REFERENCES

- Barton, G. M., Evans, R. S. & Gardner, J. A. F. (1952). *Nature, Lond.*, **170**, 249.
- Bernstein, S. & McGilvery, R. W. (1952a). *J. biol. Chem.* **198**, 195.
- Bernstein, S. & McGilvery, R. W. (1952b). *J. biol. Chem.* **199**, 745.
- Bush, I. E. & Willoughby, M. (1957). *Biochem. J.* **67**, 689.
- De Meio, R. H. & Lewycka, C. (1955). *Endocrinology*, **56**, 489.
- De Meio, R. H. & Tkacz, L. (1952). *J. biol. Chem.* **195**, 175.
- De Meio, R. H. & Wizerkaniuk, M. (1956). *Biochim. biophys. Acta*, **20**, 428.
- De Meio, R. H., Wizerkaniuk, M. & Fabiani, E. (1953). *J. biol. Chem.* **203**, 257.
- De Meio, R. H., Wizerkaniuk, M. & Schreibman, I. (1954). *Fed. Proc.* **13**, 198.
- De Meio, R. H., Wizerkaniuk, M. & Schreibman, I. (1955). *J. biol. Chem.* **213**, 439.
- Gregory, J. D. & Nose, Y. (1957). *Fed. Proc.* **16**, 189.
- Markham, R. & Smith, J. D. (1952). *Biochem. J.* **52**, 552.
- Pearlman, W. H. & De Meio, R. H. (1949). *J. biol. Chem.* **179**, 1141.
- Pincus, G. & Pearlman, W. H. (1941). *Endocrinology*, **29**, 413.
- Roy, A. B. (1956). *Biochem. J.* **63**, 294.
- Segal, H. L. (1955). *J. biol. Chem.* **213**, 161.
- Stevenson, M. F. & Marrian, G. F. (1947). *Biochem. J.* **41**, 507.

Extraction of Proteins other than Myosin from the Isolated Rabbit Myofibril

BY S. V. PERRY AND A. CORSI*

Department of Biochemistry, University of Cambridge

(Received 4 February 1957)

Although satisfactory methods now exist for the isolation of myofibrils in appreciable quantities, little systematic combined biochemical and morphological study has yet been made of the organization of the chemical components in these structures. There is now clear evidence that the myofibril is composed mainly of the proteins myosin, actin and tropomyosin, but most investigations (e.g. Hasselbach, 1953; Hanson & Huxley, 1953; Hodge, 1955) concerned with the selective extraction of these proteins have been qualitative in nature. Furthermore, with the notable exception of the work of Hasselbach (1953), few attempts have been made to characterize and clearly identify the proteins extracted by the procedures used. Thus although the studies of Hasselbach (1953), Hanson & Huxley (1953) and Finck, Holtzer & Marshall (1956) indicate that there is a high concentration of myosin in the A band they do not allow us to decide whether this protein is present in the I band as well. Szent-Györgyi, Mazia & Szent-Györgyi (1955) have provided evidence that protein other than myosin is to be found in the A band and draw the conclusion from their results that appreciable amounts of myosin are localized in the I band.

One approach to the problem of the localization of myosin and the other myofibrillar proteins is provided by the observation (Perry, 1953) that a fraction free of myosin could be extracted from

rabbit myofibrils by solutions of low ionic strength. This fraction contained two main components, one of which was shown to be tropomyosin, but the second component could not be identified with myosin or with the F or G form of actin. The present paper is concerned with the identification of the second component as a form of inactive actin and with a further investigation of the selective extraction of proteins other than myosin from the myofibril. It forms the biochemical background to the following paper (Corsi & Perry, 1958) devoted to the study of the localization of myosin, tropomyosin and actin by phase-contrast microscopy of myofibrils extracted under varying conditions of pH and ionic strength.

METHODS

Preparation of myofibrils. In general, myofibrils were prepared from fresh back and leg muscles and from glycerol-treated psoas muscle of the rabbit by the methods already described (Perry & Grey, 1956a). To prevent the occurrence of spontaneous contraction during the homogenization of glycerol-treated muscle in a medium containing 0.1 M-KCl and 0.039 M-borate buffer, pH 7.1 (0.1 M-KCl-borate), it was found necessary to store the psoas-muscle strips in 50% (v/v) aq. glycerol for 2-3 weeks at -10° to deplete the endogenous adenosine triphosphate (ATP). When fresh rabbit muscle was homogenized in 0.1 M-KCl-borate, marked contraction of the myofibrils with reversal of striation usually occurred. This spontaneous contraction was reduced, but not eliminated, by lowering the KCl concentration of this medium to 0.025 M (0.025 M-KCl-borate) and carefully maintaining the temperature at 0° .

* Present address: Istituto di Patologia Generale, Piazza S. Eufemia, 19, Modena, Italy.

Satisfactory preparations of myofibrils, in which the I and A bands were clearly visible and in which the average sarcomere length was 2.2μ , were obtained by carrying out the initial homogenization and the first resuspension of the minced muscle (Perry & Grey, 1956*a*) in 0.1 M-KCl containing 5 mM-ethylenediaminetetra-acetate (EDTA), pH 7. This concentration of EDTA induces relaxation in glycerol-treated fibres (Boxler, 1954; Watanabe, 1955) and strongly inhibits the magnesium-activated adenosine triphosphatase (ATPase) activity of myofibrils (Perry & Grey, 1956*a, b*). Subsequent washings and recentrifugings were carried out in 0.1 M-KCl-borate, which procedure removed the EDTA as judged by the behaviour of the magnesium-activated ATPase of the myofibrils to fresh applications of EDTA (Perry & Grey, 1956*b*). There was a tendency for fibrous aggregates to form during preparation by this method but they could be removed by additional short centrifugings of the dilute myofibril suspension at low-speed.

Preparation of proteins. L-Myosin was prepared by a method previously described (Perry, 1955) and actin by the method of Guba & Szent-Györgyi (1945). 'Natural' actomyosin was obtained by the extraction of myofibrils with Weber's solution (0.6 M-KCl, 0.01 M- Na_2CO_3 , 0.04 M- NaHCO_3). The extract was centrifuged for 20 min. at 10 000 *g* and actomyosin precipitated from the supernatant by dilution with 14 vol. of water at pH 7.

Tropomyosin was prepared as described by Bailey (1948), further purified by ethanol precipitation as suggested by Bailey (1951) and recrystallized twice. This preparation, which was used to obtain the standard curve for the viscometric assay of tropomyosin, was homogeneous after electrophoresis for 7 hr. in 0.5 M-KCl containing 0.05 M-phosphate buffer, pH 6.8. Likewise only one component was apparent after centrifuging for 6 hr. at 259 000 *g* in the Spinco Analytical Ultracentrifuge under the same ionic conditions.

Extraction of myofibrillar proteins. Immediately after the myofibrils were prepared, a sample of the concentrated suspension was centrifuged for 30 min. at 20 000 *g* to estimate the soluble protein in the clear supernatant obtained (usually less than 0.5% of total N of the suspension). The concentrated preparation (3–5 mg. of N/ml.) was diluted to 0.7–0.8 mg. of N/ml. with the solution to be used as extractant and dialysed against 40–50 vol. of the extractant for 24 hr. The diffusate was replaced with a similar volume of fresh extractant and 24 hr. later the myofibrillar suspension was transferred to a stoppered flask containing a trace of toluene and minimum air space. After the required interval the suspension was centrifuged for 1 hr. at 105 000 *g* on the Spinco Preparative Ultracentrifuge. The soluble fraction had essentially the same composition if the suspension was centrifuged for 15 min. at 20 000 *g*, but the higher speed was chosen for routine analysis so that the residue sedimented to a small pellet, which was less than one-eighth of the total volume of the original suspension. Under these conditions it was assumed that the percentage of protein extracted was $100 \times$ concentration of N in the supernatant divided by the concentration of N in the whole suspension. Storage and all manipulations were carried out at 0°.

Estimation of tropomyosin. This was carried out by dialysing the protein solution against 0.078 M-borate buffer, pH 7.1, and determining with an Ostwald viscometer at 0° the drop in viscosity on addition of sufficient 2 M-KCl (in 0.078 M-borate) to obtain a final concentration of 0.5 M

(Perry, 1953). The tropomyosin concentration of the solution was determined by reference to a standard curve obtained with a highly purified sample of tropomyosin treated under identical conditions over the range 0.015–0.17 mg. of N/ml.

After dialysis for 7 days against 5 mM-acetate buffer, pH 4.3, the viscosity drop obtained under the standard-assay conditions was identical with that obtained with a control sample stored for the same period in 0.078 M-borate, pH 7.1. Dialysis against 5 mM-2-amino-2-hydroxymethylpropane-1:3-diol (tris), pH 7.9, for 7 days, however, reduced the drop in relative viscosity from 0.79 to 0.70 in a tropomyosin sample containing 0.081 mg. of N/ml. For this reason tropomyosin estimations on samples obtained by prolonged periods of alkaline extraction of the myofibril tended to be somewhat low, and in these cases estimations were usually restricted to extracts less than 2 or 3 days old.

Electrophoresis. Most studies were carried out in 0.25 M-KCl containing 0.05 M-phosphate buffer, pH 6.6–6.8, with the Perkin-Elmer electrophoresis apparatus, model 38. At this ionic strength satisfactory separation of components without appreciable precipitation of protein was best obtained at pH 6.8. Protein solutions, concentrated when necessary by suspending them at 0° in a dialysis bag in front of an electric fan, were dialysed against the buffer for 1–2 days at 0°. For estimation of mobility the conductivity of the buffer used for dialysis was taken rather than that of the protein solution.

Sedimentation and diffusion constants. The sedimentation rate was determined in the Spinco Analytical Ultracentrifuge, model E. The Tiselius electrophoresis cell (Perkin-Elmer) was used to determine the diffusion constant. The diffusion curves were analysed by the method of Rarkham (unpublished work).

Total N determinations were carried out by the micro-Kjeldahl technique, and the method of Krebs & Hems (1953) was used for the chromatography of adenine nucleotides.

RESULTS

It became apparent in the earlier stages of this investigation that on prolonged treatment of myofibrils with 0.078 M-borate buffer, pH 7.1, about 25% of the myofibrillar protein passed into solution. This value was much higher than that obtained previously (Perry, 1953) and it was considered to represent the upper amount of protein which could be extracted under these conditions. An increase in the ionic strength of the borate buffer by addition of salts such as KCl considerably reduced both the amount of protein passing into solution and the marked swelling which is characteristic of myofibrils suspended in media of low ionic strength.

Whereas at pH 7.1 in the borate medium 15–20 days or longer were required for maximum extraction, with 5 mM-tris buffer, pH 7.7 or 8.3, 30–35% of the total myofibrillar protein passed into solution within 4–5 days. After such a period at the higher pH the myofibrillar structure usually became unrecognizable and extraction under these conditions

was of limited value for morphological studies (see Corsi & Perry, 1958). Even if extraction was prolonged usually little more than 35–36 % of the myofibrillar protein dissolved in the buffer. In one experiment, however, after extraction for 59 days with 5 mM-tris buffer, pH 7·7, 42 % of the total myofibrillar protein passed into solution.

Electrophoresis of the protein solution obtained by extraction of myofibrils isolated from fresh muscle with low-ionic-strength buffers (hereafter called the 'soluble fraction') showed that three main components were present (Fig. 1). These will be referred to as the *A* (tropomyosin), *B* and *C* components, in order of decreasing mobility (see Table 1). Similar results were obtained with myofibrils prepared in the 0·025M-KCl-borate and the 0·1M-KCl-borate-EDTA media (Fig. 1*a, b*).

Usually electrophoresis was carried out in 0·25M-KCl containing 0·05M-phosphate buffer, pH 6·8, under which conditions satisfactory separation of the components was obtained without precipitation of protein. Component *B* was the most abundant and after exhaustive extraction at higher pH values the amount present was about twice that of *A*, whereas *C* was somewhat variable in amount; with myofibrils isolated from fresh muscle, by inspection of the Tiselius diagrams it was roughly estimated to represent about 5 % of the whole soluble fraction. As a rule component *A* appeared as a symmetrical peak, whereas the *B* peak sometimes showed asymmetry, particularly in the ascending limb, and on occasions after prolonged electrophoresis it appeared to be splitting into two components.

Although only slight differences in the relative proportions of tropomyosin and component *B* were noted during the progress of extraction, more marked effects were observed with myofibrils prepared from fibres which had been stored for several weeks in 50 % (v/v) glycerol. Under otherwise comparable conditions the total protein extracted by 0·078M-borate was less than that obtained from fresh myofibrils (Table 2). This was reflected in the reduced amount of component *B* and the almost complete disappearance of *C*. The extraction of tropomyosin was relatively much less

Fig. 1. Electrophoresis diagrams of solutions obtained by extraction of rabbit myofibrils with buffers of low ionic strength. Electrophoresis conditions: 0·25M-KCl, 0·05M-potassium phosphate buffer. (a) From fresh muscle extracted 9 days with 0·078M-borate buffer, pH 7·1. 300 min. at pH 6·8. (b) From fresh muscle (EDTA preparation) extracted 12 days with 5 mM-tris buffer, pH 7·75. 510 min. at pH 6·8. (c) From fresh muscle extracted 45 days with 5 mM-phosphate buffer, pH 6·45. 480 min. at pH 6·8. (d) From muscle in rigor mortis extracted for 3 days with 0·078M-borate, pH 7·1. 240 min. at pH 6·7.

Table 1. *Mobilities of components A, B and C*

Conditions: 0·25M-KCl, 0·05M-sodium phosphate buffer, pH 6·8. Mobility is expressed as 10^{-5} cm.²/v/sec. Desc. and asc.: descending and ascending boundaries.

Extractant	Mobility					
	Component A		Component B		Component C	
	Desc.	Asc.	Desc.	Asc.	Desc.	Asc.
0·078M-Borate, pH 7·1	3·8	3·9	3·1	3·5	1·3	1·2
0·078M-Borate, pH 7·1 (duplicate)	4·0	4·1	3·0	3·4	1·4	1·3
5 mM-Phosphate, pH 6·45	4·0	4·2	2·8	3·2	1·4	1·6

reduced and the soluble fraction obtained from glycerol-treated myofibrils usually contained this protein as the main component (Table 2, e.g. preparation no. 164G).

As might be expected, extraction at pH 6.45 (5 or 6.7 mM-phosphate buffer) reduced the amount of protein which passed into solution. For example, after 10–15 days 8–10% of protein was extracted and electrophoretic analysis indicated that this was due mainly to the suppression of the solubility of tropomyosin and component *B* (Fig. 1c). The extraction of these two components at higher pH values was considerably reduced by the presence of 0.5 mM-MgCl₂ (Table 2).

In the previous investigation (Perry, 1953) myosin was shown to be absent from the borate-soluble fraction and likewise under conditions of more complete extraction, i.e. with buffers of pH 7.7 and 8.3, no ATPase activity passed into solution (Table 3). It appeared unlikely that components *A*, *B* and *C* were derived by breakdown of the myosin molecule for no significant amount of protein passed into solution on treatment of L-myosin with borate. This fact, together with the finding that there was no fall in ATPase activity of myofibrils during the extraction process (on the contrary there was usually a 10–15% increase in activity), suggested that the myosin molecule was not being degraded during the extraction.

Component A (tropomyosin)

It was established that component *A* is electrophoretically identical with tropomyosin (Perry, 1953). In this earlier study rabbit myofibrils isolated in 0.078 M-borate buffer contained about 4% of tropomyosin, a result which agrees well with the figure given by Bailey (1948) for the tropomyosin which could be extracted from whole adult rabbit muscle. Our present studies indicate that at least 10–12% of the total N of myofibrils, freed from other cell components by isolation in media of ionic strength ranging from 0.025 to 0.10, consists of tropomyosin (Table 2). Analysis of the composition of the soluble fraction extracted by 0.078 M-borate after varying intervals of time showed that the soluble fraction was most rapidly extracted during the first and second days of exposure to the borate solution (Fig. 2). Consequently, in the earlier study (Perry, 1953), considerable loss of the soluble fraction occurred during the washing by centrifuging and resuspension. These losses have now been avoided by dialysing the myofibril suspension against the extractant (see Methods). Somewhat similar values to those obtained for mixed rabbit-skeletal muscle were obtained for the tropomyosin content of myofibrils isolated from hen-breast and rat-leg muscles (Table 2).

Table 2. Total nitrogen and tropomyosin extracted by buffers of low ionic strength

Preparation no.	Preparation medium	Extraction medium			Extraction time (days)	Total N extracted (% total N)	Tropomyosin N (% total N)	Tropomyosin N (% of N in soluble fraction)
		Composition	pH					
150F	0.025 M-KCl-borate	0.078 M-Borate	7.1		40	24.5	8.6	35
151F	0.025 M-KCl-borate	0.078 M-Borate	7.1		130	21.7	—	—
164G	0.1 M-KCl-borate	0.078 M-Borate	7.1		17	17.5	11	68
167G	0.1 M-KCl-borate	0.078 M-Borate	7.1		19	15.5	7.6	49
163FV	0.1 M-KCl-borate + EDTA	5 mM-Tris	7.75		2	25.5	9.6	38
		5 mM-Tris	7.75		65	35.2	—	—
166FV	0.1 M-KCl-borate + EDTA	5 mM-Tris	8.3		3	30.5	11.5	37
		5 mM-Tris	8.3		7	32.8	(11.2)	—
172F	0.025 M-KCl-borate	5 mM-Tris	7.9		4	28.2	10.0	35
175F	0.025 M-KCl-borate	5 mM-Tris	7.8		3	21.4	7.5	35
174FV	0.1 M-KCl-borate + EDTA	5 mM-Tris + 0.5 mM-MgCl ₂	7.9		2	9.2	4.1	36
					10	10.2	—	—
157F	0.025 M-KCl-borate	6.7 mM-Phosphate	6.45		10	8.4	2	—
160F	0.025 M-KCl-borate	5 mM-Phosphate	6.45		45	12.4	3.2	26
168FV	0.1 M-KCl-borate + EDTA	5 mM-Phosphate	6.45		17	11.6	3.3	28

Nitrogen soluble in the fresh myofibril preparations (usually <0.5%) is included in the total extracted. Preparations 164G and 167G were glycerol-treated (see text). All preparations were from rabbits except 172F (hen) and 175F (rat). For definition of 'soluble fraction', see text.

Table 3. Protein nitrogen extracted from rabbit myofibrils by buffers of low ionic strength

 $Q_p = \mu\text{l. of P/hr./mg. of protein.}$

Preparation no.	Extraction medium	Extraction time (days)	Q_p of whole suspension	ATPase soluble (%)	Protein N extracted (as % of total N)
157F	0.078M-Borate, pH 7.1	3	792	—	—
	0.078M-Borate, pH 7.1	23	904	Nil	26.3
160F	0.1M-KCl-borate, pH 7.1	Freshly prepared	690	—	—
	5 mM-Tris, pH 7.8	2	824	Nil	34
163FV	5 mM-Tris, pH 7.75	76	1187	Nil	35

Fig. 2. Time course of extraction of proteins from rabbit myofibrils. Myofibrils were prepared in 0.025M-KCl-borate buffer and were extracted with 0.078M-borate at 0°. ○, Total N/ml. of soluble fraction; □, N/ml., precipitated by 0.75M-phosphate buffer, pH 7.2; △, tropomyosin N/ml., estimated viscometrically.

Component B

On exhaustive extraction component *B* was obtained in amounts representing 15–20% of the total myofibrillar protein and, although on quantitative grounds it seemed that this protein was derived from the myofibrillar actin, component *B* was not identical with F or G actin. Component *B* possessed pseudoglobulin properties, in that it was precipitated by 50% saturated $(\text{NH}_4)_2\text{SO}_4$ but not by dialysis against distilled water or very low ionic-strength buffers, pH 7. It seemed likely that it was some form of inactive actin but attempts to restore its ability to undergo the G-F polymerization by treatment with ATP, ascorbic acid and MgCl_2 were unsuccessful. Study of component *B* was greatly facilitated by the observation that it was selectively precipitated by phosphate buffer, pH 7.2, at con-

centrations higher than 0.6M. After precipitation and re-solution 90–95% of component *B* was re-precipitated by 0.75M-phosphate from solutions containing from 0.04 to 0.19 mg. of N/ml. It was noted that on precipitation component *B* tended to undergo irreversible changes which rendered it partly insoluble. If precipitation was carried out at 0° and the precipitate redissolved within 5–10 min. clear solutions were readily obtained. Re-solution of the precipitate was facilitated by the addition of 5 mM-ascorbic acid to the original solution of the soluble fraction. Under these conditions precipitates of component *B* could be left for longer periods without becoming insoluble. In general this protein was unstable when redissolved after precipitation. The original soluble fraction could be preserved for weeks without apparent change but solutions of precipitated component *B* progressively became turbid on standing at pH 7 and ionic strength 0.2–0.4.

F and G actin, myosin and tropomyosin were not precipitated by 0.75M-phosphate buffer, pH 7.2, but actin irreversibly depolymerized by treatment with M-KI was precipitated. Nevertheless such depolymerized F actin did not have the same mobility as component *B* when added to a preparation of the borate-soluble fraction (Fig. 3c, d).

When F actin solutions were dialysed against 0.078M-borate buffer, pH 7.1, for several days a steady increase in the amount of protein which could be precipitated with 0.75M-phosphate was obtained. The properties of this protein appeared identical with those of component *B* and when added to the borate-soluble fraction it had the same mobility as component *B* and was indistinguishable from it (Fig. 3a, b).

When 'natural' actomyosin was dialysed for 9 days against 0.078M-borate buffer, pH 7.1, a soluble fraction was obtained representing about 11% of the total protein and 70–80% of which could be precipitated by 0.75M-phosphate.

These results indicated that component *B* was a form of irreversibly inactivated actin, and the low relative viscosity of its solutions suggested that it was in the depolymerized form. After phosphate precipitation the particle weight was high because,

in a medium consisting of 0.1M-glycine, 0.1M-KCl and sufficient N-HCl to bring the pH to 1.95, component *B* sedimented as one slightly asymmetric peak, $S_{20} \sim 16$ (one determination). A single determination of the diffusion constant in the same medium gave a value of $D_{20} = 1.1$.

Component *B* was produced by the treatment with borate and was not originally present in the myofibril, for protein was not precipitated by 0.75M-phosphate buffer from extracts of freshly prepared whole myofibrils made with Weber's solution and 1.65M-LiCl.

The maximum amount of component *B* (actin) was extracted by tris buffer of low ionic strength at higher pH values (7.7–8.3) and usually amounted to 20% of the myofibrillar protein. This would represent about 80% of the total actin presumed to be present if the figure of 15% (Hasselbach & Schneider, 1951) is taken for the actin content of whole muscle and the myofibrillar protein is

assumed to account for 60% of the total muscle protein.

It was noted in the studies with 0.078M-borate, pH 7.1, that after extraction of 20–25% of the myofibrillar protein little additional protein passed into solution even if extraction was continued for several months. Some increase in the amount extracted was achieved with the more alkaline tris buffers, but also under these conditions some of the actin resisted extraction. Myofibrils from which 35% of the protein had been extracted would still contract on the addition of ATP although the process was slow, and if any organized structure was apparent before contraction certainly none could be recognized after it had occurred. Also extracts of these myofibrils made with Weber's solution showed a significant decrease in relative viscosity on addition of ATP under the conditions of the actomyosin test.

Component C

Component *C* was usually observed as a small peak representing about 2–3% of the whole myofibrillar protein when extracts of myofibrils prepared from fresh muscle were made in borate and tris buffers, pH 7.1–8.3. It was also present in extracts made from myofibrils prepared from muscle allowed to go into rigor mortis (Fig. 1*d*). The amounts of component *C* in the soluble fraction were reduced if the myofibrils were washed in larger volumes of buffer than were used normally during the preparation. This component was not usually present in 0.078M-borate extracts of myofibrils made from glycerol-treated fibres which had been stored from 2 to 3 weeks at -10° . Component *C* was apparently readily extracted at pH 6.45 from myofibrils prepared from fresh muscle, for the amounts obtained under these conditions represented about the same percentage of the total myofibrillar protein as was extracted by borate and tris buffers under more alkaline conditions (Fig. 1 and Table 2).

Extraction of soluble components together with myosin fraction

While this investigation was in progress Szent-Györgyi *et al.* (1955) reported that when the myofibril is extracted with solutions of the Guba & Straub (1943) type fortified with low concentrations of ATP (which could be expected to dissolve myosin and little actin) myosin passes into solution together with another fraction which is soluble in a medium containing 0.04M-KCl and 0.0067M-phosphate buffer, pH 7.2. These workers conclude that this soluble protein represents about 20% of the myofibril and suggest that in part it is responsible for the increased optical density of the A band.

Fig. 3. Electrophoresis diagrams of 0.078M-borate extracts of rabbit myofibrils with and without added actin preparations. Electrophoresis conditions: 0.25M-KCl, 0.05M-potassium phosphate buffer. (a) 7-day extract. 660 min. at pH 6.8. (b) Extract as used in (a) plus inactive actin obtained by prolonged dialysis of F actin against 0.078M-borate buffer followed by phosphate precipitation. 660 min. at pH 6.8. (c) Ten-day extract. 720 min. at pH 6.7. (d) Extract as used in (c) plus actin treated with 1.2M-KI. 720 min. at pH 6.7.

With the aim of relating these findings to our own observations we have extracted fresh and glycerol-treated myofibrils in a similar manner to that employed by Szent-Györgyi *et al.* (1955). With fresh myofibrils the non-dialysable N of the fraction remaining in solution after precipitation of myosin from these extracts invariably represented 6–7 % of the total myofibrillar protein; with glycerol-treated myofibrils it amounted to 3–4 % only. These experiments were carried out on myofibrils separated from other cell components and debris and well-washed by repeated centrifugings. It is worth noting that in our experience (with one exception of doubtful significance) it was never possible (15 experiments) to extract myosin entirely free of actin from fresh or glycerol-treated myofibrils. Extractions were carried out with varying concentrations of ATP and pyrophosphate and with added $MgCl_2$. ATP was shown to be present throughout the extraction by chromatographic methods. In these experiments the N extracted ranged from 40 to 70 % of the whole.

DISCUSSION

Extraction of myofibrils with solutions of low ionic strength, pH 6.5–8.3, brings into solution an inactive form of actin, tropomyosin and small amounts of an unidentified fraction provisionally called component *C*. Considerable swelling occurs at the higher pH values and apparently aids the extraction, which appears to be maximal when about 35 % of the myofibrillar protein is in solution. It is probable that the total myofibrillar content of these three components is about 40 %, and the fact that the myofibril disintegrates under exhaustive extraction suggests that the components of the soluble fraction have an important function in maintaining the continuity of the structural framework. It is of interest to note that even when all the myosin is extracted, i.e. in extracts containing 60–70 % of the total protein, the myofibril structure still shows organization despite the fact that the cross-band structure has been radically changed (Corsi & Perry, 1958).

Tropomyosin has been shown to be present in skeletal-muscle myofibrils in greater amounts than was originally considered to be the case. It is rather striking that tropomyosin and actin are invariably extracted together and in a fairly constant ratio (1:1.5–2.0) irrespective of the total amount of soluble fraction obtained from myofibrils prepared from fresh muscle. This observation could indicate a close association of the two proteins *in situ*.

The data presented indicate that extraction of actin by suspensions of myofibrils in media of low ionic strength in some way changes the protein so that it forms solutions of low viscosity and will no

longer combine with myosin to give actomyosin which can be recognized by the conventional tests.

It must be concluded that, although part of the actin is comparatively easily extracted, about 20 % resists extraction even under more alkaline conditions. This suggests that there exists along the myofibril considerable variation in the strength of association of actin either with itself or with other protein. This observation was made on myofibrils isolated from fresh muscle and is presumably of significance in the role of this protein in myofibrillar function. The decreased solubility of the actin which occurs after prolonged storage of muscle in 50 % (v/v) aqueous glycerol is probably due to denaturation changes.

Our investigations support the view that actin, tropomyosin and myosin each exist as distinct entities in the myofibril. We have no evidence to indicate that during extraction myosin is being degraded into actin and tropomyosin, which proteins Laki (see Morales, Botts, Blum & Hill, 1955) has postulated as subunits of the myosin molecule. In fact our investigations suggest the contrary and, if taken with the findings described in the next paper, clearly indicate, as has the work of previous investigators (Hasselbach, 1953; Hanson & Huxley, 1953), that these proteins have precise localization which is dependent on the physiological state.

Component *C* is the protein of the soluble fraction which was not consistently detected and on repeated washing in 0.1 M-potassium chloride-borate the amount of this protein which could be extracted from the myofibril fell. Further, the low mobility of this fraction is reminiscent of that of the group I proteins (Jacob, 1947), which are present in extracts of whole rabbit muscle made with a solution of ionic strength 0.15 and pH 7.7. Such salt solutions do not bring into solution the myofibrillar proteins but are presumed to extract the sarcoplasm, defined as the complex system of proteins in solution which fills up the spaces between myofibrils and other formed elements within the cell. The facts suggest that component *C* arises from the sarcoplasm or possibly represents a sarcoplasmic fraction closely associated with the myofibril which is not readily removed by repeated washing. It may be dangerous to assume, however, that a component removed by prolonged washing is necessarily a contaminant of the myofibrils, particularly as one of us (S. V. Perry, unpublished work) has observed that the increased washing necessary to reduce the content of component *C* can also reduce the yield of tropomyosin extracted from the myofibril. Further investigations are in progress on the nature and origin of component *C*.

We have been unable to confirm that a protein soluble in a medium containing 0.04 M-potassium chloride and 0.0067 M-phosphate buffer, pH 7.2,

which represents 20% of the total myofibrillar protein, is extracted from the myofibril together with myosin by Guba–Straub solution containing ATP. In our hands this fraction represented 3–7% of the whole and it might be supposed that it consists in part of tropomyosin, which in all makes up at least 10–12% of the myofibril. de Villafranca (1956) has further studied this fraction, and although the effect of salt on its viscosity is remarkably like the behaviour of tropomyosin the protein is precipitated at lower ammonium sulphate concentrations; he concludes therefore that it is probably not tropomyosin. If that is so then the protein studied by Villafranca must constitute part of the residue, representing 60–65% of the myofibrillar protein left after extraction of the soluble fraction. This seems rather unlikely, as the results of the analysis of whole muscle (Hasselbach & Schneider, 1951) suggest that this residue must consist almost entirely of myosin.

SUMMARY

1. On exhaustive extraction of rabbit myofibrils with buffers of low ionic strength, pH 7.1–8.3, 25–36% of the total myofibrillar protein passed into solution. This extract contained tropomyosin, an inactive form of actin and an unidentified protein provisionally named component C.

2. Tropomyosin has been shown to account for at least 10–12% of the total myofibrillar protein.

3. The inactive form of actin extracted from myofibrils was electrophoretically identical with F actin inactivated by dialysis against 0.078M-borate buffer, pH 7.1, but not with F actin inactivated by treatment with potassium iodide.

4. When the extract obtained by treating myofibril with solutions which are considered to remove the A band selectively was dialysed against

0.04M-potassium chloride containing 6.7 mM-phosphate, pH 7.2, 3–7% of the total myofibrillar nitrogen remained in solution.

We wish to express our thanks to Mr T. C. Grey for skilled technical assistance and to the Medical Research Council for a research expenses grant (to S.V.P.). We are also indebted to Mr B. R. Slater for help in the determination of the diffusion constant of component B.

REFERENCES

- Bailey, K. (1948). *Biochem. J.* **43**, 271.
 Bailey, K. (1951). *Biochem. J.* **49**, 23.
 Boxler, E. (1954). *J. gen. Physiol.* **38**, 53.
 Corsi, A. & Perry, S. V. (1958). *Biochem. J.* **68**, 12.
 de Villafranca, G. W. (1956). *Arch. Biochem. Biophys.* **61**, 378.
 Finck, H., Holtzer, H. & Marshall, J. M. (1956). *J. biophys. biochem. Cytol.* **2**, Suppl. p. 175.
 Guba, F. & Straub, F. B. (1943). *Studies Inst. med. Chem. Univ. Szeged*, **3**, 46.
 Guba, F. & Szent-Györgyi, A. (1945). *Acta physiol. scand.* **9**, Suppl. 25.
 Hanson, J. & Huxley, H. E. (1953). *Nature, Lond.*, **172**, 530.
 Hasselbach, W. (1953). *Z. Naturf.* **8b**, 449.
 Hasselbach, W. & Schneider, G. (1951). *Biochem. Z.* **321**, 462.
 Hodge, A. J. (1955). *J. biophys. biochem. Cytol.* **1**, 361.
 Jacob, J. J. C. (1947). *Biochem. J.* **41**, 83.
 Krebs, H. A. & Hems, R. (1953). *Biochim. biophys. Acta*, **12**, 172.
 Morales, M. F., Botts, J., Blum, J. J. & Hill, T. L. (1955). *Physiol. Rev.* **35**, 475.
 Perry, S. V. (1953). *Biochem. J.* **55**, 114.
 Perry, S. V. (1955). In *Methods in Enzymology*, vol. 2, p. 582. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press.
 Perry, S. V. & Grey, T. C. (1956a). *Biochem. J.* **64**, 184.
 Perry, S. V. & Grey, T. C. (1956b). *Biochem. J.* **64**, 5P.
 Szent-Györgyi, A. G., Mazia, D. & Szent-Györgyi, A. (1955). *Biochim. biophys. Acta*, **16**, 339.
 Watanabe, S. (1955). *Arch. Biochem. Biophys.* **54**, 559.

Some Observations on the Localization of Myosin, Actin and Tropomyosin in the Rabbit Myofibril

BY A. CORSI* AND S. V. PERRY

Department of Biochemistry, University of Cambridge

(Received 4 February 1957)

In the previous paper (Perry & Corsi, 1958) it has been shown that at pH 7–8, under conditions of low ionic strength, about 35% of the total protein can be extracted from the isolated myofibril. As myosin was absent from this extract, which consisted mainly of actin and tropomyosin, it seemed likely that a study of the morphological changes involved

* Present address: Istituto di Patologia Generale, Piazza S. Eufemia, 19, Modena, Italy.

during the extraction of myofibrils under these conditions would provide useful information on the localization of actin and tropomyosin on the one hand and myosin on the other. In the communication which follows, the results of such an investigation are described together with those of complementary morphological and chemical studies under conditions in which myosin was the main component to be extracted from the myofibrils.