ELSEVIER

Comparative Biochemistry and Physiology Part A 131 (2002) 775–785

CBP

www.elsevier.com/locate/cbpa

Cardiac rhythms in prenatal and perinatal emu embryos☆

K. Kato[a], K. Moriya[a], E. Dzialowski[b], W.W. Burggren[b], H. Tazawa[a,*]

[a]*Department of Electrical and Electronic Engineering, Muroran Institute of Technology, Muroran 050-8585, Japan*
[b]*Department of Biology, University of North Texas, Denton, TX 76203-5189, USA*

Received 25 April 2001; received in revised form 28 August 2001; accepted 30 August 2001

Abstract

Emu eggs weigh approximately 600 g and have an incubation duration (ID) of approximately 50 days. The egg mass is approximately 10-fold heavier than the chicken egg and the ID is approximately 2.5-fold longer. Daily changes in mean heart rate (MHR) of emu embryos were previously determined, but further measurement was needed to investigate the species-specific behavior of cardiac rhythm for comparison with other species. In the present study, we continuously measured the electrocardiogram of emu embryos while maintaining adequate gas exchange through the eggshell and determined instantaneous heart rate (IHR) during the last 2–7 days of incubation until hatching or death. The MHR over 1-min intervals was calculated from IHR data in order to present continuous developmental patterns of heart rate (HR) in a single graph and 24-h recordings of HR in a single panel, showing the HR trend over a prolonged period. However, neither circadian nor ultradian rhythms of HR were shown in these figures or by power spectrum analysis. The IHR distinctively fluctuated and the fluctuations were mainly comprised of three patterns of irregular HR accelerations in embryos that hatched. Respiratory sinus arrhythmia also occurred in perinatal embryos. During the final stages of the perinatal period, short-term, repeated, large accelerations of IHR appeared, which signaled imminent hatching and has been reported for chick embryos. IHR fluctuations in embryos that failed to hatch tended to become inactive towards death.

Keywords: Emu embryo; External pipping; Instantaneous heart rate (IHR); Mean heart rate (MHR); Developmental pattern of mean heart rate; Fluctuation of instantaneous heart rate; Heart rate acceleration; Heart rate irregularities; Prenatal and perinatal periods

1. Introduction

We have determined daily changes (i.e. developmental patterns) of embryonic mean heart rate (MHR) in 34 species of birds while maintaining adequate gas exchange through the eggshell and chorioallantoic membrane. Determinations of MHR developmental patterns were made for 20 altricial and semi-altricial birds (Burggren et al., 1994; Tazawa et al., 1994; Pearson and Tazawa, 1999a,b; Pearson et al., 1999), five semi-altricial and semi-precocial seabirds (Tazawa et al., 1991b; Tazawa and Whittow, 1994; Pearson et al., 2000) and nine precocial birds (Tazawa et al., 1991a; Pearson et al., 1998; Tazawa et al., 1998a,b, 2000). The mean egg mass ranged from approximately 1 g for zebra finch (*Taeniopygia guttata*) to approximately 630 g for emu (*Dromaius novaehollandiae*) and approximately 1400 g for ostrich (*Struthio camelus*). The developmental patterns of embryonic MHR in small altricial birds show a marked increase during the last period of incubation and become maximum during the pipping (perinatal) period. Meanwhile, those of precocial

☆ Contribution of a special issue of Comparative Biochemistry and Physiology on Perinatal Development of Control Systems in Birds, collated by Guest editors Martin Nichelmann, Barbara Tzschentke and Heike Tönhardt.

*Corresponding author. Tel.: +81-143-465526; fax: +81-143-465501.

E-mail address: tazawa@elec.muroran-it.ac.jp (H. Tazawa).

1095-6433/02/$ - see front matter
PII: S1095-6433(02)00015-6

birds ($N=9$) tend to be convex with a few exceptions, i.e. MHR becomes maximum prior to the perinatal period and tends to decrease towards pipping. The incubation time for a peak of the convex pattern to appear is likely to depend on the egg mass. The peak tends to appear late in incubation in small species, e.g. king quail (*Coturnix chinensis*) and Japanese quail (*Coturnix coturnix japonica*), and shifts to early incubation days in large species. In emu and ostrich, embryonic MHR markedly decreases to approximately 120–140 beats per min (bpm) during the last one-third of prenatal development prior to pipping. These values are significantly low compared with those of small birds, e.g. approximately 300–320 bpm in king quail and Japanese quail and approximately 260 bpm in the domestic fowl (*Gallus domesticus*). These developmental patterns of embryonic MHR may be related to oxygen requirement of the late embryos, and from these developmental patterns the allometric relationships with fresh egg mass were studied (Ar and Tazawa, 1999; Tazawa et al., 2001).

Measurements of developmental patterns were carried out for a restricted period on a given day of incubation because of the requirement for determinations from many individuals. However, they showed that the MHR over a few seconds extensively changed during a short time lapse, that is, the coefficient of variation (CV) of MHR was large, particularly late in incubation, although the daily increases in CV depended upon the species of bird (Tazawa et al., 1991a). This suggests that the beat-to-beat heart rate (HR), i.e. the instantaneous HR (IHR), fluctuates with an increase in the magnitude towards the end of incubation and that fluctuation is dependent upon the species. Recently, IHR in chick embryos was determined by measuring acoustocardiogram (Akiyama et al., 1997, 1999) or blood pressure of the allantoic artery (Höchel et al., 1998). Since then, development of IHR fluctuations has been studied in chick and duck embryos and newly hatched chicks (Höchel et al., 1999; Moriya et al., 1999, 2000; Tazawa et al., 1999). Although the developmental patterns of embryonic MHR have been investigated in many species of birds for comparison, measurement of IHR remains to be carried out in other species of birds, not only for comparative study, but also firstly for an investigation into the species-specific behavior of cardiac chronotropic activity. The present report was designed to investigate the HR trend and IHR fluctuations in emu embryos during the last stages of prenatal and perinatal development and to compare the data with those of late chick embryos.

2. Materials and methods

2.1. Incubation of eggs

Eggs were collected in breeding yards of the Cross Timbers Emu Ranch, Flower Mound, Texas and incubated there in a forced draught incubator at a temperature of 36.5 °C and relative humidity of 30%. The first day of incubation was designated as day 0. The eggs incubated for approximately 3 and 5 weeks were then transported to a laboratory of the University of North Texas, where incubation was continued at the same temperature and relative humidity in a forced draught incubator with automatic egg turning every 3 h.

The living status of eggs was ascertained by checking whether they consumed oxygen, using an O_2 analyzer. Eight living eggs and two fresh eggs were further transported to the Muroran Institute of Technology by flight and car. The eggs were put into a corrugated cardboard box and exposed to low environmental temperatures during transportation of approximately 20 h. After arrival at the laboratory in Muroran, incubation was recovered at a temperature of 36.5 °C and relative humidity of approximately 40% in a forced draught incubator, which was also used for experiments with chicken eggs. The two fresh eggs were simultaneously put into the same incubator. Egg turning was manually carried out three times a day. On the day of arrival at Muroran, five embryos were aged 41 days (designated as group I) and the remaining three embryos were 27 days old (group II) without correction of the time difference between Texas and Muroran (16 h). The two fresh eggs were day 0 (group III).

2.2. Determination of heart rate

The HR of the embryo was determined from electrocardiogram (ECG) detected by three spiral electrodes, as previously used for chicken eggs (Moriya et al., 2000). The electrodes were silver wires, 5 cm long and 1 mm in diameter. One end of the wire was wound to make a one-turn spiral and the spiral was perpendicularly bent. The spiral was approximately 1 cm across and the distance

between the tip and end of the spiral was approximately 3 mm. Three locations were marked on the eggshell, forming a triangle around the egg, so that the air cell would not be included. The egg could not be candled because of the dark green color and thick eggshell, and thus the position of the air cell was judged by putting the egg on a flat surface and identifying the slope of the egg (Tazawa et al., 2000). A hole 2 mm across was made with a hand drill through the marked eggshell. The tip of the spiral electrode was inserted into the hole and the electrode was rotated by 360°, so that the spiral part was screwed in and embedded in the egg. The electrodes were fixed onto the eggshell using epoxy glue.

The implantation of the electrodes was made at least half a day prior to the start of measurement in both Texas and Muroran, and eggs with implanted electrodes were returned to the incubators. The electrodes were connected to shield wires passing through small holes in the incubators to polygraph amplifiers. Measurement of ECG was started on day 44 and 46 in Texas and Muroran, respectively, without a particular reason for the difference, and continued until hatching or embryonic death. When embryos died within a few days of measurement, another egg was installed with the electrodes and ECG measurement was started.

IHR was determined from amplified and band-pass-filtered ECG signals with the aid of a Powerlab system in Texas and a conventional personal computer in Muroran. The determination of IHR was principally the same in both systems, except for the sampling frequency, i.e. 2000 Hz in the Powerlab system and 4000 Hz in Muroran. The ECG signals were first sampled at the above frequency. The signals sampled were compared with a threshold level set above background noise levels to determine the time when a rising deflection of the QRS complex first exceeded the threshold, and this time was stored in data files. The IHR (beats min^{-1}) was calculated from the time interval between adjacent QRS complexes.

In the present study, and as in the previous reports on the continuous measurements of IHR, the MHR during a 1-min period was also determined from IHR in order to present continuously measured HR data on a single graph. This procedure produced a continuous developmental pattern of HR and had the effect of acquiring the HR trend by a simple visual inspection.

2.3. Power spectrum analysis

In order to determine whether the HR fluctuations were periodic changes and the oscillating frequency where they oscillated, power spectrum analysis of the HR was made by a fast Fourier transform (FFT), as detailed elsewhere (Akiyama et al., 1999; Moriya et al., 1999, 2000).

3. Results

3.1. Viability of embryos

Because a single measurement was continuously made throughout several days, and the continuous measurement of IHR was also designed for hatchlings in a companion report (Moriya et al., 2002), all the incubated eggs were not always subjected to the measurement. All the eight living eggs brought to Muroran survived the transportation. Two eggs selected arbitrarily from group I were implanted with ECG electrodes on day 45 of incubation and measured for ECG. However, an ECG signal could not be detected from one embryo in a few hours and it was found to be dead. Another egg was measured for ECG until hatching. The third egg from group I was implanted with electrodes soon after the ECG could not be measured from the first egg, and ECG was measured until hatching. The remaining two eggs in group I were incubated without experiment, but were found to have died after internally pipping the chorioallantoic membrane (CAM) (internal pipping, IP) and externally pipping the eggshell (external pipping, EP), respectively. All three eggs in group II hatched, and two of them had been measured for ECG from day 46 of incubation. One egg in group III was infertile and another egg was measured for ECG from day 46 until hatching.

In Texas, two living eggs were first arbitrarily taken from the incubator and implanted with electrodes. Measurement was started on day 44 of incubation, but the ECG signal failed to be detected on day 46 and day 49, respectively. Another two living eggs were then implanted with electrodes on these days, and ECG measurement was started after temperature equilibrium. They hatched on day 51 and day 52, respectively. In the second trial, three living eggs were implanted with electrodes on day 44, but they died on days 45, 48 and 51, respectively. Another living egg was then

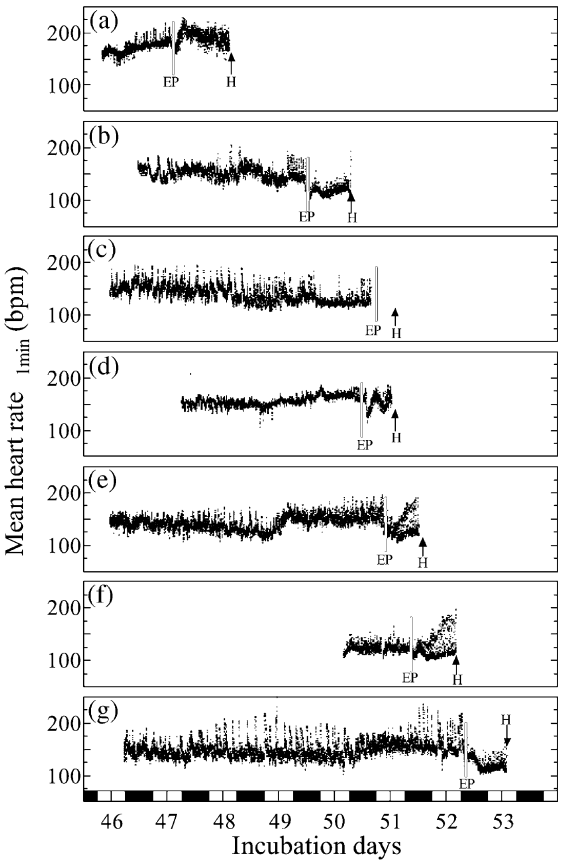

Fig. 1. The continuous developmental patterns of mean heart rate (MHR) during the last stages of prenatal and perinatal periods in seven embryos that hatched. MHR is the value in beats per min (bpm) averaged over 1-min intervals, which is shown by a single point. All measurements of heart rate (HR) were begun prior to externally pipping the eggshell. The initiation of external pipping (EP) is shown by a vertical bar and symbol EP, and the time of hatching is indicated by symbol H. The embryo c could not been measured for HR prior to EP, but EP and hatching were identified.

implanted with electrodes on day 45 and measured for ECG until hatching on day 48.

As a result, the HR of seven embryos in total was determined until hatching and six embryos died during ECG measurement.

3.2. Cardiac rhythms during the last stage of incubation in embryos that hatched

Fig. 1 shows the changes in MHR in seven embryos during the period within the last 10% of incubation (except embryo g). Embryos a–g (panels a–g) are shown in order of hatching. The period from 06:00 to 18:00 h and that from 18:00 to 06:00 on the following day are shown by open and closed bars, respectively, on the abscissa. Symbols EP and H on each panel indicate initiation of external pipping and time of hatching, respectively. Embryos b, c, e and g were measured for ECG in Muroran, and thus the actual incubation duration should be subtracted by the time difference of 16 h. After correction, the average incubation duration of seven embryos was 50.3 ± 1.5 (S.D.) days and the EP period was 17.0 ± 4.5 h.

Fig. 2 presents an example of changes in MHR during a 24-h period taken from embryo g in Fig. 1. The recording shown in the top panel is the same as that in the bottom panel of Fig. 1. The individual panel from the second shows the chang-

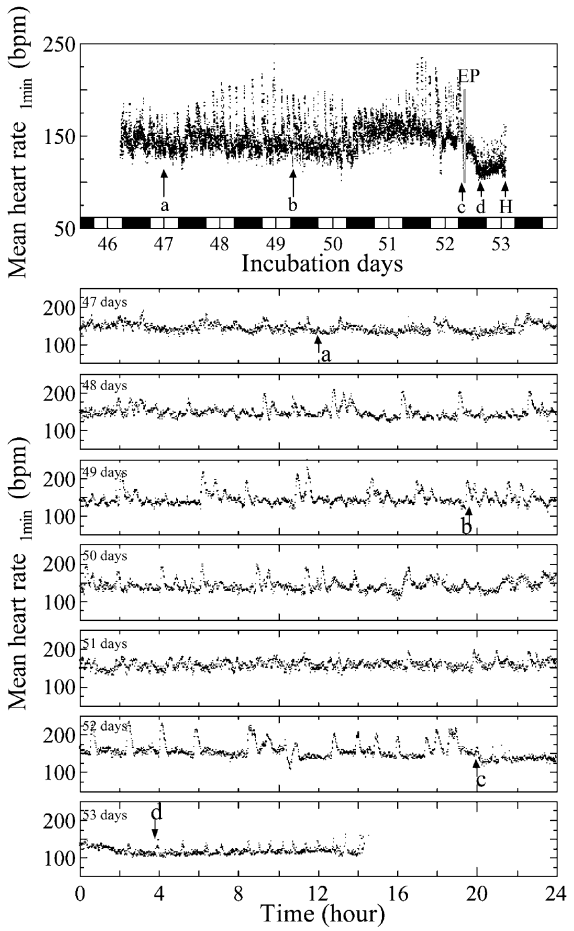

Fig. 2. The continuous developmental pattern of mean heart rate (MHR) presented in a single graph (top panel) and in the time-expanded recordings (bottom seven panels) of the hatched embryo g in Fig. 1. The number in the left-upper corner of individual panels indicates the age (days) of the embryo.

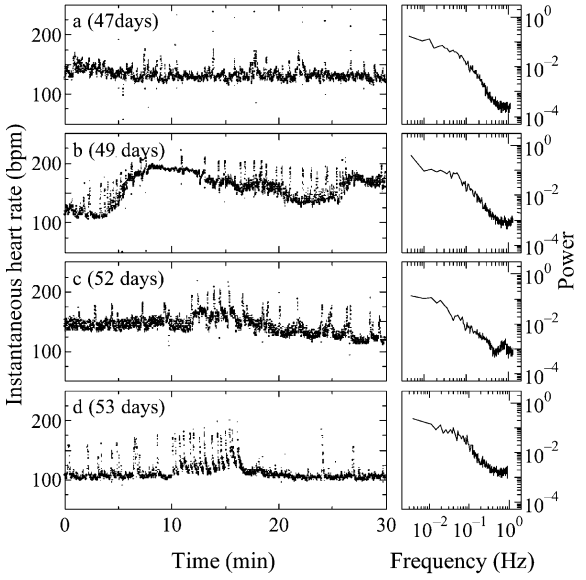

Fig. 3. The instantaneous heart rate (IHR) during the 30-min period at the time indicated by symbols a–d in Fig. 2 and the normalized power spectrum analyzed for the IHR recordings shown in the individual left panels. The number in parentheses indicates the age (days) of the embryo.

es in MHR during a 1-day period from day 47 to day 53 of incubation

Fig. 3 shows 30-min recordings of IHR extracted from the time indicated by symbols a–d in the top panel and also in four panels of the 24-h recording in Fig. 2 and their spectrum analyses. For instance, panel a is the 30-min recording at the time indicated by symbol a in the top panel and in the second panel (47 days) of Fig. 2. Panels b–d are presented in the same manner.

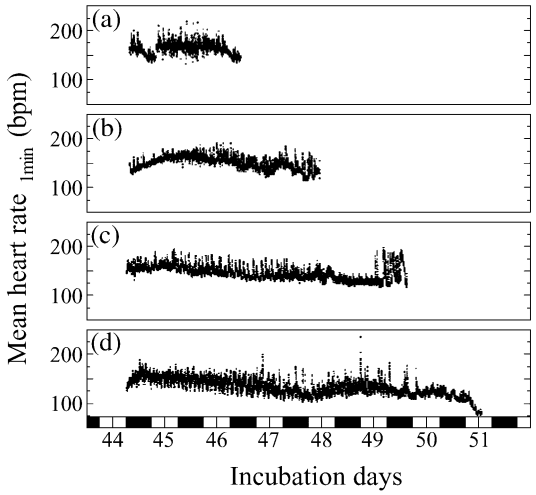

Fig. 4. The continuous developmental patterns of mean heart rate (MHR) in four embryos that died before external pipping.

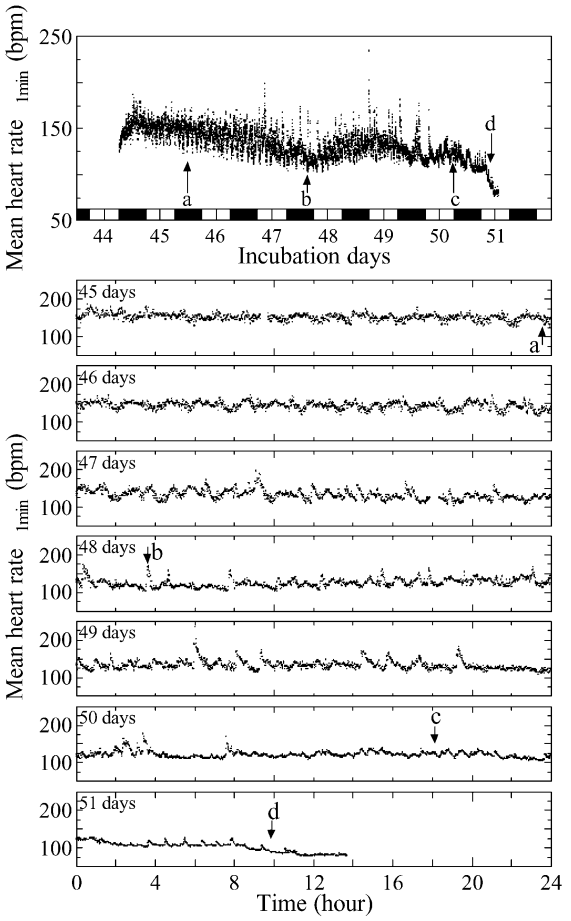

Fig. 5. The continuous developmental patterns of mean heart rate (MHR) presented in a single graph (top panel) and in the time-expanded recordings (bottom seven panels) of the failed embryo d in Fig. 4.

3.3. Cardiac rhythms in embryos that failed to hatch

Fig. 4 shows the changes in MHR in four embryos that failed to hatch before EP. Two other embryos were measured for ECG for only a few hours, and thus their MHR data are not presented. Embryos a–d (panels a–d) are shown in order of death. The time scale of the abscissa is the same as that for Fig. 1.

Fig. 5 presents the changes in MHR during a 24-h period taken from embryo d in Fig. 4. The recording shown in the top panel is the same as

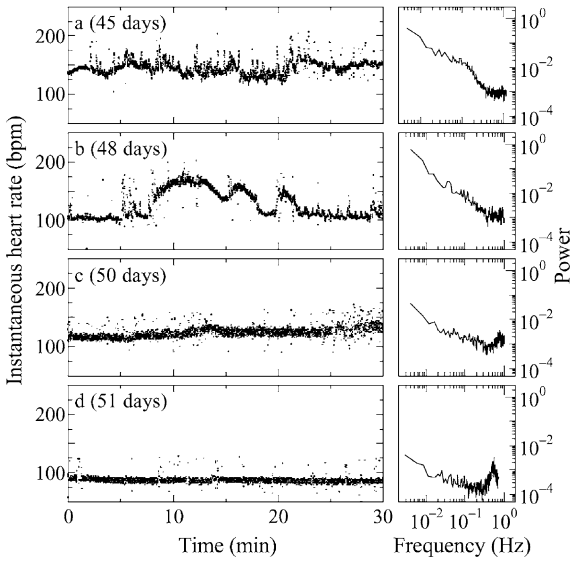

Fig. 6. The instantaneous heart rate (IHR) during the 30-min period at the time indicated by symbols a–d in Fig. 5 and the normalized power spectrum for each recording shown in the left panel.

that in the bottom panel of Fig. 4. The individual panel from the second shows the changes in MHR during a 1-day period from day 45 to day 51 when the embryo died.

Fig. 6 shows 30-min recordings of IHR extracted from the time indicated by the symbols a–d in the top panel and also in four panels of the 24-h recording in Fig. 5 and their spectrum analyses. Panel a (45 days) is the 30-min recording at the time indicated by symbol a in the top panel and in the second panel (45 days) of Fig. 5. Panels b–d are presented in the same manner.

4. Discussion

4.1. Viability of embryos

The fresh mass of the 10 eggs that were brought to Muroran was 615±32 g. One egg (mass=642 g) was infertile. Three embryos in group I died. Among them, one embryo was measured for ECG for several hours on day 46. The mass of the embryo and the yolk was 270 and 140 g, respectively (egg mass=564 g). Two other eggs were not subjected to the experiment, but they died after IP and EP, respectively, on day 53. The IP embryo absorbed the yolk and weighed 480 g (egg mass= 634 g). The EP embryo incompletely absorbed the yolk and weighed 420 g (egg mass=627 g). When they were transported, they were exposed to low environmental temperatures. Generally, avian embryos can tolerate low temperature exposure, e.g. chick embryos can survive an 8 °C environment for several hours without beating of the heart (Tazawa and Rahn, 1986). In fact, exposure of the eggs to low temperatures during transportation did not kill the embryos. Meanwhile, IP and EP were delayed in these failed embryos. It is not known whether the low temperature exposure was responsible for the delay in pipping. The remaining two embryos in group I that were measured for ECG hatched, and the hatchlings weighed 445 g (egg mass=621 g) and 430 g (egg mass=611 g), respectively. All three embryos in group II, which were 27 days old on the day of arrival at Muroran, and one embryo in group III, which was first incubated in Muroran, successfully hatched. Four hatchlings weighed 430±31 g (egg mass= 614±39 g).

The spiral electrodes were designed so that they might be inserted between the embryo and the CAM under the eggshell and have a wide contact area with the egg content. Even if they did not come into direct contact with the embryo, the ECG signal could be measured well. When the embryo grew up to the eggshell, the spiral electrodes touched the embryo and could detect ECG, even during hatching activity. However, in some cases the electrodes injured the embryos, which might have been a cause of death during the long period of measurement.

4.2. Developmental patterns of mean heart rate

In order to present daily changes in HR, which extended over 2–7 days in a single graph, MHR over 1-min intervals was calculated from IHR data and plotted against incubation days (Figs. 1 and 4). This procedure shows the daily changes in HR as a continuous developmental pattern, and visual inspection of the pattern clearly gives the HR trend. Fig. 1 shows the developmental patterns of seven embryos that hatched. The baseline of MHR was wide, although it depends on individual embryos. The MHR baseline in embryo d was 20–30 bpm in width, while that in embryos c and g extended over 50–80 bpm due to irregular episodes of abrupt HR accelerations. The level of the MHR baseline was high in embryo a, which elevated to 150–200 bpm. That in other embryos

was approximately 150 bpm. Previous measurement of MHR in emu embryos showed that the HR decreased from approximately 170 to 140 bpm during the last 30% of incubation, with a value of approximately 140–150 bpm during the last 10% of incubation (Tazawa et al., 2000). The MHR in the previous report was non-invasively determined by acoustocardiography for a ~10-min period once a day in order to show daily changes over a prolonged period (i.e. the last 30% of incubation). Thus, daily changes in MHR were presented as a discrete developmental pattern. Meanwhile, the present measurement shows a continuous developmental pattern of MHR.

Visual inspection of the continuous developmental patterns indicated no circadian rhythms of HR during the last 10% of incubation in emu embryos. Previously, it was also reported that chick embryos did not have a circadian rhythm of HR until hatching (Moriya et al., 1999, 2000). However, in chick embryos, it was shown that an ultradian rhythm of HR, with a period of 40–90 min, occurred during the last stages of prenatal development, although the origins were unknown (Akiyama et al., 1999; Moriya et al., 2000). In emu, such an ultradian rhythm of HR was not observed in the developmental patterns of embryonic MHR. Instead, abrupt HR accelerations irregularly occurred, as shown in the continuous developmental patterns of some embryos (e.g. embryo g in Fig. 2), which were not observed in chick embryos.

Fig. 2 presents the continuous developmental pattern of MHR of embryo g in Fig. 1 and individual 24-h recordings, which show more clearly the changes in MHR with time. The abrupt HR accelerations shown in the top panel were composed of triangular patterns. Visual inspection indicated that these triangular patterns occurred irregularly. In addition, the power spectrum analysis of MHR changes in each panel showed no peaks of power at any particular frequency. An ultradian rhythm with a period of approximately 1 h, as observed in some chick embryos, did not occur in this embryo, or in other emu embryos either.

This embryo began to externally pip the eggshell at the time indicated by the symbol EP (and c) in the top panel and by the symbol c in the second panel (52 days) from the bottom. Soon after EP, the HR decreased, and this trend was also observed in other embryos (Fig. 1). The embryo hatched at the time indicated by the symbol H. The EP period extended over 17 h 20 min. The average EP period for seven embryos was 17 h, which was slightly longer than that previously determined for 10 chick embryos (ca. 13.5 h) (Chiba et al., 2002). During the EP period, the developmental patterns became distinctive, i.e. the HR baseline became wide towards hatching, as particularly emphasized in embryos e, f and g in Fig. 1. These distinctive patterns originated from distinctive accelerations of IHR, which are shown in Fig. 3d.

4.3. Fluctuations of instantaneous heart rate

The IHR fluctuations were mainly comprised of irregular accelerations (Fig. 3). The representative 30-min recordings shown in Fig. 3a–d correspond to the MHR indicated by the same symbols a–d in the top panel and the second (47 days), fourth (49 days), seventh (52 days) and eighth (53 days) panels of Fig. 2. Three patterns of IHR accelerations were characteristic of late emu embryos. They were: (1) transient, brief accelerations of large magnitude, which occurred irregularly (referred to as *irregular intermittent large accelerations*); (2) relatively large accelerations lasting for 10–20 min in duration, as shown in panel b (referred to as *wide accelerations*); and (3) accelerations of relatively low frequency (ca. 2–3 per min) and large magnitude, lasting for a short period (ca. 5–10 min) as shown in panel d (referred to as *short-term repeated large accelerations*). The *irregular intermittent large accelerations* occurred throughout the last stages of development. These accelerations were also commonly observed in perinatal chick embryos (Tazawa et al., 1999: Moriya et al., 2000). The *wide accelerations* were distinctive of late emu embryos, and were not observed in chick embryos. As indicated by symbol b in the top panel and in the fourth panel of Fig. 2, the large accelerations and the triangular patterns of MHR were attributed to *wide accelerations* of IHR. The *short-term repeated large accelerations*, as shown in panel d, also occurred in chick embryos prior to hatching. These distinct acceleration patterns signaled imminent hatching in emu, and also in chickens. The MHR over 1-min intervals during the period when these short-term accelerations occurred formed an acceleration pattern of MHR as indicated by symbol d in the bottom panel of Fig. 2. As the magnitude of these MHR accelerations became large during EP, the HR baseline became wide towards hatch-

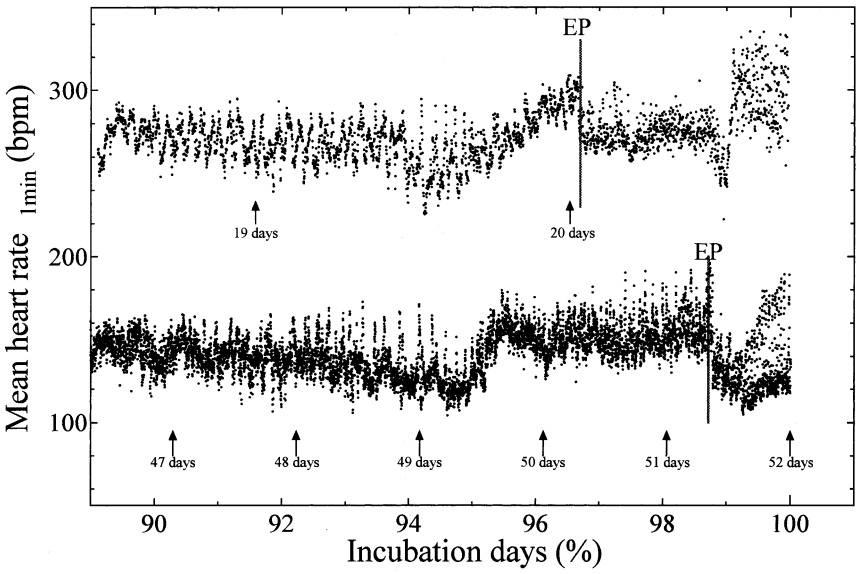

Fig. 7. Visual comparison between chicken and emu of the developmental patterns of embryonic mean heart rate (MHR) during the last 10% of incubation duration. The top recording is for a chicken embryo and the last 10% corresponds to approximately 2 days. The bottom recording is for emu embryo e in Fig. 1 and the last 10% corresponds to approximately 5 days. The number with an arrow indicates the age (days) of the embryos. Symbol EP with a vertical bar shows the onset of external pipping. Individual points indicate MHR over 1-min intervals.

ing, as observed in embryo g and particularly emphasized in embryos e and f of Fig. 1.

IHR fluctuations in emu embryos were substantially irregular, i.e. heart rate irregularities (HRI). The spectrum analysis of IHR, presented in the right panels of Fig. 3, did not show a peak of power at any frequency, except for the small peak at approximately 1 Hz in the third panel. The embryo pipped the eggshell at this time on day 52 of incubation and breathed using its lungs. As observed in chick embryos after EP, this peak indicates the breathing frequency, i.e. the wide baseline of IHR in panel c is attributed to respiratory sinus arrhythmia (RSA).

4.4. Cardiac rhythms in embryos that failed to hatch

Six embryos died after implantation of ECG electrodes and the developmental patterns of MHR acquired from four embryos are presented in Fig. 4. All these embryos did not pip the eggshell. However, embryos c and d pierced their beak into the air cell (i.e. IP). Although the baseline MHR tended to narrow and fall in embryos a and d, fluctuations occurred during the measurement, as for hatched embryos. The 24-h recordings in Fig. 5 were obtained from embryo d of Fig. 4. The continuous developmental patterns of MHR in Fig. 4 are again shown in the top panel of Fig. 5. Triangular patterns also occurred in the failed embryo. However, MHR fluctuations were depressed towards death, which was attributed to depression of IHR (Fig. 6). The depression of IHR fluctuations already occurred on day 50 in this embryo and proceeded with a fall in baseline on the following day. The depression of HR fluctuations may signal imminent death. Although the duration of the depression was short, it also occurred in all failed embryos prior to their death. The power spectrum analysis of IHR showed that the slope of the power against frequency decreased towards death (Fig. 6). The small peaks of the power at approximately 1 Hz on days 50 and 51 indicate RSA, and the slightly wide baseline was attributed to oscillation of IHR.

4.5. Comparison with chick embryos

Because the total incubation duration of emu eggs is approximately 50 days, the last 10% of incubation extends over approximately 5 days. In the present study, we attempted to measure the embryonic HR continuously from day 44 or day

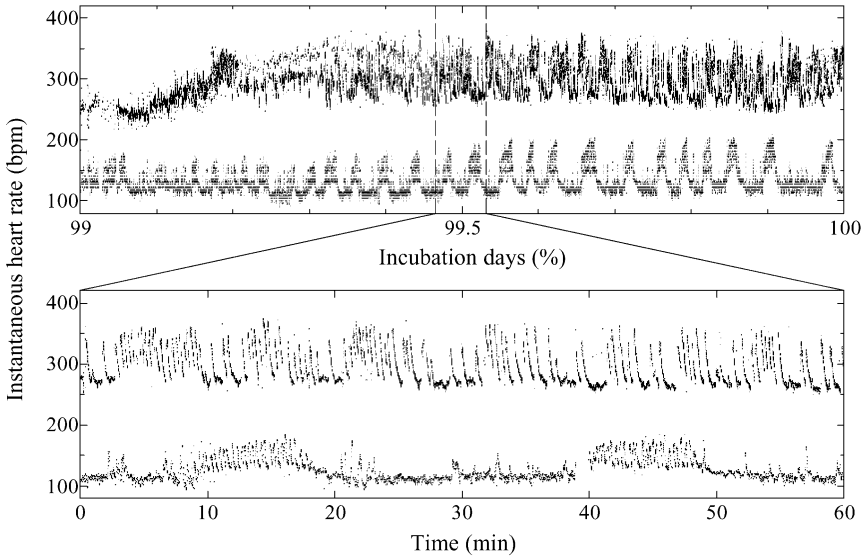

Fig. 8. The instantaneous heart rate (IHR) during the last 1% of incubation duration in the chicken and the emu (top panel), corresponding to the mean heart rate during the last 1% period in Fig. 7, and 60-min recordings of IHR extracted from the last 1% recordings (bottom panel).

46 until hatching, so that the measurement would cover the last stages of prenatal and perinatal development of emu embryos. Meanwhile, the last 10% of incubation duration in chickens is approximately 2 days. Fig. 7 presents the developmental patterns of embryonic MHR in the emu and chicken. The emu embryo was embryo e in Fig. 1 and the chick embryo was measured for developmental patterns in a previous report (Moriya et al., 2000). Visual comparison of these developmental patterns in the restricted two examples first shows that the baseline level was clearly different. The small chicken egg has markedly higher HR compared with the large emu egg. The HR fluctuation prior to EP was cyclic oscillation in the chick embryo, i.e. an ultradian rhythm with an oscillating period of approximately 42 min (Moriya et al., 2000). Meanwhile, HR fluctuation in the emu embryo was not cyclic and was attributed to the *wide accelerations*, which occurred intermittently.

The chick embryo began EP before 97% of incubation duration and the emu embryo after 98%, but the EP period was coincidentally the same in both embryos, i.e. 16 h (Fig. 7). When the embryos of both species began to pip the eggshell, the HR transiently dropped, with a subsequent increase. This trend was observed in other embryos of both species. However, the magnitude of the subsequent increase in MHR was larger in the chick than the emu embryos, as in the examples shown during the last 1% of incubation in Fig. 7. In the chick embryos, the drop in MHR baseline after the onset of EP was followed by cyclic accelerations with relatively high frequency (ca. 5 min^{-1}) and low magnitude, lasting for a relatively long period (ca. 20 min) (referred to as *relatively long-lasting cyclic small accelerations*) (Tazawa et al., 1999). The level of HR baseline was increased during the *relatively long-lasting cyclic small accelerations* in the chick embryos, which caused the elevation of HR baseline that occurred at approximately 99% of incubation time in Fig. 7. In the emu embryos, these HR accelerations were not obvious, but the magnitude of *short-term repeated large accelerations* was increased towards hatching (Fig. 8).

The elevated MHR baseline during the last 1% of incubation in Fig. 7 is presented in the time-expanded panel of Fig. 8. The top recording of the top panel in Fig. 8 shows IHR fluctuation of the chick embryo, corresponding to the MHR during the last 1% of incubation shown in the top recording of Fig. 7. Simultaneously, the IHR fluctuation shown in the bottom part of both panels in Fig. 8 is for the emu embryo, corresponding to the MHR during the last 1% of incubation shown in the

bottom recording of Fig. 7. The IHR baseline increased during the first 0.2% period in the top recording, which corresponded to *relatively long-lasting cyclic small accelerations* in the chick embryo. Then, after *irregular intermittent large accelerations* occurred during the next 0.15% period, *short-term repeated large accelerations* and *irregular intermittent large accelerations* alternately occurred until hatching. Meanwhile, in the emu embryos, *short-term repeated large accelerations* occurred intermittently during the given 1% period of incubation, the magnitude of which increased towards the end of incubation. The patterns of *short-term repeated large accelerations* in the chick and emu embryos are shown in the top and bottom recordings of the bottom panel in Fig. 8, respectively. Although the duration of the repeated accelerations was different between the two embryos in this example, these patterns were signals of imminent hatching in both species.

The chicken and the emu are both precocial birds. The egg mass is approximately 10-fold heavier in the emu than the chicken and the incubation duration is approximately 2.5-fold longer in the emu. In the present report, the developmental patterns of MHR and fluctuations of IHR were measured for emu embryos during only the last stages of prenatal and perinatal development. The physiological significance of these patterns and fluctuations was not elucidated, as elucidation needs further measurement and investigation. Continuous measurement for several weeks, corresponding to at least last half of the incubation period of the emu, remains to be carried out.

Acknowledgments

We are grateful to the Cross Timbers Emu Ranch in Flower Mound, Texas for supplying the emu eggs. The present study was supported in part by the US–Japan Co-operative Science Program (Co-operative Research) of the National Science Foundation (awarded to WWB) and the Japan Society for the Promotion of Science (awarded to HT).

References

Akiyama, R., Ono, H., Höchel, J., Pearson, J.T., Tazawa, H., 1997. Non-invasive determination of instantaneous heart rate in developing avian embryos by means of acoustocardiogram. Med. Biol. Eng. Comput. 35, 323–327.

Akiyama, R., Matsuhisa, A., Pearson, J.T., Tazawa, H., 1999. Long-term measurement of heart rate in chicken eggs. Comp. Biochem. Physiol. 124A, 483–490.

Ar, A., Tazawa, H., 1999. Analysis of heart rate in developing bird embryos: effects of developmental mode and mass. Comp. Biochem. Physiol. 124A, 491–500.

Burggren, W.W., Tazawa, H., Thompson, D., 1994. Genetic and maternal environmental influences on embryonic physiology: intraspecific variability in avian embryonic heart rates. Isr. J. Zool. 40, 351–362.

Chiba, Y., Khandoker, A.H., Nobuta, M., Moriya, K., Akiyama, R., Tazawa, H., 2002. Development of respiratory rhythms in perinatal chick embryos. Comp. Biochem. Physiol. 131, 817–824.

Höchel, J., Akiyama, R., Masuko, T., Pearson, J., Nickelmann, M., Tazawa, H., 1998. Development of heart rate irregularities in chick embryos. Am. J. Physiol. (Heart Circ. Physiol.) 275, H527–H533.

Höchel, J., Mohr, E., Nichelmann, M., Pirow, R., Tazawa, H., 1999. Development of heart rate rhythmicity in muscovy duck embryos. Comp. Biochem. Physiol. 124A, 501–510.

Moriya, K., Höchel, J., Pearson, J., Tazawa, H., 1999. Cardiac rhythms in developing chicks. Comp. Biochem. Physiol. 124A, 461–468.

Moriya, K., Pearson, J.T., Burggren, W.W., Ar, A., Tazawa, H., 2000. Continuous measurements of instantaneous heart rate and its fluctuations before and after hatching in chickens. J. Exp. Biol. 203, 895–903.

Moriya, K., Kato, K., Matsumura, M., Dzialowski, E., Burggren, W.W., Tazawa, H., 2002. Cardiac rhythms in developing emu hatchlings. Comp. Biochem. Physiol. 131, 787–795.

Pearson, J.T., Tazawa, H., 1999a. Ontogeny of heart rate in embryonic and nestling crows (*Corvus corone* and *Corvus macrorhynchos*). J. Comp. Physiol. B 169, 256–262.

Pearson, J.T., Tazawa, H., 1999b. Development of cardiac rhythms in altricial avian embryos. Comp. Biochem. Physiol. 124A, 475–482.

Pearson, J.T., Tsuzuki, M., Nakane, Y., Akiyama, R., Tazawa, H., 1998. Development of heart rate in the precocial king quail, *Coturnix chinensis*. J. Exp. Biol. 201, 931–941.

Pearson, J.T., Noma, Y., Tazawa, H., 1999. Developmental patterns of heart rate in altricial avian embryos and hatchlings. J. Exp. Biol. 202, 1545–1550.

Pearson, J.T., Moriya, K., Yanone, M., Tazawa, H., 2000. Development and regulation of heart rate in embryos and hatchlings of gulls (*Larus schistisagus* and *Larus crassirostris*) in relation to growth. J. Comp. Physiol. B 170, 429–438.

Tazawa, H., Rahn, H., 1986. Tolerance of chick embryos to low temperatures in reference to the heart rate. Comp. Biochem. Physiol. 85A, 531–534.

Tazawa, H., Whittow, G.C., 1994. Embryonic heart rate and oxygen pulse in two procellariiform seabirds, *Diomedea immutabilis* and *Puffinus pacificus*. J. Comp. Physiol. B 163, 642–648.

Tazawa, H., Hiraguchi, T., Kuroda, O., Tullett, S.G., Deeming, O.C., 1991. Embryonic heart rate during development of domesticated birds. Physiol. Zool. 64, 1002–1022.

Tazawa, H., Kuroda, O., Whittow, G.C., 1991. Non-invasive*Anous stolidus*). Auk 108, 594–601.

Tazawa, H., Watanabe, W., Burggren, W.W., 1994. Embryonic heart rate in altricial birds, the pigeon (*Columba domestica*)

and the bank swallow (*Riparia riparia*). Physiol. Zool. 40, 1448–1460.

Tazawa, H., Ar, A., Gefen, E., Moriya, K., Pearson, J.T., 1998a. Effects of incubator humidity on embryonic heart rate in the ostrich.Proceedings of the 10th European Poultry Conference, pp. 843–847.

Tazawa, H., Ar, A., Pearson, J.T., Moriya, K., Gefen, E., 1998b. Heart rate in developing ostrich embryos. Br. Poult. Sci. 39, 161–166.

Tazawa, H., Mitsubayashi, H., Hirata, M., Höchel, J., Pearson, J.T., 1999. Cardiac rhythms in chick embryos during hatching. Comp. Biochem. Physiol. 124A, 511–521.

Tazawa, H., Ar, A., Moriya, K., Gefen, E., Pearson, J.T., 2000. Embryonic heart rate measurements during artificial incubation of emu eggs. Br. Poult. Sci. 41, 89–93.

Tazawa, H., Pearson, J.T., Komoro, T., Ar, A., 2001. Allometric relationships between embryonic heart rate and fresh egg mass in birds. J. Exp. Biol. 204, 165–174.