Protein Synthesis in Skeletal Muscle, with Emphasis on Myofibrils*

R. E. WINNICK AND THEODORE WINNICK

From the Biochemistry Section, Weizmann Institute of Science, Rehovoth, Israel

(Received for publication, March 3, 1960)

Although many studies have dealt with the mechanism of muscular contraction and with the composition and structure of myofibrillar proteins, very little information exists as to the origin of these substances, or the manner in which they are assembled. Moscona (1), from the study of myogenic cells of the chick embryo, found indications that cytoplasmic granules may be the precursors of myofibrils. It is well known that protein synthesis in animals occurs more slowly in striated muscle than in such organs as liver. McLean et al. (2) have shown that in muscle, mitochondria are as active as microsomes in the incorporation of isotopic amino acids into protein. They also observed the labeling of myofibrils. It is possible that certain of the proteins synthesized by the particulate fractions of muscle may be subsequently integrated into the myofibrils. It may also be postulated that myofibrils can themselves synthesize proteins. In this connection, it is of interest that Perry and Zydowo (3) have isolated a ribonucleoprotein from myofibrils of rabbit and chicken skeletal muscle.

The incorporation of labeled amino acids into proteins of muscle has been measured under several physiological conditions. For instance, the uptake of glycine-$C^14$ into myosin of rabbit gastrocnemius muscle was found to decrease following nerve section (4). From the standpoint of the biosynthesis and inter-relationships of myofibrillar proteins, the only detailed research has been that of Velick (5). Using specific activity measurements with isotopic phenylalanine and tyrosine, he concluded that actin, H-meromyosin, and the glycolytic enzymes were derived from a common amino acid pool in skeletal muscle of the rabbit. Also there were indications that L- and H-meromyosins were independently synthesized and had different turnover rates. It appeared unlikely that tropomyosin was simply a precursor, or a form of myosin.

The present investigation deals with the rates of incorporation in vivo of radioactively labeled amino acids into proteins of myofibrils, mitochondria, microsomes, and the soluble (nonsedimentable) fraction of chick pectoral muscle. Experiments were also performed with labeled adenine, to obtain an indication of the metabolic activity of RNA in the myofibrils. The research is primarily concerned with the origin of the myofibrillar proteins.

**EXPERIMENTAL PROCEDURE**

*Isotopic Compounds*—DL-Valine-$1-C^4$ and DL-leucine-$1-C^4$ were obtained from the Radiochemical Centre, Amersham, England; adenine-$8-C^4$ was purchased from Schwartz Laboratories, Mount Vernon, New York. The compounds were used at indicated specific radioactivities and dosage.

**Animals**—Groups of 2- to 4-week-old chicks, closely matched in body weight, were given intraperitoneal injections of specified compounds. At different time intervals two birds of each group were killed, and their pectoral muscles were pooled. The chicks were not fasted beyond the first 3 hours after injection of labeled compounds.

**Isolation of Muscle Cell Components**—All preparative operations were done at $0^\circ$. The freshly excised muscle was quickly chilled and then homogenized with 0 parts of 0.1 m KCl containing 0.05 m Tris, pH 7.5 (6). This buffer did not cause agglutination of mitochondria and microsomes, as was reported for saline solutions with liver (7). Sucrose was not used, since it favors gel formation (8). Also, a slightly better yield of granules is obtained with KCl buffer, as compared to sucrose (3). The homogenization was performed with a loose fitting glass-Teflon Potter homogenizer, and then with a moderately tight apparatus. The resulting homogenate was centrifuged for 15 minutes at $600 \times g$. The supernatant phase was decanted, filtered through glass wool, and centrifuged for 20 minutes at $600 \times g$ to remove myofibrillar debris. The turbid liquid was then centrifuged for 25 minutes at $15,000 \times g$ to sediment the mitochondria, and lastly for 90 minutes at $80,000 \times g$ (Spinco ultracentrifuge, model L) to bring down microsomes. The pellets were drained free of liquid and the inside walls of the tubes were rinsed with KCl-Tris buffer. The particulate fractions were then suspended in water, and their proteins precipitated with an equal volume of 10% trichloroacetic acid. After centrifuging, the precipitates were washed twice with 5% trichloroacetic acid and twice with ethanol. A portion of the supernatant phase from the sedimentation of microsomes was likewise subjected to the above treatment for the isolation of protein.

The myofibrillar sediment from the initial centrifugation of the muscle homogenate was rehomogenized (without delay) for 3 minutes in a Waring Blender with 5 volumes of 0.025 m KCl-0.04 m borate pH 7.1, as employed by Perry and Grey (9). The procedure of these investigators was then used to purify the myofibrils. It was found advantageous to wash the final preparations on conical paper filters (Whatman No. 1) with KCl-borate buffer and gravity filtration, to further reduce contamination by mitochondria. A dilute suspension in water of a portion of myofibrils was stirred, while concentrated trichloroacetic acid

1 The term “protein,” used in connection with the different cellular fractions, includes nucleoproteins precipitated by cold trichloroacetic acid.
solution was added to a 5% concentration. The precipitated protein was then collected, and washed as in the case of the other cell fractions. In experiments with adenine-C\textsuperscript{14}, care was taken to maintain the myofibrils in trichloroacetic acid for at least 20 minutes at 0\(^\circ\)C, in order to remove bound nucleotide (10).

Treatment of Myofibrils with Sodium Deoxycholate—Operations were performed at 0\(^\circ\)C. One part of myofibrillar paste was suspended in about 5 parts of 0.05 M Tris pH 7.5, and an equal volume of 0.8% deoxycholate was added. After 10 minutes, 4 volumes of water were added, and the mixture was centrifuged for 15 minutes at 15,000 \(\times g\). The clear supernatant solution was removed, and protein was isolated from it by the method described for particulate fractions. In certain experiments, this supernatant solution was instead centrifuged for an additional 2 hours at 110,000 \(\times g\). The resulting pellets of nucleoprotein were then isolated, rinsed with water, and subjected to analysis.

Fractionation of Myofibrillar Proteins with Potassium Chloride—The procedure of Perry and Zydowo (11) was used to yield three fractions: (a) proteins insoluble in cold KCl-pyrophosphate buffer; (b) actomyosin, extracted by KCl and precipitated by dialysis; and (c) “extra protein,” also extracted, but not precipitated by dialysis.

Radioactivity Measurements—Replicate samples (5 to 15 mg) of each protein preparation (suspended in ethanol) were dried as uniform layers on tared steel planchets, with a heat lamp. The radioactivities were measured in the gas flow Geiger counter and corrected to zero layer thickness. The exact distribution of C\textsuperscript{14} in protein and nucleic acid fractions was not determined by analysis of hydrolysates in the present work. However, it has been shown that the administration of either isotopic valine or leucine to animals leads to labeling of liver proteins with only these amino acids, and no secondary radioactive components are found (12). Also, it is well established (13) that labeled adenine gives rise to labeling in only adenine and guanine of various tissue nucleic acids. Hence, it seems permissible to express the observed radioactivity values of muscle proteins in terms of \(\mu\)moles of purines or of leucine or valine, by reference to standard activities of the injected compounds.

Nucleic Acid Analysis—The RNA content of various protein fractions was determined by the orcinol procedure of Drury (14), following a 20-minute extraction at 70\(^\circ\)C with 3.5% perchloric acid. Close agreement with these results was obtained when RNA was calculated from spectrophotometric measurements at 260 nm on the same perchloric acid extracts, with the use of the factor 33.1 to convert optical density to \(\mu\)g of RNA per ml (15).

The DNA content of myofibril preparations was determined by the method of Webb and Levy (16).

**RESULTS AND DISCUSSION**

The myofibril preparations were rather uniform in appearance when examined in both the phase contrast (light) and the electron microscope. Occasional nuclei were present, but granules were rare. The myofibrillar protein contained approximately 0.6% RNA, when analyzed by either ultraviolet spectroscopy or an orcinol method (Table I). This value is in good agreement with that reported by Perry and Zydowo (11). Our myofibril preparations, however, contained from 0.2 to 0.3% DNA, which is about twice the value found by the above investigators (3). Assuming an RNA:DNA ratio of 0.1 to 0.2 for chick muscle nuclei (17), it seems unlikely that contaminating nuclear RNA accounted for more than a minor proportion of the RNA retained in the isolated myofibrils.

Viewed in the electron microscope, the mitochondria contained both large and smaller granules, whereas the microsomes appeared fairly homogeneous. In neither case were myofibrils nor cellular debris present. In appearance, the preparations resembled the corresponding fractions of liver tissue. Information comparable to that available for endoplasmic reticulum of liver is lacking in the case of sarcoplasmic reticulum. Perry and Zydowo (3) found that the bulk of the granules of rabbit skeletal muscle sedimented in 2 hours at 15,000 \(\times g\). In the present procedure, patterned after that of Simpson and McLean (18), approximately equal quantities of mitochondria and microsomes (each corresponding to about 120 to 150 mg of protein per 100 g of muscle) were obtained after 15,000 and 80,000 \(\times g\). Certain investigators, including Kitayakara and Harman (19), have stressed the heterogeneity of muscle mitochondria. However, a series of mitochondrial subfractions did not differ markedly in activity in the amino acid incorporation process (2).

In rabbit striated muscle, the RNA amounts to only about 0.4% of the total protein plus RNA (3), as compared to 5% in (rat) liver tissue (20). However, chick muscle microsomal protein contained 10.4% RNA (Table I), about the same value as that of rat liver microsomes (20). The protein of the muscle mitochondria had a lower RNA content, 4.8%.

It was found that labeled adenine was incorporated to only a very limited extent into the subcellular fractions of chick skeletal muscle (Table I), and that a considerable loss of isotope occurred between 6 and 20 hours. The highest concentrations, at 6 hours, were in proteins of mitochondria and microsomes. However, myofibrils comprise more than 50%, and soluble protein about 40%, of the total muscle cell protein. Accordingly, it can be calculated that each of these fractions accounted for approximately 40% of the incorporated C\textsuperscript{14}. This conclusion may be compared to the finding that the ribonucleoprotein component of myofibrils contains about 40% of the RNA of rabbit or hen skeletal muscle (3).

From the RNA content of the protein fractions in Table I, the specific activities per g of RNA were calculated. On this basis, soluble RNA had the highest C\textsuperscript{14} concentration; mitochondrial and myofibrillar RNA were intermediate; whereas microsomal RNA was lowest in activity, at both 6 and 20 hours. It is now well established (21) that the turnover rate of RNA is highest in the soluble nucleoprotein fraction of such tissues as liver and pancreas, and it has been suggested that an inverse relationship

<table>
<thead>
<tr>
<th>Fraction isolated</th>
<th>Purine-C\textsuperscript{14} incorporated</th>
<th>RNA content of proteins</th>
<th>Purine-C\textsuperscript{14} incorporated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 hours</td>
<td>20 hours</td>
<td>%</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>0.0133</td>
<td>0.0049</td>
<td>4.8</td>
</tr>
<tr>
<td>Microsomes</td>
<td>0.0172</td>
<td>0.0061</td>
<td>10.4</td>
</tr>
<tr>
<td>Soluble protein</td>
<td>0.0211</td>
<td>0.0008</td>
<td>0.21</td>
</tr>
<tr>
<td>Myofibrils</td>
<td>0.0015</td>
<td>0.0001</td>
<td>0.09</td>
</tr>
<tr>
<td>15,000 g supernatant of deoxycholate-treated myofibrils</td>
<td>0.0046</td>
<td></td>
<td>2.05</td>
</tr>
</tbody>
</table>
exists between rates of RNA renewal and protein synthesis (22). Our data on muscle are not adequate to examine this latter generalization, but a moderate rate of amino acid incorporation into myofibrils was anticipated and found, as will be pointed out presently.

The figures for myofibrils in Table I do not exclude radioactivity due to DNA of contaminating nuclei, nor was the isolation of nuclei undertaken in the present research. However, some additional information on the RNA of myofibrils was obtained by the use of deoxycholate. This reagent was found to extract about one-half of the RNA and 15% of the protein. It may be seen that the deoxycholate extract of myofibrils (Table I) was enriched more than 3-fold in both RNA and purine-C\textsuperscript{14} concentration. Upon further analysis, this fraction was found to have a DNA content of less than 0.1%.

The initial experiments on amino acid incorporation into muscle cell components were made with a tracer level dosage of leucine-C\textsuperscript{14} (Fig. 1). The early phase of the process resembles that found (2) after 5 minutes in rat muscle: microsomal and mitochondrial proteins were highest; soluble protein had a considerably lower activity, followed closely by myofibrils. The C\textsuperscript{14} concentration in the particulate fractions of Fig. 1 passed through a maximum at approximately 2 hours, and subsequently declined slowly. On the other hand, soluble protein and myofibrils reached maximal radioactivity at about 3 hours, and then did not change appreciably up to at least 12 hours. It is generally considered that precursor-product relationships require a crossing over of specific activity curves. This condition was not realized in Fig. 1, and the data cannot be taken as evidence that myofibrillar protein and soluble protein are derived from that of the muscle granules. However, results to be presented later in this paper (Fig. 4) suggest such a possibility.

It was of interest to determine the relative concentrations of labeled leucine in proteins of myofibrils, partially fractionated by the salt extraction procedure of Perry. The analyses of material taken from the preceding experiment are shown in Fig. 2. Highest radioactivities were consistently found in "extra protein" and the lowest values in actomyosin, whereas proteins not extracted by KCl-pyrophosphate buffer were intermediate in C\textsuperscript{14} concentration. The extra protein has been resolved (11) by chromatography into four subfractions: sarcoplasmic proteins, globulin plus water-soluble component, troponyosin, and ribonucleoprotein.

When another labeled amino acid, valine, was used at a much higher level (on a molar basis) and with somewhat younger birds (Fig. 3), the radioactivity curves of microsomal and mitochondrial proteins did not have sharp peaks as in the leucine experiment, but remained virtually horizontal after maximal activity was reached. The curves for soluble and myofibrillar proteins had the same forms as in Fig. 1. No conclusions could be drawn from this data as to the origin of the labeled proteins of the myofibrils. The high degree of incorporation of isotopic amino acid in Fig. 3, as compared to that with the tracer dose of leucine, may be noted.

Table II shows that the extra protein fraction of valine-labeled myofibrils had the highest C\textsuperscript{14} concentration, and actomyosin the lowest radioactivity, as in the leucine experiment.

In seeking further clues to the origin of myofibrillar proteins, a procedure was used which permitted the abrupt interruption of the amino acid incorporation process in particulate fractions of muscle. This was achieved by flooding the birds with a large quantity of a nonisotopic amino acid at a time when the corresponding labeled compound was being rapidly utilized for protein synthesis. Under these conditions of great isotopic dilution, it would be expected that myofibrils would not increase significantly in C\textsuperscript{14} concentration if they were dependent upon a supply of...
of labeling only slightly (if at all), as compared to the glucose-
saline control chicks. The results suggest that the myofibrils
continued to receive radioactive valine in a conjugated form
after the supply of free valine-\(^{14}C\) was rendered ineffective by
isotope dilution. If a transfer of protein from particulate frac-
tions occurred, it must have been extensive in magnitude to
account for the observed rise in specific radioactivity of the much
more abundant myofibrillar protein. A loss in isotopic concen-
tration in the microsomal and mitochondrial proteins might even
have been expected, although it could be argued that such a
decrease was masked by a continuing synthesis before the flood-
ing dose of L-valine became effective. The use of intermediates
such as RNA-bound amino acids cannot be easily invoked to
explain the results, unless there were evidence that these con-
jugates were utilized preferentially by myofibrils.

As already mentioned, the labeling of muscle nuclei was not
determined, so that the possibility cannot be excluded that
myofibrillar protein was also derived from this source.

The slight difference in slopes between the two branches of the
myofibril curve in Fig. 4 (if valid) does imply that protein syn-
thesis can occur to some extent within the myofibrillar structure,
and the suggestion has been recently made (11) that the ribo-
nucleic acid component may govern this process. Accordingly,
it was of interest to examine an RNA-protein fraction of a deoxy-
cholate extract of labeled myofibrils, separated by ultracentrif-
ugation. Myofibrils from the 1.75 hour experiment in Fig. 4
were treated as described in “Experimental.” The 110,000-g
pellet represented less than one-half of the initial RNA, and
only a minor fraction of the total protein of the myofibrils. The
RNA comprised only 14.3% of the nucleoprotein, whereas the
purified chromatographic preparation of Perry and Zydowo (3) had
50% RNA. However, the present material had a specific activ-
ity of 0.39 \(\mu\)moles of valine-\(^{14}C\) per g of protein, a value almost
4 times higher than that of the corresponding original myofibrils.

It seems likely from the fractionations with KCl buffer and
with deoxycholate that, even in relatively short time intervals,
the labeled amino acids were incorporated into all the different
protein species of the myofibrils. It is not clear whether this
process reflects the formation of new myofibrils, or primarily an
eelongation or thickening of existing ones in the fiber bundles.
It is generally considered that the granules are in intimate contact
with the myofibrils; although the former constitute only a small
part of the muscle cell substance, they still may supply a con-
siderable proportion of the matrix proteins of myofibrils, as well
as the sarcoplasmic enzymes, in the growing animal.

The exact location of the ribonucleoprotein in relation to the
myofibrillar structure is not known (3), and although its per-
centage is small, its metabolic activity may be of importance.
Further experiments in vitro may contribute toward a better
understanding of the role of myofibrils in relation to protein
synthesis in muscle.

**SUMMARY**

The rates of incorporation of labeled adenine into RNA, and
of \(^{14}C\)-labeled amino acids into the protein of skeletal motor cell
fractions in the chick, were determined under various conditions.
The results supported the view that a considerable proportion of
the myofibrillar proteins were derived from the mitochondria and
microsomes. However, the possibility that myofibrils them-
1 It was felt that this type of control was less complicated, from
the standpoint of interpretation, than the administration of
amino acids other than valine. In no case was there evidence of
injury or toxicity from the large dosages, but the fluids were only
partly absorbed from the peritoneal cavity, even after the addi-
tional 2-hour interval.

---

**Table II**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Radioactivity ((\mu)moles of valine/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extra protein</td>
<td>0.72</td>
</tr>
<tr>
<td>Protein insoluble in KCl-pyrophosphate buffer</td>
<td>0.52</td>
</tr>
<tr>
<td>Actomyosin</td>
<td>0.40</td>
</tr>
</tbody>
</table>

**Fig. 4.** Interruption of labeled valine incorporation into muscle cell fractions by massive injections of nonlabeled valine. Twelve birds (125 to 130 g body weight) were each injected at zero time with 14 \(\mu\)moles of valine-\(^{14}C\) (0.3 \(\mu\)c per \(\mu\)mole) per 100 g. After 0.75 hours, four birds were killed. At this same time, one-half of the remaining birds were injected with 1.7 \(\mu\)moles of L-valine (solid symbols) and the other half with 50 mg of NaCl plus 100 mg of glucose per 100 g (open symbols). Two chicks of each series were subsequently killed at the indicated times.

---

**Fig. 5.** Analysis of the myofibrils from the 7+ hour experiment of Fig. 3 was used.
$^{35}$S-labeled myofibrils was shown to have an augmented concentration of isotopic amino acid.

Acknowledgment. The aid of Dr. D. Danon in electron microscopic examination of cytological fractions of muscle is appreciated.

REFERENCES
Protein Synthesis in Skeletal Muscle, with Emphasis on Myofibrils
R. E. Winnick and Theodore Winnick


Access the most updated version of this article at
http://www.jbc.org/content/235/9/2657.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/235/9/2657.citation.full.html#ref-list-1