

Early *in-vitro* histological chondral differentiation

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Summary. *In vitro* chondrogenesis is possible in the chick embryo from stage 4 of Hamburger and Hamilton (1951), only 18-19 hours of incubation, before somite formation. In stage 4 of Hamburger and Hamilton (1951) the chondroblasts are placed laterally to the primitive streak and notochord cells are not necessary for cartilage differentiation.

Key words: Chondrogenesis, Chick, Primitive streak

Introduction

It is accepted that the chick (*Gallus domesticus*) is laid down as cartilage and later on ossified. In the formation of embryonic cartilage four histological stages can be recognized (Romanoff, 1960); a) mesenchymal condensation without orientation of nuclei; b) mesenchymal condensation with elongation and parallel alignment of nuclei; c) prechondral stages, with appearance of intercellular matrix in increasing amounts until it completely encircles the cells; d) metachondral or hypertrophic stage, with enlargement of cells and formation of additional matrix.

Prior to histological differentiation monoclonal antibodies can be used (George-Weinstein et al., 1988) to distinguish chondrogenic subpopulations of chick embryonic cells from stage 15 of Hamburger and Hamilton (1951). It also appears that chondroitin sulphate is associated with chondrogenesis of the chick embryo (Bellairs, 1971; Goldstein et al., 1986; Kimata et al., 1986). *In vitro*, the concentration changes in cyclic AMP and prostaglandin are the cause of chondrogenesis in chick limb mesenchyme (Biddulph et al., 1988). Retinoic acid acts in the opposite way (Biddulph et al., 1987). Furthermore, *in vitro* tenascin

causes chick chondrogenesis (Mackie et al., 1987).

Zwilling (1960) and Murillo-Ferrol et al. (1978) reported that histological chondral differentiation is possible from the area pellucida of early chick embryos in stages 4 and 5 of Hamburger and Hamilton (1951). They made enzymatic dissociation of whole chick area pellucida and grew them in organ culture, in explant culture on different substrata and on the chorioallantoic membrane of the host chick.

Further information about this specific case is not available. The aim of the present study is to find out whether an earlier chondral histological differentiation is possible and if the notochord is necessary for his differentiation in the early chick embryo.

Materials and methods

Babcock 8300 chick eggs were incubated for 18-19 hours, up to Hamburger and Hamilton (1951) stage 4, in a 38° C static incubator. The embryos were removed from the yolk and vitelline membrane and placed in Moscona's saline (1952). The area pellucida of embryos was isolated and then prenatal areas removed and discarded. Thus, only postnatal areas were used (Fig. 1A).

Thirty chick embryos were dissociated by enzymatic (Moscona, 1952) and mechanical (Auerbach and Grosbstein, 1958) techniques. Three cultures of dissociated cells were grown 24 hours in Eagle's organ culture (1959), in a 38° C rotating incubator. The three cultures were then transferred to 35 mm Falcon dishes each containing a layer of Agar-Tyrode-Equine Serum (Wolff and Haffen, 1952) about 2 mm deep and the explants were grown for 4 days. After that, the three explants were grown 6 days on the chorioallantoic membrane of the host chick, with a modification (Murillo-Ferrol et al., 1971) of the technique of Wolff (1936). Two grafts were recovered.

In a second experiment, another 240 chick embryos were excised, but only their primitive streaks were

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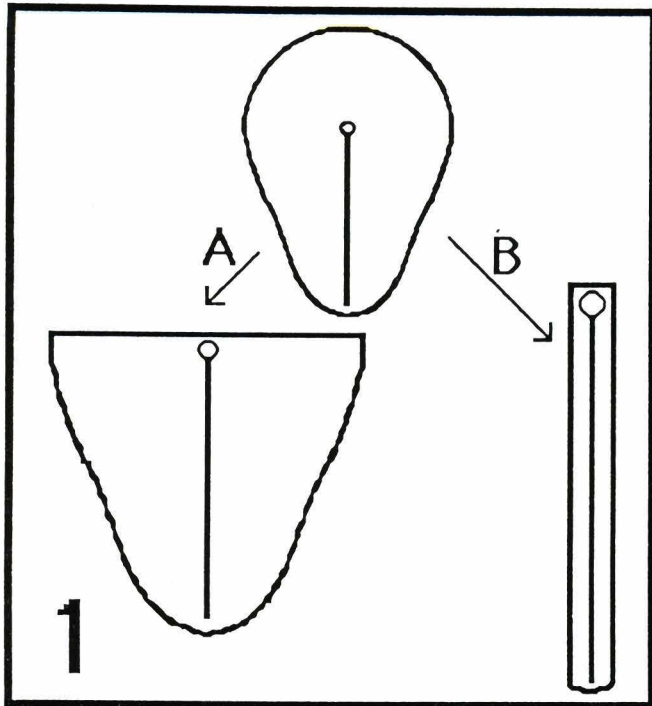


Fig. 1. Schematic diagrams of two experiments performed in stage 4 of Hamburger and Hamilton. A. Excision and removal of prenatal area. B. Primitive streak area selected.

used in the dissociation technique (Fig. 1B). Then the same steps indicated above were followed and two grafts from four 35 mm Falcon dish cultures were recovered.

These four grafts were dehydrated, paraffin-embedded and sectioned at 4 μ m. Hematoxylin and eosin was used as histological stain.

Results

From postnodal area pellucida

Histologically, plenty of cartilage was found, in some cases with muscle tissue (Fig. 2), in others around endodermic epithelium or inside the mesenchyme (Fig. 3). Sometimes the cartilage presented a segmentary shape with some of the segments already ossified (Figs. 4, 5).

Some tubular shapes showed epithelium enclosed by cartilage (Figs. 6, 7) as in the respiratory system. Cartilage surrounding the digestive epithelium was also found (Figs. 3, 8, 9) close to nephritic tubes (Fig. 10), and near glandular tissue (Fig. 11).

All the cartilage that was found was in metachondral or hypertrophic stage, with enlargement of cells and formation of additional matrix.

From primitive streak

Histologically, no cartilage was found.

Discussion

In the present study, cartilage tissue from embryos younger than those used by Murillo-Ferrol et al. (1978) was found. Zwilling (1960) like Murillo-Ferrol et al. (1978) used the whole area pellucida of the embryos; in this study, however, its prenatal region was discarded (Fig. 1). Zwilling (1960) also used chick embryos from stage 4 of Hamburger and Hamilton (1951); however we think he did not obtain a complete mechanical dissociation only when using the pipette Repeatedly.

Cellular populations from the area pellucida were lost when its prenatal region in which the notochord cells are placed was discarded; however, in absence of these cells, histological cartilage differentiation did not change. Thus, this fact demonstrates that in stage 4 of Hamburger and Hamilton (1951) prenatal cells are not necessary for *in vitro* chondral differentiation when applying dissociation and reaggregation method.

On the other hand, the results from primitive streak indicate that these cells in stage 4 of Hamburger and Hamilton (1951) cannot differentiate cartilage tissue.

In summary, the following conclusions can be made: 1) Chondral differentiation is possible from stage 4 of Hamburger and Hamilton (1951); 2) In stage 4 of Hamburger and Hamilton (1951) chondroblasts are placed laterally to the primitive streak; 3) In stage 4 of Hamburger and Hamilton (1951) notochord cells are not necessary for cartilage differentiation.

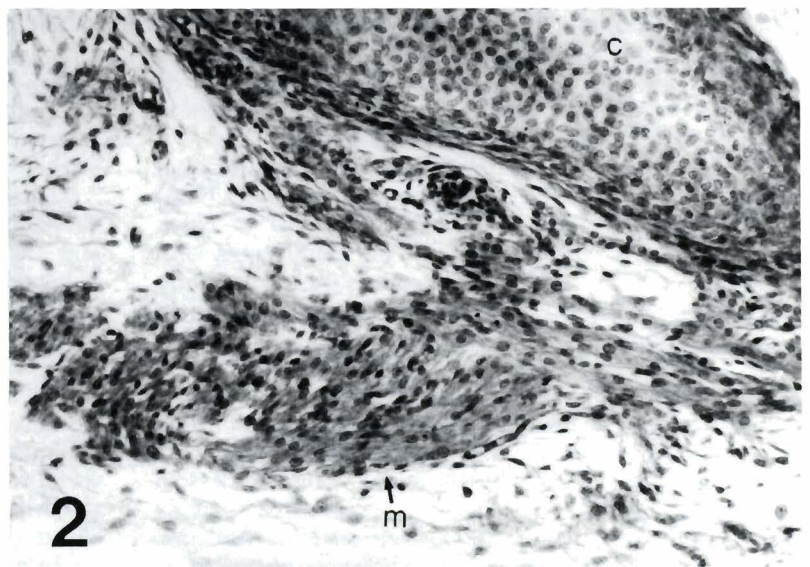


Fig. 2. Miocardial tissue (m) and cartilage (c). $\times 640$

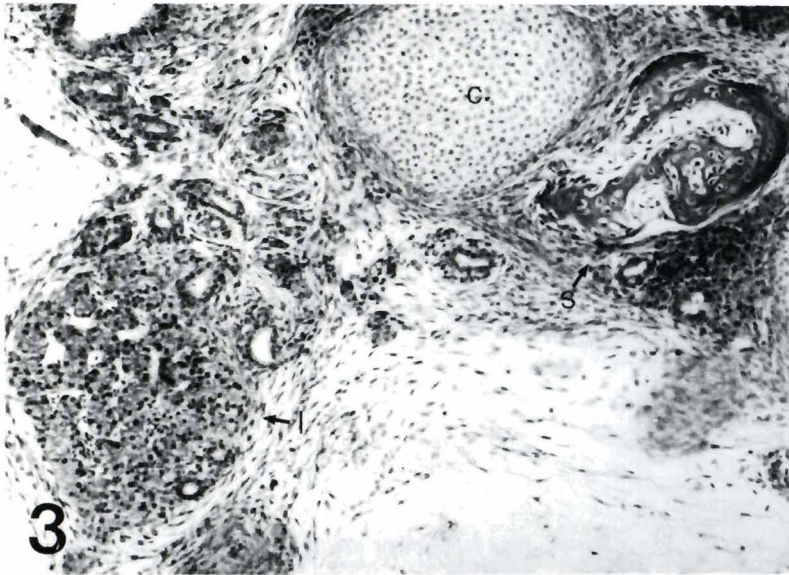


Fig. 3. Cartilage (c), bone (s) and hepatic tissue (l).
× 250

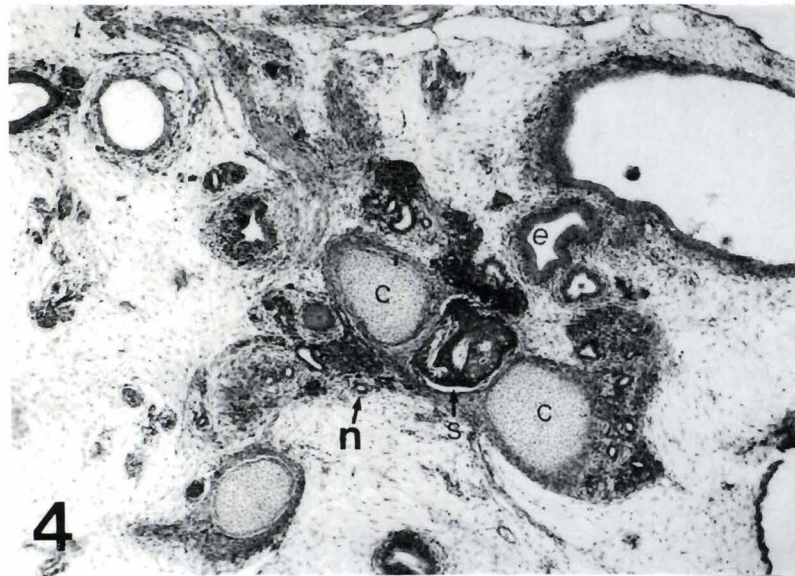


Fig. 4. Segments already ossified (s) inside cartilage (c), nephritic tube (n) and endodermic tube (e). × 160

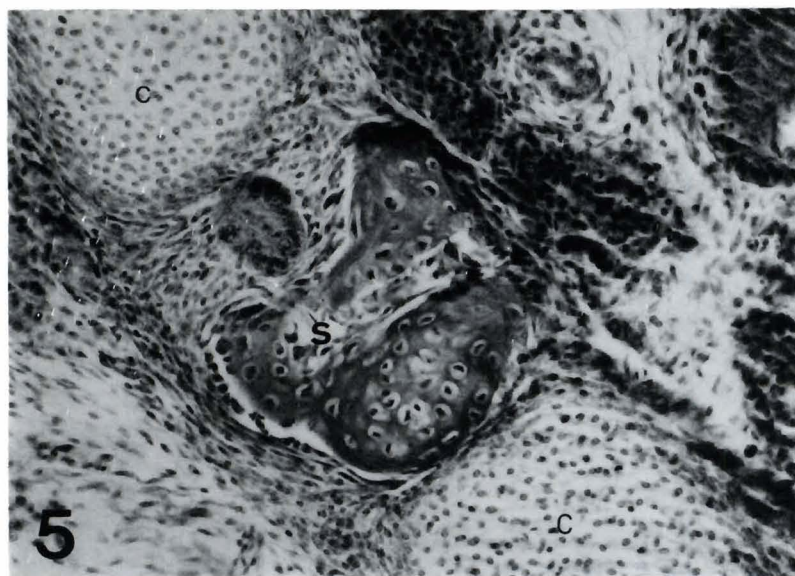


Fig. 5. Segments become ossified (s) inside cartilage (c). × 400



Fig. 6. General vision of a section from one graft. Cartilage (c), contractile tissue (a), and endodermic tubes (e). $\times 160$

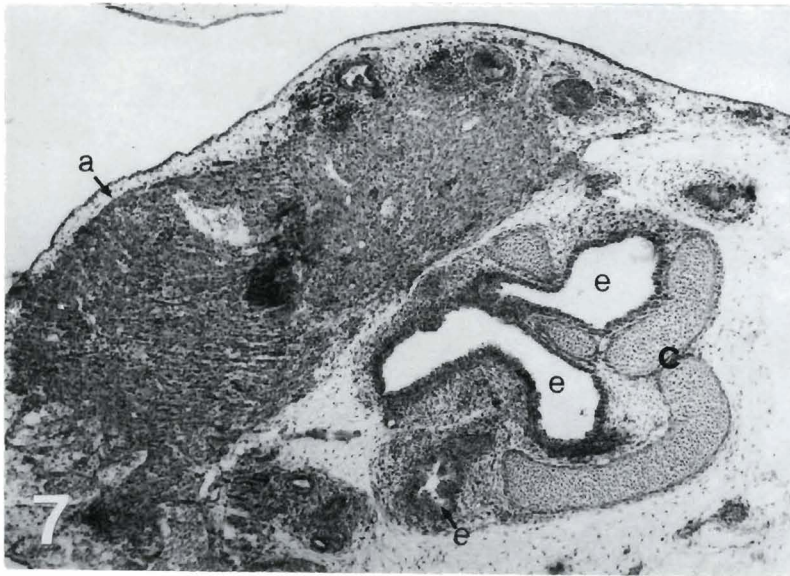


Fig. 7. Endodermic tissues (e) enclosed by cartilage (c), and contractile tissue (a). $\times 160$



Fig. 8. Cartilage (c), endodermic tube (t) and hepatic tissue (h) $\times 100$



Fig. 9. Chondrogenic node (c) next to hepatic tissue (l), and endodermic tube (e). $\times 250$

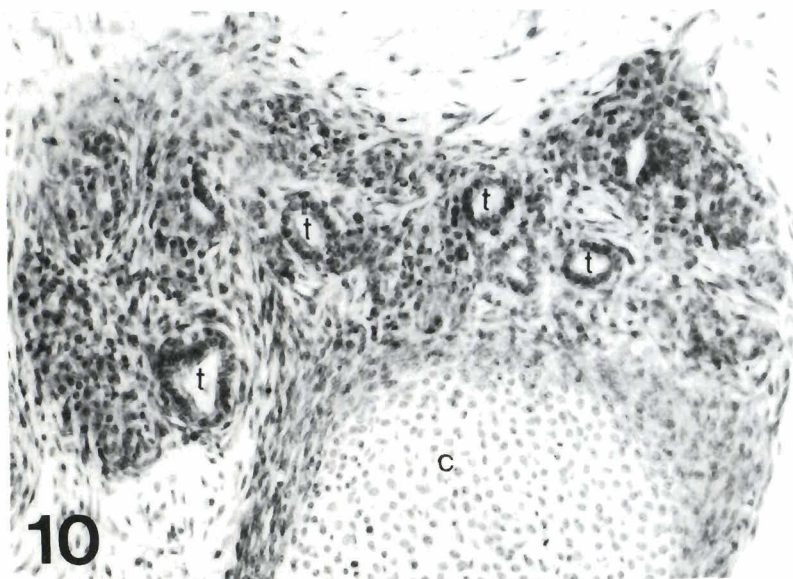


Fig. 10. Nephritic tubes (t) beside cartilage (c). $\times 450$



Fig. 11. Bone (h) and glandular tissue (g). $\times 250$

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