# AUTOGRAFTING OF WHOLE MUSCLES IN REPTILES

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Autografting (reimplantation) of the whole gastrocnemius muscle was carried out in turtles (*Testudo horsfieldi*) weighing 300-500 g. The grafts were investigated 2 weeks to 6 months after transplantation. After 5-6 months the reimplanted muscles were similar in color and shape to the intact symmetrical muscles and they amounted to 77% of their weight. Regeneration was observed in the grafts, but its course was slower than in mammals. Separation of myoblasts from disintegrating muscle fibers of the graft took place rapidly during the first month and continued until 2 months after grafting. Simultaneously, differentiation of myogenic elements into cross-striated muscle fibers and vascularization of the grafts were observed. Most of the grafts by the end of the period of investigation had a muscular type of structure, although considerable variability was observed in the diameter of the muscle fibers.

KEY WORDS: *transplantation of muscles*; *regeneration of muscle fibers.*

The possibility of free grafting of whole skeletal muscles, suitably prepared, in mammals was first demonstrated in Studitskii's laboratory [9]. It was found that a whole muscle could be successfully transplanted in adult animals only after preliminary denervation [1]. Later investigations showed that totally intact muscles can be transplanted in young rats [4]. The method of transplantation of both prepared and unprepared muscles is nowadays used by many investigators [6, 10-12, 14] studying various problems in the experimental morphology and pathology of muscles. The property of a muscle to survive at the site of grafting, to redevelop, and to construct a new muscular organ has been called its transplantation activity [8]. Processes of regeneration in damaged and transplanted muscles, it must be noted, have been studied largely in mammals [7]. To discover the character of changes in transplantation activity of muscles in different groups of vertebrates it is particularly interesting to investigate this property in members of the class of reptiles, for analysis of data in the literature shows that processes of regeneration in the tissues of reptiles have not yet been adequately studied [5, 15]. The object of this investigation was to study whether whole intact skeletal muscles can be transplanted in turtles and to study the processes of regeneration taking place in the grafts.

## EXPERIMENTAL METHOD

Experiments were performed in spring on 42 young terrapins *Testudo horsfieldi* weighing 300-500 g, kept under animal house conditions in special metal cages with continuous electric lighting (air temperature 24-26°C). The animals received a vegetable diet daily.

The test object was the gastrocnemius muscle. Under pentobarbital anesthesia the right gastrocnemius muscle was completely detached and reimplanted in its previous position, by suturing it to the remnants of the tendons and leading the tibial nerve to it. The autografts were studied from 2 weeks to 6 months after transplantation. The transplanted and symmetrical intact gastrocnemius muscles were weighed before fixation. Zenker's fluid was used as the fixative. Histological sections were stained by Mallory's method, with iron

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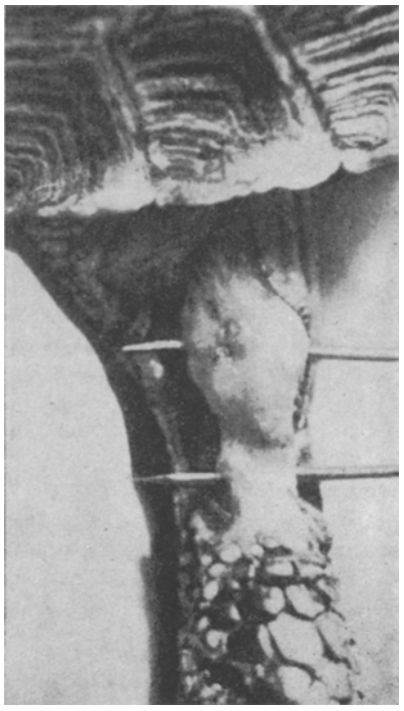

Fig. 1. Autograft of whole gastrocnemius muscle of *T. horsfieldi* 3 months after transplantation. Magnification 2×.

hematoxylin and counterstained by Mallory's method, and with azure–eosin. The numerical results were subjected to statistical analysis.

## EXPERIMENTAL RESULTS

Macroscopic investigation showed that during the first month after the operation the grafts were edematous in appearance. They were pale pink in color with yellowish stains and round in shape. After 0.5-2 months the edema subsided and the grafted muscle became darker in color. At 3 months the transplanted muscle became longer and in color and shape it resembled the intact symmetrical muscle (Fig. 1). Later (5-6 months) the grafts differed from the intact muscles only in their slightly smaller volume.

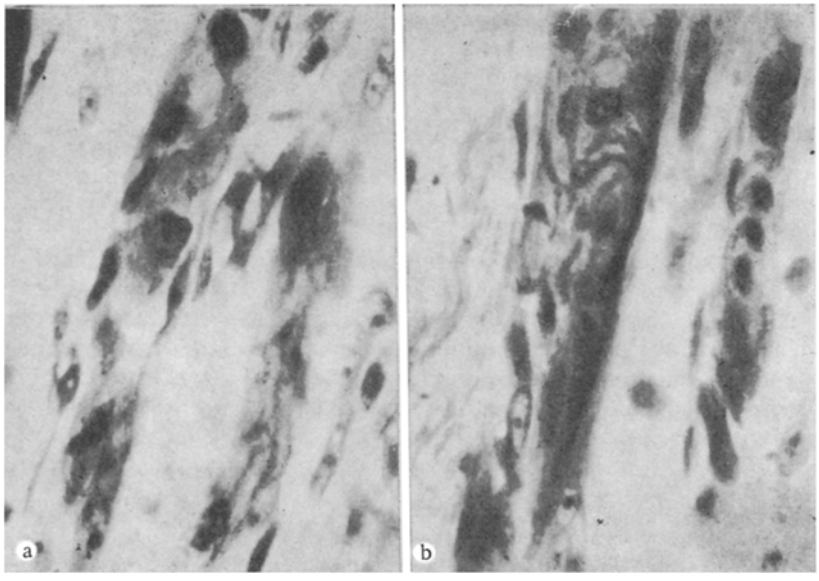


Fig. 2. Longitudinal section through 14-day-old graft of turtle gastrocnemius muscle. Zenker. Iron hematoxylin. a) Separation of myoblasts on walls of sarcolemma tube of old muscle fiber. 400×; b) mitosis in a myoblast. 400×.

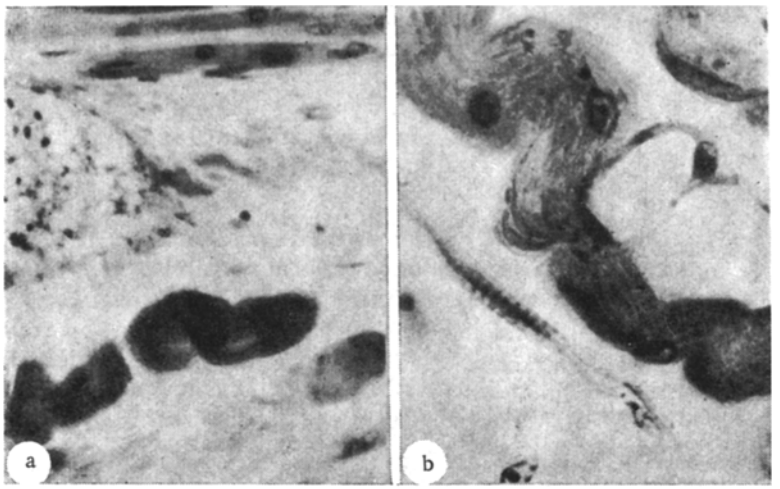

Fig. 3. Longitudinal section through 30-day-old graft of turtle gastrocnemius muscle. Zenker. Iron hematoxylin. a) Old (thick) and young (thin) muscle fibers. 200×; b) vacuolation of old muscle fibers. 400×.

Weighing the transplanted muscles showed that during the first month after transplantation the weight of the autografts increased relative to the symmetrical intact muscles, to reach 119 ± 8% by the 30th day. By 2 months, when the edema has subsided, the transplanted muscles were again equal in weight to the intact muscles. Later the weight of the grafts fell a little, and after 6 months the grafted muscles weighed 77 ± 4% of the weight of the intact muscles.

Analysis of the histological sections showed that after 2 weeks the autografted muscles of the turtles consisted largely of old muscle tissue. Some of the superficial muscle fibers still preserved their structure: Nuclei and cross-striation were still visible. In somewhat deeper fibers large vacuoles were found. In the loosely arranged muscle bundles in the central zone of the fiber cross striation was still clearly defined, but most of the nuclei were no longer visible. In some fibers discoid degeneration was observed. Meanwhile, in the peripheral zone of the grafts the reconstruction of the old muscle fibers, development of myogenic elements, and their differentiation were clearly in progress. Side by side with surviving muscle fibers with an intact structure individual muscle tubes and myosyncytia oriented in the direction of the original muscle fibers, whose place they occupied, could be seen. Between the loosely packed myogenic elements there were many polyblasts and connective-tissue cells, frequently in a state of mitosis, and also collagen fibers. Together with differentiation of the myogenic elements which appeared earlier, active myoblast formation also was observed. In many old muscle fibers separation of myoblasts, destruction of the myofibrillary apparatus and other components of the fibers, and phagocytosis of the destroyed material by macrophages could be seen on the walls of the sarcolemma tubes (Fig. 2a). Often one or more basophilic myoblasts were next to an old, incompletely destroyed muscle fiber. The myoblasts frequently divided by mitosis (Fig. 2b). Fusion of myoblasts and the formation of wide and narrow myosyncytia were observed. In the peripheral zone — the region of reconstruction of the graft material — invasion of numerous dilated capillaries carrying blood was observed. In the endothelium of the vessels in the peripheral zone mitoses were seen.

Reconstruction of the graft material continued in the later stages after transplantation, but the formation of myogenic elements took place less intensively. The grafts 1 month after transplantation now contained many young cross-striated muscle fibers. Frequently among the thin, newly formed fibers there were thick, old muscle fibers (Fig. 3a). These fibers were twisted, their cross-striation was ill defined, and they had few nuclei; small round nuclei were seen. Some fibers showed vacuolation (Fig. 3b). Separation of myoblasts from the disintegrating muscle fibers also was observed. Sometimes areas of loose and dense fibrous connective tissue, replacing dying muscle fibers, could be found in the grafts after 6 weeks. Meanwhile, thin syncytia with chains of round nuclei, containing distinct, large nucleoli, branched out from the old, thick muscle fibers.

The grafts 2 months after transplantation consisted mainly of newly formed muscle tissue. The young muscle fibers varied in diameter. The thicker fibers occupied a peripheral position, whereas the thin fibers were in the central parts of the grafts. Bundles of muscle fibers were separated from one another by small layers of fibrous connective tissue. Sometimes solitary old muscle fibers, from which myogenic elements were separating, could still be seen. Blood vessels growing into the grafts differentiated into arterioles and venules, accompanied by large, branched, pigmented cells. By 3 months, resorption of the old muscle tissue was mainly complete.

After 5-6 months the greater part of the grafts had a muscular type of structure, closely resembling intact turtle muscle in histological structure. The muscle fibers in the grafts contained numerous elongated nuclei; mainly they were regularly arranged, grouped into bundles separated from one another by bands of connective tissue. Together with thick muscle fibers, some quite thin fibers also were found in the grafts. Sometimes the muscle fibers were interwoven a little. The quantity of connective tissue in the grafts was a little increased compared with the intact muscle. The grafts were well vascularized.

Free autografting of a whole skeletal muscle without preliminary preparation is thus possible in *T. horsfieldi*. The gastrocnemius muscles of turtles were shown to possess considerable transplantation activity. After 2-3 months new muscle organs were formed from the material of the graft and they persisted until the 6th month of the investigation. Nearly all grafts had a muscular type of structure [2]. The turtle skeletal muscle, when totally transplanted, undergoes profound and complex structural changes including regeneration, a process called transplantation regeneration in the writers' laboratory [3]. Most of the old muscle tissue of the grafted turtle muscles underwent progressive reconstruction. Myoblasts separated from many of the old muscle fibers, but the myofibrillary system and other components of the muscle fibers were destroyed. According to data in the literature [13] a role of satellite cells is postulated in the regeneration of muscle fibers in another representative of the class of reptiles — the lizard. In turtles also, it seems, the role of satellite cells in the regeneration of transplanted muscles cannot be ruled out. The process of transplantation regeneration in grafted turtle muscles takes place intensively during the first month after grafting, but subsides in the second month. Young cross-striated muscle fibers are formed through the development and differentiation of the newly formed myogenic elements. A special feature of the regeneration process in grafted turtle muscles is the prolonged preservation of some of the old muscle fibers, in which the characteristic structural components could be found until 1-1.5 months. Later nearly all these fibers underwent vacuolation and were destroyed. The regenerated muscle organs did not reach the size of the intact muscles, but after 6 months they amounted to 77.4% of their weight. If these results are compared with those obtained previously in rats [3] it can be concluded that in the reptiles studied the transplanted muscles attained a greater weight than in mammals, in which the weight of the grafted muscles after 6 months was on average only 50% of the weight of the intact muscles. Meanwhile, the process of transplantation regeneration takes place more slowly in turtle muscles than in rats: In the totally transplanted rat gastrocnemius muscle no old muscle fibers not yet reconstructed still remain after 1 month, whereas in turtles their remnants were still present 1.5-2 months after the operation.

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# CHARACTERISTICS OF HEPATOCYTES CONTAINING α-FETOPROTEIN IN REGENERATING MOUSE LIVER

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After poisoning SWR mice of different ages with single or repeated doses of $CCl_4$ vapor the synthesis of the embryo-specific protein α-fetoprotein (α-FP) was induced. The greatest rise in the α-FP level was observed in mice under 1 month old. In sections through the liver regenerating after $CCl_4$ poisoning, α-FP was found in hepatocytes indistinguishable from the main population: in small cells in young animals and in large, polyploid hepatocytes in the repeatedly poisoned mice. The only distinguishing feature of the α-FP-containing cells after poisoning of the mice with different doses of $CCl_4$ was that most of them were on the boundary with the necrotic zone. A similar localization of α-FP-containing hepatocytes was observed when two other hepatotoxins were used: paracetamol and allyl alcohol.

KEY WORDS: *α-fetoprotein; immunofluorescence; hepatocytes; regeneration of the liver.*

Synthesis of the embryonic serum protein α-fetoprotein (α-FP) is renewed in the regenerating mouse liver after carbon tetrachloride poisoning [1, 3]. The writers showed previously that under these conditions α-FP is contained in typical mature hepatocytes, which amount to not more than a few percent of the total number of undamaged cells and are located chiefly at the boundary with the necrotic zone [7].

The object of this investigation was to study the localization of α-FP in the regenerating mouse liver depending on the age of the animals, the degree of maturity of the hepatocytes, and the character of liver damage.

## EXPERIMENTAL METHOD

Regeneration of the liver in SWR mice (male and female) of different ages (from 13 days to 7 months) was induced by poisoning with $CCl_4$ vapor in a concentration of 0.005 ml in 3 liters air [3] or 0.003 and 0.001 ml in 3 liters air. Allyl alcohol and paracetamol were injected intraperitoneally in physiological saline in doses of 5 and 40 mg/100 g body weight, respectively [10, 11]. On the 2nd or 3rd day after poisoning the mice were decapitated and the blood levels of α-FP determined by the precipitation reaction with a standard test system [4]. Repeated $CCl_4$ poisoning was carried out with intervals of not less than 18 days between doses. On the second to third day after each dose of the poison, blood samples were taken from the retro orbital sinus of the mice for determination of the α-FP concentration. The mice were killed after the 1st, 2nd, 3rd, 4th, 6th, 7th, and 9th doses of the poison. The localization of α-FP and γ-globulin was studied in paraffin sections (3 μ) of the liver by the indirect immunofluorescence method [12]. Pieces of liver were fixed in a mixture of ethanol with acetic acid at 4°C. The technique of processing of the material was described

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