HISTOCHEMICAL AND STRUCTURAL CHARACTERISTICS OF GROWTH AND CONVERSION OF CARTILAGE TISSUE CULTIVATED IN VIVO

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Minced cartilage of rabbit embryos and of rabbits aged 15 days and 6 months was implanted subcutaneously into rabbits aged 3 months. Histological and histochemical investigations showed that the implanted cartilage tissue passes through a cycle of conversions: a state of depression, restoration of reactivity of cambial zones of cartilage fragments, formation of perichondrium, appositional and interstitial growth, and osteogenesis. Conclusions were drawn regarding determination of the cartilage tissue and the decrease in its reactive, plastic, and inductive properties during ontogenesis.

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Numerous investigations [1, 4, 7] have revealed the pattern of conversion of cartilage when transplanted and cultivated in vitro. F. M. Lazarenko's method of tissue culture [3], together with the use of ordinary histological methods, has been used to investigate embryonic and postnatal cartilage of birds and rabbits [5] and cartilage of the air passages of rabbits [2].

In this investigation the histochemical and structural changes taking place in cartilage tissues at various stages of ontogenesis were studied.

EXPERIMENTAL METHOD

Three series of experiments were carried out on male chinchilla rabbits kept under identical conditions. The epiphyseal cartilages of long bones of embryos aged 20-28 days (series I) and the costal cartilage from rabbits aged 15 days (series II) and 6 months (series III) were removed. The tissue for investigation was minced to a size of 0.67 mm^3, mixed with pieces of neutral celloidin of the same size, and implanted beneath the skin of the anterior abdominal wall of male rabbits aged 3 months. The implanted material was extirpated after intervals of between 1 and 150 days, fixed in 10% neutral formalin and Carnoy's fluid, decalcified where necessary in 20% trilon B, pH 7.4, and then embedded in paraffin-celloidin. Serial histological sections were stained with Mayer's hematoxylin and eosin, and with Heidenhain's azan. Histochemical tests with appropriate controls were carried out to detect glycogen and neutral mucopolysaccharides (PAS reaction), acid mucopolysaccharides (Hale's method with colloidal iron, alcian blue by Steedman's method), RNA by Brachet's method, and chromotropic substances (metachromasia reaction with toluidine blue, pH 4.6; fast cresyl violet, pH 6.0; azure I and basic fuchsin). Altogether 120 specimens were studied.

EXPERIMENTAL RESULTS

Several regular patterns were observed in the implanted tissues in the first 3 series. On the 1st day of the experiment the cultivated tissues were in a state of depression. The number of proliferating cells was reduced, together with the content of glycogen, RNA, and acid mucopolysaccharides in the cells and ground substance. The fragments of cartilage were saturated with exudative fluid. On the 3rd day of the experiment a newly formed connective tissue, rich in hyaluronic acid, began to spread along the spaces between the celloidin and cartilage fragments, interacting with the implanted tissues. The reactivity of the cambial zones of the embryonic cartilage was restored. By the 4th-5th day a process of fibrillogenesis had begun: connective-tissue cells in the graft bed accumulated glycogen and chromatotropic substances, while alcian and Hale-positive granules and fibrous structures appeared in the intercellular spaces. Cells

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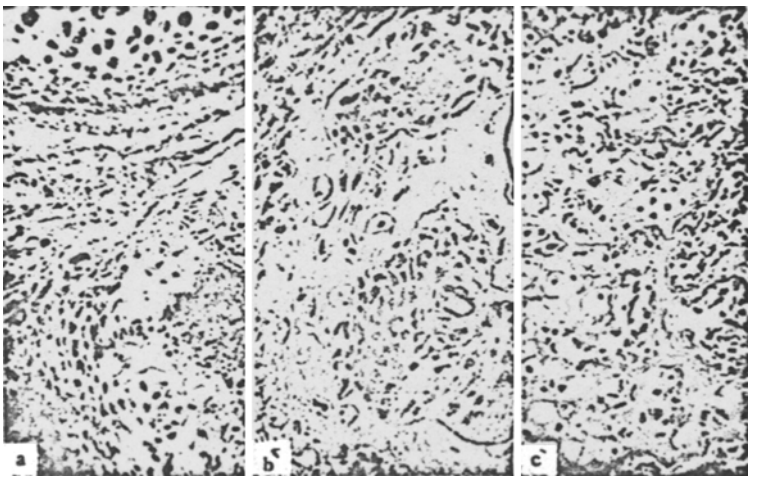

Fig. 1. Culture of embryonic cartilage. a) Distribution of chromotropic substances in cartilage fragments at different stages of differentiation. Stage of 40 days. Toluidine blue, pH 4.6. Objective 20, ocular 7; b) zone of perichondrial osteogenesis. Stage of 50 days. Mayer's hematoxylin and eosin. Objective 40, ocular 7; c) endochondral osteogenesis in center of cartilage fragments. Stage of 30 days. Steedman's alcian blue. Objective 30, ocular 7.

of the peripheral zones of the embryonic cartilage showed mitotic division, accumulated structural materials, and produced a fibrous and amorphous ground substance coming into contact with the newly formed fibrils of the connective-tissue bed. In this way a new perichondrium was formed around the fragments of embryonic cartilage.

Chondroblasts were surrounded by capsules and immersed in ground substance rich in glycogen, hyaluronic acid, and chondroitin. The flattened chondrocytes of the peripheral zones proliferated, increased in size, became round in shape, and formed isogenous groups of cells, where the precursors of the acid mucopolysaccharides became conjugated with sulfates. On staining with metachromatic dyes the areas of isogenous groups of cells were distinguished by their bright γ-metachomasia resulting from large quantities of sulfated forms of acid mucopolysaccharides: chondroitin sulfates A and C. In the next stages of the experiment, surviving pieces of cartilage had enlarged to 6-7 mm, and PAS-positive structures, not removed by amylase, and chromotropic substances appeared in their central areas.

New chondrogenic islands, around which a typical perichondrium formed, appeared in the zones of connective tissue surrounding the fragments. After the stage of 20 days of the experiment, the hyaline cartilage began to be replaced by fibers of woven bone. The newly formed bone possessed a high content of acid mucopolysaccharides, both conjugated and unconjugated with sulfates, and glycogen and gave a γ-metachromatic color when treated with fast cresyl violet. In subsequent stages the content of acid mucopolysaccharides in the ground substance of the bone decreased and the content of the chemically more stable neutral mucopolysaccharides increased correspondingly.

The great majority of growing fragments showed an endochondral type of reorganization. In the intervals between trabeculae of endochondral bone and around the invading blood vessels a typical reticular tissue was built, with hematopoiesis taking place in its loops. Less frequently osteogenesis was of the perichondrial type, with subsequent endochondral transformation of the cartilage fragments. Newly formed woven bone, with rare exceptions, was rebuilt into lamellar. Embryonic cartilage cultivated in vivo thus reflected the main stages of phylogenetic development of the supporting tissues and induced chondrogenesis and osteogenesis (Fig. 1).

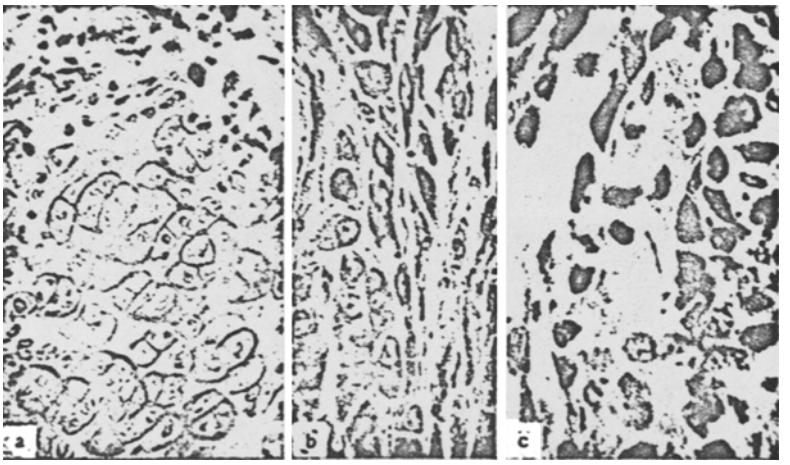

Fig. 2. Cultivation of costal cartilage of an animal aged 15 days. a) Accumulation of chromotropic substances in zone of isogenous groups of cells of surviving cartilage fragment. Stage of 12 days. 0.1% Basic fuchsin. Objective 40, ocular 7; b) osteogenic differentiation of cells of connective-tissue bed. Stage of 30 days. Methyl green – pyronin (Brachet). Objective 90, ocular 7; c) newly formed bone trabecula. Stage of 12 days. Steedman's alcian blue. Objective 90, ocular 7.

In the experiments of series II, on the 4th and 5th days the cartilage fragments were separated from the surrounding connective-tissue bed by a zone of oxyphilic material rich in neutral mucopolysaccharides. On the 6th-7th day, a perichondrium formed around the cartilage fragments. Growth of the implanted fragment took place on account of cambial cells of the narrow peripheral zone. The surrounding connective tissue gave rise (on the 8th-12th day) to cells of the osteoclastic series, forming a sleeve of bone around the cartilage. In the central zones of the cartilage fragments, osteoclastic resorption took place, and bands of woven bone were formed on the remains of the intercellular substance (Fig. 2).

The progressive changes were less marked in implanted fragments of costal cartilage from the 6-month old rabbit. Only the peripheral zone of the cartilage, under normal conditions lying next to the perichondrium, was activated. In the experiments of series III, survival of the cartilage fragments was largely dependent on integrity of their own perichondrium, and when this was absent, the cartilage took badly and was gradually absorbed [6]. The surviving perichondrium soon established connections with the connective tissue of the graft bed and became indistinguishable from it. Mature areas of the fragments showed degenerative changes by the 3rd-4th day, and osteoclastic resorption by the 6th-7th day. Bone tissue of woven type was formed at the site of the resorbed cartilage.

Analysis of the results showed that the cartilage tissue after implantation passed through a cycle of histogenetic and histochemical transformations reflecting the stages of ontogenetic development of the supporting tissues. The reactive, structural, and inductive properties and morphogenetic powers of cartilage tissue diminish with age. The fact that osteogenesis takes place in cultivated cartilage and in new cartilage formed during cultivation is evidence of its determination.

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