# EFFECT OF COBALT COMPLEXES ON REGENERATION OF MUSCLE TISSUE IN IRRADIATED ANIMALS

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UDC 616.74-003.93-02:615.777.953/-06:617-001.28

In previous investigations [1, 2] the author showed that certain cobalt complexes stimulate the regeneration of bone tissue and skin, increase the number of blood cells and restore the normal clotting power of the blood, intensify protein synthesis, and increase the natural and artificial immunity factors of irradiated animals.

The object of the present investigation was to study the effect of certain complexes of cobalt with various vitamins (with nicotinamide—coamide; with vitamin $B_1$—Co-8, with vitamin $H_1$—Co-9, and also Co-30) on the regeneration of a wound of muscle in irradiated animals.

## EXPERIMENTAL METHOD

Experiments were carried out on 150 male albino rats weighing 200-250 g, receiving a single whole-body exposure to x rays from the RUM-11 x-ray therapy apparatus in the following conditions: voltage 180 kV, current 10 mA, filter 0.5 mm Cu, focus distance 50 cm, dose rate 18.5 R/min, dose 800 R.

The irradiated rats were divided into two groups. The animals of group 1 (30) did not receive the test substances (control I), and those of group 2 (120) were treated with the cobalt complexes. As an additional control (control II), 20 unirradiated rats were used.

On the fifth day after irradiation, in aseptic conditions a transverse incision of the skin and the biceps muscle down to its middle was made at the level of the middle third of the right thigh, with a safety razor blade, the wound then being closed with a fine capron suture. The skin wound was closed without drainage and its surface covered with cleol.*

Treatment began on the day of injury. The test preparations were injected intramuscularly in the form of a 1% solution (coamide, Co-8, and Co-9) or 0.1% solution (Co-30) in a dose of 2-3 mg/kg body weight daily for 20-25 days.

The process of healing of the wound was studied clinically and microscopically. Material for histological treatment was taken between 5 and 50 days after the operation. The rats were decapitated in batches of 2-4 at a time. The material for study was fixed in 14% neutral formalin and celloidin sections were stained with hematoxylin-eosin.

## EXPERIMENTAL RESULTS

All the irradiated rats developed severe radiation sickness. In the first week after irradiation the weight of the animals fell on the average by 20-25%, and it was restored slowly. During 5-10 days after irradiation the leukocyte count fell to 1500-1000 per ml blood, and in a few cases to 600-450. The mortality among the irradiated animals in the control group in the first three weeks reached 43.5%.

No gaping of the wound edges was observed. In most of the control irradiated rats the edema and infiltration of the tissue in the region of the wound persisted much longer than in the unirradiated animals and also in the experimental (treated) animals.

During the morphological study of the preparations, the following were taken into account: the time of sloughing of the necrotic elements, the appearance of myoblasts and muscle syncytia in the wound, the

*A mixture of rosin, ethyl alcohol, ether, and sunflower oil.

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 63, No. 5, pp. 93-97, May, 1967. Original article submitted May 6, 1965.

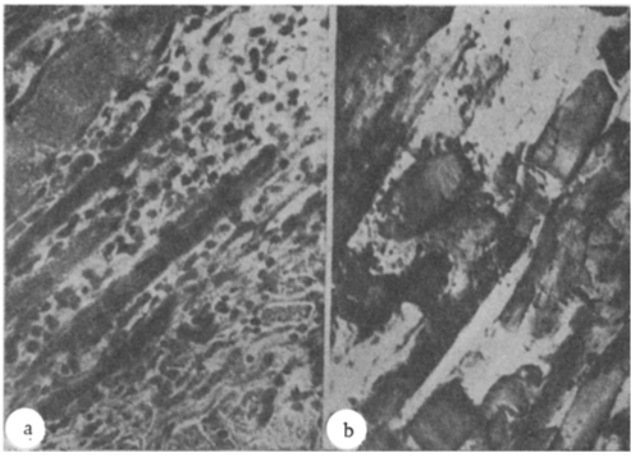

Fig. 1. Region of the muscle defect 5 days after the operation: a) appearance of muscle syncytia in the wound of an experimental rat treated with coamide; b) lumpy degeneration of muscle fibers in a control irradiated rat. Hematoxylin-eosin. Objective 20×, ocular 10×.

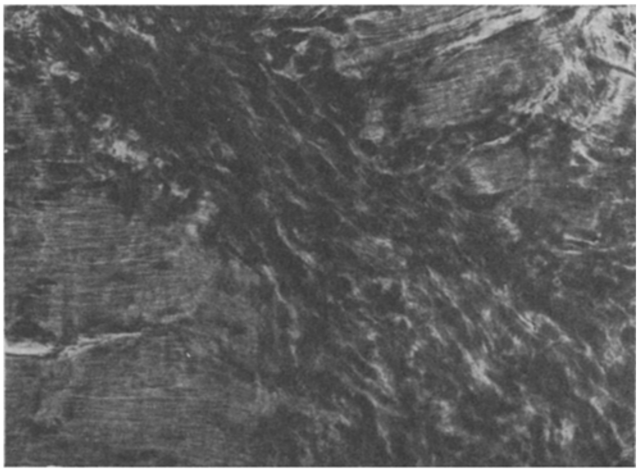

Fig. 2. Region of the muscle defect 50 days after the operation in a control irradiated rat. The defect is filled with scar connective tissue. Hematoxylin-eosin. Objective 20 ×, ocular 10×.

formation (differentiation) of muscle tubes, the formation of young muscle fibers, and the filling of the defect with mature muscle fibers or connective tissue.

In the unirradiated rats on the fifth day after injury the necrotic masses of muscle in the region of the defect as a rule were almost completely resorbed, and in places only inflammatory foci with aggregations of blood cells were seen, accompanied by exudation and edema. Many connective-tissue cells appeared. Young muscle cells—myoblasts and syncytia—appeared at the borders of the defect. Meanwhile dedifferentiation of the ends of the divided, viable muscle fibers took place, and muscle buds developed in them, becoming visibly longer. Conversely, in the irradiated control animals on the fifth day the necrotic muscle fibers, which had not undergone resorption, were completely intact (Fig. 1, b). Whereas the control irradiated animals were characterized by limitation of spread of the destructive processes in the

Fig. 3. Region of the muscle defect 25 days after the operation in an experimental rat treated with coamide. The defect is filled with chaotically arranged young muscle fibers. Hematoxylin-eosin. Objective 20×, ocular 10×.

injured part, in the irradiated rats the region of the degenerative changes, with lumpy degeneration of the muscle fibers extended further. The divided muscle fibers had disintegrated throughout their length, and because of this, the defect as a rule was much wider than in the unirradiated animals.

On the tenth day differentiation of muscle syncytia into muscle tubes was observed in the unirradiated rats. At the periphery of the defect, connective tissue was proliferating in the space between the muscle tubes. Signs of inflammation in the wound were almost completely absent.

In the irradiated control animals on the tenth day after wounding the defect in the muscle was filled with necrotic masses of a waxy character. Delay in the resorption of the necrotic masses was evident. The cellular and phagocytic reactions were still supressed. Most animals showed no signs of regeneration, and in only a few were clusters of connective-tissue cells visible. In some areas both in the region of the defect and in the subcutaneous areolar tissue, hemorrhages were seen.

On the fifteenth-twentieth day the connective-tissue of the wound in the unirradiated animals had become loose, the muscle tubes were more completely differentiated, and the wound defect was filled by them and by young muscle fibers containing many nuclei. At this time in the control irradiated rats the wound was cleared of necrotic masses and activation on the fifteenth day. At the edges of the defect the connective-tissue was developing, but proliferation of the fibroblasts was appreciably depressed. The muscle buds, syncytia, and tubes appearing near this time developed slowly and sluggishly, and at times were lost in a mass of connective tissue.

On the twenty-fifth to thirtieth day the formation of muscle fibers in the unirradiated rats was largely complete. The defect was completely filled with chaotically arranged muscle fibers. In the control irradiated animals at this time the muscle defect in most cases was filled with loose connective-tissue, but in some animals the muscle fibers present were chaotically arranged. Healing of the wound in the animals of the control irradiated group was mainly completed by the filling of the defect with scar connective-tissue (Fig. 2).

A single exposure of rats to whole-body irradiation in a dose of 800 R thus gave rise to marked depression and distortion of regeneration of the skeletal muscle tissue. In all probability this took place as a result of depression of the migration of leukocytes, considerable slowing of resorption of the necrotic part of the muscle fibers, and most important of all, the state of depressed reactivity of the organism.

In the experimentally treated animals the process of regeneration of the muscle tissue followed a sharply different course from that in the control irradiated animals.

On the fifth day after the beginning of treatment, the defect in the muscle was almost completely cleared of the necrotic mass. Only in a few animals edema was present in the central part of the wound and the necrotic focus was not completely absorbed. The cellular reaction was somewhat less intensive than in the unirradiated animals, but it was clearly defined. At the edges of the defect connective tissue was developing actively. Groups of myoblasts and muscle symplasts appeared, and muscle buds were seen at the ends of the preserved muscle fibers (Fig. 1, a).

On the tenth-fifteenth day the defect was completely freed from all degenerated elements, and was filled with muscle syncytia and differentiated muscle tubes, with many nuclei, against a background of connective tissue.

On the twentieth to twenty-fifth day the defect in the muscle was filled mainly with chaotically arranged young muscle fibers, in which the cross striations were clearly visible (Fig. 3). In some cases the development of a zone of regeneration, composed of muscle and connective-tissue, was observed in the region of the defect, and later (on the thirtieth-fortieth days) the individual parts of this zone were separated by bands of fat.

These results show that the cobalt preparations stimulated the development of reparative regeneration after muscle trauma almost equally, but from the pattern of differentiation of the muscle elements which they produced in the wound, coamide and Co-9 were more effective than Co-8 and Co-30.

After 3 or 4 injections of these preparations the general condition of the experimental animals began to improve noticeably: their appetite returned and they began to regain their lost weight quickly. The state of the peripheral blood also improved. The mortality rate was lowered.

## LITERATURE CITED

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