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Protein turnover measured *in vivo* and *in vitro* in muscles undergoing compensatory growth and subsequent denervation atrophy

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The rapid growth (1–6 days) of the functionally overloaded soleus muscle, in response to tenotomy of the synergist gastrocnemius, was found to correlate with increases in both the protein synthetic and degradative rates, the change in the former being greater than that of the latter. These conclusions were drawn from two different methods used to measure (*in vivo* and *in vitro*) the average rates of protein synthesis and protein breakdown in these soleus muscles. Although the basal rates of synthesis were higher when measured *in vivo*, and the degradative rates higher in isolated muscle preparations incubated *in vitro*, both methods gave good agreement concerning the changes in protein turnover induced by tenotomy of the gastrocnemius. The possible involvement of passive stretch in inducing this additional growth is discussed. As an antagonist to the soleus, growth of the extensor digitorum longus muscle was decreased under the same conditions, presumably because of less usage. At 3 days after the cutting of the sciatic nerve, the previously normal or overloaded soleus muscles underwent rapid atrophy. Although in both cases RNA and protein were lost, while protein synthesis decreased and protein breakdown increased, denervation induced larger changes within these parameters of the formerly overloaded muscle. The slowing of growth in the tenotomized gastrocnemius, and its subsequent rapid atrophy after additional denervation, were explained by large increases in protein breakdown, with little or no change in the synthetic rate.

The plasticity of skeletal muscle is now well accepted (Pette, 1980; Jolesz & Sreter, 1981), with several chemical and mechanical factors known to influence both the developmental and adaptive growth of this tissue (Waterlow *et al.*, 1978; Goldspink, 1981). One particular experimental approach has been extensively used in studying the adaptive growth of muscle. This method involves cutting the tendon of one muscle (e.g. the gastrocnemius) and studying the changes induced in a remaining functionally overloaded synergist (e.g. soleus) during its compensatory growth (Hamosh *et al.*, 1967; Gutmann *et al.*, 1970; Goldberg, 1971). With the benefit of hindsight and improvements in techniques, some of the earlier experimental findings have been re-evaluated. For example, a large proportion of the increased RNA and DNA in such overloaded synergist muscles has now been attributed to the proliferation of connective tissue rather than to changes within the muscle fibres themselves (Jablecki *et al.*, 1973; Fleckman *et al.*, 1978). The induced fibre hypertrophy and metabolic adaptations (Goldberg, 1971; Baldwin *et al.*, 1977; Ianuzzo & Chen, 1979) during such situations of compensatory growth have been attributed to both an increase in the rate of protein synthesis (Hamosh *et al.*, 1967; Goldberg, 1969) and a complementary decrease in the rate of protein breakdown (Goldberg, 1969). The latter observation has more recently been questioned on technical grounds (Waterlow *et al.*, 1978). Techniques that measure the rate of protein breakdown *in vivo* from the decay of prelabelled proteins are probably subject to error because of the high extent of reutilization of most tracer amino acids and the non-exponential decay of label in mixed proteins (Waterlow *et al.*, 1978).

In the present study we have investigated the induced changes in protein turnover during compen-

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satory growth of the soleus muscle after tenotomy of the gastrocnemius. Measurements were also made in a remaining functional antagonist, the extensor digitorum longus and in the tenotomized gastrocnemius itself. Where possible, we have employed methods both *in vivo* and *in vitro* for measuring protein synthesis and protein breakdown, thereby enabling a direct comparison of these two techniques. In addition, we have simultaneously examined the suggestion (Hnik *et al.*, 1974) that the newly synthesized proteins in the overloaded soleus muscle are of a more 'labile' nature and consequently more susceptible to degradation on denervation of the tissue.

Materials and methods

All experiments involved the use of young male rats (CD strain, 49 ± 0.8 g) obtained from Charles River, Manston, Kent, U.K. Under halothane (I.C.I., Alderley Park, Cheshire, U.K.) anaesthesia the distal tendon of the gastrocnemius muscle was carefully separated from those of the soleus and plantaris to the point where they fuse to form the achilles tendon. The gastrocnemius was then tenotomized by removal of as much tendon as possible to minimize the likelihood of the tenotomized muscle reattaching to the skeleton or surrounding muscles. Denervations involved the removal of approx. 1 cm of the sciatic nerve at a point close to its exit from the spinal cord. Muscles of the unoperated contralateral limb were used as internal controls, since it had previously been shown (Goldspink, 1978) that this was valid for experiments of short duration.

All rats were killed by cervical dislocation. For measurements of protein synthesis and protein breakdown *in vitro*, control and experimental extensor digitorum longus or soleus muscles were rapidly dissected out and placed in oxygenated (O_2/CO_2, 19:1) Krebs–Ringer bicarbonate buffer (DeLuca & Cohen, 1964). Excess buffer was blotted off and the wet weights of the tissues were determined. Average rates of protein synthesis and protein breakdown were measured in these intact isolated muscles by a slight modification of the method of Fulks *et al.* (1975). This method makes use of the fact that the amino acid tyrosine is neither synthesized nor degraded by skeletal muscle. Protein synthesis was determined by measuring the incorporation of tyrosine into muscle proteins after a 3 h incubation at 37°C in 3 ml of Krebs–Ringer bicarbonate buffer, containing glucose (10 mM), 0.05 μCi of L-[U-^{14}C]tyrosine (483 mCi/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.), L-tyrosine hydrochloride (0.5 mM), amino acid at 5 times the plasma concentrations (Mallette *et al.*, 1969) and insulin (0.03 unit; Duncan, Flockhart and Co., London E2 6LA, U.K.). Both the medium and the atmosphere above it in the stoppered flasks were gassed with O_2/CO_2 (19:1). Actual calculation of the nmol of tyrosine incorporated was made by dividing the [^{14}C]tyrosine bound in protein by the specific radioactivity of the intracellular tyrosine pool (Fulks *et al.*, 1975).

Average rates of protein breakdown were determined independently of protein synthesis by measuring the release of tyrosine (Waalkes & Udenfriend, 1957) into intracellular amino acid pools and the surrounding medium (3 ml) after 3 h incubation at 37°C in oxygenated (O_2/CO_2, 19:1) Krebs–Ringer bicarbonate buffer, containing glucose (10 mM), amino acids at 5 times the plasma concentrations and insulin (0.03 unit). Cycloheximide (50 μM) was added to the medium in this case to block protein synthesis, thus preventing reutilization of the tyrosine released by degradation of muscle proteins.

For measurements of protein synthesis *in vivo*, each rat was given an intravenous injection, which was administered via a lateral tail vein. This injection contained 150 μmol of phenylalanine, including 65 μCi of L-[4-^{3}H]phenylalanine (sp. radioactivity 24 Ci/mmol; from The Radiochemical Centre), in 1 ml of 0.9% NaCl per 100 g body wt. Animals were decapitated 10 min after the start of the injection, and the appropriate leg muscles were rapidly dissected free under ice-cold NaCl and frozen in liquid nitrogen. Muscles were subsequently homogenized in 0.2 M-$HClO_4$, and the specific radioactivity of both the phenylalanine in the 'flooded' intracellular pool (i.e. S_A, the mean of values measured at 2 and 10 min; McNurlan *et al.*, 1979) and covalently bound in protein (S_B) determined as described by Garlick *et al.* (1980). This involved the prior hydrolysis of the washed protein pellet in 6 M-HCl at 110°C for 24 h and the conversion of phenylalanine into β-phenethylamine (Garlick *et al.*, 1980). All measurements of radioactivity were made in a Packard scintillation counter in a Triton X-100/xylene-based scintillant. The fractional rate of synthesis, K_S, as a percentage of the protein mass synthesized per day, was calculated as

$$K_S = \frac{S_B}{S_A t} \times 100$$

where S_A and S_B are the specific radioactivities of phenylalanine in the precursor pool and protein respectively, and t is the time in days.

Muscle growth was determined as the net accumulation of protein over a maximum of 3 days either immediately before, or spanning, the time points at which protein synthesis was measured. This was then expressed as a percentage change in the protein content per day. Muscle protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin (Sigma, Kingston upon Thames,

Surrey, U.K.) as a standard. The extraction and assay procedures for measuring muscle RNA and DNA have been described elsewhere (Goldberg & Goldspink, 1975).

Results

Changes in the growth patterns and protein turnover of three hind-limb muscles have been studied under different experimental conditions. These muscles included the soleus and gastrocnemius, which normally function synergistically in extending the ankle, and one of their antagonists, the extensor digitorum longus, which dorsi-flexes the foot. Although little oedema was evident after the surgical procedures, muscle growth was always measured as the accumulation of protein with time, rather than as changes in muscle wet weight.

Compensatory growth of the soleus muscle after tenotomy of the gastrocnemius

Functional overloading of the soleus muscle was achieved by tenotomizing the gastrocnemius in the same leg. This induced a rapid additional growth of the soleus, over and above the linear growth of its control within the contralateral limb. This adaptive growth was particularly pronounced over the first 3 days, slowing thereafter (Fig. 1*a*). The total RNA (Fig. 1*b*) and DNA (Fig. 1*c*) contents also increased dramatically in the surgically overloaded soleus. RNA per unit wet wt. ($230 \pm 5\,\mu g$ of RNA P/g of muscle) increased ($P < 0.01$) by 24% and 18% in the 3- and 6-day-overloaded soleus muscles respectively. The amount of DNA per unit wet wt. was not, however, significantly changed throughout ($159 \pm 3\,\mu g$ of DNA P/g of muscle).

In order to explain the additional growth in the overloaded soleus, the induced changes in protein synthesis and protein breakdown were studied, firstly by incubation *in vitro*. With this technique it has been usual to express rates of synthesis as nmol of tyrosine incorporated/3 h per mg of muscle. However, since the muscle weight changed considerably during the course of the experiment, rates were also expressed as nmol of tyrosine incorporated/3 h per whole muscle. In keeping with several earlier studies (Hamosh *et al.*, 1967; Goldberg, 1969, 1971), the compensatory growth of the soleus corresponded to an increase in synthesis when expressed either way. The rate per whole muscle was increased by 95–100% 1 and 3 days after tenotomy of the gastrocnemius, but by only 27% at 6 days: control values were respectively 4.0 ± 0.2, 6.9 ± 0.5 and 10.4 ± 0.7 nmol of tyrosine incorporated/3 h. Similarly, when expressed per unit wet weight (Fig. 2), the rate was significantly ($P < 0.01$) greater than control values by 45% at day 1 and 22% at day 3, but by day 6 was indistinguishable from the control.

Protein breakdown in the larger overloaded soleus muscles was also found to be significantly higher (80–95%) than in control tissues at all experimental time points when the rate was expressed per whole muscle. Although remaining consistently higher, when normalized to values per unit wet wt., the rate of degradation was only significantly ($P < 0.01$) elevated 6 days after surgery (Fig. 2). Hence the changes in protein degradation were if anything antagonistic, rather than complementary (Goldberg, 1969), to the increased rates of protein synthesis and muscle growth.

Since there are many technical difficulties associated with measuring the rates of protein turnover *in vitro* (Waterlow *et al.*, 1978), we

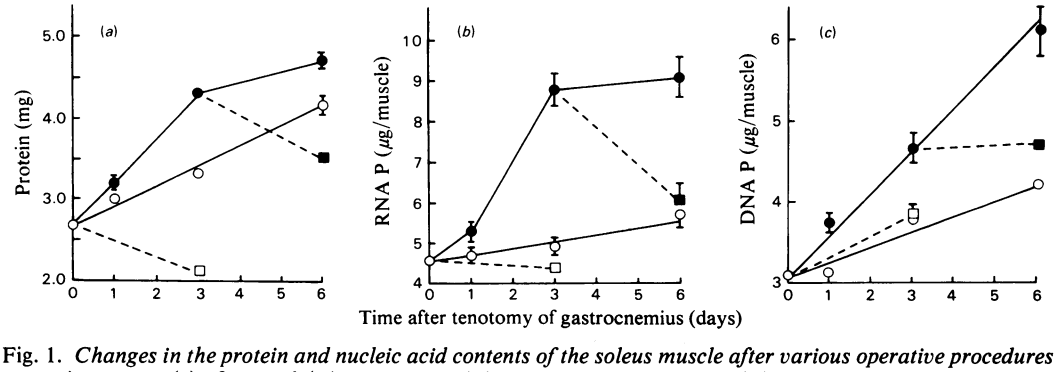

Fig. 1. *Changes in the protein and nucleic acid contents of the soleus muscle after various operative procedures*
The protein content (*a*) of control (○), denervated (□), functionally overloaded (●) and overloaded plus denervated (■) soleus muscles was measured by the method of Lowry *et al.* (1951). RNA (*b*) and DNA (*c*) from the same muscles were extracted and assayed as described elsewhere (Goldberg & Goldspink, 1975). Each value is the mean ± S.E.M. for at least six individual control or experimental muscles.

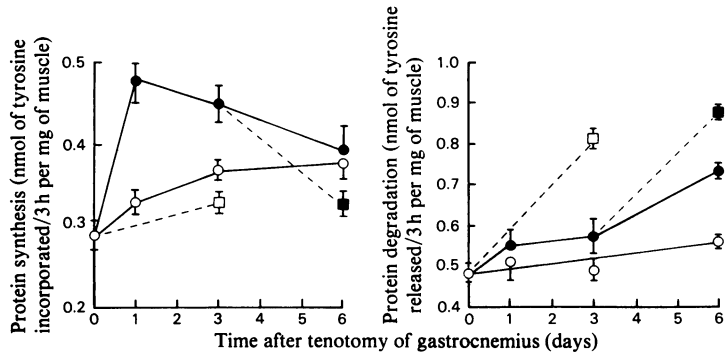

Fig. 2. *Rates of protein synthesis and protein breakdown in vitro in the soleus muscle after various operative procedures*
Average rates of protein synthesis or protein breakdown were measured (Fulks *et al.*, 1975) in the isolated soleus muscle described in Fig. 1. The nmol of tyrosine incorporated into, or released from, muscle proteins after a 3 h incubation are expressed per mg of muscle. Symbols are as for Fig. 1. The values are presented as means ± S.E.M. for at least six individual muscles.

Table 1. *Measured changes in protein turnover in vivo in the soleus muscle*
The fractional rate of synthesis (K_s, i.e. the percentage of the protein mass synthesized within 24 h) was measured 10 min after an intravenous injection of a massive dose of phenylalanine (Garlick *et al.*, 1980). The growth rate was measured as the daily accumulation (+) or loss (−) of protein over 3 days and is expressed as the percentage change in the protein content. The fractional rate of protein breakdown (K_b) was calculated by subtracting the mean growth rate from the mean fractional rate of synthesis for that particular muscle. Where possible, statistical analyses were performed by Student's *t* test (*$P < 0.01$), with each measurement representing the mean ± S.E.M. of six control or experimental soleus muscles.

	K_s, fractional rate of synthesis (%/day)	Growth rate (%/day)	K_b, calculated rate of breakdown (%/day)
(*a*) Control	20.4 ± 0.9	10.6	9.8
3-day sham-operated	20.7 ± 0.9	9.4	11.3
% change	+1		+15
(*b*) Control	19.6 ± 1.8	9.7	9.9
3-day overloaded	29.9 ± 1.2	17.5	12.4
% change	+53*		+26
(*c*) Control	17.4 ± 0.5	8.5	8.9
3-day overloaded + 3-day denervated	20.9 ± 1.2	−6.6	27.5
% change	+20*		+209

complemented this study with measurements of protein synthesis *in vivo* (McNurlan *et al.*, 1979; Garlick *et al.*, 1980). With this technique results have usually been expressed as fractional rates (Table 1), i.e. the percentage of the tissue protein that is renewed per day. This is convenient for calculating breakdown rates (see below), but is not appropriate for comparison of rates obtained *in vivo* and *in vitro*. Rates of synthesis in μg of protein per day per whole muscle were therefore calculated by multiplying the fractional rate by the protein content of the muscle. The rate of synthesis in control soleus muscles *in vivo* was considerably higher than that measured *in vitro*, but despite this the measurements *in vivo* confirmed the elevated rate of synthesis seen *in vitro* after 3 days of overloading.

Table 1 shows, in addition to the fractional rates of synthesis, the fractional rate of growth in the protein mass and the fractional rate of protein breakdown, calculated from synthesis minus growth. After 3 days of overloading, the increase in the fractional rate of synthesis was in fact more than that required to account for the additional growth of the soleus. Thus the fractional rate of protein degradation also increased slightly. The rate of

breakdown, expressed as μg of protein per day per whole muscle, is shown in Table 2 and shows an increase of 59% after 3 days of overloading. Hence in the 3-day-overloaded soleus there exists good agreement between the increased calculated rate of protein degradation (*in vivo*: Table 1) and the enhanced rate as measured *in vitro* (Fig. 2).

Both techniques therefore point to an accelerated turnover of proteins within the overloaded muscle. However, the additional growth was still made possible by a further widening of the relative rates of synthesis and breakdown. Neither the surgery nor associated stresses seemed to contribute to any significant extent to these changes in protein turnover, as can be seen by comparing the soleus muscles from control and sham-operated animals (Tables 1 and 2).

Denervation of normal and overloaded soleus muscles

As found in an earlier study (Goldspink, 1976), the normal soleus underwent a substantial true atrophy (22%) 3 days after cutting of the sciatic nerve (Fig. 1*a*). At the same time after denervation, a similar proportion (20%) of the total protein mass was also lost from the previously overloaded soleus (Fig. 1*a*). However, since the two muscles differed considerably in their size immediately before nerve section, this actually represented a much greater loss of protein from the formerly overloaded tissue, i.e. 285μg of protein/day compared with 190μg of protein/day from the previously normal muscle. Denervation-induced losses of RNA were even more pronounced (920ng compared with 70ng of RNA P/day) in the wasting 'overloaded' muscle (Fig. 1*b*). The DNA content of the normal muscle continued to increase as in the control after denervation, whereas DNA was merely maintained at the pre-denervation value in the formerly overloaded soleus (Fig. 1*c*). The overall effects of denervation on RNA and DNA were therefore more marked in the previously overloaded and hence enlarged tissue, thus verifying its greater susceptibility to this type of induced atrophy.

The denervation-induced atrophies in both the normal and overloaded soleus muscles correlated with decreased rates of protein synthesis measured *in vitro*, compared with their respective controls. This was true regardless of whether the rates were expresed per whole muscle or per unit wet weight (Fig. 2). Rates of protein breakdown measured *in vitro* were also increased in these denervated tissues, particularly when normalized to values per unit wet weight of tissue (Fig. 2). Measurements *in vivo* also confirmed the increase in degradation of proteins in the denervated but formerly overloaded muscle. The fractional rate of breakdown (K_b) increased from 12 to 28%/day after nerve section (Table 1), and the rate, in μg of protein/day per whole muscle, increased by 80% from 530 to 955 in a similar manner to the increase (30%) observed *in vitro* (Table 2). This change, together with the decrease in the fractional rate of synthesis from 30% to 21% (Table 1), accounts for the rapid atrophy of this denervated muscle (Fig. 1*a*). Hence, once again the adaptive changes in muscle size were effected through regulating both protein synthesis and breakdown. In compensatory growth the induced changes in synthesis and breakdown were antagonistic, but after cutting the sciatic nerve the reversal of the change in synthesis and the further potentiation of the degradative process(es) were complementary, and switched the tissue from a state of growth to one of atrophy.

Table 2. *Comparison of the rates of synthesis and breakdown of protein in the soleus muscle measured in vitro and in vivo*

The total amounts of protein synthesized or degraded are derived either from the rates *in vitro* (Fig. 2), assuming the bound tyrosine content in muscle protein to be 3.5% (Turner & Garlick, 1974), or the product of the fractional rates (*in vivo*; Table 1) and the protein content of the muscle (*$P < 0.01$).

	Total protein synthesized (μg/day)		Total protein breakdown (μg/day)	
	in vitro	*in vivo*	*in vitro*	*in vivo*
(*a*) Control (3 day)		682 ± 44		328
3-day sham-operated		714 ± 55		387
% change		+5		+17
(*b*) Control (3 day)	340 ± 24	654 ± 59	569 ± 32	336
3-day overloaded	686 ± 54	1282 ± 120	850 ± 74	533
% change	+102*	+96*	+50*	+59
(*c*) Control (6 day)	513 ± 36	724 ± 33	783 ± 50	370
3-day overloaded + 3-day denervated	522 ± 45	726 ± 80	1110 ± 65	955
% change	+2	0	+42*	+158

Changes within the tenotomized gastrocnemius

Cutting the distal tendon of the gastrocnemius led to a slowing of growth over the ensuing 3 days (Table 3). In keeping, the RNA content, but not that of DNA, was found to be significantly lower (23%) in the 3-day-tenotomized tissue, compared with its normal control. This change did not, however, represent a net loss of RNA, but rather a slower accumulation of this nucleic acid over this period (Table 4). RNA per unit wet wt. was also decreased after tenotomy, suggesting a slower accumulation of RNA relative to the general gain in muscle mass. The more marked effect on RNA inevitably led to a fall in the RNA/DNA ratio within the tenotomized muscle, compared with a gradual increase in this value in the control muscles (Table 4).

Since the size and thickness of the gastrocnemius precluded any studies on protein turnover *in vitro*, the method *in vivo* alone was employed to determine the changes induced by tenotomy. Interestingly, the fractional rate of protein synthesis was not significantly changed by cutting the distal tendon (Table 3). Hence, the overall decrease in the amount of protein being synthesized was more a reflection of the decreased protein content of the tenotomized muscle. The accelerated degradation of proteins would appear to be the principal change explaining the lower rate of growth in this tissue. This same basic conclusion remained essentially true after subsequently denervating the tenotomized muscle. The fractional synthetic rate was decreased, but only by a further 8%, whereas the rate of breakdown (K_b) increased by a further 145% (Table 3). Hence an elevated rate of breakdown accounted for not only

Table 3. *Measured changes in protein turnover in vivo in the gastrocnemius muscle*
All experiments were made on at least six control or experimental gastrocnemius muscles in a manner identical with that described in Tables 1 and 2; $*P < 0.01$.

	K_s, fractional rate of synthesis (%/day)	Total protein synthesized (mg/day)	Growth rate (%/day)	K_b, calculated rate of breakdown (%/day)	Total protein degraded (mg/day)
(*a*) Control (3 day)	12.7 ± 0.9	5.2 ± 0.4	10.7	2.0	0.8
3-day sham-operated	12.6 ± 0.7	5.0 ± 0.4	10.5	2.1	0.8
% change	−1	−3		+9	+1
(*b*) Control (3 day)	14.5 ± 0.3	5.9 ± 0.3	10.8	3.7	1.5
3-day tenotomized	13.4 ± 0.6	4.6 ± 0.4	6.4	6.9	2.4
% change	−8	−22*		+86	+58
(c) Control (6 day)	15.0 ± 0.5	7.4 ± 0.6	10.5	4.5	2.2
3-day tenotomized + 3-day denervated	12.3 ± 0.6	3.7 ± 0.4	−4.6	16.9	5.1
% change	−17*	−51*		+281	+127

Table 4. *Changes in nucleic acid content of the gastrocnemius muscle after tenotomy and subsequent denervation*
RNA and DNA were extracted and assayed (Goldberg & Goldspink, 1975) from control, tenotomized and denervated gastrocnemius muscles as a function of time. Each value represents the mean ± S.E.M. of at least five muscles in each group. The statistical significance of the differences between the means of age-related control and experimental muscles ($*P < 0.01$) was evaluated using Student's *t* test.

	RNA P		DNA P		RNA P / DNA P
	(μg/muscle)	(μg/g of muscle)	(μg/muscle)	(μg/g of muscle)	
Control (day 0)	39 ± 1.6	265 ± 6	19 ± 0.6	134 ± 4	1.9 ± 0.7
Control (day 1)	44 ± 0.6	271 ± 2	21 ± 0.5	130 ± 3	2.1 ± 0.04
1-day tenotomized	41 ± 1.0*	252 ± 2*	21 ± 0.5	130 ± 4	1.8 ± 0.08*
Control (day 3)	53 ± 1.3	257 ± 4	25 ± 1.2	121 ± 7	2.1 ± 0.1
3-day tenotomized	41 ± 1.6*	223 ± 7*	24 ± 1.1	131 ± 2	1.7 ± 0.07*
Control (day 6)	60 ± 2.3	250 ± 5	26 ± 0.8	107 ± 3	2.3 ± 0.03
3-day tenotomized + 3-day denervated	39 ± 2.2*	254 ± 10	25 ± 1.2	163 ± 6*	1.6 ± 0.03*

Table 5. *Changes in nucleic acid concentrations and protein turnover in the extensor digitorum longus muscle*
Each value represents the mean ± S.E.M. for six muscles; with internal innervated controls (Con.) to denervated (Den.) muscles, or extensor digitorum longus muscles 3 or 6 days after tenotomy of the gastrocnemius (Ten.), followed in some cases (3 Ten. + 3 Den.) by 3 days denervation. Values in parentheses are the percentage differences from measurements in control muscles (*$P < 0.01$, **$P < 0.05$).

		3 days			6 days		
		Con.	Ten.	Den.	Con.	Ten.	3 Ten. + 3 Den.
Protein synthesis (nmol of tyrosine incorporated/3 h)	per muscle	8.4 ± 0.2	7.8 ± 0.5 (−7)	10.5 ± 0.7 (+26*)	9.6 ± 0.4	10.2 ± 0.4 (+13)	12.2 ± 0.6 (+27*)
	per mg of muscle	0.33 ± 0.01	0.03 ± 0.02 (+2)	0.41 ± 0.01 (+25*)	0.30 ± 0.02	0.34 ± 0.01 (+14**)	0.39 ± 0.03 (+31*)
Protein breakdown (nmol of tyrosine released/3 h)	per muscle	7.7 ± 0.6	8.8 ± 0.2 (+14**)	20.7 ± 1.0 (+168*)	11.5 ± 0.7	11.4 ± 0.5 (−1)	22.2 ± 0.7 (+100*)
	per mg of muscle	0.29 ± 0.02	0.34 ± 0.01 (+19**)	0.76 ± 0.02 (+163*)	0.38 ± 0.02	0.41 ± 0.02 (+8)	0.74 ± 0.03 (+94*)
RNA P	μg/muscle	4.9 ± 0.3	5.1 ± 0.2 (+4)	6.2 ± 0.3 (+27*)	5.8 ± 0.3	5.0 ± 0.2 (−14)	7.3 ± 0.2 (+27*)
	μg/g of muscle	210 ± 7	221 ± 7 (+5)	271 ± 9 (+29*)	192 ± 8	168 ± 5 (−13)	246 ± 15 (+28*)
DNA P	μg/muscle	3.0 ± 0.1	2.9 ± 0.1 (−4)	3.2 ± 0.1 (+7)	3.9 ± 0.1	3.8 ± 0.1 (−3)	4.3 ± 0.1 (+10*)
	μg/g of muscle	129 ± 2	126 ± 2 (−2)	139 ± 3 (+8)	133 ± 2	133 ± 3 (0)	154 ± 4 (+16*)

the slower growth of the tenotomized muscle, but also its subsequent rapid atrophy after nerve section.

Changes in the extensor digitorum longus muscle after tenotomy of the gastrocnemius

As a functional antagonist to the soleus and gastrocnemius muscles, the extensor digitorum longus muscle was also studied under the various experimental conditions described above. Growth of the extensor digitorum longus was slightly decreased after tenotomy of the gastrocnemius, suggesting less usage of the former muscle. This change in the growth rate, however, was slight and only significantly different from control tissues at day 6 (Fig. 3). No significant changes were found throughout (i.e. at 3 or 6 days after tenotomy) in the nucleic acid concentrations or the rates of tyrosine incorporation into proteins *in vitro* (Table 5). A small increase (14–19%) in the measured rates of protein breakdown *in vitro* was, however, found after 3 but not after 6 days (Table 5). As in an earlier study (Goldspink, 1976), cutting the sciatic nerve in either the normal or tenotomized limbs merely slowed the subsequent growth of the extensor digitorum longus muscles (Fig. 3). Despite this slowing of growth the denervated tissues significantly increased their concentration of RNA, both per whole muscle and per g of tissue (Table 5). DNA increased similarly after combining the effects of tenotomy and denervation (i.e. '3 Ten. + 3 Den.', Table 5). In keeping with the increased nucleic acid concentrations after denervation, the rate of protein synthesis measured *in vitro* increased, both per whole muscle and per unit wet wt. (Table 5). These anabolic trends, however, were opposed by even larger increases in the rates of protein breakdown, such that the net effect would lead to the slowing of growth in these

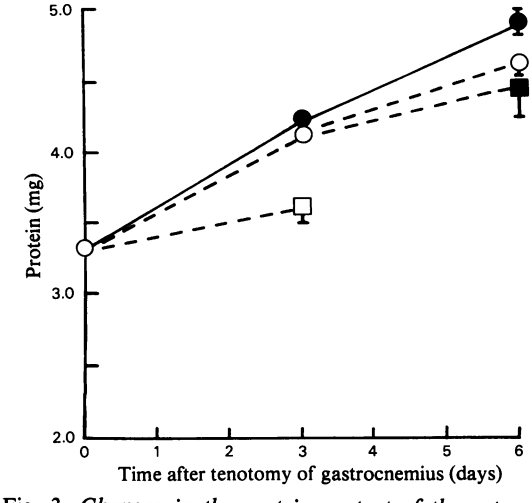

Fig. 3. *Changes in the protein content of the extensor digitorum longus muscle after various operative procedures*
The protein content of control (●), denervated (□), functionally underused extensor digitorum longus as a consequence of tenotomizing the gastrocnemius (○), or the underused muscles followed by denervation (■), was measured by the method of Lowry *et al.* (1951). Each point is the mean ± S.E.M. for six control or experimental muscles.

denervated tissues, compared with their rapidly growing controls (Fig. 3).

Discussion

Few direct comparisons have been made of the different methods currently used for measuring the rates of protein synthesis and protein breakdown in body tissues. The soleus muscle in the young rats used here is small and thin enough to allow for adequate diffusion of nutrients *in vitro* and is large enough as a single tissue in which to measure protein synthesis *in vivo*.

As in several studies with isolated muscles, the soleus exhibited a negative nitrogen balance *in vitro*, with the rate of breakdown exceeding that of synthesis (Fig. 2 and Table 2). This clearly is not a true reflection of the rapid growth of this muscle within the animal (Fig. 1*a*). The rate of synthesis *in vitro* was one-half to two-thirds of that measured *in vivo*, and the same was true, but in reverse, for the rates of breakdown (Table 2). The rates measured *in vitro* depend on, and can be readily altered by, the various constituents of the incubation medium. For example, out of all the plasma hormones, only insulin was added to the medium. Probably of equal importance is the loss of mechanical factors, such as contractile activity and passive stretch, which are known to influence protein turnover (Goldspink, 1981). Although the loss of such individual factors is often difficult to detect within short incubations (2–3 h), these effects may be additive. The isolated extensor digitorum longus muscle is, however, more nearly maintained in a positive nitrogen balance *in vitro* (Table 5) than is the soleus. Since the former muscle is less frequently recruited within the body, its isolation and subsequent loss of activity is less of a deprivation than it is for the normally highly active soleus muscle (Goldspink, 1981). In addition, this measurement of protein synthesis *in vitro* assumes an intracellular location for the precursor amino acid pool(s). Although this appears to hold for tyrosine and skeletal muscle (Li *et al.*, 1973), it remains a controversial issue (Waterlow *et al.*, 1978). Such problems in identifying the precursor pool are largely overcome in this method *in vivo* (McNurlan *et al.*, 1979; Garlick *et al.*, 1980), since the specific radioactivity of the free phenylalanine in the muscles is very similar to that of the plasma (Garlick *et al.*, 1980) and declines little (about 5%), if at all, between 2 and 10 min after injection, the latter being the time at which protein synthesis was measured. Protein breakdown *in vivo* was calculated as the difference between the measured rates of protein synthesis and tissue growth. The accuracy of this calculated value therefore depends on the accuracy of the two measured parameters. Of these the growth rate is most likely to be in error. To minimize as far as possible daily fluctuations in growth under the different experimental conditions, growth rates were measured over short time intervals (i.e. 2–3 days) close to, and around, the times of measuring the synthetic rates. The sources of error with this technique have been discussed in some detail by Garlick *et al.* (1979) and Waterlow *et al.* (1978). Although standard errors of the breakdown rates cannot be obtained using this method, the large differences between the calculated rates in control and experimentally manipulated muscles are likely to be real. Furthermore, in the soleus muscle such calculated differences (*in vivo*) are well supported by statistically significant changes when measured directly *in vitro* (e.g. Table 2).

Despite the differences in the basal rates measured *in vitro* and *in vivo*, good agreement was found throughout in the qualitative changes in protein synthesis and breakdown, as induced by the various experimental conditions. For example, the methods complemented each other in showing an increase in both the rates of protein synthesis and protein breakdown in the overloaded soleus muscle after tenotomizing the gastrocnemius (Table 2). The rapid additional growth and increased rate of protein synthesis of the soleus after the functional elimination of its synergist are now well-known features of this experimental model (Hamosh *et al.*, 1967; Goldberg, 1969, 1971). The increased rate of protein breakdown, as indicated by both the measurement *in vitro* and its calculation *in vivo* (Table 2), is, however, in conflict with the early work in this field (Goldberg, 1969). This difference may possibly be related to the high extent of re-utilization of the amino acid leucine (Waterlow *et al.*, 1978), which was used as a tracer in the early study (Goldberg, 1969), even though attempts were made to minimize such problems by chasing with large doses of unlabelled leucine.

The increased turnover of proteins in the overloaded soleus is similar to the changes induced by pressure and volume overloading in the heart (Morkin *et al.*, 1972) and in stretched innervated (Goldspink, 1977*a*; Laurent *et al.*, 1978) and denervated (Turner & Garlick, 1974; Goldspink, 1978) skeletal muscles. Passive stretch rather than, or in addition to, an increase in activity may well be an early factor involved in inducing the rapid growth of the overloaded muscles (Gutmann *et al.*, 1971). It is probable that immediately after tenotomy of its synergist the remaining functional soleus is initially unable to compete effectively with its intact antagonists (e.g. extensor digitorum longus and tibialis anterior) in the plantar flexion of the foot. Hence the soleus may be temporarily held in a lengthened/stretched state. Simultaneously on the other side of the leg the extensor digitorum

longus would be held in a shortened state. Predictably from studies on immobilized muscles, such a situation should lead to an additional growth of the soleus and a slowing of growth in the extensor digitorum longus (Goldspink, 1977*a*). This was indeed found to be the case (Figs. 1*a* and 3). Also, the type of changes induced in protein synthesis and protein breakdown were similar in both these (Fig. 2) and immobilized (Goldspink, 1977*a*) muscles. If increased muscle activity does represent the major initial stimulus for growth in the overloaded soleus, then a decreased rate of protein breakdown, and not an increase (Fig. 2), might have been expected (Goldspink, 1977*b*). Further supporting the possible involvement of stretch in inducing the compensatory growth of the overloaded soleus has been the finding of increased longitudinal growth involving the addition of more sarcomeres in series (Williams & Goldspink, 1973). Once again, this is a feature found in muscles immobilized in a lengthened/stretched state (Williams & Goldspink, 1973). Such longitudinal growth and fibre hypertrophy (Goldberg, 1971) may be complementary changes assisting the muscle in its adaptation to new functional requirements. With increasing fibre hypertrophy, the acute influence of stretch may be gradually lifted, such that the extra sarcomeres now present in series are lost (Williams & Goldspink, 1973). This subsequent decrease in longitudinal growth may possibly explain the smaller chronic-phase enlargement of the overloaded muscle, relative to its larger acute response (Mackova & Hnik, 1973).

At first sight, an increased turnover of proteins may seem wasteful and even counter-productive in a situation where rapid growth is called for. This may, however, represent an internal remodelling of the tissue, requiring changes in its proportion of fibre types (Ianuzzo & Chen, 1979), profiles of metabolic enzymes and polymorphic forms of its contractile characteristics (Gutmann *et al.*, 1970). Alternatively, the increased breakdown of proteins may be related to the splitting of fibres, which is known to occur in grossly overloaded muscles, but which may represent more of a pathological than a physiological response of the tissues (Hall-Craggs, 1970).

After 3 days of denervation of the normal soleus and extensor digitorum longus, the changes were consistent with those reported earlier by Goldspink (1976), the soleus undergoing a true atrophy and the extensor digitorum longus a decreased rate of growth (Figs. 1 and 3). At this particular time the atrophy of the soleus correlates with both a decrease in protein synthesis and an increase in protein breakdown (Fig. 2). At longer times after denervation, however, these changes became more reminiscent of those found in the extensor digitorum longus (Table 5) and diaphragm, where protein synthesis and the concentrations of nucleic acids increase, as well as protein breakdown (Turner & Garlick, 1974; Goldspink, 1976). Such anabolic trends have previously been attributed, at least in part, to the passive stretching of denervated leg muscles, arising out of the alteration in the gait of the animal after nerve section. An even greater loss of protein, RNA and DNA (Fig. 1) was found on denervating the formerly overloaded soleus. Although generally less dramatic, these findings do confirm the earlier observations of Hnik *et al.* (1974). Protein breakdown was increased after denervating both the normal (Fig. 2) and the overloaded tissues (Fig. 2 and Table 2). The more marked atrophy of the formerly overloaded soleus (Fig. 1*a*) can therefore be explained by this increase in protein breakdown and the complementary decrease in protein synthesis (Fig. 2 and Table 2). The former change may suggest that the proteins of the overloaded tissue are indeed more 'labile' (Hnik *et al.*, 1974), i.e. more susceptible to proteolysis.

Finally, the decreased growth rate of the gastrocnemius after tenotomy (Table 3) presumably relates to the muscle's failure to shorten against a load when activated. Subsequent denervation, thereby rendering the muscle inactive, caused the tissue to undergo a rapid atrophy. Interestingly, in both experimental situations the induced changes in protein synthesis were small, thereby necessitating substantial changes in the degradative rates in order to account for both the slowing of growth and muscle atrophy (Table 3). This observation, as in the study as a whole, further demonstrates the importance of protein breakdown as well as protein synthesis in regulating tissue growth.

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