Location of pacemaker in chick embryo heart at the time of initiation of heartbeat

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VAN MIEROP, LODEWYK H. S. Location of pacemaker in chick embryo heart at the time of initiation of heartbeat. Am. J. Physiol. 212 (2): 407-415. 1967.—Electrograms and intracellular action potentials were recorded of the hearts of chick embryos during the 1st week of incubation. All studies were carried out with the embryos in situ and with spontaneously beating hearts. It has been possible to show that the pacemaker even before initiation of the heartbeat (0 somite embryos) is already located in the sinoatrial portion of the heart, i.e., there is no pacemaker shift from ventricle to sinus venosus during the early phases of cardiac development, as is often stated. The (sino)atrial conduction time has been found remarkably constant at 90-100 msec throughout development. Short bursts of sinoatrial ectopic beats often occurred on impaling a sinoatrial cell. Such tachycardia never resulted from impalement of a ventricular cell. Ventricular action potentials rarely exhibited prepotentials, and then only in the younger specimens.

action potentials of chick embryonic heart; initiation of heartbeat in a chick embryo; initiation of electrical phenomena in chick embryo heart

THE EARLY PRESOMITE vertebrate embryo is represented by little more than a disc or ellipsoid which is spread over its food supply, the yolk sac, and which consists initially of two and, somewhat later, three cell layers. The cardiovascular system develops very early, and consists at first of a bilaterally symmetrical system of endothelial tubes on either side of the midline. As the embryo grows, it pulls away from the yolk sac. The embryonic ellipsoid folds under all along its border and gradually acquires a more tubular appearance. Part of the yolk sac is pinched off in the process and comes to lie within the embryonic body to form the primordium of the gut. Thus, the originally lateral portions of the ellipsoid become the ventral body wall of the embryo, and the endothelial tubes they contain are brought closer and closer together toward the midline. Left and right components of the ventral body walls fuse with each other in a craniocaudal direction to form the floor of the foregut. This is followed shortly by fusion of those parts of the endothelial tubes from which the heart will develop (Fig. 1A). Fusion of these cardiac primordia also takes place in a craniocaudal direction, and in a chick embryo of about 9-10 somites the bulboventricular part of the embryonic heart has been established as a single, medially located tube (Fig. 1B). Its wall consists at this stage of an inner, single layer of endothelial cells, an outer layer, the epicardial mantle, which is one to three cell layers thick and is derived from the splanchnopleuric mesoderm, and a third acellular layer inbetween which, because of its appearance in microscopic sections, is material that has been called the cardiac jelly (Fig. 2A).

The more caudal, sinoatrial portion of the heart is still unfused and only partly invested by splanchnic mesoderm. It is at this stage of development of the chick embryo that Sabin (22) for the first time noted the occurrence of contractile activity of the bulboventricular part of the heart. This observation has since been confirmed by Johnstone (11), Olivo (17), and many others. Patten and Kramer (20) made careful studies on chick embryos cultured in vitro, employing microcinematographic techniques. They completely confirmed Sabin and Johnstone's findings and amplified them. Very faint contractions were seen to appear first along the right border of the conoventricular portion of the tubular heart of the 9- to 10-somite embryo. The active area rapidly spread until the entire embryonic ventricle beat synchronously. No contractions were observed in the splanchnic mesoderm (future epicardial mantle) partially covering the paired atrial portions of the heart.

It has been shown that initially the heart rate is slow and increases as the heart continues to develop. It has also been observed that there exists a cephalocoal gradient in myocardial contraction rate throughout the heart tube: the caudal portions of the ventricle have a faster inherent rate than the cranial portions, the atrium faster than the ventricle, and the sinus venosus faster than the atrium.

Fragmentation experiments carried out by Patten and...
FIG. 1. Approximately frontal sections through the hearts of embryos of 8 somites (A), 10 somites (B), 12 somites (C), and 26 somites (E), and sagittal section through the heart of an embryo of 15 somites (D). In A, fusion of the bilateral cardiac primordia is still incomplete, as is the lumen. In B, remnants of the medially opposed endocardial layers are still present. C shows early bending of the heart tube to the right. In D and E note the scant amount of cardiac jelly in the sinoatrial part of the heart. Early trabeculations are present in the ventricle of the 26-somite embryo and the amount of cardiac jelly here is greatly reduced.
Kramer (20), Cohn (3), Barry (1), and others have shown that the part of the heart with the highest contraction rate sets the pace for the whole organ.

The fact that the fastest beating part of the heart dominates the rhythm of the entire organ, and the observation that newly added portions of the heart have a higher intrinsic rate than the earlier formed parts, led Patten (19) to conclude that there is during development a succession of pacemaking "zones" in the tubular heart (Fig. 3). In other words, since the bulboventricular part of the heart beats first, the pacemaker is located initially in the ventricle. As fusion of the cardiac tubes progresses and new zones of atrium and later sinus venosus are added on, the pacemaker keeps shifting in a craniocaudal direction, while at the same time the heart rate increases. Patten emphasized that the process is a gradual and continuous one (Fig. 3A) and points out that the commonly seen description of a pacemaker which shifts from chamber to chamber as these chambers develop and begin to beat (Fig. 3B) is an oversimplification which, although convenient, is incorrect.

Electrophysiologic studies have been hampered until very recently by technical problems. The currents generated by the tiny embryonic hearts are of extremely small magnitude, and sophisticated electronic equipment is
necessary to demonstrate their presence. Since Wertheim-
Salomonson (23) in 1913 was able for the first time to
obtain electrocardiograms in embryos of about 60 hr
incubation, technical improvements have made possible
the recording of electrocardiograms at progressively
younger ages. Hoff et al. (9) recorded slow sinusoidal
waves in embryos of about 36 hr incubation (15 somites);
Olivo et al. (17) obtained similar records in 9 somite
embryos. A spike preceding the slow waves was seen in
slightly older embryos, and a P wave was demonstrable in
embryos of 20–30 somites. The early sinusoidal curve
and the somewhat later appearing spike have been inter-
preted as being of ventricular origin, since no P
waves were seen and the atrium was not yet differ-
entiated. In other words, these studies seem to bear out
the view that the pacemaker is located initially in the
ventricle and later shifts to the sinoatrial portion of the
heart.

The small size of the early embryonic heart and its
subdivisions makes it impossible to obtain electrocardio-
graphic records which reflect events in sharply circum-
scribed areas. The introduction of the intracellular ultra-
microelectrode by Ling and Gerard (14) has made pos-
sible the study of local electrical events, since the elec-
trode tip is so very small and the potentials recorded are
from the one cell impaled by the electrode.

It has been possible to record intracellular action po-
tentials from both in situ and explanted chick embryo
hearts (7, 13, 15) and even from trypsin-dispersed and
cultured heart cells (4, 6, 12). Such studies have shown
that already, very early in embryonic life, there exists a
functional difference between atrial and ventricular cells.
Marked qualitative differences in action-potential con-
figuration were demonstrated by Lieberman (13) for
sinus venosus, atrial, and ventricular cells of the 72-hr
chick embryo, and by Meda and Ferroni (15) for sino-
atrial and ventricular cells of the 13-somite embryo. In
Lieberman's study the action potentials from sinus veno-
sus cells showed a prepotential; no such prepotentials
were recorded from atrial or ventricular cells. Prepo-
tentials were present in sinoatrial action potentials re-
corded by Meda and Ferroni from 13 somite embryos;
none were seen in ventricular action potentials.

In this paper I will present evidence which shows that:
1) electrical activity is present before there is any visible
heartbeat, and 2) the pacemaker is not initially located
in the bulboventricular part of the heart but is in the
sinoatrial portion from the beginning (Fig. 3C).

MATERIALS AND METHODS

White Leghorn eggs of an average weight of 60 g were
incubated in a forced-draft incubator at a temperature
of 37.7 C. At the beginning of the 2nd day of incubation
the eggs were turned with their blunt ends up, and fur-
ther incubation was carried out in that position. This
caused the embryos to be located below the air chamber,
which allowed for rapid entry of the egg at any time with-
out danger of injury to the embryo. After removing the
shell over the air chamber the inner shell membrane
could be peeled off easily. All studies were carried out
with the embryos in situ. Where necessary, in older em-
byros, the amnion was opened. In all the heart was ex-
posed by removing the body wall and parietal pericar-
dium. In embryos of 20–30 somites the atrium could not
be approached adequately in this way since it was hidden
by the conus cordis. Instead embryos lie face down
on the yolk and cover the entire heart. Furthermore,
these embryos are so small and translucent that they are
difficult to see against the yellow yolk. Accordingly, in
all embryos of 26 somites or younger a cut was made
somewhat more than halfway around the circumference
of the blastoderm and a small rectangular piece of saline-
soaked black paper laid on top of the blastoderm on the
opposite side of the embryo. The cut edge of the blasto-
derm was then grasped with two fine forceps and the
embryo flipped over onto the paper. It was now easy to
see the whitish, translucent embryo against the black
background, and the heart could be exposed without
much difficulty by opening the pericardial cavity. All
manipulations were carried out with the aid of a stereo-
scope. A few drops of warm chick-Ringer solution
kept the superficially located embryos from drying out.
The egg itself acted as a reservoir of heat; heat loss was
retarded by placing the egg in a cup made of paraffin.
The egg was allowed to cool until the heart rate (of
older embryos) was reduced to about 90–130 beats/min.
Total time elapsed between removal of the egg from the
incubator and termination of each experiment was gen-
era1ly less than 15 min, not enough to cause a significant
further drop in temperature, particularly since the am-
bient temperature was kept high.

Both surface electrodes and ultramicroelectrodes were
used. The surface electrodes consisted of a drawn-out
glass pipette manufactured of the same capillary tubing
and in the same fashion as the ultramicroelectrodes, but
with the tip broken off to a diameter of about 50-75 $\mu$.

A length of fine platinum wire, somewhat longer than the pipette, was placed within its lumen with one end of the wire close to the tip of the pipette. The other end was bent over the blunt end of the pipette, which was then pushed into one end of a piece of polyethylene tubing of appropriate diameter, leaving about 1 1/4 cm of platinum wire free onto which a lead wire could be clipped. The opposite end of the polyethylene tube was pushed over a hypodermic needle. A small sleeve of heat-shrinkable plastic tubing was placed over each of the two junctions. Heating these sleeves insured perfectly firm and airtight connections. Thus, a type of microsuction electrode was fashioned. Because of the small size of the heart and the very thin myocardium it cannot be used as a suction electrode in embryos of less than 3 days incubation age. Used singly or in pairs such electrodes were found to be extremely useful and valuable, however, for the recording of electrograms and for the purpose of immobilizing a small portion of the vigorously beating hearts of embryos of 12 somites and older. Very slight suction was used, only enough to make the electrode "stick" to the epimyocardial mantle. Not only is it possible to record electrograms very easily, necessitating only moderate amplification, but the electrode tip remained at the same spot of the heart, no matter how vigorously it was beating. This was important since the electrograms were used for timing purposes. Frequencies lower than 20 cycles/sec were filtered out electronically.

The methodology used in recording intracellular action potentials has been adequately described previously by others (10) and will not be discussed here. Best results were obtained if the resistance of the electrodes was about 40 megohms.

As reference electrode for both types of recording electrodes a silver-silver chloride wire, introduced somewhere in the periphery of the egg, was used. All measurements were carried out on spontaneously beating hearts.
RESULTS

Initial experiments were carried out on embryos of 4-6 days incubation age (stages 23-28 (8)). In these embryos the sinus venosus, atrium and ventricle were anatomically well defined and all three cardiac subdivisions were beating vigorously. Configuration and amplitude of the action potentials recorded from the hearts of 4- to 6-day embryos were virtually identical (Fig. 4) to those reported by Moore (16) for the adult fowl. Duration of the action potentials as given by Moore are somewhat longer (ventricle 200 msec, atrium 120 msec), almost certainly due to slower (60 beats/min) heart rates.

The configurations of the ventricular action potentials in the 4- to 6-day old embryo varied little. The upstroke was very rapid, the apex somewhat rounded without a distinct phase 1. Phase 2 was merely indicated. Clear-cut phases 1 and 2 were only seen with slow heart rates and action potentials of long duration (200 msec or more). Furthermore, in these studies the heart was approached from the epicardial side rather than from the endocardial surface and Purkinje type fibers, which show phases 1 and 2, were probably rarely if ever impaled. The average duration of the ventricular action potentials measured at 50% of total amplitude was 170 msec at cardiac rates of 90-130 beats/min. The amplitude was in the order of 100 mv, varying between 70 and 125 mv.

As has been the experience of others, the lower values were less frequently encountered with increasing experience and refinement of technique. It is very likely, therefore, that the amplitude of the ventricular action potentials of embryos of this age group is already similar to that of the adult bird. Prepotentials were never seen and impaling a ventricular cell never caused periods of tachycardia.

Atrial action potentials were much less uniform in configuration. Action potentials from the periphery of the atrium showed a very rapid upstroke, only sometimes a distinct phase 1, and phases 2 and 3 were rapid. Action potentials of this type were obtained by Moore (16) from the adult parietal atrial wall. At times no distinction could be made between phases 2 and 3 and the action potential had a narrow, peaked appearance similar to those recorded by Moore from atrial pectinate muscle. The duration of the atrial action potentials was always much less than that of the ventricle (avg 73 msec) but the amplitude was similar. Closer to the sinoatrial groove the configuration of the atrial action potentials was often quite different. The total amplitude was less, but the duration was again about 70-75 msec. The upstroke was much less rapid and a distinct prepotential of about 15 mv was present which terminated in the following upstroke either abruptly or more gradually. The appearance of these action potentials (Figs. 4, 5) was nearly identical to those recorded by Moore (16) from the adult, right sinoatrial valve.

In embryos of between 48 and 72 hr incubation (stage 15-19) the ventricular action potentials resembled those obtained in older specimens, but their average duration was less (125 msec). The amplitude again was in the order of 100 mv (Fig. 6). An occasional ventricular cell exhibited a prepotential of low magnitude, always terminating abruptly, however, in a rapid upstroke. Atrial action potentials were generally similar to those obtained in older embryos from the area near the sinoatrial groove. There was a distinct prepotential and the transition into a rather slow upstroke was rounded. Duration was only slightly greater, the amplitude lower. Since the heart, particularly the atrial portion, in embryos younger than 48 hr could be approached easier from the left side, all embryos of stage 16 and younger were inverted as described above.

In embryos of about 48 hr incubation (stage 16, 26 somites) the ventricular action potentials were again similar in configuration and amplitude as those described above, but their duration was less (avg 110 msec) (Fig. 7). Low-magnitude prepotentials were recorded from a few ventricular cells.

Atrial action potentials were also similar to those of the slightly older embryos but their amplitude was less and their duration was greater and only slightly less than that of the ventricular action potentials. Impaling an atrial cell not infrequently caused a transient increase in cardiac rate.

Action potentials in embryos of 40 hr incubation (stage 13, 19 somites) and younger were recorded with increasing difficulty. The ventricular myocardium in these very young specimens is only 1-3 cell layers thick and the individual cells are small, containing relatively large nuclei. The decreasing vigor of the heartbeat, on the other hand, in part compensated for these disadvantages, making it easier to immobilize a small portion of the heart, and it was possible to obtain satisfactory records (Fig. 8). The amplitude of the ventricular action potentials decreased progressively and their duration became somewhat more variable. Occasionally, as in the slightly older specimens, prepotentials were seen. The transition between such a prepotential and the following upstroke was always abrupt, and impalement of a ventricular cell was never seen to cause an increase in heart rate.

Nineteen-somite embryos were the youngest specimens in which the atrioventricular junction was anatomically well defined and in which the atrium was always seen to beat. Atrial beats always preceded ventricular contractions. Simultaneous recording of a ventricular electrogram clearly showed the atrial action potentials to precede the ventricular action potentials by about 100 msec. The duration of the atrial action potentials was about the same as that of the ventricular action potentials.

Before proceeding further a brief digression is in order here.

The ventricular part of the heart in these and all younger stages is not trabeculated and a thick layer of cardiac jelly separates the thin myocardial layer from the endocardium. It is further of interest that the char-
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FIGS. 5-11. Sinoatrial and ventricular action potentials and electrograms recorded from hearts of embryos of 6 days, 54 hr (90 somites), 48 hr (80 somites), 40 hr (19 somites), 33 hr (13 somites), 31 hr (11 somites), and 28 hr (8 somites) incubation age. Paper speed 25 mm/sec; time lines, where present, 100 msec in Figs. 6 and 8-11, 1 sec in Fig. 5. Crosshatching = trabeculated portion of ventricles. A, atrium; AVC, atrioventricular canal; RCCV, rt common cardinal vein; SV, sinus venosus; V, ventricle; E = position of surface electrode.
acter of the ventricular contractions is no longer synchronous, but peristaltoid, and electrograms recorded from different points in the ventricle showed the conduction velocity to be rather slow. This slow intraventricular conduction velocity has to be taken into account in estimating the actual conduction delay across the atrioventricular junction.

In embryos possessing 15–16 somites the atrioventricular junction could no longer be accurately determined. However, the atrium did beat, and again its beat preceded that of the ventricle. In 33-hr embryos (stage 11, 13 somites) no atrial beat could be seen. If a sinoatrial cell was impaled, however, its action potential, taking into account the slow intraventricular conduction velocity, was again seen to precede a ventricular action potential by about 100 msec (Fig. 9). The duration of the atrial action potentials showed a further increase. Here, and in all younger embryos, impalement of a sinoatrial cell was usually followed by a brief period of tachycardia.

In 31-hr embryos (stage 10, 10 somites) the bulboventricular part of the heart was always seen to beat, the sinoatrial part never. Here again, however, sinoatrial action potentials could be recorded which were followed after an interval of about 100 msec by a ventricular action potential and a peristaltoid ventricular contraction (Fig. 10). Both the action potentials and the contraction waves at times occurred in groups separated by pauses lasting several seconds. This phenomenon has been referred to as “Lucciani grouping” (19). A bigeminal rhythm was also occasionally observed in these and younger specimens. Such bigeminy always involved both the sinoatrial and bulboventricular parts of the heart.

Twenty-nine-hour (9-somite) embryo hearts were occasionally seen to beat faintly; no visual heartbeat was ever seen in 38-hr (8-somite) embryos. Yet, in most of the 9-somite embryos and some of the 8-somite specimens it was possible to record ventricular electrograms and sinoatrial and bulboventricular action potentials, and in both the sinoatrial action potentials preceded the ventricular action potentials by about 100 msec (Fig. 11). No electrical activity was ever recorded in embryos younger than 8 somites.

DISCUSSION

Patten's concept on the development of the heartbeat and the localization of the pacemaker are based in part on the assumption that the pacemaker is located in whichever cardiac chamber (or portion thereof) is actually beating. If the heart tube of a 12- to 13-somite embryo is cut, e.g., at the atrioventricular junction, and only the ventricular segment continues to pulsate, it is obvious that this segment must now have acquired its own pacemaker. It does not necessarily mean, however, that the atrial, quiescent segment does not possess a pacemaker. It only indicates that its myocardium is not able as yet to contract. De Haan (5) has postulated that there are two populations of cells in the heart, one concerned chiefly with impulse generation and conduction, the other with mechanical contraction. Meda and Ferroni (15) reported qualitatively different action potentials from the ventricular and sinoatrial portions of the 13-somite embryo heart. Action potentials from the sinoatrium showed features which are at present considered to indicate pacemaker activity and strongly suggest that the pacemaker in these 13-somite embryos is located in the sinoatrium. Their study did not prove, however, that this is so, since sinoatrial and ventricular action potentials could not be related to each other in time. It would still be possible that the ventricle had its own pacemaker and was beating independently. Meda and Ferroni's data show some peculiarities. The heart rate of the 13-somite embryo as calculated from the illustration given, and using the timing bars shown, would be either about 70 beats/min or about 160 beats/min. Both of these values, particularly the latter, seem rather high for such a young embryo. The ventricular action potential duration calculated from the illustration (less than 100 msec) is far less than that given in the table (249 msec ±10). Finally, it is rather surprising that with an incubation temperature of 38°C a 42-hr embryo only possesses 13 somites. My own data and those of others (2, 18, 21) show embryos of that age to have 18–20 somites at an incubation temperature of 37.5°C. Conversely, a 13-somite embryo would be only 33–36 hr old. Meda and Ferroni's data as to age and number of somites, however, agree with those given by Hamburger and Hamilton (8).

The data presented in this paper show that cardiac electrical activity is present in embryos as young as 8 somites, before there is any discernible contractile activity. Moreover, the pacemaker is located in the sinoatrial portion of the heart from the beginning and the ventricle simply follows it even if arrhythmias are present. It is interesting that in the very young embryos impalement of a sinoatrial cell so often induced a period of tachycardia lasting for a few seconds. These short bursts of sinoatrial ectopic beats were always faithfully followed by contractions of the bulboventricular part of the heart.

The constancy of the atrioventricular conduction time throughout embryonic development is remarkable. The values found in the very young embryo (90–100 msec) are similar to those recorded in older specimens (85–105 msec) and agree very well with those reported by others both for embryos (2, 23) and adult fowl (16).

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REFERENCES


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