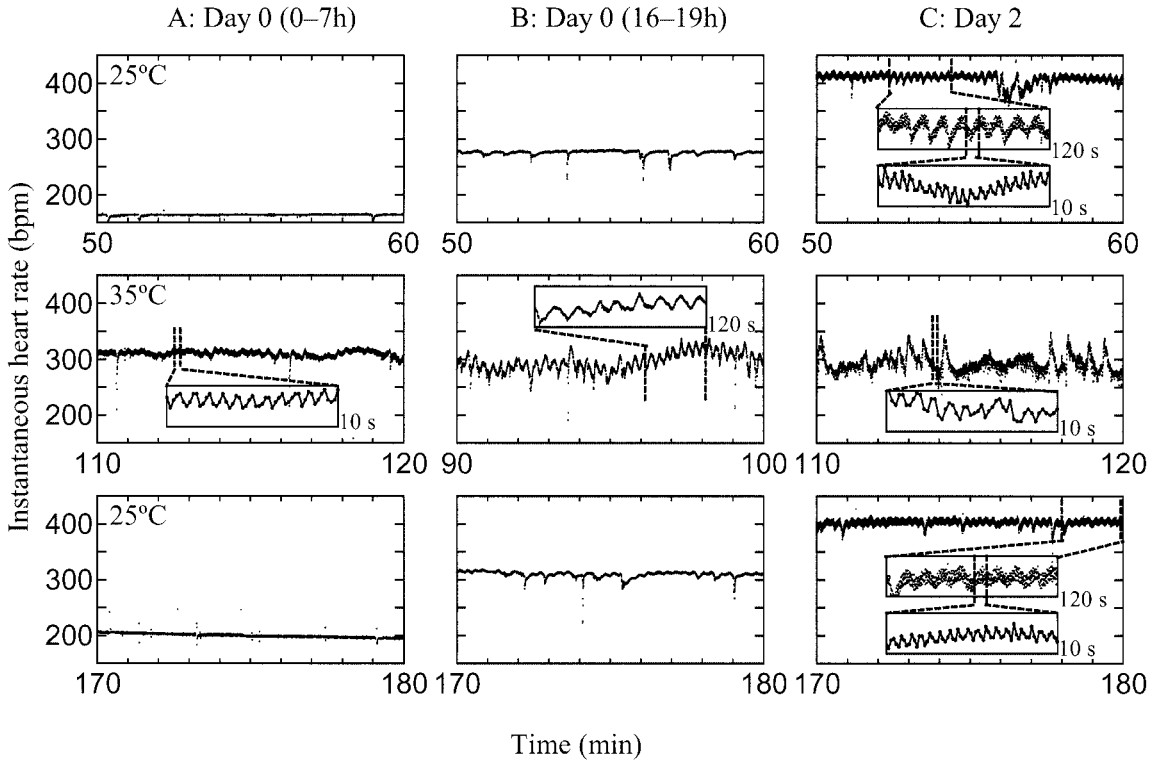

Fig. 5. Time-expanded 10-min recordings of IHR in 3 hatchlings that were exposed to Ta of 25°C (*top*), 35°C (*middle*), and 25°C (*bottom*) for 1 h, respectively. *A*: last 10 min of individual exposures to 3 altered Ta values for a chick of *group 0–7 h* on *day 0* (*chick 11* in Fig. 1*A*). *Inset:* (35°C) a time-expanded 10-s recording indicated by dashed lines. Span of the ordinate corresponds to 20 beats/min. *B*: last 10 min of individual exposures to 3 altered Ta values for a chick of *group 16–19 h* on *day 0* (*chick 12* in Fig. 1*C*). *Inset:* (35°C) a time-expanded 2-min recording indicated by dashed lines. Span of the ordinate corresponds to 70 beats/min. *C*: rightmost 3 recordings were extracted from the second panel of Fig. 2 (*chick 3* on *day 2*) for the 10-min period of individual exposures to 3 altered Ta values. *Top inset* at *top*: time-expanded 2-min recording indicated by dashed lines; span of the ordinate corresponds to 30 beats/min. *Bottom inset* at *top*: time-expanded 10-s recording extracted from the *top inset*; span of the ordinate corresponds to 20 beats/min. *Inset* at *middle*: time-expanded 10-s recording indicated by dashed lines; span of the ordinate corresponds to 20 beats/min. Two *insets* at *bottom* are also time-expanded 2-min and 10-s recordings as indicated by dashed lines, respectively. Spans of the ordinate correspond to 30 and 20 beats/min for the *top* and *bottom insets*, respectively.

Table 2. *Frequency and occurrence of high-frequency heart rate oscillations (type I) before, during, and after warming in a 35°C thermal environment*

		Thermal Environment		
Age	N_T	25°C	35°C	25°C
Day 0				
0–7 h	13	—	1.06±0.53(3)	—
8–15 h	15	1.33±0.35(7)	0.95±0.33(11)*	1.5±0.27(7)
16–19 h	13	1.59±0.29(10)	0.91±0.16(12)*	1.75±0.31(10)
20–23 h	20	1.65±0.38(14)	0.79±0.19(18)*	1.53±0.33(16)
Day 1				
24–35 h	13	1.70±0.23(11)	0.71±0.12(12)*	1.38±0.27(12)
36–47 h	11	1.68±0.41(9)	0.89±0.32(9)*	1.47±0.25(9)
Day 2	10	1.75±0.35(10)	0.93±0.20(10)*	1.68±0.31(10)
Day 3	7	1.84±0.14(5)	0.97±0.13(5)*	1.73±0.17(5)
Days 4–7	12	1.85±0.14(12)	1.15±0.17(12)*	1.75±0.23(12)

Values (in Hz) are mean ± SD (*n*). Values in parentheses are the *n* out of N_T exhibiting the oscillation. *Significantly different from other values for a given age at $P < 0.05$.

Table 3. *Frequency and occurrence of low-frequency heart rate oscillations (type II) before, during, and after warming in a 35°C thermal environment*

		Thermal Environment		
Age	N_T	25°C	35°C	25°C
Day 0				
0–7 h	13	—	—	—
8–15 h	15	—	0.05±0.00(4)	—
16–19 h	13	—	0.06±0.01(2)	—
20–23 h	20	—	0.06±0.00(1)	0.06±0.02(3)
Day 1				
24–35 h	13	—	0.06±0.01(3)	0.06±0.02(6)
36–47 h	11	0.05±0.01(3)	0.06±0.01(2)	0.06±0.02(6)
Day 2	10	0.06±0.01(8)	0.05±0.01(6)	0.06±0.01(8)
Day 3	7	0.07±0.01(6)	0.06±0.00(2)	0.07±0.02(6)
Days 4–7	12	0.08±0.01(12)	0.06±0.01(11)*	0.08±0.01(12)

Values (in Hz) are mean ± SD (*n*). Values in parentheses are the *n* out of N_T exhibiting the oscillation. *Significantly different from other values for a given age at $P < 0.05$.

the same heat transfer as another area of the skin, the changes in temperature measured would provide at least a relative measure of changes in Tb in response to a warming and cooling bout. The difference between Ts and Ta indicates the relative development of thermoregulatory ability of the hatchling. The advance of homeothermic capacity was apparent in the deceased change in ΔTs with development of the hatchlings.

During the entire first 1-h exposure to 25°C, Ts baseline remained constant and elevated above 25°C to different degrees in all the hatchlings that were acclimated to the low-temperature environment regardless of age. This elevated Ts suggests that hatchlings of all ages have some ability to maintain an elevated Tb beginning on the first day posthatch.

Ts_{ref} of chicks exposed to the mild thermal stress changed with age (Figs. 1–3). The development of thermoregulatory competence can be seen in the increase in Ts_{ref} with age (Fig. 3). Ts_{ref}, which was ~28°C in the *group 0–7 h* chick on *day 0*, increased to ~33°C in the *group 20–23 h* chick on the same day and further increased to 39°C in the 7-day chick. During the first 2 days of posthatch life, the hatchlings appear to rapidly develop the ability to thermoregulate in a cool thermal environment. By *day 2* posthatch, the chicks exhibit fully functional homeothermy under the conditions tested.

The difference in Ts (ΔTs) between the initial cooling and the warming decreased with elapse of time (Figs. 1 and 2). ΔTs decreased from ~9°C in the *group 0–7 h* chick on *day 0* to 1.4°C in the 7-day chick. Increases in T_{ref} and T_{35} that were accompanied with decreases in ΔTs were predominant and in progress on *day 0*, indicating that thermoregulatory competence of newly hatched chicks progressed predominantly on the day of hatching. These changes may be due to increased insulation and mass in the older chicks along with greater control of local blood flow to the surface of the chick and increased metabolic rates/heat production. In chickens, it was reported that during the 5-h period of exposure to Ta of 24°C, the oxygen uptake was maintained at ~40% of the control (Ta of 37°C) value up to hatching time (i.e., temperature coefficient, Q_{10}, was ~2), but thereafter rapidly increases to 70–95% by *day 1* (22). Hatchlings of precocial species rapidly develop the ability to maintain Tb under minor thermal stress (2, 7, 8, 12, 17, 20, 22, 28). This is shown in the extreme case where emu hatchlings were able to maintain Ts ~12°C above Ta even on the first day of posthatch life (21).

Further evidence of the thermoregulatory competence is seen in the rate of change in Ts on warming and cooling. On exposure to Ta of 35°C, Ts increased in an exponential fashion (i.e., the first-order response) during the 1-h warming period on *day 0* and *1* (Figs. 1 and 2). During cooling to 25°C for the next 1-h period, Ts decreased but did not return to Ts_{ref}. During both the warming and cooling bouts, Ts did not reach steady state during the 1-h period in any of the 0-day chicks. Given more time in these environments, the change in Ts may have been even greater than that reported, suggesting that these hatchlings had not yet fully developed thermoregulatory competence. In contrast, 2-day and older chicks all reached a steady-state Ts during the 1-h exposure period to both the initial warming and the subsequent cooling (Fig. 2).

HR responses. In reference to thermoregulatory responses of Ts to warming and cooling bouts, development of HR regulation in relation to thermoregulation was elucidated. The response of MHR_{ref}, MHR_{35}, and MHR_{25} during the first day of posthatching life corresponded with the rapid development of thermoregulatory competence in chick hatchlings (Fig. 3*B*). HR of developing chicks in the warm environment (MHR_{35}) moderately increased from ~260 beats/min on *day 0* to 360 beats/min on *days 4–7*, whereas that in the cool environment (MHR_{ref}) predominantly increased from ~150 beats/min for the first 8-h period of *day 0* to 470 beats/min on *days 4–7* (Fig. 3*B*). In contrast to the increase of ~100 beats/min in MHR_{35} during the first 1 wk of posthatching life, MHR_{ref} gained ~320 beats/min during the same period. Consequently, the values of MHR_{ref} and MHR_{35} became almost identical during the last quarter of *day 0* and reversed afterward; that is, ΔMHR became negative in response to warming. Before this period, MHR_{ref} increased to MHR_{35} in parallel with the increase in Ta, and thus ΔMHR was positive during warming. Meanwhile, MHR_{35} decreased to MHR_{25} in parallel with the decrease in Ta for the first half of *day 0*, and thus ΔMHR was negative during cooling. For the last half of *day 0*, MHR_{35} increased to MHR_{25} against the decrease in Ta, with subsequent augmented increase on advanced days, producing positive ΔMHR during cooling. These reversed responses of ΔMHR to the change in Ta indicate that thermoregulatory competence against mild change in Ta progresses on the day of hatching in the chicken, although Ts responses to altered Ta did not indicate it (Fig. 3). As a result, HR responses initially exhibited a thermoconformity pattern, but during the second half of *day 0* the thermoregulatory ability appears to develop as indicative of the increased HR in the cool environment. In contrast, MHR of emu hatchlings exhibit a typical homeothermic HR response to increases in Ta as early as *day 0* posthatch (21).

Changes in the relationship between ΔTs and ΔMHR show a clear progression in the development of a homeothermic response of HR to changes in Ta of 10°C (Fig. 4). Although the slope of ΔMHR to ΔTb may indicate quantitatively the thermoregulatory ability as gain, in the present experiment, where we measured Ts instead of Tb, the relationship between ΔMHR and ΔTs was defined as apparent gain. During the first half of *day 0*, the slope of the relationship between ΔMHR and ΔTs was positive (Fig. 4), indicating no thermoregulatory competence when experiencing a mild change in Ta. The apparent gain decreased to nearly zero with further development during the rest of the first day of posthatching. Beginning on *day 1* posthatch, the apparent gain became negative with subsequent augmentation with development through *days 4–7* posthatching. This development of a negative gain suggests that the hatchlings were developing the ability to upregulate HR during cooling periods and a greater ability to maintain Tb, suggesting an ability to increase metabolic rate and subsequent heat production. It remains to be seen if metabolic rate follows the same developmental pattern after hatching.

The observed developmental changes in the response of MHR to thermal stress may be mediated by further maturation of the autonomic nervous system during the first 2 days of posthatch life. Neurohumoral pathways of the autonomic nervous system are major regulators of HR in the chicken. Before hatching on *day 21* of incubation, chick embryos show an adrenergic tone (4) and have a functional cholinergic tone (3, 10; E. M. Dzialowski, unpublished observation). It was previously reported that administration of propranolol to 8-h posthatch chicks before a thermal stress of 8°C resulted in a similar

decrease in Tb as the control chicks, but by 34 h posthatch, propranolol-treated chicks had a significantly lower Tb than the controls (28). This suggests that between hatching and *day 2* of posthatching life the adrenergic pathways mature and contribute to the thermoregulatory response. We observed a similar developmental change in HR from *day 0* to *day 1* (Fig. 3), suggesting that adrenergic pathways may potentially provide greater control of HR under thermal stress. As the chicks get older, HR in the cool environment increases due to an increase in sympathetic responsiveness. A change in contribution of cholinergic inputs to regulation of HR baseline during warming and cooling is not excluded. It is possible that during the first day of posthatching life, further autonomic control of HR may develop, resulting in the homeothermic response of HR to mild changes in Ta.

IHR fluctuations during warming and cooling. IHR fluctuations were present in all ages of chicks, but differed in their responses to thermal stress. Before the experiment, hatchlings were acclimated to the low-temperature environment (24–27°C) and measurement of IHR was begun at low Ta (25°C). In this low-temperature environment, HR baseline was narrow in newly hatched chicks on *day 0* and it was widening in advanced hatchlings (Figs. 1, 2, and 5). HR baseline widened when hatchlings were exposed to high Ta. Because narrow baseline implied lack of HR fluctuation, low Ta suppressed HR fluctuations and high Ta induced them in 0-day chicks and in other advanced chicks. This trend was evident in 0-day chicks that were provided with poor thermoregulatory ability and had low Ts in low-temperature environment. It may be assumed that HR fluctuations occur in chick hatchlings that live in thermal environment favorable to their thermoregulatory competence. Accordingly, the low-temperature environment at 25°C may be severe for 0-day chicks to live and they prefer warm environment at 35°C accompanied with occurrence of HR fluctuations. As chicks develop greater thermoregulatory capacity, the 25°C environment may not be severe to live, but advanced embryos still prefer the environment at 35°C in reference to HR fluctuations.

The HR of hatchlings in this study fluctuated with both LF and HF oscillations (Tables 2 and 3). The HR fluctuations with these LF and HF have been previously reported in chick embryos and hatchlings (14, 15). In a previous study, HR oscillations with a mean oscillatory frequency of 0.74 Hz [range 0.4–1.2 Hz (14) and Table 2] were designated as type I HR variability, which is of a high frequency and produced by breathing; i.e., respiratory sinus arrhythmia (RSA) (16).

Type I HR oscillations (RSA) appeared in three hatchlings at high Ta in the youngest hatchlings (0–7 h of *day 0*), and in the three older groups of *day 0*, it appeared at both low Ta and high Ta with different oscillatory frequency in at least half of the animals tested (Table 2). HR baseline that was widened during warming in the *group 0–7 h* chick on *day 0* (*chick 11* in Fig. 1) was due to RSA with frequency of 1.1 Hz (Fig. 5*A*, *inset*). In advanced hatchlings (53 1- to 7-day hatchlings), it appeared during the 3-h exposure to low Ta with increased frequency (1.76 ± 0.27 Hz) and to high Ta with decreased frequency (0.96 ± 0.28 Hz). The increase in frequency with cooling suggests that the breathing of these hatchlings was rapid in the low-temperature environment compared with high-temperature environment. These changes in breathing frequency at low Ta may indicate an increased metabolic rate during cooling. It is interesting to note that although mean HR on *day 0* followed a different pattern during warming and cooling than in the older chicks, the changes in frequency of type I HR oscillations were not different from the older chicks. Thus it appears that the control of HR oscillations may develop before the development of mean HR control.

Preliminary studies suggest that type I HR oscillations in the chick hatchling are governed by vagus nerve function. Type I HR oscillations were eliminated by atropine (H. Tazawa, unpublished data). This is in agreement with the vagal origin of RSA in mammals (1). Because RSA is mediated by vagus nerve function, the cholinergic contribution to thermoregulatory competence may be important for the downregulation of HR when the chick is exposed to higher temperatures.

LF oscillations designated as type II HR fluctuation with a mean oscillatory frequency of 0.07 Hz [range 0.04–0.10 Hz (14) and Table 3] were also observed in hatchlings frequently as they aged. It has previously been shown that this frequency was produced by exposure of advanced hatchlings to lowered Ta and was abolished by exposure to elevated Ta (27). HR baseline that was widened during warming in the *group 16–19 h* chick on *day 0* (*chick 12* in Fig. 1) was due to LF oscillation with frequency of 0.06 Hz (Fig. 5*B*, *inset*). HR baseline that was widened with elapse of time in the 2-day chick in low-temperature environment (*chick 3* in Fig. 2) was due to both RSA (1.9 Hz) and LF oscillation (0.07 Hz; Fig. 5*C*, *top* and *bottom*, *insets*). It should be noted that LF oscillation of this hatchling was eliminated by exposure to high Ta and RSA decreased oscillatory frequency (1.0 Hz) at high Ta (Fig. 5*C*, *middle*).

In a previous study, LF oscillation of HR was inferred to be a phenomenon related to thermoregulation of chick hatchlings (27). It appeared in seven hatchlings on *day 0* and three hatchlings on the first half of *day 1* only during warming (Table 3). Afterward, it appeared during cooling in 32 hatchlings, and in eight hatchlings the LF oscillation disappeared during warming. Judging from the appearance of LF oscillations, in advanced hatchlings thermoregulatory competence functions in the low-temperature environment (25°C) and also in the high-temperature environment (35°C). However, in newly hatched chicks, low temperature of 25°C was so severe that thermoregulatory competence that appeared at high Ta was overwhelmed. In mammals, LF and very LF oscillations are controlled by both the sympathetic and parasympathetic systems (1) and may have a thermoregulatory role (11). It remains to be seen if type II oscillations in the chicken are controlled by both the sympathetic and parasympathetic system. However, if they are autonomic in origin then the gradual appearance of these oscillations with time after hatching suggests that the responsiveness of these systems to changes in Ta continue to develop 1 or 2 days after hatching.

Perspectives

During the first day of egg-free life, the thermoregulatory competence of newly hatched chicks develops rapidly. The ability to maintain Tb increases with development during the first 2 days of posthatch life. Newly hatched chicks exhibit an ectothermic response in HR. The regulation of HR under thermal stress transitions from an apparent ectothermic response to the typical endothermic pattern during the first day of

posthatch life. This transition is most likely mediated by the development of both the sympathetic and parasympathetic branches of the autonomic nervous system. By *day 1* posthatch, HR of the chick responds in a similar fashion as the older chicks. The development of HR regulation during this time period correlates to an increased capacity for heat production under thermal stress. Maturation of the autonomic responsiveness on HR, exhibited as type I and II oscillations, occurred rapidly on hatching. The exact role of the sympathetic and parasympathetic system in the regulation of HR baseline and HR variability when exposed to a thermal stress remains to be investigated. While we have begun to elucidate the maturation of HR regulation in regard to thermoregulation, the developmental changes in metabolic control that occur during the day of hatching and their influence on thermoregulation remain to be examined.

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