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Influence of muscle dimensions on economy of isometric exercise in rat medial gastrocnemius muscles in situ

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Summary. The effect of muscle dimensions on economy (force-time integral divided by the amount of energy utilized) was investigated in male rats (body mass range 95—490 g), anaesthetized with pentobarbital. The medial gastrocnemius muscle in situ performed 6 maximal isometric contractions of 350 ms duration ($1 \cdot s^{-1}$) at twitch optimum length at 35° C. The areas under the 6 time-force curves were added to obtain force-time integral of the experiment. Differences of concentrations of ATP, phosphocreatine and lactate between experimental and contralateral (resting) muscles were used to calculate high-energy phosphate consumption due to stimulation. Muscle mass and cross-sectional area increased (approximately +400% and +300%, respectively) over the rat body mass range studied. Muscle length and length of the most distal fibre bundle increased by approximately 17 mm and 4 mm, respectively. Force-time integral (N · s) increased proportional to cross-sectional area whereas high-energy phosphate consumption (μmoles) increased proportional to muscle mass. The relative fraction of the total energy consumption utilized for force-independent processes was independent of rat body mass. The economy of the actomyosin system was unaffected during growth, whereas economy of the whole muscle decreased during growth by approximately 30% ($p < 0.001$). The effect of muscle dimensions on economy is discussed with respect to human endurance capacity measured by voluntary isometric contractions.

Key words: High-energy phosphates — Isometric exercise — Muscle dimensions

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Introduction

In studies of human muscle function endurance capacity is often assessed by measuring the time that subjects can sustain a submaximal proportion of the maximum isometric voluntary contraction. There are however large inter-individual variations in this measurement for which it is difficult to account (de Haan et al. 1985; Parker 1985; Maughan et al. 1986).

Clearly differences in the rate of energy utilization could affect endurance capacity and this will be reflected in the muscle economy: that is, the relationship between external output (force × time) and the energy utilized (Goldspink 1978). A number of factors have been reported as influencing muscle economy including temperature (Biewener et al. 1983), stimulation duration, and relative muscle length (de Haan et al. 1986). Since muscle economy is the calculated quotient of force output and energy input, changes in economy may be reflecting an influence on only one, or on both of these determinants. Furthermore because force generation and energy consumption are not determined by the same muscle parameters several factors related to muscle morphology may influence economy e.g. fibre composition (Goldspink 1978), and possibly muscle architecture and dimensions. Kushmerick and Paul (1976) showed that when comparing force and energy data, the variation in muscle economy was decreased twofold by introducing muscle length as the normalizing factor. This indicates that at least one morphological factor may influence muscle economy.

Unfortunately, in experiments involving the intact human it is difficult to estimate the effect of differences in muscle architecture on muscle economy. Therefore we have sought to examine

the problem using an 'in situ' animal pennate muscle as our model. Using rats which had a wide range of body masses, morphological and isometric force parameters of medial gastrocnemius muscle in situ were obtained. The effects of variation in muscle dimensions on muscle economy was investigated by comparison of the total high-energy phosphate consumption of a series of repetitive contractions with force integrated over time for these contractions. In addition we looked to see if the fraction of the total high-energy phosphate consumption utilized for processes independent of actin-myosin interactions was affected by muscle dimensions.

Methods and material

Male Wistar rats (body mass 95—490 gram; $n=34$) were anaesthetized with pentobarbital (i.p.; 60 mg · kg body mass^{-1}). After surgical preparation (about 40 min after the first anaesthesia) a second dose of pentobarbital (24 mg · kg body mass^{-1}) was administered. The medial head of the gastrocnemius muscle was carefully exposed in both legs. Muscle length and fibre length were measured with a pair of compasses at muscle optimum length (length yielding highest twitch force). The index of architecture was taken as the ratio fibre length/muscle length at muscle optimum length. Cross-sectional area was calculated as the quotient of muscle volume and fibre length (Woittiez et al. 1983). Muscle volume was calculated as muscle mass divided by the density, 1.072 g · cm^{-3} according to Gollnick (1981).

The origin of the medial gastrocnemius muscle on the femur was left intact and the femur was fixed by means of a metal clamp. Distally the calcaneus was cut and connected to a force transducer. Muscle ambient temperature was maintained at 35°C by a water-saturated air flow. Contractions were induced by stimulation (Neurology Systems; pulse height 1 mA; pulse width 0.5 ms) of the distal end of the severed tibial nerve, with only it's branch to the medial gastrocnemius muscle left intact. To identify muscle optimum length 10 twitches were used (one each minute at different muscle lengths). With the muscle set at optimum length maximal isometric force was measured with a short tetanic contraction (duration 0.2 s; pulse frequency 100 Hz). The stimulus frequency used in this study (100 Hz) was based on observations in previous experiments with rats weighing approximately 250 g. In those experiments lower stimulus frequencies resulted in lower peak forces; higher frequencies showed slightly (5—10%) higher peak forces but the rate of force decay was faster.

After a recovery period of 10 min the muscle performed 6 maximal isometric tetanic contractions (each of 0.35 s duration at the rate of one per second) at optimum length. The force decreased by 5—10% independent of body mass. The areas under the force-time curve of the 6 contractions were added to obtain total force-time integral (FTI) for each muscle.

Experimental protocol

The contralateral (resting) muscle was sampled first by freeze-clamping the whole muscle with a pair of tongs precooled in liquid nitrogen. About 10 s before the start of stimulation the blood supply to the experimental muscle was interrupted (by ligating the femoral artery and vein) to minimize aerobic metabolism and prevent leakage of metabolites out of the muscle. Some slight replenishment of energy stores may still occur during the stimulation period as a result of small amounts of O_2 trapped in the muscle. However, this replenishment is likely to be very small in relation to total energy utilization, especially when account is taken of the short time course of the experiment. We have therefore made no attempt to correct for it.

Immediately after the 6th contraction the experimental muscle was freeze-clamped. The freeze-clamped muscles were weighed, ground in a precooled mortar with continuous addition of liquid nitrogen and were freeze-dried (Breda-scientific). Duplicate extractions were made by homogenizing the dry muscle tissue (15 mg) in 0.5 ml cold 1.7 N perchloric acid (Braun Potter S). The homogenate was centrifuged at 0°C (MSE Mistral 4 L 18000 × g). The supernatant was neutralized with potassium carbonate and potassium hydroxide. The neutralized extract was then centrifuged to remove potassium perchlorate (Eppendorf 10000 rpm) and stored at −18°C until analysis which was carried out within 3 days.

Enzymatic determinations of phosphocreatine, creatine, ATP, ADP and lactate were performed as described by Bergmeyer (1970) on a double-beam spectrophotometer (UV-190, Shimadzu). From the differences in concentrations of ATP, phosphocreatine and lactate between experimental and contralateral (resting) muscle, high-energy phosphate consumption (HEPC) was calculated (HEPC = $-\Delta$phosphocreatine $-\Delta$ATP + 1.5 × Δlactate). In a previous study it was shown that the use of twitches to determine optimum length followed by 10 min recovery did not affect metabolite concentrations in the experimental compared with the contralateral muscle (de Haan et al. 1986). A dry weight/wet weight ratio of 0.23 was used (de Haan et al. 1986).

It is possible that the measurement of HEPC could have been slightly affected by the variation in muscle mass between experiments, since it may have taken marginally longer to effectively freeze the whole of the larger, thicker muscles during clamping. Clearly a marked difference in freezing time could affect the measured concentrations of phosphocreatine and inorganic phosphate (Meyer et al. 1985). However, it seems unlikely that differences in freezing times affected metabolite levels in our study, since no effect of body mass was found on the phosphocreatine/creatine ratio in the contralateral (rest-

Table 1. Mean values and SD (μmoles · g ww^{-1}) of metabolite concentrations in the contralateral resting medial gastrocnemius muscle of the rat ($n=34$)

Metabolite	Mean	SD
PC	20.4*	1.9
Cr	12.1*	1.3
PC + Cr	32.5*	2.4
PC/Cr	1.70	0.22
ATP	6.70	0.53
ADP	1.18	0.18
ATP + ADP	7.87	0.65
ATP/ADP	5.83	0.88
lactate	1.93	0.30

* indicates significantly correlated with rat body mass ($p<0.05$)

ing) muscles (Table 1). This ratio would be a sensitive indication of any extra hydrolysis of phosphocreatine which resulted from slower freezing of the larger muscles.

Muscle economy was calculated as FTI (N · s) divided by HEPC (μmoles). To assess growth effects on the actin-myosin interaction FTI was normalized for cross-sectional area yielding tension-time integral, which is expressed in kPa · s ($1\ kPa = 10^3 \cdot N \cdot mm^{-2}$). HEPC was normalized for wet muscle mass (HEPC, μmoles · g ww^{-1}) and economy was calculated from these normalized values.

Myofilament overlap-independent energy consumption can be estimated from experiments with muscles stretched to different lengths to decrease the overlap between actin- and myosin filaments (Crow and Kushmerick 1983). Experiments were carried out at various lengths greater than muscle optimum length to obtain overlap-independent HEPC by extrapolation of the regression line between HEPC and FTI to zero FTI. Estimation of filament overlap-independent HEPC was made in a second series of experiments with 4 groups of rats having different body masses (means ±SD: 109±13, 204±16, 302±29 and 465±19 g; $n=7$, 6, 11, and 5, respectively).

Enzymes and nucleotides were obtained from Boehringer Mannheim, Federal Republic of Germany. The other chemicals were purchased from BDH-Chemicals (UK) and Sigma Chemical Co. (USA).

Linear regression analysis was carried out by the least-squares method. A 95% confidence interval was chosen to test significance of the Pearson's correlation coefficient.

Results

The changes in morphology of medial gastrocnemius muscle with body mass are summarized in Fig. 1. In the group of rats studied there was a direct proportional relationship between muscle mass and body mass. Expressed in relation to the values seen in the lightest rats both parameters increased by +400%. Cross sectional area of medial gastrocnemius muscle increased by an almost equal amount (+300%), but increases in muscle length and fibre length were rather small (+70% and +30%, respectively). As a result of the larger increase of muscle length (17 mm) when compared to fibre length (4 mm) there was a decrease of the index of architecture (fibre length/muscle length) with increasing body mass (Fig. 1). These results are very similar to the results of Woittiez et al. (1986).

Twitch tension (force per cross-sectional area) was independent of body mass ($n=34$). Mean twitch tension was 44.7±6.3 kPa. However, a slight but significant positive correlation ($r=0.64$) was found between tetanic tension (range 171—319 kPa) and body mass. Consequently, there was a negative correlation between body mass and the ratio twitch tension/tetanic tension ($r=-0.73$).

Mean values and standard deviations of metabolic parameters for the contralateral resting medial gastrocnemius muscle are summarized in Table 1. Significant correlations ($p<0.05$; $n=34$) were found between body mass and concentrations of phosphocreatine ($r=0.43$), and creatine

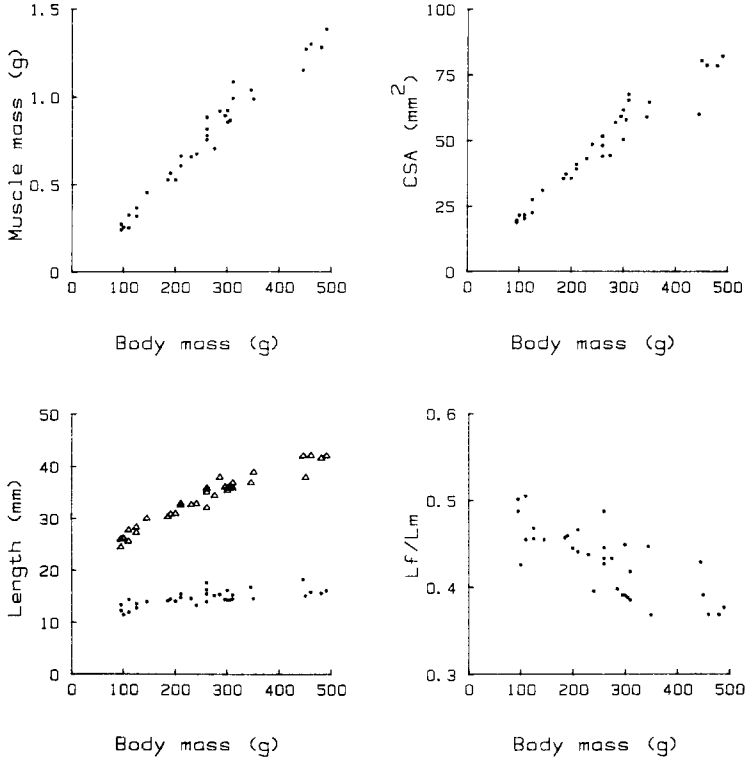

Fig. 1. Dimensions of medial gastrocnemius muscle in relation to body mass of male Wistar rats ($n=34$). Data are shown for (1) Muscle mass, $r=0.98$. (2) Length of the muscle, Δ, $r=0.96$, and Length of the most distal fibre bundle, ●, $r=0.69$, both measured at optimum length for twitch force. (3) Cross-sectional area (CSA) calculated as described in the Methods, $r=0.96$. (4) The index of architecture *(Lf/Lm)* that is the ratio of fibre length to muscle length, $r=-0.76$

($r=0.43$) and the sum (phosphocreatine + creatine; $r=0.57$), whereas the ratio phosphocreatine/creatine was independent of body mass. Adenine nucleotide and lactate concentrations were also independent of body mass. All metabolite concentrations were comparable with chemical determinations of human (Sahlin and Henriksson 1984) and rat muscles (Spriet et al. 1985) which contain predominantly fast-twitch fibres. However, the concentrations of phosphocreatine were somewhat lower than reported for ^{31}P-NMR analyzed muscles (Meyer et al. 1985).

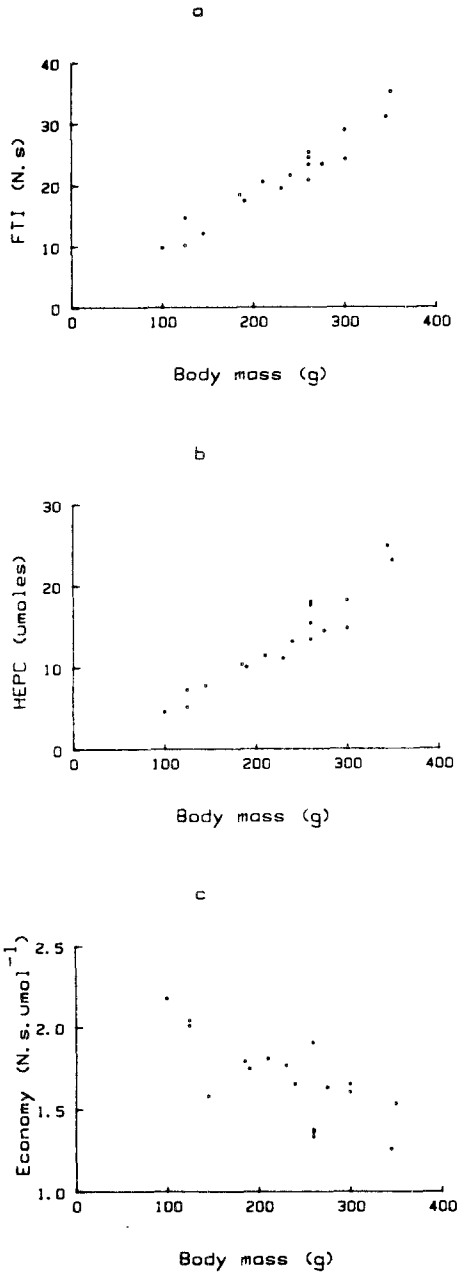

Fig. 2a—c. Data in relation to body mass from isometrically exercised (6 contractions; each 0.35 s duration; rate $1 \cdot s^{-1}$) medial gastrocnemius muscles of male Wistar rats ($n=18$). **a** Force integrated with respect to time *(FTI; $r=0.96$)*. **b** High-energy phosphate consumption (HEPC; $r=0.95$). **c** Economy calculated as FTI/HEPC ($r=0.74$). *Note:* All values are for the whole muscle

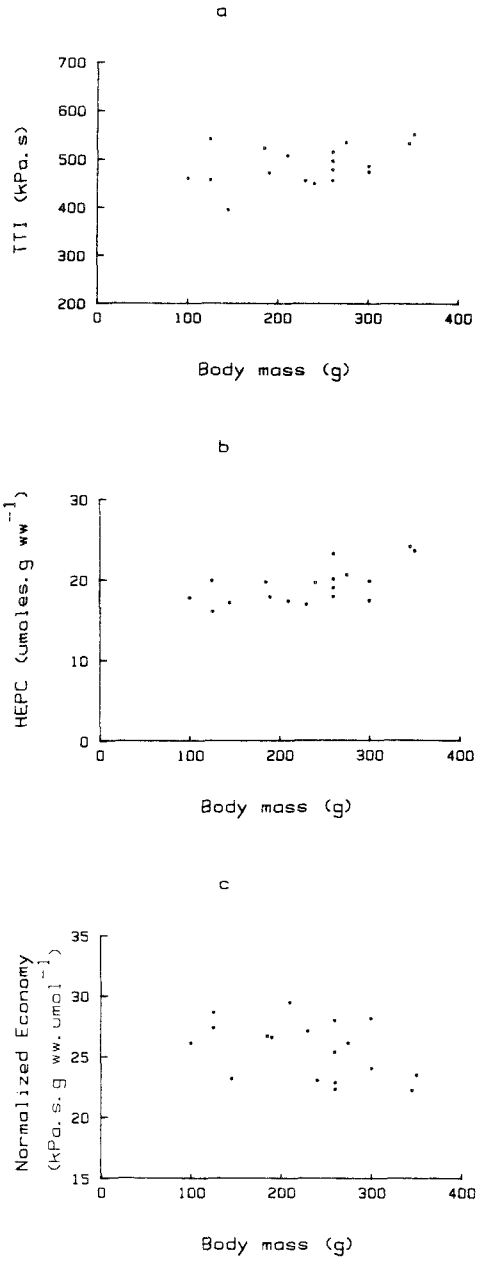

Fig. 3a—c. Data in relation to body mass for isometrically exercised muscles after appropriate normalization. **a** Tension integrated with respect to time *(TTI)* is the force time integral normalized for cross-sectional area and expressed as $kPa \cdot s$ ($1\ kPa = 10^3 \cdot N \cdot mm^{-2}$). **b** High-energy phosphate consumption (HEPC) is normalized for muscle mass. **c** Normalized economy is calculated from the normalized values (TTI and HEPC)

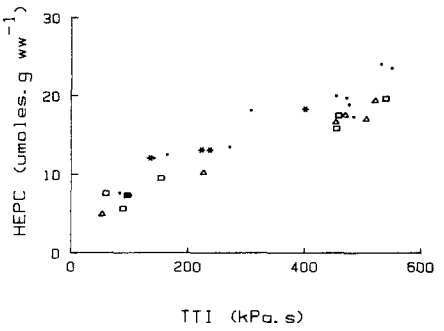

Fig. 4. Relation between force production and total high-energy phosphate consumption in isometrically exercised medial gastrocnemius muscles of 4 groups of male Wistar rats ($n=34$). Mean body masses (g) of the 4 groups were 109 (□); 204 (△); 302 (●) and 465 (*). Exercise consisted of 6 isometric tetanic contractions (350 ms, $1 \cdot s^{-1}$) at different muscle lengths (equal or greater than twitch muscle optimum length). High-energy phosphate consumption (HEPC) and time integral of tension *(TTI)* are normalized for muscle mass and cross-sectional area, respectively

The force-time integral of 6 tetanic contractions (FTI, N · s) as well as high-energy phosphate consumption (HEPC, μmoles) increased with body mass (Fig. 2a, b), but not in the same proportion. This resulted in a decreased economy for the whole muscle ($N \cdot s \cdot \mu mol^{-1}$) with increasing body mass (Fig. 2c).

After normalization for cross-sectional area, the tension-time integral (kPa · s) showed no significant correlation with body mass (Fig. 3a). When HEPC was normalized for muscle mass ($\mu moles \cdot g\ ww^{-1}$) there was still a slight positive correlation with body mass (Fig. 3b). The calculated normalized economy ($kPa \cdot s \cdot g\ ww \cdot \mu mol^{-1}$) was independent of body mass (Fig. 3c).

In a seperate series of experiments actin-myosin filament overlap-independent HEPC was estimated in 4 groups of rats having different body masses. With increasing body mass filament overlap-independent HEPC for the whole muscle increased. However, HEPC normalized for muscle mass was independent of body mass (Fig. 4). The amount of high-energy phosphate utilized for filament overlap-independent processes (5.6 $\mu mol \cdot g\ ww^{-1}$) was 30% of the total utilization at muscle optimum length.

Discussion

Maximal tension

Twitch tension (that is, force normalized for cross-sectional area) was independent of body mass, which is in agreement with Woittiez et al. (1986). Maximal tetanic tension did however increase slightly with body mass. This increase was not caused by continuing differentiation of muscle fibres since this process is virtually complete in the rat by 30 days of age, after which only relatively small changes are observed (Close 1964): As the smallest rat in this study weighed 95 g and from the growth curve of Wistar rats it was known that animals of 30 days old weigh approximately 82 g we believe that differentiation of fibres would have been completed.

The small increase seen in maximal tetanic tension with body mass may have been the result of slight, but systematic, changes in the optimum stimulation frequency with body mass. Since Close (1964) has reported such a decrease in the optimum during growth of rat EDL muscle. If the optimum frequency for medial gastrocnemius muscle of younger, smaller animals was higher than that applied in this study, lower forces would have been obtained and this may explain the small increase of the maximal tetanic tension with increasing body mass.

Economy

Force-time integral (FTI) and high-energy phosphate consumption (HEPC) increase with body mass (Fig. 2a, b) but not in the same proportion. This is because they are dependent on different aspects of muscle size. FTI will increase in proportion to the number of force generating elements in parallel, effectively expressed as the cross-sectional area of the muscle. When normalized for this parameter tension-time integral was found to be independent of body mass (Fig. 3a) and the coefficient of variation was reduced fourfold from 32% to 8%. By contrast HEPC will be determined by the total number of active force generating sites in parallel and in series, expressed as muscle wet weight. When normalized in this way the coefficient of variation fell from 41% to 12%, and although in our data there was still a slight positive correlation with body mass (Fig. 3b), it must be noted that this is critically dependent on the two heaviest rats.

When economy was calculated from the normalized data for FTI and HEPC there was no systematic change with body mass (Fig. 3c) and the coefficient of variation for our rats ($n=18$) was 9%. This finding taken together with the observation that HEPC of filament overlap-independent processes was independent of body mass leads one to the conclusion that the economy of the ac-

tomyosin system was not itself affected by growth. This constancy of economy of the actomyosin system is in contrast to the economy expressed for the whole muscle where there was a significantly correlated decrease of 30% over the body mass range studied (Fig. 2c). It is to be expected that when muscle increases in size by the addition of sarcomeres in series (i.e. increase in length) then there should be a reciprocal and proportional decrease in economy. Thus on first consideration of our data the increase in *muscle* length (approx. +45% over the body mass range 95—350 g) would seem to account for the decrease in economy illustrated in figure 2c. This is however misleading, since in the pennate medial gastrocnemius muscle the relative increase in fibre length is less than the relative increase in muscle length (Fig. 1; Woittiez et al. 1986), and it is fibre length at muscle optimum length which is thought to reflect the number of sarcomeres in series (Herring et al. 1984).

These observations may have implications for human studies in which endurance time for a sustained isometric contraction (% MVC) is used to characterise muscle function. Using this approach large inter-subject variations have often been reported for which it is difficult to account and which have made it difficult to demonstrate significant differences as a result of e.g.; administration of drugs (de Haan et al. 1985); training (Parker 1985); or muscle fibre type (Maughan et al. 1985). In these studies subjects have usually been tested at the same relative isometric force (% MVC). However, this is not necessarily the same *metabolic* load for the subjects due to the dependence of metabolic cost on muscle mass rather than cross-sectional area. Thus longer muscles with similar cross-sectional areas will have a higher energy utilization at the same % MVC since they will have more sarcomeres in series, which will utilize energy but not enhance force output. Such a higher metabolic cost might have particular significance for measurements of endurance capacity where blood flow through the muscle is not or only partly occluded. This is the case in sustained contractions at low % MVC and during intermittent contractions. Indeed dimensional factors may help to explain the differences reported between males and females under these conditions (Maughan et al. 1986).

It could also be expected that muscle dimensions might influence the metabolic cost during dynamic exercise in both animals and in man and clearly this is an area which merits further investigation.

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