The Journal of Experimental Biology 203, 895–903 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
JEB2445

CONTINUOUS MEASUREMENTS OF INSTANTANEOUS HEART RATE AND ITS FLUCTUATIONS BEFORE AND AFTER HATCHING IN CHICKENS

K. MORIYA[1], J. T. PEARSON[1], W. W. BURGGREN[2], A. AR[3] AND H. TAZAWA[1,*]

[1]*Department of Electrical and Electronic Engineering, Muroran Institute of Technology, Muroran 050-8585, Japan,* [2]*Department of Biology, College of Arts and Sciences, University of North Texas, PO Box 305189, Denton, TX 76203-5189, USA* and [3]*Department of Zoology, Faculty of Life Sciences, Tel Aviv University, PO Box 39040, Ramat-Aviv, Tel Aviv 69978, Israel*

*Author for correspondence (e-mail: tazawa@eee.elec.muroran-it.ac.jp)

Accepted 30 December 1999; published on WWW 10 February 2000

Summary

There has been considerable interest in heart rate (f_H) fluctuations in relation to cardiovascular control systems and foetal conditions during pregnancy in mammals. Prominent fluctuations in f_H also occur in avian embryos, which are an important experimental model for studying developmental physiology. The present study determined the instantaneous f_H of seven chick embryos continuously from the last stage of prenatal development (day 18), throughout the pipping (perinatal) period (days 19–21) until hatching and, subsequently, of newly hatched chicks (up to day 2).

The distinctive patterns of instantaneous f_H fluctuations took the form of specific changes within a broad mean f_H baseline. Cyclic oscillations (ultradian rhythm) occurred until an early stage of the perinatal period, when the f_H baseline started rising. Subsequently, the baseline dropped and respiratory arrhythmia began to appear concomitant with external pipping. During the final stage of external pipping, when the f_H baseline rose again prior to hatching, three unique patterns of instantaneous f_H fluctuations were evident: relatively long-lasting cyclic small accelerations, irregular intermittent large accelerations and short-term repeated large accelerations. Furthermore, repeated alternate occurrences of the latter two types of acceleration formed an additional oscillating pattern with a period of 10–15 min.

During the early period after hatching, when the f_H baseline reached its maximum, instantaneous f_H changed relatively slowly accompanied by transient rapid decelerations, probably due to augmented vagal tone. Subsequently, the mean f_H baseline dropped to its minimum, and a circadian rhythm and three types of previously reported f_H fluctuations (types I–III) appeared.

Developmental patterns of mean f_H and the appearance of distinctive patterns of instantaneous fluctuations in f_H and circadian rhythms were not influenced by an ultimate failure of hatching after a normal development.

The demonstration of complex, repeatable patterns of f_H fluctuation that change during development suggests that the avian embryo model should be useful in studying the phenomenon of f_H fluctuation and its underlying causes.

Key words: circadian rhythm, developmental pattern, heart rate fluctuation, instantaneous heart rate, mean heart rate, prenatal embryo, perinatal embryo, postnatal chick, ultradian rhythm, *Gallus gallus domesticus*.

Introduction

Heart rate (f_H) has long been recognized as a general, easily measured indicator of cardiovascular performance, as demonstrated in a recent special issue of *Comparative Biochemistry and Physiology* devoted to the topic of f_H and the information derived from it (Tazawa et al., 1999a). Beat-to-beat f_H (i.e. instantaneous f_H) fluctuations have been subject to considerable attention in relation to autonomic nervous function and cardiovascular status in mammalian adults and foetuses (Akselrod et al., 1981; Ferrazzi et al., 1989; Fleisher, 1996; Groome et al., 1994; Lindecrantz et al., 1993; Moser et al., 1994; Pagani et al., 1986). The patterns of f_H fluctuations have also been studied in foetuses to evaluate their relationships with foetal movements and with conditions during pregnancy (Aladjem et al., 1977; Brubaker and Garite, 1988; Hammacher et al., 1968; Pillai and James, 1990; Sorokin et al., 1982; Wheeler and Murrills, 1978). The prominent fluctuations in f_H are substantial phenomena not only in mammalian foetuses but also in avian embryos. Avian embryos develop within the confines of an eggshell independently of the maternal body, and their physiological functions are not influenced by maternal functions. The patterns of fluctuation in f_H recorded from embryos originate in their own cardiac pacing activities uninfluenced by maternal conditions and are characteristic of developing embryos. We have measured instantaneous f_H in avian embryos, while maintaining adequate gas exchange through the eggshell, and also in hatchlings

(Akiyama et al., 1997, 1999; Höchel et al., 1998; Pearson et al., 1998; Pearson and Tazawa, 1999; Moriya et al., 1999; Tazawa et al., 1999b). Among eggs of altricial and precocial birds in which f_H fluctuations have been measured, those of the chicken (*Gallus gallus* var. *domesticus*) have been the most frequently used for experimentation because of their easy availability and relatively large size. In addition, a thorough investigation of chicken eggs and hatchlings is needed to elucidate the development and origins of fluctuations in f_H because they provide an experimental model for understanding f_H fluctuations in other avian species and in mammalian foetuses.

In chickens, instantaneous f_H has been determined both by acoustocardiography (Akiyama et al., 1997, 1999; Rahn et al., 1990; Wang et al., 1990) and using a catheterization method (Höchel et al., 1998; Tazawa, 1981; Tazawa et al., 1980) for prenatal embryos during the last half of incubation and by electrocardiography for postnatal chicks (Moriya et al., 1999). For continuity of investigation of f_H fluctuations from prenatal embryos to postnatal chicks, the instantaneous f_H of perinatal (pipped) embryos during the internal and external pipping period (perinatal period) was measured by electrocardiography using wire electrodes inserted into the egg (Tazawa et al., 1999b). Three unique acceleration patterns of instantaneous f_H were found during the external pipping period. Respiratory arrhythmia with a period of 1–1.5 s was also observed during the external pipping period, and additional oscillating patterns with a period of 10–15 min were found in some perinatal embryos (Tazawa et al., 1999b). Generally, the electrocardiogram (ECG) was disturbed frequently by embryonic activity, rendering impossible continuous measurements of instantaneous f_H for a period of hours or a few days (Akiyama et al., 1997; Tazawa et al., 1999b). In the experiments described above with perinatal embryos, the electrodes were pushed into the egg contents through the eggshell and chorioallantoic membrane so as to touch the embryonic body and therefore to measure the ECG directly from the body and thus minimize disturbance. This method made it possible to measure additional patterns of f_H fluctuations (Tazawa et al., 1999b). However, it was still difficult to measure instantaneous f_H continuously throughout the hatching period, because the embryo moved in the egg during hatching and the electrodes thus became detached from the body or pierced it. The present study was designed to cope with these difficulties and to elucidate developmental patterns of f_H and changes in instantaneous f_H fluctuations from the last stages of prenatal incubation to the postnatal period. The resulting data were used to elucidate the unexplored perinatal period and the patterns of f_H fluctuation that typify it.

Materials and methods

Eggs and their incubation

Fertile eggs of broiler chickens (*Gallus gallus*) were purchased from the local hatchery that had provided us with the broiler eggs used in our previous experiments (Akiyama et al., 1997, 1999; Höchel et al., 1998; Moriya et al., 1999; Tazawa et al., 1999b). The eggs were incubated in a forced draught incubator at 38 °C and at a relative humidity of approximately 60 %. The first day of incubation was designated day 0. Eggs were turned automatically every hour for 16 days to avoid the known adverse effects of lack of turning on embryonic oxygen consumption, growth and f_H (New, 1957; Tazawa, 1980; Deeming, 1989a,b; Pearson et al., 1996).

Electrode preparation and experimental protocol

Eggs were candled on day 17 of incubation to confirm normal development of the embryo and to mark the air cell. ECG electrodes formed from three silver wires, 3 cm long and 0.6 mm in diameter, were then implanted. One end of the wire was wound round a rod (3 mm in diameter) to make a one-turn spiral, and the tip of the wire was bent into a ‘fish hook barb’. The distance between the tip and end of the spiral was approximately 1–2 mm. Three locations were marked on the eggshell, forming a triangle around the egg. Two sites were on the under side of the egg between the air cell and the equator of the egg and made up the bottom of a triangle when the egg was placed in the horizontal position. The third site was marked on the top of the egg. A tiny piece of eggshell (<2 mm across) was removed by a sharp blow with the tip of a 20 gauge hypodermic needle, which pierced the shell membranes and the chorioallantoic membrane. 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 then fixed onto the eggshell using epoxy glue.

The egg with implanted electrodes was placed in a still-air incubator at 38 °C, and the electrodes were connected to shield wires passing through a small hole in the incubator to a polygraph amplifier. The ECG was recorded continuously from day 18 until hatching in the still-air incubator at 38 °C. To avoid external disturbances affecting measurements, the egg was not touched and the incubator doors were not opened. The egg in the incubator was viewed through glass windows (10 cm wide and 30 cm long) in the doors.

When a chick hatched, it was removed briefly (for less than 20 min) from the incubator for the connection of new electrodes. Three flexible Ag/AgCl electrodes were attached to the skin of the chick using adhesive gel on the lateral thoracic wall under both wings and on the ventral abdomen caudal to the sternum (Moriya et al., 1999). The chick was then returned to the incubator, which was reset at 35 °C. The first day after hatching was designated day 0. Measurements of the ECG were continued for three more days after hatching in the same incubator at 35 °C. Throughout the period of measurement from day 18 of incubation to day 2 of postnatal life, only natural light was allowed to enter the incubator.

Signal processing

Instantaneous f_H was determined from the amplified and

bandpass-filtered ECG signals with the aid of a computer (Moriya et al., 1999). In brief, ECG signals were sampled at 4000 Hz by an analog-to-digital converter. The sampled signals were compared with a threshold level set on the computer above background noise levels to detect the rising deflection of the QRS complex. The times at which the magnitude of sampled QRS waves exceeded the threshold for the first time were stored in sequential order in data files. Instantaneous f_H (beats min^{-1}) was then calculated from the time between adjacent QRS complexes.

To determine the oscillatory period of cyclic f_H fluctuations, power spectrum analysis of instantaneous f_H was made using a fast Fourier transform (FFT; Moriya et al., 1999). When the cyclic f_H fluctuations contained discrete changes, the oscillatory period could not always be calculated using FFT. In these instances, the number of cyclic changes (n beats) was counted during the period when they appeared (T min), and the approximate period of the cyclic change was then calculated as $60T/n$. To present the developmental patterns of f_H from day 18 of incubation to day 2 of postnatal life, mean f_H over a 1 min period (referred to as $\overline{f}_{H1min}$) was calculated from instantaneous f_H data every 1 min and plotted in sequence in a single graph.

Results

Developmental patterns of heart rate in eggs that hatched

Continuous measurements of instantaneous f_H were made in seven chicken eggs, but one failed to escape from the egg even on day 24 of incubation. The developmental patterns of f_H and changes in instantaneous f_H fluctuations with time were similar among the six eggs that hatched, although the time and duration of individual changes in the developmental patterns and the hatching time were different. Fig. 1 presents the developmental pattern of $\overline{f}_{H1min}$, before and after hatching, of a single representative individual. Measurements were started at 12:00 h on day 18 of incubation. $\overline{f}_{H1min}$ fluctuated with time, producing a wide baseline (i.e. a wide fluctuation in f_H). The $\overline{f}_{H1min}$ baseline tended to fall towards the end of prenatal development and then rose during the second half of day 19 (12:00–00:00 h, marked as segment B), with a subsequent fall. Following the fall, which lasted for approximately the first half of day 20, the $\overline{f}_{H1min}$ baseline rose sharply at around 12:00 h, and an elevated $\overline{f}_{H1min}$ baseline was maintained until hatching.

The embryo depicted in Fig. 1 hatched at approximately 18:00 h on day 20. The $\overline{f}_{H1min}$ baseline of the newly hatched chick, which was initially low after the electrodes had been

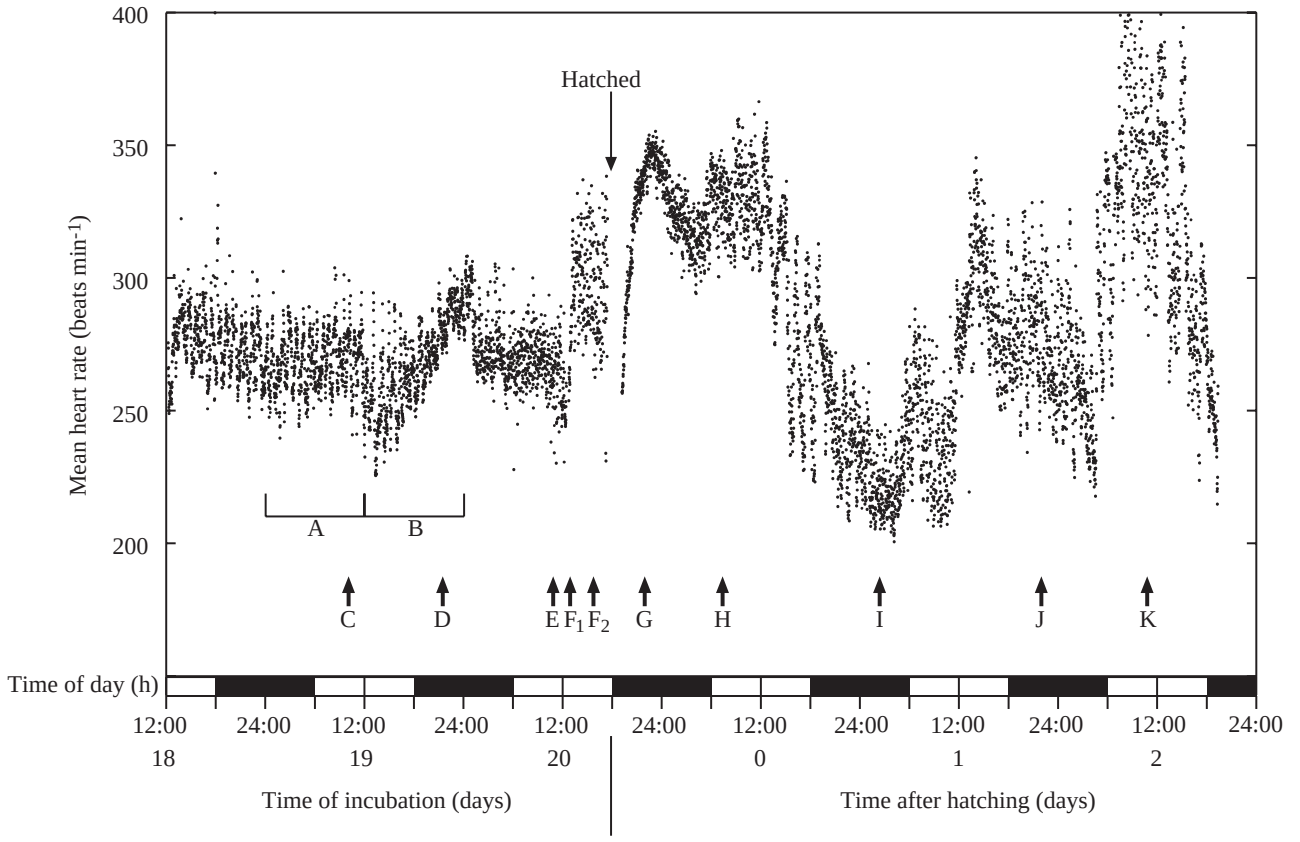

Fig. 1. Developmental pattern of mean heart rate (f_H) of a single chick before and after hatching; this example is typical of the chicks observed. Each point indicates the mean f_H over a 1 min period determined from the continuous recording of instantaneous f_H. The time between 06:00 and 18:00 h is conventionally termed the daytime phase and is shown by open rectangles, and that from 18:00 to 06:00 h of the following day is termed the night-time phase (filled rectangles). Actual daytime was from approximately 04:50 to 18:20 h in this experiment. The downward-pointing arrow indicates the time of hatching. The upward-pointing arrows labelled with letters show the time of 10 min recordings of instantaneous f_H presented in the following figures.

positioned, rose to a peak that was similar to the maximal perinatal level. Although the $\overline{f_{H1min}}$ baseline then dropped to some extent, the high level of the baseline was maintained for the first half of the following daytime phase (06:00–12:00 h on day 0), with a subsequent fall during the remaining daytime phase (12:00–18:00 h). The $\overline{f_{H1min}}$ baseline dropped further during the next night phase (18:00–06:00 h). The $\overline{f_{H1min}}$ baseline during the first half of the daytime phase (06:00–12:00 h) on day 1 dropped after transiently rising in the early morning. It then exhibited a prominent rise during the remaining half of the daytime phase (12:00–18:00 h on day 1), dropped during the following night phase (18:00–06:00 h) and subsequently rose during the daytime phase (06:00–18:00 h) on day 2. A prominent circadian rhythm of f_H began to occur.

Changes in heart rate fluctuations over time in eggs that hatched

The baseline of the developmental pattern of $\overline{f_{H1min}}$ was very wide as a result of various types of f_H fluctuations. Fig. 2 presents two 12 h recordings of $\overline{f_{H1min}}$. Fig. 2A and Fig. 2B correspond to segments A and B in Fig. 1 and cover 24 consecutive hours of recording on day 19. $\overline{f_{H1min}}$ fluctuated cyclically during the first 12 h (Fig. 2A) and over the next 5 h period (Fig. 2B) and was independent of diurnal phase. Spectral analysis of instantaneous f_H data corresponding to Fig. 2A indicated that the fluctuation was cyclic with an oscillatory period of 42 min, i.e. that there was an ultradian rhythm that oscillated with a period of less than 24 h.

Instantaneous f_H fluctuations consisted of various distinctive patterns before and after hatching. Fig. 3 presents examples of instantaneous f_H fluctuations recorded for the 10 min period before hatching. Fig. 3A–E corresponds to the periods indicated by C, D, E, F_1 and F_2, respectively, in Fig. 1. During the ultradian rhythm and the following increase in f_H shown in Fig. 2, rapid transient decelerations and accelerations occurred frequently (Fig. 3A,B). When f_H decreased prior to the final increase during the last stage of the perinatal period, the instantaneous f_H baseline occasionally became wide (Fig. 3C). After this, the instantaneous f_H began to oscillate, and the f_H baseline rose again to the final maximal level (Fig. 3D). Because f_H accelerations occurred rapidly, and thus discretely, the oscillatory period could not be determined by spectrum analysis, and it was therefore approximated by counting the oscillations. The peaks of oscillation appeared 37 times during the 10 min period, corresponding to an oscillatory period of approximately 16 s. During the last stage of the perinatal period when f_H peaked, the distinctive acceleration patterns of f_H appeared (Fig. 3E); the pattern consisted of intermittent irregular accelerations and large accelerations that were repeated for short periods no longer than 5 min. These distinct acceleration patterns signalled imminent hatching not only in this embryo but also in the other five embryos that hatched.

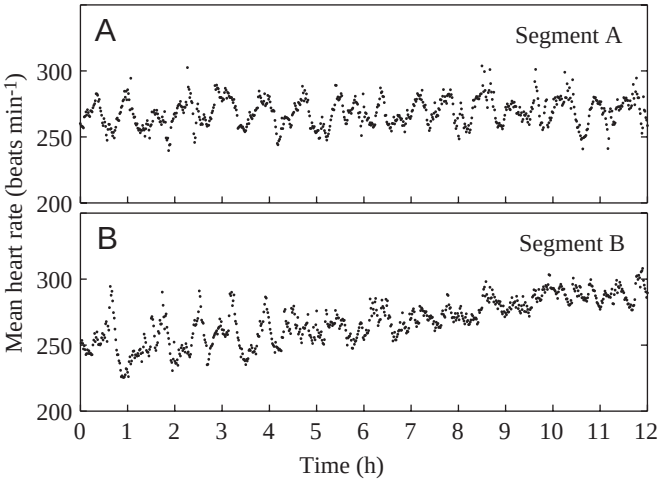

Fig. 2. (A,B) Recordings over periods of 12 h of heart rate for segments A and B in Fig. 1. Each point is the heart rate averaged over a 1 min period.

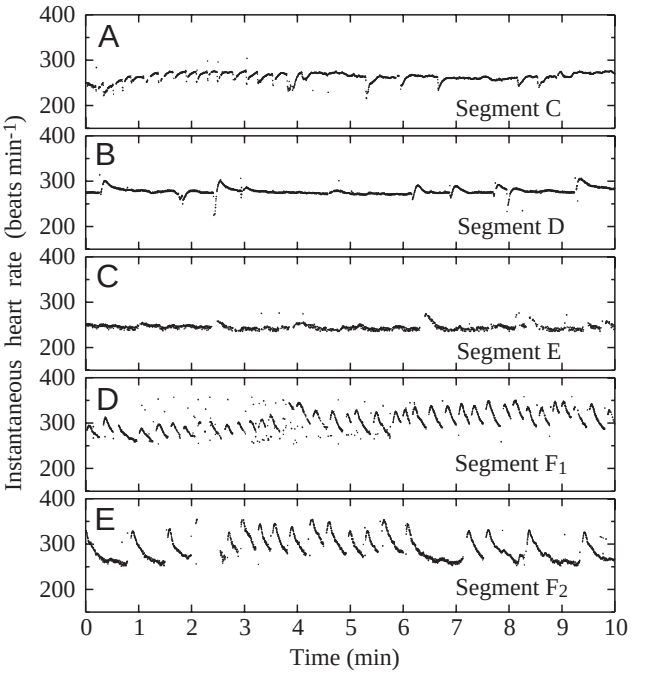

Fig. 3. 10 min periods of instantaneous heart rate fluctuations recorded before hatching. Panels A–E correspond to the times indicated by segments C–F_2 in Fig. 1.

Distinctive patterns of instantaneous f_H fluctuations continued after hatching. Fig. 4A–E presents 10 min recordings of instantaneous f_H during the periods indicated by G, H, I, J and K in Fig. 1. Instantaneous f_H fluctuations (Fig. 4A,B) were recorded while f_H remained high during the early period after hatching. The instantaneous f_H baseline changed irregularly and slowly compared with other periods, and the intermittent decelerations were predominant. When f_H decreased, the baseline broadened (Fig. 4C). Irregular acclerations and oscillations of f_H occurred (Fig. 4D,E), widening further the $\overline{f_{H1min}}$ baseline on day 2 (see Fig. 1).

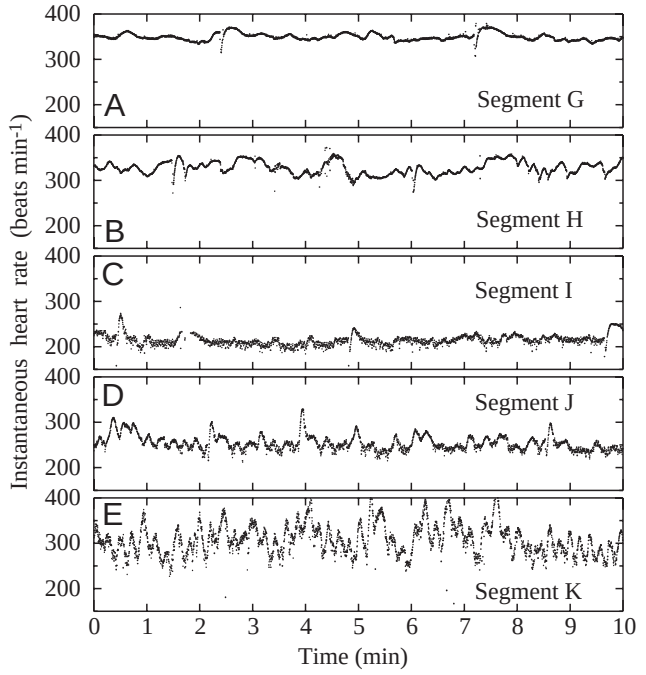

Fig. 4. 10 min periods of instantaneous heart rate fluctuations recorded after hatching. Panels A–E correspond to the times indicated by segments G–K in Fig. 1.

Developmental patterns of heart rate and heart rate fluctuations in an embryo that failed to hatch

Among the seven eggs investigated in the present study, one embryo failed to hatch fully after pipping the eggshell on day 21 and remained in the egg. Fig. 5 shows the developmental pattern of $\overline{f_{H1min}}$ in this unhatched embryo. The pattern was essentially similar to that of the hatched embryo shown in Fig. 1, although the time and duration of f_H changes were different and the $\overline{f_{H1min}}$ baseline dropped on days 23–24. The $\overline{f_{H1min}}$ baseline tended to fall from day 18 of incubation and then began to rise at approximately 12:00 h on day 20 with a subsequent drop during the first half of day 21. The $\overline{f_{H1min}}$ baseline then rose sharply at approximately 12:00 h of day 21 and remained at a high level.

This embryo was supposed to hatch at approximately 18:00 h on day 21 (marked by a downward-pointing arrow in Fig. 5), judging from changes in the distinctive patterns of instantaneous f_H fluctuations, as shown below. f_H remained high during the early period soon after the predicted hatching time (18:00–00:00 h on day 21) and the first half of day 22, with a subsequent drop at approximately 14:00 h. The low level of the $\overline{f_{H1min}}$ baseline was maintained for approximately the next half day until f_H increased again at around 02:00–03:00 h on day 23. Although the $\overline{f_{H1min}}$ baseline dropped gradually during the daytime phase on day 23, it dropped further, remained at the lowest level during the night phase, and then rose again in the early morning of day 24. Interestingly, a circadian rhythm occurred in this unhatched embryo as in the hatched embryos.

The wide f_H baseline during the last stage of the prenatal period was also due to f_H oscillations, just as in hatched embryos. Fig. 6A,B presents the changes in the $\overline{f_{H1min}}$ baseline during the periods shown by segments A and B in Fig. 5. Spectrum analysis of instantaneous f_H data during segment A indicated that the oscillatory period was 40 min, i.e. an ultradian rhythm. The changes in instantaneous f_H fluctuations of this unhatched embryo were also similar to those of hatched embryos. Fig. 7A–E shows instantaneous f_H fluctuations recorded for 10 min periods at the times indicated by C, D, E, F_1 and F_2 in Fig. 5. During the period when the $\overline{f_{H1min}}$ baseline oscillated with a period of 40 min, rapid decelerations of instantaneous f_H were predominant (Fig. 7A). When the f_H baseline rose (Fig. 7B), f_H accelerations with intermittent decelerations occurred. The f_H baseline then dropped, during which time the baseline of instantaneous f_H broadened (Fig. 7C). The $\overline{f_{H1min}}$ baseline then rose sharply, during which time instantaneous f_H began to oscillate with a period of approximately 10 s (Fig. 7D) and distinctive patterns of instantaneous f_H accelerations occurred (Fig. 7E). The other embryos hatched after these typical acceleration patterns had appeared repeatedly for several hours, and the hatching time of this embryo was therefore assumed to be close. Approximately 4 h later, the pattern of instantaneous f_H fluctuations changed to that shown in Fig. 8A. Each panel of Fig. 8A–E corresponds to instantaneous f_H recorded for a 10 min period indicated by G, H, I, J and K in Fig. 5. The large irregular accelerations occurred continuously for approximately half a day when the f_H baseline stayed high after the predicted hatching time (Fig. 8B). When the f_H baseline subsequently dropped, the baseline of instantaneous f_H broadened (Fig. 8C). Subsequently, cyclic oscillations (Fig. 8D) and irregular accelerations (Fig. 8E) occurred.

Discussion

Previous measurements of daily changes in mean f_H ($\overline{f_H}$) of late chick embryos and hatchlings indicated that $\overline{f_H}$ decreased towards the end of prenatal incubation, increased markedly during the external pipping period and then decreased after hatching, with subsequent daily increases (Tazawa et al., 1991, 1992). These previous studies of changes in $\overline{f_H}$ in embryos and hatchlings, which comprised brief periods of recordings, led us to determine continuously the developmental patterns of f_H before and after hatching. The present study reveals for the first time the developmental patterns of f_H and changes in instantaneous f_H fluctuations with time during hatching.

One embryo in the present study failed to hatch. This anomalous occurrence was instructive in many ways when the results were compared with those of the other six embryos that hatched. In hatched chicks, f_H immediately after hatching was initially low but increased swiftly with time (Fig. 1). Newly hatched chicks were temporarily removed from the incubator and briefly exposed to a lower temperature (approximately 25 °C) during the placement of ECG electrodes. The feathers were still wet, and heat loss from the chick must have decreased the body temperature because of the incomplete capacity for thermoregulation in precocial birds (Tazawa and

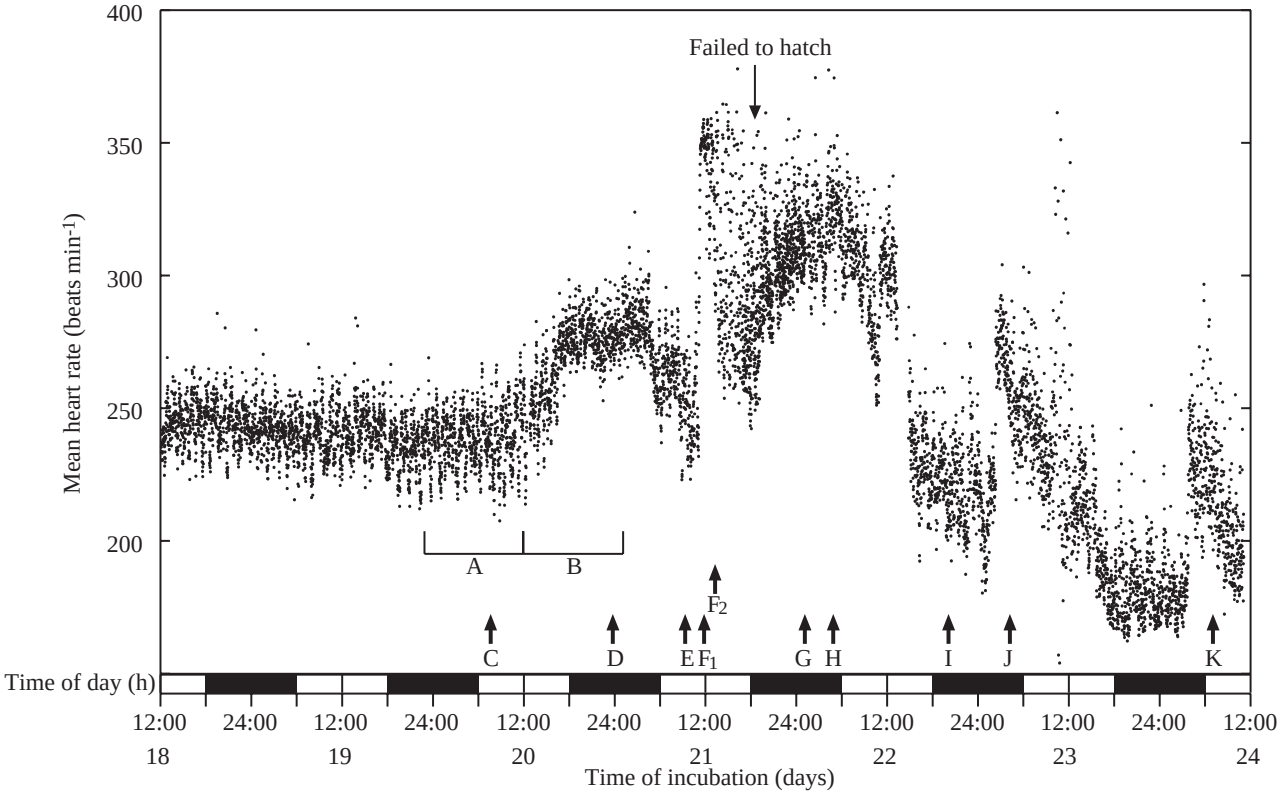

Fig. 5. Developmental pattern of mean heart rate (f_H) of an embryo that failed to escape from the egg before the end of recording on day 24. Open and filled rectangles on the abscissa represent the day and night phases, as in Fig. 1. Actual daytime was from approximately 04:50 to 18:27 h in this experiment. The downward-pointing arrow indicates the predicted time of hatching, which was judged from the specific changes in instantaneous f_H fluctuation patterns.

Rahn, 1987; Whittow and Tazawa, 1991). f_H must presumably have decreased in accordance with lowered body temperature. Interestingly, however, the unhatched embryo was kept in the incubator, and its f_H remained at the same level as the perinatal f_H even after the predicted hatching time (Fig. 5). This suggests that hatching may not change the $\overline{f_H}$ baseline as long as the environmental temperature does not change, although evaporative cooling of the wet chick is considered to be another possibility that could provide the stimulus for a change in f_H. Another difference between hatched chicks and the embryo that failed to hatch was the level of the $\overline{f_H}$ baseline 1–2 days after actual or predicted hatching (23–24 days in the unhatched embryo). The unhatched embryo was restrained from normal development in the egg and became weak, as reflected in a low $\overline{f_{H1min}}$. Nevertheless, a circadian rhythm of f_H appeared in the unhatched embryo just as in hatched chicks. In addition, changes in f_H fluctuations after the predicted hatching time were similar to those of hatched chicks (segments I, J and K in Figs 4C–E, 8C–E). These similarities between the hatched chicks and the unhatched embryo suggest that the development of patterns of f_H was not influenced by the failure to hatch, but was related to incubation time and probably to the development and maturity of the embyro. The development of thermoregulatory capacity was not influenced by the failure to hatch (Tazawa et al., 1988).

Because our f_H measurements were made without interruption, including opening of incubator doors, neither internal nor external pipping was confirmed directly. However, it can be inferred that changes in patterns of $\overline{f_{H1min}}$ are related to internal and external pipping events. In prenatal embryos, the cyclic oscillations in f_H with a period of 40–90 min were found to appear at approximately day 16–17 of incubation, and their magnitude increased with time (Akiyama et al., 1999). However, in that study, these f_H ultradian rhythms could not be measured continuously after day 18–19 because of signal disturbances caused by embryonic activity. The present measurements showed that these ultradian rhythms continued until internal pipping, which probably occurred at the time of the increase in f_H shown in segment B in Figs 1 and 5. If the subsequent decrease in f_H were initiated by the hatching activities of the embryo, then external pipping might be a possible factor initiating this decrease, because respiratory arrhythmia occurred during this period in both hatched and unhatched embryos (Figs 3C, 7C). The wide baseline of instantaneous f_H in these figures was a result of respiratory arrhythmia. Previous experiments with newly hatched chicks have shown that the broad baseline of instantaneous f_H was caused by lung ventilation and consisted of cyclic oscillations with a frequency of 0.4–1.2 Hz, which was the same as that of ventilatory movements (Moriya et al., 1999). It has also been

confirmed previously that the wide baseline of instantaneous f_H, signalling respiratory arrhythmia, occurred in external pipping embryos (Tazawa et al., 1999b). Thus, the external pipping period seemed to consist of two phases; the first was the period when the $\overline{f}_{H1min}$ baseline dropped, and this was followed by the second phase which began with a sharp rise in the $\overline{f}_{H1min}$ baseline.

During the second phase of external pipping, instantaneous f_H began to accelerate cyclically with a period of approximately 16 s in the hatched embryo (Fig. 3D) and of approximately 10 s in the unhatched embryo (Fig. 7D). The increase in f_H was approximately 50 beats min^{-1} in the former and 30 beats min^{-1} in the latter. In other hatched embryos, the increase was also no more than 50 beats min^{-1}, and the period of these cyclic acceleration patterns ranged from 10 to 24 s. The cyclic accelerations lasted for several tens of minutes with a rise of f_H baseline and subsequent plateau. The instantaneous f_H baseline then dropped, and irregular intermittent large accelerations occurred. With time, irregular intermittent large accelerations occasionally turned into repeated acceleration patterns lasting for a short period, no longer than 5 min (referred to as short-term repeated large accelerations; Figs 3E, 7E). When the irregular intermittent large accelerations occurred, the baseline tended to fall for several minutes. During the period of short-term repeated large accelerations, the baseline tended to rise. Thus, the repeated occurrences of irregular accelerations and repeated accelerations formed additional oscillatory patterns with a period of 10–15 min (top panel in Fig. 5 of Tazawa et al., 1999b). As a result, three patterns of instantaneous f_H accelerations characterized the last phase of external pipping period: relatively long-lasting cyclic small accelerations, which appeared first (Figs 3D, 7D), irregular intermittent large accelerations and short-term repeated large accelerations (Figs 3E, 7E). The appearance of the latter two patterns was an indication of imminent hatching.

The $\overline{f}_{H1min}$ baseline widened further after hatching because of augmented fluctuations in f_H. During the period when the $\overline{f}_{H1min}$ baseline reached a peak soon after hatching, the instantaneous f_H baseline changed relatively slowly, and intermittent f_H decelerations dominated (Fig. 4A). With the passage of time, the changes in instantaneous f_H baseline became fast and augmented, but f_H decelerations were still dominant (Fig. 4B). These f_H fluctuations with marked intermittent decelerations were similar in all chicks. When the $\overline{f}_{H1min}$ baseline reached a minimum, respiratory arrhythmia became apparent (Fig. 4C). Subsequently, the instantaneous f_H fluctuations were increasingly augmented by irregular accelerations (Fig. 4D) and cyclic accelerations (Fig. 4E). The fluctuations in f_H shown in Fig. 4C, D and E correspond to those grouped into three types (types I, III and II, respectively) categorized in the previous report (Moriya et al., 1999): type I is categorized as a widespread baseline f_H (20–50 beats min^{-1}) due to respiratory arrhythmia with a mean oscillatory frequency of 0.74 Hz (range 0.4–1.2 Hz); type III is categorized as non-cyclic irregularities, dominated by frequent transient accelerations; and type II is categorized as low-frequency oscillations of the instantaneous f_H baseline at a mean frequency of 0.07 Hz (range 0.04–0.1 Hz).

The development of instantaneous fluctuations in f_H after the predicted hatching time in the unhatched embryo was substantially the same as that in hatched eggs except for the period soon after the predicted hatching time (segments G and H in Figs 1, 5). During this period, while the intermittent decelerations were dominant and instantaneous f_H baseline changes were relatively slow and small in hatchlings (segments G and H in Fig. 1), in the unhatched embryo the f_H baseline changed swiftly and frequently, forming large and wide

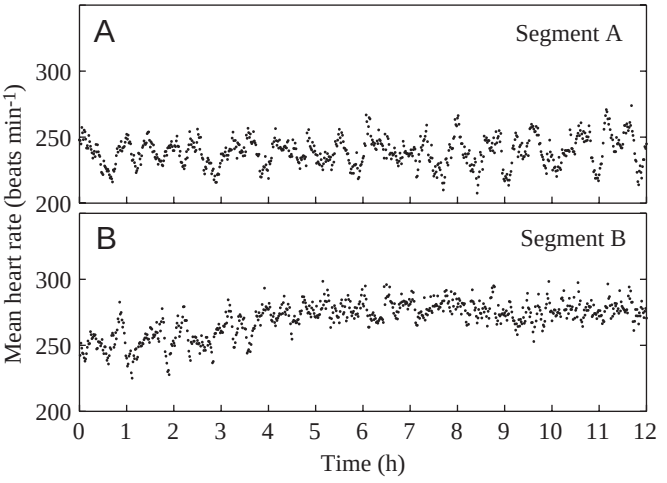

Fig. 6. (A,B) Recordings over periods of 12 h of mean heart rates averaged over 1 min periods, corresponding to segments A and B in Fig. 5.

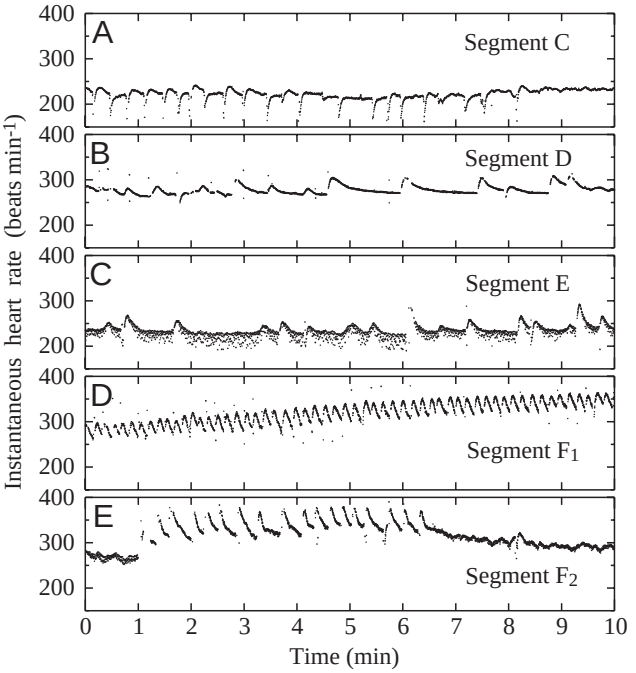

Fig. 7. 10 min periods of instantaneous heart rate fluctuations recorded before the predicted time of hatching. Panels A–E correspond to the times indicated by segments C–F_2 in Fig. 5.

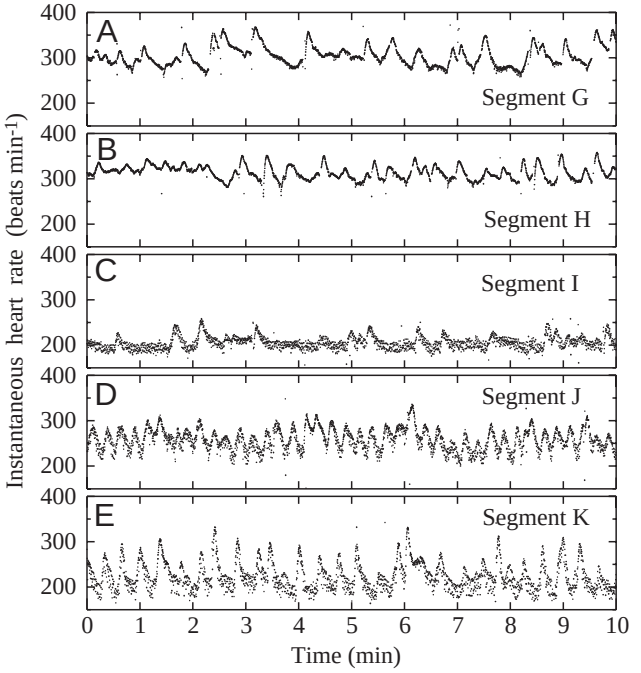

Fig. 8. 10 min periods of instantaneous heart rate (f_H) fluctuations recorded after the predicted time of hatching. Panels A–E correspond to the times indicated by segments G–K in Fig. 5. The instantaneous f_H baseline in D (segment J) oscillates with a period of 15 s, and mean f_H during the 10 min period is approximately 260 beats min^{-1}.

irregular accelerations, although decelerations also occurred. It has previously been shown that f_H decelerations disappeared in response to venous administration of atropine in prenatal embryos and were therefore mediated by parasympathetic nervous function (Höchel et al., 1998). This mechanism may also operate in newly hatched chicks. During the period soon after hatching when the f_H baseline was elevated and accompanied by intermittent f_H decelerations, parasympathetic nerve function may dominate sympathetic nerve function. In the unhatched embryo, augmentation of parasympathetic function during this period may be small compared with that in hatchlings, allowing accelerations in f_H. With the passage of time, the f_H baseline dropped and three types of f_H fluctuation appeared, as in the hatchlings (Fig. 8C,D,E). Respiratory arrhythmia in this embryo, which was confined inside the eggshell, was dominant not only during the period when the f_H baseline was lowered (Fig. 8C), but also during the period of type II (Fig. 8D) and type III (Fig. 8E) fluctuations in f_H. The combined patterns of types I and II and types I and III have been reported previously in developing chick hatchlings (Moriya et al., 1999). The low-frequency oscillations of instantaneous f_H baseline (0.09 Hz on average) were also found in young and adult quail (Pearson et al., 1998). Type II f_H fluctuations may be due to baroreflexes, as suggested in mammals (Sayers, 1973), but this seems unlikely, since the response time is too slow to be effective (e.g. in Fig. 8D, a period of 15 s for a mean f_H of 260 beats min^{-1} is equivalent to every sixty-fifth heart beat). Non-cyclic irregularities, dominated by frequent transient accelerations (type III), may be mediated by sympathetic nervous function, although venous administration of sympathomimetic and sympathetic blocking agents in prenatal chick embryos did not show a clear relationship with type III fluctuations (Höchel et al., 1998). Another possibility is a transient decline in parasympathetic nervous function resulting from an antagonistic balance with sympathetic nervous function. The origins of types II and III fluctuations in f_H remain to be studied.

References

Akiyama, R., Matsuhisa, A., Pearson, J. T. and Tazawa, H. (1999). Long-term measurement of heart rate in chicken eggs. *Comp. Biochem. Physiol*. **124**, 485–492.

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

Akselrod, S., Gordon, D., Ubel, F. A., Shannon, D. C., Barger, A. C. and Cohen, R. J. (1981). Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science* **213**, 220–222.

Aladjem, S., Rest, J. and Stojanovic, J. (1977). Fetal heart rate responses to fetal movements. *Br. J. Obstet. Gynaecol*. **84**, 487–491.

Brubaker, K. and Garite, T. J. (1988). The lambda fetal heart rate pattern: an assessment of its significance in the intrapartum period. *Obstet. Gynecol*. **72**, 881–885.

Deeming, D. C. (1989a). Characteristics of unturned eggs: critical period, retarded embryonic growth and poor albumen utilisation. *Br. Poultry Sci*. **30**, 239–249.

Deeming, D. C. (1989b). Failure to turn eggs during incubation: development of the area vasculosa and embryonic growth. *J. Morph*. **201**, 179–186.

Ferrazzi, E., Pardi, G., Setti, P. L., Rodolfi, M., Civardi, S. and Cerutti, S. (1989). Power spectral analysis of the heart rate of the human fetus at 26 and 36 weeks of gestation. *Clin. Phys. Physiol. Meas*. **10** (Suppl. B), 57–60.

Fleisher, L. A. (1996). Heart rate variability as an assessment of cardiovascular status. *J. Cardiothorac. Vasc. Anesth*. **10**, 659–671.

Groome, L. J., Mooney, D. M., Bentz, L. S. and Singh, K. P. (1994). Spectral analysis of heart rate variability during quiet sleep in normal human fetuses between 36 and 40 weeks of gestation. *Early Human Dev*. **38**, 1–10.

Hammacher, K., Hüter, K. A., Bokelmann, J. and Werners, P. H. (1968). Foetal heart frequency and perinatal conditiion of the foetus and newborn. *Gynaecologia* **166**, 349–360.

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

Lindecrantz, K., Cerutti, S., Civardi, S., Hokegard, K. H., Lilja, H., Rosen, K. G., Signorini, M. G. and Widmark, C. (1993). Power spectrum analysis of the fetal heart rate during noradrenaline infusion and acute hypoxemia in the chronic fetal lamb preparation. *Int. J. Biomed. Comput*. **33**, 199–207.

Moriya, K., Höchel, J., Pearson, J. and Tazawa, H. (1999). Cardiac rhythms in developing chicks. *Comp. Biochem. Physiol*. **124**, 463–470.

Moser, M., Lehofer, M., Sedminek, A., Lux, M., Zapotoczky, H.-

G., Kenner, T. and Noordergraaf, A. (1994). Heart rate variability as a prognostic tool in cardiology: a contribution to the problem from a theoretical point of view. *Circulation* **90**, 1078–1082.

New, D. A. T. (1957). A critical period for the turning of hens' eggs. *J. Embryol. Exp. Morph.* **5**, 293–299.

Pagani, M., Lombardi, F., Guzzetti, S., Furlan, R., Pizzinelli, P., Rimoldi, S., Sandrone, G., Malfatto, G., Dall'Orto, S., Picaluga, E., Turiel, M., Baselli, G., Cerutti, S. and Malliani, A. (1986). Power spectral analysis of a beat-to-beat heart and blood pressure variabilities as a marker of sympathovagal interaction in man and conscious dog. *Circ. Res.* **59**, 178–193.

Pearson, J. T., Haque, M. A., Hou, P.-C. L. and Tazawa, H. (1996). Developmental patterns of O_2 consumption, heart rate and O_2 pulse in unturned eggs. *Respir. Physiol.* **103**, 83–87.

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

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

Pillai, M. and James, D. (1990). The development of fetal heart rate patterns during normal pregnancy. *Obstet. Gynecol.* **76**, 812–816.

Rahn, H., Poturalski, S. A. and Paganelli, C. V. (1990). The acoustocardiogram: a noninvasive method for measuring heart rate of avian embryos *in ovo*. *J. Appl. Physiol.* **69**, 1546–1548.

Sayers, B. McA. (1973). Analysis of heart rate variability. *Ergonomics* **6**, 17–32.

Sorokin, Y., Dierker, L. J., Pillay, S. K., Zador, I. E., Schreiner, M. L. and Rosen, M. G. (1982). The association between fetal heart rate patterns and fetal movements in pregnancies between 20 and 30 week's gestation. *Am. J. Obstet. Gynecol.* **143**, 243–249.

Tazawa, H. (1980). Adverse effect of failure to turn the avian egg on embryo oxygen exchange. *Respir. Physiol.* **41**, 137–142.

Tazawa, H. (1981). Measurement of blood pressure of chick embryo with an implanted needle catheter. *J. Appl. Physiol.* **51**, 1023–1026.

Tazawa, H., Ar, A., Rahn, H. and Piiper, J. (1980). Repetitive and simultaneous sampling from the air cell and blood vessels in the chick embryo. *Respir. Physiol.* **39**, 265–272.

Tazawa, H., Burggren, W. W. and Ar, A. (1999a). Introduction: on the significance of cardiac rhythms. *Comp. Biochem. Physiol.* **124**, 367–368.

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

Tazawa, H., Mitsubayashi, H., Hirata, M., Höchel, J. and Pearson, J. T. (1999b). Cardiac rhythms in chick embryos during hatching. *Comp. Biochem. Physiol.* **124**, 513–523.

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

Tazawa, H., Takami, M., Kobayashi, K., Hasegawa, J. and Ar, A. (1992). Non-invasive determination of heart rate in newly hatched chicks. *Br. Poultry Sci.* **33**, 1111–1118.

Tazawa, H., Wakayama, H., Turner, J. S. and Paganelli, C. V. (1988). Metabolic compensation for gradual cooling in developing chick embryos. *Comp. Biochem. Physiol.* **89**A, 125–129.

Wang, N., Butler, J. P. and Banzett, R. B. (1990). Gas exchange across avian eggshells oscillates in phase with heartbeat. *J. Appl. Physiol.* **69**, 1546–1552.

Wheeler, T. and Murrills, A. (1978). Patterns of fetal heart rate during normal pregnacy. *Br. J. Obstet. Gynaecol.* **85**, 18–27.

Whittow, G. C. and Tazawa, H. (1991). The early development of thermoregulation in birds. *Physiol. Zool.* **64**, 1371–1390.