Biochem. J. (1978) **174**, 595–602
Printed in Great Britain

The Influence of Passive Stretch on the Growth and Protein Turnover of the Denervated Extensor Digitorum Longus Muscle

By DAVID F. GOLDSPINK
Department of Physiology, Medical Biology Centre, The Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, U.K.

(*Received* 9 *January* 1978)

At 7 days after cutting the sciatic nerve, the extensor digitorum longus muscle was smaller and contained less protein than its innervated control. Correlating with these changes was the finding of elevated rates of protein degradation (measured *in vitro*) in the denervated tissue. However, at this time, rates of protein synthesis (measured *in vitro*) and nucleic acid concentrations were also higher in the denervated tissue, changes more usually associated with an active muscle rather than a disused one. These anabolic trends have, at least in part, been explained by the possible greater exposure of the denervated extensor digitorum longus to passive stretch. When immobilized under a maintained influence of stretch the denervated muscle grew to a greater extent. Although this stretch-induced growth appeared to occur predominantly through a stimulation of protein synthesis, it was opposed by smaller increases in degradative rates. Nucleic acids increased at a similar rate to the increase in muscle mass when a continuous influence of stretch was imposed on the denervated tissue. In contrast, immobilization of the denervated extensor digitorum longus in a shortened unstretched state reversed most of the stretch-induced changes; that is, the muscle became even smaller, with protein synthesis decreasing to a greater extent than breakdown after the removal of passive stretch. The present investigation suggests that stretch will promote protein synthesis and hence growth of the extensor digitorum longus even in the absence of an intact nerve supply. However, some factor(s), in addition to passive stretch, must contribute to the anabolic trends in this denervated muscle.

Denervation has frequently been used to induce a state of muscle inactivity. After deprivation of its nerve supply, the disused tissue either grows at a slower rate (Zak, 1962; Lewis, 1973; Goldspink, 1976), or undergoes atrophy (Gutmann, 1962; Goldberg *et al.*, 1974; Goldspink, 1976). Although a variety of morphological and biochemical changes (Gutmann, 1962; Weinstock & Iodice, 1969; Goldspink *et al.*, 1971; Goldberg *et al.*, 1974) have been found to correlate with the decrease in muscle size and concomitant losses of intracellular protein, by no means all of the observed changes after denervation are in keeping with wasting processes (Zak, 1962; Manchester & Harris, 1968; Goldberg *et al.*, 1974; Turner & Garlick, 1974). In a recent study (Goldspink, 1976) it was found that the synthesis of new proteins in the extensor digitorum longus and soleus muscles decreased for the first 2 days after cutting the sciatic nerve. This particular change complemented the increased rates of protein degradation, thus giving rise to the smaller muscles after denervation. However, 7 days after nerve section, protein synthesis had increased again in the denervated tissues and was now significantly higher than the rates measured in their innervated controls. Other workers (Pater & Kohn, 1967; Turner & Garlick, 1974) have also reported higher rates of protein synthesis in other muscles after denervation. These increased rates of protein synthesis together with higher concentrations of nucleic acids (Gutmann & Zak, 1961; Manchester & Harris, 1968; Goldspink, 1976) are more in keeping with a highly active tissue undergoing growth (Hamosh *et al.*, 1967; Goldberg *et al.*, 1974; Goldberg *et al.*, 1975; Goldspink, 1977*b*) than an inactive muscle. It has been suggested (Sola & Martin, 1953; Goldspink, 1976) that some of the anabolic trends, including the high rates of protein synthesis, found in denervated muscles might be related to the stretching of these tissues. It has been shown that innervated muscles grow to a greater extent (Sola *et al.*, 1973; Goldspink, 1977*a*) and add on new sarcomeres in series (Williams & Goldspink, 1973) when stretched. Further, this stretch-induced growth appears to be primarily due to a stimulation of protein synthesis (Goldspink, 1977*a*,*b*). In the previous study (Goldspink, 1976) it was suggested that the denervated extensor digitorum longus and soleus muscles may be stretched to a greater extent by the more frequent dragging of the ankle in an extended position after denervation. Certainly the

extensor digitorum longus muscle should experience a greater incidence of stretch with the ankle held for longer periods in an extended position (Goldspink, 1977*a*).

The present study was undertaken to test the hypothesis that stretching a denervated muscle would stimulate protein synthesis, and thus promote the tissue's growth. If true, this might also provide an explanation for the high rate of protein synthesis in the extensor digitorum longus after denervation. Induced changes in protein turnover were therefore studied in the extensor digitorum longus muscle of a denervated limb that was carried freely by the animal. These changes were then compared with events found in similarly denervated muscles, but that had also been immobilized either under a maintained influence, or in the absence, of stretch.

Materials and Methods

All experiments involved the use of young male rats (CD strain, 59±0.8g) obtained from Charles River, Manston, Kent, U.K. Under halothane (I.C.I., Alderley Park, Cheshire, U.K.) anaesthesia the extensor digitorum longus muscle of one hind limb was denervated by the removal of approx. 1cm of the sciatic nerve at a point close to the spine. The contralateral limb was simultaneously sham-operated and its extensor digitorum longus muscle used as an internal control for the denervated tissue. Several animals, of carefully selected body weights (45g), were treated in this manner and then randomly divided into three groups. In the first group the animals were simply allowed to recover from the anaesthetic, thus permitting the use of the denervated limbs without any subsequent restraint. In the second and third groups, before recovery the ankle joints of the denervated limbs were immobilized with Gypsona plaster-of-Paris bandages (Goldspink, 1977*a*). When dry, the casts so formed were painted with picric acid to deter the rats from biting and removing them. In one group of the joint-immobilized animals, the ankle joints of the denervated limbs were restrained in an extended position, thus immobilizing the denervated extensor digitorum longus muscles in a lengthened and stretched state. By contrast, the ankles of the denervated limbs of rats in the remaining group were restrained in a dorsi-flexed position, thus immobilizing the denervated extensor digitorum longus muscles in a shortened, unstretched state.

A fourth group of 12 normal, non-operated rats, carefully matched for body weights with the animals of the other three groups, were used to provide external-control muscles for comparisons with the appropriate internal control extensor digitorum longus muscles.

All of the rats in the four groups were killed by cervical dislocation 7 days after initiation of the respective treatments. The appropriate extensor digitorum longus muscles were rapidly dissected out and placed in the 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 determined (reproducibility less than 3%) on a torsion balance with an accuracy of ±0.4%.

Average rates of protein synthesis and protein breakdown were measured in these intact, isolated muscles according to a slightly modified 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 protein during a 3h incubation in 3ml of Krebs–Ringer bicarbonate, containing glucose (10mM), 0.05μCi of L-[U-^{14}C]tyrosine (483mCi/mmol; The Radiochemical Centre, Amersham, Bucks, U.K.), L-tyrosine hydrochloride (0.1mM), amino acids at 5-fold the plasma concentrations (Mallette *et al.*, 1969) and insulin (0.03 i.u.; Duncan, Flockhart and Co. Ltd., London E2 6LA, U.K.) at 37°C. Both the medium and atmosphere above it in the stoppered flasks were gassed with O_2/CO_2 (19:1). Actual calculation of the tyrosine incorporated was made by dividing the incorporated [^{14}C]tyrosine radioactivity by the specific radioactivity of the intracellular tyrosine pool. The latter value was obtained as follows:

$$\text{C.p.m./nmol} = \frac{(\text{acid-soluble c.p.m./mg of muscle}) - (10^{-2} \times \text{E} \times \text{c.p.m./}\mu\text{l of medium})}{(\text{acid-soluble nmol of tyrosine/mg of muscle}) - (10^{-2} \times \text{E} \times \text{nmol of tyrosine/}\mu\text{l of medium})}$$

where E is the percentage extracellular space of the muscle, and was determined as previously described (Goldspink, 1976).

Average rates of protein breakdown were determined independently of protein synthesis by measuring the release of tyrosine (Waalkes & Undenfriend, 1957) into intracellular amino acid pools and into the surrounding medium (3ml) after 3h incubation in oxygenated (O_2/CO_2, 19:1) Krebs–Ringer bicarbonate, containing glucose (10mM), amino acids at 5-fold the plasma concentrations, insulin (0.03i.u.), and L-tyrosine hydrochloride (5μM) at 37°C. Cycloheximide (0.5mM) was added to the medium in this case to block protein synthesis, thus preventing reutilization of the tyrosine released by degradation of muscle proteins.

The extraction and assay procedures for measuring

muscle RNA, DNA, or DNA synthesis have been described elsewhere (Goldspink & Goldberg, 1973). 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.

Results

In an attempt to determine whether passive stretch promotes growth in the denervated extensor digitorum longus muscle, three different operative procedures were used. In the first, the extensor digitorum longus muscle of one hind limb was denervated and the limb subsequently allowed to be carried freely by the animal. It has been previously suggested (Goldspink, 1976) that under such conditions the ankle of the denervated limb is dragged more frequently when the animal moves, such that the denervated extensor digitorum longus muscle probably experiences a greater incidence of stretch than its innervated counterpart in the contralateral limb. In the two remaining procedures, instead of the denervated limb being carried free by the animal, the ankle joint of this limb was immobilized either in an extended or dorsi-flexed position by means of a plaster cast. Immobilization in extension restrains the extensor digitorum longus muscle in a lengthened and stretched state, thus mimicking the situation where the denervated foot is dragged. The two systems are not, however, identical. With immobilization in this position, a continuous influence of stretch is imposed on the denervated muscle. However, in the denervated limb without immobilization, the ankle can still be mechanically flexed as the animal moves. In this latter case the influence of stretch must necessarily be intermittent since the stretch is temporarily removed whenever the ankle is flexed. When the ankle is immobilized in the dorsi-flexed position, there is probably little or no influence of stretch on the denervated extensor digitorum longus, because in this position the tissue is held in a shortened state and the foot is unable to assume the dragged position.

The use of internal control muscles, where possible, should minimize some of the problems associated with animal-to-animal variations. However, the longer the duration of experiments of this nature, the greater is the possibility that such internal controls may be subjected to changes in their work load as a consequence of the operative procedure performed on the opposite limb. Hence the controls may themselves undergo compensatory changes in size. Preliminary experiments were undertaken to determine whether internal control muscles could be used as valid controls to the denervated or denervated and immobilized extensor digitorum longus muscles. All of the data collected on the respective internal controls were compared with data derived from external control extensor digitorum longus, i.e., taken from normal, non-operated animals. None of the seven different parameters in the internal control muscles differed significantly either from the external control tissues, or from each other (Tables 1–3). Hence no evidence of compensatory growth or atrophy was found in the internal control tissues 7 days after the operations. In a related study the same was also found to be true of the soleus muscle, examined 7 days after the same operative procedures as used here (D.F. Goldspink, unpublished work).

Although no changes in muscle weight were found within the groups of control tissues, significant changes were found with the denervated muscles (Table 1). In keeping with the previous study (Goldspink, 1976), the 7-day-denervated extensor digitorum longus muscle was significantly smaller than its innervated control. However, when this denervated muscle was immobilized under a continuous influence of stretch, it grew to a greater extent, even though it still remained smaller than its innervated control (Table 1). In contrast, immobilization of the denervated tissue without stretch produced an even smaller muscle, both relative to its internal control and the denervated tissue subjected to the proposed intermittent influence of stretch (Table 1). Direct comparisons of the weights of the denervated muscles immobilized with or without the influence of continuous stretch revealed a significant difference of 6 mg after the 7-day study (Table 1).

Although the induced changes in the amounts of intracellular protein correlated with the changes in muscle size after each respective procedure (Table 1), the protein composition remained between 17 and 19% of the tissue wet weight. Since the total amount of protein present in a muscle represents the balance between the rates of protein synthesis and protein degradation, both parameters were measured to determine their respective contributions to these adjustments of tissue protein after denervation, with or without immobilization. From the data in Table 2 the fractional rates of protein synthesis and breakdown in the control tissues (i.e. the percentage of the total protein synthesized or degraded per day) were calculated to be approximately 12 and 8% respectively, assuming a bound-tyrosine content of 3.5% for mixed muscle proteins (Turner & Garlick, 1974). However, these basal rates changed significantly 7 days after nerve section (Table 2).

The increased rates of protein degradation in the denervated extensor digitorum longus without immobilization, correlate with the slower growth of this denervated tissue compared with its innervated control (Table 1 and Goldspink, 1976). However, the higher rates of protein synthesis in this same denervated tissue at this time (Table 2) suggest an anabolic

Table 1. *Changes in the wet weight and protein content of the extensor digitorum longus muscle after 7 days of denervation with or without passive stretching*

The wet weights and protein contents were measured for external and internal, innervated control and 7-day-denervated extensor digitorum longus muscles. The denervated tissues were taken from three groups of animals, one in which the denervated muscles were immobilized in a lengthened, stretched state, another with the tissues immobilized in a shortened, unstretched state, and the third with no immobilization whatsoever (i.e. with proposed intermittent stretching). Each value is the mean±S.E.M. of determinations made on at least ten muscles. Statistical significances (*$P<0.005$; **$P<0.025$) between these values were determined with Student's t test.

	External control	Internal control	Denervated	Internal control	Denervated+ immobilized with stretch	Internal control	Denervated+ immobilized without stretch
Muscle wet wt. (mg) ...	23.9±0.6	23.2±0.4	17.1±0.5	24.9±0.4	21.0±0.6	24.8±0.6	15.0±0.5
Percentage difference from:							
Control			−26*		−15*		−40*
Denervated					+23*		−12*
Continuous stretch							−29*
Protein (mg) ...	4.5±0.1	4.6±0.1	3.1±0.2	4.7±0.2	3.7±0.2	4.4±0.2	2.9±0.1
Percentage difference from:							
Control			−31*		−20*		−34*
Denervated					+20**		−7
Continuous stretch							−22*

trend, a trend not present at earlier times (e.g., 2 days) after denervation (Goldspink, 1976). It was previously suggested (Goldspink, 1976) that the stimulation of protein synthesis in this disused tissue may be related to the passive stretching of the denervated muscle. Supporting this hypothesis was the finding that the elevated rates of synthesis, whether expressed per whole muscle or per g of tissue, increased still further with the imposition of a continuous rather than an intermittent influence of stretch (Table 2). In contrast, these rates decreased significantly with the removal of stretch (Table 2). These findings, together with the direct comparison of the influence of immobilization with and without stretch (Table 2), suggest that passively stretching the denervated extensor digitorum longus stimulates protein synthesis in the denervated tissue. Further, this stimulation is greater than, or in advance of, the general gain in muscle mass (Table 2). It is, however, noteworthy that although immobilization without stretch caused the denervated tissue to waste (Table 1), with breakdown now exceeding synthesis, the synthetic rate per g of muscle still remained significantly higher than in the innervated, internal control (Table 2).

As in the previous study (Goldspink, 1976), the importance of allowing for changes in the specific radioactivity of the free-tyrosine pool when measuring synthetic rates became apparent after denervation. The specific radioactivity of the extensor digitorum longus muscle (200±19c.p.m./nmol of tyrosine) decreased significantly (35%; $P<0.001$) 7 days after nerve section. Such a change after denervation would clearly tend to underestimate the size of the increase in synthetic rates (Table 2) if only the incorporation of radioactive tyrosine into protein had been measured. This decrease in the specific radioactivity was probably due to both a 12% decrease ($P<0.01$) in amino acid transport (as verified by the use of the non-metabolized amino acid, cycloleucine, as well as measuring the accumulation of free [^{14}C]tyrosine in the acid-soluble phase) and a 23% increase ($P<0.001$) in the size of the free intracellular tyrosine pool (234±9nmol of tyrosine/g of muscle). The increase in the pool size is presumably related to the enhanced rates of protein catabolism after denervation (Table 2). The presence or absence of continuous stretch did not appear to produce any further significant changes in the tyrosine pools/g of the denervated extensor digitorum longus.

The elevated rates of protein breakdown per g of muscle 7 days after denervation were not altered further by either the continuous application or removal of stretch (Table 2). Breakdown in the whole muscle did, however, change (Table 2) in parallel with the general gains or losses in muscle mass (Table 1). It appears then that the influence of passive stretch in promoting the growth of the denervated extensor digitorum longus (Table 1) is predominantly achieved through a stimulation of protein synthesis opposed by a smaller enhancement of the degradative rates (Table 2).

Changes in the nucleic acid concentrations were simultaneously investigated in the various muscles (Table 3). Although denervation alone produced increases in both RNA and DNA per g of muscle, additional immobilization of the denervated extensor digitorum longus failed to cause any further significant changes (Table 3). However, the nucleic acid concentrations expressed per whole muscle changed

Table 2. *Changes in the rates of protein synthesis and protein breakdown of the extensor digitorum longus after 7 days of denervation with or without passive stretching*
Average rates of (*a*) protein synthesis and (*b*) breakdown were measured in intact isolated extensor digitorum longus muscles. Synthesis was measured as the incorporation of tyrosine from the medium into muscle proteins after a 3 h incubation. The specific radioactivity of the intracellular tyrosine pool was measured and this value incorporated into the final calculation of rates of synthesis. Breakdown was measured as the release of tyrosine into intracellular amino acid pools and into the surrounding medium after a 3 h incubation. Each value is the mean ± S.E.M. for at least six control or 7-day-denervated muscles (see Table 1). Statistical significances (*$P<0.01$ and **$P<0.025$) between these values were determined with Student's *t* test.

	External control	Internal control	Denervated	Internal control	Denervated+ immobilized with stretch	Internal control	Denervated+ immobilized without stretch
(*a*) Protein breakdown							
(nmol of tyrosine released/3 h per muscle) ...	7.6 ± 0.4	8.0 ± 0.3	15.0 ± 1.0	8.1 ± 0.8	17.0 ± 0.8	7.3 ± 0.6	11.0 ± 0.7
Percentage difference from:							
Control			+82*		+111*		+52*
Denervated					+15**		−23**
Continuous stretch							−35*
(nmol of tyrosine released/3 h per g of muscle) ...	314 ± 6	338 ± 13	874 ± 78	347 ± 36	806 ± 52	288 ± 23	800 ± 24
Percentage difference from:							
Control			+159*		+132*		+178*
Denervated					−8		−8
Continuous stretch							−1
(*b*) Protein synthesis							
(nmol of tyrosine incorporated/3 h per muscle) ...	11.1 ± 1.0	10.4 ± 0.1	14.7 ± 1.0	11.6 ± 0.8	19.4 ± 2.0	12.0 ± 1.0	10.0 ± 0.9
Percentage difference from:							
Control			+41*		+67*		−16
Denervated					+32**		−32*
Continuous stretch							−50*
(nmol of tyrosine incorporated/3 h per g of muscle) ...	464 ± 26	449 ± 14	844 ± 28	437 ± 11	935 ± 74	485 ± 15	684 ± 49
Percentage difference from:							
Control			+88*		+114*		+41*
Denervated					+11		−19**
Continous stretch							−26*

Table 3. *Changes in the content of (a) RNA and (b) DNA in the extensor digitorum longus after 7 days of denervation with or without passive stretching*

RNA and DNA were isolated and measured (Goldspink & Goldberg, 1973) in control and 7-day-denervated extensor digitorum longus muscles (see Table 1). Each value is the mean ± S.E.M. of measurements made on a minimum of six control or denervated tissues. Statistical significances (*$P<0.001$; **$P<0.025$) between these values were determined with Student's *t* test.

	External control	Internal control	Denervated	Internal control	Denervated+ immobilized with stretch	Internal control	Denervated+ immobilized without stretch
(*a*) RNA (as phosphorus)							
(μg/muscle) ...	5.0±0.2	4.7±0.2	4.9±0.3	4.9±0.3	5.9±0.3	4.9±0.2	4.0±0.1
Percentage difference from							
Control			+4		+22*		−18*
Denervated					+22**		−17**
Continous stretch							−32*
(μg/g of muscle) ...	208±8	205±5	286±7	189±8	289±5	205±5	282±6
Percentage difference from							
Control			+42*		+53*		+38*
Denervated					+1		−1
Continous stretch							−2
(*b*) DNA (as phosphorus)							
(μg/muscle) ...	2.0±0.1	1.9±0.1	2.0±0.1	2.2±0.1	2.4±0.1	2.1±0.1	1.8±0.1
Percentage difference from							
Control			+3		+9		−15
Denervated					+19**		−9
Continuous stretch							−24*
(μg/g of muscle) ...	83±2.0	83±1.4	117±2.2	87±2.0	112±3.7	85±2.1	120±4.2
Percentage difference from							
Control			+41*		+30*		+40*
Denervated					−4		+3
Continous stretch							+7

in parallel with the induced changes in muscle size in the presence or absence of stretch (Table 3).

Discussion

None of the various measurements made on the internal and external control muscles (Tables 1–3) differed significantly, suggesting that no compensatory growth had occurred as a result of increased work demands being placed on the internal control tissues. This is not altogether surprising, since the denervated limb, with or without a plaster cast, can be seen to participate actively in supporting the animal's body weight. These data appear to validate the use of internal control muscles, at least under these experimental conditions.

The changes in muscle weight, protein, protein turnover and nucleic acid concentrations (Tables 1–3) 7 days after denervation are in excellent agreement with the previous investigation (Goldspink, 1976). However, an interesting point arises from a comparison of the rates of protein synthesis and degradation in these two studies. In the previous study, all muscles, including the controls, when incubated *in vitro* were found to be in negative nitrogen balance, i.e., with rates of breakdown exceeding those of synthesis. However, in the present investigation, amino acids and insulin (and tyrosine for protein breakdown) have been added to the appropriate incubation media (see above). These additions to the media help maintain the control tissues in a positive nitrogen balance (Table 2) by increasing synthesis and decreasing breakdown. Under these latter conditions the muscles more aptly reflect their probable situation *in vivo* in these young animals, and these rates are now in good agreement with measurements *in vivo* reported by other investigators (Turner & Garlick, 1974; Millward *et al.*, 1973). However, more important is the fact that these rates measured *in vitro* do allow for a net increase of approximately 200 μg of protein per day. Such increases are in good agreement with the measured accumulation of protein in the innervated extensor digitorum longus muscle (Goldspink, 1976) over a 10-day period. However,

although the basal rates of the control tissues varied in accord with these manipulations of the media, the percentage changes induced by denervation (Table 2; Goldspink, 1976) remained remarkably similar in both studies. It was found (D. E. Goldspink, unpublished work) that the 7-day-denervated extensor digitorum longus is only slightly less responsive than the control tissue to the addition of amino acids and insulin. For example, protein synthesis was enhanced 112% in the control and 70% in the denervated extensor digitorum longus by these additions. These findings differ from the reported loss of insulin sensitivity that occurs in the denervated diaphragm (Harris & Manchester, 1966), although this does appear to be a time-dependent phenomenon. These induced changes are further supported by studies *in vivo* that indicated increased rates of protein breakdown after denervation (Goldberg, 1969; Goldspink, 1976). These induced changes in protein synthesis and protein degradation, as measured *in vitro* by these techniques, probably do therefore provide a good, if indirect, indication of the events taking place in the intact animal.

The dual-immobilization techniques for applying, or removing, stretch strongly suggest that passive stretch can cause an increased accumulation of protein and hence promote muscle growth (Table 1), even in the absence of an intact nerve supply. It has previously been either suggested (Stewart *et al.*, 1972), or shown (Sola *et al.*, 1973; Williams & Goldspink, 1976) that the passive stretching of other denervated muscles by various techniques also promotes longitudinal growth. The present findings also support the concept that there is probably some additional passive stretching of the denervated extensor digitorum longus arising from the more frequent dragging of the foot. Further, this stretch can either be removed, or applied in a more continuous form, influencing the muscle's growth and protein synthesis accordingly (Tables 1 and 2).

The stretch-induced growth appears to occur predominantly through a stimulation of protein synthesis (Table 2). It has previously been shown that stretch stimulates protein synthesis in the papillary muscles of the heart (Peterson & Lesch, 1972), the innervated soleus and extensor digitorum longus muscles (Goldspink, 1977*a*,*b*) and the denervated extensor digitorum longus (Buresova *et al.*, 1969). The calculated fractional rate of synthesis was found to increase from approx. 12% in the control to 20% in the denervated, and to 23% in the stretch-denervated tissues. However, the increase above control values was less marked (16%) when the ankle was immobilized so as to prevent the foot from being dragged, i.e., unstretched. In all of these experimental situations the fractional rate of degradation rose and remained at approx. 20%. These fractional rates are adequate to explain the differences in protein accumulation within the tissues subjected to the various degrees of stretch (Table 1). Further, these data are in close agreement with the fractional rates measured, or calculated, for the denervated hemidiaphragm (Turner & Garlick, 1974). In the latter study, as here (Table 2), protein turnover increased after nerve section. The subsequent periods of growth or atrophy of the denervated hemidiaphragm also occurred predominantly through modifications of the synthetic rates, with breakdown remaining fairly constant at a new and higher value (Turner & Garlick, 1974). It has been suggested (Feng *et al.*, 1962; Stewart *et al.*, 1972) that the transient hypertrophy of the denervated hemidiaphragm may be attributable to the rhythmic passive stretching of this tissue by the contractile activity of its innervated counterpart. In the light of the present findings (Table 2), it seems probable that the changes in synthetic rates could indeed be attributed to such passive stretching of the tissue and its subsequent adaptive growth. One marked difference between these two studies is seen in the extent of the growth of the denervated tissues. Although the 7-day-denervated diaphragm grew more than its innervated control (Manchester & Harris, 1968), the denervated leg muscle, even when maintained under a continuous influence of stretch, failed to keep pace with the growth of its control (Table 1). This may be related to apparent differences in the initial responses of these muscles to the interruption of their respective nerve supplies. Protein synthesis initially fell to below control values in the extensor digitorum longus 1 and 2 days after cutting the sciatic nerve, thereafter increasing and becoming higher in the disused tissue (Goldspink, 1976). From the available information (Harris & Manchester, 1966; Turner & Garlick, 1974) synthesis appears only to increase after section of the phrenic nerve, hence possibly explaining the greater growth of the diaphragm. Alternatively there may be different amounts of stretch, or other anabolism-promoting factors, imposed on the two denervated muscles.

This stretch-induced longitudinal growth with the addition of new sarcomeres in series in the denervated muscle (Williams & Goldspink, 1973, 1976) probably occurs against a background of atrophy, evidenced as a decrease in fibre diameters (Alder *et al.*, 1959; Muscatello *et al.*, 1965). Although stretch, arising from the dragging of the foot, appears to be an important factor contributing to the stimulation of protein synthesis, clearly other additional factors must be contributing to the anabolic trends in the 7-day-denervated extensor digitorum longus. For example, the removal of stretch from the denervated tissue did not bring synthesis per gram of muscle back to, or below, the rate measured in the control tissue (Table 2). Further, the elevated rates of protein breakdown (Table 2) and higher nucleic acid concentrations (Table 3) per gram of denervated muscle

were not changed further by the addition or removal of stretch. It has been suggested (Stewart, 1972) that the stretching of skeletal muscle by the rapid growth of the skeleton may be a factor involved in the normal growth and development of the tissue. Although there is appreciable ($18\pm0.9\%$, $P<0.001$) elongation of bones such as the tibia and fibula in these young animals during the period covered by the present study, it appears unlikely that this form of stretch is quantitatively very important. This conclusion is based on the finding that very similar changes in protein synthesis and breakdown were found in the denervated extensor digitorum longus of both young (Table 2 and Goldspink, 1976) and adult (Goldspink, 1978) animals. In the latter case, bone growth should have effectively ceased. Other possible factors known to correlate in time with the onset of these anabolic changes in the denervated muscle include spontaneous fibrillation (Thesleff, 1963) and other changes in electrical and mechanical properties (Lewis, 1972).

I am grateful to Paul Anderson and Mrs. Patricia Verner for their excellent technical assistance in this investigation. This research was made possible by the financial support of the Muscular Dystrophy Group of Great Britain and the Medical Research Council.

References

Alder, A. B., Crawford, G. N. C. & Edwards, R. G. (1959) *Proc. R. Soc. London Ser. B* **151**, 551–562

Buresova, M., Gutmann, E. & Klicpera, M. (1969) *Experientia* **25**, 144–145

DeLuca, H. F. & Cohen, P. P. (1964) in *Manometric Techniques* (Umbreit, W. W., Burris, R. H. & Stauffer, J. F., eds.), p. 132, Burgess, Minneapolis

Feng, J. P., Jung, H. W. & Wu, W. Y. (1962) *Acta Physiol. Sin.* **25**, 304–311

Fulks, R. M., Li, J. B. & Goldberg, A. L. (1975) *J. Biol. Chem.* **250**, 290–298

Goldberg, A. L. (1969) *J. Biol. Chem.* **244**, 3223–3229

Goldberg, A. L., Jablecki, C. &. Li, J. B. (1974) *Ann. N. Y. Acad. Sci.* **228**, 190–200

Goldberg, A. L., Etlinger, J. D., Goldspink, D. F. & Jablecki, C. (1975) *Med. Sci. Sports* **7**, 248–261

Goldspink, D. F. (1976) *Biochem. J.* **156**, 71–80

Goldspink, D. F. (1977*b*) *J. Physiol. (London)* **264**, 276–282

Goldspink, D. F. (1977*a*) *J. Physiol. (London)* **264**, 283–296

Goldspink, D. F. (1978) *Comp. Biochem. Physiol.* in the press

Goldspink, D. F. & Goldberg, A. L. (1973) *Biochim. Biophys. Acta* **229**, 521–532

Goldspink, D. F., Harris, J. B., Park, D. C., Parsons, M. E. & Pennington, R. J. (1971) *Int. J. Biochem.* **2**, 427–433

Gutmann, E. (ed.) (1962) *The Denervated Muscle*, Czechoslavakian Academy of Science, Prague

Gutmann, E. & Zak, R. (1961) *Physiol. Bohemoslov.* **10**, 493–500

Hamosh, M., Lesch, M., Baron, J. & Kaufman, S. (1967) *Science* **157**, 935–937

Harris, E. J. & Manchester, K. L. (1966) *Biochem. J.* **101**, 135–145

Lewis, D. M. (1972) *Nature (London) New Biol.* **241**, 285–286

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Mallette, L. E., Exton, J. H. & Parks, C. R. (1969) *J. Biol. Chem.* **244**, 5713–5723

Manchester, K. L. & Harris, E. J. (1968) *Biochem. J.* **108**, 177–183

Millward, D. J., Garlick, P. J., James, W. P. T., Nnanyelugo, D. O. & Ryatt, J. S. (1973) *Nature (London)* **241**, 204–205

Muscatello, V., Margreth, A. & Aloisi, M. (1965) *J. Cell Biol.* **27**, 1–22

Pater, J. L. & Kohn, R. R. (1967) *Proc. Soc. Exp. Biol. Med.* **125**, 476–481

Peterson, M. D. & Lesch, M. (1972) *Circ. Res.* **31**, 317–327

Sola, O. M. & Martin, A. W. (1953) *Am. J. Physiol.* **172**, 324–332

Sola, O. M., Christensen, D. L. & Martin, A. W. (1973). *Exp. Neurol.* **41**, 76–100

Stewart, D. M. (1972) in *Regulation of Organ and Tissue Growth* (Goss, R. J., ed.), pp. 77–100, Academic Press, New York and London

Stewart, D. M., Sola, O. M. & Martin, A. W. (1972) *Z. Vgl. Physiol.* **76**, 146–167

Thesleff, S. (1963) in *The Effect of Use and Disuse on Neuromuscular Functions* (Gutmann, E. & Hnik, P., eds.), pp. 41–51, Elsevier Publishing Co., Amsterdam

Turner, L. V. & Garlick, P. J. (1974) *Biochim. Biophys. Acta* **349**, 109–113

Waalkes, T. P. & Undenfriend, S. (1957) *J. Lab. Clin. Med.* **50**, 733–736

Weinstock, I. M. & Iodice, A. A. (1969) in *Biology and Pathology* (Dingle, J. T. & Fell, H. B., eds.), vol. 1, pp. 450–460, Elsevier Publishing Co., Amsterdam

Williams, P. E. & Goldspink, G. (1973) *J. Anat.* **116**, 45–55

Williams, P. E. & Goldspink, G. (1976) *J. Anat.* **122**, 455–465

Zak, R. (1962) in *The Denervated Muscle* (Gutmann, E., ed.), pp. 273–332, Czechoslovakian Academy of Science, Prague