Short Communications

Satellite cells in the regenerated and regrafted skeletal muscles of rats

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Summary. Soleus (SOL) muscles were grafted into extensor digitorum longus (EDL) muscle beds (EDL-first-graft). Sixty days later, some mature EDL-first-grafts were regrafted into their own beds (EDL-second-grafts). Fully regenerated muscle fibers and satellite cells were observed in both types of mature grafts. The ratios of satellite cell nuclei per total nuclei (myonuclei and satellite cell nuclei) were 4.81 ± 0.47 % for EDL-2nd graft, 4.26 ± 0.51 % for EDL-1st-graft, 4.30 ± 0.33 % for control SOL, and 3.30 ± 0.18 % for control EDL. It is thought that satellite cells are required for the repeated activity of muscle fiber regeneration. The persistance of satellite cells in EDL-second-grafts suggests that satellite cells are not depleted during the first grafting, making second-grafts possible.
Key words. EDL-first-grafts; EDL-second-grafts; satellite cells; muscle regeneration.

Free muscle grafting has been used experimentally to study the regeneration of skeletal muscle. After grafting, the original muscle fibers degenerate due to the interruption of neurovascular supply. From the degenerating muscle fibers, however, myogenic myoblasts will emerge and will bring about the regeneration of new muscle fibers [1, 2]. The origin of the myogenic myoblasts has been debated for more than two decades [3, 4]. It is now generally accepted that these myogenic cells are derived from the undifferentiated, reserved satellite cells located between the sarcolemma and the basal lamina of muscle fibers [5, 6].

How remarkable this regenerative feat can be was demonstrated in recent experiments in which the regenerated muscle fibers repeated the degeneration and regeneration processes when the free graft (first-graft) was re-transplanted (second-graft) [7, 8]. Since the regeneration processes are essentially the same in the first and second grafts, it is likely that satellite cells in the regenerated muscle are responsible for the second wave of regeneration. Satellite cells in the regenerated muscle fibers have not been investigated, however. The purpose of the present investigation, therefore, is to see if satellite cells are present in the regenerated and re-grafted muscles.

Material and methods. Twenty male Sprague-Dawley rats (130 ± 5 g) were used in this experiment. Under chloral hydrate anesthesia (40 mg/100 g i.p.), the soleus (SOL) and extensor digitorum longus (EDL) muscles were removed. The SOL was then sutured to the tendon stumps of the EDL muscle left in situ and, thus, became the EDL-first-graft. Sixty days later, all rats were re-anesthetized. In one group (10 rats), the EDL-first-grafts as well as contralateral SOL and EDL muscles were removed for electron microscopy. In another group (10 rats), the EDL-first-grafts were removed but regrafted into their own beds by suturing to the tendons left in situ. These became the EDL-second-grafts. Sixty days later, the EDL-second-grafts and the contralateral SOL and EDL muscles were removed for electron microscopy. The muscles as well as various grafts were divided into proximal, middle, and distal segments. Each segment was then divided into small pieces, fixed in 4% glutaraldehyde, postfixed in 1% osmium tetroxide and processed for routine transmission electron microscopy. Ultrathin sections were obtained for observation using Zeiss electron microscope (Model 10 A/B). One section from each tissue block and 5–7 blocks from each segment (proximal, middle, or distal) of muscles or grafts were used to count the numbers of satellite nuclei and myonuclei. The ratio of satellite cell nuclei per total nuclei counted was calculated. This method of arithmetic calculation is in comformity with previous studies of satellite cells in various muscles [9, 11, 12, 14]. Since the purpose of the present investigation is not to compare the ratios of satellite cells and myonuclei among regenerated, regrafted and normal muscles, detailed stereological measurements such as nuclear density, number of nuclei per area of muscle cross section and the mean length of nuclei, etc. were omitted [15, 16].

Results. Fully regenerated skeletal muscle fibers were observed in both EDL-first-grafts and EDL-second-grafts. In general, the caliber of muscle fibers in the graft was smaller than that of normal control muscle. Occasionally a few muscle fibers contained two motor endplates on the same fiber with or without satellite cells in the vicinity. Satellite cells were identified as cells having large nuclei with dense heterochromatin and scanty cytoplasm. Also, a few small mitochondria and some rough endoplasmic reticulum could typically be seen in the satellite cells. The entire satellite cell lies between the sarcolemma and the basal lamina of muscle fibers (figs 1, 2). There were some satellite-like cells in which the basal lamina of muscle fiber penetrated to some extent into the space between the sarcolemma and the cell membrane of these cells. These cells were not counted due to the ambiguity as to whether they were true satellite cells. The table 1 shows the quantification of satellite cell nuclei and myonuclei in the grafts and in the control muscles. The total

Number of satellite cell nuclei and ratio of satellite cell nuclei per total nuclei (satellite cell nuclei and myonuclei) of control and grafted muscles (P: proximal; M: middle; D: distal).

	Satellite cell nuclei				Myonuclei				Total	Satellite cell nuclei per total nuclei % ± SE	Range %	Total thin section counted
	P	M	D	Sub-total	P	M	D	Sub-total				
EDL-second-graft	34	17	25	76	572	518	451	1541	1617	4.81 ± 0.47	1.9–8.8	15
EDL-first-graft	26	20	27	71	576	522	500	1598	1669	4.26 ± 0.51	1.7–8.2	15
Control SOL	15	16	17	48	336	294	343	973	1021	4.30 ± 0.33	2.2–7.1	16
Control EDL	14	11	10	35	353	319	369	1041	1076	3.30 ± 0.18	1.6–4.4	21

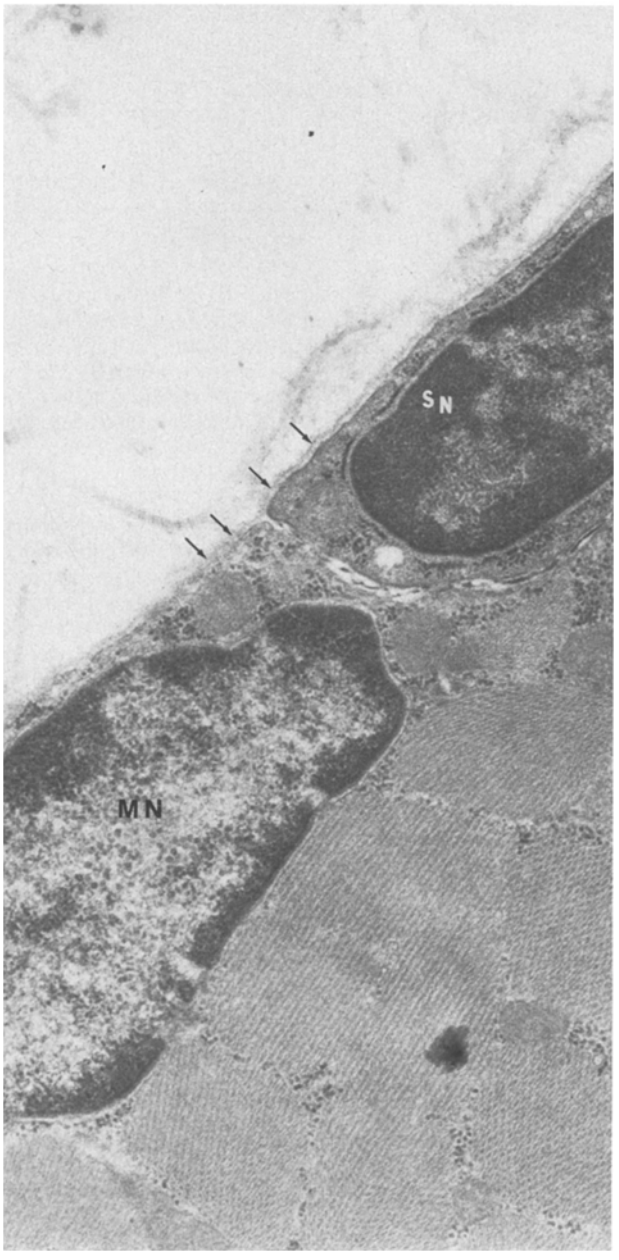

Figure 1. Satellite cell nucleus (SN) and myonuclei (MN) of EDL-second-graft. Arrows indicated that the basal lamina of myofiber is continuous over the surface of satellite cell. ×10,000.

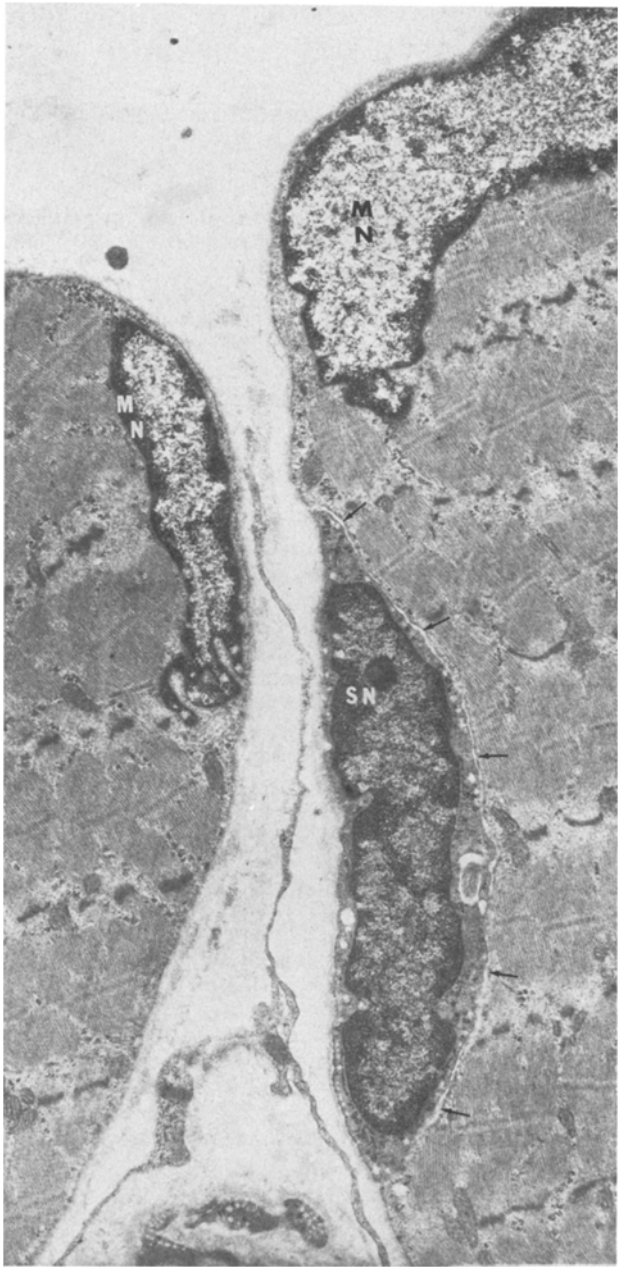

Figure 2. Satellite cell nucleus (SN) and myonuclei (MN) of EDL-first-graft. The space (indicated by arrows) between satellite cell membrane and sarcolemma is elearly seen. ×4000.

nuclei (satellite cell nuclei and myonuclei) counted were 1617 for EDL-second-grafts, 1669 for EDL-first-grafts, 1076 for EDL muscles, and 1021 for SOL muscles. The ratios of satellite cell nuclei per total nuclei counted were $4.81 \pm 0.47\%$ for EDL-second-grafts, $4.26 \pm 0.51\%$ for EDL-first-grafts, $3.30 \pm 0.33\%$ for EDL muscles, and $4.30 \pm 0.18\%$ for SOL muscles. The range of ratios and the total number of thin sections used for quantification are shown in the table also.

Discussion. The present investigation showed that skeletal muscle fibers could undergo, at least twice, degeneration and regeneration as previously shown [7, 8]. It also showed that, in EDL-first-grafts and EDL-second-grafts, there were satellite cells. Since satellite cells are believed to be the source of regenerating myoblasts [5, 6], it is likely that if EDL-second-grafts were grafted again, the muscles fibers in them could exhibit a third wave of regeneration phenomenon. It should be pointed out that success of muscle grafting did not solely depend on the availability of satellite cells and myoblasts. Fibroblasts proliferation in the graft is faster than satellite cells and myoblasts. The increased amount of connective tissues in the grafts renders the contact between the regenerating muscle fibers and the regenerating axon terminals difficult. The regenerated muscle fibers may secondarily undergo atrophy or degenerate due to the lack of innervation. Thus, the success of long-term results of third or fourth grafting is not guaranteed despite the fact that satellite cells are present in the regenerated muscle fibers.

Previous studies [9, 10] showed that slow muscles (SOL) contained more satellite cells than fast muscles (EDL). The result of the present study is in conformity with the previous

findings, although the ratio of satellite cells in control EDL muscle in this study is higher than previously reported. The difference in sample size may be a factor for such controversy. The ratios of satellite cells in the EDL-first-graft and EDL-second-graft are similar to each other and to SOL muscles suggesting that the grafts retain the satellite cell frequency of the original muscle, rather than being transformed to a frequency found in EDL muscles. However, this similarity should not be viewed as absolute and be interpreted that the satellite cell content in these two types of grafts and SOL muscles were comparable for several reasons. First, the sample size may not be large enough. Snow[11] has quantified 4711 nuclei (total nuclei) in the SOL muscle of adult mouse and has suggested large sample size is needed to assure statistical significance of the ratios. What optimal sample size would be considered large has not been determined. In the present study, a total of 1617 nuclei for the second-grafts and 1669 nuclei for the first-grafts were counted. These numbers are obviously small compared to 4711. Secondly, the degree of innervation in grafts may be related to the number of satellite cells. Denervation has been shown to increase the number of satellite cells in the muscle[12]. In the graft, the innervation is never as complete as that of normal muscle[1,7]. Therefore, some regenerated muscle fibers are in the denervated state which may contribute to the variation of satellite cell numbers. Thirdly, there are satellite-like cells which were included in the quantitative analysis by previous workers[13,14] but not included in the present study due to its ambiguity. Finally, the study of ratios of satellite cell nuclei per total nuclei has to consider the factors such as nuclear dimensions (width, length, volume), cross-sectional areas of muscle fibers, nuclear density etc. as indicated by recent communications[15,16] using stereological analysis so that morphometric comparison is accurate and meaningful. Though the above-mentioned uncertainties make the frequency of satellite cells in the regenerated muscle fibers less precise, the present investigation showed that satellite cells were present in grafted and regrafted muscles indeed. The persistance of these satellite cells means that they are not depleted in the first-grafts, and that second- and third-grafts are possible.

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Morphological effects of serotonin and ketanserin on embryonic chick skin in vitro

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Summary. Cell and organotypical cultures are used to study the direct effect of serotonin and of ketanserin, a serotonin antagonist, on dermal and epidermal cells of embryonic chick skin. Ketanserin stimulates the increase in cell number and inhibits the differentiation, whereas serotonin stimulates differentiation and inhibits the increase in cell number.
Key words. Serotonin; ketanserin; fibroblast culture; organotypical culture.

Ketanserin, a newer 5-hydroxytryptamine$_2$ (and also α_1) antagonist in vascular muscle is used as an antihypertensive drug[1-4]. Recent clinical observations[5] suggest that ketanserin promotes the healing of chronic skin ulcers. This healing effect could be due to the indirect effect of vasodilatation. To study a possible direct effect of serotonin (5-HT) and ketanserin on dermal and epidermal cells, we have used cell and organotypical cultures of embryonic chick skin[6].

Methods. Organotypic culture. The skin of the dorsal region of 6.5-day-old embryonic chicks was dissected and fragments of 0.3 cm diameter were punched out. These fragments were transferred to minimal Eagle's medium with Hanks' salts, supplemented with 10% fetal calf serum and antibiotics. This medium served as a control medium. Test media were prepared by adding serotonin or ketanserin, in concentrations similar to those used in experiments for testing the antihypertensive effect of ketanserin[7]: 10 µg/ml serotonin and 5 µg/ml ketanserin. The skin fragments were explanted on the medium described above, made semisolid by adding 0.5 mg/ml agar, or on the bottom of a Falcon plastic dish submerged in the fluid medium. Twelve series of organotypical cultures were incubated at 37 °C. On days 1, 2, 5 and 6, eight series of cultures were photographed in order to measure the area covered by the skin fragment and its outgrowths. After an incubation of 2, 4, 7 or 12 days, the skin fragments were fixed in a Bouin-Hollande solution. Some of the organotypical cultures were stained in toto with Giemsa. Seven series of cultures were prepared for 6-µm paraffin cross-sections and stained with Hematoxylin-Eosin or with a specific keratin stain following Ayoub-Shklar[8].

Cell culture. In addition to the organotypic cultures, ten series of cell cultures of dermal fibroblasts were also prepared. The dermal stroma of the skin fragments, mechanically separated from the epidermal layer, was placed in a trypsin