THE EFFECT OF DISUSE ON NUCLEIC ACID CONTENT AND RNase ACTIVITY IN SKELETAL MUSCLE

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1. Introduction

The observations that skeletal muscle undergoes conspicuous weight loss induced by 'no use' have made it necessary to elucidate to what degree this change is involved in cell destruction, viz. metabolic changes.

The quantitative changes related to the immobilization of muscle have been described for protein [1]. It has been proved that the weight loss of atrophied muscle is due to the decrease of protein synthesis with simultaneous increase of protein catabolism. In these respects the biological systems regulating protein metabolism seem to be interrelated [2]. Plaster cast immobilization of limbs has a profound effect on the nucleic acid metabolism of skeletal muscle [3]. The synthesis of various proteins is not affected in the same way by injury. According to [4], in the case of muscular dystrophy, actin and myosin synthesis decreases but the synthesis of tropomyosin, troponin and the light chain of myosin remains unchanged.

Our experiments were designed to elucidate the shift in balance between the synthesis and degradation of muscle proteins. Thus we have investigated quantitative changes of the DNA and RNA of the muscle tissue and changes in the activity of enzymes involved in their metabolism.

2. Materials and methods

Experiments were performed using 3000 ± 200 g New-Zealand male white rabbits. The right hind limb was immobilized in full extension with a plaster cast [5]. In our experiments M. soleus and M. gastrocnemius were used after 7, 14 and 28 days of immobilization. After each treatment period the muscles of 4 animals were analysed. As controls the same muscles of 6 untreated animals were analysed.

Animals were exsanguinated, the muscles rapidly excised, freed from connective tissue, weighed and immediately processed.

One part of the muscle was frozen in liquid N_2 and the RNA and DNA extracted by Schmidt-Thannhauser and Schneider method adapted for the muscle [3]. The RNA content of the extract was determined with orcin as in [6]. The DNA content of the extract was determined with diphenylamine as in [7].

The other part of the muscle sample was used for measuring the RNase and RNase inhibitor. The muscle was chilled in a solution containing 0.25 M sucrose, 50 mM Tris–HCl, 25 mM KCl and 10 mM $MgCl_2$ (pH 7.6). The sample was then homogenized in 9 vol. solution of the same composition and the particulate and cytosol fractions were separated by the method in [8]. The alkali RNase activity and the RNase inhibitor activity were measured by the method in [9] as modified [10]. For quantitative determination of nucleic acids DNA–Na salt (isolated from calf thymus, Sigma, MO) and RNA (isolated from yeast, Serva, FRG) were used as standards. In RNase measurements the unit activity was defined by crystalline pancreatic ribonuclease (Reanal, Hungary).

3. Results

DNA concentration compared to the control showed a 3-fold increase by the 28th day (fig.1). But

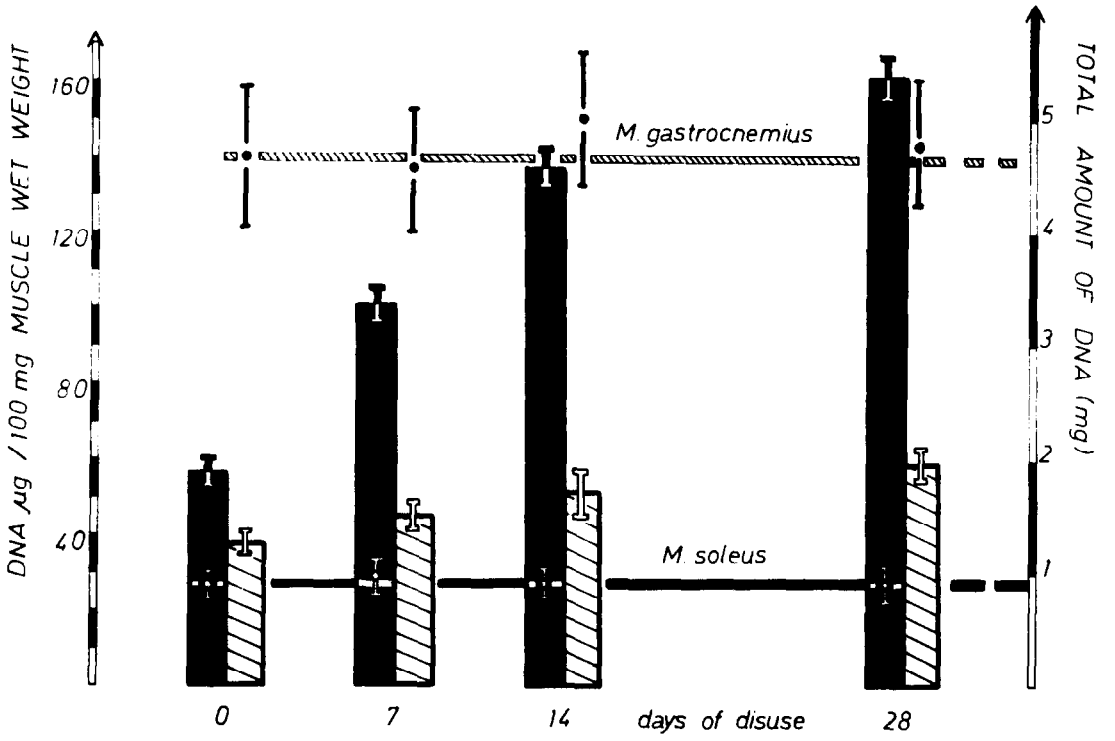

Fig.1. The effect of disuse on the DNA concentration and content of M. soleus and M. gastrocnemius.

if we consider the DNA content of the whole M. soleus and M. gastrocnemius, respectively, and take into account the decreased weight of the muscle [1], we get a nearly constant value. We may conclude that during immobilization the DNA was 'concentrated'. With M. gastrocnemius the DNA concentration does not increase as much as with M. soleus, but for the whole muscle DNA content we obtained nearly constant values, because with M. soleus weight decrease is also less.

With M. soleus we obtained nearly constant values for the RNA concentration (fig.2.). Here the RNA content of the whole bundle of muscle was also calculated showing a tendency to decrease. With M. gastrocnemius a slight increase of the RNA concentration can be encountered, but is not significant. The whole RNA content also decreases with M. gastrocnemius.

Supposing that the change in the RNA contents is due to increased RNase activity, the non-specific alkali RNase activity and the activity of the RNase inhibitor regulating its function have been measured.

The RNase activity was measured in the particulate fraction, and the activity of the RNase inhibitor in the cytosol fraction. One unit of RNase activity was defined as that giving the same ΔA increase as 2.5 ng crystalline pancreatic RNase at pH 7.8 and similar ionic strength. One unit of inhibitor activity was defined as that giving 50% inhibition of 2.5 ng crystalline pancreatic RNase, under these conditions [10].

We found that RNase activity increased with increasing immobilization time (fig.3A). The activity of RNase inhibitor found in the cytosol fraction to decrease with increasing immobilization with reference to the amount of the inhibitor (fig.3B). This result is in accordance with the definitions in [11] for the balance of the RNase and RNase inhibitor.

4. Discussion

By comparing the increase in DNA concentration

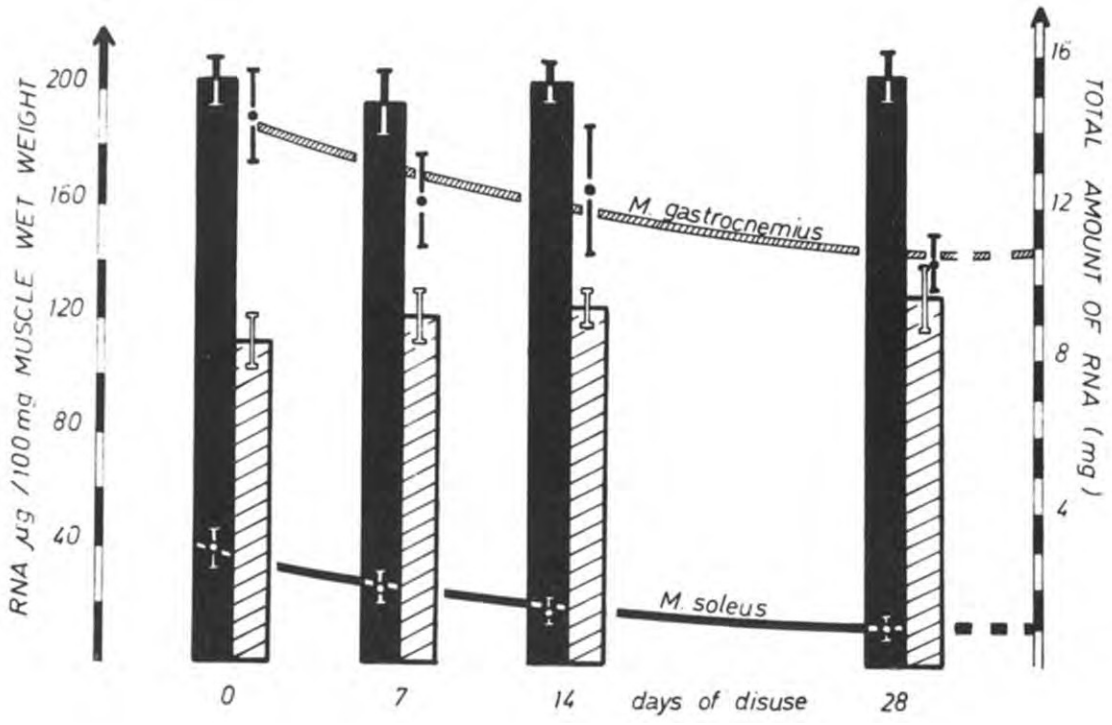

Fig.2. The effect of disuse on the RNA concentration and content of M. soleus and M. gastrocnemius.

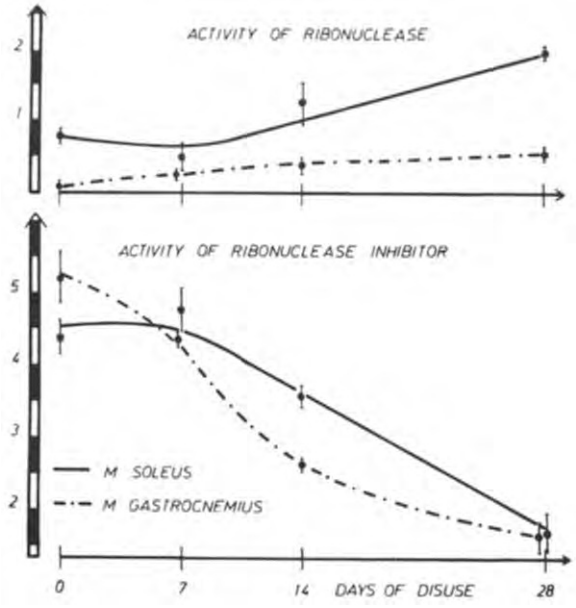

Fig.3. (A) The effect of disuse on the activity of RNase (units of activity/mg protein). (B) The effect of disuse on the activity of RNase inhibitor (units of inhibitor activity/mg protein). Units of activity were determined as in section 2.

with the decrease in weight encountered during immobilization we obtained constant values for DNA content. These findings indicate that during immobilization the genetic reservoir is not impaired with the decrease of proteins, and no considerable cellular death seems to occur during the 'no use' period.

The decrease is characteristic for the quantitative change of RNA. Our results indicate that this may be due to the increase of RNase activity, the quantity of the inhibitor decreases as proteolytic enzymes become more active during immobilization causing a shift in balance of RNase inhibitor, RNase and RNA.

The considerable loss of protein described is manifest in the decrease in muscle weight. This protein decrease results from the increased activity of proteolytic enzymes and some inhibition of protein synthesis. It may be assumed that immobilization plays no role in transcription regulation but that the increase of RNase activity may result in post-transcriptional damage of protein synthesis.

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