EFFECT OF THYROCALCITONIN ON REPARATIVE OSTEOGENESIS IN ANIMALS

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The effect of the new thyroid hormone thyrocalcitonin (TCT) on the course and character of repair in bone tissue was studied in experimental animals. It was shown by several methods that TCT considerably accelerates consolidation of bone fragments. The stimulant effect of the hormone is reflected in more intensive proliferation and differentiation of osteoblasts, more rapid formation of cancellous bone tissue, and the development of a rich network of capillaries. The increased osteoblast activity leds to the early formation of a collagen matrix, to the nucleation of hydroxyapatite crystals in that matrix and, thus, to the formation of normal bone tissue 45-50 days after the fracture.

The discovery of the new hormone thyrocalcitonin (TCT), produced by the pale cells of the thyroid gland, has provided new ways for studying neuro-hormonal regulatory mechanisms of reparative osteogenesis [1-4, 14-17]. The hormone is known to inhibit the resorption and demineralization of bone tissue, with a connected hypocalcemic effect [5-9]. Morphological changes in the bones after administration of TCT consist of the inhibition of osteocytic osteolysis [13] and increased osteoblastic activity [10-12, 18, 19].

Since TCT affects not only the resorption, but also the formation of bone,the study of its effects on the course and character of repair in bone tissue is of practical interest.

EXPERIMENTAL METHOD

Experiments were carried out on 108 noninbred male rats with a mean weight of 167 g. The animals were divided into two groups: control and experimental. The rats of the experimental group received TCT

TABLE 1. Indices of Repair in Bone Tissue after TCT Administration

Group of animals	Stage of consolidation (in days)			Statistical index	Width of trabecular structures (in μ)		
	periosteal reaction	primary bony callus	definitive bony callus		10 days	20 days	30 days
Control	20	40	60	M	27,0	38,4	59,0
				$\pm m$	1,09	2,42	2,28
				n	25	25	25
Exptl. (administration of	10	20	50	M	51,2	48,3	69,0
				$\pm m$	2,40	1,95	3,25
				n	25	25	25
				P	0,001	0,005	0,02

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Fig. 1

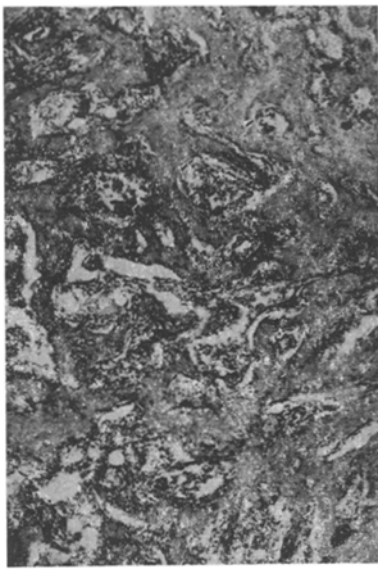

Fig. 2

Fig. 1. Region of regenerating bone in control animal 30 days after fracture. Here and in Figs. 2 and 3: hematoxylin-eosin, 120×.

Fig. 2. Newly formed cancellous bone with trabeculae of lamellar structure 30 days after operation in rat receiving thyrocalcitonin.

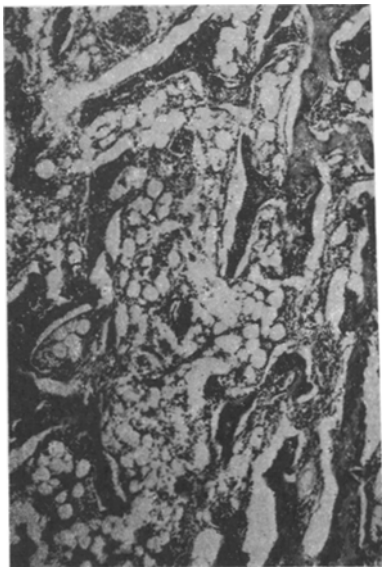

Fig. 3. Formation of medullary canal after 40 days in rat receiving thyrocalcitonin.

by intraperitoneal injection twice a day in a dose of 2.5 units/100 g body weight after fracture of the bone and throughout the rest of the experiment. Regeneration of bone tissue was studied after fracture of the tibial diaphysis. The bone was exposed and fractured in its middle part by special forceps. The fibula was used as a natural splint. Conclusions regarding the rate of repair in the bone tissue were based on the results of macroscopic, microscopic, and fluoroscopic investigations of the region of the fracture followed by roentgenography of the newly formed bone tissues. Biopsy was performed every 5 days for 60 days. Longitudinal histological sections, 8-10 μ in thickness, were stained with hematoxylin and eosin and by Van Gieson's method. To estimate the rate of development and maturation of the bone the width of the bony trabeculae was determined with an ocular micrometer. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The roentgenological investigations showed that 10 days after fracture the fragments in the control animals were in the correct position and separated by a complete transverse cleft. The outlines of the fragments were less sharply defined. A small shadow surrounded the region of the regenerating bone and the ends of the old fragments with a delicate cuff. The periosteal response became clearly apparent in the region of the bone defect after 20 days (Table 1). The shadow due to callus was clearly marked and zones of decalcification could be seen. Partial union of the fragments was observed roentgenologically after 30 days. Gradual reconstruction of the periosteal callus took place. After 50 days the ends of the fragments were joined together by primary bony callus, with the appearance of a homogeneous structure. After 60 days the fracture was united and a medullary canal had formed.

Analysis of the roentgenograms taken of the fracture region in animals receiving TCT showed acceleration of consolidation. Ten days after the fracture the bone fragments were surrounded by a small

but clear zone of periosteal callus. Later (15 days) further growth of the periosteal callus was observed, and at that stage it surrounded the region of the regenerating bone as a dense homogeneous shadow, extending as far as the ends of the old bone fragments. Primary bony callus began to be formed 20 days after the beginning of healing. After 30 days all the features of formation of cancellous bony tissue could be seen, so that the structure of the bone was homogeneous. By the 50th day of the experiment that region of the fracture was apparent only from the wider growth of the bone tissue in the region of the periosteum. Consolidation of the proximal and distil fragments of the bone was followed by complete restoration of the medullary canal throughout its extent.

The roentgenological picture reflecting the rate of repair in the control animals and in the animals receiving TCT matched the histological changes developing in the region of the fracture.

After 10 days the formation of osteoid tissue and large accumulations of cartilage could be seen in the region of the fracture. The bony trabeculae were still very thin – only 27.0 ± 1.09 μ in thickness. The central part of the callus was filled with granulation and young connective tissue. After 15 days the distance between the fragments was reduced. The tissue between the fragments consisted of young bony trabeculae (31.9 ± 1.10 in thickness) and islets of chondroid tissue. Nearer to the center of the defect the callus was formed by cartilage cells at different stages of maturity, and by layers of young connective tissue and granulation tissue. After 30 days young cancellous bone tissue with residual areas of cartilage tissue was seen in the region of the injury (Fig. 1). After 40 days bony callus was formed; it consisted of trabeculae with a lamellar structure, with cavities of fatty bone marrow among them. Definitive bony callus and portions of the medullary canal were formed 55-60 days after the beginning of repair in the control rats.

The morphological picture of healing of the fractures in the animals receiving TCT showed a much more rapid succession of the individual phases of osteogenesis although they continued to appear in the same order. Only 10 days after the fracture the region of injury was filled with large numbers of mature bony trabeculae of considerable thickness (51.2 ± 2.40 μ), surrounded by a layer of large osteoblasts. The early appearance of osteoblasts led to the more rapid formation of osteogenic tissue and was accompanied by an active periosteal reaction. It was observed that periosteal and endosteal callus were formed simultaneously and with equal intensity under these conditions. Closer to the central part of the callus the tissue was mixed chondroid or cartilaginous in type, while the center consisted of connective tissue. Conversion of osteogenic tissue into osteoid took place more rapidly under the influence of TCT than in the control and and it led to the formation of young cancellous bone 15 days after injury. The blood vessels increased considerably in number and formed large vascular plexuses among the areas of newly formed cancellous bone. The central part of the callus was occupied by a narrow band of cartilage tissue. As a result of this intensification of the proliferation and differentiation of osteogenic elements, 30 days after injury, cancellous bony callus with features of compact bone formation could be seen in the region of regenerations (Fig. 2). The bony trabeculae were flatter and wider (69.0 ± 3.25 μ). Proliferating osteoblasts were located at the edges of the bony trabeculae and filled all the intertrabecular spaces. The histological picture of healing 40 days after injury showed further progress in bony callus formation (Fig. 3). The trabeculae filling the lumen of the medullary canal were gradually absorbed and replaced by large cavities filled with fatty bone marrow. Later in the investigation (after 45-50 days) complete restoration of the bone as an organ was observed. The periosteal bone had merged with the compact substance of the bone fragments. The newly formed medullary canals had joined together throughout their extent.

Analysis of the experimental results thus shows that administration of TCT leads to marked activation of repair in bone tissue. This is reflected in the more intensive proliferation and differentiation of the osteoblasts in the regenerating bone, the more rapid formation of cancellous bone tissue, and the development of a rich network of capillaries. Osteoblastic activity maintains a high intensity of enzymic reactions in the regenerating zone, leading to the early formation of a collagen matrix, the nucleation of hydroxyapatite crystals in that matrix, and the consequent formation of bone tissue.

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