Simultaneous acquisition of electrocardiogram, ballistocardiogram and arterial blood pressure of chicken eggs.

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Abstract

Developing techniques to measure minute motions of the eggshell attributable to cardiac contractions of the embryo, we attempted to assemble techniques for simultaeous acquisition of ballistocardiogram (BCG), electrocardiogram (ECG) and arterial blood presstre. The procedure for implanting electrodes and catheter fulfilled requirements for the gas exchange through the eggshell to be maintained adequate. Despite of these appendages in the egg, the ballistic throw of the embryo could be detected from the eggshell employing the audio cartridge. The cartridge comprises a small magnet installed on a cantilever and moving in a core. The motion of the magnet in the core changes density of magnetic flux and produces electrial output proportionate to a velocity of its motion. Simultaneous recordings of BCG from multiple sites on the eggshell show that the amplitude of BCG waves is dependent upon a site of detection and the BCG is directional wave. Isochronal comparison between ECG , BCG and blood presssure indicates that the ballistic throw of the embryo body is caused by not only the ventricular contraction , but also atrial excitation ; that is, the BCG comprises basic throws occurring during ventricular presystolic, isometric and ejection periods. Patterns of BCG waves which are composed of these basic throws are various among eggs and even in the same egg when its heart rate is altered with ambient temperature. The isometric contraction period remains constant irrespective of the heart rate, which is about 60 msec.

1. Introduction

In contrast to mammalian fetuses whose devlopment and life-support depend upon functional environments of maternal body, physiological capacities for development of avian embryos are directly influenced by external environments. Embryos exchange respiratory gases and heat with surroundings through the eggshell which comprises protective barrier for themselves. The gas exchange across the shell and the respiratory and O_2 transport properties of blood have been intensively studied not only in normal conditions of environments, but also by determining responses of these respiratory variables to altered environments (see Tazawa, 1987). In connection with the investigations on avian egg's respiration, some physiological properties of cardiovascular system have been studied in chicken eggs. Previously, the direct measurements of allantoic blood flow were attempted employing a conventional electromagnetic flow probe (Tazawa *et al.*, 1985) and the arterial blood pressure was measured with elaborate techniques of micropipette and catheterization (Van Mierop and Bertuch, 1967; Girard, 1973a, 1973b; Tazawa, 1981a, 1981b). The circuit model analysis, dyedilution and isotope-labeled microsphere techniques were employed to elucidate the cardiovascular shunt and cardiac output distribution (White, 1974; Tazawa and Mochizuki, 1977; Rahn *et al.*, 1985; Tazawa and Johansen, 1987). Recordings of electrocardiogram (ECG) and ballistocardiogram (BCG) were made to count the cardiac beatings in normal and altered environments (Cain *et al.*, 1967; Laughlin *et al.*, 1976; Tazawa and Rahn, 1986). Despite of these exquisite techniques developed to acquire information on the cardiovascular functions of chicken eggs, a isochronal relationship between electrical and mechanical events of embryo heart remains to be studied. For instance, in spite that the technique has been developed to record the ECG and blood pressure, respectively, both waves have never been registered simultaneously. The present study therefore concerns itself with developing techniques to record simultaneously ECG, arterial blood pressure and mechanical event relating to the cardiac beating in order to investigate the electro-mechanical relationships of embryo cardiac functions.

2. Requirements for measurements

The gas exchange of chicken eggs takes place by molecular diffusion across the porous eggshell and further through the vascularized chorioallantoic membrane. The O_2 uptake of the embryo is therefore affected by diffusive conductance of the shell and diffusing capacity of the chorioallantoic membrane (Rhan and Paganelli, 1982; Wakayama and Tazawa, 1987). The diffusive conductance is a function of the shell surface area, and the diffusing capacity depends upon the allantoic blood flow (Tazawa *et al.*, 1976). In order to minimize possible disturbances induced by implantation of electrodes and catheters on the gas exchange of embryos, the following are required. 1) When the eggshell is removed to implant electrodes and/or catheter in the egg, the removal of the shell should be restricted as much as possible so that the shell diffusive conductance is not reduced. 2) The removed area must be re-covered after implantation procedure to minimize the disturbances of the diffusive gas exchange and the internal environment of the egg. 3) The blood flow through the allantoic circulation and the chorioallantoic capillary plexus should not be impeded by implantation procedure.

3. Acquisition of individual signals

Electrocardiogram The embryo developing within the amniotic sac is further covered with the allantoic membrane. The cardiac electrical activity propagates through the body of embryo and the extraembryonic fluids (amniotic and allantoic fluids) to the chorioallantoic membrane spreading out whole inner surface of the eggshell. In order to minimize adverse effects of invasion on the embryo and interanl environment and to fulfill requirements for implantation of electrodes, the electrical activity of the heart should be detected just beneath the eggshell without opening the egg and inserting electrodes up to the embryo. The electrode is a 26-gauge hypodermic needle 25 mm long and is bent at right angle about 3 mm from the tip. The bore is occluded with clay. The egg is placed on the horizontal position and three points are marked on the eggshell so that they form a triangle with side of about 2cm long. The sharp end of the needle is pointed perpendicularly to the shell at points marked previously and pushed into the egg. The tip (about 3 mm long) penetrates the eggshell and reaches the chorioallantoic membrane. The needles are then glued to the eggshell at the bent part and the free ends are connected to fine shielded electrode wires. The electrical signal is amplified with a differential amplifier provided with a band-pass filter. Because the electrical activity of the heart is not led directly from the embryo, noises originating from large body motion of the embryo cradling in the extraembryonic fluids and white noises interfere with it. While the band-pass filter partly cuts off these noises, the averaged response processing is made employing a microcomputer to improve a signal to noise ratio.

Ballistocardiogram While the body motion of the embryo offers electrical disturbances to the electrocardiogram, it propagates through the extraembryonic fluids to the eggshell, swinging the egg. Similarly, in association with cardiac beating, a minute motion of the embryo body which is caused by a recoil and impact of contraction should also propagate in the egg and throw the eggshell. This minute motion of the egg originated from the ballistic throw is referred to as ballistocardiogram (BCG) which is detected employing an audio cartridge (model AT-100, audio-technica). The cartridge comprises a small magnet installed on a cantilever and moving in a core. The motion of the magnet in the core changes density of magnetic flux and produces electrical output proportionate to a velocity of its motion. The cartridge is supported on the stand; the height and angle are controlled. The free end of cantilever has a pin set and the pinpoint is gently applied at right angle on the eggshell. The electrical output is amplified by an AC amplifier with variable time constant to minimize noises caused by random body motion and filtered by a lowpass filter with 200 Hz cut-off frequency. For actual recording of the BCG, both the egg and cartridge are placed on the identical flaoting platform to reduce disturbances caused by environmental vibrations. The averaged response processing is also made for the BCG wave to improve a S/N ratio.

Arterial blood pressure The chorioallantoic membrane spreading out whole area under the eggshell is highly vascularized and serves as the respiratory organ of the embryo. The O_2-poor blood is supplied through the allantoic artery which forms a single stem when it leaves the embryo's body via the allantoic stalk. The stem bifurcates to the right and left branches on to the allantois, either of which is subjected to catheterization for measurement of blood pressure. The blood vessel floating in the allantoic fluid is located by candling the egg. The allantoic blood flow should not be occluded by an implantation of the catheter so that gas exchange through the chorioallantoic capillary plexus is well maintained. In order to fulfill this requirement, the catheter comprises a 26-gauge hypodermic needle 15 mm long connected with polyethylene tubing 5 cm long. The outside diameter of the needle is thinner than the artery, and the needle is bent at risht angle about 3 mm from the tip so that the incision faces outwards. The catheter is filled with heparin to avoid blood clotting and the free end of tubing is plugged with clay. A small area of the eggshell marked previously by candling is removed with sharp needle and forceps together with two shell membranes. The chorioallantoic membrane is torn with needle about 5 - 8 mm long, and the allantoic artery is gently lifted by forceps from the allantoic fluid. The tip of the catheter needle bent at right angle is inserted into the artery pointing upstream. After insertion and repositioning the catheterized vessel in the allantoic fluid, the catheter is fixed with clay and epoxy to the edge of the hole in the shell. The removed area is re-covered with tape and epoxy glue to minimize disturbances of the diffusive gas exchange and of the internal environment. The polyethylene tubing , filled with heparin, emerging from the egg is connected to a Statham electromanometric transducer (model P23ID) through another polyethylene tubing filled with saline solution . The connection of two tubings is made with a short piece of a needle.

4. Procedure for simultaneous measurements of ECG, BCG and blood pressure

The incubating egg has first the allantoic artery catheterized. The procedure for catheterization takes about 5 min and the egg along with catheter is placed in a 38℃ environment until the epoxy glue used for fixing the catheter and closing the hole gets solid. Second, three needle ECG electrodes are inserted into the egg one after another and glued on the eggshell. The egg which is now catheterized and has ECG electrodes implanted is placed on a smooth surface of the platform which is suspended in a temperature-controlled chamber. On the same platform are placed the electromanometric transducer and the cartridge. The clay plug of the catheter is cut off with scissors and immediately the polyethylene tubing is connected with that of the electromanometric transducer using a short piece of a needle. The length of tubing from the tip of the catheter to the membrane surface of the manometric transducer is about 12 cm and the height of the transducer is adjusted so that the center of the membrane surface comes to a height corresponding to the tip of catheter. The free end of ECG electrodes are then connected to thin wires employing a IC transistor socket, and wires are plugged in the differential amplifier. Finally, the cartridge holder is manipulated so that the pinpoint of cantilever gently comes into contact with the eggshell. The waves of blood pressure, ECG and BCG are monitored on the oscilloscope and stored on the magnetic tape recorder. These to be analyzed are fed into the microcomputer (PC 9801 Vm, Nippon Elec.) through a 12 bit A/D converter at sampling rate of 500 Hz.

5. Results and discussion

Based upon the model analysis of the cardiovascular shunt and blood flow distribution in chick embryos, it was reported that the cardiac output of 16-day-old embryos is about 20 ml/min (Tazawa and Takenaka, 1985; Tazawa and Johansen, 1987). The stroke volume is thus estimated to be about 70 - 80 μl. Similar values have also been reported (Hughes, 1949). The recoil and impact of the cardiac contraction producing that stroke volume are propagated to the eggshell, causing minute motion. In Fig. 1 are shown velocity waves of eggshell motion recorded simultaneously from three different points (BCGs 1-3) along with ECG. All the recordings show waves synchronizing with QRS complexes of ECG, apparently indicating that the waves are originated from the cardiac contraction. BCGs 1 and 3 are recorded from opposite sites of latitudinal axis and their main deflections are inverse each other. BCG 2 shows velocity of egg motion recorded from right angle to the axis connecting former two sites. The largest deflection common to three recordings appears with QRS complexes of ECG, but even prior to QRS waves the deflection , though small, occurs.

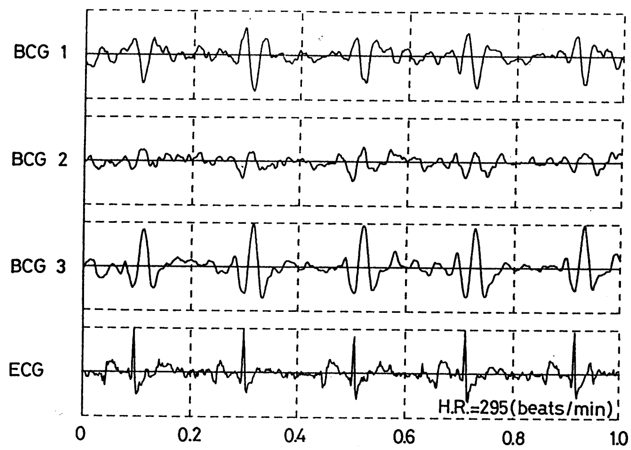

Fig. 1. Ballistocardiograms recorded simultaneously from three sites of the egg along with electrocardiogram. The embryo was 19-day-old, and the average heart rate was 295 beats/min. The egg was placed on the vertical position. BCGs 1 and 3 were measured from two ends of the latitudinal axis facing each other, and BCG 2, from a site at right angle to the axis connecting former two sites.

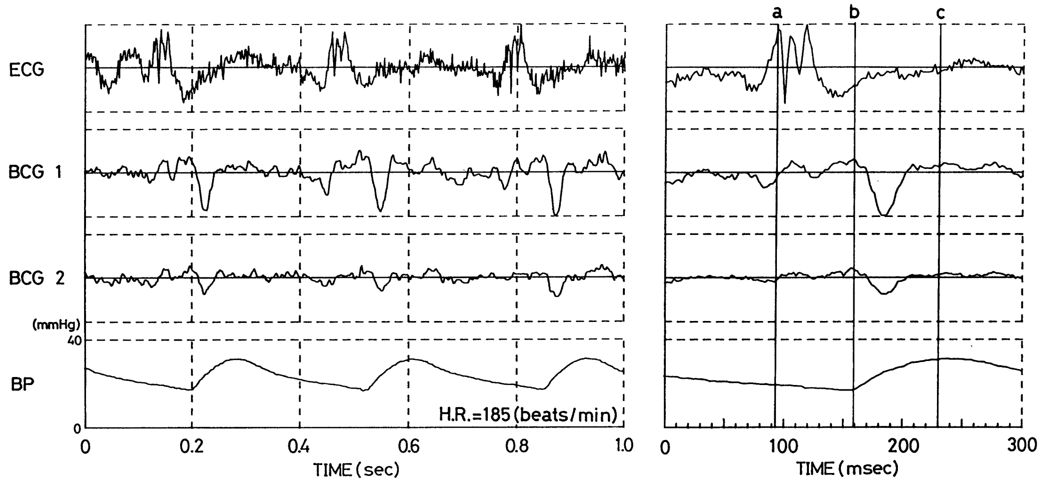

Fig. 2. Simultaneously recorded waves of electrocardiogram, ballistocardiograms (BCGs 1 and 2), and blood pressure of the allantoic artery (BP) (left panel) and averaged waves (N=10, right panel). The embryo was 18-day-old, and the average heart rate was 185 beats/min. The egg was placed on the horizontal position. BCGs 1 and 2 were recorded from two sites facing the center of the egg at right angles each other. Main deflection of BCG appears after onset of ventricular systolic pressure.

This indicates that ballistic motion of the embryo is produced by not only the blood ejection from the ventricle, but also atrial contraction and isometric contraction of the ventricle. Patterns of BCG waves are various among the eggs and even in the same egg when its heart rate is altered with ambient temperature.

The following are some examples for the simultaneous recordings of ECG, BCGs and arterial blood pressure, sorted out on the basis of representative patterns of BCG. The BCG was measured from two places locating at right angle each other. Fig. 2 represents the BCG of which main deflection appears after onset of the ventricular systolic pressure. Blood ejection from the ventricle into the aorta contributes to this ballistic wave. The averaged response processing (N=10) further implies a minute motion between oneset of QRS complex (isochronal line a) and that of the systolic pressure (isochronal line b) as well as one preceding the ventricular electrical excitation . The period between two lines a and b corresponds to the isometric phase of the ventricular contraction, and the ballistic motion caused by the isometric contraction is more apparent in Fig. 3 . The recordings were acquired from the same embryo as for Fig. 2 at an increased ambient temperature. The cardiac beating was faster than the previous record. Soon after electrical excitation of the

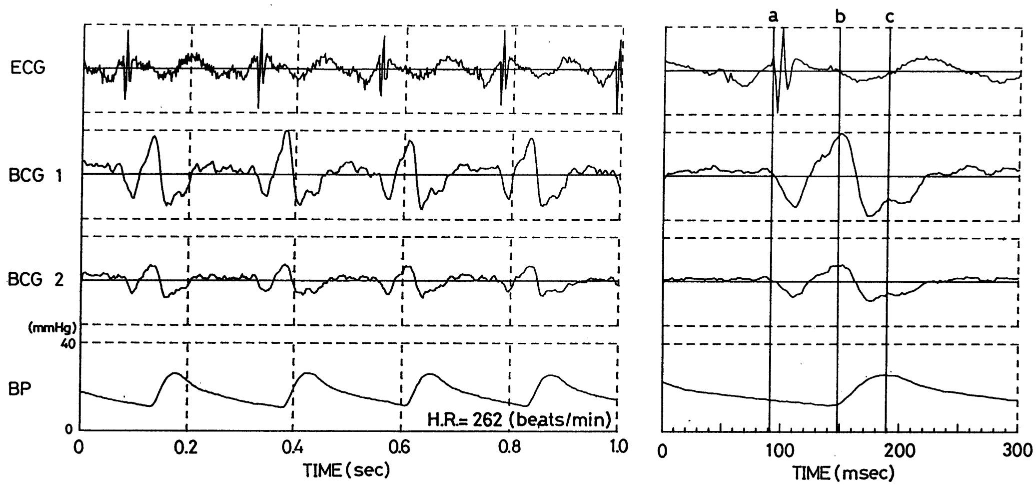

Fig. 3. Simultaneous recordings ECG, BCG and arterial blood pressure (left panel) and averaged waves (N=10, right panel) acquired from the same embryo as for Fig. 2. The ambient temperature was increased and the cardiac beating counted 262 beats/min. Additional deflection occurred during isometric contraction of the ventricle.

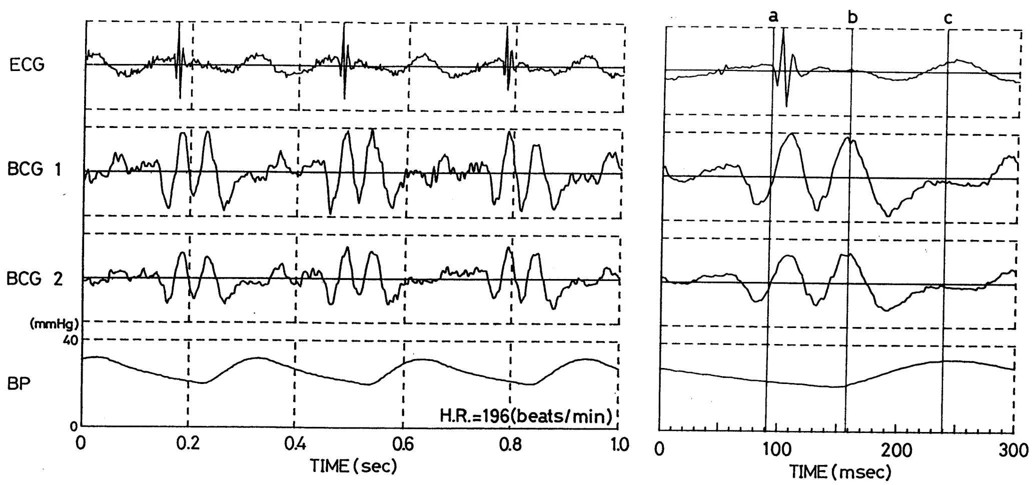

Fig. 4. Simultaneous recordings of ECG, BCG and arterial blood pressure (left panel) and averaged waves (N=10, right panel) acquired from the 18-day-old embryo. The ambient temperature was lowered and the average heart rate was 196 beats/min. In addition to large throws during ventricular systolic period, another deflection appeared during atrial contraction.

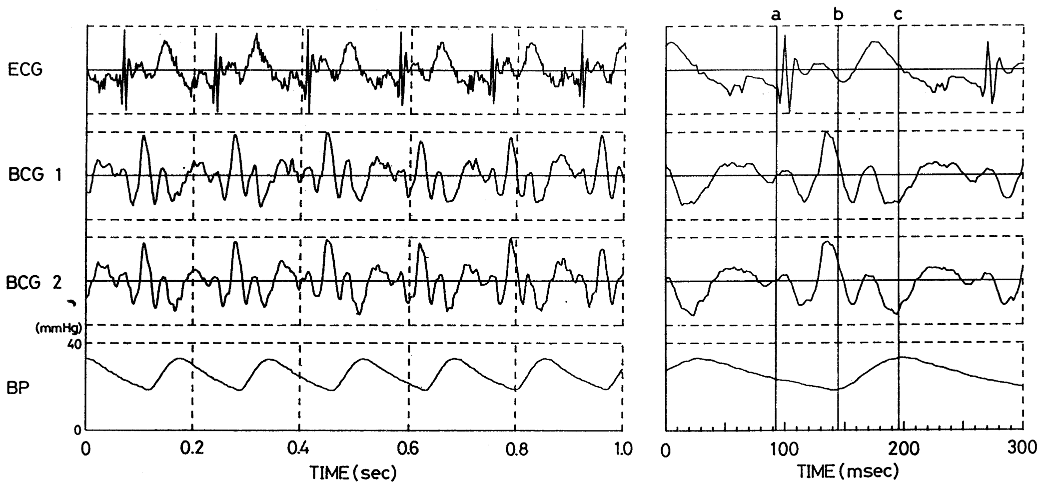

Fig. 5. Simultaneous recording of ECG, BCG and arterial blood pressure (left pancl) and averaged waves (N=10, right panel) acquired from the 18-day-old embryo of which heart rate was increased to 353 beats/min by raising an ambient temperature. The ballistic swings appeared during ventricular presystolic and systolic periods.

ventricle occurred, the egg was throwed with prominent recoil and again after ventricular ejection with little recoil. The pattern of ECG altered, for it was not a direct recording from the embryo . The apparent ballistic motion during ventricular presystolic period is presented in Fig. 4. The egg was throwed first with recoil prior to onset of electrical excitation of the ventricle, which was apparently attributed to atrial contraction as indicated by the P wave in the averaged ECG. The ventricular isometric contraction produced the second throw of the egg accompanying with large recoil, and the third throw occurred after opening of the aortic valve. Fig. 5 shows another example of simultaneous recordinsg acquired from the embryo when the egg was exposed to increased ambient temperature. The heart rate counted 353 beats/min which the embryo at control temperature (38℃) had never encountered. The minute swing occurred prior to onset of QRS waves, followed by large swings during isometric contraction and again after blood ejection from the ventricle into the aorta.

The isochronal line C is drawn at a time of the systolic pressure reaching the maximum. Because the blood pressure wave has no incisure in chick embryos, the period between two isochronal lines b and c is conveniently defined as main ejection period. While this period is changed with the heart rate; particularly it is prolonged with decreasing heart rate below the control (250-270 beats/min), isometric contraction period remains unaltered with heart rate (Fig. 6). The present technique developed for acquiring ECG, BCG and arterial blood pressure simultaneously from incubating chicken eggs makes possible isochronal comparison between these waves, which will contribute to investigations on the electromechanical relationships of cardiac contraction in chicken embryos.

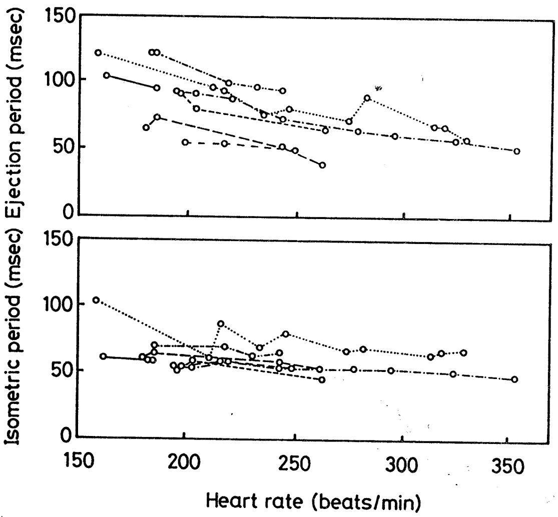

Fig. 6. The isometric contraction (lower panel) and ejection periods (upper panel) of the ventricle as a function of the heart rate. The ejection period is conveniently defined as a time beginning from onset of systolic pressure and ending at a maximum pressure.

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