DIVERGENT DIFFERENTIATION OF SKELETOGENIC TISSUES IN IMPLANTS

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The mesenchyme of 8- and 12-day,and cartilage of 18-day rabbit embryos were implanted into male rabbits under the subcutaneous muscle. At the stages of the 8th and subsequent days of embryogenesis the skeletogenic tissues of the rabbit are already determined, although they remain capable of modifications of development. Growth of the newly formed pieces of cartilage is organotypic in character. Differentiation of skeletogenic elements depends on the conditions of vascularization.

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The distinctive properties of skeletogenic elements are clearly manifested during delayed growth, when signs of differentiation predominate [3]. Amprino, Saunders, and other workers [5, 6, 10], in experiments on chick embryos, studied differentiation of supporting tissues after transplantation of limb analagen and showed that the factors influencing morphogenesis of the limbs have not been precisely established. According to Kreutz [9] and Krompecher and co-workers [8], differentiation of skeletogenic tissues depends on the intensity of vascularization.

The object of the present investigation was to define the stages of determination and to study the differentiation of skeletogenic elements by using Lazarenko's method of tissue and organ cultivation [2, 4].

EXPERIMENTAL METHOD

The mesenchyme and cartilage tissue of rabbit embryos at the stages of 8 (series I), 12 (series II), and 18 (series III) days of intrauterine development were implanted beneath the subcutaneous muscle of the anterior abdominal wall of male chinchilla rabbits aged 4 months. The control and experimental material was fixed in Peisakhovich's fluid and 10% neutral formalin solution. Altogether 52 implants were studied in the course of the experiment from 12 h to 150 days after implantation. The specimens were stained by the usual histological methods. Carbohydrates were identified histochemically (PAS reaction by McManus's method, Hale's colloidal iron reaction, alcian blue by Steedman's method), chromotropic substances were detected by the metachromasia reaction at different pH values with toluidine blue, with fast cresyl violet in my modification [1], with basic fuchsin,and with azure I. Nucleoproteins were determined by Feulgen's and Brachet's method, and with chrome lake by Einarson's method, and calcium ions were detected by Dahl's reaction with alizarin red [7].

EXPERIMENTAL RESULTS

On the first day of the experiment a profuse exudation developed in the zone of the implant, and large numbers of PAS-positive granules, diffusely distributed throughout the implant, appeared in the fibrillary structures of the grafted pieces of tissue (similar to the control findings). Fibrinous clots formed loose PAS-positive membranes between the transplanted pieces and intercelloidin spaces filled with plasma and blood cells.

On the 2nd-3rd day, proliferation of cells of the graft bed and of the implanted structures began. Inflammation at the periphery of the implant moved into the fibroplastic stage.

On the 4th-6th day of the experiment a mutual ingrowing took place of the newly formed connective tissue of the transplanted pieces and the tissues of the graft bed. Perichondrium formed around the pieces of cartilage, and a process of divergent differentiation of the transplanted mesenchyme began. In the foci of chondrogenesis the cells multiplied rapidly, Hale-positive and alcian-positive zones of ground substance

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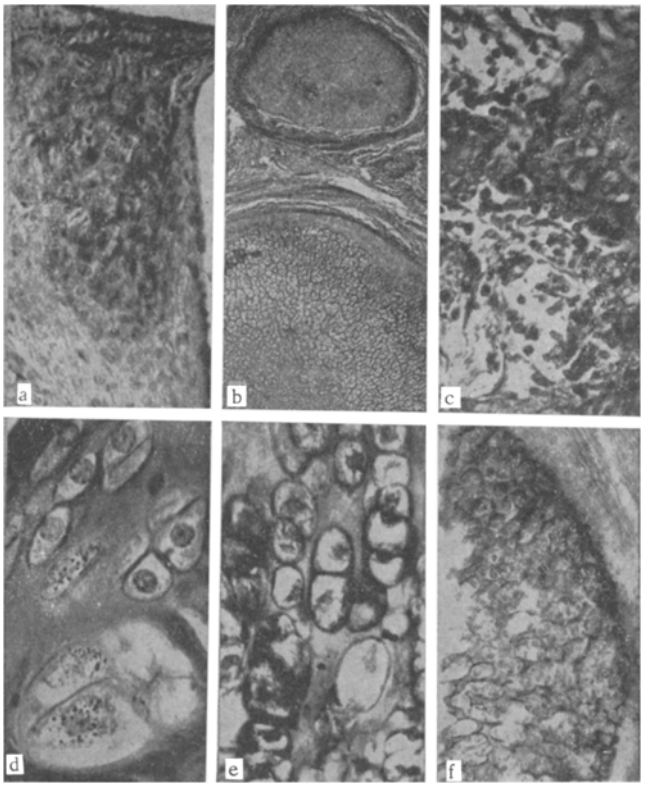

Fig. 1. Implants of mesenchyme and cartilage tissue at different stages of the experiments. a) Implant of mesenchyme of 8-day rabbit embryo (duration of experiment 16 days; avascular zone of implant; area of chondrification; reaction of Ritter and Oleson, 280 ×); b) implant of mesenchyme of 12-day rabbit embryo (duration of experiment 60 days; newly formed pieces of cartilage; Mayer's hematoxylin and eosin, 21 ×; c) implant of cartilage tissue of 18-day rabbit embryo (duration of experiment 90 days; blood vessels in zone of enchondral osteogenesis; Heidenhain's azan, 140 ×); d) glycogen granules in chondrocytes of newly formed piece of cartilage (duration of experiment 60 days; PAS reaction, 630 ×); e) formation of zone of vesicular chondrocytes in growing piece of cartilage (duration of experiment 60 days; metachromatic reaction with fast cresyl violet, pH 4.6, 280 ×); f) areas of calcified cartilage in newly formed cartilage islands (duration of experiment 60 days; Dahl's reaction with alizarin red, 140 ×).

were formed, and the newly formed pieces acquired an organotypic structure and gave a clear γ-metachromatic reaction with chromotropic dyes. In other parts of the implant the mesenchymal cells differentiated into fibroblasts, forming loose connective-tissue bands with very slight metachromasia at sites of fibrillogenesis.

In the experiments of series I fibrillogenesis was delayed. The intercelloidin spaces on the 15th day of the experiment consisted of loose connective tissue with abundant amorphous ground substance and a few cells and fibrous structures. In the foci of chondrogenesis, by the 20th-25th day of the experiment nonsulfated, followed by sulfated forms of acid mucopolysaccharides accumulated. With invasion of the zone of implantation by blood vessels, the newly formed pieces of cartilage were replaced by woven bone tissue by a process of perichondrial osteogenesis with the formation of a primary medullary cavity. In the next stages of the experiment the islets of bone grew in size through the formation of layers of osteoblastic tissue.

In the experiments of series II, by the 6th-12th days the connective-tissue skeleton of the implant was formed, composed of fibrous structures, cells, and a small quantity of amorphous substance. Zones of chaotically growing cartilage were formed mainly in avascular areas. The pieces of cartilage grew by apposition and intussusception, to attain a diameter of 2-8 mm by the 30th-60th days of the experiment. Cells of the peripheral zone of the cartilage fragments synthesized and accumulated glycogen and RNA intensively. Besides these substances, Hale-positive granules were visible in the cells of isogenous groups, suggesting continuing differentiation of the cells of the newly formed fragments of cartilage. An organotypic reorganization took place in the central areas of the islands of cartilage: the cartilage calcified, zones of vesicular and columnar chondrocytes were formed, large quantities of sulfated forms of acid mucopolysaccharides were detected in them histochemically, and a line of resorption and osteogenesis of enchondral type was formed. The surface cartilage grew by apposition and formed a boundary zone between the bed of the graft and the calcified cartilage (Fig. 1).

The pieces of embryonic cartilage (experiments of series III) underwent organotypic reconstruction by the 12th-15th days of the experiment. At later stages the residual cartilage fragments underwent calcification, followed by lysis or, if the blood supply was good, with the formation of woven bone tissue by a process of enchondral osteogenesis.

The material described above shows that, starting from the stage of 8 days of embryogenesis, the skeletogenic tissues of the rabbit are determined, but within certain limits they may develop differently.

Divergent differentiation of the skeletogenic elements of the mesenchyme and the graft bed is dependent on local conditions: if vascularization is minimal, fibrous connective tissue and cartilage are formed, but if it is maximal, bone results. Growth of newly formed areas of cartilage is organotypic in character.

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