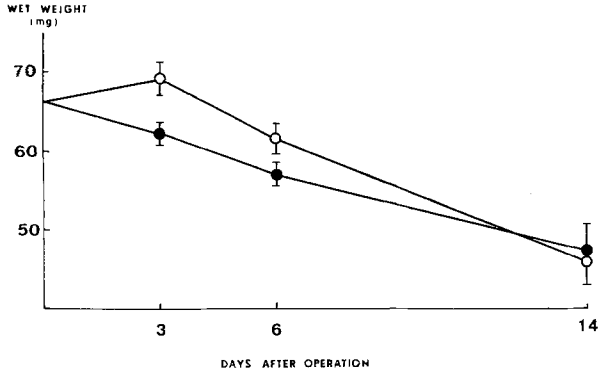
Hypertrophy of Skeletal Muscle Induced by Tendon Shortening

Passive tension has been implicated in skeletal muscle growth during development and in the maintenance of muscle weight in the adult animal[1]. Complete release of tension by tenotomy is accompanied by muscle atrophy; conversely, under certain circumstances, increased passive tension may even induce hypertrophy of denervated muscles[2]. The transient hypertrophy of the denervated rat hemidiaphragm, which has been extensively utilized as an experimental model of skeletal muscle hypertrophy, is apparently dependent on rhythmic stretching exerted by the normally innervated contralateral hemidiaphragm[3-5]. Postdenervation muscle hypertrophy, however, does not appear to represent an adequate model of stretch hypertrophy due to the complications introduced by denervation per se. The hypertrophy of the denervated hemidiaphragm has been shown to be the resultant of contrasting changes of different fibre populations, some undergoing hypertrophy, others atrophy even in the earliest stages[4]. This combination of denervation atrophy and stretch hypertrophy makes the interpretation of the process difficult and ambiguous. We have previously shown that the compensatory hypertrophy of skeletal muscles induced by synergist elimination can be largely accounted for by increased passive tension and may thus provide a useful model of stretch hypertrophy[6,7]. We present here a new experimental system which may also prove of value for analyzing the effect of stretch. In this system, an increased tension is applied to normally innervated or denervated muscles by shortening their tendons.

Materials and methods. Experiments were performed on male Wistar rats weighing about 100 g. In the first group of animals (group 1) the distal tendon of the plantaris muscle was shortened in one leg and a sham-operation was performed on the contralateral limb. While keeping the foot in full plantarflexion, the tendon of the plantaris was looped around a metal needle 1.35 mm thick and a suture was made at the base of the loop. The needle was then withdrawn and the suture reinforced. A segment of tendon of constant length (about 5 mm) was thus functionally excluded. To obtain a precise evaluation of the degree of extension produced in the plantaris by this experimental procedure, sarcomere length was measured in fibres from operated and control muscles. The knee and ankle joints were immobilized in a position intermediate between flexion and extension and the muscles were fixed in situ in 5% glutaraldeyde in 0.1 *M* phosphate buffer. Small fascicles dissected from different regions of the muscles were postfixed in 1% OsO_4 in phosphate buffer and embedded in Epon. Sarcomere length, measured on electron micrographs of longitudinal sections, averaged 2.38 ± 0.07 μm (SE) in the control plantaris and increased to 2.78 ± 0.10 μm (approximately + 17%) in muscles fixed immediately after the operation.

In a second series of experiments (group 2) shortening of the plantaris tendon was combined with section of the tibialis nerve which supplies the plantaris and the other ankle extensors. Simple denervation was performed on the contralateral limb.

Results and comments. Wet and dry weight changes of the plantaris muscle were determined at various time intervals after tendon shortening. The water content of the operated muscles was never significantly different from that of the controls. The Table shows that there is a significant increase in weight of about 10% in stretched muscles with respect to control 3 days postoperatively. Thereafter, the growth rate of the hypertrophied muscles declines, falling to control values by the 14th postoperative day. The transient hypertrophy of the stretched muscles is apparently due to an increase in size of the constituent fibres: the mean cross-sectional area of fibres of hypertrophied muscles, as determined by plani-



Weight changes of the plantaris muscle after denervation (●) and after tendon shortening plus denervation (○).

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Dry weight changes of the plantaris muscle after tendon shortening[a]

Experimental group[b]	Days after operation		
	3	6	14
1	+ 9.9 ± 2.0 *P* 0.01	+ 6.8 ± 1.2 *P* 0.01	− 0.1 ± 2.0 *P* 0.1
2	+ 14.0 ± 3.2 *P* 0.01	+ 8.0 ± 3.0 *P* 0.05	− 2.0 ± 4.2 *P* 0.1

[a] Changes expressed as percentage of contralateral control ± SE. Each value represents the average of 8 animals.
[b] Group 1: tendon shortening (control: sham-operation). Group 2: tendon shortening plus denervation (control: denervation).

metric measurements, was 11.2 ± 3.6% larger than in the controls 3 days after the operation. The effect of tendon shortening is also apparent in denervated muscles (Table). The relative hypertrophy of stretched and denervated muscles, when compared to their denervated controls, is actually due to a transient delay in denervation atrophy induced by tendon shortening (Figure).

The findings appear to indicate that an increase in passive tension can produce hypertrophy of mammalian skeletal muscles through a direct effect not mediated by nerves. The transient nature of the hypertrophic process in this experimental system is presumably due to softening of the tendon tissue at the site of the suture and consequent release of tension. Sarcomere length in the plantaris muscle 3 days after the operation was in fact, on the average, 2.34 ± 0.06 μm, i.e. not significantly different from control[8].

Riassunto. L'accorciamento del tendine distale del muscolo plantare del ratto produce una transitoria ipertrofia muscolare con un massimo aumento in peso a tre giorni dall'operazione. L'accorciamento tendineo combinato con sezione del nervo risulta in un temporaneo ritardo nella comparsa dell'atrofia da denervazione.

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Retention of Noradrenaline-^{3}H in Brain and Preferential Extraction of Labeled Metabolites by Glutaraldehyde Fixation

All radioautographic investigations aimed at the ultrastructural examination of noradrenaline-^{3}H (NA-^{3}H) uptake and storage sites, in peripheral or central nervous tissue, have relied on the use of primary fixation with glutaraldehyde to preserve the tracer in situ[1]. Thus, selective accumulations of radioactivity have been found characteristic of catecholaminergic nerve cell bodies and axon terminals in the adult rat brain, following intracerebral administration of NA-^{3}H[2–4]. Moreover, such reactions could be specifically attributed to the presence of NA-^{3}H rather than labeled metabolites, provided that monoamine oxidase was inhibited[3].

In these and other studies, prevailing experimental conditions precluded the conclusive answering of several outstanding questions: 1. what is the proportion of brain NA-^{3}H actually retained in tissue after primary fixation with glutaraldehyde? 2. are some metabolites of NA-^{3}H also bound to brain tissue by this fixative? 3. is there any validity in the assumption that NA-^{3}H metabolites might be preferentially extracted in the course of glutaraldehyde fixation?

These problems were tackled by simple experiments on newborn rats, where it was deemed feasible to: a) obtain reproducible measurements of NA-^{3}H and labeled metabolites from brains of different animals by introducing NA-^{3}H via the bloodstream[5]; b) alter the respective brain contents of NA-^{3}H and labeled metabolites to a significant degree, using enzymatic inhibitors[6–8]; c) estimate the retention of radioactivity in nervous tissue, and extraction by the fixative[9], following immersion of brain slices in glutaraldehyde.

Material and methods. 23 newborn Sprague-Dawley rats of both sexes were each given 100 μCi of NA-^{3}H, within 24 h after birth (DL-noradrenaline-7-H^3, specific activity: 9.6–20 Ci/m*M*, obtained chromatographically pure from Amersham/Searle). The tracer, contained in 50 μl of aqueous solution, was injected in 15 sec into an exposed jugular vein. 1 group of rats (11) also received a combined pretreatment with both an inhibitor of monoamine oxidase (IMAO: Catron, 10 mg/kg s.c., 45 min before NA-^{3}H) and catechol-*O*-methyl transferase (ICOMT: Pyrogallol, 200 mg/kg s.c., 45 min before NA-^{3}H and hourly thereafter).

All animals were decapitated 3 h after administration of the tracer. Their entire brains were rapidly removed and frozen on dry ice. Several specimens from control and pretreated rats were homogenized in perchloric acid (0.4 *N*), and measured for total radioactivity (^{3}H) and NA-^{3}H contents, using liquid scintillation spectrometry before and after chromatography on aluminium oxide respectively[10]. The other brains were cut into 1 mm-thick slices, fixed for 1 h by immersion in four 15 min baths of 12.5 ml of 3.5% glutaraldehyde in 0.05 *M* phosphate buffer (Sorensen sol. A) at room temperature, and dissolved in Soluene. The amounts of ^{3}H retained in tissue and extracted by the fixative were then measured by scintillation. All values were corrected for quenching, with internal standards, and expressed per mg wet weight of tissue.

Results and discussion. The main results of these experiments are summarized in the Table. 3 h after i.v. injection of NA-^{3}H in newborn rats, radioactivity is found in appreciable quantity within the brains of animals pretreated or not with enzymatic inhibitors. In pretreated rats, total brain ^{3}H is significantly elevated, amounting to 1.6% of the initial dose administered compared to 0.7% in control rats, presumably due to preservation of higher levels of the exogenous amine in the bloodstream[11,12].

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