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IMMUNOHISTOCHEMICAL STUDY OF THE EXTRACELLULAR MATRIX DURING SOMITOGENESIS IN THE CHICK EMBRYO

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Five dozen selected fertilized eggs of *Gallus gallus dom*. L (White leghorn) were purchased from Granja Tolomei, Rio de Janeiro, and incubated at 38°C, in a humidified egg incubator, for periods between 24 and 72 h in order to study the somitic embryo at stage 14 of Hamburger & Hamilton (1951, *J. Morphol., 88*: 49-92), which enables the observation of four different phases of somite development: a) caudally situaded unsegmented mesoderm; b) transitional mesoderm; c) newly formed somite; d) cephalic mature somite.

Early blastoderms were excised from their yolk in PBS solution and the vitelline membranes were removed with filter paper rings as previously described (J. W. Lash, 1967, *J. Exp. Zool., 165*: 47-56). Embryos were washed and replaced in PBS solution in 35 mm petri dishes for observing and staging by criteria of Hamburger & Hamilton (loc. cit.) and soon after they were immersed in a solution containing Bouin's fixative. After 1 h of fixation, the specimens were transversely cleaved either at the level of the segmental plate or last pair of somites or 20th pair of somites or 12th pair of somites and remained 1 h more in the fixative solution.

Then, the fragments were washed for 20 min in running water, dehydrated in ethanol of several concentrations (starting on 50% alcohol and finishing on absolute alcohol), cleared in two changes of xylene and infiltrated plus embedded in melted paraffin. Five micrometer sections of the blocks were deparaffinized, hydrated to 70% ethanol and subsequently were treated with 70% methanol containing 30% hydrogen peroxide and then incubated with 1% goat serum for 2 h and exposed for 1 to primary antibodies to type I, III, IV and VI collagens (policlonals), fibronectin (monoclonal), heparan sulfate (monoclonal), chondroitin sulfate (monoclonal) and elastic microfibril (monoclonal HB8). After treatment of 1 h with rabbit or mouse antiserum, and of 1 h with avidin-biotin-peroxidase complex, the sections were incubated with diaminobenzidine containing hydrogen peroxide and then washed, stained with hematoxylin, dehydrated, cleared and mounted with coverslip using neutral Canada balsam. Specificity controls included: a) substitution of primary antibody by PBS; b) mouse and quail tissues including muscles, cartilages, nervous tissue and embryonic tegument.

Positive reactin for type IV collagen in basement membranes and for type I, III and VI collagens between somite cells in later stages of somitogenesis has been observed. Also strong reaction for heparan and chondroitin sulfates has been accompanied by weaker positivity for fibronectin. Negative reaction for microfibrils suggests that such a component of extracellular matrix is only expressed in a further stage of somite development.

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