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TRANSPOSITION OF THE SOLEUS INTO THE BED OF THE EXTENSOR DIGITORUM LONGUS MUSCLE IN THE RAT

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SUMMARY

In 38 rats the slow soleus muscle was transposed, with its neurovascular bundle intact, into the bed of the fast extensor digitorum longus muscle. Transposed muscles underwent degeneration and regeneration like free muscle grafts. Both contractile and histochemical properties remained those of the soleus muscle, supporting the conclusion that the nerve to a slow muscle is pre-eminent in determining the functional and histochemical characteristics of the muscle.

Among the factors and tissue interactions that determine the structural, functional and metabolic properties of a muscle, the effects of the functional environment have proven to be among the most difficult to assess. The experimental method of muscle transposition [6] has facilitated analysis of these effects because it permits one to dissociate effects produced by the motor nerve to a muscle from those that are environmentally induced.

Transposition of a muscle is accomplished by cutting both of its tendons and lifting the entire muscle, with its motor nerve and associated vascular bundle, from its own bed and placing it into the bed of another muscle, which had been previously removed. This procedure differs from cross innervation [1], in which the muscle remains intact in its own bed and the motor nerve supply is exchanged with that of another muscle. In cross-transplantation, a muscle is disconnected from its tendons of origin and insertion as well as its neurovascular supply and then freely grafted into the bed of another muscle [7].

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**Address correspondence to: Bruce M. Carlson, Department of Anatomy, 4622 Medical Sciences II, School of Medicine, University of Michigan, Ann Arbor, Mich. 48109 (U.S.A.) After this procedure, the muscle graft becomes reinnervated by the nerve to the muscle in whose bed it was placed, and it also becomes anatomically integrated into the functional environment of the host muscle bed.

This experiment was carried out on 38 two-month old male rats of the inbred AVN strain that is maintained at the Institute of Physiology in Prague. The fast extensor digitorum longus (EDL) muscle was removed from one leg, and the stump of its motor nerve was embedded into another muscle. Then the proximal and distal tendons of the slow soleus muscle were cut and the muscle was carefully moved over into the bed of the EDL so that its neurovascular bundle remained intact. The proximal and distal tendons of the transposed soleus were sutured to the corresponding tendon stumps of the EDL, and the overlying fascia and the skin were sutured closed in layers. This experiment consisted of two series: one in which the soleus muscle was transposed as described above and another in which the soleus was injected with 0.75% Marcaine (Winthrop) before transposition. Injection with Marcaine produces a nearly complete degeneration and subsequent regeneration of the muscle fibers within the muscle [2, 8].

At intervals of 7, 14, 30 and 60 days after transposition, both the transposed and the contralateral normal soleus muscles were removed from anesthetized rats. The muscles were placed into an oxygenated culture medium and set up for direct electrical stimulation and recording of contractile properties in vitro [3]. After determination of the optimal resting tension, the following contractile characteristics were recorded: twitch and tetanic tensions, latency period, full contraction time (time to peak tension) and half relaxation time. After analysis of contractile properties, the muscles were examined histochemically for succinic dehydrogenase (SDH) and myofibrillar adenosine triphosphatase (ATPase) activity.

There was relatively little difference between the series in which the normal soleus muscle was transposed and that in which the muscle was first treated with Marcaine. Therefore only data from the former series will be presented here. In both groups there was morphological evidence of substantial degeneration and subsequent regeneration of muscle fibers within the transposed muscles. It is apparent that whatever vascular supply was preserved in the neuro-vascular bundle was not sufficient to preserve the structural integrity of the muscle fibers in the transposed normal soleus muscles.

Contractile properties (Table I). The low twitch (Tw. T.) and tetanic tensions (Tet. T.) of the transposed soleus muscles reflected the breakdown and subsequent regeneration of muscle fibers in the transposed muscles. In these experiments the muscles remained weak, a characteristic of regenerating and freely grafted soleus muscles in the rat [5]. The full contraction time (FCT) and half relaxation time (HRT) remained slow and did not differ significantly from the values of the contralateral soleus (Table I). The full contraction time of the normal EDL and also 30- or 60-day free grafts of this muscle is three times faster than that of the transposed soleus [5].

Histochemical properties. Sections through 7- and 14-day transposed muscles

TABLE

CONTRACTILE PROPERTIES OF THE SOLEUS MUSCLE, TRANSPOSED WITH INTACT NERVES, IN PLACE OF THE EDL MUSCLE IN TWO-MONTH-OLD AVN RATS

Days	Muscle	Tw.T .	FCT	HRT	Tet. T.	Muscle wt.	Rat wt.
(No. of rats)		(g)	(msec)	(msec)	(g)	(mg)	(g)
7 days (n=4)	Transposed Control	2.53 ± 1.34 12.83 ± 2.98	27.58 ± 3.57 30.98 ± 2.25	32.90 ± 1.97 38.63 ± 3.46	10.70 + 5.86 77.45 ± 13.56	$\begin{array}{rrrr} 60.0 & \pm \ 6.92 \\ 82.5 & \pm \ 4.25 \end{array}$	151.5 ± 4.29
14 days	Transposed	4.78 ± 0.41	30.52 ± 0.92	38.22 ± 2.02	28.26 ± 3.02	63.7 ± 4.85	158.5 ± 2.33
(n=5)	Control	14.53 ± 1.18	32.30 ± 1.69	36.63 ± 0.96	98.60 ± 7.27	84.75 ± 3.42	
30 Days	Transposed	6.28 ± 1.34	24.13 ± 1.27	33.80 ± 1.93	41.90 ± 14.01	85.0 ± 6.12	183.0 ± 9.51
(n=4)	Control	13.33 ± 1.92	29.73 ± 1.44	37.83 ± 1.15	109.50 ± 9.69	102.50 ± 4.79	
60 Days (n=7)	Transposed Control	$\begin{array}{c} 4.51 \pm 1.34 \\ 17.38 \pm 1.11 \end{array}$	32.83 ± 1.35 35.38 ± 0.76	31.71 ± 2.54 38.27 ± 1.71	45.21 ± 14.77 128.57 ± 3.73	94.0 ± 19.75 97.5 ± 3.1	177.0 ± 10.0

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showed clear evidence of degeneration and regeneration in the central areas of transposed muscles. Surviving peripheral muscle fibers retained their histochemical staining characteristics and remained much larger than the newly regenerating muscle fibers, which stained uniformly for both SDH and ATPase activity. By 30 days dark and light muscle fibers were clearly distinguishable in ATPase preparations, and sections stained for SDH activity showed heterogeneous staining, as well. Sixty day muscles appeared to be stable with respect to histochemical staining characteristics. The outstanding characteristic of these muscles was their similarity to the contralateral control soleus muscles (Fig. 1). The transposed muscles retained the 'checkerboard' or mosaic pattern of staining. In only restricted areas were there small 'type-groups' of homogeneously staining fibers. SDH activity in the transposed muscles was high and the overall staining pattern resembled closely that of the normal soleus muscle.



Fig. 1. Myofibrillar ATPase preparations of a normal soleus muscle (A) and a soleus transposed into the bed of the EDL for 60 days (B). The staining pattern characteristic of the normal soleus is retained in the transposed muscle. $\times 250$.

It is well known from studies of both cross union of nerves [1] and crosstransplantation of muscles [7] that the quality of the motor nerve determines to a large extent the contractile and histochemical properties of a muscle. In these experimental models the conversion between fast and slow properties is not always complete, and the degree of influence of the local functional environment has not been entirely clear.

Earlier, Gutmann [6] transposed the EDL muscle into the bed of the soleus in rats. He found that the contractile properties of the transposed muscles remained fast, but that within the transposed EDL muscles the histochemical pattern of SDH activity became converted to a predominantly high oxidative type, like that of the soleus muscle. In the present study not only did the the contractile properties and staining pattern of ATPase activity retain the characteristics of the slow soleus muscle, but the histochemical pattern of oxidative activity, as indicated by SDH staining, remained that of the soleus muscle. After cross-transplantation of the soleus muscle there was an almost complete conversion of both the contractile and histochemical properties to those of the EDL, into the bed of which the soleus was grafted [7]. Thus, the properties of the fast muscles into which they were placed, suggesting that in this case the quality of the motor nerve is the dominant force in determining the functional characteristics of the muscle.

As was the case of the EDL muscle grafted into the bed of the soleus [6], this transposition model should be looked upon as a free graft with an attached nerve, because after transposition considerable degeneration and subsequent regeneration occur in a pattern closely resembling that of a true free graft [4, 5]. The outstanding difference is the preservation of the original histochemical mosaic of muscle fiber types, reflecting the dispersal of the muscle fibers within the motor unit. In a typical free muscle graft, in which the nerve is also cut, the muscle fibers of a mature graft are clustered into similar type groups, a phenomenon interpreted by Karpati and Engel [9] as indicative of innervation. The other major difference between the transposed and the freely grafted soleus muscle was the retention of distinct differences in staining characteristics (especially ATPase) by the peripheral surviving muscle fibers and the differentiation of clearly distinct histochemical fiber types within the regenerating muscle fibers as early as 30 days. In a free graft the histochemical differences among the denervated surviving muscle fibers become diminished, and the differentiation of muscle fiber types in areas of regeneration occurs about a week later.

It is apparent that the motor nerve to the soleus is able to maintain the functional and histochemical differentiation of the muscle despite the muscle's being transposed into a foreign functional environment.

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