NONINVASIVE DETERMINATION OF EMBRYONIC HEART RATE DURING HATCHING IN THE BROWN NODDY (*ANOUS STOLIDUS*)

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ABSTRACT.—We used an audiocartridge system, which measures the ballistic movements of the egg attributable to embryonic cardiac contractions, to determine the heart rate (HR) of the Brown Noddy (*Anous stolidus*) before and during the prolonged hatching process. The average heart rate at 36°C in 8 embryos (mean fresh egg weight = 38 g) was 262 beats/min (bpm) on the last day of prepipping, and 264 bpm on the first day of shell fracture (external pipping). Heart rate increased to 291 bpm after penetration of the air cell by the beak (internal pipping), and increased to 310 bpm on the last day of incubation. With a few exceptions, the heart rate, which did not change systematically during the last stage of prepipping, remained unchanged early in external pipping and then increased markedly with the initiation of pulmonary respiration. Changes in heart rate during hatch may be related to increases in the oxygen consumption of the embryo. The heart rate was highly variable even at constant ambient temperatures. It changed according to a temperature coefficient (Q_{10}) of 2 at all developmental stages, when the ambient temperature was modified 2°C from the control (36°C). *Received 26 February 1990, accepted 29 December 1990.*

MANY seabirds breeding in the Hawaiian Archipelago pass through a prolonged hatching process (Pettit and Whittow 1983a, b). Hatching begins with shell fracture (external pipping) and is followed by penetration of the air cell by the beak through the chorioallantoic membrane (internal pipping), which initiates pulmonary respiration (Pettit and Whittow 1982a, b). Although the gas exchange during the hatching process of seabirds has been well studied (see references *in* Rahn et al. 1985), there are scanty data on the circulatory system.

Noninvasive systems to measure embryonic heart rate (HR) take advantage of the calcareous hard shell of the egg, which moves periodically because of the cardiac contractions of the embryo (Cain et al. 1967; Suzuki et al. 1989; Tazawa et al. 1989a, b; Hashimoto et al. 1991). Measurements are based on ballistocardiography and various transducers are employed: piezoelectric transducer (Cain et al. 1967), moving-magnet type audiocartridge (Suzuki et al. 1989, Tazawa et al. 1989a), laser speckle meter (Tazawa et al. 1989b), and laser displacement meter (Hashimoto et al. 1991). Although the physical measurements determined with these transducers are different (Hashimoto et al. 1991), in each case the heart rate of the embryo can be counted from the cardiogenic ballistic waves produced (referred to as the *ballistocardiogram*).

We used an audiocartridge measuring system to elucidate the changes in heart rate of the Brown Noddy (*Anous stolidus*) during development, particularly just before and during the prolonged pip-to-hatch period. We also estimated the effect of ambient temperature change on the embryonic heart rate. Previously, the initiation of embryonic thermogenesis in the Brown Noddy was determined from the O_2 consumption before, during, and after hatching (Matsunaga et al. 1989). We used the Brown Noddy because O_2 consumption data were available as a reference point.

MATERIALS AND METHODS

Eggs were collected on Manana Island, a small offshore island of Oahu in the main Hawaiian Islands. They were brought to the laboratories at the University of Hawaii within 3 h of collection and numbered on the eggshell to distinguish individuals. We measured the length (L, in cm) and maximum breadth (B, in cm) with a micrometer to 0.01 cm, and estimated the fresh mass (W, in g) of each egg from the equation (Hoyt 1979), $W = 0.534 \cdot LB^2$. Eggs were incubated at 36°C and 55–60% relative humidity in a forced-draft incubator and turned twice daily (morning and eve-

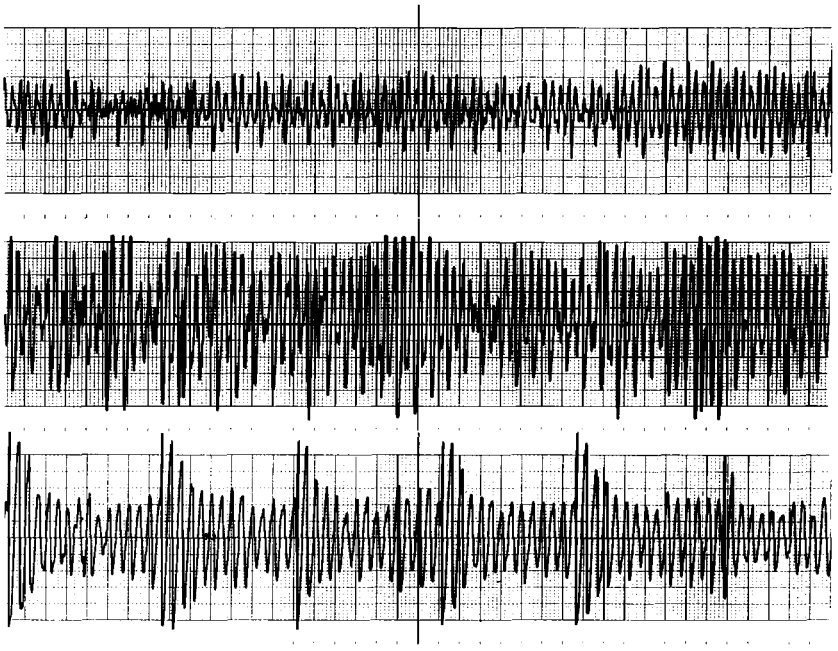

Fig. 1. Ballistocardiograms recorded with the audiocartridge measuring system during external pipping (top panel) and internal pipping (center and lower panels). Each record is of 8-s duration.

ning). Regular turning was continued until external pipping.

The measuring system was described elsewhere (Suzuki et al. 1989, Tazawa et al. 1989a) and is briefly summarized here. Eggs were transferred from the incubator to a Hotpack environmental chamber every day. The chamber was kept at 36°C and contained a floating platform suspended from a frame. An audio-

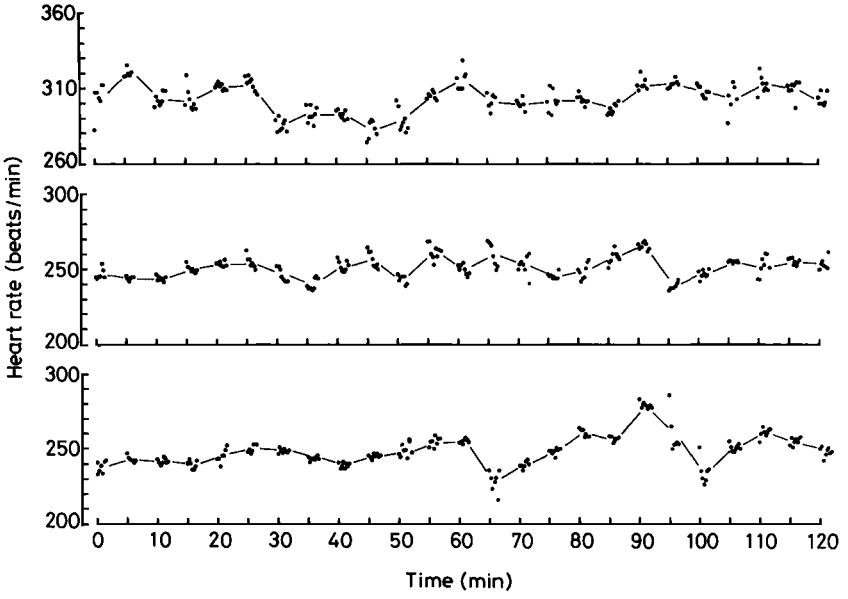

Fig. 2. Variability of 4-s average heart rates during a 2-h period in internally pipped (top), externally pipped (center), and unpipped (bottom) eggs.

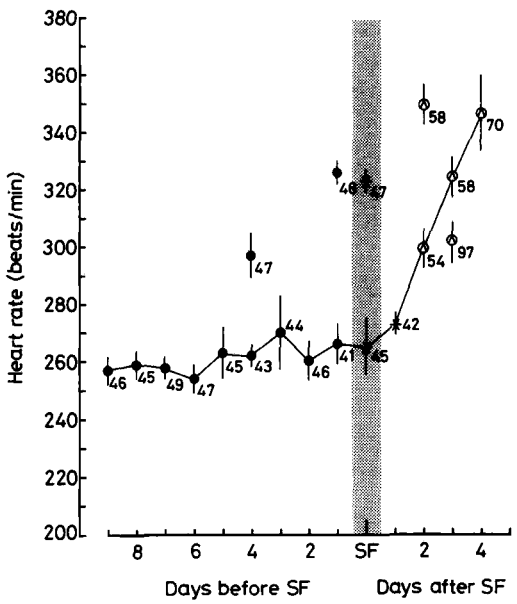

Fig. 3. The mean heart rate (HR) before and during pipping of one egg. The HR increased markedly 2 days after the onset of pipping (third day of pipping) when the embryo penetrated the air cell and rebreathed air-cell gas. The egg hatched soon after the last measurement was made on the fifth day of pipping. A solid line connects the HRs at 36°C. An isolated point above the line indicates the HR at 38°C, and a point below the line shows the HR at 34°C, measured 5 h after exposure to the altered temperature, at a given stage of development of the same embryo. The number adjacent to each point is the number of 4-s average heart rates. Closed circle = unpipped; asterisk = star-fractured (externally pipped); circle with hat = internally pipped; SF = star fracture. The vertical lines represent ±SD; the shaded column identifies the beginning of pipping.

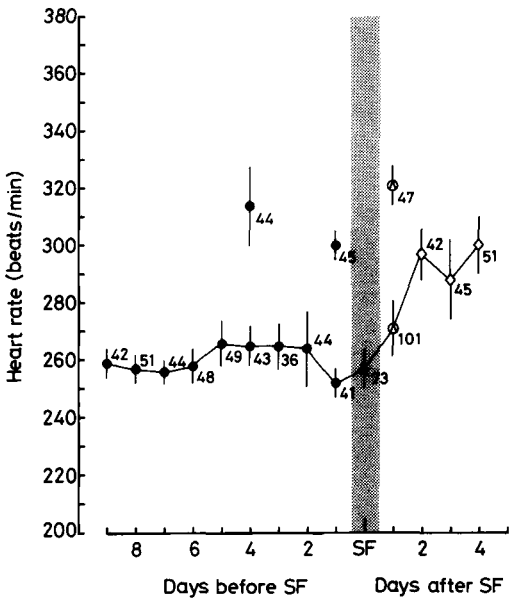

Fig. 4. The mean heart rate (HR) before and during pipping of another egg. The embryo pipped the chorioallantoic membrane (internal pipping) on the second day of pipping, causing a slight increase in the HR. The diamond indicates that the embryo was making a pip-hole; other notations as in Figure 3.

cartridge (model AT150E, Audio-Technica, Tokyo) installed on a manipulator-holder and a concave glass plate were placed on the platform. The plate provided the egg with a smooth surface and kept it in position. The platform attenuated extrinsic vibrations (Suzuki et al. 1989; Tazawa et al. 1989a, b). The stylus pin of the cartridge was gently placed in contact with the egg positioned in the center of the plate. The electrical output from the cartridge was amplified by approx. 80 dB with a conventional AC amplifier provided with an input time constant of 0.03 s and lowpass filter with a cutoff frequency of 30 Hz. Because the cardiogenic ballistic movement of the egg was directional (Tazawa et al. 1989a), the output signal from the cartridge was monitored on an oscilloscope. We chose a site for detection that produced clear spike waves. The ballistocardiogram (BCG) was recorded on a pen recorder with a chart speed of 25 mm/s, for 30 s or more, every 5 min for 30 min, or 2 h in a few measurements. After measurements of BCG, the egg was returned to the 36°C incubator.

The average heart rate over 4 s (referred to as 4-s average HR) was counted every 4 s from each 30-s recording. This procedure was repeated every 5 min for 30 min or 2 h. Finally, we averaged all the 4-s average HRs to calculate the mean HR of the embryo on a given day of incubation.

For 6 eggs, the BCG measurement was repeated at 38°C or 34°C at an arbitrary day of development. The eggs were kept in the experimental chamber after measurement at 36°C and the temperature of the chamber was changed to 38°C or 34°C. The BCG was measured after 5 h of exposure to an altered temperature. The average HR was calculated from the record. After measurements of the BCG, we returned the eggs to the 36°C incubator and the temperature of the experimental chamber was restored to 36°C. The temperature of the experimental chamber was controlled within ±0.2°C of a set temperature and monitored with a calibrated thermistor probe 5 cm from the egg. For the eggs whose HR was measured at 34°C or 38°C, we calculated the temperature coefficient (Q_{10}) of HR from the equation,

$$Q_{10} = [HR(36)/HR(T)]^{10/(36-T)},$$

where T is a temperature of 38°C or 34°C, HR(36)

represents the average heart rate measured at 36°C and HR(T) is the average at T°C. For calculation of Q_{10}, we used the chamber temperature determined with the probe during the BCG measurement.

Whenever the eggs were turned and handled, they were checked for fracture of the shell and sounds of cheeping or breathing.

Results

The ballistocardiograms (BCG) were measured during the first 3 weeks of July 1988. Eggs were collected twice (1 and 9 July). We studied 8 eggs from a prepipping stage to hatching. Because the age of eggs was not known on the day of collection, age was defined in terms of days before the first day of shell fracture.

The BCG changed with time and age (Fig. 1). The embryo represented in the top record (Fig. 1) had fractured the eggshell (external pipping). The center and lower records were obtained from older embryos that had ruptured both the eggshell and the chorioallantoic membrane (internal pipping). The periodic, large downward deflection in the top and center records corresponded to each heart beat. In the lower record, the ballistic waves attributed to each heart beat appeared every 2 pulses. In addition to the cardiogenic movements, we simultaneously detected signals produced by breathing as large spike waves appearing periodically (Fig. 1: lower). These were directional movements and thus their presence was dependent upon the site of the transducer. For most records, we selected a site that did not record respiratory movements.

The 4-s average heart rate (HR) was 263 and 261 beats/min (bpm) for the first and last 4 s in the top record (Fig. 1). In the center record, the 4-s average HR was 340 bpm (first) and 370 bpm (last). Similarly, it was 303 bpm and 298 bpm for the first and last 4 s in the lower record.

Heart rates varied over a 2-h period (Fig. 2). Each point (Fig. 2) indicates a 4-s average HR determined every 4 s from a record taken for approximately 30 s. The mean values of 4-s average HRs counted from a 30-s record are connected by a solid line. All values for 4-s averages were averaged to give the mean HR for a day of measurement. From the top panel to the bottom, the mean HR (±SD) was 303 ± 11 bpm (n = 187), 251 ± 8 bpm (n = 193) and 249 ± 11 bpm (n = 190). The HR during internal pipping (Fig. 2: top) was fast compared with the prepipped embryo (Fig. 2: bottom). The shell-fractured embryo (Fig. 2: center) had an average HR similar to that of a prepipped embryo.

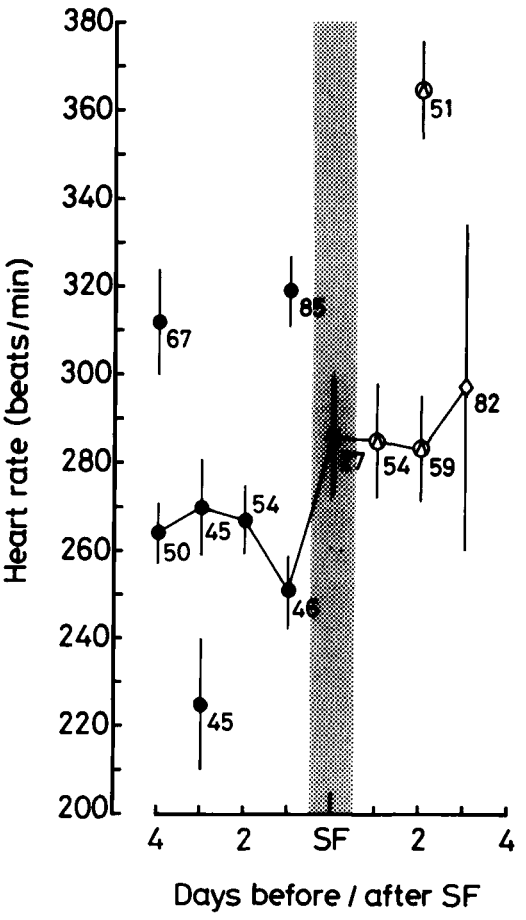

Fig. 5. The mean heart rate (HR) before and during pipping of another egg. The first shell fracture caused a marked increase in the HR. Notations as in Figures 3 and 4.

The HR did not change systematically during prepipping, but it increased prominently during pipping (Figs. 3–5). The mean HR for 8 embryos on the last day of prepipping development was 262 ± 7 bpm (Table 1). On the following day, 7 embryos pipped the shell first, but the mean external pipping HR remained unchanged (Table 1). Three embryos began internal pipping the following day, and three others pipped the chorioallantoic membrane 2 days later. The remainder took 3 days to pip internally. Seven embryos took 4–6 days from the onset of external pipping to hatch (Fig. 6, Table 1). Only one embryo began internal pipping first, fractured the shell the following day, and

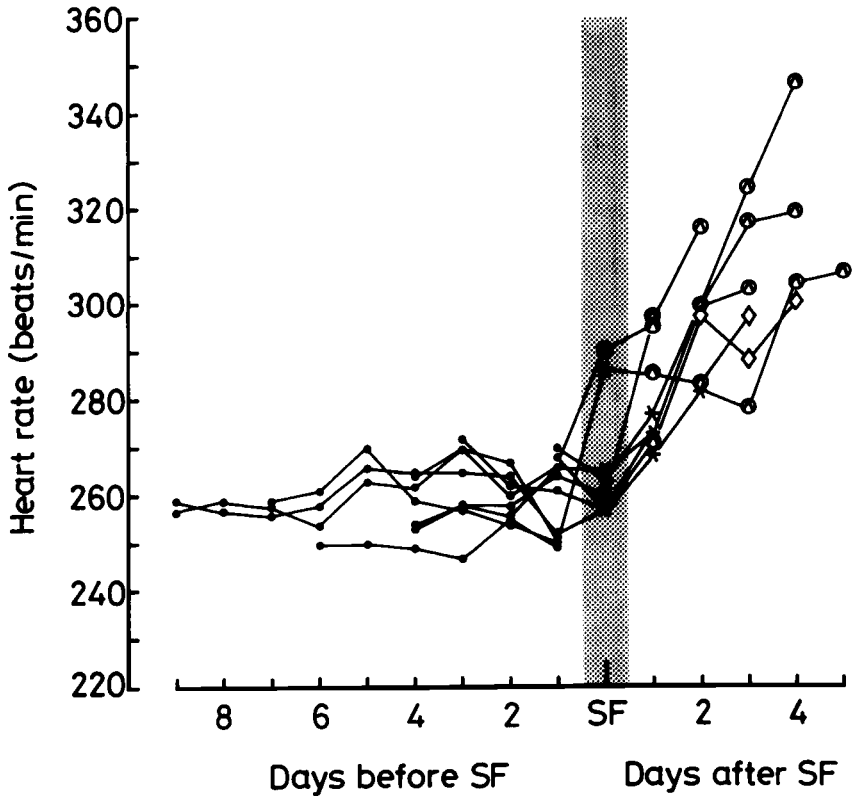

Fig. 6. The mean heart rate (HR) of 8 embryos before and during pipping. With a few exceptions, the HR was constant during the prepipping stage, unchanged on the first day of external pipping and increased slightly on the second day, then rose markedly from the following day onward. Notations as in Figures 3 and 4.

hatched 3 days after the onset of internal pipping. The mean HR of 8 embryos on the first day of internal pipping increased significantly compared with the prepipping HR (Table 1). The HR increased further and was maximal on the last day of incubation in all embryos (Table 1).

Irrespective of the developmental stages, the HR changed markedly 5 h after we altered ambient temperature by 2°C (Figs. 3–5). In one egg

TABLE 1. Fresh egg mass (g) and heart rate (beats/min) on the last day of prepipping development (Pre-pip), on the first day of shell fracture (EP), on the first day of internal pipping (IP), and the maximum heart rate (MHR). Numbers in parentheses represent days after pipping begins, the first day was designated as day 0.

		Heart rate			
Egg	Mass	Pre-pip	EP	IP	MHR
1	40.1	266	265 (0)	299 (2)	346 (4)
2	37.2	270	263 (0)	296 (2)	319 (4)
3	36.8	268	257 (0)	297 (2)	303 (3)
4	37.8	261	257 (0)	294 (3)	306 (5)
5	42.5	252	257 (0)	271 (1)	300 (4)
6	35.9	264	260 (0)	297 (1)	316 (2)
7	35.6	251	286 (0)	285 (1)	297 (3)
8	36.9	266	—	290 (0)	295 (1)
Mean ± SD	37.9 ± 2.2	262 ± 7	264 ± 10	291 ± 9	310 ± 16

TABLE 2. Temperature coefficient (Q_{10}) of the heart rate measured at an ambient temperature 2°C above or 2°C below (*) the control temperature of 36°C during the prepipped ($n = 10$), externally pipped ($n = 3$) and internally pipped ($n = 9$) stages. The two numbers in parentheses are the heart rate at the altered ambient temperature (38°C or 34°C) over the heart rate at control temperature (36°C). Other notations as in Table 1.

Egg	Pre-pip		EP	IP	
1	1.9 (297/262)	2.6 (326/266)	2.5 (323/265)	2.1 (349/299)	1.4* (302/324)
2	2.2 (323/271)	2.8 (316/257)	2.2 (316/268)	2.1 (345/296)	2.4* (266/317)
3	1.6* (233/262)		2.0 (307/267)	2.9 (345/278)	
4	2.3 (314/265)	2.4 (300/252)		2.3 (321/271)	
5				1.9 (364/320)	2.1* (294/347)
6	2.2 (312/264)	2.8 (319/251)		3.5 (364/283)	
	2.1* (225/262)				
Mean ± SD	2.3 ± 0.4		2.2 ± 0.2	2.3 ± 0.6	

(Fig. 3; Table 2), the mean HR of 324 bpm measured at 36°C during internal pipping (3 days after star fracture) decreased to 302 bpm 5 h after exposure to 34°C, a Q_{10} of 1.4. In another egg (Fig. 5; Table 2), the HR during internal pipping (2 days after star fracture) increased to 364 bpm at 38°C 5 h from 283 bpm at 36°C, a Q_{10} value of 3.5. These Q_{10} values, small at lowered temperature (Fig. 3) and large at increased temperature (Fig. 5), might be due to natural fluctuation rather than increases in HR during the 5-h exposure.

DISCUSSION

Changes in heart rate during hatching.—The heart rate in 3 embryos remained unchanged during the first day of the external pipping, increased slightly during the second day of star fracture, and then increased markedly on the third day of pipping when the embryo initiated internal pipping and breathed atmospheric air (Fig. 3). In a fourth egg, the HR increased on the third day while the embryo still remained at the stage of external pipping. A fifth egg had already initiated internal pipping on the second day of pipping without a marked increase in HR, but it increased HR on the third day when the shell fracture became a pip-hole (Fig. 4). Consequently, the marked increase in HR occurred on the third day of the pipping process in 5 embryos, independently of whether they pipped externally or internally, but for the most part after internal pipping.

Of the remaining 3 eggs, HR increased in one embryo during internal pipping, which occurred on the second day of the pipping process. Another initiated the pipping process with external pipping (Fig. 5) and the other with internal pipping. The HR of these 2 embryos increased during the first day of pipping. Apart from these exceptions, HR tended to remain almost identical with the prepipping value during the first day of external pipping, then increased with internal pipping, and reached maximum at the end of incubation (Fig. 6).

Relationship with oxygen consumption.—The oxygen consumption ($\dot{V}O_2$) measured previously for the Brown Noddy during the pipping and hatching process also reached a maximum at the end of incubation (Matsunaga et al. 1989). Oxygen consumption increased ca. 15% after the last day of prepipping development, 64% on the first day of internal pipping, and 156% at the end of incubation. The greatest increase in $\dot{V}O_2$ during pipping accompanied internal pipping and pulmonary respiration, while shell fracture (external pipping) caused only a minor increase in $\dot{V}O_2$. Although the increase in $\dot{V}O_2$ was much larger than in HR, the increase in both variables occurred coincidentally with internal pipping. Shell fracture caused little or no increase in either variable.

Until external pipping, the prepipping HR

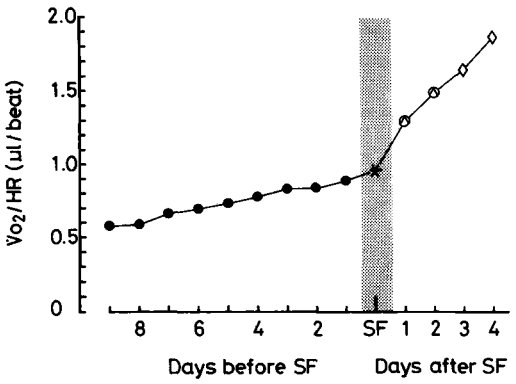

Fig. 7. The ratio of mean oxygen consumption to mean heart rate before and during pipping (SF = star fracture). Data for oxygen consumption was taken from Matsunaga et al. (1989). Notations as in Figures 3 and 4.

remained nearly constant, while the $\dot{V}O_2$ increased gradually (Matsunaga et al. 1989). When the embryo pipped externally and fractured the shell, oxygen was still absorbed through the vascularized choriallantoic membrane. The gradual increase in $\dot{V}O_2$ during the late prepipping and external pipping periods thus depends on an increase in the chorioallantoic blood flow ($\dot{Q}_a$) and blood O_2 transport capacity. If an increase in $\dot{Q}_a$ depends on cardiac output, the increase in cardiac output may be accomplished by an increase in stroke volume. Changes in the ratio of $\dot{V}O_2$ to HR were calculated from mean values of $\dot{V}O_2$ determined previously (Matsunaga et al. 1989) and HR measured in this study (Fig. 7). If it is assumed that the oxygen extraction by the embryonic tissues changes little during development, this implies that the stroke volume gradually increases towards external pipping and that the increase is augmented during internal pipping. The increase in HR and stroke volume may contribute to the cardiac output, which should be augmented to match the increase in $\dot{V}O_2$.

Temperature coefficient of heart rate.—The HR was variable even over short periods (Figs. 1 and 2) and dependent upon environmental temperature. Because of wide fluctuations of the HR, we anticipated difficulty in evaluating the Q_{10} value by only a 2°C change. With a few exceptions, however, the averaged value of Q_{10} from 6 eggs was roughly 2, irrespective of the developmental stages and differences between control heart rates at 36°C (Table 2). Q_{10} in Brown Noddys for $\dot{V}O_2$ after prolonged exposure to a temperature lowered by 6°C was 2 until hatching, and the Q_{10} became <2 soon after hatching (Matsunaga et al. 1989). This implies that metabolism in Brown Noddys exposed to cooling was affected primarily by temperature, and no compensatory metabolic response existed before hatching. The temperature coefficient of heart rate we determined before hatching also indicates that the change in HR due to ambient temperature alteration is primarily the direct effect of temperature on cardiac pacemaker cells.

ACKNOWLEDGMENTS

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