ON THE PRINCIPLES OF CARTILAGE REGENERATION ASSOCIATED WITH ITS CULTIVATION IN THE ORGANISM*

Z. S. Khlystova

From the Department of Histology and Embryology (Head: Prof. Z. S. Khlystova)
of the Orenburg Medical Institute (Director: S. S. Mikhailov)
(Presented by Active Member of the Akad. Med. Nauk SSSR A. V. Lebedinskii)
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In the opinion of some authors, transplanted cartilage tissue can only regenerate from perichondrium which is retained [11, 15, 7] or from the surrounding connective tissue [2, 3, 12]. There are indications that the cartilage has an inductive effect on the connective tissue [1, 8, 10, 13, 16**]. Other investigators [3, 4, 5, 7, 14] believe that cartilaginous tissue can regenerate not only from perichondrium and surrounding connective tissue, but also through multiplication of the cartilage cells themselves. K. E. Gromtseva [6] considers that the outcome of cartilage transplantation depends on the age of the donor. Progressive changes are possible only in young transplanted cartilage.

The controversy in the data found in the literature on such an important question makes it necessary to apply new investigative methods in order to establish the rules and conditions of cartilage regeneration. In connection with this, we adopted the method of tissue cultivation in the organism developed by F. M. Lazarenko [9]. This method has been very effective in demonstrating the properties of other tissues.

METHOD AND RESULTS

Homotransplantation was carried out using ground costal cartilage from the donor together with fragments of celloidin as a stimulus; the material was placed in the subcutaneous layer of the recipient. The experiments were carried out on chickens and rabbits. As donors we used embryos in the last days of development, young and old animals, while the recipients were young and old animals. This permitted us to clarify the age peculiarities in the regeneration of implanted cartilage. The implants were extirpated at intervals of from 1 to 65 days, fixed in Zenker's solution, imbedded in celloidin, and sections stained with Carazzi's hematoxylin, eosin and picrofuchsin.

Introduction of the implant into the subcutaneous layer caused an aseptic inflammatory reaction in the tissues surrounding the implant on the first day. Both in the chickens and in the rabbits the hyaline cartilage of the donor in the implants was present under the same conditions – in the focus of the aseptic inflammation.

With implantation of the cartilage from the embryos all the fragments of the tissue that lacked perichondrium on their surface or had only part of it survived completely on the first day of the experiment; the cartilage cells entered a depressed state.

On the second day of the experiment the cartilaginous tissue in the fragments retaining their perichondrium and the fragments lacking this tissue became impregnated with inflammatory exudate, and their cells began to proliferate. The cartilage cells along the edge and in the middle of the fragment began to divide mitotically, and the dimensions of the implanted piece of cartilage increased as a result of internal growth.

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** As in Russian original – Publisher.

The cells of the partially retained embryonal perichondrium, present in certain fragments, became similar to the connective tissue cells of the recipient surrounding the implant. The intensity with which they did this depended on the degree of stimulation. The perichondrial tissue of the donor in the implant responded to the regulatory mechanisms present in the organism of the adult recipient, and grew as though it were the tissue of that organism. These and other cells moved into the interspace between the particles of celloidin, and took part in the structure of the connective tissue in the intercelloidin layers and the capsule around the celloidin. All the cartilage fragments initially bearing perichondrium on their surfaces, as well as those without it, were seen to be surrounded with connective tissue which arose from the elements of the donor and the recipient.

On the 4th-5th days of the experiment highly vascularized intercelloidin connective tissue layers arose in the implant. A typical network of fibroblasts developed anew around all the cartilage fragments. It assumed the function of the perichondrium, giving rise to new cartilage cells. Through this the dimensions of the implanted fragments of cartilage increased by appositional growth (Fig. 1). Thus, a new perichondrium arose around the implanted fragments of cartilage from the donor, out of the tissues of the donor and of the recipient.

On the 6th-12th days of the experiment new isogenous groups appeared in the cartilage, earlier in the birds, later in the rabbits. When the fragments of cartilage became coarse and the nutrition of the central cells deteriorated, layers of connective tissue containing new blood vessels grew into the cartilage from the periphery, out of the new perichondrium (Figs. 1 and 2). The mitotic activity of the cartilage cells around the new blood vessels increased.

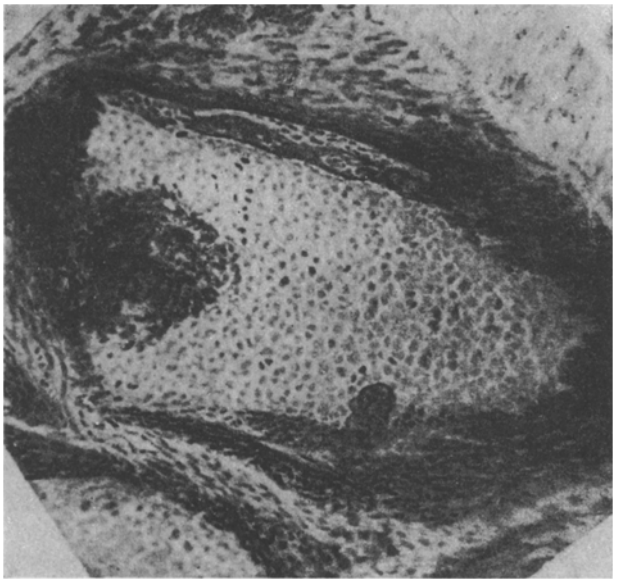

Fig. 1. Hyaline cartilage culture. Six-day stage. Stained with hematoxylin-eosin. Photomicrograph. Magnification: obj. 3×, oc. 10×.

tilage from the embryos of birds and mammals retained their viability and acquired complete similarity to the cartilage of the adult organism. At their periphery there was a zone of appositional growth and in the center of the fragment there were coarse isogenous groups containing from 6 to 10 cells.

Having obtained the survival of the embryonic cartilage in all cases when the material was cultivated in the organism of birds and mammals, and having observed the new formation of perichondrium from the tissues of the donor and the recipient with regeneration of cartilage tissue, we decided, in the same manner, to ascertain the presence of these properties in postnatal cartilage depending upon the age of the organism. To do this, we cultivated the costal cartilage of young and 2-year-old rabbits, both with preservation of the perichondrium and with its removal.

With cultivations of the cartilage from the young animals both with and without the perichondrium the cartilage tissue entered a depressed state on the 1st day of implantation. On the 2nd day, viable elements and cells appeared, having arisen in the process of reverse development. The latter were in greater number in the fragments lacking perichondrium and having undergone especially severe trauma.

In the cartilage implants with the perichondrium taken from the young animals, the perichondrial cells were activated (just as with implantation of the embryonal cartilage), proliferated on the sides, and, together with the cells of the recipient, took part in the structure of the intercelloidin connective tissue layers. The cartilage cells distributed under the perichondrium and deeper, but not in the center, were activated by the effect of the inflammation, and began to divide mitotically and amitotically, while the most central cells perished. In the cartilage fragments implanted without perichondrium the cartilage cells were also activated and divided, but here a much larger number of cells underwent reverse development than in the first case.

On the 6th-8th days of the experiment all the fragments in the cartilage implants containing portions of perichondrium became covered with new perichondrium consisting of tissues from the donor and the recipient. In the implants of cartilage without perichondrium only those fragments which also covered themselves with new perichondrium survived, while the fragments that were most traumatized during the operation did not acquire

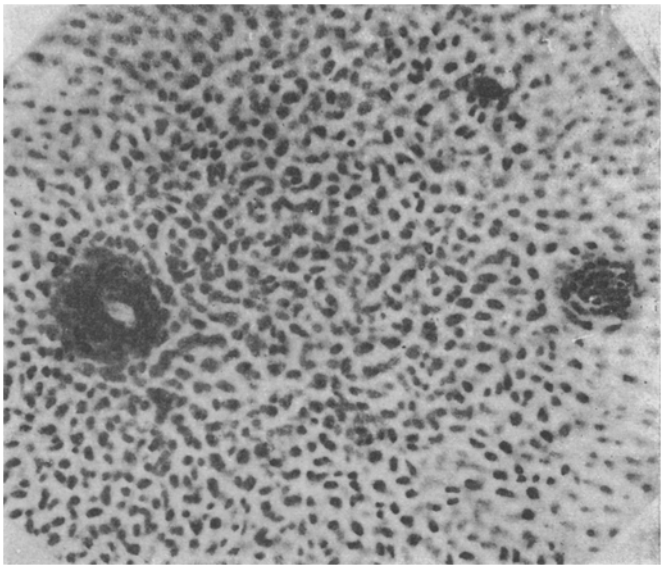

Fig. 2. Hyaline cartilage culture. Ten-day stage. Stained with hematoxylin-eosin. Photomicrograph. Magnification: obj. 6 ×, oc. 2 ×.

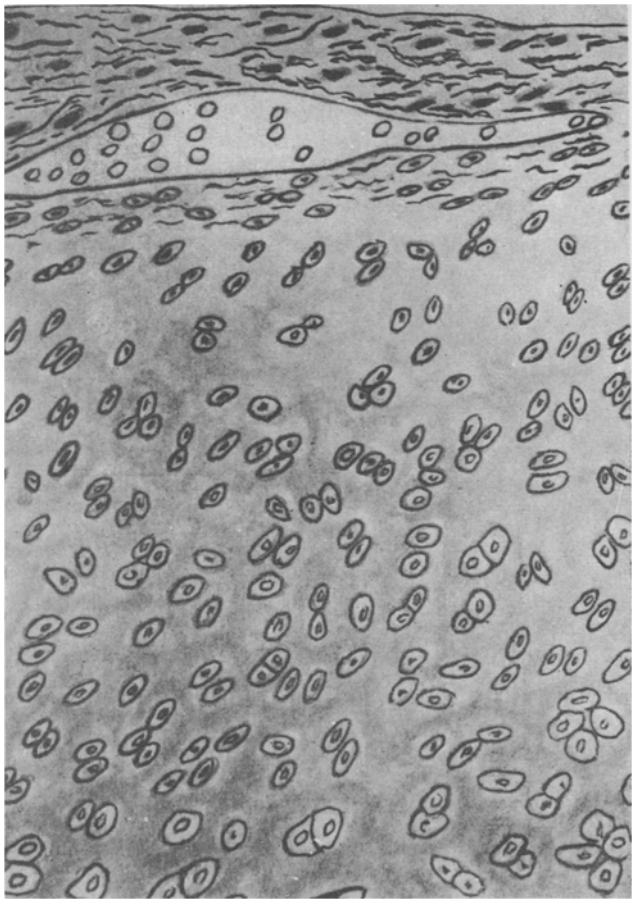

Fig. 3. Hyaline cartilage culture. Eight-day stage. Drawn from a photomicrograph at a magnification of: obj. 6 ×, oc. 2 ×. Stained with hematoxylin-eosin.

perichondrium and underwent reverse development. Following the development of the perichondrium (Fig. 3), growth of the tissue was completely similar in all the fragments, i.e., was both appositional and intussusceptive. The cartilage cells divided both mitotically and amitotically, and the dimensions of the implanted cartilage fragments increased.

The old isogenous groups in the central portions of the large growing cartilage fragments began to die. At this time a non-uniformity appeared in the isogenous groups and in the cells within a single isogenous group. Along with mass destruction of cartilage cells there were isogenous groups with fully developed cells that survived. Within a single isogenous group there were differentiating cells along the periphery and mitotically dividing cells in the center. It gave the impression that the isogenous group consisted of a collection of cells at varying stages of differentiation, securing support for the structure of the isogenous group as the basic element of the cartilaginous tissue. With age the proportion of cells dividing mitotically and amitotically changed within the cartilage tissue of the implant. Beginning with the 20-25-day stage of implantation the number of mitoses in the cartilage decreased and the number of amitoses increased.

The costal cartilage of the 2-yr-old donor showed high sensitivity to trauma, and rapidly lost its viability. A smaller portion of the implanted fragments in which the perichondrium was partially retained remained alive. They became overgrown with perichondrium from all sides, but no new cells appeared from

them, while the old ones began the process of reverse development. A large portion of the cartilage fragments with perichondrium, undergoing major trauma during the maceration of the material, perished. At the same time, the fragments that were implanted without perichondrium all died, even before the formation of new perichondrium around them. Remnants of the dead implanted cartilage of the donor were retained for a very long time, and during the formation of the intercelloidin layers they became overgrown with a dense connective tissue capsule, like a foreign body. Empty cavities remained at the sides of the dead cells in the non-living cartilage; the intercellular substance acquired a pink tone with staining, and developed a fibrous appearance. Projections of connective tissue grew out from the capsule into this intercellular substance, and organization of the non-living cartilage began which was accomplished at a very slow rate.

Thus, our data show that cartilage cells from embryos and young animals manifest mitotic activity in cultures within the organism.

With cultivation of hyaline cartilage from an embryo or young animal without the inclusion of perichondrium, new functioning perichondrium arises from the connective tissue of the recipient. At late stages of the implantation layers of connective tissue containing blood vessels grow deep into the large developing fragments of cartilage from the new perichondrium, and improve the nutrition of the cartilaginous tissue.

Inasmuch as mitotic activity of the cartilage cells and the new formation of perichondrium were observed by us in the cartilage implants of both birds and mammals, we are obviously dealing here with general established principles for the regenerative processes of cartilage tissue.

With aging of the animal the regenerative properties of the cartilage decrease, and its sensitivity to injury increases. The cartilage of old animals rapidly undergoes reverse development secondary to traumas and to the impaired nutrition of the implant.

SUMMARY

Hyaline cartilage of chicken and rabbit ribs was implanted by F. M. Lazarenko's method. As shown, hyaline cartilage of embryos and young animals may regenerate after removal of the perichondrium. The cartilage cells, becoming activated under the effect of inflammation in the transplant, multiply by mitosis and amitosis. New functional perichondrium, formed from the surrounding connective tissue of the recipient, appears around the pieces of donor cartilage. At late stages of implantation the cartilage tissue nutrition improves as a result of blood vessels growing into the large newly formed pieces of cartilage and perichondrium. The regenerative ability of the cartilage is less, the older donor animal. Implanted cartilage obtained from two-year-old animals does not grow.

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