EXPERIMENTAL BIOLOGY

CRANIAL BONE REGENERATION EFFECTED BY TRANSPLANTATION OF GROUND MAMMAL BONES

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Surgeons usually employ alloplasty, i.e., transplantation of nonliving plates (metal, horn, plastic) to replace defects in nonregenerating, integumentary, human cranial bones. However, foreign material cannot avoid causing some harmful effect on healthy living tissues and organs – for example, the brain; therefore biological methods for covering defects with living bony tissue should be developed. Autoplastic or homoplastic transplantation of developed bone or cartilage is difficult and is attended by the resorption of the transplantates. In previous works [8,9,10], we proposed three biological methods, one of which was used successfully in another laboratory [2,3].

In this work, we propose a new, simpler method and also present data regarding the dependency of mammalian integumentary cranial bones' regeneration on age.

When setting up the experiments, we started from the following premises. In cases where a defect is artificially induced in the integumentary cranial bones of mice, dogs and other animals, although regeneration over the actual edge of the undecayed bone in the periosteum is absent, slight signs of osteogenesis are nevertheless evident [10]. Organs regenerate more efficiently and completely when their component tissues are destroyed [9]. The tails and extremities of mature axolotls can regenerate when their tissues are completely destroyed [8]. A. N. Studitsky [12] also obtained complete regeneration after destroying muscles capable of regeneration in roosters and rats. We proposed that slightly decaying mammalian integumentary cranial bones, nonregenerating under ordinary conditions, can be stimulated to regeneration by means of artificial intensification of their decay by destruction.

EXPERIMENTAL METHODS

A total of 54 animals were used in the experiment. Destruction experiments were conducted on 11 adult white mice and 11 white rats, and experiments to determine the dependency of regeneration on age, on 4 five-day old kittens and on 8 eight-day old rats. Besides this, a cranial roof defect was artificially induced in 10 adult mice and 10 adult rats, which were used as the control. The operation was performed under sterile conditions. After ether anesthesia had been administered and the fur had been plucked and shaved, an incision was made into the skin of the head to the left of the center line. Rectangular pieces, constituting about half of the surface of the parietal bones, were extirpated from the cranium on the right; the pieces were 6×3 mm in the mice, 8×5 mm in the rats and 20×15 mm in the kittens. In the rats and kittens, the bone was sawed out with a special saw, but a scalpel was used for the operation on the mice. Special care was taken to prevent damage to the cerebral membranes, although they were slit in one or two places in a few cases. In the destruction experiments on the mice and rats, the sectioned cranial roofs were ground into a powder on a cutting grinder of V. E. Sokolov's design, the bone dust was moistened with a solution of penicillin prepared in a physiological solution, and the resulting thick paste was transplanted homoplastically in a thick layer on to the region of the

cranial defect, partially covering the edge of the old bone. After the operation, the region of the wound was drenched with penicillin, the skin was sutured, the wounds were covered with streptocide, and the animals were injected with penicillin in doses of 2,000 to 10,000 units for a period of 2-5 days. All the animals lived. In the destruction experiments, the mice were sacrificed on the 10th, 16th, 60th, 84th and 120th days, and the rats, on the 23rd, 80th and 150th days after the operation. In the experiments examining the age factor, the kittens were killed on the 34th, 60th and 96th days, the rats on the 60th, 102nd and 124th days after the operation. pieces were fixed in 10% formalin, Zenker's fluid with added formalin or acetic acid, decalcined in 5% nitric acid, imbedded in celloidin-paraffin, cut into lateral sections 10 microns thick and stained by Mallory's method or with hematoxylin-eosin.

EXPERIMENTAL RESULTS

In the destruction experiments, unfortunately, the paste sometimes fell out of the defect region while the skin was being sutured. When it was displaced, a thin, supple, fibrous layer appeared in the region of the defect, just as in the control, with the artifical inducement of a simple defect. In the places where the paste remained in place, the cranial defect was completely or partially filled with newly formed, regenerated bone. The regeneration process of the cranial bone differed somewhat in the mice and rats. In both, a reddening of the skin and a severe inflammation of the tissue in the region of the wound was observed the first days after the operation. This abated on the 10th day in the mice; in the region of the defect, between the edges of the cranial bone, a thick layer of immature connective tissue formed, made up of small and somewhat larger bone particles, distributed at random, which became oriented along the line of tension (Fig. 1). The cells had disappeared in almost all of the particles, and the bony substance was partially dissolved. Such a picture was ob-

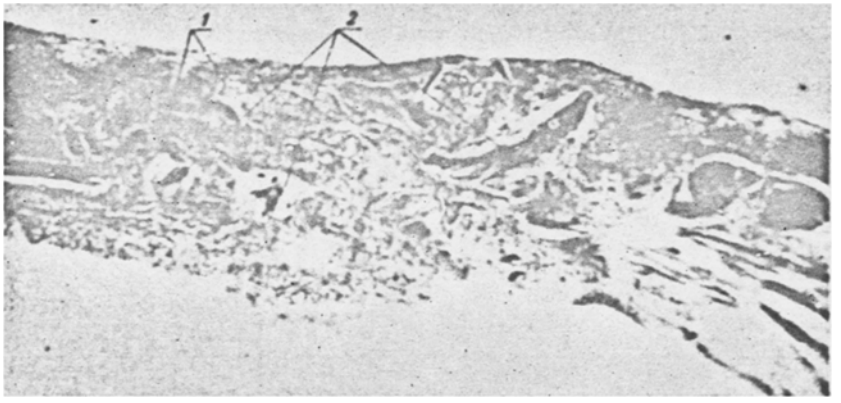

Fig. 1. Destroyed tissue in the region of the defect in a parietal bone of a mouse on the 10th day after the operation.
1) connective tissue; 2) bone particles.

served in both the central and peripheral parts of the transplanted mass, where the particles lay on the edge of the old bone. On the 16th day after the operation, the connective tissue and the whole destroyed mass began to thicken. On the 80th day and 120th day, the destroyed mass became firm and hard, like normal cranial bone, and consisted of newly formed, coarse-fibered bone, in which many acellular bone particles appeared, sealed into it, colored like normal bone and showing no signs of necrosis. The newly formed bone was especially thick and dense in the parts adjoining the old bone (Fig. 2). It had no bone marrow cavities and was considerably younger than the old bone, which had a laminated, dense structure with many bone marrow cavities. The edges of the old bone adjoining the destroyed mass and the newly formed bone were somewhat more rejuvenated than the more distant portions of the old bone. Many bone cells appeared in the bone particles lying on the old bone; the particles were enveloped in newly formed, dense bone, which also contained osteocytes, and were organically fused with the surrounding bony tissue. The periosteum on the old bone, where the bone particles lay, disappeared and was changed into newly formed bone. A new periosteum, covering the upper surface of the newly formed bone, which had replaced the destructive mass, formed from connective tissue.

The picture was similar to that observed by T. P. Vinogradova [4] in experiments establishing that, in plastic operations performed on humans, pieces of hyaline cartilage stimulate the surrounding connective tissue to metamorphose into a periosteum. In cases where the transplanted tissue had either fallen out or been completely resorbed, i.e., when it was not retained, new, thick, coarse-fibered bone nevertheless formed in the region of the defect, but its calcification was somewhat retarded.

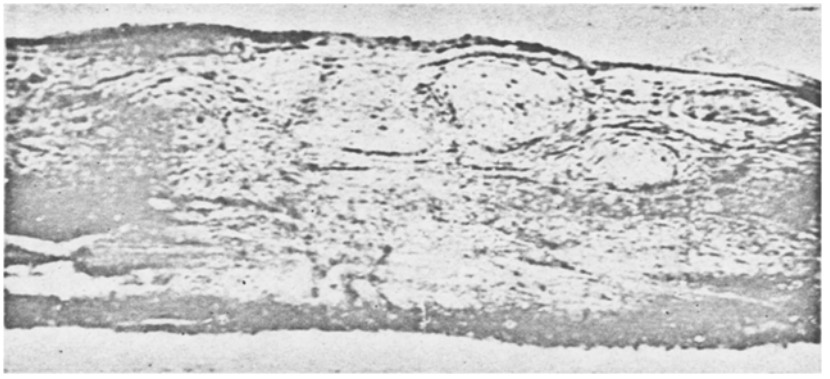

Fig. 2. Dense, newly-formed bone in the region of a cranial defect in a mouse on the 8 th day after transplantation of the destroyed bone.

In the rats, the destroyed mass was fully resorbed by the 23rd day after the operation, and, at the same time, new young bone formed in the middle of the layer while regeneration occurred from the edges of the old bone. On the 84th and 150th days after the operation, the newly formed bone looked like well-developed laminae, connected with the edges of the old bone and like small plates distributed over the rest of the defect surface. Histological study showed that the regenerating bone was thinner and younger than the old cranial bone.

In the rats, the destroyed bone tissue was not incorporated into the regenerate, but stimulated the surrounding connective tissue to regenerative, new formation just as cartilage, or extracts from it, stimulate connective tissue to become cartilage [7,13]. In the mice, the destroyed tissue was itself incorporated into the newly formed bone besides, and took part in its construction. Was this tissue living? Evidently it was, since many bone particles in the mice contained live bone cells, but even if there were no live bone cells, their basic bone substance would nevertheless have been preserved and would have stained the same as normal bone. This question will be resolved by special study.

The special feature of this work was the use of the destruction method to obtain regeneration of the integumentary cranial bones in several kinds of adult mammals, which ordinarily are nonregenerating, and obtained a positive result. A similar method has been used by surgeons successfully on tubular bones [6].

This simple and effective method of cranial bone destruction must be perfected on dogs, after which, it may be considered for clinical use. It does not cause inflammatory phenomena or commissures in the cerebral membranes or in the brain.

In the experiments examining the influence of the age factor on the regeneration of the integumentary cranial bones, complete regeneration occurred in all of the kittens after the cranial bone piece had been removed (Fig. 3). Regeneration was most intense along the course of the large blood vessels lying in the grooves between the convolutions of the cortex of the cerebral hemispheres, then spread over the entire surface of the dura mater in the region of the defect. On the 34th day after the operation, the connective tissue stage of cranial development was reached, and approximately one-third of the regenerate surface was filled with young, newly-formed bone. On the 60th day, ossification had extended over the entire surface of the defect, and on the 96th day, the still-developing bone had become very thick. The bone regenerated by new formation, similar to bone development in normal embryogenesis. The edges of the thick, old bone were noticed with numerous bone marrow lacunae and sharply delimited from the regenerating bone, although both bones were indirectly

joined in many places with connective tissues which had not yet ossified. The shape of the regenerated bone followed that of the brain – the ridges and the prominences on the inside surface of the regenerated cranial bones always conformed exactly and strictly to the surface of the underlying brain and vessels lying in the grooves between the brain convolutions. In one case where, due to intracranial pressure, the brain with the membranes protruded like a knob into the bone defect, the bone regenerate formed exactly the same prominence.

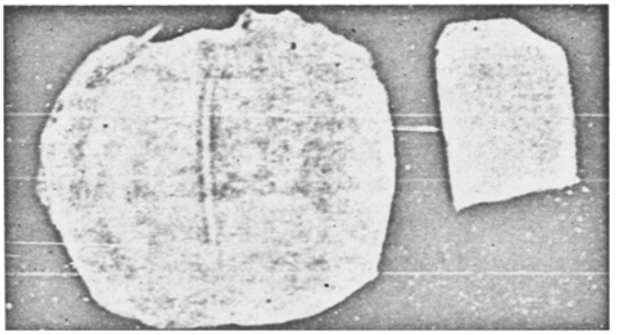

Fig. 3. Complete cranial bone regneration in a kitten on the 96th day after removal of the bone piece, which is shown next to cranium. Seen from below.

In the experiments on the baby rats which lasted up to 124 days, the cranial bone only regenerated in 2 cases; in 6 cases, there was no regeneration, but the defect was overgrown with a supple fibrous layer. The regenerated bone was not uniform in thickness; it was thick at the edges of the old bone and thin in other places, but sometimes formed thick plates. In our preceding experiments, it was shown that the cranial bones can regenerate completely in 26 days in puppies a month old [1,5,8,9,10,11]. The present data show that complete regeneration of the cranial roof bones can also occur in 5-day old kittens, although more slowly than in the dogs. The cranial bones do not regenerate well in 8-day old baby rats (2 positive cases versus 6 negative cases), but can regenerate. The cranial bones do not regenerate in adult dogs, rats and mice, as we were able to conclude earlier. Consequently, the example afforded us by the cranial bones of mammals confirms one of the principal rules of regeneration, i.e., that the ability to regenerate decreases as the animal grows older. Cranial bone regeneration in the destruction experiments was similar to that occurring in young animals, which have the ability to regenerate a given organ.

Therefore, homoplastic transplantation of freshly-destroyed material obtained from the integumentary cranial bones causes the bone in the region of the cranial bone defect to be completely restored in adult mice and rats, whose integumentary bones cannot regenerate.

The regeneration of newly-formed bone is effected by the influence of the destroyed material on the connected tissue, which it stimulates to become bone (in rats). In mice, the destroyed material also participates directly in the formation of the new bone.

Unlike adult animals, which cannot regenerate the integumentary bones of the cranium, young animals, 5-day old kittens and 8-day old baby rats, like one-month old puppies, can regenerate these organs completely. Regeneration of the integumentary cranial bones proceeds similarly to the new formation of bone which occurs in embryogenesis.

Cranial bone regeneration artificially effected by the destruction method is similar to that occurring after simple extirpation of a cranial bone piece in young animals.

SUMMARY

In order to stimulate regenerative bone formation in adult mammals experiments were conducted in replacing cranial bone defects by ground cranial bone. In rats the homotransplanted bone grindings resolved in

three weeks. In mice – more slowly – they were preserved for several months. Bone grindings stimulated transformation of local connective tissue into genuine bone tissue, and replaced cranial bone defects both in rats and mice.

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