The Synthesis of Myosin, Actin and the Major Protein Fractions in Rabbit Skeletal Muscle

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New Zealand White rabbits were infused with [^3H]tyrosine for periods of 5–6 h and then different methods of extraction were applied for the purification of the main muscle proteins and protein fractions. Myosin (I), prepared from salt extraction of muscle mince, consistently had a higher specific radioactivity than did myosin (II), isolated by dissociation of actomyosin. Actins (IA) and (IB), extracted from acetone-dried powders prepared by different treatments of myosin-extracted muscle mince, gave specific radioactivities approx. 0.6 that of myosin (I) and 0.7 that of myosin (II). Actin (II), isolated by dissociation of actomyosin, had a specific radioactivity similar to that of myosin (II) from the same source, but higher than those of actins (IA) and (IB). The differences between the specific radioactivities of the proteins, in particular actin, purified by the various methods, are attributed to the loss of newly synthesized material of high specific radioactivity during the initial extraction procedures. It is suggested that actin (II) and myosin (II) are representative preparations for the total population of each protein and that, on this basis, myosin and actin have similar rates of synthesis. Total muscle protein, myofibrils, actomyosin and sarcoplasm were all found to have very similar specific radioactivities at the end of a 6 h infusion.

Skeletal muscle is the most extensive tissue in the body and its major structural feature, the myofibril, accounts for 35–40% of the total protein in an adult animal. The magnitude of this contribution makes a knowledge of the rate and manner by which this organelle is synthesized, assembled and degraded necessary for the understanding of the processes involved in the control of both growth and homeostasis. Evidence from several studies of the turnover of individual proteins of the myofibril has been contradictory. Thus, if comparisons are restricted to the main proteins myosin and actin, then there are reports that suggest that their fractional rates of synthesis are similar if not identical (Bidinost, 1951; Zak *et al.*, 1971; Perry, 1974); that actin is synthesized more slowly than myosin (Velick, 1956; Swick & Song, 1974; Koizumu, 1974; Zak *et al.*, 1977); or that the reverse is the case (Low & Goldberg, 1973). Recently the resolution of this problem has become even more important with the introduction of urinary elimination of N^T -methylhistidine as a measure of muscle catabolism in humans (Bilmazes *et al.*, 1978) and in animals (Harris & Milne, 1978). N^T -Methylhistidine is found almost exclusively in muscle (Haverburg *et al.*, 1975), where it is confined to the polypeptide chains of myosin and actin and, at

present, its use as an index of catabolism assumes that myosin and actin are degraded at the same rate and, further, that this is similar to that for the other proteins of muscle. If myosin and actin have rates of turnover that are dissimilar and/or are different from the rates of catabolism of the other muscle proteins, then the use of this technique would be limited.

The measurement of protein-synthesis rates by incorporation of labelled amino acids involves special difficulties when applied to the isolation of proteins from highly ordered structures, such as the myofibril. The present study was undertaken, therefore, to examine what effect, if any, different methods of isolation of myosin and actin had on their apparent rates of synthesis. In addition, the average specific radioactivities of the proteins in the major muscle fractions were compared.

Experimental

Animals

New Zealand White rabbits from the Rowett Research Institute colony were individually caged and fed *ad lib* from weaning on a pelleted semisynthetic diet (Nicholas *et al.*, 1977). Animals were studied at ages from weaning (500 g) to early maturity (3.5 kg), and both sexes were used.

Abbreviations used: SDS, sodium dodecyl sulphate.

(a) Trayer & Perry (1966); 15 mM-2-mercaptoethanol was added to all solutions.

(b) Carsten & Monmaerts (1963).

(c) Johnson *et al.* (1967); 0.2 mM-ascorbate was replaced by 0.2 mM-dithiothreitol.

(d) Serradarian *et al.* (1967); Scheme II; 0.2 mM-ascorbate was replaced by 0.2 mM-dithiothreitol.

(e) Perry & Zydowo (1959); 15 mM-2-mercaptoethanol was added to all solutions.

(f) Schaub & Perry (1971); the precipitate of natural actomyosin was allowed to settle for 18 h, then collected by centrifugation at 3000g for 20 min.

(g) Detailed in the Experimental section.

Scheme 1. Extraction of muscle protein fractions and myosins (I) and (II) and actins (IA), (IB) and (II). All procedures were carried out at 4°C.

Materials

L-[side chain-2,3-³H]Tyrosine (22 Ci/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks. ATP (sodium salt) was obtained from the Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan. Other materials were AnalaR-grade chemicals or the purest commercially available grade.

Constant infusion of [³H]tyrosine

The procedure adopted was that described by Nicholas *et al.* (1977). Total known dosages, in the range 0.3–1.0 mCi/kg body wt., were infused via a peripheral ear vein for 5 or 6 h.

Protein separation

Immediately after termination of the infusion the skeletal muscles of the back and hind limbs were excised, minced and stored on ice at 4°C. This mince of mixed muscle was then used as the starting material for all subsequent protein purifications, details of which are shown in Scheme 1.

Light- and heavy-chain fractions of myosin

Myosin was dissociated into light- and heavy-chain fractions with 5 M-guanidine hydrochloride as described by Perrie & Perry (1970).

Dissociation of natural actomyosin [actin (II) and myosin (II)]

The pellet of natural actomyosin was dispersed with a hand homogenizer in 10 vol. of 0.6 M-KCl/5 mM-TrisATP/5 mM-MgCl₂/0.2 mM-dithiothreitol/0.1 M-phosphate buffer, pH 7.0, to produce a 'saturated' solution of actomyosin, which was clarified by centrifugation at 40 000g for 1 min. The supernatant was centrifuged for 3 h at 75 000g, and the pellet of actin was then removed and analysed on SDS/polyacrylamide gels. Usually only a single band, corresponding to actin (II), was present, but if traces of other material were found, then a repeat treatment with the dissociation buffer, as above, was sufficient to yield a pure product. The supernatant was diluted to 1/0.04 and centrifuged at 3000g for 30 min. The sediment was then suspended in 5 vol. of 0.28 M-KCl and centrifuged at 3000g for 30 min. The supernatant was then diluted to 1/0.04 and the precipitated myosin (II) removed by centrifugation at 3000g for 20 min.

Sarcoplasm

The supernatant from the first extraction of muscle mince in 0.025 M-KCl/0.039 M-borate, pH 7.0, used in the preparation of myofibrils was centrifuged at 130 000g for 1 h. The supernatant contained the proteins of the sarcoplasm, and this was removed and treated as detailed below.

Analytical techniques

Total muscle extracts and individual proteins were treated, hydrolysed and subjected to ion-exchange chromatography to isolate the peptide-bound radioactive tyrosine as described by Nicholas *et al.* (1977). The pooled fractions were assayed for tyrosine content with a Locarte amino acid analyser. Portions (2.5 ml) were added to 10 ml of liquid scintillator (NE 260; Nuclear Enterprises (G.B.) Ltd., Edinburgh, Scotland, U.K.) and the radioactivity measured on a Beckman LS-345 liquid-scintillation spectrometer. Corrections for quenching were applied from standard curves prepared against the 0.2 M-citrate buffer, pH 4.25, used in the chromatographic procedure.

A 7% (w/v) analytical SDS/polyacrylamide-gel system based on that of Weber & Osborn (1969) was used, with the replacement of the 0.1 M-phosphate buffer with a solution of 2.5% Tris, 2.5% boric acid and 0.1% SDS (all w/v).

Results

Comparison of myosin extractions

The electrophoresis patterns of myosin (I) and (II) were very similar, and occasional traces of actin were removed by additional actomyosin precipitation at 1/0.28. There were slight but significant differences in the specific radioactivities of the two preparations, with myosin (I) always the greater (Table 1). These could not be attributed to the presence of the ribonuclear fraction, since, after removal of this contaminant by chromatography on DEAE-Sephadex A-50 (Richards *et al.*, 1967), no change in the specific radioactivity of either type of preparation was detected. Neither could large differences between the specific radioactivities of the heavy and light chains of either myosin (I) or (II) account for the result, because after dissociation of the heavy and light chains of myosin (I) from two animals (nos. 15 and 16), the specific radioactivity ratio between the two respective fractions was only 1.05 and 1.07. Similarly a preparation of myosin (II) from rabbit no. 16 gave a specific radioactivity ratio for heavy to light chains of 0.96.

Comparison of actin extractions

The methods of Carsten & Mommaerts (1963) and Seraydarian *et al.* (1967) are both based on extraction with ATP of an acetone-dried preparation from minced muscle. Actins (IA) and (IB) exhibited a single band on electrophoresis with sample loadings up to 60 µg, and both preparations had specific radioactivities that were considerably less than those of the myosins isolated from the same muscle mince (Table 2). On average the ratio of the specific radioactivities of actin (IA) and (IB) to the corresponding preparations of myosin (I) was 0.6, and, although there was

Table 1. *Specific radioactivities of myosins (I) and (II)*

The myosins were prepared and treated as described in the Experimental section. The ratios ($\times 100$) of specific radioactivities for the comparable preparations are given ± 1 s.d. Probabilities for differences in treatment were analysed by Student's *t*-test for pairs.

		Specific radioactivity of isolated tyrosine (d.p.m./nmol)													
Rabbit no.	...	8	9	10	13	14	15	16	34	47	50	57	59	66	
Myosin (I)		9.64	5.03	5.21	6.99	4.42	1.12	4.34	2.84	5.63	12.41	12.65	3.28	9.80	
Myosin (II)		9.09	4.23	4.66	5.53	4.16	1.00	3.66	2.68	5.51	10.44	11.40	3.01	9.58	

$100 \times [\text{Myosin (I)/myosin (II)}] = 111.1 \pm 7.4$ (13); by *t*-test, $P < 0.01$.

Table 2. *Specific radioactivities of myosin (I) and actins (IA) and (IB)*

Myosin (I) and actins (IA) and (IB) were isolated and treated as described in the Experimental section. The ratios ($\times 100$) of specific radioactivities for comparable preparations are given ± 1 s.d. Probabilities for differences in treatment were analysed by Student's *t*-test for pairs.

		Specific radioactivity of isolated tyrosine (d.p.m./nmol)								
Rabbit no.	...	7	9	10	14	17	34	47	50	66
Myosin (I)		0.53	5.03	5.21	4.42	7.32	2.84	5.63	12.41	9.80
Actin (IA)		0.33	3.56	3.16	2.91	3.02	1.19			
Actin (IB)								3.57	8.56	5.39

$100 \times [\text{Actin (IA)/myosin (I)}] = 57.1 \pm 12.5$ (6); by *t*-test, $P < 0.02$.

$100 \times [\text{Actin (IB)/myosin (I)}] = 62.3 \pm 7.0$ (3); by *t*-test, $P < 0.02$.

Table 3. *Specific radioactivities of natural actomyosin, myosin (II), actin (II) and actin (III)*

Natural actomyosin, myosin (II) and actin (II) were isolated and treated as described in the Experimental section. Actin (III) was prepared as described in the Results section and treated by the same procedures as the other proteins. The ratios ($\times 100$) of specific radioactivities for comparable preparations are given ± 1 s.d. Probabilities for differences in treatment were analysed by Student's *t*-test for pairs.

Rabbit no.	Specific radioactivity of isolated tyrosine (d.p.m./nmol)										
	...	9	14	15	16	47	52	59	60	66	76
Natural actomyosin		4.64	4.82	1.00	3.99	5.62	6.93	3.39	24.01	9.37	3.31
Myosin (II)		4.23	4.16	1.12	3.66	5.51	6.83	3.01	22.50	9.58	3.44
Actin (II)		4.47	5.16	0.84	3.38	5.77	6.90	3.38	22.33	9.65	3.28
Actin (III)								2.48	17.70	3.39	1.43

$100 \times [\text{Myosin (II)/natural actomyosin}] = 96.6 \pm 7.9$ (10); by *t*-test $P < 0.10$.

$100 \times [\text{Actin (II)/natural actomyosin}] = 96.9 \pm 7.6$ (10); by *t*-test, $P > 0.30$.

$100 \times [\text{Actin (II)/myosin (II)}] = 101.0 \pm 12.8$ (10); by *t*-test, $P > 0.30$.

considerable variation between individual preparations, the differences were significant (Table 2).

Actin (II), isolated from natural actomyosin, was of similar purity to actins (IA) and (IB), but the specific radioactivity of this type of preparation was always higher than that of the comparable extraction of either actin (IA) or (IB) (Table 3). There were, however, no significant differences between the specific radioactivities of myosin (II) and actin (II), and both types of preparation gave values similar to the original natural actomyosin (Table 3).

In order to ascertain whether the state of the original extract was an important factor in the differences found between the actins, an acetone-dried powder of natural actomyosin was prepared. The wash treatment with NaHCO_3 was omitted, as it resulted in the solubilization of large quantities of the natural actomyosin. The natural actomyosin was acetone-dried directly and then actin (III) extracted by the method of Seraydarian *et al.* (1967). Although the final yield of actin (III) was very low, and its purity variable, the final specific radioactivities were,

in all cases, lower than that of actin (II) and in one case (no. 66) lower even than actin (IA) (Table 3).

The age of the actomyosin sample also influenced the yield and the specific radioactivity of the isolated proteins. In two experiments (nos. 49 and 50), instead of the natural actomyosin being immediately dissolved in ATP solution and dissociated, the pellet of actomyosin was stored at 4°C for 48 h before the dissociation procedure was begun. In both cases the yield, especially of actin, was low, although the purity was similar to that achieved by the normal procedure. However, their specific radioactivities were 43 and 60% higher respectively than that of the natural actomyosin, although there was no concomitant rise in that for myosin (II) (Table 4).

In view of the report of Etlinger *et al.* (1975) that recently synthesized, loosely bound material could be dissociated from the bulk of the myofibrils, two experiments were carried out to determine whether such a removal would contribute to the differences in

specific radioactivity that were observed. The muscle mince was equally divided and myofibrils were prepared by the normal procedure and by the method described by Etlinger *et al.* (1975), where ATP is included in the initial extraction buffers. For the first experiment (no. 73), in which only the natural actomyosin was prepared, the difference between the two samples was within experimental error (Table 5). In the second experiment (no. 76) the myosin (II) and actin (II) were prepared by dissociation with ATP of natural actomyosin from both sets of myofibrils, and again the differences in specific radioactivity between the two procedures were within the experimental error (Table 5).

Comparison of the major protein fractions of muscle

Table 6 shows values of specific radioactivity for total muscle protein and its main constituent fractions, myofibrils, natural actomyosin and sarco-

Table 4. Effect of delayed dissociation of natural actomyosin on the specific radioactivity of myosin (II) and actin (II)

A pellet of natural actomyosin was prepared as described in the Experimental section and stored at 4°C for 48 h. It was then dissociated into myosin (II) and actin (II) by the normal procedure and treated as described in the Experimental section.

	Rabbit no.	...	Specific radioactivity of isolated tyrosine (d.p.m./nmol)	
			49	50
Natural actomyosin			18.36	11.89
Myosin (II)			20.42	10.44
Actin (II)			26.18	18.97

Table 5. Effect of method for preparation of myofibrils on the specific radioactivity of the subsequent natural actomyosin, actin (II) and myosin (II)

Muscle mince was equally divided. Myofibrils were isolated from one half by the method of Perry & Zydowo (1959) (a) and from the other half by the method of Etlinger *et al.* (1975) (b). Natural actomyosin, myosin (II) and actin (II) were isolated and treated as described in the Experimental section.

	Rabbit no.	...	Specific radioactivity of isolated tyrosine (d.p.m./nmol)			
			73		76	
			(a)	(b)	(a)	(b)
Natural actomyosin			5.31	5.41	3.31	3.49
Myosin (II)					3.44	3.42
Actin (II)					3.28	3.29

Table 6. Specific radioactivity of major muscle-protein fractions

Each fraction was isolated and treated as described in the Experimental section. The ratios ($\times 100$) of specific radioactivities for the comparable preparations are given $\pm$ s.d. Probabilities for differences were analysed by Student's *t*-test for pairs.

Preparation no.	...	Specific radioactivity of isolated tyrosine (d.p.m./nmol)								
		9	10	14	15	16	47	66	73	76
Mixed muscle		5.33	4.86	4.79	1.17	4.84	5.89	10.41	5.59	3.49
Myofibrils		5.06	5.10	5.15	1.14	—	6.07	8.42	—	3.48
Natural actomyosin		4.64	4.59	4.42	1.00	3.99	5.62	9.37	5.31	3.49
Sarcoplasm		5.24	5.41	5.06	1.09	4.48	5.70	11.82	5.66	2.96
Sarcoplasm residue									6.65	5.10

$100 \times (\text{Myofibrils/muscle}) = 98.3 \pm 8.8$ (7); by *t*-test, $P > 0.3$.

$100 \times (\text{Natural actomyosin/muscle}) = 91.3 \pm 5.6$ (9); by *t*-test, $P < 0.01$.

$100 \times (\text{Sarcoplasm/muscle}) = 99.7 \pm 9.3$ (9); by *t*-test, $P > 0.3$.

plasm (the supernatant from centrifugation at 130000g for 1 h). The specific radioactivity of isolated natural actomyosin was significantly lower than that of total muscle protein (by Student's *t*-test, $P < 0.01$), whereas the differences for myofibrils and sarcoplasm were not significant (Table 6). In contrast the pellet obtained by the centrifugation of the soluble fraction at 130000g for 1 h was analysed in two preparations (nos. 73 and 76) and on both occasions was found to have a specific radioactivity above that of the other fractions and even higher than the total muscle protein itself (119 and 146% respectively).

Discussion

Most radiochemical-incorporation experiments, whether by pulse injection or constant infusion, are of a sufficiently short duration to minimize the problems caused by reutilization of the radioactive amino acid released from the breakdown of protein. The disadvantage of these brief periods of exposure is, however, that only a small proportion of the protein becomes labelled. From the present experiments (results not shown) and a previous study (Nicholas *et al.*, 1977), it can be calculated that, for adult rabbits, only about 0.5% of the muscle-protein molecules contain radioactive tyrosine at the end of a 6 h infusion. Even in the weanling animals, where the rate of protein synthesis is higher, less than 4% of the protein molecules became labelled during a similar period. Although this presents no problem provided that a quantitative recovery of the relevant protein(s) can be achieved, most of the normal isolation procedures for myosin and actin give a less than complete yield (see Scheme 1). Consequently, it is crucial that the final preparation for analysis should be representative of the total protein.

Myosin synthesis

Myosins (I) and (II) are composites of the light and heavy chains with associated ribonuclear material. There was no change in the specific radioactivity of myosin after removal of the ribonuclear fraction, and under the conditions of radioisotope administration used, the average specific radioactivity of the three light chains was similar to that for the heavy chains. Thus, the extracted myosins could be studied directly without further fractionation. Other workers have isolated light- and heavy-chain fractions with different specific radioactivities and suggested that this indicates the existence, in muscle, of a pool of light chains not directly associated with myosin heavy chains (Morkin *et al.*, 1973; Zak *et al.*, 1977).

Etlinger *et al.* (1975) showed that newly synthesized molecules of myosin are either associated with loosely bound myofilaments or are themselves so loosely bound to the myofilaments that they will readily disaggregate and solubilize during the initial extrac-

tions. It follows, then, that myosin solubilized by direct treatment of a muscle mince with solutions of high ionic strength will probably contain a disproportionate amount of the newly synthesized molecules and have a higher specific radioactivity than that for the myosin population as a whole. The reverse holds for material extracted from preparations of myofibrils or natural actomyosin, where the loosely bound proteins are probably removed when the myofibrils are washed with buffers of low ionic strength and so the residual proteins will have a lower average specific radioactivity. These considerations are consistent with the observations that the specific radioactivity of myosin (I) was invariably higher than that of myosin (II), but although these differences were highly significant, they were also slight (Table 1). The myosins extracted after a 6 h exposure to the labelled amino acid only differed by, on average, 11%. This suggests that, in rabbit skeletal muscle, the time required for integration of new protein into the myofibril is much shorter than in rat muscle for which Etlinger *et al.* (1975) demonstrated that loosely bound material had a specific radioactivity twice that of the residual myofibrils 3 h after injection.

Although the methods of preparation for myosin (I) and (II) are different, as are the yields obtained, the similarity in the specific radioactivity of the final samples indicates that these values are probably close to that for the total myosin.

Actin synthesis

The situation for actin was more complex, as the various methods resulted in actin preparations that were considerably different in specific radioactivity both between themselves and compared with myosin. Actin (II), prepared by dissociation of actomyosin, was obtained in the highest yield and must represent material firmly integrated into the myofibrillar structure. It can, therefore, be compared directly with myosin (II) isolated from the same source, and the differences between the specific radioactivities of the two proteins were not significant. On this basis then, and assuming that the two proteins are produced from the same amino acid precursor pool, actin and myosin appear to have identical rates of synthesis.

In comparison with actin (II), actins (IA) and (IB) have low specific radioactivities and, in consequence, lower apparent fractional-synthesis rates. The differences in specific radioactivity could be due to the preferential loss of either labelled protein during the isolation of actins (IA) and (IIB) or unlabelled material from the actin (II) preparation. The spatial arrangement of the I-filaments and the differences in yield between the various methods (see Scheme 1) make it unlikely, however, that preferential loss of at least 20% of pre-existing, unlabelled molecules had occurred during the isolation of actin (II). It has

been suggested that newly synthesized protein is added to the periphery of the myofibrils (Morkin, 1970), and, if this is the case, it is possible that loss could also occur more easily from these sites. During the preparation of the acetone-dried powder, small quantities of actin are occasionally removed during the initial extraction of myosin and, in addition, the wash treatment with NaHCO_3 can result in the denaturation of actin (Barany *et al.*, 1957) and/or the solution of actomyosin. If these losses contain more of the newly synthesized polypeptides, then the residual actin [(IA) and (IB)] in the powder will have a lower specific radioactivity. The magnitude of these losses need not be very great; even for a method yielding a near quantitative recovery, less than 0.2% of the total actin needs be removed to account for the observed differences (Tables 2 and 3).

The difficulties associated with extraction need not be exclusive to the acetone-dried preparations, as actin (II) of high specific radioactivity was obtained in low yield from aged samples of natural actomyosin (Table 4). This indicates that these preparations contained a higher-than-usual proportion of newly synthesized molecules. This is consistent with the hypothesis that the most easily extracted material is that most recently synthesized. It is not clear, however, why the myosin (II) from the same preparation should have a specific radioactivity similar to that of the natural actomyosin but lower than that of the actin (II). The yield of myosin (II), although less than usual, was still greater than that of the actin (II), and, therefore, actin from aged natural actomyosin is possibly more susceptible to modification or denaturation, which could affect the extraction properties. For example, the actin may not dissolve in the solution of ATP, and instead an extract of natural actomyosin and myosin would be obtained. Alternatively, the actin may dissolve in the solution of ATP but not then be able to repolymerize to F-actin nor recombine with myosin, to give actomyosin, when the ionic strength is decreased and consequently remains in the residual liquor. This problem requires further investigation.

Comparison of actin and myosin

On the basis of the present observations it would appear that although the specific radioactivity of myosin is affected little by changes in extraction procedure, the same is not true for actin. Conclusions as to whether or not actin and myosin have similar rates of synthesis will depend, therefore, on which methods of isolation for the former are used. For example, Bidinost (1951) and Perry (1974), by using preparations based on dissociation of the actomyosin complex, both found that myosin and actin had similar rates of synthesis, consistent with the data presented here for myosin (II) and actin (II). Conversely those workers who prepared actin from

acetone-dried powder usually reported the protein to have a lower specific radioactivity than myosin (Velick, 1956; Swick & Song, 1974; Koizumi, 1974). In these cases the specific radioactivity of the actin was 0.39–0.77 that of myosin, with most of the data in the range 0.61–0.77, close to the average value of 0.57 found for the ratio of actin (IA) to myosin (I). Myosin was found to have a lower synthesis rate than actin *in vitro* (Low & Goldberg, 1973), but these conditions may not simulate the situation *in vivo*.

Although the above observations seem to account satisfactorily for most of the differences reported in the literature, Zak *et al.* (1977) have separated the myofibrillar proteins on SDS/polyacrylamide gels and found that actin was synthesized more slowly than myosin in rat cardiac muscle. The reason for the discrepancy between their results and those reported here for actin (II) and myosin (II) is not clear. Certainly it could not be attributed to differences in the way the myofibrils were prepared (see Table 5), and, as the subsequent isolation of proteins, either by dissociation of actomyosin or by separation on SDS/acrylamide gels, should both give high yields, then difficulties associated with preferential extraction should be decreased. The detection of short-term variations in the anabolic processes will be affected, however, by the mode of administration of the tracer amino acid. For instance, Zak *et al.* (1977) gave a pulse injection of radioactive leucine and examined radiochemical incorporation into the proteins after 10 min and 4 days. A period of 10 min is only slightly longer than the time required to synthesize a myosin heavy-chain polypeptide (Morris *et al.*, 1972), and during this period the specific radioactivity of the precursor aminoacyl-tRNA will be decreasing rapidly (Martin *et al.*, 1977). Furthermore, Etlinger *et al.* (1975) have shown that integration of newly synthesized proteins into the myofibrillar structure can be slow. Consequently, any temporal differences in either the sequence in which the proteins are synthesized or in their assembly into myofilaments will be highlighted by the use of pulse injections and short-time studies. In the 4-day treatment, when the above effects should have been minimized, exactly the same result was obtained for the synthesis rates of myosin and actin as in the 10 min procedure, although it is noteworthy that the synthesis rate for tropomyosin was apparently different at the two time intervals (Zak *et al.*, 1977). The constant-infusion technique used in our study will only give information for the total 5–6 h and would not detect any effects lasting only a few minutes. It should also be considered that there might be tissue- and/or species-related differences in the biosynthesis of the myofibrillar proteins between rat heart and rabbit skeletal muscle.

The finding that the specific radioactivity of the total sarcoplasmic-protein fraction is similar to that of both the myofibril and total muscle protein (Table

6) is in agreement with the observations of other workers (Winnick & Winnick, 1960; Sender & Garlick, 1973; Laurent *et al.*, 1978). The pellet obtained by centrifugation at 130000g of the soluble protein of muscle contains mitochondria and 'microsomes' as well as interstitial cells and any, or all, of these could contribute towards the high specific radioactivity of this fraction. Winnick & Winnick (1960) found that both mitochondrial and microsomal fractions from muscles had specific radioactivities that were higher than those of the myofibrillar and sarcoplasmic fractions.

If the sarcoplasmic and myofibrillar proteins do share a common amino acid precursor for protein synthesis, then on average their fractional-synthesis rates are almost identical in the skeletal muscle of the rabbit. This again raises the question whether control of myogenesis involves the addition or replacement of whole areas of the muscle, and, if so, at what level of organization this occurs. This question can only be answered with further knowledge of the turnover of the individual proteins in the myofibril, the sarcoplasm and the other organelles of the cell.

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