孵化中の鶏胚における心拍リズム

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Cardiac Rhythms in Chick Embryos During Hatching

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Avian embryos develop within a hard eggshell, which permits the measurement of heart rate while maintaining an adequate gas exchange through the chorioallantoic membrane. Heart rate has been determined from cardiogenic signals detected either noninvasively, semi-invasively or invasively with various transducers. Firstly, we reviewed these previously-developed methods and experimental results on heart rate fluctuations in prenatal embryos. Secondly, we presented new findings on the development of heart rate fluctuations during the last stages of incubation, with emphasis on the perinatal period, which remained to be studied. Three patterns of acceleration of the instantaneous heart rate were unique to the external pipping period: irregular intermittent large accelerations, short-term repeated large accelerations and relatively long-lasting cyclic small accelerations. Besides these acceleration patterns, respiratory arrhythmia, which comprized oscillating patterns with a period of 1-1.5 sec, appeared during the external pipping period. Furthermore, additional oscillating patterns with a period of 10-15 min were found in some externally pipped embryos.

Keywords: Chick embryo, Heart rate fluctuations, Perinatal period, External pipping, Hatching, Noninvasive determination, Catheterization, Autonomic nervous system

1 INTRODUCTION

A hard calcareous porous eggshell is a protective barrier for the avian embryo from bacterial infection and physical changes in the environment and also provides an airway for respiratory gases[57,58]. From the viewpoint of data acquisition, the eggshell is advantageous for measurements of respiratory and circulatory parameters while maintaining adequate gas exchange through the chorioallantoic membrane. The respiratory, circulatory and thermoregulatory functions of late chick embryos within the eggshell have been intensively studied[30,34-36,45,51,55,59]. With regard to embryonic heart rate (HR), various methods and systems have been developed to detect cardiogenic signals through the eggshell. These include electrocardiography, impedance cardiography, ballistocardiography, acoustocardiography, catheterization of allantoic blood vessels and pulse oximetry. Each method has its advantages and disadvantages and should be used individually depending upon the goal of investigations of embryonic HR.

With respect to the instantaneous heart rate (IHR) which is determined by the beat-to-beat intervals of the heart, Akiyama *et al.*[1,3] measured the beat-to-beat fluctuations of HR noninvasively in chick embryos using acoustocardiogram (ACG) and Höchel *et al.*[9] studied the IHR of chick embryos by measuring arterial blood pressure. Following their studies, Moriya *et al.*[17] investigated HR fluctuations after hatching, for continuity of data from embryos to hatchlings. However, measurements of both ACG and arterial blood pressure become difficult toward the end of incubation due to augmented embryonic activities and respiratory movements, which disturb the ACG signal, and due to shrinkage of the allantoic artery, which makes it difficult to implant a catheter. Toward the end of incubation, the embryo pierces the air cell with its beak through the chorioallantoic membrane and the inner shell membrane (which is termed internal pipping and referred to as IP) and thereafter breaks the eggshell with its egg tooth (which is termed external pipping and refered to as EP). During IP and EP period, which is defined as the perinatal (or

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paranatal) period, the embryo begins to breathe air and the gas exchange is switched from the chorioallantoic membrane to the lungs. IHR and HR fluctuations during the last stages of the prenatal and perinatal periods still remain to be investigated.

The present report concerns 1) a brief review of previously developed methods to detect the cardiogenic signals of embryos residing inside the eggshell, and the experimental results from these HR measurements of prenatal embryos, and 2) new findings on embryonic HR fluctuations during the perinatal period.

2 PRENATAL EMBRYOS

2.1 Detection of cardiogenic signals through the eggshell

2.1.1 Electrocardiography. The electrical activities of the heart in chick embryos within an eggshell were measured by Bogue as early as 1932[4]. Two small holes were drilled through the shell and silver electrodes installed on a specially designed manipulator were guided through the holes, piercing the shell membrane without puncturing blood vessels. The electrocardiogram (ECG) was measured by a string galvanometer and the average HR was determined from day 1 to day 19 of incubation and also after hatching. Cain *et al.*[6] also measured ECG with wire electrodes implanted by drilling small two holes in the shell. The HR determined from the ECG measurement was compared with the HR determined by ballistocardiography[6]. In order to prevent water loss through drilled holes, Laughlin *et al.*[14] developed vapor-sealed electrodes with 3 rubber tubes. The rubber tubes were attached to the upper surface of the egg and silver electrodes were pushed into each rubber tube until it penetrated the shell membranes. This technique was used for monitoring HR in a population of chick embryos during the last week of incubation[15], and for investigating the effects of restricted gas exchange through the eggshell on embryonic HR [13].

More simply, Tazawa and Rahn[49] used 26 gauge hypodermic needles as ECG electrodes. The tip of each electrode was bent 3 mm from the end and inserted through the eggshell by pushing it with the thumb, and the bent part was glued to the shell with epoxy to isolate the inside of the egg from the atmosphere. The three electrodes were inserted in a triangular pattern on top of the horizontal egg. Using this technique, Tazawa and Rahn[49] studied the tolerance of chick embryos to low temperature exposure. Thereafter, needle electrodes have been used to measure embryonic ECG in order to compare with other cardiogenic signals[7,43,50] and to determine the HR in small avian eggs[23]. Three thin copper or silver wires were also used as ECG electrodes to measure HR as a reference signal for the acoustocardiogram[25,56].

Pirow *et al.*[24] used gold-plated electrodes, 10 mm long and 1 mm diameter as ECG electrodes. The eggshell in small two areas (10 mm x 2 mm) was removed without damaging the shell membrane, and the tips of the electrodes were inserted between the shell and outer shell membrane. The ECG signal, which was weak and noisy because of disturbance by embryonic movements, was amplified by a factor of 10^4-10^5, band-pass filtered and processed by a computer to achieve on-line long-term recording of embryonic HR in the Muscovy duck[10,24].

2.1.2 Impedance-cardiography. Cardiac contractions and blood ejection by the embryo change intra-embryonic electrical impedance, which can be detected by an impedance converter (model 2992, UFI, California), as has been used in other adult animals. The converter requires two electrodes to supply a carrier signal and detect an amplitude-modulated carrier signal. Haque *et al.*[7] employed the impedance converter to measure the impedance-cardiogram (ICG) and determine the HR of chick embryos, together with electrocardiography, ballistocardiography and acoustocardiography. The same type of needle electrodes as used for ECG were fixed to two points on the egg in the same manner as for ECG. Then, the effects of these different methods on HR were examined for young (12-day-old) and late (16- and 18-day-old) chick embryos. The results indicated that none of these four methods significantly influenced the HR measurement, and that can be used as a simple means for determination of embryonic HR[7].

Because only two thin wires (e.g., copper wire 0.1 mm in diameter) are needed as electrodes, the ICG could be used to determine the embryonic HR of small eggs such as swallows and pigeons[5,53] and the HR of early chick embryos[2]. Taking advantage of the ICG, that allowed HR to be measured remotely from the outside an incubator, Howe *et al.*[11] investigated fixed patterns of bradycardia during late embryonic development in domestic fowl with C locus mutations. The C locus contains the structural gene for tyrosinase, and defects at this site can result in albinism [11].

2.1.3 Ballistocardiography. Cardiac contractions and blood ejection from the heart of the avian embryo are associated with minute movements of the entire egg[26,50]. The ballistic movement imparted by the embryonic heartbeat is designated as the ballistocardiogram (BCG) of the egg. Cain *et al.*[6] reported measurements of BCG in chicken eggs using an ultra-sensitive piezoelectric momentum transducer. Thereafter, several methods and techniques have been developed to measure the egg's BCG. These include measuring systems utilizing an audiocartridge [27,50], laser speckle meter[43], laser displacement meter[8], piezoelectric film[42]

and electromagnetic induction coil[19]. All of these ballistocardiographies measured embryonic HR completely noninvasively during the late period of incubation in domesticated birds[7,20,21,23,44,52], seabirds[46,54] and altricial birds[22,53], under normal incubation conditions and in altered environments. In this way, daily changes (i.e., developmental patterns) of embryonic HR in precocial and altricial birds and their HR responses to altered environments have been elucidated by the BCG method[21,22,23,44,46,53,54].

2.1.4 Acoustocardiography. Rahn *et al.*[25] showed that the gas pressure in a tightly sealed box containing an intact chicken egg oscillates in phase with the ECG. These cardiogenic pressure changes were detected by a condenser microphone[25] or a differential pressure transducer[56], and termed the acousto-cardiogram (ACG). When the microphone was sealed directly and hermetically to the eggshell, it recorded the ACG as the pressure changes in the air space sealed between the eggshell and the surface of the microphone[7,25]. Even from an egg with a fractured shell, the ACG signal could be detected by selecting a suitable measuring position on the eggshell[38]. This finding may support a previously suggested mechanism originating in the pressure oscillation due to temporal pulsatile variations in net gas exchange through the eggshell[56]. The microphone often failed to detect ACG during the last stages of prenatal incubation and perinatal period. It is speculated that blood flow through the chorioallantoic capillaries under the shell covered with the microphone diminishes locally during IP and EP, and thus gas exchange also diminishes locally, or the blood flow is shunted to other areas under the eggshell where gas exchange is not blocked. If the microphone is placed such that it covers an area of the chorioallantoic capillaries where local blood flow remains large enough to maintain gas exchange, ACG can still be detected even during the perinatal period[38].

Acoustocardiography is a noninvasive system and ACG is relatively unaffected by embryonic activities, compared with other cardiogenic signals such as ECG, ICG and BCG. It was successfully used for the continuous measurement of HR in chick embryos during the last half of incubation (ca. 200 hours)[3] and elucidated the occurrence of cyclic changes in HR with periods of 40-90 min, in chick embryos during days 16-18 of incubation[1].

ACG is also relatively free from external noises compared with other cardiogenic signals, which made it possible to measure the embryonic HR during routine operation of commercial incubation in an ostrich hatchery[37,39]. The developmental patterns of embryonic HR in emu were also determined by acoustocardiography[38].

2.1.5 Catheterization of allantoic vessels. In order to obtain blood samples from the chorioallantoic capillaries, a small part of the shell was removed and the syringe needle quickly inserted into the allantoic blood vessel[28,29]. However, after a single sample was obtained from an egg, it had to be discarded. Thus, catheterization of the allantoic blood vessels was developed to obtain samples repeatedly from the same egg without impeding adequate gas exchange through the chorioallantoic membrane and eggshell[33,40,48]. This catheterization technique was then applied to blood pressure measurement[31,32,50] and determination of HR in chick embryos[9,41].

A catheter consisted of a hypodermic needle 15 mm long (24 gauge for the allantoic vein and 26 gauge for the artery) and polyethylene tube 5 cm long. The needle was bent at a right angle 2-3 mm from the tip, and the other end of the needle was inserted into the polyethylene tube. The catheter was filled with heparin solution and the free end of the tube was plugged with clay. A small area, marked previously on the shell through candling (less than 1 cm^2), was removed together with the shell membranes. The allantoic vessel was gently lifted by forceps from the allantoic fluid through a tear in the chorioallantoic membrane. The tip of the catheter was inserted into the vessel, pointing upstream. After repositioning the catheterized vessel in the allantoic fluid, the catheter was fixed to the edge of the hole in the shell, and the removed area was re-covered with tape and epoxy. For measurement of blood pressure, the polyethylene tube emerging from the egg was connected to a strain gauge manometer through another polyethylene tube (8 cm long) filled with saline solution. Mean and instantaneous HR were calculated from the blood pressure waves[9,41]. As the blood pressure was free from the artefacts, which occasionally interfered with ACG waves, the measurement of arterial blood pressure was made as a complementary method to ACG to determine the instantaneous HR and substantiate the HR irregularity patterns in prenatal chick embryos[9].

2.1.6 Pulse oximetry. Taking advantage of the pulse oximeter, which is conventionally used to detect pulsatile changes in oxygen saturation of blood passing through the tongue or the finger in domesticated animals or human beings, Lewin *et al.*[16] examined its applicability to the determination of the HR of chick embryos. Two holes of 6 by 8 mm each were made in the eggshell 1 cm apart without damaging the outer shell membrane. Then, two probes of the oximeter; a light source and a photodetector, were fastened on the eggshell edges around the holes with an adhesive tape. The oxygen saturation curve, which was nearly sinusoidal in shape and synchronous with the ECG, could be detected from 18-day-old embryos.

The mean HR was determined by power spectral density analysis. Pulse oximetry will open opportunities for simultaneous measurements of HR and blood oxygen saturation in developing avian embryos in normoxic and also hypoxic and hyperoxic environments.

2.2 Developmental patterns of embryonic heart rate during incubation

Because of easy availability, chicken eggs have been well studied for the daily changes in mean HR (developmental patterns) by measuring the ECG (from day 1 to day 19 by Bogue[4]; from day 15 to day 21 by Laughlin *et al.*[13,15]; from day 6 to day 20 by Tazawa and Rahn[49]), the ICG (from day 3 to day 9 by Akiyama *et al.*[2]; from day 7 to day 20 by Howe *et al.*[11]), the BCG (from day 4 to day 19 by Cain *et al.*[6]; from day 11 to day 21 by Tazawa *et al.*[44]; from day 12 to day 21 by Pearson *et al.*[21]), the ACG (from day 12 to day 20 by Akiyama *et al.*[1]) and blood pressure (from day 13 to day 17 by Tazawa[32]; from day 14 to day 16 by Tazawa[31]; from day 12 to day 18 by Tazawa and Nakagawa[47]; from day 10 to day 20 by Höchel *et al.*[9]).

These various methods have also elucidated the developmental patterns of embryonic HR in other domesticated birds: king quail[23], Japanese quail, duck, turkey, peafowl and goose[44], emu[38] and ostrich[37,39], seabirds: brown noddy[46], wedge-tailed shearwater and Laysan albatross[54], and altricial birds: bank swallow and pigeons[5,53], and great tit, marsh tit, varied tit, Japanese tree sparrow, house martin, Japanese bunting, red-cheeked myna, brown-eared bulbul, cockatiel, zebra finch, Bengalese finch, carrion and jungle crows[23].

Despite many studies on developmental patterns of embryonic HR during the late stages of incubation, the pattern early in incubation still remains to be investigated in many species of birds. In general, however, the embryonic HR steadily increases during the early period of incubation when the primordial tubular heart forms the four-chamber configuration, followed by relatively slow changes in HR during the late incubation period. There are following three gross developmental patterns of HR late in incubation including the pipping period. 1) Embryonic HR tends to decrease toward the end of prenatal incubation with subsequent increase during the perinatal period, which is observed in many precocial birds. 2) Embryonic HR tends to be kept with a narrow range until pulmonary respiration is initiated as observed in semi-precocial seabirds. The perinatal period of the seabirds is generally long in comparison with that of the precocial birds, and the HR tends to increase or remains unchanged depending upon species during the pipping period. 3) Embryonic HR still continues to increase toward hatching and becomes maximal during the pipping period, which is observed in many altricial birds. These developmental patterns of late embryonic HR may mainly reflect the changing metabolic requirements toward hatching and be related to embryonic growth rate[22].

2.3 Heart rate fluctuations in prenatal embryos

Continuous measurements of HR in chick embryos throughout the last half of incubation by acoustocardiography revealed that the baseline HR began to oscillate on day 16-17 of incubation with subsequent augmentation on day 18[1]. The period of the cyclic oscillations in the baseline HR was variable, ranging from about 40 min to 90 min depending upon individual embryos. The magnitude of the oscillations was also variable and exceeded 50 bpm in some embryos. In a few embryos, the HR oscillations were vague. Because the recording of the ACG was disrupted by embryonic activities and respiratory movements toward the pipping period, the HR oscillations during the last stages of embryonic development, and their mechanism, remain to be studied.

Noninvasive measurement of instantaneous heart rate (IHR) in developing chick embryos by taking advantage of ACG also showed that the embryonic HR began to change irregularly on day 13-14 of incubation and the heart rate irregularities (HRI) became augmented with embryonic development[1,3]. Höchel *et al.*[9] studied in more detail the development of HRI in chick embryos during the last half of prenatal incubation by the catheterization technique. Figure 1 shows examples of IHR in 6 embryos aged from 11 days to 16 days, determined by blood pressure measurement[9]. On day 11 of incubation, changes in IHR were small and baseline HR was stable. On the following day, baseline HR tended to fluctuate, but no particular pattern of HRI appeared. On day 13, small rapid deceleration patterns of IHR appeared a few times during the 30-min recordings. On the following day, the spontaneous rapid deceleration patterns appeared with increasing frequency and magnitude. Subsequently, the HRI were augmented by additional irregular patterns during the last stages of prenatal development[9].

2.4 Effect of the autonomic nervous system on HRI

Höchel *et al.*[9] also investigated the effects of administration of autonomic drugs on the HR fluctuations in prenatal chick embryos. In addition to the arterial catheter, they implanted another catheter into the allantoic vein in 14- to 17-day-old embryos. Arterial blood pressure was measured in the embryos during the first 30 min to obtain control IHR and during the next one hour after administration of a drug through the venous catheter. The drugs which were examined for their effects on the HRI were acetylcholine (Ach, parasympathomimetic drug), atropine (parasympathetic blocking agent), nor-

epinephrine and isoprenaline (sympathomimetic drugs), phentolamine (.-adrenergic blocking agent) and propranolol (.-adrenergic blocking agent).

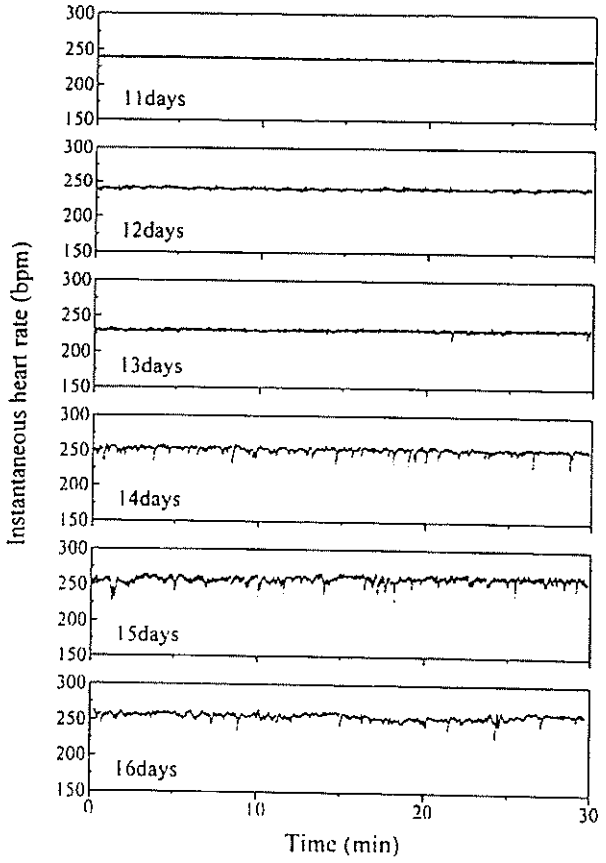

Fig. 1. Instantaneous heart rate determined from arterial blood pressure of prenatal embryos aged from 11 days to 16 days. The heart rate (HR) baseline was stable on days 11-12 of incubation, began to decelerate transiently on days 13-14, and fluctuated with irregular changes in HR afterwards. The transient decelerations of HR were dominant on days 14-16.

Figure 2 presents examples of IHR responses to administration of autonomic drugs. Figure 2A shows the IHR of a 16-day-old embryo before and after administration of atropine. The rapid, transient decelerations of IHR that appeared frequently during the 30-min period before administration were blocked by atropine and the baseline HR was elevated. When embryos were given Ach, the heartbeat stopped with subsequent swift recovery to the original baseline HR. In other embryos whose heartbeat was reduced by Ach, the HR reached a nadir within a few heartbeats, with subsequent recovery to the original baseline, resembling the rapid, transient deceleration patterns. It is likely that the first occurrence of deceleration patterns of IHR corresponds to functional initiation of the parasympathetic innervation, and the rapid, transient deceleration of embryonic HR is mediated by the parasympathetic nervous system.

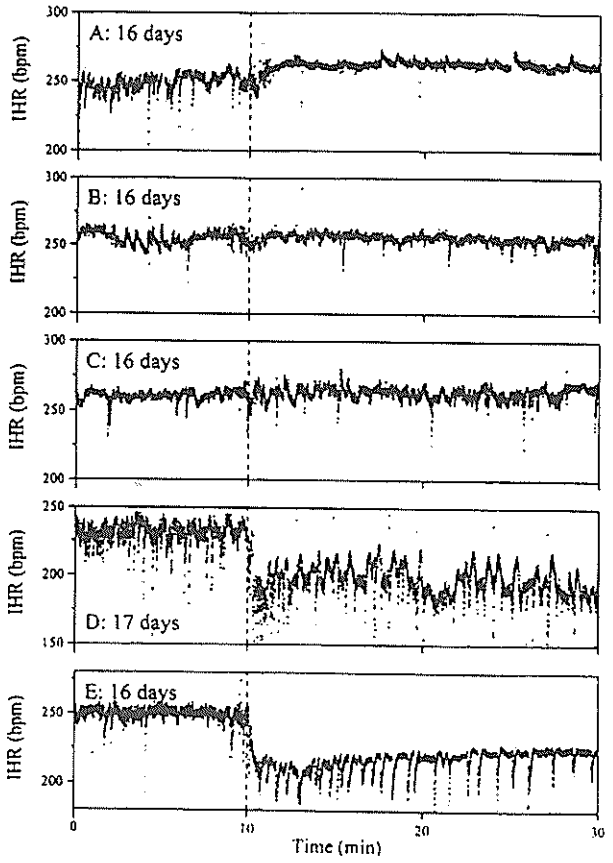

Fig. 2. Instantaneous heart rate (IHR) determined before and after administration of autonomic drugs. The first 10-min is control period and then a drug was infused through the venous catheter at the dotted line.

The IHR did not always clearly respond to administration of sympathomimetic and sympathetic blocking agents. Figure 2B shows the IHR of a 16-day-old embryo, which was given norepinephrine. Significant changes in the IHR did not occur, as in other embryos examined, except for one 14-day-old embryo, which responded to norepinephrine with a raised baseline HR, but without the accompanying marked HRI patterns[9]. Figure 2C shows the IHR of a 16-day-old embryo, which was treated with isoprenaline; the response was slight. In another 17-day-old embryo, rapid acceleration of HR occurred just after drug administration, with subsequent raising of the baseline HR[9]. However, remaining embryos treated with isoprenaline failed to show marked changes in the HRI patterns. Figure 2D shows the IHR of a 17-day-old embryo, which was given phentolamine. The baseline HR dropped by about 70 beats/min soon after administration of phentolamine and the HRI were augmented by spontaneous acceleration patterns. In another 16-day-old embryo, the baseline HR dropped transiently (about 5 min) and in a

shallow fashion (about 20 beats/min) soon after phentolamine administration, accompanied by rapid, transient deceleration patterns of HR. However, these changes in HRI did not occur in the remaining embryos examined with phentolamine. Figure 2E shows the IHR of a 16-day-old embryo given propranolol. The baseline HR was depressed, and rapid, transient decelerations of IHR occurred frequently. Baseline HR was depressed in two other embryos without accompanying marked changes in HRI patterns. In one embryo, the marked, rapid accelerations of HR which occurred intermittently during the 30-min control period did not occur after propranolol administration.

Although the responses of IHR to sympathetic drugs were not always clear, in some embryos in which acceleration patterns occurred, the pattern disappeared after administration of sympathetic blocking agents. However, it still remains to be investigated whether the accelerated HRI coincide with the initiation of sympathetic nervous function and whether the acceleration patterns are mediated by the sympathetic nervous system.

3 INSTANTANEOUS HEART RATE IN PERINATAL EMBRYOS

3.1 Materials and methods

Fertile eggs of broiler chickens were incubated at a temperature of 38°C and relative humidity of about 60% in a forced draft incubator. The eggs were turned automatically every hour until day 16 of incubation when they were transferred to a still air incubator at 38°C, which was used as a measuring chamber. Measurements of IHR were made in embryos aged from 17 days to 21 days, to make a continuous study of development of HRI in prenatal and perinatal embryos. On the morning of the experiment, eggs were candled to check whether they had pipped the chorioallantoic membrane and pierced the air cell with their beaks (i.e., internal pipping).

IHR was determined by electrocardiography. Electrodes were 3 silver rods, 3 cm long and 1 mm across. One end of the rod was filed to make a flat surface, and at the other end was soldered a small connector. Three locations were marked on the eggshell so that they made a triangle about 2 cm apart. Then, a tiny piece of the eggshell (<2 mm across) was removed by the sharp blow of a 20 gauge hypodermic needle and the tip of the needle pierced the shell membranes and the chorioallantoic membrane to make a hole. The flat end of the electrode was pushed into the hole with the gentle, steady force of the thumb and forefinger, until it reached the body of the embryo. Then the electrode was attached to the eggshell with clay and epoxy glue, and the egg was placed back in the incubator. The electrodes were connected to the lead wires of a polygraph amplifier through the connectors. IHR was determined from the amplified ECG signals with the aid of a computer, as described elsewhere[17].

3.2 Heart rate fluctuations during the last stages of incubation

Figure 3 presents examples of 30-min recordings of the IHR determined from 6 embryos. The upper 3 embryos, aged 17, 18 and 19 days, had not pipped the chorioallantoic membrane; thus they were prenatal embryos. The lower 3 recordings were taken from perinatal embryos; one had pipped the chorioallantoic membrane and pierced the air cell with the beak (IP), and two others had pipped the eggshell with the egg tooth (EP). Figures 1 and 3 show continous development of HRI during the last half of incubation, including the perinatal period. Comparison of the two figures clearly shows that the HR fluctuations increased with embryonic development, particularly toward the end of incubation, with addition of augmented acceleration patterns. The frequency and magnitude of rapid, transient deceleration patterns also tended to increase during the last stages of the prenatal period up to the perinatal period. One externally pipped embryo showed a series of deceleration patterns continuing throughout a 30-min recording period (5th panel in Fig. 3). However, such continuous occurrence of deceleration patterns was not common in many other perinatal embryos. Rather, the frequency of appearance of deceleration patterns tended to decrease, and instead, acceleration patterns of HRI were augmented during the EP period, as shown in the bottom panel of Figure 3. The perinatal HR fluctuations were characterized by the intermittent appearance of repeated patterns of large accelerations continuing for 4-5 min (referred to as 'short-term repeated large accelerations'). In addition, respiratory arrhythmia began to appear during the EP period and was augmented when the baseline HR was decreased (Fig. 3).

Figure 4 shows other examples of HR fluctuations in an externally pipped embryo. This perinatal embryo was measured for IHR for 8 consecutive hours on day 20 of incubation. Ten-min recordings of IHR were intermittently sampled from the 8-hour recording to show the development of various patterns of HR fluctuations. The first 3 panels show the HRI patterns which were frequently observed even in prenatal embryos, except for the occurrence of respiratory arrhythmia. The acceleration and deceleration patterns recorded in the first 3 panels are similar to those in prenatal embryos, which are shown in Figure 3. Respiratory arrhythmia appeared during the first 10-min recording (top panel) and became augmented with the lapse of time and a fall in the baseline HR during the next 3 hours (2nd and 3rd panels). The acceleration patterns tended to be intermittently cyclic. Two

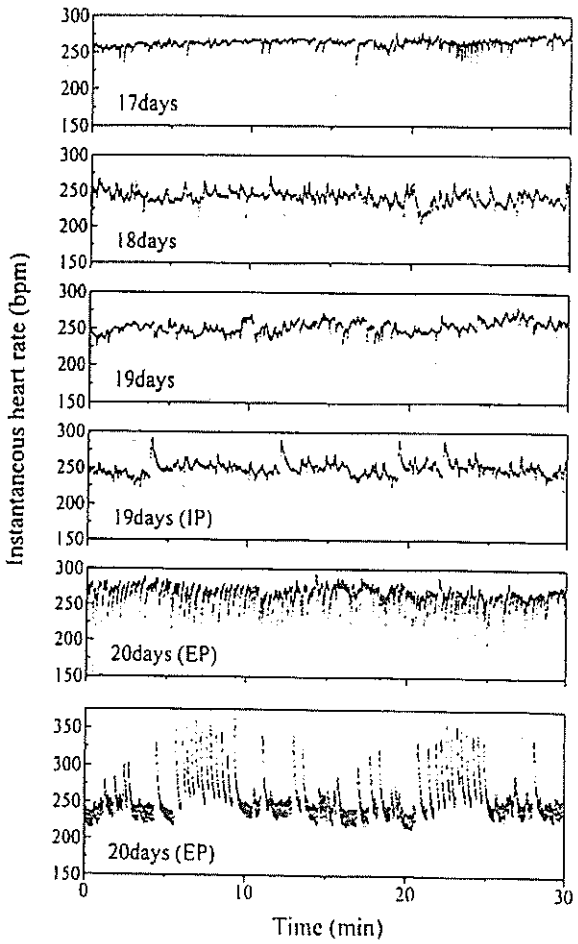

Fig. 3. Instantaneous heart rate determined from the electrocardiogram of the prenatal (the first three panels) and the perinatal (other three panels) embryos. IP and EP are internally pipped and externally pipped (perinatal) embryos, respectively. The transient heart rate (HR) decelerations were still evident on day 17, and subsequent acceleration patterns on and after day 18 augmented further the HR fluctuations. During the external pipping period, the accelerated patterns were predominant (bottom panel), but exceptionally the rapid, transient decelerations occurred repeatedly (2nd panel from the bottom). The widespread baseline in the bottom panel was due to respiratory arrhythmia of the externally pipped embryo.

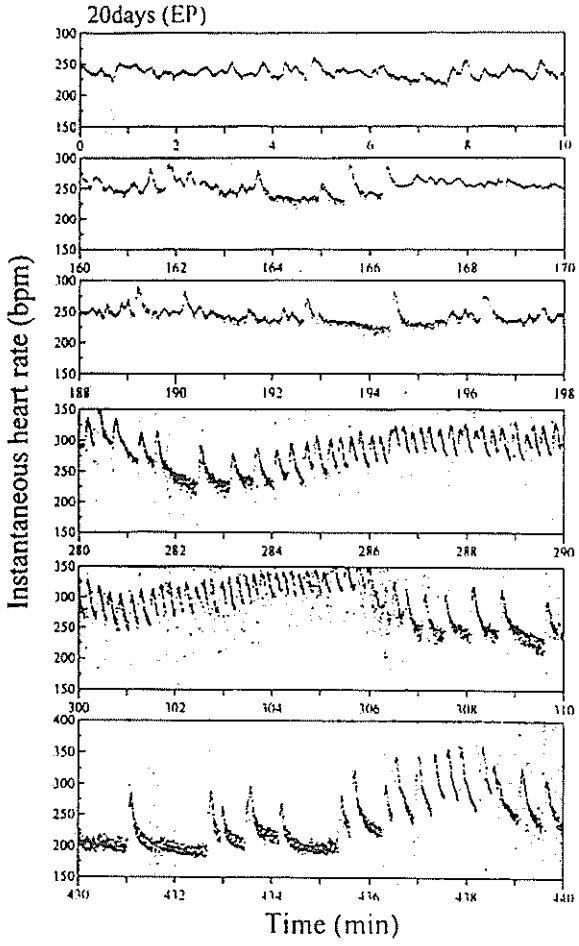

Fig. 4. Instantaneous heart rate of a 20-day-old externally pipped embryo determined from the electrocardiogram. Measurements were made for 8 consecutive hours, and six 10-min recordings were sampled to show transitions of various acceleration patterns. The first three panels show the acceleration patterns also observed in prenatal embryos, and also episodes of respiratory arrhythmia; e.g., widespread baseline during a recording between 6-8 min in the top panel, between 164-166 min in the second panel and between 191-197 min in the third panel. The last three panels show transitions in three acceleration patterns.

patterns of cyclic, accelerated HR fluctuations appeared with different frequencies and durations; one with relatively high frequency (ca. 5 times per min) and low magnitude, lasting for a relatively long period (ca. 20 min), as shown in the 4th and 5th panels in Figure 4 (referred to as 'relatively long-lasting cyclic small accelerations), and the other is of relatively low frequency (ca. 2-3 times per min) and large magnitude, lasting for a short period (ca. 4-5 min), as shown in the bottom panel in Figure 4. The latter HR fluctuation is the same acceleration pattern as shown in the bottom panel in Figure 3.

Figure 5 shows that the cyclic fluctuations of perinatal embryonic HR sometimes occur with a long period, over several hours (ca. 12 min in top panel), medium period (ca. 20 sec in middle panel), or short period (ca. 1.5 sec in bottom panel), indicating a fractal-like phenomenon in the HR fluctuations during hatching. The cyclic HR fluctuations shown in the bottom panel in Figure 5 were frequently observed in newly hatched chicks, which are known as respiratory arrhythmia[17].

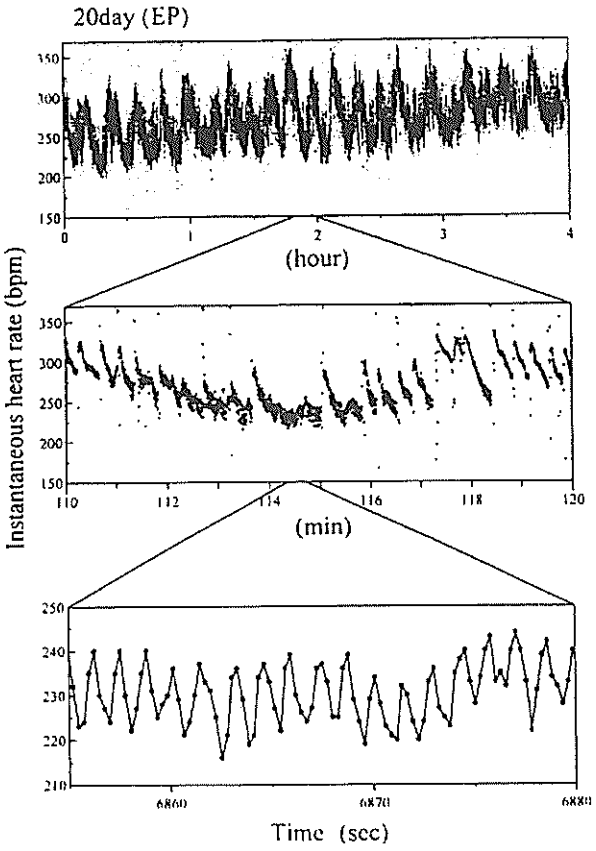

Fig. 5. Continuous recording of instantaneous heart rate for a 4 hour-period in a 20-day-old externally pipped embryo, showing three cyclic oscillations. Top panel presents continuous 4-hour recording. Second panel shows a 10-min recording extracted from the top panel at around 2 hours. Bottom panel is a 25-sec recording extracted from the second panel at around 114.5 min. The cyclic oscillations shown in the bottom panel are known as respiratory arrhythmia, but the mechanisms of the other oscillations are not known.

4 DISCUSSION

Avian embryos develop within the confines of the eggshell independent of the maternal physiological functions unlike the mammalian fetuses. The eggshell separates the embryo from the environment, and a partial removal of the eggshell makes an easy access to the early embryo. Thus the early chick embryo is used as a favorable experimental model in many pharmacological and physiological studies, particularly in development of cardiovascular functions[12,18]. Meanwhile, from a viewpoint of embryonic gas exchange, the hard porous eggshell provides an airway for respiratory gases between ambient air and capillary blood in the chorioallantoic membrane. Thus the eggshell plays an important role in the gas exchange of the embryo and also provides a unique opportunity for noninvasive measurements of embryonic HR. The embryonic HR can be measured without impeding adequate gas exchange through the eggshell and the chorioallantoic membrane. Cardiac contractions of the embryo produce ballistic movements of the eggshell, which can be detected by various means as the BCG [6,8,19,26,27,42,43,50]. The developmental patterns of embryonic mean HR in various species of birds have been investigated nonivasively by taking advantage of the BCG[5,6,21,22,23,44,46,53,54]. Additionally, in association with cardiac contractions, acoustic pressure changes occur outside the eggshell, which can be detected by a condenser microphone attached hermetically on the eggshell as ACG[25,55]. The ACG is less contaminated by the embryonic activities compared with the BCG[7]. Akiyama *et al.*[1,3] measured the HR of chick embryos noninvasively throughout the last half of incubation and noted the HRI and cyclic changes in HR during the last stages of prenatal development. Toward the end of prenatal incubation and the initiation of internal pipping, embryonic activity and respiratory movements are augmented and the microphone detects these non-cardiogenic movements. Detection of the respiratory movements of prepipping and perinatal embryos provides an additional new possibility for investigation of the ontogeny of pulmonary respiration in relation to changes in cardiac rhythms in late embryos. Both the BCG and the ACG can be detected sometimes even during the perinatal period when the embryos are quiescent, provided an adequate position for detection is found on the eggshell for placement of the transducers[38,44,46,54]. However, neither method can detect the cardiogenic signals from early embryos because the BCG and ACG signals are weak or are not produced during the early incubation period. Alternatively, the ECG and ICG can be detected from early embryos, although the implantation of electrodes injure, albeit minutely, the eggshell[2,11,23]. In order to minimize injury to the egg, the electrodes are inserted just under the chorioallantoic membrane or placed on the outer shell membrane[5,6,7,11,14,15,21-24,49]. Because the electrodes do not contact directly the body of embryo, the ECG and ICG signals are also disturbed by embryonic activities as embryos grow. Although the computer-aided signal processing technique partially solves the problem produced by the embryonic activities, the ECG and ICG methods work comparatively well during the early period of incubation when embryonic activities are small and less frequent[2].

Although the eggshell is partially removed and the procedure is invasive, catheterization of the allantoic blood vessels makes it possible to measure the blood pressure and IHR relatively free from the embryonic activities[32,41]. Taking advantage of catheterization, which can be made through a

small hole opened in the eggshell, Höchel *et al.*[9] determined the IHR during prenatal development and confirmed the previous findings by the ACG method made by Akiyama *et al.*[1,3]. The baseline HR, which was stable until day 12-13 of incubation, began to become irregular with transient, rapid decelerations of HR on day 13-14 and a subsequent increase in their frequency and magnitude. The acceleration patterns appeared on day 15-16 and IHR became more and more irregular with additional, spontaneous deceleration and acceleration patterns toward the end of prenatal development.

New experimental data were included in the present report in order to elucidate further the development of HR fluctuations from the last stages of the prenatal period to the internal and external pipping (perinatal) period. The measurement of IHR was made by the ECG method, but it differed from the previously used method with respect to the implantation of the electrodes. In the previous method the electrodes were implanted just under the choriallantoic membrane or on the outer shell membrane; in the measurement described in this report they were inserted into the egg until they touched the body of the embryo. Although the invasion of the electrodes was large relative to the previous methods, the ECG was measured directly from the embryo and the IHR could be counted from the R-R intervals of the ECG recordings relatively free from embryonic activity

The HR fluctuations may be referred to as heart rate variability (HRV) and heart rate irregularities (HRI), depending on the magnitude of the changes and their periodicity (cyclic or non-cyclic). In general, HRV is a fluctuation which tends to be oscillating and cyclic, while HRI are large fluctuations, comprising irregular, brief decelerated and/or accelerated HR. As found previously by the catheterization method[9], the HRI were further augmented by additional occurrences of the HR acceleration on day 18-19 (Fig. 3). The rapid increase in IHR within a few heart beats, followed by a slower recovery to the baseline, which was found in the late prenatal period, was also recorded during the internal pipping period in the present measurement (the fourth panel in Fig. 3). The HRI patterns observed in the pre-pipping period remained in the internal pipping period. In contrast, unique patterns of HR fluctuations began to appear during the external pipping period. Although the HR deceleration patterns occurring continuously for a 30-min recording period, as shown in the fifth panel of Fig. 3, were exceptional and occasional, the large acceleration patterns occurred intermittently and frequently in externally pipped embryos (bottom panel in Fig. 3). Unique patterns of HRI were irregular intermittent large accelerations; the increase in IHR extended to no less than 100 bpm within a few heartbeats and the HR returned swiftly to the baseline. Occasionally, the HR accelerations repeatedly occurred prior to the recovery of the baseline and became cyclic for a period of a few min, consisting of short-term repeated large accelerations, which are categorized as HRV (bottom panels of Fig. 3 and Fig. 4). In addition to these (single or repeated) large acceleration patterns, another cyclic acceleration pattern occurred in externally pipped embryos (4th-5th panels in Fig. 4). The acceleration of HR was about 50 bpm with a period of 12-15 sec, continuing for various periods: e.g., no less than 20 min in Fig. 4 -relatively long-term cyclic small accelerations. Such cyclic acceleration patterns were not observed in the prenatal embryos, but frequently recorded in newly hatched chicks[17]. Although the rapid deceleration of HR was believed to be caused by parasympathetic nerve activity, the relation between HR accelerations and sympathetic nerve activity has not been clearly shown. Further investigations using various autonomic drugs will elucidate the mechanisms for the various patterns of HR accelerations.

Another unique characteristic of the IHR in externally pipped embryos is the appearance of respiratory arrhythmia, which was recorded as widespread baseline (Figs. 3, 4 and 5). In newly hatched chicks[17], the IHR changed with breathing and the respiratory arrhythmia was recorded as widespread HR baseline as in Figures 3, 4 and 5. Although the gas exchange of externally pipped embryos switches from a diffusive process through the chorioallantoic membrane to a convective process by the lungs, it is interesting to note that the IHR is already subject to the breathing movements of the lungs.

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孵化中の鶏胚における心拍リズム

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概要

鳥類胚は堅い卵殻の中で成長し、このことは絨毛尿膜によるガス交換を適正に保って心拍数を計測することを可能としている。心拍数は、種々の変換器を用いて心起因性信号を無侵襲、半侵襲あるいは侵襲法で検出し、計測されてきた。最初に、これらの方法と嘴打ち前の胚について明らかにされてきた心拍ゆらぎに関する実験結果について述べる。次いで、これまでには明らかにされなかった孵卵後期、特に嘴打ち中の心拍ゆらぎの発達について新たな計測結果を基に述べる。外嘴打ち中には瞬時心拍リズムに三つの頻脈パターンが特異的に現れる；不規則で間欠的に起こる大きな頻脈、短期間に繰り返し起こる大きな頻脈及び比較的長い期間持続する周期性の小さい頻脈である。これらの頻脈パターン以外にも、1-1.5 秒の周期を持つ別の振動パターンも幾つかの外嘴打ち胚に見いだされている。

キーワード：ニワトリ胚、心拍ゆらぎ、孵化近期、外嘴打ち、孵化、無侵襲測定、カテーテル法、自律神経機能

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