EFFECT OF DESOXYCORTICOSTERONE ON REPARATIVE REGENERATION OF BONE TISSUE

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After operative resection of part of the radius from rabbits, injection of the adrenal hormone desoxycorticosterone led to a marked acceleration of callus formation, as shown by histological and histochemical data and also by investigation of fluorescence after injection of tetracycline.

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An important aspect of the study of regeneration of bone tissue is the investigation of endocrine effects on callus formation [1-6].

Osteogenesis was studied in rabbits after administration of the adrenal hormone desoxycorticosterone.

EXPERIMENTAL METHOD

Experiments were carried out on 56 chinchilla rabbits from which part of the radius 0.5 cm in length was resected. No splinting was carried out, because immobilization was provided by the ulna. Twenty rabbits acted as controls and 36 received desoxycorticosterone acetate (DOCA) in a dose of 1 mg/kg body weight daily for 10 days after the operation. To determine the general course of regeneration, roentgenograms were taken of the bone defect every 10 days until the 60th day. Histological and histochemical studies of the callus were carried out on the 10th, 20th, and 30th day. To judge the degree of maturity and development of the bone structures, as well as assessment of the general histological picture, two quantitative indices were used: the width of the bone trabeculae measured with an ocular micrometer (100 determinations in each experiment) and the number of osteocytes in a given area of bone tissue (100 fields of vision, each of 1110 μ^2).

To study some aspects of the process of callus formation, especially maturation and mineralization of the ground substance of the bone, the ability of tetracycline to accumulate in osteogenic structures of the callus and to give fluorescence was utilized. Tetracycline hydrochloride was injected intramuscularly in a dose of 10 mg/kg daily for 5 days before callus was taken for fluoresence investigation. Fluorescence of the structures of the callus was investigated in frozen sections in the ML-2 luminescence microscope, and the same sections were further studied in phase contrast and also after histological staining. These sections were also treated with 3% TCA solution for 4-5 h and reexamined in the luminescence microscope.

EXPERIMENTAL RESULTS

In the control rabbits a roentgenologically visible faint shadow of callus partly filled with defect only after the 20th day. By the 50th day, the continuity of the bone was restored, and after the 60th day a medullary canal began to be formed. In the experimental animals receiving DOCA, the contrast with the controls, a small, faint shadow of callus partly filled the defect by the 10th day, and on the 20th day the defect was completely filled. Reorganization of the callus with the formation of a medullary canal was observed after the 40th day. On the 50th day the continuity of the bone was restored and a well marked cortical layer and medullary canal were present.

Histologically, in the control series of experiments on the 10th day the callus appeared as an undifferentiated fibrous tissue, composed of thin fibers, and islands of young cartilage. It contained a few

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TABLE 1. Width of Bone Trabeculae and Number of Osteocytes in Them

Group of animals	Width of trabeculae (in μ)		Number of osteocytes per 100 fields of vision	
	20th day	30th day	20th day	30th day
Control	43.55 ± 1.47	71.42 ± 4.82	232	157
Experimental	71.36 ± 3.68	73.50 ± 3.48	200	128

small areas of osteoid tissue, forming irregularly shaped struts with closely packed and randomly arranged osteocytes. The nuclei of the osteocytes were polymorphic when stained for DNA. The ground substance of the osteoid islands stained intensely for mucopolysaccharides (MPS). The osteoblastic layer was ill defined.

In animals receiving DOCA the callus on the 10th day after operation consisted of areas of fibrous tissue with foci of hyaline cartilage and well formed bony trabeculae of woven character. The ground substance of the osteoid trabeculae, when stained by Van Gieson's method, was fuchsinophilic. It gave a PAS reaction of average intensity. The osteoblastic layer was well defined. The callus contained numerous large osteoclasts, lying between the trabeculae at the periphery of the cartilage islands.

On the 20th day the control callus consisted of cartilage and areas of woven bone tissue, forming anastomosing trabeculae. Fibrous tissue was present only at the periphery, where it was grouped as in the periosteal layer. Remnants of cartilage were present inside the callus in the form of islands immured in the struts of bone. The ground substance of the trabeculae and cartilage gave a strongly positive reaction for MPS. The formation of lamellar structures could be seen in some trabeculae.

In an experiment on the 20th day the callus consisted almost entirely of anastomosing bone trabeculae. In contrast to the control, cartilage tissue was found only in some rabbits as solitary islands or individual cell groups, immured in the bone tissue. The bone trabeculae in the center contained unorganized fibrous tissue and polychromatically stained ground substance. The periphery of the trabeculae was formed by lamellar structures. Osteon formation took place around some lacunae, which was not observed in the control animals at this period.

On the 30th day the callus in the control series was formed by interwoven bone trabeculae with a lamellar structure. The boundaries between the lamellae were irregular, indicating the immaturity and incomplete formation of the structures. In sections stained for neutral MPS, a deeper color was observed in the center of the trabeculae, i.e., at the sites of remnants of cartilage and osteoid tissue. The lamellar structures gave a weak reaction.

Foci of golden yellow fluorescence were seen in the luminescence microscope: in some areas they were distributed in the same manner as the bone trabeculae, anastomosing with each other; in others the foci of fluorescence consisted of unorganized areas with a cellular structure. In phase-contrast and histological investigation the zones of fluorescence corresponded to the arrangement of the bone trabeculae, and the cellular areas to the zone of replacement of cartilage by bone. Fibrous and cartilaginous tissue gave no fluorescence. After treatment of frozen sections with 3% TCA for 4-5 h the fluorescence disappeared.

On the 40th day of the experiment callus resembling spongy bone was formed by the interwoven bone trabeculae. Islands of cartilage were found in a few experiments. In some sections the formation of a medullary canal could be seen under the microscope. The bone tissue was mature in appearance, and rich in dense fuchsinophilic ground substance. In sections stained for MPS the trabeculae gave a strongly positive PAS reaction. The osteoblastic layer was clearly defined. In some areas of the lacunae collections of osteoclasts were present, indicating reorganization of the bone.

During investigation in the luminescence microscope the areas of fluorescence were much larger and more intense than in the control. Fluorescence of the bone trabeculae was homogeneous. Foci of

honeycombed fluorescence were seen, consisting of very small islands of calcified cartilage, being replaced by bone tissue. Dark areas of irregular shape were seen between the foci.

Comparison of the results of the experiments in which hormone was injected and the controls shows clearly that saturation of the rabbit with DOCA leads to significant acceleration of callus formation. This is reflected in the results of roentgenologic and histological investigation. In addition, as Table 1 shows, after injection of the hormone, development of the bone structures and the accumulation and maturation of the ground substance of the bone tissue took place much more intensively and rapidly than in the control. A study of the character of fluorescence in the ossified callus structures suggests that under the influence of DOCA calcification of the osteogenic structures of the callus takes place more rapidly and to an increased degree. The method of tetracycline labeling is very sensitive and can be used to study the process of osteogenesis experimentally. The results suggest that fluorescence is due to the formation of a complex of tetracycline with calcium compounds.

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