EFFECT OF REGENERATION OF BONE TISSUE IN THE EARLY STAGES BY THE TETRACYCLINE LABEL METHOD

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After operative removal of part of the radius in rabbits the early stages of osteogenesis were studied by the tetracycline label, histological, and histochemical methods. In this phase of formation of the organic matrix at the site of the defect fluorescence was shown to be due to the presence of calcified, necrotic areas of bone, while in the phase of mineralization it was due to processes of oscification of the osteogenic structures of the callus. The tetracycline label method is very sensitive and can be used to judge the character of reconstruction of osteogenic structures during callus formation.

The tetracycline label method was used previously [1, 2] to assess the progress of calcification of osteogenic structures of regenerating bone starting from the 10th day of callus formation. The results showed that tetracycline is fixed and retained by bony structures at different stages of reparative regeneration of bone tissue. The intensity of fluorescence increases parallel with the progress of oscification.

In the investigation described below osteogenesis was studied in the early stages of regeneration by the use of the tetracycline label method.

EXPERIMENTAL METHOD

Twenty chinchilla rabbits weighing 2.5-3 kg were used. Under aseptic conditions subperiosteal resection of 0.5 cm of the radius in the middle third of the diaphysis was carried out. The skin wound was sutured and a dry sterile dressing applied. Tetracycline sulfate was injected subcutaneously in a dose of 10 mg/kg body weight 2 days before removal of the callus for fluorescence analysis.

The callus for investigation was taken by operation at times between and 1 and 11 days. Fluorescence of the structures was investigated in frozen sections in the ML-2 luminescence microscope and the same sections were additionally studied in the phase-contrast microscope and after staining with hematoxylin-eosin.

Some material was fixed in 10% neutral formalin and embedded in paraffin wax. The sections were stained by Van Gieson's, Shueninov's, Brachet's, and Feulgen's methods and with toluidine blue.

EXPERIMENTAL RESULTS

The results showed that during the first 4 days after the operation the defect in the bone was filled with a blood clot consisting of fibrin and leukocytes with many wandering cells (macrophages and lymphocytes) and small areas of necrotic bone tissue, located chiefly in the peripheral zones, deeply stained with hematoxylin or weakly pyroninophilic.

In some experiments small areas of connective tissue with swollen, activated fibroblasts, with an increased content of RNA and DNA, revealed by staining by Brachet's and Feulgen's methods, were present at the edge of the callus.

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The study of frozen sections of the callus under the luminescence microscope revealed small, irregularly circular foci of golden-yellow fluorescence. On investigation of the same section in phase contrast and after staining with hematoxylin-eosin the foci of fluorescence were found to correspond to calcified, necrotic areas of bone tissue.

Starting on the 6th day the fibrinous and leukocytic masses of the callus became denser and gave a positive PAS reaction but thin-fibered connective tissue containing many small blood vessels proliferated at their edges. The connective-tissue cells were arranged haphazardly and contained large nuclei with clearly outlined nucleoli. The ground substance consisted of thin fibers and was weakly fuchsinophilic when stained by Van Gieson's method. The fibrous structures and amorphous substance reacted positively for mucopolysaccharides.

Examination in the luminescence microscope, just as during the first days, revealed small and larger, irregular homogeneous foci of fluorescence corresponding to necrotic, calcified areas of bone tissue.

After the 8th day the bony callus contained a diversity of tissue structures. At the periphery the connective tissue was rich in blood vessels, dense and collagenized in places, and fuchsinophilic when stained by Van Gieson's method. The fibroblasts were elongated with condensed nuclei. In other areas the tissue consisted of thin fibers and fibroblasts with swollen nuclei and an increased content of RNA and DNA. Pictures of cell division could be seen. Immature thin-fibered tissue could be seen particularly abundantly along the course of the ingrowing vessels. In the middle of the connective-tissue mass were islands of hyaline cartilage, irregular in shape, with young osteoid tissue growing in between them. Cartilage cells were close together and surrounded by dense capsules and their cytoplasm was filled with glycogen. The intercellular substance was basophilic and reacted weakly for neutral mucopolysaccharides. Young bone tissue lay side by side with the islands of cartilage and penetrated among them as interweaving trabeculae. The marginal zones of the bony trabeculae consisted of oxyphilic ground substance. The osteoblastic layer around the trabeculae was discontinuous and the osteoblasts were oval in shape, with basophilic cytoplasm and large, swollen nuclei.

In the luminescence microscope the sections showed areas of golden-yellow luminescence of different sizes, corresponding to the trabeculae of newly formed bone, as histochemical investigation confirmed.

By the 10th day the bony callus consisted of fibrous and chondro-osteoid tissue. The cartilaginous areas had the appearance of large islands replaced at the periphery by bands and trabeculae of osteoid tissue of coarsely woven character. A sharp line could not always be drawn between the cartilaginous and osteoid tissues. An osteoblastic layer formed at the periphery of the foci of oscification. The osteocytes were irregularly round in shape and were separated by an oxyphilic ground substance. Wide areas of undifferentiated connective tissue, rich in cells, could be seen between all the structures mentioned above. A continuous layer of dense fibrous tissue was situated at the periphery of the callus.

Large, homogeneous areas of golden yellow luminescence, appearing like bony trabeculae in some places, could be seen in the luminescence microscope. At the edges of the luminescent areas there were small, angular foci of fluorescence. In phase contrast and on histological examination of these sections the fluorescent areas were found to correspond to trabeculae of newly formed bone, while the small foci of luminescence corresponded to zones of replacement of the destroyed cartilage by bone. Cartilaginous and fibrous tissue, clearly detectable in phase contrast, did not exhibit fluorescence.

The results thus showed that in the early periods of reparative regeneration, in the phase of formation and restoration of the organic matrix of the regenerating bone, fluorescence of the bony callus was connected with the presence of calcified, necrotic areas of bone. Later (9-11 day), in the phase of mineralization, the fluorescence was due to commencing processes of oscification of certain osteogenic structures of the callus.

The tetracycline label method is simple, readily available, and provides objective evidence of the character of reorganization of the osteogenic structures during callus formation in its early stages.

LITERATURE CITED

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2. N. F. Krut'ko, Trudy Gor'kovsk. Med. Inst., No. 32, 298 (1970).