EFFECT OF GALANTHAMINE ON REPARATIVE REGENERATION OF MUSCLES IN TAILLESS AMPHIBIANS

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The distal end of the quadriceps femoris muscle was removed in frogs. Injection of galanthamine considerably stimulated regeneration of the muscle and led to better development of the bony tuberosity formed at the site of operation to which the muscle fibers were attached.

Very few investigations have been made into the possibility of reparative regeneration of skeletal muscle in tailless amphibians. In most cases reparative regeneration of cross-striated muscles has been studied after transverse incisions to the muscle or injury to a localized area [6, 8]. In these cases regeneration of the muscle as an organ was not studied at all, and only the tissue processes taking place during regeneration were considered.

There is some evidence that the skeletal muscles of tailless amphibians possess very low ability for reparative regeneration,for these animals lead a sedentary mode of life [9]. However, according to one view, if the appropriate conditions are created reparative regeneration of the skeletal muscles can take place [3].

There is much experimental evidence of the important role of nerves in the development of regeneration in skeletal muscles, for after injury to the nerve, regeneration of muscles is arrested in the initial phases of the process. However, the mechanism of this effect of the nervous system on muscle regeneration is unknown [1, 2, 4, 10].

The object of the investigation described below was to study reparative regeneration of a skeletal muscle in tailless amphibians. Since it could be expected that the intensity of such regeneration would be low, galanthamine was used to stimulate regeneration, in view of its anticholinesterase action [5].

EXPERIMENTAL METHOD

Adult frogs of the species Rana temporaria and Rana ridibunda were used. The quadriceps femoris muscle in the right hind limb was cut through transversely at its midpoint and the distal end was removed along with the tendon of its attachment to the knee joint. After the operation the animals were divided into two groups. The animals of group 1 acted as the control and received no further treatment. The frogs of group 2 began to receive subcutaneous injections of 0.25% galanthamine solution one week after the operation and continuing for 10 days, starting with a dose of 0.1 ml and gradually increasing the dose to 0.4 ml (a dose of 0.6 ml proved to be toxic). The mortality among the frogs of both groups was high. They died, not from the operation, but evidently because of the unsatisfactory conditions in which they were kept (the frogs were

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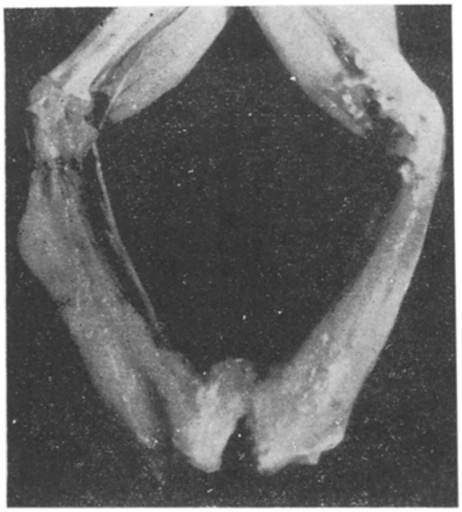

Fig. 1

Fig. 2

Fig. 1. Total preparation of both hind limbs. Bony tuberosity can be seen clearly on the right.

Fig. 2. Section through bony tuberosity.

kept in glass jars with water). Of a large number of frogs undergoing the operation, 18 survived for a long time (four frogs lived four months, two from each group). The animals were sacrificed 120 days after the operation. The muscle together with the bone was fixed in formalin and decalcified in nitric acid. The pieces of tissue were embedded in paraffin wax and sections 7-8 μ in thickness were stained by VanGieson's method.

EXPERIMENTAL RESULTS

On palpation of the frog's thigh through the skin 1-1.5 months after the operation, a small bony tuberosity (1-2 mm high) could be felt at the junction between the lower and middle thirds of the femur. By 2-2.5 months after the operation it was considerably enlarged and raised the skin. By the end of the experiment the height of the tuberosity was 2.5-3 mm, and in two cases it actually reached 4-4.5 mm.

The formation of bony tuberosities was observed in both groups of animals, but they were somewhat lower in the control frogs,and their borders with the surrounding bone were smoother. Four months after the operation, after sacrifice of the frog and removal of bone from the limb, new muscle tissue could be seen to be formed in the experimental animals and was distinguished by its paler color. Newly formed muscle fibers ran toward the bony tuberosity of the femur and were attached to its summit (Fig. 1). Some fibers passed alongside the bony tuberosity and ran toward the knee joint. On examination of the control animals, no young muscle fibers were seen to be formed. However, connective-tissue fibers were observed, starting from the muscle stump and attached to the bony tuberosity. In the animals of both groups the nerve grew into the muscles attached to the bony tuberosity. Histological examination of the material showed that the bony tuberosity consisted largely of young cartilage tissue, which was still continuing to develop. In the base of the tuberosity there were large cavities filled with bone marrow cells (Fig. 2).

In the distal part of the muscle stump young muscle fibers were formed, and in some places their cross-striation could be clearly distinguished. In the young muscle fibers there were many nuclei, frequently arranged in chains and forming clusters at the end of the muscle fibers. Newly formed muscle fibers were attached to the bony tuberosity of the femur by connective-tissue fibers. Some muscle fibers ran to the knee joint without attachment to the bony tuberosity.

In the control animals the bony tuberosity was much smaller. Only slight formation of new muscle fibers was observed, and these also ran to the tuberosity.

It can accordingly be concluded that under the influence of galanthamine (i.e., evidently as the result of the more prolonged action of acetylcholine), regeneration of skeletal muscle in tailless amphibians is accelerated. However, the muscle was not completely restored four months after the operation. The conditions favoring development of the bony tuberosity and attachment of the muscles to it are not completely clear. Investigations have shown that prolonged and systematic contractions of muscles lead to changes in bone metabolism and ultimately to working hypertrophy of bone [7]. This is a possible explanation of the formation of the bony tuberosity on the femur in these experiments.

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