5. Specimens from Prof. C. J. Watson's laboratory have been examined. The uroporphyrin methyl ester I had m.p. 292–293° and behaved as uroporphyrin I containing a trace of uroporphyrin III on paper chromatography. The 208° ester behaved chromatographically as a hexacarboxylic porphyrin.

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The Protein Components of the Isolated Myofibril

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The myofibril is unique among the organized components of cells in that it requires a common substrate, adenosine triphosphate (ATP), for enzymic and mechanical activity. Both these properties survive when the myofibril is removed intact from the cell, and although it is true that the main protein component, actomyosin, has similar characteristics, extraction of this complex completely disorganizes the myofibrillar structure and results in modification of both enzymic and mechanical behaviour.

Schick & Hass (1951) investigated the solubility in salt solutions of varying ionic strength of myofibrils obtained by a procedure which involved tryptic digestion of the muscle tissue. These workers used an arbitrary method of microscopic examination to determine when solution was complete, but in view of the pronounced effect of trypsin on myosin (Gergely, 1950; Perry, 1951), and on proteins

generally, it is difficult to decide to what extent the constituent proteins were modified during the preparation of the myofibrils. Information about the protein components of the myofibril other than actomyosin is scanty; for example nothing is known about the nature of the various banded features, all of which must play a part in the function of the contractile unit. Earlier electron-microscope investigations (Rosza, Szent-Györgyi & Wyckoff, 1950; Perry & Horne, 1952) have provided evidence for the washing out of some band components with no apparent modification of the underlying basic filamentous structures.

The present communication is concerned with further investigations of the properties of myofibrils prepared without the use of enzymes. In particular, the nature of the protein components extracted from the myofibril under varying ionic conditions has been investigated.

METHODS

Preparation of myofibrils. Myofibrils were prepared as previously described (Perry, 1952), using as the medium for suspension 0.078M-borate buffer, pH 7.1 (30 ml. 0.05M-borax and 970 ml. 0.2M-boric acid diluted to 2.5 l.; Palitzsch, 1922). In certain cases, to reduce the amount of protein leaching out of the myofibrils, the preparation was carried out in a solution of 0.025M-KCl and 0.039M-borate buffer, pH 7.1. All manipulations were carried out in the cold room.

Extraction of myofibrils. Myofibrils were treated at 0° with the appropriate salt solutions so that extraction was carried out at protein concentrations ranging from 0.5 to 1.0 mg./ml. The original suspension was always in borate buffer and in consequence a low concentration of the latter was usually present during the first extraction. After a given extraction time, the myofibrillar residues were sedimented by centrifugation at 0° for 10 min. at 10000 g. To determine the amount of insoluble fraction, the residue was resuspended in salt solution and the extraction repeated 2-3 times. Finally total N estimations were carried out on the appropriate fractions.

Viscosity measurements. The usual procedure was to mix 5 ml. of extract with 2 ml. of 0.16M-phosphate buffer, pH 7.0, and take 3 ml. of this solution for viscosity determination at 0° in an Ostwald viscosimeter (time of flow for water, 40-50 sec.). Total concentration of protein in the viscosimeter was 3-4 mg./ml. ATP (0.03 ml.) was added in the viscosimeter to give a final concentration of 0.0004M.

Adenosine triphosphatase activity. The method was essentially that described earlier (Perry, 1951); glycylglycine buffer was used at pH 7.4 and glycine at pH 9.0.

Collagen estimation. Myofibrils were treated with 0.1N-NaOH for 20 hr. at 15°, centrifuged for 10 min. at 10000 g and N determinations carried out on the clear supernatant and the original whole extract. Nitrogenous material not rendered soluble by this procedure was considered to consist of collagen and elastin (Lowry, Gilligan & Katersky, 1941; Robinson, 1952).

Estimations. The acid-labile P content of the myofibril and derived fractions was estimated as described in an earlier communication (Perry, 1952). Total nucleic acid (including phosphoprotein) P values were determined by the method of Schmidt & Thannhauser (1945), and N by a micro-Kjeldahl technique. Protein concentration was arbitrarily taken as 6 × nitrogen concentration.

Materials. The ATP was prepared routinely in this department by Mr E. J. Morgan. It was freed from heavy-metal impurities by treatment with 8-hydroxyquinoline, stored as the barium salt and converted to the sodium salt for use. Dr T.-C. Tsao kindly provided the electrophoretically pure, recrystallized sample of tropomyosin.

Electrophoresis. Experiments were carried out with the usual Longworth & McInnes Tiselius apparatus, and also with the Perkin-Elmer model 38 instrument. Conductivities were determined on the protein solutions as prepared for electrophoresis.

RESULTS

Myofibril proteins soluble in 0.078M-borate, pH 7.1

Freshly prepared myofibril suspensions in 0.078M-borate buffer contained very little dissolved protein, for the addition of 10% (w/v) trichloroacetic acid

(TCA) to an equal volume of the clear supernatant obtained when the suspensions had been centrifuged for 10 min. at 10000 g, usually gave no detectable turbidity. During storage, at 4° in the presence of toluene, protein passed into solution with the result that after 15 days 11-12% of the total nitrogen of the suspension had become soluble. Continued storage under sterile conditions brought little additional nitrogen into solution, and dialysis of the solution showed that it did not contain nitrogen compounds of low molecular weight. This solution of myofibrillar proteins is subsequently referred to as the soluble fraction.

Fig. 1. Rate of solution of protein N on storing myofibril suspensions in 0.078M-borate buffer, pH 7.1, at 4°. The results obtained with five preparations are shown, each preparation being indicated by a different set of points.

Fig. 1 illustrates the results obtained with five different myofibril preparations and shows that after a comparatively rapid solution of protein in the first day or two, the rate of extraction decreases until after 15 days it is very slow. When a suspension of myofibrils, which had been stored until 11.2% of the total protein had gone into solution, was centrifuged down and resuspended in fresh borate buffer, the rate of solution was not increased. This indicates that the process was not a distribution of myofibrillar proteins between the gel and sol phases but rather the removal from the myofibril of one fraction more soluble in the buffer than the bulk of the myofibrillar proteins.

The soluble fraction was obtained as a clear, slightly viscous solution of protein with minimum solubility in the range pH 4.8-5.4. At 41% saturation with ammonium sulphate little protein remained in solution, and precipitation was practically complete at 50% saturation.

This fraction showed certain properties which suggested that it might consist, in part, of the globulin X complex. According to Weber & Meyer (1933) globulin X is precipitated at ionic strength < 0.005 and there is evidence that in living muscle

only part is in solution in the sarcoplasm. Dialysis of the soluble fraction against distilled water adjusted to pH 7, or against phosphate buffers containing equimolar amounts of KH_2PO_4 and K_2HPO_4 and ranging in total ionic strength from 0.001 to 0.1, did not precipitate any significant amount of protein. On this evidence it was concluded that the soluble fraction did not contain globulin X.

Adenosine triphosphatase activity of soluble fraction. No adenosine triphosphatase (ATPase) activity was shown by the soluble fraction in the presence of calcium ions at pH's 7.4 and 9.0, nor in the presence of magnesium ions at pH 7.4. For example, when 12–14% of the total protein had leached out of the myofibril, the whole suspension still contained powerful ATPase activity which was entirely localized in the readily centrifuged myofibrillar residue. These facts indicate that myosin was not a component of the soluble fraction.

Absence of F-actin. Precipitation of the soluble fraction with 0.01M-acetate buffer, pH 5.1, and dissolution in a small volume of distilled water with sufficient 0.1M-sodium bicarbonate to bring the pH back to 7, gave a very viscous solution. This was not F-actin as it did not form actomyosin on addition to a solution of freshly prepared myosin in 0.5M-potassium chloride, i.e. ATP did not produce a drop in viscosity when added to the combined solutions.

Tropomyosin and the soluble fraction. Addition of potassium chloride to the soluble fraction, as obtained from the myofibrils by centrifugation, or to the concentrated solution obtained after isoelectric precipitation, gave a marked drop in viscosity (Fig. 2). This salt effect was reversible and strongly suggested the presence of tropomyosin, the only protein so far isolated from muscle which exhibits these properties and which is presumed to be myofibrillar in origin on the basis of the procedure employed for its extraction from washed muscle mince (Bailey, 1948).

As usually obtained, the soluble fraction contained 1.5–2.0 mg. protein/ml., and concentration was necessary before electrophoretic investigation could be undertaken. This was accomplished by suspending the solution (containing 0.05M-phosphate buffer, pH 7.0) in a dialysis bag in front of an electric fan. When this process was carried out in the cold room at 4° the volume was reduced to one-sixth in 36 hr. without any significant amount of denaturation. Examination of this concentrated solution in the Tiselius electrophoresis apparatus revealed two main peaks (Fig. 3). These were invariably seen, but in some preparations another component, moving more slowly than the two main peaks, and present in much smaller amount, could be detected. The slower of the two main components was estimated to be present in twice the amount of the faster. Addition of a sample of recrystallized

tropomyosin to the soluble fraction did not result in the appearance of a new peak but, as Fig. 3 clearly shows, the fast component increased in amount relative to the slower. This was considered as good evidence for the identity of the fast component with tropomyosin. In Table 1 are presented the mobilities of the two main components of the soluble fraction.

Fig. 2. Effect of KCl on the viscosity of a solution of the soluble fraction in 0.078M-borate buffer, pH 7.1. Protein concentration approx. 0.5 mg./ml.

Fig. 3. Electrophoresis diagrams of the soluble fraction extracted from rabbit myofibrils, after 270 min. (a) Soluble fraction; (b) soluble fraction with added tropomyosin. Composition of buffer: 0.087M-phosphate, pH 7.1; 0.25M-NaCl.

On addition of potassium chloride to a final concentration of 0.5M the viscosity of the soluble fraction dropped to a very low level (Fig. 2), and in view of the electrophoretic evidence it was assumed that at low salt concentrations the viscosity of the extract was almost entirely due to the tropomyosin present and that the main component contributed

little. On this assumption a value for the tropomyosin concentration of the soluble fraction was obtained by comparing the reduction of viscosity obtained on addition of 0.5M-potassium chloride with that produced under identical conditions with

Table 1. *Electrophoretic mobilities of components of the soluble fraction of rabbit myofibrils*

(Electrophoresis carried out at 0° in 0.05M-sodium phosphate buffer, pH 6.5, and 0.22M-KCl. Values are the average of two independent determinations.)

Component	Mobility (10 ⁻⁵ sq.cm./v/sec.)	
	Ascending value	Descending value
Fast	5.0	4.9
Slow	3.7	3.4

a sample of pure tropomyosin. Table 2, showing the results of such determinations on several preparations, indicates that about 30% of the soluble fraction consists of tropomyosin. This proportion

Table 2. *Tropomyosin extracted from myofibrils stored in 0.078M-borate buffer, pH 7.1*

Prep. no.	Period of extraction (days)	Drop in relative viscosity	Protein concn. of soluble fraction (mg./ml.)	Deducted tropomyosin concn. (mg./ml.)	Tropomyosin (as % soluble fraction)
45	17	0.562	1.88	0.57	30.3
50	14	0.344	1.42	0.44	31.0
51	11	0.320	1.55	0.43	27.7

stayed fairly constant during the extraction of the soluble fraction, and the maximum amount of tropomyosin extracted represented 4.0% of the

Table 3. *Nucleic-acid phosphorus content of myofibril fractions*

(All fractions were extracted with cold trichloroacetic acid and fat solvents according to the procedure of Schmidt & Thannhauser (1945). The P figure represents the total amount of ribonucleic and deoxyribonucleic acids and phosphoprotein in the fraction analysed.)

Myofibril prep. no.	Fraction	Total P (%)
45	Whole myofibrils	0.042
45	Whole myofibrils after removal of the soluble fraction	0.040
45	Soluble fraction	0.081
47	Soluble fraction	0.064
63	Soluble fraction	0.073

total proteins of the myofibril. On the basis of his organic solvent extraction method Bailey (1948) estimated tropomyosin to account for 2.6% of the total proteins of rabbit muscle, i.e. 4.0% of the myofibril if the latter contains 65% of the total protein of muscle.

Phosphorus content of the soluble fraction. Hamoir (1951) has shown that tropomyosin from carp and rabbit muscle can form a crystallizable complex with nucleic acid, but it is not yet clear whether this protein exists *in situ* as such a complex or whether the nucleic acid is picked up during the extraction procedure. Consequently, it was of interest to determine the amount of nucleic-acid phosphorus in the soluble fraction, for under the conditions of extraction tropomyosin is removed from the myofibril without exposure to contamination by the nucleic acid of intracellular components such as nuclei and sarcosomes.

The soluble fraction contained variable amounts of free inorganic phosphate which presumably had leached out of the myofibril. Total nucleic-acid phosphorus values also showed some variation, the highest value obtained being 0.081% (see Table 3). If, in the extreme case, which is rather unlikely, all this phosphorus occurred as nucleic acid associated with tropomyosin in the soluble fraction, the complex would contain 2.5-3.0% nucleic acid. Nucleo-tropomyosin isolated by Hamoir (1951) from carp

muscle contained considerably more nucleic acid than this.

Effect of low concentrations of potassium chloride on the extraction of the soluble fraction. To investigate the effect of low concentrations of potassium chloride on the extraction of the soluble fraction, samples of

Table 4. *Protein passing into solution on storing myofibrils in media of low ionic strength*

Composition of medium		Turbidity on addition of TCA to the supernatant
Borate buffer pH 7.1 (M)	KCl (M)	
0.039	0.20	++ +
0.039	0.15	++ +
0.039	0.10	++
0.039	0.05	++
0.039	0.025	+
0.039	0.01	++
0.039	Nil	++ +
0.078	Nil	++ +

a freshly prepared myofibril suspension were left for 15 days in various solutions, the compositions of which are shown in Table 4. At the end of this period, the suspensions were centrifuged for 10 min. at 10000 g and a sample of each supernatant added

to an equal volume of 10% TCA. The relative turbidities obtained, which represent approximately the relative amounts of protein which had gone into solution, are shown in Table 4. It was readily apparent that the minimum amount of protein had leached out when the myofibrils were stored in a solution containing 0.039M-borate buffer, pH 7.1, and 0.025M-potassium chloride.

Fig. 4. Electron micrograph of rabbit myofibrils after extraction for 17 days at 4° with 0.078M-borate buffer, pH 7.1. Gold-palladium shadowed. Magnification, $\times 9250$.

More precise investigation indicated that only 4% of the total myofibrillar protein passed into solution during storage for 15 days in this medium. This solution was adopted for routine preparations when it was necessary to reduce the leaching out of myofibrillar protein to a minimum. It was noted that myofibrils prepared in this manner sedimented faster and formed a more compact mass in the centrifuge tube than those prepared in 0.078M-borate, and it was more difficult to free the preparations from nuclei and other rapidly sedimenting cell components. During preparation in 0.078M-borate the myofibrils became increasingly difficult to centrifuge down at the speeds used (Perry, 1952) and presumably in this buffer some hydration and swelling occurred.

Microscopic examination

Myofibrils from which the soluble fraction had been extracted were similar in general microscopic appearance to the freshly prepared material. If methylene blue was used as an aid for more careful

observation, the contrast between the heavily stained bands (which frequently appeared as a double band, see below) and the lightly stained bands was greater in the stored than in the fresh preparations. This appearance of increased contrast was possibly due to the fact that, compared with the fresh material, myofibrils from which the soluble fraction had been extracted stained very lightly indeed in the less dense part of the structure.

Fig. 5. Electron micrograph of rabbit myofibrils after 30 days extraction with 0.078M-borate buffer, pH 7.1. Gold-palladium shadowed. Magnification, $\times 7600$.

Electron-microscope examination revealed that although there was evidence of longitudinal filamentous structures within the myofibril, fine details could no longer be recognized. A characteristic feature of these extracted myofibrils was the appearance of regular dense bands (Figs. 4, 5). Two of these bands occurred in each sarcomere, although sometimes a pair was close enough together to appear as one broader band. These bands are considered to correspond to the outer edges of the A band or perhaps to the A substance which has moved into the I band, for myofibrils isolated by the method described are usually slightly contracted (Perry, 1952). Features of the A band such as the M line and H sub-lines (Perry & Horne, 1952) were never seen in electron microscope examinations of myofibrils stored long enough to extract the soluble fraction. Further work needs to be done to decide the precise location within the myofibril of the proteins of the soluble fraction.

Double refraction of the myofibril. Examination of a number of myofibril preparations was kindly carried out with the polarizing microscope by

Table 5. *Effect of ATP on the viscosity of Weber's solution extracts of myofibrils*

Prep. no.	Age (days)	Extraction time (hr.)	Relative viscosity		Remarks
			- ATP	+ ATP	
50	1	20	3.05	2.25	—
50	1	1.25	2.49	2.02	—
50	13	19	2.24	1.82	—
50	13	19	2.24	1.87	Soluble fraction removed before extraction
51	0	1	2.19	1.77	—
51	0	20	2.75	1.98	—

Dr M. Mitchinson of the Zoology Department. These investigations were of a qualitative nature but showed that all preparations had pronounced double refracting bands. No obvious diminution of the relative intensity of these bands could be seen in preparations which had been stored at 4° for 11–50 days. This suggests that the proteins of the soluble fraction are not responsible for the birefringent properties of the myofibril.

Proteins extracted by salt solutions of higher ionic strength (> 0.5)

Treatment of myofibrils with salt solutions > 0.5M extracted considerable amounts of protein to give a viscous, sometimes slightly turbid, solution. These solutions were shown to contain actomyosin by the fall in viscosity produced on the addition of low concentrations of ATP. Myosin almost free from actin is obtained by short extractions of minced fresh whole muscle with 0.5M-potassium chloride, whereas the isolated myofibrils produced viscous actomyosin solutions immediately they were treated with potassium chloride at this or higher concentrations. In addition, a solution containing 0.47M-potassium chloride, 0.01M-sodium pyrophosphate and 0.05M-phosphate buffer, pH 6.3, such as was used by Hasselbach & Schneider (1951) to extract myosin free from actin from whole muscle, was found to extract considerable amounts of actomyosin from the myofibril in 20 min. Weber's solution (0.6M-potassium chloride, 0.01M-sodium carbonate, 0.04M-sodium bicarbonate) was a very efficient agent for the extraction of actomyosin. This medium also extracted actomyosin from myofibrils which had been stored for 13 days and which had been washed free from the soluble fraction (Table 5).

When the process of salt extraction of the myofibrils was followed microscopically, cross striations were seen to disappear, although the myofibrils persisted in outline for some time, and finally the residues or ghosts which remained could be centrifuged down by high-speed centrifugation. Lithium chloride, which Bate-Smith (1937) found to be an efficient extractant for the proteins of whole muscle, brought an average of 89 % of the total myofibrillar

protein into solution and less (82 %) when used in conjunction with 0.067M-dipotassium hydrogen phosphate. Potassium chloride was less effective than lithium chloride and similarly its power of bringing myofibrillar protein into solution was reduced by 0.067M-dipotassium hydrogen phosphate (Table 6). In general, both these salts extracted rather more protein from myofibrils which had been stored for some time under sterile conditions. Weber's solution, or salt solutions containing potassium iodide (Table 6), were the most efficient in extracting protein from the myofibril. Exhaustive extraction with these solutions reduced the insoluble residue to 2–4 % of the total nitrogen of the myofibril.

Salt extraction, other than with potassium iodide and Weber's solutions, invariably left a residue which centrifuged down as a jelly-like mass and which was shown by electron microscope examination to be composed mainly of fibrous material which could not be characterized. No myofibrils could be recognized, however, if the extraction had been exhaustive, e.g. with lithium chloride. Although potassium chloride readily extracted actomyosin, the process was by no means complete; even after fifteen extractions with M-potassium chloride the residue still showed myosin-ATPase activity. Lithium chloride was much more effective, for a short extraction with this salt brought the whole of the myosin-ATPase into solution. The residue of lithium chloride-insoluble material, averaging 11 % of the nitrogen of the whole myofibril, contained 16.7 % total nitrogen and 0.14 % total phosphorus.

The nature of this fraction is unknown, but it probably consists of components of the banded structures of the myofibril, e.g. from the A band and the Z membrane. The presence of actin in this residue seems unlikely, as lithium chloride brings into solution all the labile phosphorus, and therefore presumably all of the actin of the myofibril (Table 7). If the residue was treated with acetone and proceeded with as for the preparation of actin (Straub, 1942), none of this protein could be prepared. Attempts to prepare actin from fresh myofibrils by the Straub method were uniformly unsuccessful. It

Table 6. *Protein extracted from myofibrils by salt solutions of higher ionic strength*

Prep. no.	Time after prep. (days)	Extractant	No. of times extracted	Insoluble (%)
49	13	0.90 M-LiCl, 0.067 M-K ₂ HPO ₄	4	17
49	21		4	16.7
50	0		4	18.9
51	0		4	18.8
51	10	0.90 M-LiCl	1 (2 hr.)	8.6
58	0		1 (3 days)	8.8
49	13		1 (20 hr.)	14.5
47	53	1.25 M-KCl, 0.067 M-K ₂ HPO ₄	3	13.6
48	0		3	25.4
49	0		3	22.7
51	8	1.25 M-KCl	1 (1.25 hr.)	17.1
51	10		1 (2 hr.)	15.2
51	0	Weber's solution	4	2.3
62	1		1 (22 hr.)	7.4
62	1		3	4.0
58	0	0.4 M-KCl, 0.6 M-KI, 0.067 M-K ₂ HPO ₄	4	3.1
49	4	1.25 M-KCl, 0.1 M-K ₂ HPO ₄ , 0.01 M-Na ₂ P ₂ O ₇	4	22.3
62	6	0.47 M-KCl, 0.01 M-Na ₂ P ₂ O ₇ , 0.05 M-phosphate buffer, pH 6.23	4	24.9

Table 7. *Effect of salt extraction on the bound nucleotide of the myofibril*

(The extractions were carried out at 0° and the residue separated and washed with extractant once or twice by centrifugation for 10 min. at 10 000 g.)

Prep. no.	Age (days)	Bound nucleotide		Nucleotide extracted (%)	Conditions
		Whole (mg. 10-min. P/ml.)	Residue (mg. 10-min. P/ml.)		
50	16	1.08	0.81	25	1.25 M-KCl, 1 hr.
50	16	1.08	0.16	85	0.90 M-LiCl, overnight
51	3	1.05	0.32	79	1.25 M-KCl, 2 hr.
51	3	1.05	0.09	91	0.90 M-LiCl, 2 hr.
61	3	1.43	0.29	80	1.0 M-KCl, 1 hr.
61	3	1.43	0.05	97	0.90 M-LiCl, 1 hr.
61	10	1.37	1.36	1	Soluble fraction removed and 10-min. P estimated; residue by difference
62	22	1.62	1.49	8	

appeared that most of the actin is removed by the salt extraction of the myofibrils in the first stage of the actin preparation. This is yet another example of the differences in extractability of the proteins actin and myosin in the isolated myofibril as compared with fresh minced muscle.

Collagen content of myofibril preparations. Under the conditions of estimation a very small amount of material remained insoluble, but as the nitrogen of myofibrils prepared both by the collagenase and the non-enzymic methods was rendered completely soluble by 0.1 N-sodium hydroxide the preparations were considered free from collagen and elastin.

The labile phosphorus of the myofibril

In an earlier communication (Perry, 1952) it was shown that nucleotide, mainly in the form of adenosine diphosphate, was closely associated with the myofibril and was responsible for the small acid-

labile phosphorus content of these preparations. On storage of myofibril suspension in borate buffer this labile phosphorus remained constant in amount and was invariably associated with the insoluble, myofibril fraction. Table 7 shows that even after storage until 13 % of the total protein had gone into solution no significant amount of labile phosphorus could be detected in the soluble fraction. This labile phosphorus was stable in the presence of lithium chloride and potassium chloride, although the extraction of the myofibril by these salts did affect its distribution between the soluble and the insoluble fractions. Lithium chloride was very effective in extracting the bound nucleotide. Treatment for a period of 1 hr. at 0° was adequate to bring 85–100 % of the labile phosphorus into solution, whereas potassium chloride was less effective and in various experiments under similar conditions with this salt, 25–80 % was extracted.

DISCUSSION

One of the objects of the present investigation was to obtain a quantitative assessment of the various proteins present in the myofibril isolated free from sarcoplasm. Unfortunately, the fractionation methods which have been used for such studies on whole muscle, e.g. see Hasselbach & Schneider (1951), could not be satisfactorily applied to the isolated myofibril and this aim has not yet been achieved. Whereas myosin practically free from actin can be obtained from fresh whole muscle mince without much difficulty, extraction of the isolated myofibril under similar conditions produced actomyosin rich in actin.

The conventional method of actomyosin preparation involves the prolonged extraction of whole muscle and produces a very impure product. As a starting material for actomyosin preparation, isolated myofibrils have many advantages; for example, one short extraction produces an actomyosin completely free from sarcoplasmic proteins. In fact, if methods can be devised for obtaining myosin and actin independently from the isolated myofibrils, the preparation of these proteins in the purified form would be considerably simplified.

It is generally assumed that the presence of ATP in the muscle cell is necessary for the extraction of myosin free from actin. This explanation follows naturally from the behaviour of ATP with the isolated proteins, but it is not entirely satisfactory as the endogenous ATP is rapidly broken down in muscle homogenates (Bailey & Marsh, 1952). It may be that in the cell myosin can only be extracted without appreciable contamination with actin when active phosphorylation systems are working to maintain the bound nucleotide of the myofibril (Perry, 1952) in the maximally phosphorylated state, i.e. mainly as ATP. This state of affairs would be characteristic of relaxed muscle or of muscle which has been freshly minced, where the oxidative and glycolytic systems are still directed into the production of energy-rich phosphate. It would not apply to muscle which has gone into rigor or to the isolated myofibril; indeed, in the latter case, the bound nucleotide exists mainly as adenosine diphosphate. The nature of the mechanisms involved in the phosphorylation of the bound nucleotide is now under investigation.

Of the two main components which passed into solution on continued extraction of myofibrils with solutions of low ionic strength, one has unequivocally been shown to be tropomyosin. Little nucleic acid is associated with this fraction and, as it is unlikely that extraction with 0.078M-borate buffer, pH 7.1, would dissociate a tropomyosin-nucleic acid complex, it is reasonable to conclude that tropomyosin does not occur in the myofibril as

a complex containing large amounts of nucleic acid such as has been isolated by Hamoir (1951) from carp muscle. The other component which was extracted with tropomyosin has not yet been identified. It was clearly neither myosin nor the normal form of G-actin. The absence of actin from the soluble fraction was further suggested by the fact that no labile phosphorus appeared in this fraction during the extraction process. The possibility cannot be excluded that this unidentified protein was a form of inactive or denatured actin, but the fact that it was always found in constant amount relative to the tropomyosin present argues against this explanation. It may be that this component is identical with that protein invariably found in Straub's actin preparations and which has been assumed, without any clear reason, to be inactive actin.

Repeated extraction with neutral salts such as potassium chloride or lithium chloride failed to bring 9–17% of the total myofibrillar nitrogen into solution, but if Weber's or potassium iodide solutions were used 96–98% of the nitrogen became soluble, and indicated that the myofibril must contribute little if anything to the so-called stroma protein which accounts for 17% of the total nitrogen of rabbit white muscle and 27% in the red variety (Weber & Meyer, 1933; cf. Hasselbach & Schneider, 1951). The sarcoplasmic granules, or sarcosomes, which are very resistant to agents of solution and not easily removed from the muscle residue, probably contribute to the insoluble stroma fraction. In mixed rabbit muscle the sarcosomes make 3–4% of the total muscle protein nitrogen, but in red muscle such as that obtained from pigeon breast, the figure is considerably higher (Chappell, 1952). This fact might account, in part at least, for the higher stroma values for red muscle. In rabbit white muscle the bulk of the stroma protein must be derived from insoluble nuclear residues, the sarcolemma and extracellular tissue. It is very likely that part of this stroma fraction consists of protein which has been denatured during the attainment of the degree of comminution necessary for adequate extraction. Disintegration, particularly in media of low ionic strength, can produce very appreciable denaturation of the myofibrillar components.

SUMMARY

1. On prolonged extraction of isolated rabbit-muscle myofibrils with 0.078M-borate buffer, pH 7.1, approximately 13% of the myofibrillar protein passed into solution.

2. This fraction soluble in 0.078M-borate buffer contained two main components, one of which was tropomyosin, whereas the other has not yet been identified. Proteins shown not to be present in the

soluble fraction were globulin X, myosin, F-actin and G-actin.

3. The bound nucleotide of the myofibril remained associated with the myofibril on prolonged extraction with 0.078M-borate at pH 7.1, but passed into solution on extraction with salt solutions of higher ionic strength.

4. Exhaustive extraction with potassium chloride

and lithium chloride failed to dissolve 9–17% of the myofibrillar protein, whereas extraction with Weber's and potassium iodide solutions reduced the insoluble residue to 2–4%.

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A New Colorimetric Reagent for Carbohydrates

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Methods for the estimation of carbohydrates, and particularly polysaccharides, have been somewhat tedious till the introduction by Dreywood (1946) and Morris (1948) of the reagent anthrone in concentrated sulphuric acid. This provided a sensitive, facile and general method for carbohydrate analysis, since simple dilution of the reagent (2 volumes) by the test sample (1 volume) produces a strongly acid medium sufficiently hot to hydrolyse a polysaccharide, which then gives a colour reaction with the anthrone equivalent to that of its constituent units. Thus the reagent may be calibrated against standards of the constituent monosaccharide, since the colorimetric yield is known to be quantitative.

Unfortunately the anthrone reagent is somewhat unstable, necessitating frequent preparations, including standards for calibration. A search was therefore made for a similar but stable reagent. Various simple polyphenols, substituted phenols and aromatic amines were tried, but those which were chromogenic were also unstable in sulphuric acid. In an endeavour to stabilize resorcinol, one of the most promising reagents when used in place of anthrone, the hydroxyl groups were protected

by benzylation. When resorcinol dibenzoate in ethanolic solution and sulphuric acid were added in succession to test samples, the benzoyl groups were removed by hydrolysis with the development of a satisfactory colour, but benzoic acid crystallized out on cooling. The ditoluene-*p*-sulphonate proved resistant to hydrolysis and gave scarcely any colour. *O*-Substitution having failed, the nuclear-substituted derivative resorcinol-4:6-disulphonic acid (RDSA) was prepared, and found to be stable in aqueous solution, although insoluble in concentrated sulphuric acid. The disulphonic acid was about as sensitive a colorimetric reagent as the parent phenol, giving an intense golden-brown colour with carbohydrates. This reaction has been investigated so as to provide a reliable and simple method for carbohydrate estimation which would be useful when only very small volumes or very dilute solutions were available for analysis. Under the conditions of the reaction all the isomeric hexoses tested gave a peak at 490 m μ , but the sensitivity varied with the individual hexose (Fig. 1). For any series of determinations, therefore, the appropriate hexose must be used as standard