The effect of intermittent changes in tension on protein and collagen synthesis in isolated rabbit muscles

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Isolated intact rabbit muscles were incubated in a medium containing radioactive proline. The rates of synthesis of collagen and total muscle protein after incubation with a constant tension or intermittent mechanical stretching were compared with the rates *in vivo*. Muscles incubated under a constant tension synthesized protein at 22% of the rate observed *in vivo*; intermittent mechanical stretching resulted in an increase of 73% in the rate of protein synthesis, to 38% of that found *in vivo*. Collagen synthesis was affected in the same way as total protein synthesis by both types of incubation, therefore the relative rates of collagen and total protein synthesis were unchanged. ATP concentration in the isolated muscles and the uptake of glucose from the medium were increased by intermittent mechanical stretching. Incubating the muscles with a gas phase containing 5% O₂ decreased the rate of protein synthesis, abolished the effect of intermittent mechanical stretching, lowered the concentration of ATP and increased the lactate concentration. The rate of protein synthesis in muscles maintained with a constant or intermittently applied tension was not affected by a previous period of incubation with the other type of stimulus.

There is considerable interest in the possible interrelationship between the rates of turnover of proteins in skeletal muscle and muscular activity. Because the physical activity of specific muscles is difficult to regulate *in vivo*, there has been a tendency to study protein synthesis during inactivity, by the immobilization of limbs at different degrees of extension (Goldspink, 1977; Booth & Seider, 1979) or by changing the resting tension applied to otherwise mobile muscles (Laurent *et al.*, 1978a).

Some of the limitations experienced *in vivo* can be overcome by the incubation of isolated muscles. The effect of single treatments may be observed without the complication of interactions between muscles or other physiological changes which can occur *in vivo*. In the present paper we describe the effect on synthesis of collagen and total protein of physical activity simulated by intermittent mechanical stretching of isolated intact muscles.

However, the removal of a muscle from its natural environment and the resultant loss of nervous and vascular supply may result in a depression in rates of protein synthesis and other metabolic processes. In addition, only small muscles are suitable for study

intact *in vitro*, owing to the reliance on diffusion for a supply of oxygen (Trowell, 1959). In spite of these drawbacks, studies *in vitro* can provide useful information, particularly if a comparison is made with measurements in the intact animal. Therefore the present work also attempts to compare the rates of protein synthesis in isolated intact rabbit forelimb muscles with values obtained *in vivo* in similar muscles from the donor animals. In addition the concentration of adenosine phosphates, lactate, pyruvate and glycogen have been compared in muscles from the intact animal and after incubation.

Experimental

Materials

L-[5-³H]Proline (12 Ci/mmol), L-[U-¹⁴C]proline (300 Ci/mol) and inulin-[¹⁴C]carboxylic acid (7 mCi/mmol) were purchased from The Radiochemical Centre (Amersham, Bucks., U.K.). Test Combination kits for the measurement of lactate, pyruvate and adenosine phosphates were obtained from Boehringer-Mannheim (London) Ltd. (Lewes, Sussex, U.K.). Materials used in the preparation of media and all other chemicals were purchased from Gibco Bio-Cult (Paisley, Scotland, U.K.), British

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The muscle was stretched by placing an electromagnetic coil (24 V, 500 Hz; I.T.T. Controls, MacLaren Division, Glasgow, Scotland, U.K.) around the base of the culture vessel. This coil was energized for a duration of 100 ms every 3 s, pulling the magnetic follower with a force equivalent to an 8 g increase in weight. When this force was applied, the muscle was stretched to 110% of its resting length.

The medium used in all incubations was based on Trowell's (1959) formulation; the modifications, including addition of the non-essential amino acids and the omission of insulin, have been described previously (Reeds *et al.*, 1980a). The amino acids were present in the medium in concentrations similar to those in rabbit blood; the concentration of proline was 200 μM . [^3H]- or [^{14}C]-proline was added to give a concentration of 1–2 $\mu\text{Ci/ml}$ of medium. Immediately before use, the medium was sterilized by filtration through an Acrodisc (0.2 μm pore size; Gelman-Hawksley, Lancing, Sussex, U.K.). Unless otherwise stated the muscles were incubated for a period of 6 h under O_2/CO_2 (19:1) at $37 \pm 0.1^\circ\text{C}$. The volume of medium in each incubation vessel was 3.5–4 ml.

Muscles required for the analysis of free and protein-bound amino acids were removed from the incubation medium, washed briefly (1–2 s) in 0.9% NaCl at 4°C , blotted dry and stored at -20°C . The medium was also stored at -20°C .

Muscle samples were homogenized in 5% (w/v) trichloroacetic acid. Hydroxyproline and proline were isolated by ion-exchange chromatography as described previously (Reeds *et al.*, 1980a). Analysis of the isolated hydroxyproline was by the method of Firschein & Shill (1966) and of the proline by automated ion-exchange chromatography (Locarte Co., London, U.K.) as described previously (Reeds *et al.*, 1980). Radioactivity in the isolated amino acids from the medium, homogenate pool and muscle protein was measured with a two-channel Beckman LS45 liquid-scintillation counter by using NE260 scintillator (Nuclear Enterprises, Edinburgh, Scotland, U.K.). Under typical conditions ^{14}C radioactivity was counted in one channel with an efficiency of 67%; in the other channel ^3H radioactivity was counted with an efficiency of 33% and a spillover of 22% of ^{14}C .

Natural actomyosin was isolated by a small-scale adaptation of the method of Schaub & Perry (1971).

The concentrations of lactate, pyruvate, AMP, ADP and ATP were all determined by using Boehringer-Mannheim Test Combinations.

Histochemical examination

Sections of muscle were prepared and stained for Ca^{2+} -dependent ATPase activity at pH 9.4 and for cytochrome oxidase as described by Loble *et al.* (1977).

Expression of results

Protein-synthesis rates have been calculated *in vivo* and *in vitro* from the ratio of the specific radioactivity of the protein-bound amino acid to that of either the extracellular or homogenate pool. When the specific radioactivity of the extracellular pool was used, the rate of protein synthesis *in vitro* was calculated as $(S_B/S_M)(24/t)$, where S_B is the specific radioactivity of the protein-bound proline, S_M is the specific radioactivity of the medium and t is the time of incubation (h). The equation derived by Nicholas *et al.* (1977) was used to calculate the rate of protein synthesis *in vivo* and also for the calculation of the rate *in vitro* when the homogenate pool was the basis of the calculation. The value of the rate constant for the mono-exponential rise in the specific radioactivity of free proline was taken as 32 day^{-1} in blood (Loble *et al.*, 1980) and 18 day^{-1} in muscle (Palmer *et al.*, 1980).

The statistical significance of differences between means was assessed by Student's *t* test for paired results. Values of $P < 0.05$ were taken as statistically significant.

Results

Viability of muscles maintained *in vitro*

Three criteria have been employed in assessing viability: (i) the concentration and rate of equilibration of the intracellular pool of proline; (ii) the concentrations of ATP, glycogen and lactate before and after incubation; (iii) histochemical observations.

(i) Muscles incubated in medium containing radioactive proline showed a first-order change in the specific radioactivity of free proline (Fig. 2). The rate constant for the rise in specific radioactivity was 16–18 days^{-1} . After 4 h incubation the specific radioactivity had risen to a near-constant value, and at the end of the incubation the ratio of the specific radioactivity of the homogenate free proline to that of the medium was approx. 0.5:1 (Table 1), a value similar to that found *in vivo* between blood plasma and muscle at the end of a constant infusion. In addition, the concentration of free proline in the muscle homogenate was unaffected by incubation for 6 h under constant tension (Table 1).

(ii) The concentrations of ATP, ADP, AMP, lactate and pyruvate in muscles after 6 h of incubation under constant tension were compared with values for freshly dissected muscles (Table 2). No significant differences were observed in the amounts of ADP (31–40 nmol/mg of N), AMP (3.4–5.1 nmol/mg of N) or pyruvate (6.0–6.6 nmol/mg of N). The concentration of ATP was also unaffected, but the tissue lactate concentration was more than twice that observed in fresh muscle, and there was a consequent rise in the lactate/pyruvate ratio (Table

Fig. 2. Change in the specific radioactivity of tissue free proline during a 6 h incubation

The concentration of proline in the medium was 200 nmol/ml for all incubations; 1–2 μ Ci of [3 H]- or [14 C]-proline was added at the start of the incubation. The specific radioactivity attained by the homogenate pool is expressed relative to that of the medium (S_H/S_M). No significant differences were found between the values obtained after the two different types of incubation; values for both constant tension and intermittent mechanical stretching were combined. Values for 1–4 h are the means $\pm$ 1 S.E.M. for eight observations; the line represents the equation

$$\frac{\text{specific radioactivity of tissue free proline at time } t}{\text{maximum specific radioactivity of tissue free proline}} = 0.5(1 - e^{-\phi t})$$

where $\phi = 18 \text{ day}^{-1}$.

2). There was also a 2-fold increase in the amount of glycogen stored by the muscle during a 6 h incubation.

(iii) Fresh muscles showed a staining pattern similar to that reported for extensor digitorum longus (Lobley *et al.*, 1977), with a preponderance of fast-twitch glycolytic fibres. After 6 h incubation neither the Ca^{2+} -ATPase nor the cytochrome oxidase activity was apparently diminished, and there was no indication of central fibre necrosis.

Comparison of rates of total protein and collagen synthesis *in vivo* and *in vitro*

Rates of protein synthesis in muscles incubated at a constant tension (10g) or with intermittent mechanical stretching were compared with the rate determined *in vivo* after a constant infusion of similar duration (Table 1). Muscles maintained *in vitro* under constant tension synthesized protein at 22% of the rate *in vivo*. The synthesis of both collagen and total protein was unaffected by varying the constantly applied tension from 0 to 10g, but intermittent mechanical stretching increased significantly the rates of both total protein and collagen synthesis. The mean increase in total protein synthesis achieved by intermittent stretching was 73%, so that under this condition the rate achieved *in vitro* was, on average, 38% of that *in vivo*. It is likely that the deliberate omission of insulin is a contributory factor in the decrease in protein-synthesis rates below those found *in vivo*. However, it is noteworthy that the addition of insulin at high physiological concentrations (100 μ units/ml) had a small stimulatory effect (about 10%) on protein synthesis under both constant tension and inter-

Table 1. Fractional rates of synthesis of muscle proteins *in vivo* and *in vitro*

The fractional synthesis rates of total muscle protein, actomyosin and collagen are expressed as k_{S_M} (where calculated from the specific radioactivity of the medium or blood) and k_{S_H} (where calculated from the specific radioactivity of the homogenate pool). Values are shown as means $\pm$ S.E.M. with numbers of experiments in parentheses. The rate constants used in the calculations were 32 day^{-1} for blood and 18 day^{-1} for muscle homogenate. The concentration of proline in the homogenate pool and the ratio of the specific radioactivities in the intracellular and extracellular pools are also shown. Abbreviation: N.D., not determined.

	<i>In vivo</i>	<i>In vitro</i>	
		Constant tension	Intermittent stretch
S_H/S_M	0.49 ± 0.07 (6)	0.52 ± 0.04 (21)	0.51 ± 0.03 (21)
Free proline (nmol/g fresh wt. of muscle)	409 ± 21 (6)	421 ± 36 (6)	415 ± 21 (6)
Total protein synthesis (%/day)			
k_{S_M}	4.92 ± 0.62 (6)	0.84 ± 0.08 (21)	1.62 ± 0.16 (21)
k_{S_H}	11.0 ± 1.38 (6)	2.27 ± 0.20 (21)	3.96 ± 0.38 (21)
Actomyosin synthesis (%/day)			
k_{S_M}	N.D.	0.91 ± 0.19 (4)	1.74 ± 0.22 (4)
Collagen synthesis (%/day)			
k_{S_M}	2.14 ± 0.32 (6)	0.38 ± 0.05 (14)	0.70 ± 0.09 (14)
k_{S_H}	4.86 ± 0.72 (6)	0.91 ± 0.12 (14)	1.73 ± 0.22 (14)

Table 2. Concentrations of lactate, pyruvate, glycogen and adenosine phosphates in muscles before and after incubation: lactate release and glucose uptake during incubation

The concentrations of ATP, lactate and glycogen are expressed per mg of N, $\pm$ s.e.m. No significant differences were found between treatments in the concentrations of ADP, AMP or pyruvate (see text), therefore only the ratios [ATP]/[ADP] + [AMP] and [lactate]/[pyruvate] are shown. Lactate release into the medium and glucose uptake from the medium are expressed in $\mu\text{mol/h}$ per mg of muscle N; both measurements were made on the medium after a 6 h incubation. A measured dose of [$1\text{-}^{14}\text{C}$]inulin was added to the medium at the start of the incubation to enable possible evaporation losses to be estimated. For all estimations $n = 4$. * Significantly different from constant tension, $P < 0.05$. † Significantly different from freshly dissected muscle, $P < 0.02$.

	ATP (nmol/mg of N)	[ATP] [ADP] + [AMP]	Tissue lactate (nmol/mg of N)	Tissue lactate pyruvate	Glycogen ($\mu\text{mol/mg}$ of N)	Glucose uptake ($\mu\text{mol/h}$ per mg of N)	Lactate release ($\mu\text{mol/h}$ per mg of N)
Freshly dissected muscle	534 $\pm$ 17	15.5 $\pm$ 1.1	10.2 $\pm$ 0.8	1.7 $\pm$ 0.4	35 $\pm$ 10		
Constant tension	538 $\pm$ 55	13.8 $\pm$ 1.0	23.4† $\pm$ 2.2	3.5† $\pm$ 1.4	115† $\pm$ 6.7	1.1 $\pm$ 0.1	0.38 $\pm$ 0.03
Intermittent stretching	684*† $\pm$ 55	15.5 $\pm$ 1.0	21.5† $\pm$ 2.4	3.4† $\pm$ 0.6	119† $\pm$ 11.0	2.0* $\pm$ 0.3	0.43 $\pm$ 0.05

mittent mechanical stretching, and did not decrease the effect of intermittent mechanical stretching on the rate of protein synthesis (R. M. Palmer, P. J. Reeds & R. H. Smith, unpublished work).

Under both incubation conditions the specific radioactivity of protein-bound proline and collagen hydroxyproline showed a linear increase with time (Fig. 3). The specific radioactivity of the hydroxyproline in the muscle hydrolysates was invariably lower than that of the proline, but the hydroxyproline/proline specific-radioactivity ratio (0.40–0.44:1) was similar to that *in vivo*. In addition, the synthesis rates of actomyosin after incubation under the two types of stimulation were similar to those of muscle protein under the same incubation conditions (Table 1).

Effect on synthesis rates of alteration in the type of stimulus during the incubation

Pairs of muscles were incubated with either constant tension or intermittent mechanical stretching for 5 h in the absence of radioactive amino acids. After this preliminary incubation the type of stimulus applied to one muscle of each pair was reversed, i.e. constant tension to intermittent stretching or intermittent stretching to constant tension. At the same time [^3H]proline was added to all incubation vessels and the incubation was continued for a further 5 h. Muscles maintained initially under a constant tension and subsequently subjected to intermittent stretching showed an elevated rate of protein synthesis similar to that obtained in the muscles that were intermittently stretched throughout the 10 h incubation (Table 3). Muscles that had initially been incubated with intermittent stretching and then maintained under a constant tension for the second half of the experiment with [^3H]proline synthesized protein at a rate that was not significantly different from that in muscles maintained under a constant tension for the whole incubation.

Fig. 3. Increase in the specific radioactivity of muscle proteins during a 6 h incubation

Muscles were incubated as described in the Experimental section. All values are expressed as

$$\frac{\text{specific radioactivity of protein-bound proline (or hydroxyproline) at time } t}{\text{specific radioactivity of protein-bound proline after 6 h constant tension}}$$

Each point for 1–4 h represents the mean $\pm$ 1 s.e.m. for four values: Δ , protein-bound proline, constant tension; $\square$, protein-bound proline, intermittent mechanical stretching; $\circ$, hydroxyproline, constant tension; $\bullet$, hydroxyproline, intermittent mechanical stretching.

Incubations under low oxygen concentration

In the standard incubation conditions the gas phase contained 95% O_2 (i.e. O_2/CO_2 , 19:1). Under

Table 3. *Effect on protein synthesis of changing the type of stimulation during incubation*

Pairs of muscles were incubated with constant tension or intermittent stretching in a radioisotope-free medium. After 5 h the treatment given to one muscle of each pair was reversed, [^3H]proline was added to the medium and incubation continued for a further 5 h. The muscles were then analysed in the usual way to determine the rates of protein synthesis during the latter half of the incubation. Fractional rates of total protein and collagen synthesis were calculated from the specific radioactivity of the medium and expressed in %/day ($n = 4$ for all treatments). * Significantly different from constant tension, $P < 0.01$ (paired t tests).

	Collagen synthesis, %/day (k_{S_M})	Total protein synthesis, %/day (k_{S_M})
Constant tension	0.41 ± 0.05	0.98 ± 0.12
Constant tension after 5 h intermittent stretching	0.35 ± 0.04	0.80 ± 0.10
Intermittent stretching	$0.71 \pm 0.06^*$	$1.78 \pm 0.15^*$
Intermittent stretching after 5 h constant tension	$0.75 \pm 0.04^*$	$1.71 \pm 0.10^*$

Table 4. *Effect of incubation under 5% O₂ on glucose uptake and the concentration of adenosine phosphates, lactate and pyruvate*

Muscles were incubated with a gas phase of O₂/CO₂/N₂ (1:1:18); simultaneously muscles from the same animal were incubated under O₂/CO₂ (19:1). Glucose uptake was measured as described in Table 2, and there was a statistically significant difference between the two types of incubation. The differences in concentration of lactate and ATP were not statistically significant between treatments under 5% O₂; there were, however, significant differences from the corresponding treatments under 95% O₂ (Table 2). †Significantly different from constant tension, $P < 0.02$ (paired t test). Significance of difference from corresponding treatment under 95% O₂ (Table 2) (paired t tests): ** $P < 0.02$, *** $P < 0.01$.

	Constant tension	Intermittent stretching
Glucose uptake ($\mu\text{mol/h per mg of N}$)	1.81 ± 0.02	$2.98 \pm 0.40^\dagger$
ATP (nmol/mg of N)	$299 \pm 72^{**}$	$335 \pm 24^{**}$
[ATP]	11.4 ± 1.5	10.8 ± 1.1
[ADP] + [AMP]	$52.8 \pm 4.2^{**}$	$49.8 \pm 4.1^{**}$
Tissue lactate (nmol/mg of N)	$13.1 \pm 2.3^{***}$	$10.5 \pm 3.3^{***}$
Tissue pyruvate		

these conditions of incubation, intermittent mechanical stretching increased ATP contents above those found after incubation with a constant tension

Table 5. *Effect of incubation under low concentrations of oxygen on protein-synthesis rates*

Muscles were incubated with a gas phase containing 5 or 10% O₂, 5% CO₂ and 90 or 85% N₂. The rates of total protein and collagen synthesis were calculated from the specific radioactivity of the medium and expressed in %/day ($n = 4$ for all treatments). †Significantly different from constant tension, $P < 0.05$.

Type of stimulation	O ₂ (%)	Total protein synthesis (%/day)	Collagen synthesis (%/day)
Constant tension	10	0.40 ± 0.04	0.18 ± 0.02
Intermittent stretching	10	$0.61 \pm 0.11^\dagger$	0.19 ± 0.03
Constant tension	5	0.32 ± 0.01	0.15 ± 0.02
Intermittent stretching	5	0.33 ± 0.04	0.19 ± 0.04

or in freshly dissected muscles (Table 2). The uptake of glucose from the medium was also increased to twice that observed in incubations with constant tension. The influence of oxygen partial pressure on adenine nucleotide concentrations and glucose uptake was investigated in muscles incubated with a gas phase containing 5% O₂ (O₂/CO₂/N₂, 1:1:18). The results (Table 4) indicate that hypoxia resulted in a decrease in the concentration of adenine nucleotides, a decrease in the ratio [ATP]/([ADP] + [AMP]) and an increase in that of [lactate]/[pyruvate]. The increase in ATP concentration associated with mechanical stimulation under 95% O₂ (Table 2) was not maintained at 5% O₂, whereas the increase in glucose uptake caused by intermittent mechanical stretching was still apparent (Table 4). However, the rate of protein synthesis in muscles maintained under a constant tension at 5% O₂ was depressed by 65% and the effect of intermittent stretching was abolished (Table 5). The rate of protein synthesis in muscles maintained under constant tension at 10% O₂ (O₂/CO₂/N₂, 2:1:17) was also decreased (by 54%). However, at this concentration of O₂ the effect of intermittent stretching on protein synthesis was still statistically significant (Table 5).

Discussion

Validity of comparison of protein-synthesis rates under different conditions

Controversy persists about the site of the precursors for the measurement of true rates of protein synthesis (Hider *et al.*, 1969; Fern & Garlick, 1974; Fulks *et al.*, 1975; Lobley *et al.*, 1980). Two factors require consideration, the specific radioactivity of the precursor and the rate at which that specific radioactivity rises to a constant value. In the present

paper all experiments *in vitro* have been performed in a medium in which the concentrations of amino acids are close to those in rabbit blood. The advantage of this approach, when measurements of the rate of protein synthesis are to be made both *in vitro* and *in vivo*, is that the homogenate pool rises to a constant specific radioactivity *in vitro*, with a similar rate constant to that observed *in vivo* (Palmer *et al.*, 1980). In addition, the same value for the final specific radioactivity (relative to that of the extracellular pool) is observed *in vivo* (Lobley *et al.*, 1980) and *in vitro*. It is also apparent that the size of the homogenate pool (400 nmol/g of tissue) is unaffected and that the isolated muscle is able to maintain its intracellular proline concentration at normal values against a 2-fold concentration gradient. These considerations simplify a comparison of the results obtained *in vivo* and *in vitro*, for, if it is assumed that the same pool of amino acids is used for protein synthesis in both the isolated muscle and in the whole animal, calculation of the relative synthesis rates will be unaffected by the choice of the possible precursor.

Similar considerations apply to collagen, although the true precursor for collagen synthesis is procollagen. At the end of a constant infusion of [^3H]proline, the specific radioactivity of the procollagen in rabbit muscle is approx. 40% of that of tissue free proline (Palmer *et al.*, 1980), and the true rate of collagen synthesis, calculated from the specific radioactivity of procollagen, is similar to that of total muscle protein. Calculations based on the specific radioactivity of either the homogenate or extracellular pool of proline will therefore underestimate the true rate of collagen synthesis. However, the relative rates of collagen and total protein synthesis *in vivo* and *in vitro* seem to be very consistent. Despite the generally accepted view that a discrete population of fibroblast-like cells within a muscle is responsible for most of the collagen synthesis and the evidence that collagen and total protein are synthesized from different precursors (Palmer *et al.*, 1980), it is apparent that both the isolation of the muscle and mechanical stimulation affect the synthesis of collagen and total muscle protein equally. This is not a uniform finding. In arterial smooth-muscle cells, cyclic stretching affects collagen synthesis to a greater extent than total protein synthesis (Leung *et al.*, 1976), and a similar observation has been made in fowl muscle undergoing stretch-induced hypertrophy (Laurent *et al.*, 1978b).

Two different comparisons have been attempted in the present work, a comparison of protein-synthesis rates after incubation either with intermittent mechanical stretching or under constant tension, and a comparison of protein-synthesis rates *in vivo* and *in vitro*. In the former of these there was

a 70% increase in protein synthesis when intermittent stimuli were applied to muscles, and an essentially similar response has been observed in arterial smooth-muscle cells subjected to cyclic stretching (Leung *et al.*, 1977). In contrast with the observations of Vandeburgh & Kaufman (1979), we have been unable to demonstrate a stimulation of protein synthesis in muscles subjected to a constant tension varying from 2 to 10 g (Reeds *et al.*, 1980b).

A number of factors that may affect the rate of protein synthesis have been investigated. Most directly, changes in amino acid transport or concentration could possibly affect protein synthesis, but we have found no evidence for changes in the transport or intracellular concentration of proline. Furthermore, under the incubation conditions described, the transport and intracellular concentration of leucine and tyrosine are also unaltered (R. M. Palmer, P. J. Reeds & R. H. Smith, unpublished work). It is also possible that lower oxygen partial pressure within the tissue may influence the amount of energy available for protein synthesis and that the effect of intermittent mechanical stretching may be due to increased penetration of oxygen. The increase in the [lactate]/[pyruvate] ratio indicates that a degree of hypoxia may occur during a normal incubation. Incubations under 5% O_2 have shown that protein synthesis can be inhibited and the effect of intermittent stretching abolished when very low concentrations of O_2 are provided. However, the ATP concentrations in muscles incubated under constant tension with 95% O_2 were the same as those in freshly dissected muscle (see also Keul *et al.*, 1972), and it is noteworthy that the glycogen content of the muscle rose during the incubation. Although intermittent mechanical stretching increased the concentration of ATP, it was not associated with a significant decrease in the [lactate]/[pyruvate] ratio. It seems unlikely therefore that the supply of O_2 or the availability of energy are major factors in determining the response to intermittent stretching.

The experiments in which the type of stimulus was changed halfway through the incubation were all continued for 10 h. It appears that the viability of the muscles during these 10 h incubations was unimpaired, since the rates of protein synthesis during the second 5 h were not significantly different from those observed for the same treatment in a normal (6 h) incubation. The rate of protein synthesis measured during incubations with mechanical stretching after a preincubation under a constant tension was similar to that observed in a normal 6 h incubation with intermittent stretching. Thus the ability of intermittent stretching to increase protein-synthesis rates is not abolished by initially maintaining the muscle at a constant length for several hours. Conversely it would appear that intermittent

stretching influences protein synthesis only for the period during which it is applied.

This failure of intermittent stretching to influence the subsequent rate of protein synthesis appears to contradict previous observations that alterations in tension induced in a donor animal affect the rate of protein synthesis measured *in vitro* (Goldspink, 1977). Most experiments of this type, however, have involved changes of tension *in vivo* applied for several days, resulting in increases in the size, protein content and nucleic acid content of the tensioned muscle (Goldberg *et al.*, 1975); significant changes of this nature would not be expected to occur in 5–6 h.

Caution has to be exercised in relating occurrences *in vitro* to those in the intact animal. Although the isolated intact muscles synthesize protein at a rate which is approximately twice that found in cut muscle (Reeds *et al.*, 1980a), relative to the rate *in vivo* there is still a large decrease. No doubt the removal of the muscle from its normal blood supply, depriving it of the normal circulating factors in the blood, may account for much of the fall in synthesis rates. The ability partially to reverse this depression by a form of stimulation which is purely mechanical indicates that the method may be both a useful and humane way of studying the mechanisms of work-induced changes in protein-synthetic rates.

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