STIMULATION OF REGENERATION OF JOINT CARTILAGE BY COD LIVER OIL AND ASD* (3RD FRACTION)

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There is still disagreement as to how the repair of joint cartilage is brought about; the problem has not been sufficiently studied, and published reports are contradictory. Some investigators [2, 8] deny that repair can take place, while others [1, 3, 4, 9, 10] think that the damaged cartilage is able to regenerate.

Our previous investigations[5, 6] on the repair of joint cartilage which has been damaged as deep as the cancellous tissue of the epiphysis convinced us that it can regenerate completely. We found however that regeneration takes place extremely slowly, and that at least one year is required for the recovery of an area measuring 1 x 1 x 0.8 cm. Then, not only is new tissue formed, but the process is directed to form an organ.

In these experiments we noticed the important conditions which undoubtedly exerted a stimulating influence on the regenerating cartilage. It was found that the most important condition was to maintain the function of the joint after inflicting the damage, and it was equally important that fragments of cartilage should remain in the wound.

In view of the stimulant action of preparation ASD and cod liver oil on bone [7], we have studied their influence on the regeneration of joint cartilage.

EXPERIMENTAL METHOD

The experiments were carried out on 20 dogs aged $2\frac{1}{2}$ to 9 months kept under identical conditions. We inflicted an aseptic wound with forceps on the bone and cartilage of the lower epiphysis of the femur in both knees. The wound included the internal and external condyles, and measured 1 x 1 cm, extending to a depth of 0.6-0.8 cm. At the same time, near the wound a further scalpel cut extending up to the axial zone was made in the cartilage, without damage to the underlying bone, in order to find the effect on the regeneration of cartilage. At operation, all the animals received a 5% solution of preparation ASD (3rd fraction) in cod liver oil into the right joint, some dogs received cod liver oil, while the right joint of other dogs served as control. Next the cuts in the bursa and skin were sewn with knotted silk sutures. During the next four months, five injections of the stimulant substances were made into the joints.

Biopsy specimens for microscopical study were taken 3, 4, 10 and 20 days after the operation, and again after 5-6 months to determine the remote effects. The samples were treated by the normal histological methods.

EXPERIMENTAL RESULTS

The following histological changes were observed microscopically.

On the third and fourth day after the wound had been inflicted, in the control joints there was a blood clot consisting of threads of fibrin interspersed with blood cells. Some of the fibrin threads formed a plexus, but most of them lay parallel to each other at the surface of the wound. The erythrocytes showed degenerative changes, but the leukocytes appeared normal. In the wounds into which 3rd-fraction ASD had been injected, the blood clot was large and it contained a large number of neutrophils; a considerable proportion of the leukocytes had gathered at the cartilagenous edge of the wound. At the bottom of the wound there were thin-walled vessels of the capillary type, and regeneration of the bony trabeculae was taking place (Fig. 1).

* Translator's note: The abbreviation ASD indicates Dorog's antiseptic stimulator prepared from the decomposition products of microorganic, animal, and plant proteins.

The appearance was almost the same in the right joint which had received the fish oil.

In the control joints, the outgrowth of the vessels and migration of fibroblast cells had not occurred until the tenth day after the operation. At this time regeneration of the boney trabeculae had occurred. The new tissue had then filled the wound to one third of its depth.

At the corresponding times in the joints receiving cod liver oil, the new tissue filled one half of the wound, and in joints receiving ASD it filled two thirds, and in some animals almost the whole of the wound was filled with this new tissue. With stimulation by ASD, the tissue filling the wound consisted of three distinct layers. The superficial layer was made up of fibrous tissue containing spindle-shaped cells lying chiefly between the fibers. This layer

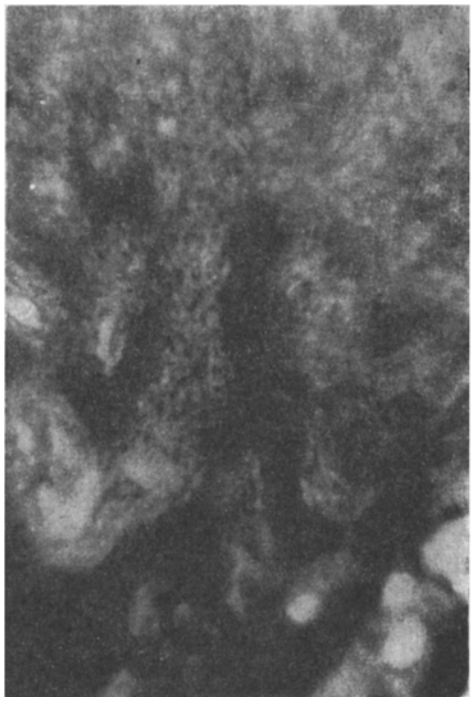

Fig. 1. Formation of new trabeculae on the fourth day after operation. 5% solution of ASD injected. Magnification: eye-piece 10×, objective 27×.

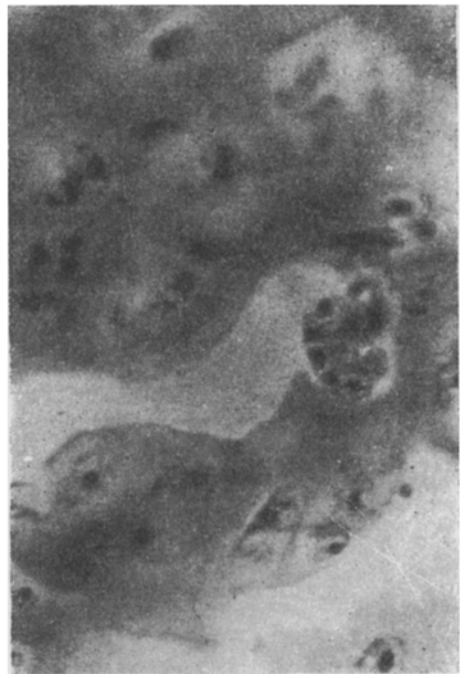

Fig. 2. Absorption of the degeneraged area, and migration of cartilagenous cells away from their clusters on the tenth day after the operation. ASD injected. Magnification: eye-piece 10×; objective 40×.

was probably a continuation of the perichondrium which had hypertrophied. The middle layer consisted of a small amount of the fibrous clot where remains of thick fibers could be seen, and between them there were numerous vessels and cells of different shapes, as well as droplets of cod liver oil. The deepest layer was thicker than the other two, and consisted of fibrous tissue and a large number of almost undifferentiated cells of various shapes having a basophil cytoplasm and an intensely-staining nucleus. Here, there were a large number of vessels and trabeculae, and the latter were equal in size to those of the cancellous substance of the epithysis.

By the 3rd-4th day, in all the experimental and control joints, at the edge of the wound in the cartilage two zones could be distinguished. The first, or edge zone, was an area of degeneration; it was about 30μ thick and the cartilagenous cells and ground substance stained weakly; here the ground substance was being absorbed. The second zone lay beneath the zone of degeneration, and contained an increasing number of cells arranged in groups, and the nuclei of the cartilagenous cells stained more deeply. We refer to this area as the reactive zone.

By the tenth day, the edge zone had increased in thickness to 40-50μ, and absorption had increased, so that the surface of the wound had become uneven, as though eaten away.

Reabsorption was more vigorous in the joints which had received cod liver oil, and most vigorous of all when ASD had also been given. In these cases, in certain areas, the necrotic cartilage had been completely absorbed, so that the regenerating portion was in direct contact with the reactive zone. During absorption of the degenerative zone, the multiplying cartilagenous cells were set free from the capsule, and moved out into the tissue, so filling up the wound (Fig. 2).

On the fourth day after injecting cod liver oil and to a greater extent after injecting ASD, at the surface of the cartilage at the edge of the wound, a thin layer of tissue was formed having a structure typical of perichondrium. By the tenth day, this layer had spread rapidly towards the wound, and had covered the whole of the regenerating portion.

In the control joints, by the tenth day, regeneration was restricted to the formation of tissue which had the same structure as that formed after the injection of the stimulant substances, and this new-formed tissue took the form of a small ridge projecting towards the wound. In puppies, on the 3rd-4th day after the operation, there was a pin-shaped outgrowth of epiphysial cartilage directed towards the wound.

By the 20th day, in the control sections, the regenerated portion extended further than it had done on the tenth day. More blood vessels had formed, and the ground substance consisted of fibrous oxyphil structures, and there were groups of round and spindle-shaped cells. In their shape and staining properties the cells resembled those of cartilage, and we considered that the tissue was in fact cartilage.

In the damaged joints which had received two injections of cod liver oil, the microscopic appearance resembled that of the control animals, except that the regeneration of the trabeculae had proceeded further.

In joints which had received two injections of ASD, the wound was filled with a more mature cartilagenous tissue, and the bone consisted of plates separating this tissue from the spongy substance of the epiphysis.

By the 20th day, in the control joints, the edge zone of the wound in the cartilage had been almost completely reabsorbed, in the same way as had occurred in the joints which had received the cod liver oil.

In the reactive zone both in the control and experimental animals there had been a multiplication of cells in isogenous groups. However, regeneration had been most intense in the joints which had received ASD; then the number of cartilagenous cells in the isogenous groups was as high as 50 in a single transverse section.

Five and six months after the operation, in both experimental and control animals, the wounds had been filled with cartilagenous tissue, and the only difference was that in the control joints it was less mature and did not fill the whole wound, and the surface of the regenerated portions had a small saucer-shaped depression 2 mm deep. In some of the control joints there were furrows running from the wound to the outer part of the joint, so the surface was deformed. We attribute this deformation of the joint to shrinkage at the edges of the wound, due to insufficiently rapid regeneration.

Microscopical studies of the cartilage in the region of the superficial incisions, made with a scapel, showed regenerative changes and considerable reabsorption of cartilage at the edges of the cuts. Here reabsorption had taken place more rapidly in the joints injected with ASD. Later, after six months, the cartilage around the incision had been completely absorbed, leaving a small depression in its place.

SUMMARY

Intracapsular injuries were made in the bone cartilage of the lower epiphysis of the femur. The experiments were performed on 20 dogs aged less than 9 months. It was found that cod liver oil and particularly ASD(3rd fraction) dissolved in cod liver oil had a marked stimulating effect on the regeneration of joint cartilage, but only when the defect extended as far as the spongy substance of the bone. Growth of blood vessels into the clot filling the wound was then accelerated; the resorption of the injured edge of bone cartilage was also intensified, thus favoring the escape of chondrocytes (from the isogenous groups), which later changed into chondroblasts.

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