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Developmental Bio
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Cytokine induced angiogenesis in chick embryos
Based on procedure developed by Katelyn Johnson, F&M College '02
Dev Bio Lab CD
file://localhost/Volumes/Bio24_CD/DB_lab/Chick/KJohnson/Chick_blood.html

Introduction

Several growth factors are involved in the development of the blood system in both developing chick embryos and in the yolk sacs themselves (Gilbert, 2003). Basic fibroblast growth factor (bFGF, FGF2) functions in vasculogenesis, the process during which blood vessels are created (Gilbert, 2003; Johnson, 2001). It is this process which is responsible for the appearance of "blood islands" in the yolk sac (Gilbert, 2003). Prior to the actual appearance of these islands, specified mesoderm cells must first from cells called hemangioblasts, a specification brought about by bFGF (Gilbert, 2003). It is these cells that form the blood islands and give rise to either angioblasts or hematopoietic stem cells (Gilbert, 2003). These cells, then, respectively, form endothelial cells and blood cells, in time resulting in the formation of capillaries (Gilbert, 2003). Following vasculogenesis, the process of angiogenesis gives rise to a more complex vessel system derived from these established capillaries (Gilbert, 2003; Johnson, 2001).

Vasculogenesis and angiogenesis, in particular, are key within the chorioallantoic membrane (CAM) of the developing embryo (Johnson, 2001). This membrane is located beneath the shell membrane removed from embryos in previous labs. It is here that calcium, needed for the formation of the skeleton of the chick embryo, is absorbed into the blood vessels in contact with the eggshell (Gilbert, 2003; Johnson, 2001).

Objective

This experiment seeks to determine the effect of the growth factor bFGF on blood vessel formation within the CAM membrane.

Gilbert, Scott. 2003. *Developmental Biology*, 7th ed. Sinauer Associates, Inc., MA, pp. 501-505; 517.

Johsnon, Katelynn. "Cytokine induced angiogenesis in chick embryos." DB Lab. 17 January 2004. Comp. Judith Cebra-Thomas. Swarthmore Server. 27 January 2004 file://localhost/Volumes/Bio24_CD/DB_lab/Chick/KJohnson/Chick_blood.html

Materials

1 Petri dish
10 day old chick embryos (4)
Magic Scotch Tape
3MM Filter Paper (3mm x 3mm disks)
Forceps, Scissors
4 mL of either bFGF (1.5 μg/m) or DMEM

Procedure

1) Obtain 4mL of either bFGF (1.5 μg/m) or DMEM* (control). Each member of the group is responsible for one of these factors. Pour the solution into a petri dish that is labeled with the name of the compound you are working with. Soak four filter paper disks (3mm x 3mm) in the solution for 30 minutes.

2) After the filter paper has been prepared, obtain four ten-day-old chick embryos and wash them in 70% ethanol. Label the eggs as either DMEM or bFGF using a pencil. Open the chick embryos at the blunt end of the egg as demonstrated in class. Peel back the shell membrane, being careful not to damage the CAM membrane.

3) Drop the filter paper over the embryo in the area with the least number of visible blood vessels. Cover the hole in the shells with Scotch tape, and place in the incubator (labeled Humidaire) for four days at 37°C.

4) After the four-day period, remove the tape covering the holes and extract the filter paper using a pair of forceps. Examine the filter paper for the presence of blood vessels. Count all blood vessels on the filter paper and record the numbers within your lab notebook. Compare to the embryos treated with the other compound.

* DMEM is defined as a "tissue culture medium" (Johnson, 2001).