In Vitro Culture of Embryonic Chicken Hearts

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Objective

The goals of this lab are to identify the anatomy of the developing chick heart, determine the direction of blood flow through the developing heart, and to observe how the heart develops over time.

Introduction

The chicken is a classic organism used to illustrate the principles of basic embryology. One of the developmental systems that has been examined in great detail is the circulatory system. In the developing embryo, the circulatory system is the first functional unit and the heart is the first functional organ. The embryonic chick (and mammalian) heart first forms as a single loop (Figures 1 & 2) on the outside of the embryo. This will enable us to clearly observe the ongoing contraction and relaxation of the heart.

The heart of the chicken embryo develops from the fusion of paired precardiac mesodermal tubes, forming a straight anterior to posterior ventricular tube. After fusion is complete, the heart tube has four distinct regions: bulbus cordis, ventricle, atrium, and sinus venosus. Pulsations in the heart starts while the paired primordial cells fuse. The sinus venosus is the pacemaker of these initial contractions (Gilbert 2010). After approximately 33 hours the heart tube bends to form an "S" shape structure with a single atrium and a single ventricle. By 2 days the heart has folded upon itself forming a single loop. This moves the sinus venosus and atrium to a position anterior and dorsal to the ventricle and the bulbus cordis. In 3 day-chick embryos, the atrium has begun to expand to the left in preparation of the division into the right and left atria. Although the heart still has two chambers at this time, communication between the sinus venosus and the atrium occurs through the right side of the atrium. Times of development may vary. Eventually, when the atrium and ventricle divide to develop a typical four-chambered heart, the sinus venosus will be incorporated into the right atrium. The bulbus cordis will eventually give rise to the aorta.

In this experiment, we will remove hearts from 3-day chick embryos and maintain them under specific conditions that allow for their development. By this time the two-chambered heart should be visible as well as the blood flow entering the lower chamber and being pumped out through the aorta.

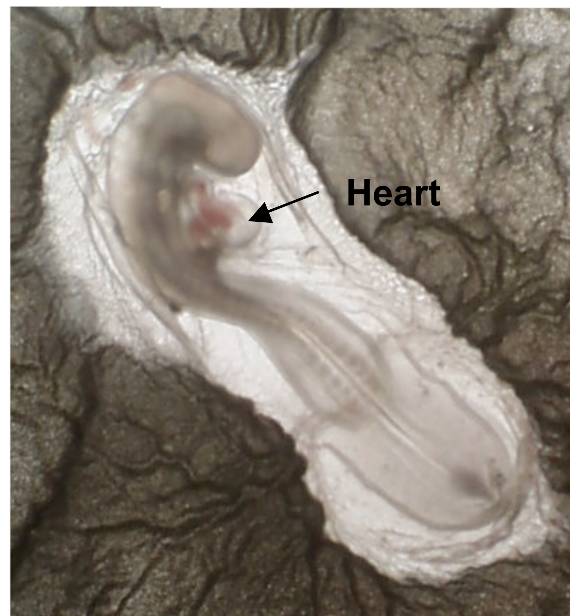

Figure 1. Three-day chick embryo, H&H stage 15.

Materials

2-day and 3-day chick embryos
70 % ethanol
PVC pipe section or finger bowl
100 mm and 60 mm Petri dishes
24-well tissue culture plate
scissors and fine forceps
Sterile Howard's Ringers with 50 μg/ml gentamicin
Dulbecco's Modified Eagles's Medium (DMEM) with 2 % fetal calf serum (FCS) and
50 μg/ml gentamicin

Procedure

1. Prepare a clean working area. Wipe off tools with 70% ethanol. Obtain 1 large Petri dish and PVC pipe section or finger bowl. Set up several small Petri dishes with Howard's Ringers.

2. Wipe off egg with 70% ethanol; rotate so that embryo moves freely. Set egg down on its side for several minutes; the embryo should float to the upper surface. Crack the 'lower' surface of the egg on the PVC pipe ring and open egg into large Petri dish. Allow egg to sit for a few minutes to allow the albumin to flow off of the top half of yolk.

3. Drop a filter paper disc around embryo. Hold on to the filter paper with fine forceps and cut around the ring with sharp scissors. Gradually lift the paper disc with the forceps, the embryo should remain within the center of the disc. Transfer to warm Howard's Ringers solution. Dissect away the extra-embryonic membranes.

3a. The 3-day embryo can also be removed using a spoon. Puncture egg at wide end (above air space) using point of sharp forceps. Carefully remove shell above air space. Using forceps, peel back the shell membrane to expose the embryo. Keeping hold of the embryo with the forceps, cut around it with fine scissors. Pick up a spoon with your cutting hand and slide it under the embryo. Continue to hold onto the embryo, lift with the spoon and transfer to the petri dish filled with Howard's Ringers solution.

4. Using fine forceps, sever the trunk above and below the heart region. Then, carefully remove the dorsal region of the embryo, leaving the heart intact.

5. Identify the sinus venosus, atrium and ventricle. Measure the heart rate, and determine the direction of blood flow.

6. Transfer the heart to tissue culture media (2% FCS in DMEM) in 24-well plate. Check for a change in heart beat rate or pattern.

7. Incubate in CO_2 incubator overnight. Check for beat and evidence of development the next day, and thereafter.

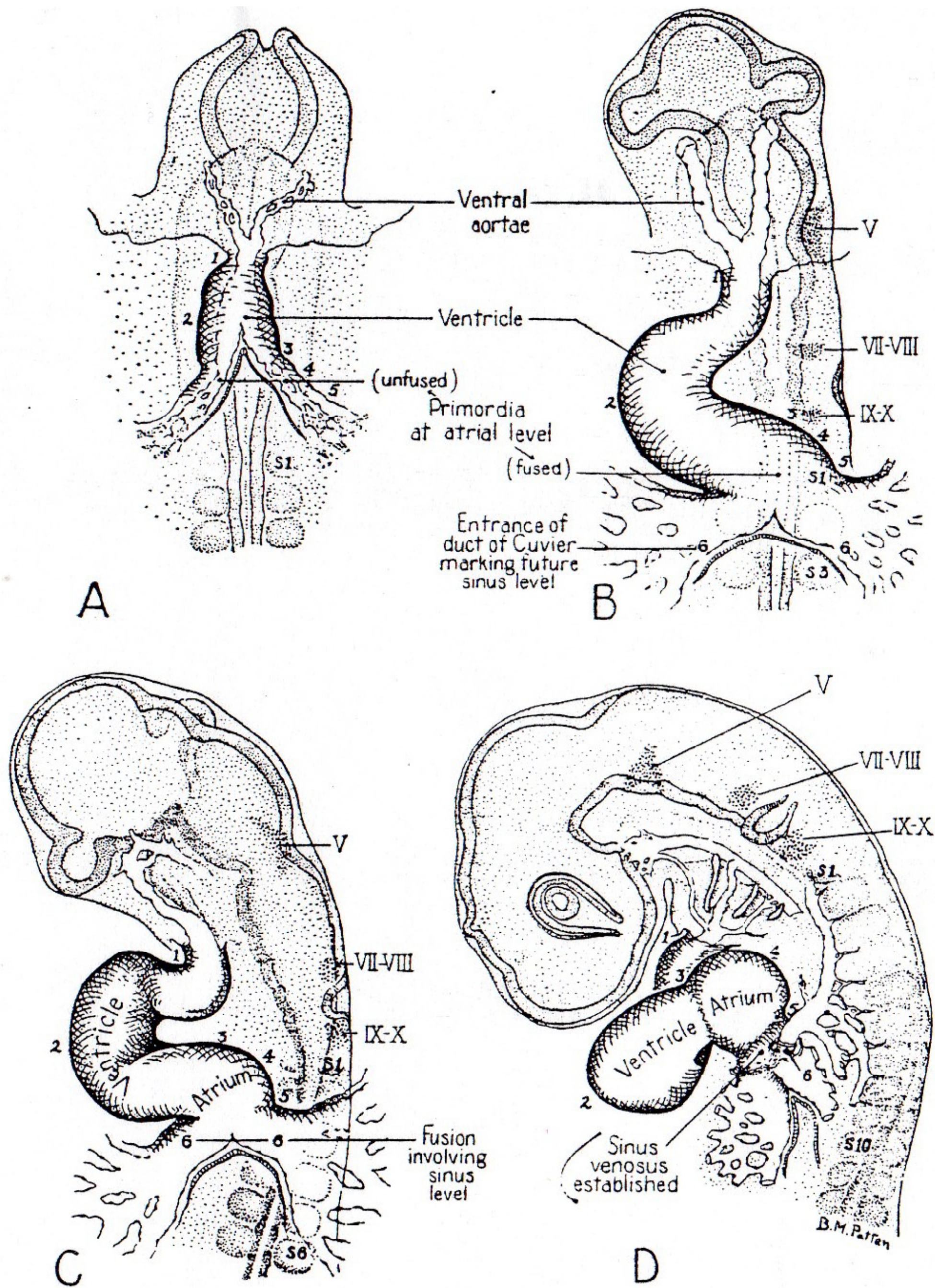

Figure 2. Chick heart development. From Patten, 1951.

References

http://www.millersville.edu/~jcebrathomas/cebra_thomas/DB_lab/Chick/Aaronhearlab.html

Gilbert, Scott. 2010. *Developmental Biology*, 9th ed. Sinauer Associates pp. 446-454.

Patten, Bradley M., *Early Embryology of the Chick*, 4th edition, McGraw-Hill, New York, 1951.

Instructor's notes

1. Fine forceps (e.g. Dumont #5) are very valuable for microdissection.
2. Tissue culture supplies are needed which make this lab more expensive. Many reagents shared with chick skin culture lab.
3. Pen/strep could be substituted for gentamicin sulfate.
4. Culture of hearts at loop stage is more technically difficult for the students, but in some cases evidence of organogenesis can be observed.

Order information

DMEM (Fisher MT-17-204-CI, 6 x 100 ml, $76)

Fetal calf serum (Fisher BW14507E, 100 ml, $89)

Costar 24 well plates (Fisher 09-761-146, case of 50, $183)

Gentamicin sulfate, 10 mg/ml (Fisher 50841714, 10 ml $32; Teknova G3625, 10 ml, $18) or 50 mg/ml (Invitrogen, 15750060, 10 ml, $48.50)

<u>Howard Ringer's solution</u>

14.4 g	NaCl
0.34g	$CaCl_2$ (anhyd.) or 0.46g $CaCl_2.2H_2O$
0.74g	KCl

dH_2O to 2 liter