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Endochondral Ossification of Chick Embryonic Femora *in vitro* and on Chorioallantoic Membrane

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We observed angiogenesis and endochondral ossification of the femora of chick embryos *in vitro*, on chorioallantoic membrane (CAM), and *in vivo* to clarify the cellular processes of avian endochondral ossification. We found that the inside of the *in vitro* femora was still filled with chondrocytes, despite being cultured for 10 days, while calcification of the diaphysis was not observed. As well, only the cartilage tissue of the epiphysis was enlarged. By contrast, blood vessels invaded into the diaphysis and the marrow cavity was formed in CAM-cultured femora. Cartilage canals extended from the marrow cavity and reached to the resting chondrocyte zone, with normal endochondral ossification occurring as *in vivo*. This study demonstrates that endochondral ossification occurs in femora in CAM culture similar to that in *in vivo* femora, but not in *in vitro* femora. The ossification is dependent on vascular invasion into the embryonic femora. In conclusion, for the endochondral ossification of long bones it is essential to supply the embryo with blood vessels. CAM culture system was found to be a superior endochondral ossification model of the embryonic femur.

Keywords: <u>chick embryo</u>, <u>chorioallantoic membrane</u>, <u>culture</u>, <u>endochondral ossification</u>, <u>femur</u>

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