# LOWERED PROPORTION OF POLYSOMES AND DECREASED AMINO ACID INCORPORATION BY RIBOSOMES FROM DENERVATED MUSCLE

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## 1. Introduction

Denervation of skeletal muscle usually leads to a progressive loss of muscle proteins. Measurements of the incorporation of labelled amino acids *in vivo* have suggested that the rate of protein synthesis in muscle is decreased by denervation (reviewed in [1]). A study of the rate at which labelled protein is replaced in denervated muscle has also indicated that a decrease in gross protein synthesis is associated with an increased rate of protein breakdown [2]. In other conditions, e.g. in diabetes and starvation, where protein synthesis in muscle is diminished it has been shown that isolated muscle ribosomes have a diminished capacity for amino acid incorporation and contain a lower proportion of polysomes [3–6]. This paper reports similar changes in ribosomes isolated from denervated gastrocnemius muscles of young rabbits.

## 2. Methods

Denervation of the gastrocnemius muscle was performed under ether anaesthesia on male New Zealand White rabbits at the age of seven weeks. The sciatic nerve was divided in the thigh on the right side leaving the left gastrocnemius as the control.

To prepare ribosomes [7] the muscle was minced and dispersed by blending for 30 sec in medium A (250 mM KCl–10 mM $MgCl_2$–20 mM Tris-HCl (pH 7.4)–5 mM β-mercaptoethanol), and the homogenate was centrifuged for 15 min at 12,000 *g*. The supernatant, after adding sodium deoxycholate (final conc. 1%) and lubrol (final conc. 0.5%) was layered over a discontinuous sucrose gradient (medium A containing 0.7 M sucrose in the upper layer and 2.0 M sucrose in the lower layer) and centrifuged for 16 hr at 80,000 *g*. The pellet of ribosomes was gently resuspended in 250 mM sucrose–100 mM KCl–5 mM $MgCl_2$–20 mM Tris-HCl (pH 7.4)–10 mM β-mercaptoethanol and used at once for measurement of amino acid incorporation and sucrose density gradient analysis.

The incorporation of [$^{14}$C]leucine was measured at 37° in the presence of saturating amounts of dialysed cell sap and pH 5 enzyme prepared from rabbit liver. The tubes contained in 125 μl: ribosomes 20 μg of RNA, liver cell sap (1 mg of protein), pH 5 enzyme (containing approx. 30 μg of tRNA), sucrose (125 mM), KCl (100 mM), $MgCl_2$ (5 mM), Tris-HCl, pH 7.4 (50 mM), β-mercaptoethanol (10 mM), ATP (1 mM), GTP (0.25 mM), phosphoenolpyruvate (5 mM), pyruvate kinase (5 μg), L-amino acids except leucine (0.1 mM), and L-[U-$^{14}$C]leucine (0.05 mM, 50 cpm/pmole). Under these conditions the incorporation of radioactivity into protein was linear for 15–20 min and was proportional to the amount of ribosomes added. Hot trichloroacetic acid-insoluble material was collected on glass fibre disks and the radioactivity was counted in a liquid scintillation system (efficiency approx. 75%).

To determine the proportion of polysomes, the ribosome suspension (1–2 mg/ml) was adjusted to 250 mM KCl and 10 mM $MgCl_2$, and 0.15 ml was layered over a 9 ml linear 15–40% sucrose gradient in 250 mM KCl–10 mM $MgCl_2$–20 mM Tris-HCl (pH 7.4). The gradient was centrifuged at 0° for 80 min at 30,000 rpm in the M.S.E. 3 × 10 swing-out rotor, and the distribution of ribosomes was determine by pumping the gradient from the bottom of the tube

through a 0.25 ml Gilford flow cell (light path 5 mm). The extinction at 260 nm was continuously recorded with a Gilford model 2000 recording spectrophotometer.

When free ribosomes were measured as 60 S and 40 S subunits, the suspension of ribosomes was adjusted to 300 mM KCl and treated with pancreatic RNAase (10 μg/ml, 10 min at 25°). This suspension was then layered over a 9 ml linear 5–20% sucrose gradient in 300 mM KCl–3 mM $MgCl_2$–10 mM Tris-HCl (pH 7.4)–1 mM dithiothreitol and centrifuged for 200 min at 30,000 rpm. The positions of 60 S and 40 S particles were determined by centrifugation under identical conditions of subunits prepared from liver ribosomes [8].

## 3. Results and discussion

In young animals denervated muscles may show little change in weight or may grow at a diminished rate [9, 10]. Gastrocnemius muscles denervated in rabbits aged 7 weeks decreased only slightly in weight but the amount and concentration of RNA and DNA in these muscles increased (table 1). An increase in the total amount of DNA following denervation has been reported [13], and in the special case of the denervated hemi-diaphragm net synthesis of both RNA and DNA is associated with hypertrophy [12].

The fact that the denervated muscles decreased in weight despite a net increase in RNA suggested that protein synthesis was inhibited. Table 2 shows the incorporation of [$^{14}$C]leucine into protein *in vitro* under conditions where the incorporation was proportional to the amount of ribosomes added. Compared with the controls, the incorporation by ribosomes from muscles 19 days and 32 days after denervation fell to 70% and 50%, respectively.

Since active ribosomes engaged in protein synthesis are associated with messenger RNA in the form of polysomes the proportion of free ribosomes to polysomes was studied. Fig. 1 shows that the proportion of polysomes decreased, and free ribosomes increased 19 days after denervation. This result was found to be independent of the method of preparing the ribosomes but could have resulted from an increased degradation of polysomes by RNAase in denervated muscle. Fig. 1 shows that polysomes were degraded to 80 S structures by added pancreatic RNAa and in other experiments homogenates of denervated muscle were found to have an increased RNAase activity. This may reflect the increased number of lysosomes present in muscle after denervation [14].

To overcome this objection the active ribosomes were measured after degrading the polysomes to 80 S ribosomes by mild RNAase digestion [15]. Active ribosomes retain nascent protein in the form of peptidyl-tRNA, and after mild RNAase digestion they appear as stable 80 S ribosomes when centrifuged on high ionic strength sucrose gradients, while ribosomes not engaged in protein synthesis dissociate into 60 S and 40 S subunits [16–18]. The data in fig. 2

Table 1
Rabbit gastrocnemius: wet weight and nucleic acid concentration.

| Time after denervation (days) | Condition of muscle | Gastrocnemius wet weight (g) | Nucleic acid concentration (μg/g wet weight) | |
|---|---|---|---|---|
| | | | RNA | DNA |
| 0 | N | 6.3±0.5 | 830±11 | 330±13 |
| 19 | N | 10.0±0.1 | 835±19 | 315± 4 |
| | D | 5.5±0.1 | 1310±73 | 395±12 |
| 32 | N | 13.3±0.4 | 930±25 | 325±5 |
| | D | 5.6±0.1 | 1770±21 | 860±16 |

Data (mean ± S.E.M.) for groups of three rabbits with the gastrocnemius muscle denervated at 7 weeks. N and D refer to normal (control) and denervated muscles, respectively. Individual muscles were taken for estimation of RNA [11] and of DNA [12]. Values for right and left muscles of unoperated rabbits were essentially the same. The weights (g) of the rabbits at 0, 19 and 32 days, respectively, were 1280 ± 33, 1630 ± 60 and 2070 ± 54.

Table 2
Incorporation of [$^{14}$C]leucine by ribosomes from normal and denervated muscle.

| Incubation time (min) | [$^{14}$C]leucine incorporated (pmoles) | | | |
|---|---|---|---|---|
| | *19 days* | | *32 days* | |
| | Control | Denerv-ated | Control | Denerv-ated |
| 10 | 24 | 17 | 26 | 11 |
| 20 | 41 | 29 | 42 | 19 |
| 40 | 59 | 39 | 51 | 23 |

Ribosomes (20 μg of RNA) were incubated as described in the text in the presence of saturating amounts of liver cell sap and pH 5 enzyme. The values (expressed as pmoles/20 μg of ribosome RNA) are averages of duplicates which agreed within 5%, and are corrected for the radioactivity (equivalent to 2 pmoles of [$^{14}$C]leucine) found with unincubated controls.

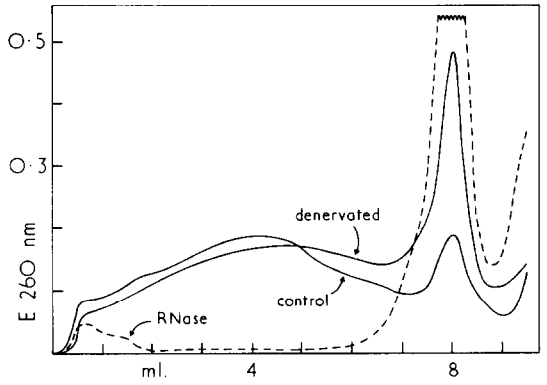


Fig. 1. Proportion of ribosomes and polysomes. Muscle ribosomes from animals 19 days after operation were fractionated on sucrose density gradients as described in the text. (– – –) Represents ribosomes from normal muscle after treatment with pancreatic RNAase (2 mg ribosomes and 10 μg RNAase/ml, 10 min at 25°). The bottom of the gradient is on the left.

confirm those in fig. 1. From 19 to 32 days after denervation there was a progressive increase in the proportion of subunits and a decrease in the 80 S ribosomes which represent active ribosomes originally associated with messenger RNA. From the areas under the curves the active ribosomes accounted for about 70% of the total ribosomes in normal muscle, but only about 50% and 35%, respectively, of the total in 19 day and 32 day denervated muscle.

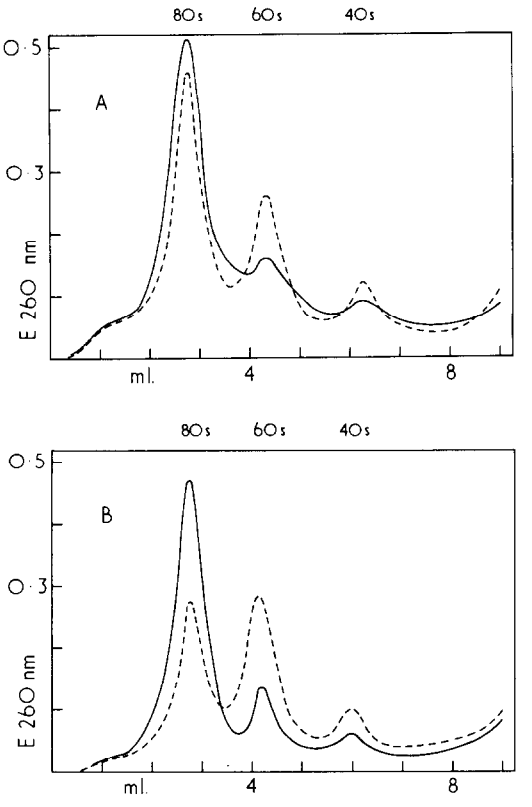


Fig. 2. Dissociation of free ribosomes into subunits. Muscle ribosomes were treated with RNAase and centrifuged on sucrose density gradients as described in the text. Ribosomes from denervated muscle are shown by the interrupted line. A) 19 days after operation; B) 32 days after operation.

The decrease in the proportion of messenger-associated ribosomes (approx. 50% after 32 days) was about the same as the decline in amino acid incorporation in table 2, suggesting that this decline was due mainly to the fall of the proportion of ribosomes in the form of polysomes. In this respect the denervated muscle of a young rabbit resembles certain non-growing as compared with growing cells [15, 19].

The low proportion of active ribosomes could have resulted from an intrinsic incapacity of part of the free ribosome population to attach to messenger RNA. This has been shown to be unlikely in several systems by demonstrating that low concentrations of cycloheximide which partially inhibit protein synthesis and slow down the movement of ribosomes over the messenger RNA cause a recruitment of free ribosomes

onto the polysomes [19–21]. Similar results were obtained with denervated muscle. Cycloheximide (5 mg/Kg body weight), sufficient to cause a partial inhibition of protein synthesis [22, 23], was administered by intraperitoneal injection 2 hr before killing the rabbits. Under these conditions the proportion of active ribosomes in the form of polysomes (measured as described in fig. 2) increased to about 90% in the control muscles and about 65% in muscle 32 days after denervation. This indicates that the free ribosomes in denervated muscle were potentially still capable of attaching to messenger RNA, and suggests that the decrease in polysomes was due primarily to a fall in the amount of messenger RNA or to some other limitation in the attachment of ribosomes to messenger.

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