lysis and complement binding. The complement-fixation and transfer test (*15*) should help to quantitate the complement ability of the 7*S* subunits.

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Enhanced Protein Synthesis in a Cell-Free System from Hypertrophied Skeletal Muscle

Abstract. *Hypertrophy of the rat soleus muscle was induced by tenotomy of the synergistic muscles. Four days after the operation, the weight of the muscle had increased by 30 percent. The hypertrophied muscle had an increased concentration of DNA and RNA, when compared to the contralateral control soleus. Although the amount of myofibrillar protein increased during the 4-day period, the concentration of this component decreased. Microsomes prepared from hypertrophied muscle had an increased RNA concentration. The combined microsomes and pH-5 enzyme from hypertrophied muscle supported a faster rate of protein synthesis in vitro than the same system prepared from an equal weight of contralateral muscle which was used as a control.*

Hypertrophy of skeletal muscle in response to exercise indicates that protein metabolism in muscle is regulated by function. The hypertrophied heart in vivo (*1*) and the overloaded, perfused heart *in situ* (*2*) exhibit increased amino acid incorporation into protein. Kendrick-Jones and Perry (*3*) have reported an increased incorporation of amino acid into protein of an isolated frog muscle in response to repeated isometric contractions of the muscle. However, increased amino acid-incorporating activity has not been demonstrated in a cell-free system. Such a system should presumably be more amenable to biochemical studies.

We now describe initial studies of protein and nucleic acid metabolism in red skeletal muscle undergoing rapid compensatory hypertrophy induced by tenotomy of the synergistic muscles (see *4*).

We have compared some chemical characteristics of hypertrophied and normal muscle and shown that a cell-free system from hypertrophied muscle is much more active than the system from normal muscle in supporting incorporation of amino acid into protein.

The left gastrocnemius and plantaris tendons of male, Sprague-Dawley rats (250 to 300 g) were severed after intraperitoneal injection of chloral hydrate (35 mg per 100 g of body weight). A sham operation was performed on the contralateral limb. Four days after operation, the animals were killed by decapitation. The soleus muscle from each leg was dissected free. All procedures including killing of the animals were carried out at 4°C, unless otherwise stated. Muscles used for nitrogen and nucleic acid analysis were immediately frozen and pulverized in liquid nitrogen and then stored at −70°C until analyzed.

Paired groups of control and hypertrophied muscles were pooled to give sufficient quantities for analysis. Since histological examination of the muscles revealed a localized cellular infiltrate at the operative site in the hypertrophied muscles, RNA and DNA concentrations were also determined on the histologically normal, proximal segment of the hypertrophied muscles. Tissue nucleic acid was determined (*5*) after lipid extraction (*6*). The RNA determination was corrected for glucose (*7*). Dry weights were determined after lyophilization to constant weight.

Incorporation of labeled amino acid into protein by cell-free extracts was measured (*8*). After the incubation, protein was purified (*9*), and the radioactivity of the solubilized protein was determined (*10*) in a Packard Tri-Carb liquid-scintillation spectrometer. Protein (*11*) and RNA (*12*) content of the microsomes were determined (*11*). For the reason noted above, the concentrations of microsomal protein and RNA and the rates of the incorporation of amino acid in vitro were determined on fractions obtained from the whole muscle and from the proximal segment in the hypertrophied muscles. That the amino acids were incorporated into interior peptide chains was confirmed by dinitrophenylation (*8*). Free amino acids present after dinitrophenylation were determined by the method of Yemm and Cocking (*13*). The amounts of amino acid in whole muscle and in the subcellular fractions were also determined (*14*).

Four days after tenotomy the mean wet weight for solei from the tenotomized leg was 151 mg compared to 116 mg for solei from the control leg (Table 1).

The tissue nitrogen was fractionated into myofibrillar, sarcoplasmic, and stromal-protein nitrogen and nonprotein nitrogen on the basis of differential solubilities (*15, 16*). The nitrogen contents, determined by the micro-Kjeldahl method, of these fractions in five sets of paired hypertrophied and control solei (Table 1) show the concentration of nonprotein, sarcoplasmic, and stromal-protein nitrogen to be similar in both groups. The total tissue nitrogen and myofibrillar nitrogen concentration are both significantly lower in the hypertrophied muscle. From Table 1, it can be calculated that a hypertrophied muscle contains 2.32 mg of myofibrillar protein nitrogen per mus-

cle as compared to 2.11 mg per muscle for the controls. Although there was a net increase of 10 percent in the myofibrillar protein fraction, this increase was insufficient to maintain a normal concentration of this component in the hypertrophied muscle during the period studied.

In contrast, Helander (*16*) found that after guinea pigs were exercised for a long period there was an increase in the total nitrogen content and myofibrillar nitrogen fraction in the skeletal muscle. Meerson *et al.* (*17*) found an increase of the total nitrogen content and nonprotein nitrogen fraction in hypertrophied canine myocardium. However, Helander's animals were exercised for 100 days, and the dogs in Meerson's study were killed 2.5 years after surgical creation of aortic stenosis. It is difficult to compare such long-term experiments with our short-term experiments.

A statistically significant increase in the amount of RNA and DNA in the tissues was found in the hypertrophied muscle (Table 1). The RNA concentration increased by 21 to 50 percent and the DNA, by 24 to 30 percent. These results are similar to the 30-percent increase in RNA and DNA reported by Meerson in his long-term experiments with hypertrophied myocardium (*17*). Arutyunov reported an increase in nuclear size, nuclear DNA content, and nuclear DNA concentration in hypertrophied rabbit myocardium within 2 weeks after creation of surgical aortic stenosis (*18*). The amounts of DNA in rat skeletal muscle increase during the first 80 days of life (*19*). Related to the findings of Meerson and Arutyunov is the report that the degree of polyploidy is increased in hypertrophied human heart muscle (*20*).

To determine whether the hypertrophied muscle has an enhanced capacity for protein synthesis, the incorporation in vitro of radioactive amino acids into protein was measured (*8*). In each case, microsomes and *p*H-5 fractions were prepared from equal weights of hypertrophied and control solei. Experiments in which the radioactive protein was carried through the dinitrophenylation procedure demonstrated that less than 10 percent of the radioactivity was removed; the data in Table 2 and Fig. 1 have not been corrected for this factor.

The recombined microsomes and *p*H-5 fraction from hypertrophied muscles is considerably more active than the system prepared from control muscles (Fig. 1). Although not shown in the figure, the amino acid-incorporating system prepared from the proximal half of the hypertrophied muscle was also more active than its control. The muscle from the sham-operated control has about the same amino acid-incorporating activity as muscle from an unoperated animal.

The amino acid-incorporating systems from the hypertrophied and control muscle have the usual characteristics (Table 2).

L-Phenylalanine is also incorporated into protein at a faster rate by the hypertrophied muscle both in the absence and in the presence of polyuridylic acid (polyU).

The enhanced activity in hypertrophied muscle has been observed over a fivefold concentration of adenosine triphosphate (ATP) ($2 \times 10^{-4}M$ to $1 \times 10^{-3}M$), and over a 16-fold concentration of microsomal protein (0.06 to 1.0 mg of microsomal protein).

In order to identify the source of the greater activity in the hypertrophied muscle system, microsomes and *p*H-5 fractions from hypertrophied and control muscles were mixed. The increased activity resides almost exclusively in the microsomal fraction (Table 2, experiments 8 and 9).

Additional evidence pointing to the microsomes as the source of the increased activity comes from protein and RNA analysis of the microsomal fractions from the two types of muscle. Microsomal RNA is markedly increased in hypertrophied muscle, the

Table 1. Chemical composition of control and hypertrophied skeletal muscle.

Properties	Control	Hypertrophied	*n**	*P*†
Weight (mg)	116 ± 15	151 ± 21	20	< .001
Dry weight percentage	24	21.9	9	< .01
Total N‡	33.9 ± 0.78	30.1 ± 0.78	5	< .01
Nonprotein N‡	2.82 ± .11	2.32 ± .15	5	< .1
Sarcoplasmic protein N‡	5.10 ± .54	5.29 ± .14	5	< .4
Myofibrillar protein N‡	18.2 ± 1.0	15.3 ± 1.7	5	< .01
Stromal protein N‡	7.6 ± 0.85	6.9 ± 0.99	5	< .3
RNA-P§ (whole muscle)	111 ± 5.6	171 ± 14.9	5	< .01
RNA-P§ (proximal half)	112 ± 15.6	135 ± 19.8	7	< .01
DNA-P§ (whole muscle)	84 ± 5.4	109 ± 20.4	6	< .01
DNA-P§ (proximal half)	88 ± 5.7	109 ± 9.0	7	< .01

* *n*, Number of determinations; † Student's *t*-test for paired values; ‡ Nitrogen reported as milligrams per gram of wet tissue ± S.D.; § Nucleic acid reported as micrograms of RNA-phosphorus or DNA-phosphorus per gram of wet tissue ± S.D.

Table 2. Incorporation of labeled amino acids into protein by a soleus microsome-*p*H-5 precipitate preparation. Incubation tubes contained adenosine triphosphate (ATP), 10^{-3} mole/liter; creatine phosphate, 2×10^{-2} mole/liter; guanidine triphosphate, 10^{-4} mole per liter; 18 unlabeled amino acids, 10^{-6} mole/liter each; creatine kinase, 50 μg/ml; sucrose, 0.25 mole/liter; tris-HCl, 0.05 mole/liter, *p*H 7.8; $MgCl_2$, 0.01 mole/liter; KCl, 0.08 mole/liter; uniformly labeled L-leucine-C^{14}, 13.5 nmole (2μc) [or uniformly labeled L-phenylalanine-C^{14}, 13.5 nmole (2 μc)]; microsomes from 0.2 g muscle and *p*H-5 fraction from 0.2 g muscle in a final volume of 1.0 ml. Incubation for 15 minutes at 37°C with shaking.

Assay system	Amino acid incorporated per 0.2 g equivalent microsome-*p*H-5 fraction per 15 minutes	
	Hypertrophied (pmole)	Control (pmole)
L-Leucine-C^{14}, uniformly labeled		
1. Complete	3.48	1.90
2. Omit ATP	0.52	0.42
3. Omit ATP, creatine phosphate, creatine kinase	.55	.42
4. Complete plus 500 μg ribonuclease	.73	.66
5. Complete plus $10^{-3}M$ puromycin	.54	.53
6. Omit microsomes	.68	.51
7. Omit *p*H-5 fraction	.45	.29
8. Microsomes from hypertrophied muscle plus control *p*H 5 fraction	3.33	
9. Control microsomes plus *p*H-5 fraction from hypertrophied muscle		1.95
L-Phenylalanine-C^{14}, uniformly labeled		
10. Complete	2.25	1.40
11. Omit ATP	1.37	0.88
12. Add 200 μg polyU	43.70	10.00
13. Add polyU, omit ATP	1.10	0.81

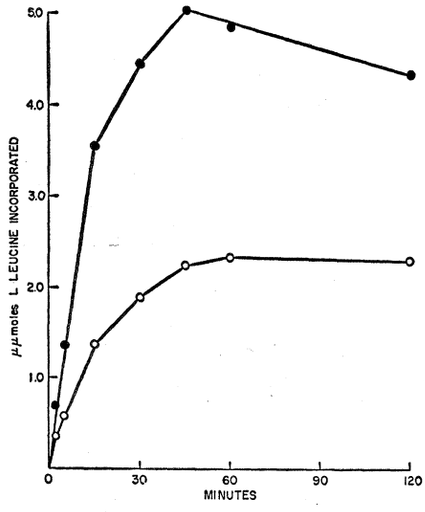

Fig. 1. Amino acid incorporation into protein by microsomes and *p*H-5 fraction from hypertrophied and control muscle. ○, control; ● hypertrophied. Microsomes and *p*H-5 fraction equivalent to 0.2 g of muscle, for both preparations. Assay system same as for Table 2.

average value for four preparations being 73 ± 11 μg of RNA per gram of hypertrophied muscle and 27 ± 4.3 μg of RNA per gram of control muscle. Protein in the microsomal fraction, on the other hand, is only slightly higher (1.8 ± 0.3 mg of protein per gram of hypertrophied muscle versus 1.7 ± 0.4 mg of protein per gram of control muscle). The corresponding values for RNA and protein concentrations in microsomes prepared from the proximal half of the hypertrophied muscle and its control are 41 μg of RNA per gram of hypertrophied muscle and 20 μg of RNA per gram of control muscle; and 1.5 mg of protein per gram of hypertrophied muscle and 1.4 mg of protein per gram of control muscle. These results indicate that the increased rate of protein synthesis in the hypertrophied muscle in vitro is due to an increase in the amount of ribosomes.

The increased activity observed with the system from hypertrophied muscle is probably not due to differences in the concentration of amino acids in the hypertrophied and control muscles. Concentrations of free valine, leucine, and phenylalanine were too low in the microsomes for determination by the method of Stein and Moore (*14*); there was less than 0.3 nmole of these amino acids per 0.34 mg of microsomal protein, which is the amount of the microsomes used in the amino acid-incorporation experiments. In the supernatant fraction obtained after centrifugation at 105,000*g*—the fraction from which the *p*H-5 enzyme is precipitated—the concentration of phenylalanine was identical for control and experimental muscle, whereas the concentrations of valine and leucine were greater in the hypertrophied muscle by about 20 percent.

Our results permit several tentative conclusions concerning the biochemical events leading to skeletal muscle hypertrophy. The higher rate of protein synthesis in vitro with the hypertrophied muscle indicates that the net increase in protein content of the hypertrophied muscle cannot be due solely to a decreased rate of protein degradation. The localization of the higher incorporating activity in vitro in the microsomal fraction is probably related to the higher RNA content of that fraction. It is known, however, that the increased activity in vitro is not due exclusively to increased amounts or availability of messenger RNA since the increase is still apparent, and even enhanced, in the presence of an artificial messenger, polyuridylic acid.

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Vertical Diurnal Migration and Endogenous Rhythmicity

Abstract. *Experimental studies of mixed populations of marine zooplankton have demonstrated that internal rhythms, synchronized by a light-dark cycle, are of dominant importance for the vertical migration of several species of crustaceans. For certain other organisms, the vertical migrations observed in the experiments can be accounted for as direct responses to light intensity only. Performances intermediate between these extremes were also observed, as well as behavior based on biological timing mechanisms that are not rhythms in the usual sense.*

The vertical diurnal migration of zooplankton represents one of the most conspicuous and widespread natural manifestations of daily rhythmicity that can be observed in animal activities. It is now well established for terrestrial organisms that internal biological rhythms are the primary mechanisms responsible for cyclic activity under field conditions, although concurrent environmental stimuli also play important roles in modifying that activity (*1*). The hypothesis that endogenous rhythmicity contributes to the phenomenon of vertical migration of plankton, however, has been largely neglected. The current viewpoint, as reflected in reviews and summaries of the subject (*2*), is that the animals are responding primarily, if not exclusively, to concurrent environmental stimuli, that is, that the rhythmic behavior is exogenous only. While this conclusion derives support from several lines of evidence, it has by no means been rigorously established.

Extensive laboratory studies leave no doubt that light intensity as well as other environmental factors such as temperature, hydrostatic pressure, and