Embryonic Heart Rate in Altricial Birds, the Pigeon (*Columba domestica*) and the Bank Swallow (*Riparia riparia*)

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Abstract

Ballistocardiography or impedance cardiography was used to measure on a daily basis the heart rate (f_H) of embryos of altricial pigeons (Columba domestica) *and bank swallows* (Riparia riparia). *The patterns of* f_H *changes during development were investigated. Embryonic* f_H *of domestic and homing pigeons increases slowly during the middle stages of development and then increases sharply toward the end of incubation. Fantail pigeons show a different pattern of embryonic* f_H *change; that is,* f_H *remains constant until 1–2 d prior to external pipping and increases thereafter. Heart rate of the bank swallow increases continually throughout the last half of the incubation period. The developmental patterns of* f_H *changes in pigeons and swallows generally are different from those of most precocial birds, in which embryonic* f_H *decreases during the last stages of prepipping development.*

Introduction

The patterns of change in many physiological variables during embryonic development differ between precocial and altricial bird species. Unlike oxygen consumption in altricial birds, for example, oxygen consumption in precocial birds reaches a plateau value before pipping (Vleck, Hoyt, and Vleck 1979). Growth patterns differ similarly between these groups. Reflecting these differences in growth and oxygen consumption and differences

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in thyroid development (McNabb 1987), the ontogenetic development of thermoregulatory capacity is also dissimilar in precocial and altricial bird species (Kuroda et al. 1990; Whittow and Tazawa 1991).

Although differences in embryonic oxygen consumption between altricial and precocial birds could be related to differences in development of allantoic blood flow, no studies have compared circulatory variables in precocial and altricial species. Indeed, almost all cardiovascular studies seem to have focused on precocial birds. For example, stroke volume, arterial blood pressure, and heart rate (f_H) have been measured during embryonic development of the domestic fowl (Hughes 1949; Cain, Abbott, and Rogallo 1967; Van Mierop and Bertuch 1967; Girard 1973; Laughlin, Lundy, and Tait 1976; Tazawa 1981; Tazawa and Nakagawa 1985; Clark 1991; Tazawa et al. 1991*a*). Only f_H has been measured for the embryos of semiprecocial seabirds until shortly before hatching (Tazawa, Kuroda, and Whittow 1991*b*; Tazawa and Whittow 1994).

The present experiments investigate the developmental pattern of f_H changes in two species of altricial birds to allow comparison with that of precocial birds. We report on daily changes in embryonic f_H of the pigeon, which lays two eggs per clutch, and the bank swallow, which lays four to five eggs per clutch.

Material and Methods

Acquisition of Eggs

Eggs were collected at a variety of sites in both Japan and the United States. They were brought to a laboratory of the Muroran Institute of Technology or the University of Massachusetts—Amherst for incubation and f_H measurements.

1. *Fantail Pigeon (*Columba domestica*).* Six eggs were acquired from a local zoo in Sapporo in June 1989. The eggs were collected on the day they were laid, but were not identified as to which clutches they originated from.

2. *Domestic Pigeon (*Columba domestica*).* Eight eggs of domestic pigeons were collected from four nests in a farmyard near Amherst, Massachusetts, in June 1990 (designated group 1). Because the day the eggs were laid was not known, the date of collection was penciled on the eggshell instead. Heart rate data from all eggs were then compared by plotting them against the number of days prior to external pipping (EP). Sibling eggs were identified. Another eight eggs were also taken from four nests from a clock tower in Amherst, Massachusetts, in June 1991 (designated group 2).

3. *Homing Pigeon (*Columba domestica*).* Twelve eggs of homing pigeons were supplied by a local pigeon rearer in western Massachusetts in June 1991. They were removed from the nests while birds were incubating them, so sibling eggs could be identified. The age of eggs at the time of removal was not known, but, as for the fantail pigeons, f_H data from all eggs were compared by plotting them against the number of days prior to EP.

4. *Bank Swallow (*Riparia riparia*).* The eggs of bank swallows were collected from their burrow nests in cliffs along the Connecticut River (western Massachusetts) in June 1991. Thirty-nine eggs in total were collected from the nine nests. Sibling eggs were identified. The age of the eggs was not known, but f_H was plotted and compared as described previously for the other bird groups.

Incubation

Egg mass (measured to 0.01 g) was measured immediately after eggs were brought to the laboratories. Eggs were then put in still-air incubators maintained at 38°C. Because the development of embryos could be influenced by the lack of turning during incubation, as observed in the domestic fowl (Tazawa 1980; Tullett and Deeming 1987), all eggs were turned manually on a schedule appropriate for measurements in individual groups. The fantail pigeon eggs were turned regularly three times a day until day 10 of incubation, when the measurement of f_H was begun. These eggs and those of domestic pigeons (group 1), whose f_H was measured by ballistocardiography (see below), were then turned at each daily f_H measurement. The eggs of other birds were turned twice a day before and after f_H measurement by impedance cardiography (see below).

Measurements of f_H

The f_H of individual embryos during incubation was determined with either ballistocardiography or impedance cardiography. Measurements were made at 38°C inside the incubator.

1. *Ballistocardiography.* An egg moves very slightly but rhythmically because of heartbeats of the embryo confined within the eggshell. This cardiogenic ballistic movement of the egg (referred to as a ballistocardiogram, or BCG) can be detected by various means (Cain et al. 1967; Suzuki, Musashi, and Tazawa 1989; Tazawa, Suzuki, and Musashi 1989*b;* Tazawa et al. 1989*a;* Hashimoto, Narita, and Tazawa 1991; Tazawa et al. 1993). We used an au-

diocartridge measuring system (Suzuki et al. 1989; Tazawa et al. 1989*b*), as was used previously for measuring the embryonic f_H of domesticated birds (Tazawa et al. 1991*a*). In brief, the incubator was provided with a floating platform on which the audiocartridge was installed. The platform was used to attenuate vibrations coming from the outside of the incubator. The egg, handled with a gloved hand, was put on the platform so as to contact a stylus pin of the cartridge. The position of the stylus pin on the eggshell was then adjusted, with care taken not to jostle the egg, in case the change of position was necessitated to provide an optimal signal. After these procedures, the door of the incubator was closed and measurement of BCG was begun at least 1 h after door closure so that the eggs would be thermally equilibrated at the incubation temperature. Ballistocardiography was used to determine f_H of fantail pigeon and domestic pigeon eggs (group 1).

2. *Impedance Cardiography.* While ballistocardiography provides complete noninvasive measurements of embryonic f_H, it required a subsequent period for thermal reequilibration after setting up the egg and also necessitated moving the eggs to make a measurement. In order to expedite f_H measurements from many eggs in a single day, we used the more invasive (but less time-consuming) technique of impedance cardiography. This technique detects changes in electric impedance of the embryo produced by cardiac contraction and blood ejection (impedance cardiogram, or ICG). Two thin copper wires, 0.1 mm and 0.3 mm in diameter for swallow eggs and pigeon eggs, respectively, were introduced into an egg through a hole in its shell produced with the tip of a 26-gauge needle. After inserting 1.0 mm of the wire into the hole, the hole was closed by a small droplet of cyanoacrylate glue. All eggs were wired on the day of collection and were placed in the incubator. Each pair of electrode wires, which was 70 cm long, was labeled to identify individual eggs. The free ends of all electrode wire pairs were led out past the closed door of the incubator. To measure f_H, an electrode wire pair was connected to an impedance converter (model 2992, UFI, Morro Bay, Calif.), which detected the ICG. The impedance method for f_H measurement did not require touching the eggs or even opening the door of the incubator. Eggs were handled only during egg turning, as described above.

3. *Calculation of* f_H. Individual bird embryos typically show a large variability of f_H (Tazawa et al. 1991*a*, 1991*b*). In order to determine a representative value of average daily f_H, it is necessary to follow a data-sampling regime that prevents sampling bias as much as possible. Because there will necessarily be an arbitrary aspect to establishing time periods for measurement,

the procedure for determination of f_H from the BCG or ICG was basically that of Tazawa et al. (1991*a*) for the domesticated fowl. In this procedure, recordings of BCG or ICG were made for 30 s, every 5 min over a 30-min period each day. The 30-s recording was divided arbitrarily into 4-s intervals, which would typically include about 15–18 heartbeats. The average f_H for the approximate 4-s period was then determined and referred to as "4-s average f_H." All the 4-s average f_H's counted over the 30-min period were then averaged to give a mean f_H of the embryo for that day of development.

Diurnal rhythms may exist in embryonic f_H, and to avoid possible introduction of additional variance due to such rhythms, f_H measurements were made at the same time each day for each egg.

External pipping and hatching did not necessarily occur at the same time in siblings. As described above, the averaged f_H of individual embryos was plotted against the number of days prior to EP so that the daily changes in f_H could be chronologically coincident for all the embryos.

Statistical Analysis

Two levels of statistical analysis were employed. In preliminary experiments to assess the nature of the variability in f_H within a single individual, the significance of f_H differences measured over time in a single bird was assessed by a one-way ANOVA for repeated measures. Subsequently, mean daily f_H values from groups of birds were analyzed and compared by ANOVA for repeated measures. A significance level of $P < 0.05$ was adopted as a fiduciary limit.

Results

Variability of Embryonic f_H

Figure 1 presents an f_H recording of an embryonic domestic pigeon and shows an example of variability of f_H representative of the species and within-species groups investigated in this study. Individual points in this 60-min recording of f_H show the 4-s average f_H. Somatic activity or posture changes of the embryo, which interfered with BCG, made it impossible at times to determine f_H, which accounts for the small gaps in this recording.

The mean value (± 1 SD) of 4-s average f_H's, counted from 30-s recordings taken every 5 min over the 30 min period, was 263 ± 14 (from seven measurements of the 4-s average f_H), 255 ± 5 (from six measurements), 250 ± 6 (from seven measurements), 253 ± 7 (from seven measurements), 265 ± 6 (from four measurements), and 267 ± 8 (from five measurements) beats

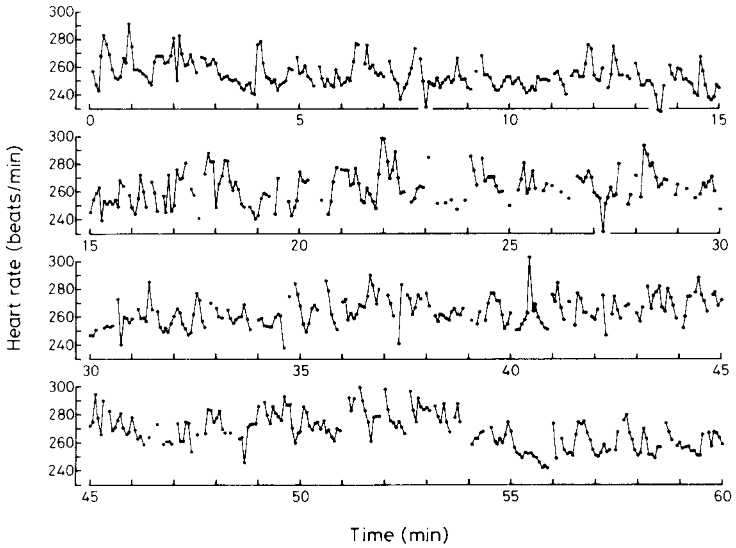

Fig. 1. Variability in embryonic f_H *of a domestic pigeon recorded over a 1-h period. Ballistocardiogram was recorded while the embryo pipped the eggshell externally. The solid line connects 4-s average* f_H*. Gaps in the lines indicate the inability to determine* f_H *due to somatic activity or postural changes of the embryo.*

per minute (bpm). ANOVA for repeated measures indicated that the difference between these six means was statistically significant, indicating real, short-term variation in f_H. The mean value of all these 4-s average f_H's, which gave the f_H value of this embryo, was 257 ± 11 bpm ($N = 36$). The mean value of all the 4-s average f_H's was 258 ± 12 bpm ($N = 380$) for the first 30 min and 262 ± 15 bpm ($N = 758$) for the 1-h period. The difference between the former and the latter two was not significant. Overall, the mean value of f_H's taken for the 30-min period represents an average f_H for a given age more closely than that for the 30-s period.

Daily Changes in Embryonic f_H

Figure 2 summarizes and compares the average f_H during embryonic development of each of the species and groups studied. Heart rate in all five populations examined increased significantly with development ($P < 0.01$).

1. *Fantail Pigeon.* Eggs were removed from the nests on the day when they were laid, so the incubation days of this group were known. The embryos hatched on day 18 of incubation (one hatched on day 19). The measurement

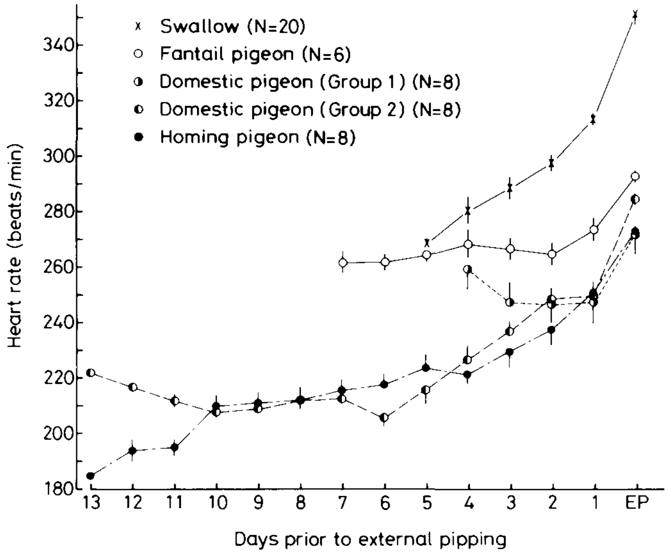

Fig. 2. A summary of the patterns of f_H *change during embryonic development of the pigeon and the swallow. The* N *in parentheses indicates the maximum number of eggs investigated for each group. Mean values ± 1 SE are given.*

of BCG was begun on day 10 of incubation and continued until EP (day 17). In four of six embryos, daily f_H did not change markedly until 2–3 d before hatching, when it then increased as EP approached. Heart rate of two eggs decreased by about 30 bpm between day 4 and day 2 before EP, before showing pronounced increase from 1 d before EP. Essentially, f_H in all embryos increased above previous levels immediately before EP. Overall, f_H increased from 265 bpm to 295 bpm during the period of measurement (fig. 2).

2. *Domestic Pigeon.* Heart rate was measured only for the last 5–6 d of incubation in the embryos of domestic pigeons (group 1). Although f_H varied widely among the eight embryos monitored, overall f_H increased significantly during development.

Daily changes in embryonic f_H were also measured in another group of domestic pigeons (group 2) collected at a much earlier stage of development. One embryo died following a gradual fall in f_H during development. All others developed and hatched. Heart rate was relatively low prior to the last week of incubation, but increased significantly as EP approached.

Overall, the daily f_H of domestic pigeon eggs (group 2) increased markedly from about 210 to 285 bpm during the last 10 d of incubation, increasing most sharply in the last 5 d of incubation. The mean daily f_H during the last 5 d was significantly different between group 1 and group 2.

3. *Homing Pigeon.* Heart rate was measured initially in 12 embryos, but four embryos failed to develop and died at early stages. In general, f_H was lowest in early stages of incubation, increasing significantly with development toward EP. Mean f_H of the eight surviving embryos over the last 10 d of incubation increased significantly by about 60 bpm from about 210 to 270 bpm (fig. 2).

4. *Bank Swallow.* Of 26 eggs instrumented, f_H was successfully measured from 20 embryos during the 6-d period ending with the conclusion of EP. The other embryos either died during incubation or developed normally but failed to give a reliable f_H signal. The day when each clutch was laid was not known. However, because incubation of swallow eggs is longer than 10 d (12–14 d, generally), the f_H patterns over the last 6 d of development almost certainly represent the daily changes over the last half of the incubation period. Heart rate increased significantly during the measurement period in a progressive fashion, increasing most sharply in the last day before EP. The average f_H of swallow embryos increased from about 270 to 350 bpm during the last 6 d of embryonic development (fig. 2).

Discussion

Incubation and Technique for f_H *Measurement*

Except for the eggs of fantail pigeons, artificial incubation was begun at different stages of embryonic development in different groups and clutches. Effects, if any, of artificial incubation with constant temperature and humidity on embryonic f_H were not determined in the present study and remain unknown for any bird. In addition, the adequacy of the egg-turning frequency we employed during incubation was not determined. Unfortunately, the effect of failure to turn eggs on embryonic f_H remains to be studied even in the domestic chicken, but it is known that the lack of egg turning during incubation seriously affects development and gas exchange of the embryos. Consequently, we elected to risk regular, minimal disturbance to the eggs induced by turning to minimize the possibly much greater effects of leaving them unturned. Certainly, that successful hatching occurred suggested that the turning procedures were adequate.

The results of the present study may be criticized at one level for employing two different techniques to determine f_H (ballistocardiography and impedance cardiography). For ballistocardiography, at least a 1-h measurement recess was observed after initially handling the eggs and closing the door of the incubator. This allowed for thermal equilibration and contributed to the return of f_H to resting values (if it ever departed from resting values). While ballistocardiography is a completely noninvasive method for measuring f_H, in impedance cardiography two electrode wires penetrated the shell and chorioallantoic membrane and an extremely weak electrical current passed through the embryo. In chicken eggs, impedance cardiography employing either 24-gauge needles or 40-gauge wire for the electrodes produced f_H data not significantly different from data derived noninvasively (Haque et al. 1994; R. Howe, W. W. Burggren, and S. Warburton, personal observation). It is not known, however, whether impedance cardiography had any effect on the smaller eggs of pigeons and swallows. A few embryos of homing pigeons died during development. Mortality of swallow embryos was higher than that of pigeon embryos. Inspection of dead swallow eggs indicated that the normally white shell became a faint gray around the inserted wire electrode. This was possibly due to the allantoic fluid oozing through an incision under the eggshell or into the shell membranes, which would eventually interfere with gas exchange of the embryo through that region of the chorioallantoic membrane, with perhaps subsequent bacterial infection. The color of the shell membrane of the great majority of embryos that hatched remained unchanged before and after penetration of electrodes into the allantoic fluid. The gas exchange of these embryos appeared unimpaired and the influence on embryonic f_H presumably negligible. The main reason for the death of embryos in the egg is probably related to an impairment of gas exchange through the eggshell and chorioallantoic membrane, causing hypoxia, though developmental anomalies in the embryo itself could also contribute. In the domestic fowl, hypoxia decreases embryonic f_H (Laughlin et al. 1976; Tazawa 1981). A domestic pigeon embryo from group 2 that died during incubation showed a decreased f_H, which might be mainly due to hypoxia caused by the impairment of gas exchange.

The criticisms directed at our techniques and protocol should be explored experimentally. However, we emphasize that although there were different treatments regarding egg turning and some different results obtained with the two techniques of determining f_H, the general patterns in f_H (i.e., an increase in f_H during the late stages of development) were observed in all altricial birds that we studied, regardless of technique or protocol.

Variability of f_H

Embryonic f_H in precocial and semiprecocial birds becomes variable toward the end of incubation (Tazawa et al. 1991*a*, 1991*b*). Heart rate of the altricial pigeon embryo was also very variable (fig. 1). The individual points in figure 1 show an average f_H of about 15–18 beats every 4 s. During the first 30-s period of the recording presented in figure 1, for example, the 4-s average f_H changed from about 240 to 280 bpm with a mean value of 263 ($N = 7$) bpm. The six mean values determined in the same manner every 5 min over the 30-min period were significantly different. This indicates that f_H should be counted and averaged for a prolonged period to give a representative value for a given age.

Developmental Changes in Embryonic f_H *of Altricial Birds*

During the last half of the incubation period, the embryos of the precocial quail and chicken show a temporarily increased f_H with a subsequent decrease toward the end of prepipping development. Heart rate in turkey embryos changes similarly, except for the EP period, during which f_H changes are not pronounced. Duck and goose embryos decrease f_H during the last stages of prepipping development. A gradual increase in f_H toward the end of incubation occurs in the peafowl embryo (Tazawa et al. 1991*a*). The semiprecocial seabirds the albatross (*Diomedea immutabilis*), the shearwater (*Puffinus pacificus*), and the noddy (*Anous stolidus*), keep f_H constant during the last several days of prepipping development. The particularly dramatic changes in f_H of the latter two species occur during the prolonged perinatal period (Tazawa et al. 1991*b;* Tazawa and Whittow 1994).

The averaged patterns of daily changes in embryonic f_H of the altricial pigeon and swallow, presented in figure 2, were quite different from those of precocial domesticated birds and semiprecocial seabirds. Judging from the experimental results of the domestic pigeon (group 2) and homing pigeon eggs, it seems that f_H increases only slowly during the middle stages of development (13 to 7 d prior to EP) and then increases sharply toward the end of incubation. The change in f_H of these two species seems to be in parallel with curves of growth and oxygen consumption during the last several days of incubation. While increases in f_H during EP are in common with altricial birds, the fantail pigeon and domestic pigeon (group 1) showed slightly different patterns of f_H change. The prominent changes in f_H occurred only during the few days prior to hatching. The embryonic f_H of domestic pigeons belonging to group 1 was particularly variable compared with another group of domestic pigeons (group 2) and other pigeons. This implies that geographically separated populations of breeding pigeons might produce embryos each with their own characteristic developmental patterns of f_H.

Assuming that the incubation period of bank swallow eggs is 12–14 d, embryonic f_H of this species increases continually throughout the last half of the incubation period, with the greatest rate of increase occurring during EP, as found in the other altricial birds.

Figure 3 shows regressions between prepipping f_H and egg mass in precocial and altricial birds. While differences apparently exist, a direct comparison of embryonic f_H of altricial and precocial birds of similar egg mass for the purposes of studying the allometry of embryonic f_H currently is difficult because of the lack of extensive overlap in egg mass between the two bird types. Measurements of f_H in altricial and precocial birds with a view to determining allometric equations for f_H should be the focus of future studies.

In the domestic fowl, f_H changes gradually from embryonic to posthatching level, with the greatest increase occurring during EP (Tazawa et al. 1992). The increase is supposedly due to the process of the maturation of the heart and circulation during EP. The cardiovascular system at EP may be less mature in altricial bird embryos than in the precocial embryos of the domestic fowl. It would be interesting to see whether f_H, which increased

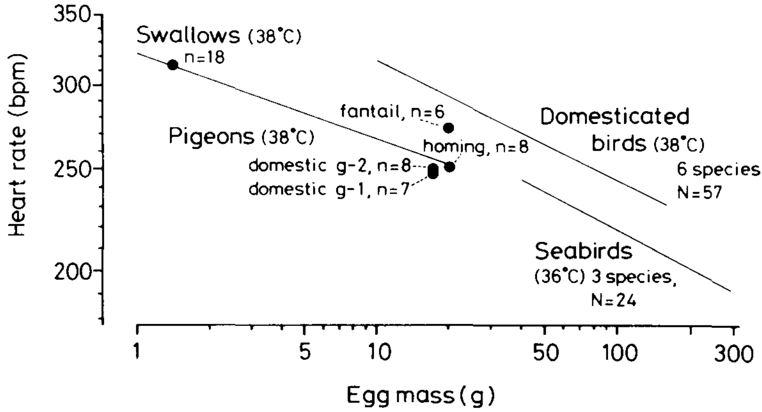

Fig. 3. The relationship between egg mass and f_H *immediately before pipping in altricial and precocial birds. Regressions for mean prepipping* f_H *data (at normal incubation temperatures) are indicated for 57 individuals of six species of precocial domesticated birds (Tazawa et al. 1991a), for 24 individuals of three species of precocial seabirds (Tazawa and Whittow 1994), and for 33 individuals of two species of altricial birds from the present study. The range of body mass overlap between precocial and altricial bird embryos for which* f_H *is known is quite small, which precludes meaningful allometric analysis until further data are available (see text for further discussion).*

during EP, increases further after hatching in a fashion similar to developmental curves for growth and oxygen consumption.

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