The Effects of Cutting or of Stretching Skeletal Muscle *in vitro* on the Rates of Protein Synthesis and Degradation

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Rates of protein synthesis were significantly lower in the cut soleus and extensor digitorum longus muscles than in their uncut counterparts. Rates of protein degradation were significantly higher in cut soleus muscles, but not in cut extensor digitorum longus muscles as compared with their uncut controls. Concentrations of ATP and phosphocreatine were significantly lower in cut soleus and extensor digitorum longus muscles after incubation *in vitro* in contrast with their respective control uncut muscles. These data indicate that cutting of muscle fibres alters rates of protein synthesis and degradation, in addition to altering concentrations of high-energy phosphates. Since these findings stressed the importance of using intact muscles to study protein metabolism, additional studies were made on intact muscles *in vitro*. Stretched soleus muscles had higher concentrations of high-energy phosphates at the end of an incubation period than did unstretched muscles. However, the length of the soleus, extensor digitorum longus and diaphragm muscles during incubation did not affect rates of protein degradation.

Skeletal-muscle preparations with cut muscle fibres have been used to study protein metabolism (Manchester & Krah, 1959; Wool & Krah, 1959; Li *et al.*, 1973; Buse *et al.*, 1973; Odessey *et al.*, 1974; Fulks *et al.*, 1975; Palaiologos & Felig, 1976; Goldspink & Goldspink, 1977; Chang & Goldberg, 1978). However, it is unknown whether cutting muscle fibres alters the rates of either protein synthesis or protein degradation, or both. Others have previously reported that preparations with cut muscle fibres have greater extracellular spaces (Kipnis & Cori, 1957), higher glycogen concentrations (Gross *et al.*, 1976) and increased response of glucose uptake to exogenously applied cyclic AMP (Tarui *et al.*, 1976). Thus, one purpose of the present study is to clarify whether the rates of protein synthesis and/or the rates of protein degradation are altered by cutting muscle fibres.

There is a difference in the directional responses of protein degradation when comparing the stretched muscle *in vitro* and *in vivo*. Others (Goldberg & Odessey, 1974; Goldberg *et al.*, 1974, 1975; Goldberg, 1979) have observed a decrease in the rate of protein degradation in the stretched muscle *in vitro*. However, it has been observed that stretching muscle *in vivo* increases the rate of protein degradation (Turner & Garlick, 1974; Goldspink, 1977; Laurent *et al.*, 1978a,b). Thus, we raise the question

whether the differences in the directional responses of protein degradation observed *in vivo* and *in vitro* might be related, in part, to ATP concentrations. Muscles *in vivo* have relatively small diffusion distances for O₂ because of their intact blood supply. In contrast, the isolated muscle depends upon oxygenation from the incubation medium. Therefore, we decided to investigate whether oxygenation of muscles *in vitro* is adequate to maintain muscle ATP concentration during incubation. Furthermore, we were concerned whether the stretching of flaccid muscles might better maintain ATP concentrations during their incubation because stretched muscles are thinner and thus have relatively shorter diffusion distances for O₂ than the flaccid muscles. Thus the second purpose of the present study was to test whether stretching altered concentrations of ATP and phosphocreatine in intact skeletal muscles *in vitro*.

Materials and Methods

Animal care

Male rats of a Wistar strain, 35–45 g body wt., from Charles River (Wilmington, MA, U.S.A.) were provided with Purina lab chow and water *ad libitum*. When they reached 60–80 g body wt., they were randomly divided into control and experimental groups.

Tissue preparation

Animals were anaesthetized with ether and soleus or extensor digitorum longus muscles were dissected and immediately incubated. Diaphragms were dissected from animals killed by cervical dislocation. Further manipulations of the dissected tissue were performed in a tissue bath at 37°C containing Krebs–Ringer bicarbonate buffer (pH 7.4) gassed with O₂/CO₂ (19:1). Soleus and extensor digitorum longus muscles were dissected with minimal tendinous tissue and no visible damage to muscle fibres. The intact diaphragm was dissected into halves as described by Kipnis & Cori (1957).

After their dissection, muscles were incubated at 37°C in Krebs–Ringer bicarbonate buffer (pH 7.4), which was continuously gassed with water-saturated O₂/CO₂ (19:1). Muscles were stretched to the approximate resting length found *in vivo* during incubation by placing no. 27-gauge needles through tendons (soleus or extensor digitorum longus) into a Dow–Corning Silastic strip (protein-synthesis experiments) or into a Dow–Corning Sylgard layer (protein-degradation experiments). Incubation vessels were covered with a Parafilm sheet during incubation.

Assay procedures

Protein-synthesis rates in isolated muscles were estimated by procedures previously described by Li *et al.* (1973) and Fulks *et al.* (1975), with the modifications that the extracellular space of each experimental muscle was measured, and that the incubation medium was continuously bubbled with water-saturated O₂/CO₂ (19:1). Muscles were incubated at 37°C in Krebs–Ringer bicarbonate buffer containing 10 mM-glucose, 100 m-i.u. of insulin/ml, 5 × the plasma concentration of the branched-chain L-amino acids (Mallette *et al.*, 1969), 0.05 mM-L-tyrosine, 0.05 µCi of L-[¹⁴C]tyrosine/ml, 0.05 µCi of [³H]methoxyinulin/ml and 100 i.u. of penicillin/ml. After 2 h of incubation, the muscles were quickly weighed and homogenized in 10% (w/v) trichloroacetic acid and centrifuged. The resulting precipitate was washed two times in 10% (w/v) trichloroacetic acid and once in ethanol/ether (1:1). The final wash always had background radiation. The washed precipitate was dissolved in NCS (Amersham–Searle), to which was added Aquasol for counting in a dual-channel liquid scintillation counter. The supernatant of the first trichloroacetic wash of the muscle, as well as an aliquot of the incubation medium, were both analysed for tyrosine concentration (Waalkes & Udenfriend, 1957) and counted for radioactivity. The incorporation of tyrosine into protein was estimated by dividing the ¹⁴C counts in protein by the intracellular specific activity of tyrosine, as previously described (Li *et*

al., 1973). The intracellular specific activity was calculated from the inulin space by methods previously described (Li *et al.*, 1973; Fulks *et al.*, 1975). Unincorporated free tyrosine in skeletal muscle has been shown to be the immediate precursor for protein synthesis in skeletal muscle (Li *et al.*, 1973).

Tyrosine release from isolated muscle in the presence of cycloheximide was used as an index of the rate of protein degradation, as previously described by Fulks *et al.* (1975). Muscles were incubated, as described above, in a Krebs–Ringer bicarbonate buffer (pH 7.4) containing 10 mM-glucose, 0.5 mM-cycloheximide, 100 m-i.u. of insulin/ml and 100 i.u. of penicillin/ml. At the times indicated for individual experiments, 0.5 ml portions of the incubation medium were used for assay of tyrosine by the fluorometric procedure of Waalkes & Udenfriend (1957). At the end of incubation, muscles were quickly weighed and homogenized in 10% (w/v) trichloroacetic acid and then centrifuged. The resulting supernatant was assayed for tyrosine, and the post-incubation concentration of unincorporated tyrosine in the muscle was then calculated. In some experiments, the unincubated, contralateral muscle was homogenized in 10% (w/v) trichloroacetic acid and the pre-pool concentration of unincorporated free tyrosine in the muscle was obtained as described above for the post-pool tyrosine. Data were calculated, as described by Fulks *et al.* (1975) and by Li & Goldberg (1976), and the absolute values thus obtained were similar to previous reports (Fulks *et al.*, 1975; Li & Goldberg, 1976; Libby & Goldberg, 1978; Goldberg, 1979; Kameyama & Etlinger, 1979).

ATP and phosphocreatine were assayed as described by Lowry & Passonneau (1972). The procedure of Lowry *et al.* (1951) was used for protein determination using bovine serum albumin as a standard.

Experimental design

Muscle fibres in intact isolated muscles were cut by various amounts, depending upon the individual experiment. Cuts were made in a perpendicular direction to the longitudinal orientation of the muscle fibres, either 50 or 100% through the muscle. For experiments in which a single cut was made, the cut was made in the belly of the muscle. When two cuts were made, each cut was made opposite to the other by being on opposite sides of the muscle belly, in addition to being on opposite sides of the longitudinal orientation.

In order to investigate the effects of stretching on the rate of protein degradation in skeletal muscles *in vitro*, one of the experimental designs that we employed utilized two sets of controls. One of these sets of controls was a paired muscle to the treated

muscle. For example, during the first incubation period, treated and control muscles were maintained at approximately the resting length found *in vivo*. After the first period, treated muscles were stretched to approx. 120% of their resting length while controls remained at 100%. After the stretching treatment, stretched muscles were returned to a length that was approximately the same length as the control muscle. The above design also permitted the treated muscle to serve as its own control. Measurements were made at the end of each of the three periods described above.

Results

The rate of protein synthesis was significantly lower in the cut soleus and extensor digitorum longus muscles than in their respective uncut muscles (Table 1). This reduction was related specifically to the significantly lower specific activity in the washed trichloroacetic acid precipitate of the cut soleus and extensor digitorum longus muscles as compared with the specific activity of the uncut muscles.

No significant differences were observed between the first and second hour of incubation for the measurement of protein-degradation rates when soleus muscles remained uncut for both hours of incubation (Table 2). A single cut, which was one-half way through the soleus muscle, did not significantly alter the rate of protein degradation when pre- and post-cut values were compared for the same muscle. A minimum of two cuts, of which each

cut was one-half way through the soleus muscle, was required in order for a significant increase in the rate of protein degradation to occur (Table 2). The concentration of unbound tyrosine in the trichloroacetic acid-soluble soleus muscle material did not significantly change as a result of cutting the soleus muscle (Table 2).

In contrast with the soleus muscle, cutting did not alter the protein-degradation rate in the extensor digitorum longus muscle. However, the interpretation of the effect of cutting on the rate of protein degradation in the extensor digitorum longus muscle is a little more complicated than that of the soleus muscle. First, a significant decline in tyrosine release occurred between the first and second hours of incubation for uncut extensor digitorum longus muscles (Table 2). Second, a significant decrease in tyrosine concentration in the trichloroacetic acid-soluble material, i.e., the unbound, intracellular tyrosine pool, occurred when the extensor digitorum longus muscle was cut during incubation (Table 2). Correcting tyrosine release for these two alterations only increased its release 11–12% in the cut extensor digitorum longus muscle.

Values for tyrosine release varied with shipments of rats and these variations contributed to the magnitude of the standard error of the means. We have no explanation for these observations; others have had similar findings (Fulks *et al.*, 1975). Thus we did not compare tyrosine-release rates between different groups. However, since each muscle served at its own control, i.e., tyrosine release was measured before and after cutting in the same muscle, day-to-day variation had less impact upon our

Table 1. *Rates of protein synthesis in cut and uncut soleus and extensor digitorum longus muscles*

The incubation medium was a Krebs–Ringer bicarbonate buffer (pH 7.4) containing 10 mM-glucose, 100 m-i.u. insulin/ml, 5 × the plasma concentration of branched-chain L-amino acids, 0.05 mM-L-tyrosine, 0.05 μ Ci of L-[14 C]tyrosine/ml, 0.05 μ Ci of [3 H]methoxyinulin/ml and 100 i.u. of penicillin/ml. All muscles were incubated for 2 h. Soleus and extensor digitorum longus muscles designated as cut had two cuts made through one-half of the muscle. Cuts were made perpendicular to the orientation of the muscle fibres, after dissection and immediately before incubation for 2 h. Muscles were incubated at lengths approximately equal to 100% of the resting length which was determined when the ankle joint was at an angle of approx. 120° and the knee joint at an angle of approx. 115° (Booth & Kelso, 1973). Further details for incubation conditions, assay procedures, and calculations are given in the Materials and Methods section. Values are means $\pm$ s.e.m. for six animals. Means were compared with a paired two-tailed Student's *t* test. *, *P* < 0.02 from uncut; **, *P* < 0.01 from uncut.

	Soleus muscle		Extensor digitorum longus muscle	
	Uncut	Cut	Uncut	Cut
Protein-synthesis rate (nmol of tyrosine/h per g wet wt.)	134 $\pm$ 13	80 $\pm$ 9*	103 $\pm$ 9	76 $\pm$ 7**
Specific radioactivity of precipitate (d.p.m./2 h per mg of protein)	79 $\pm$ 10	44 $\pm$ 6**	86 $\pm$ 8	64 $\pm$ 5**
Specific radioactivity of supernatant (d.p.m./nmol of tyrosine)	298 $\pm$ 38	273 $\pm$ 15	427 $\pm$ 39	439 $\pm$ 44
Unincorporated tyrosine (nmol/g wet wt.)	130 $\pm$ 6	127 $\pm$ 9	129 $\pm$ 10	119 $\pm$ 7
Extracellular space (μ l/mg wet wt.)	0.324 $\pm$ 0.039	0.454 $\pm$ 0.031**	0.266 $\pm$ 0.032	0.380 $\pm$ 0.031**

Table 2. *Protein-degradation rates and post-incubation intracellular tyrosine concentrations in uncut and cut soleus and extensor digitorum longus muscles of the rat*

All muscles were incubated for 2h. After 1h of incubation in an uncut condition, muscles were cut as described in the Materials and Methods section. Cuts were made perpendicular to the orientation of muscle fibres. After cutting, muscles were incubated for 30min before their transfer to a jar with fresh Krebs–Ringer bicarbonate buffer containing ingredients described in text. Tyrosine release was then measured for 60min. The incubation medium contained Krebs–Ringer bicarbonate buffer (pH 7.4) containing 10mM-glucose, 0.5mM-cycloheximide, 100m-i.u. of insulin/ml and 100i.u. of penicillin/ml. Muscles were stretched to 115% of resting length throughout the incubation period. The resting length *in vivo* was defined to be 100% in limbs with ankle and knee angles at approx. 120° and 115° respectively (Booth & Kelso, 1973). Values are means $\pm$ S.E.M. for the numbers of observations (*n*) given in parentheses. Differences for tyrosine release between uncut and cut muscles were tested with paired two-tailed Student's *t* test because each muscle served as its own control: *, $P < 0.01$; **, $P < 0.001$. Differences for tyrosine pool sizes between uncut and cut muscles were tested with unpaired Student's *t* test: †, $P < 0.05$.

Muscle	Description of cut	Tyrosine released (nmol/h per g wet wt.)		Post-incubation intracellular tyrosine concentration (nmol/g wet wt.)	<i>n</i>
		First hour (uncut)	Second hour		
Soleus	Uncut control	139 $\pm$ 10	135 $\pm$ 16	122 $\pm$ 8	(6)
	One cut made after 1h	189 $\pm$ 12	213 $\pm$ 14	119 $\pm$ 11	(4)
	Two cuts made after 1h	190 $\pm$ 12	251 $\pm$ 16**	127 $\pm$ 7	(4)
	Muscle cut into halves after 1h	160 $\pm$ 7	241 $\pm$ 11**	—	(6)
Extensor digitorum longus	Uncut control	143 $\pm$ 13	107 $\pm$ 11**	100 $\pm$ 8	(6)
	Two cuts made after 1h	140 $\pm$ 8	139 $\pm$ 8	80 $\pm$ 5†	(6)
	Muscle cut into halves after 1h	127 $\pm$ 7	144 $\pm$ 7*	79 $\pm$ 3†	(6)

Table 3. *Concentrations of ATP and phosphocreatine in uncut and cut soleus and extensor digitorum longus muscles after 120 min of incubation*

Values are means $\pm$ S.E.M. for four muscles. Soleus and extensor digitorum longus muscles were cut as described in the Materials and Methods section, and then incubated at a length approximately 100% of resting length in Krebs–Ringer bicarbonate buffer, pH 7.4, gassed with O₂/CO₂ (19:1). The angles of ankle and knee joints were approx. 120° and 115° respectively, when 100% of resting length *in vivo* was determined. The buffer contained 10mM-glucose, 100m-i.u. of insulin/ml, and 100i.u. of penicillin/ml. These experiments were performed and analysed at a different time from the experiment in Table 2. Means were compared with an unpaired two-tailed Student's *t* test: *, $P < 0.02$ from the uncut group; †, $P < 0.001$ from the uncut group.

	Concentration (nmol/mg of protein) in			
	Soleus		Extensor digitorum longus	
	ATP	Phospho- creatine	ATP	Phospho- creatine
Uncut	20.9 $\pm$ 1.1	107.0 $\pm$ 6.9	31.6 $\pm$ 2.7	211.0 $\pm$ 19.8
Cut	10.3 $\pm$ 0.7†	38.0 $\pm$ 3.0†	19.8 $\pm$ 1.7*	125.1 $\pm$ 11.4*

Table 4. *Effect of time of incubation in vitro on concentrations of ATP and phosphocreatine in soleus and extensor digitorum longus muscles at resting length*

Values are means $\pm$ S.E.M. There are four muscles/mean, except for the 1h incubation of the extensor digitorum longus which had three observations. Muscles were incubated in Krebs–Ringer bicarbonate buffer, pH 7.4, gassed with O₂/CO₂ (19:1). The buffer contained 10mM-glucose, 100m-i.u. of insulin/ml, and 100i.u. of penicillin/ml. Muscle fibres were not cut and muscle lengths approximated to resting lengths, which were defined as the muscle length in limbs with ankle and knee joints at approx. 120° and 115° respectively (Booth & Kelso, 1973). *, $P < 0.05$ from the 1h value; **, $P < 0.05$ from the value *in situ*.

Metabolite	Time of incubation	Concentration (nmol/mg of protein) in	
		Soleus	Extensor digitorum longus
ATP	<i>in situ</i>	28.3 $\pm$ 3.9	45.0 $\pm$ 4.7
	1h	32.4 $\pm$ 1.5	39.4 $\pm$ 1.6
	2h	20.9 $\pm$ 1.1*	31.6 $\pm$ 2.7**
Phospho- creatine	<i>in situ</i>	150 $\pm$ 28	243 $\pm$ 14
	1h	160 $\pm$ 18	266 $\pm$ 12
	2h	107 $\pm$ 7*	211 $\pm$ 20

present data. Day-to-day variation was probably not caused by variations in the tyrosine standard curve, since the coefficient of variation for the slope of the standard curve was 6% among days.

The concentrations of ATP and of phosphocreatine in both the soleus and extensor digitorum longus muscles were significantly lower in muscles that had been cut 2h previously than for contra-

lateral uncut muscles that were also incubated for 2 h (Table 3).

Concentrations of ATP and phosphocreatine in the uncut soleus muscle at resting length were

Table 5. *Effect of muscle length on the concentrations of ATP and phosphocreatine in the rat soleus muscle after 2 h of incubation*

Values are means $\pm$ S.E.M. There are five muscles/mean, except for 115% which has four muscles/mean. Muscles were incubated in Krebs-Ringer bicarbonate buffer, pH 7.4, gassed with O₂/CO₂ (19:1). The buffer contained 10 mM-glucose, 100 m-i.u. of insulin/ml and 100 i.u. of penicillin/ml. One soleus muscle was placed at a muscle length that approximated to 70% of resting length while the contralateral soleus muscle from the same rat was placed at a muscle length that was either about 100% or 115% of resting length depending upon the experiment. Resting length was defined at 100% in limbs with ankle and knee angles at approx. 120° and 115°, respectively (Booth & Kelso, 1973). *, $P < 0.05$ from the 100% of resting length; **, $P < 0.01$ from the 115% of resting length.

Stretch(%)	Concentration (nmol/mg of protein) of	
	ATP	Phosphocreatine
115	21.6 $\pm$ 0.9	114 $\pm$ 10
70	15.9 $\pm$ 1.0**	79 $\pm$ 3**
100	19.2 $\pm$ 1.6	89 $\pm$ 8
70	13.7 $\pm$ 1.5*	68 $\pm$ 8

significantly lower after 2 h of incubation *in vitro* compared with those after 1 h of incubation (Table 4). ATP concentration in the uncut extensor digitorum longus muscle at resting length was significantly less than values obtained *in situ* after 2 h of incubation *in vitro* (Table 4). Concentrations of ATP and phosphocreatine were significantly lower after 2 h of incubation *in vitro* in the uncut soleus muscles that were at 70% of resting length than in contralateral paired muscles that were at 115% of resting length (Table 5). Likewise, ATP concentrations were significantly lower in the uncut soleus muscles at 70% of resting length as compared with the contralateral muscles at 100% of resting length after 2 h of incubation *in vitro* (Table 5).

In degradation experiments, comparisons were made *in vitro* between groups of muscles whose sole experimental variable was the amount of stretch placed upon them during incubation. One pair of muscles from a rat was divided into a non-manipulated control group and a treatment group. Muscles studied were quarter-diaphragms, soleus and extensor digitorum longus muscles. Rates of protein degradation between groups of paired muscles were identical when both groups were incubated at resting length during time period 1 (Table 6). At time period 2, treated muscles were stretched. No significant differences in protein-degradation rates existed between groups of stretched muscles and groups of muscles at resting

Table 6. *Effect of stretch on protein degradation in skeletal muscles*

Values are means $\pm$ S.E.M. Values in parentheses are expressed as a percentage of tyrosine release in time-period 1, with time-period 1 equal to 100%. There are 5-6 paired muscles at each time period with one pair at resting length while the other is stretched to 120% of resting length. Resting length *in vivo* was defined to be 100% in limbs with ankle and knee angles at approximately 120° and 115° respectively (Booth & Kelso, 1973). The duration of each time period was 50 min for the soleus and extensor digitorum longus muscles. For the quarter-diaphragm, the durations of time periods 1 and 3 was 30 min and of time period 2 was 60 min. Muscles were incubated in Krebs-Ringer bicarbonate buffer (pH 7.4) gassed with O₂/CO₂ (19:1) and the buffer contained 10 mM-glucose, 100 m-i.u. insulin/ml, 100 units penicillin/ml and 0.5 mM-cycloheximide (Fulks *et al.*, 1975). No significant differences were observed between muscles at resting and stretched length.

Muscle	Amount of stretch at time period 2	Time period	Tyrosine released (nmol/h per g muscle wet wt.)		
			... 1 (All muscles at resting length)	2 (One muscle stretched)	3 (All muscles at resting length)
Quarter diaphragm	Resting length		355 $\pm$ 39 (100)	322 $\pm$ 68 (91)	399 $\pm$ 73 (112)
	120% of resting length		369 $\pm$ 39 (100)	338 $\pm$ 37 (92)	313 $\pm$ 29 (85)
Soleus	Resting length		160 $\pm$ 9 (100)	115 $\pm$ 13 (72)	126 $\pm$ 16 (79)
	120% of resting length		140 $\pm$ 9 (100)	104 $\pm$ 5 (74)	109 $\pm$ 4 (78)
Extensor digitorum longus	Resting length		129 $\pm$ 6 (100)	83 $\pm$ 4 (64)	84 $\pm$ 6 (65)
	120% of resting length		125 $\pm$ 11 (100)	97 $\pm$ 18 (78)	107 $\pm$ 30 (86)

length at time period 1. At time period 3, treated muscles were returned to their initial length and no significant differences in protein-degradation rates were found between groups of these post-stretch muscles and their respective controls.

The above data conclusively show that 1 h periods of stretch do not significantly change rates of protein degradation *in vitro* in muscles from rats. Previously published data (Goldberg & Odessey, 1974; Goldberg *et al.*, 1974, 1975; Goldberg, 1979) disclosed a significant decrease in protein degradation *in vitro* in muscles that were stretched for 2 h. We therefore decided to perform further experiments in which muscles were stretched for longer than 1 h. Extending the period of stretching *in vitro* to 2 h did not result in any significant difference in tyrosine release between unstretched and stretched soleus or hemidiaphragm muscles.

Discussion

The results of the present study show that cutting of skeletal-muscle fibres alters the rates of both protein synthesis and protein degradation. It is unlikely that this finding invalidates earlier data about qualitative effects of hormones and other factors on protein metabolism in preparations with cut muscle fibres (for references, see introduction). However, the quantification of changes in the transfer of amino acids into and out of protein in response to hormones and other agents is probably incorrect for muscles with cut fibres. For example, in the present study, rates of protein degradation are 2–3 times the rates of protein synthesis in cut skeletal muscles (Tables 1 and 2). In contrast, rates of protein synthesis are nearly equal to rates of protein degradation in skeletal muscles without cut muscle fibres.

A possible explanation for the decline in the rate of protein synthesis after cutting muscle fibres may be related to the fall in the concentration of endogenous high-energy phosphates. For example, the requirement of multiple molecules of high-energy phosphate for each amino acid added to the peptide chain is well known (White *et al.*, 1978). On the other hand, the cause of the decline in the concentration of high-energy phosphates after skeletal-muscle fibres are cut is not known. However, declines in both ATP and phosphocreatine concentrations in skeletal muscles have been observed during respiratory and circulatory shock (Karlsson *et al.*, 1975; Bergström *et al.*, 1976; Chaudry *et al.*, 1976). According to one of these reports, not all of the decline in ATP concentrations of skeletal muscles could be attributed to hypoxia alone (Bergström *et al.*, 1976). It is possible that injury or insult to muscle produces an unknown sequence of

events which contributes to the decline in high-energy phosphates. Further studies are needed to resolve these issues.

The identity of the possible cause of the increase in the rate of protein degradation in slow-twitch muscle fibres after cutting is less certain than that of the potential reason for the decline in protein-synthesis rate. It is unlikely that the decline in ATP explains this change. Although high-energy phosphate concentrations declined in both soleus and extensor digitorum longus muscles after muscle fibres were cut, protein-degradation rates increased in the soleus muscle, but not the extensor digitorum longus muscle. A possible explanation for the differential response in the rate of protein degradation between these two muscles after cutting is that these muscles are different types of skeletal muscle with different rates of degradation (Millward, 1978). The soleus is a slow-twitch tonic muscle whereas the extensor digitorum longus is a fast-twitch phasic muscle (Ariano *et al.*, 1973).

Apparently other differences exist between the extensor digitorum longus muscle and the soleus muscle. For example, the intracellular concentration of tyrosine declines after cutting the extensor digitorum longus muscle, but not in the soleus. We do not know the etiology of these differences between slow- and fast-twitch muscles.

We chose tyrosine as a marker for rates of protein synthesis and degradation because tyrosine is neither synthesized nor oxidized by skeletal muscles (Fulks *et al.*, 1975; Guroff & Udenfriend, 1960; McGee *et al.*, 1972). Thus tyrosine transfer into protein represents an estimate of the rate of protein synthesis. Also, when cycloheximide is present during measurements, amino acid re-utilization in protein synthesis is blocked, and thus tyrosine release from protein is an estimate of the rate of protein degradation. Nonetheless, we must recognize that cycloheximide does lower the rate of protein degradation (Jefferson *et al.*, 1977).

One problem generated by the present study is the difference between our present results and those results reported previously (Goldberg & Odessey, 1974; Goldberg *et al.*, 1974, 1975; Goldberg, 1979; Kameyama & Etlinger, 1979). Whereas we observed that stretching soleus muscles *in vitro* did not change rates of protein degradation, others have reported that stretching muscles *in vitro* lowers rates of protein degradation (Goldberg & Odessey, 1974; Goldberg *et al.*, 1974, 1975; Goldberg, 1979; Kameyama & Etlinger, 1979). It is unlikely that the methodological difference between earlier and present studies is the explanation for the variation in the results between their findings and our findings. They gassed the atmosphere above the incubation medium only before the experimental period (Goldberg & Odessey, 1974; Goldberg *et al.*, 1974, 1975;

Goldberg, 1979; Kameyama & Etlinger, 1979), whereas we bubbled O₂/CO₂ (19:1) into the incubation medium throughout the experiment.

The thickness of intact skeletal muscle during incubation was inversely related to the concentration of ATP and phosphocreatine at the end of incubation. This suggests, but does not prove, that the fibres at the centres of the thicker muscles were more poorly oxygenated during incubation *in vitro*. Hill (1965) reported that the critical diffusion distance at which O₂ partial pressure in muscle becomes zero is 0.54 mm. In the soleus muscles used in the present experiments, muscles stretched to 115% of their resting length were about 1 mm thick, and those at 70% of their resting length were 2–3 mm thick. In the latter case, it is possible that fibres at the centre of the muscle might have low ATP and phosphocreatine concentrations, that fibres between the centre of the muscle and the peripheral muscle might have high ATP and low phosphocreatine concentrations and fibres at the periphery of the muscle would have high ATP and phosphocreatine concentrations. When all these concentrations are added together, the total for the whole muscle would be less than normal.

However, in contrast with the changes in ATP concentration, altering the thickness of intact muscles via stretching did not change the rate of protein degradation in these muscles during their incubation *in vitro*. This observation suggests that there is no direct relationship between moderate changes in ATP concentrations and protein-degradation rate. However, in a review of the literature, Goldberg & St. John (1976) concluded that metabolic energy is indirectly involved in the process of protein degradation in many types of cells.

The present study does not identify the factors responsible for the directional difference in the response of protein-degradation rate between stretching skeletal muscles *in vivo* (Turner & Garlick, 1974; Goldspink, 1977; Laurent *et al.*, 1978a,b) and *in vitro* (Goldberg & Odessey, 1974; Goldberg *et al.*, 1974, 1975; Goldberg, 1979). Although we hypothesized that muscles *in vitro* might have poorer oxygenation and thus lower ATP concentrations than *in vivo* and that this difference might account for the differences in the response of protein degradation to stretching, different ATP concentrations did not alter rates of protein degradation in the present experiments.

In summary, our present findings demonstrate that cutting skeletal-muscle fibres alters many aspects of muscle metabolism, especially high-energy phosphate concentrations, protein-synthesis rates and rates of protein degradation. Future interpretations of quantitative changes in certain aspects of muscle metabolism in preparations of cut muscle fibres will have to be made judiciously. We also

observed that, although concentrations of ATP and phosphocreatine were less in unstretched than in stretched intact soleus muscles after incubation, rates of protein degradation were similar in these two groups. This observation suggests that moderate decreases in ATP concentration do not alter rates of protein degradation of intact muscles *in vitro*.

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