Purification of desmin from adult mammalian skeletal muscle

John M. O'SHEA, Richard M. ROBSON,* Michael K. HARTZER, Ted W. HUIATT,†
Wayne E. RATHBUN, and Marvin H. STROMER

*Muscle Biology Group, Departments of Animal Science, Biochemistry and Biophysics and Food Technology
(Co-operating), Iowa State University, Ames, IA 50011, U.S.A.*

(Received 14 July 1980/Accepted 8 January 1981)

A method has been developed for preparation of purified desmin from mature mammalian (porcine) skeletal muscle. A crude desmin-containing fraction was prepared by modification of procedures used for isolation of smooth-muscle intermediate-filament protein [Small & Sobieszek (1977) *J. Cell Sci.* 23, 243–268]. The desmin was extracted in 1M-acetic acid/20mM-NaCl at 4°C for 15h from the residue remaining after actomyosin extraction from washed myofibrils. Successive chromatography on hydroxyapatite and DEAE-Sepharose CL-6B in 6M-urea yielded desmin that was routinely more than 97% 55000-dalton protein and that had no detectable actin contamination. Removal of urea by dialysis against 10mM-Tris/acetate (pH 8.5)/1mM dithioerythritol and subsequent clarification at 134000g (r_{av} , 5.9cm) for 1h results in a clear desmin solution. Dialysis of purified desmin against 100mM-NaCl/1mM-MgCl₂/10mM-imidazole/HCl, pH 7.0, at 2°C resulted in the formation of synthetic desmin filaments having an average diameter of 9–11.5nm. The present studies demonstrate that the relatively small amount of desmin in mature skeletal muscle can be isolated in sufficient quantity and purity to permit detailed studies of its properties and function. Although 10nm filaments have not been unequivocally demonstrated in mature muscle *in vivo*, that the purified skeletal-muscle desmin will form 10nm filaments *in vitro* lends support to their possible existence and cytoskeletal function in mature skeletal-muscle cells.

The term 'intermediate-sized' filament was introduced by Ishikawa *et al.* (1968) to describe a new set of filaments approx. 10nm in diameter that were observed between striated myofibrils in cultured myogenic cells. Since then it has become evident that most, if not all, vertebrate cells contain, in addition to microtubules and microfilaments, this less-characterized cytoplasmic filament class having diameters of about 10 (7–12)nm (Uehara *et al.*, 1971; Goldman & Knipe, 1973; Dahl & Bignami, 1975; Steinert *et al.*, 1976; Franke *et al.*, 1978; Gilbert, 1978; Hynes & Destree, 1978; Goldman *et al.*, 1979). Although the 10nm filaments from a wide variety of cell types share some common structural and chemical properties, there is considerable

evidence, based primarily on polypeptide analysis by one- and two-dimensional gel electrophoresis and on immunofluorescence microscopy, of as many as five major classes of 10nm filaments composed of distinct subunit proteins (Franke *et al.*, 1978; Davison *et al.*, 1977; Schlaepfer and Lynch, 1977; Bennett *et al.*, 1978a; Fellini *et al.*, 1978; Steinert *et al.*, 1978; Lazarides & Balzer, 1978). In general, each class is characteristic of a particular cell type, although some cells have two (Franke *et al.*, 1979; Gard *et al.*, 1979; Lazarides, 1980; Tuszynski *et al.*, 1979) or more (Wang *et al.*, 1980) co-existing classes.

Studies in our laboratory (Schollmeyer *et al.*, 1976; Huiatt *et al.*, 1978, 1980; O'Shea *et al.*, 1979) and in others (Cooke, 1976; Lazarides & Hubbard, 1976; Small & Sobieszek, 1977; Bennett *et al.*, 1978b; Fellini *et al.*, 1978) have suggested that a protein having a subunit molecular weight of approx. 55000 [termed 'desmin' by Lazarides & Hubbard (1976) and 'skeleton' by Small & Sobieszek (1977); the term 'desmin' will be used throughout the present

Abbreviation used: SDS, sodium dodecyl sulphate.

* To whom all correspondence and requests for reprints should be addressed.

† Present address: Rosenstiel Basic Medical Sciences Center, Brandeis University, 415 South Street, Waltham, MA 02154, U.S.A.

paper] is a major component of 10 nm filaments present in vertebrate smooth-muscle cells. The desmin filaments constitute one of the currently recognized classes of 10 nm filaments (Bennett *et al.*, 1978a; Lazarides & Balzer, 1978; Bennett *et al.*, 1979). By immunofluorescence (using antibodies produced against the smooth-muscle protein) (Lazarides & Hubbard, 1976; Lazarides, 1978; Bennett *et al.*, 1978b, 1979; Campbell *et al.*, 1979) and by two-dimensional gel-electrophoretic analysis (Izant & Lazarides, 1977), desmin has also been identified in differentiated cardiac- and skeletal-muscle cells. The immunofluorescence studies (Lazarides & Hubbard, 1976; Lazarides, 1978; Lazarides & Granger, 1978; Granger & Lazarides, 1978) suggest that desmin is located at or near the Z-line of differentiated skeletal-muscle cells, and it has been suggested (Granger & Lazarides, 1978; Lazarides & Granger, 1978) that desmin may serve one or more important roles involving striated-muscle Z-lines. The properties and function of skeletal-muscle desmin remain unclear, however. In particular, the clear identification of a distinct set of 10 nm filaments in this region has not been demonstrated in electron-microscopic studies of mature skeletal muscle (see discussion in Bennett *et al.*, 1979). This discrepancy might indicate a difference in physical properties of skeletal- and smooth-muscle desmins and/or differences in ability of the two desmins to assemble into 10 nm filaments. To answer these questions, as a part of our continuing studies on the structural composition and biochemistry of the Z-line and Z-line-like structures (Robson *et al.*, 1970; Robson & Zeece, 1973; Dayton *et al.*, 1976; Schollmeyer *et al.*, 1976; Suzuki *et al.*, 1976; Singh *et al.*, 1977; Davies *et al.*, 1978; Yamaguchi *et al.*, 1978; O'Shea *et al.*, 1979; Zeece *et al.*, 1979), it was necessary to develop a method for purification of desmin from skeletal muscle, and to determine if the purified protein was capable of self-assembly. In the present paper we outline the purification of the skeletal muscle protein and show that it is capable of assembling into 'synthetic' 10 nm-diameter filaments.

A preliminary account of this work was presented at the American Society for Cell Biology Meeting at San Antonio, TX, U.S.A., on 4–8 November 1978.

Materials and methods

Unless specified otherwise, all procedures were done at 0–4°C or on ice with precooled solutions prepared with double-deionized distilled water that had been redistilled in glass and stored in polyethylene containers. Unless noted otherwise, adjustment of buffer pH was done at 4°C by using a combination electrode.

Source of muscle and preparation of crude desmin extract

Porcine semitendinosus and biceps femoris muscles were excised immediately after death from 90–110 kg pigs, immersed in ice, trimmed free of fat and connective tissue, ground in a precooled meat grinder, and used for preparation of desmin. The purification scheme developed in the present study for preparation of crude desmin is as follows.

Step I. Coarsely ground muscle (most preparations were initiated with 600 g of ground muscle, and all solution volumes are based on the wet weight of ground tissue used in Step I unless noted otherwise) was suspended in 6 vol. (v/w) of standard salt solution (100 mM-KCl/1 mM- NaN_3 /2 mM-EGTA/2 mM- MgCl_2 /20 mM-potassium phosphate, pH 6.8) by four 15 s homogenizations in a Waring blender at 12 000, 12 000, 14 000 and 18 000 rev./min respectively, with 15 s pauses between each, and then centrifuged at 2000 g (r_{av} 29.7 cm) for 10 min (centrifugation conditions in Steps I–IV were identical). A portion of each supernatant resulting from Steps I–X was saved for electrophoretic analysis and the remainder was discarded.

Step II. The sediment was resuspended in 5 vol. of standard salt solution containing 1% Triton X-100 by homogenization for 10 s in a Waring blender at 12 000 rev./min (the method of homogenization in Steps II–XI was identical) and then centrifuged.

Step III. The sediment was resuspended in 5 vol. of 50 mM-Tris/HCl (pH 7.6)/5 mM-EDTA/1% Triton X-100 by homogenization and then centrifuged.

Step IV. The sediment was resuspended in 5 vol. of 150 mM-KCl/5 mM-EDTA/1% Triton X-100 by homogenization and then centrifuged.

Step V. The sediment (washed myofibrils) was resuspended in 5 vol. of 500 mM-NaCl/5 mM-EDTA/5 mM-ATP/1 mM-cysteine/40 mM-imidazole/HCl, pH 7.1, by homogenization, stirred gently on a magnetic stir-plate for 30 min, and then centrifuged at 13 800 g (r_{av} 15.2 cm) for 30 min (centrifugation conditions were identical in Steps V–VIII).

Step VI. Step V was repeated, except that the time on the magnetic stir-plate was increased to 4 h.

Step VII. The sediment was stored at –25°C overnight. The sediment was then removed from the centrifuge tubes while still in the frozen state, resuspended in 5 vol. of 1 M-KI/20 mM-imidazole/HCl, pH 7.1, by homogenization, stirred gently on a magnetic stir-plate for 2 h, and centrifuged.

Step VIII. The sediment was resuspended in 5 vol. of 1 M-KI/20 mM-imidazole/HCl, pH 7.1, and treated as in Step VII.

Step IX. The sediment was resuspended in 5 vol. of distilled water by homogenization and then centrifuged at 13 800 g (r_{av} 15.2 cm) for 15 min.

Step X. Repeat of Step IX.

Step XI. The sediment was resuspended in 3.3 vol.

of 1M-acetic acid/20mM-NaCl, by homogenization, stirred gently overnight at 4°C, and then centrifuged at 13800g (r_{av} , 15.2cm) for 1h. A portion of the sediment was saved for electrophoretic analysis and the remainder was discarded.

Step XII. The supernatant was filtered through Whatman 541 paper and then adjusted to pH 5.5 by the addition of 10M-NaOH. $(NH_4)_2SO_4$ was slowly added (14.4g/100ml of supernatant), the suspension was left standing for 30min, and then centrifuged at 13800g (r_{av} , 15.2cm) for 15min. A portion of the supernatant was saved for electrophoretic analysis and the remainder was discarded.

Step XIII. The sediment was dissolved in 40–50ml of 6M-urea/1mM-dithioerythritol/10mM-imidazole/HCl, pH 7.1 (the pH was re-adjusted to 7.1 as the sediment dissolved) by homogenization in a Dounce tissue grinder, dialysed overnight against the same solution, and centrifuged at 95500g (r_{av} , 7.0cm) for 2h. A portion of the sediment was saved for electrophoretic analysis and the remainder was discarded. The supernatant is the 'crude desmin.' Additional descriptions of the isolation of the crude desmin and subsequent purification are given in the Results section.

Column chromatography

Desmin was purified from the crude desmin extract by column chromatography by using procedures adapted from those described for purification of avian smooth-muscle desmin by Huiatt *et al.* (1980). Hydroxyapatite chromatography was done with Bio-Gel HT (Bio-Rad Laboratories, Richmond, CA, U.S.A.). The hydroxyapatite was suspended in 6M-urea/1mM-dithioerythritol/10mM-imidazole/HCl, pH 7.2, packed into a column (1.6cm × 30cm), and equilibrated with 3 column vol. of the same solution. The protein sample (crude desmin that had been dialysed against the equilibration solution) was applied to the column and eluted at a flow rate of 6ml/h with a linear 0–60mM-sodium phosphate gradient in the presence of 6M-urea/1mM-dithioerythritol/10mM-imidazole/HCl, pH 7.2. Fractions (3ml) were collected. The concentration of phosphate in selected fractions was measured by the method of Taussky & Shorr (1953).

Ion-exchange chromatography was done with DEAE-Sephacrose CL-6B (Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A.). The column material was suspended in 6M-urea/1mM-dithioerythritol/10mM-imidazole/HCl, pH 7.2, packed into a column (1.6cm × 30cm), and equilibrated with 3 column vol. of the same solution. Partially purified desmin resulting from chromatography of crude desmin on hydroxyapatite (fraction 3, Fig. 2) was concentrated in an ultrafiltration cell (Amicon Corporation, Lexington, MA, U.S.A.) with a PM-10

membrane and dialysed against 6M-urea/1mM-dithioerythritol/10mM-imidazole/HCl, pH 7.2. The protein was applied to the column and eluted at a flow rate of 6ml/h with a linear 0–250mM-NaCl gradient in the presence of 6M-urea/1mM-dithioerythritol/10mM-imidazole/HCl, pH 7.2. Fractions (3ml) were collected. Additional details on chromatography are given in the Figure legends. The NaCl concentration in selected fractions was determined by titrating chloride with 0.1M-AgNO₃, with K₂CrO₄ as an internal indicator as described by Robson *et al.* (1970). The eluted fractions were analysed by SDS/polyacrylamide-gel electrophoresis and pooled as appropriate. Urea was removed from the DEAE-Sephacrose-purified desmin by extensive dialysis against 10mM-Tris/acetate (pH 8.5)/1mM-dithioerythritol. The sample was clarified by centrifugation at 134000g (r_{av} , 5.9cm) for 1h and could be stored for at least 2 weeks under N₂ and toluene vapour at 0°C without noticeable degradation.

One-dimensional electrophoresis

Electrophoresis was performed on SDS/polyacrylamide slab gels (17cm × 16cm × 0.15cm) by using a discontinuous Tris/glycine buffer system (Laemmli, 1970). The concentrations of acrylamide (bisacrylamide/acrylamide, 1:30) used were 5% (w/v) in the stacking gel and 8% in the separating gel. All insoluble samples (myofibrils etc.) were dissolved in 5% (w/v) SDS/1% 2-mercaptoethanol/10mM-Tris/acetate, pH 8.5, and heated in a boiling-water bath for 10min. Samples were then centrifuged for 20min at 25°C at 2000g (r_{av} , 17.0cm) and filtered through Whatman 541 filter paper to remove any small amount of remaining insoluble material. The samples were then dialysed against several changes of 0.1% SDS/10mM-sodium phosphate, pH 6.8. Protein concentrations were measured and adjusted to 1mg/ml by addition of 0.1% SDS/10mM-sodium phosphate, pH 6.8. Samples were prepared for electrophoresis before loading as described by Bechtel (1979). A constant current of 12mA was applied until the Bromophenol Blue tracking dye reached the bottom of the gel. Gels were stained overnight in 50% (v/v) methanol/7% acetic acid/0.1% Coomassie Brilliant Blue R250. Gels were initially destained by gentle agitation in 50% methanol/7% acetic acid for 30min, followed by gentle agitation in several changes of 7% acetic acid until the background colour was decreased. Molecular weights were estimated by extrapolation of results obtained with proteins having known subunit molecular weights (myosin heavy chain, α -actinin, bovine serum albumin, avian smooth-muscle desmin, actin, tropomyosin and carbonic anhydrase).

Two-dimensional electrophoresis

Isoelectric focusing was done in the first dimension in the pH range 3.5–10.0 in 4%-acrylamide gels (120 mm long, 2.5 mm diameter) as described by O'Farrell (1975). Insoluble samples were first extracted overnight with 8 M-urea at 2–4°C and then centrifuged at 106 000 g (r_{av} , 5.9 cm) for 1 h. The supernatants were then equilibrated in sample buffer as described for soluble samples (O'Farrell, 1975). Electrophoresis in the second dimension was done in the presence of SDS on 10% polyacrylamide slab gels as described by Allen *et al.* (1978) by using the Laemmli (1970) buffer system. No stacking gel was used. Gels were stained and destained as described for cylindrical gels. Porcine skeletal actin, which migrates as the α -variant, and purified gizzard actin, which migrates primarily as the γ -variant, (Rubenstein & Spudich, 1977; Zechel & Weber, 1978) were used as standards. The two actins were purified from acetone-dried powders by the method of Spudich & Watt (1971).

Electron microscopy

Purified desmin, at a concentration of 1 mg/ml, was dialysed overnight against 100 mM-NaCl/1 mM-MgCl₂/10 mM-imidazole/HCl, pH 7.0, and then diluted to 0.2 mg/ml with the same solution. A drop of the diluted desmin suspension was placed on a copper grid that had been covered with carbon support film, left for 1–2 min, washed gently with water, and stained with aq. 1.5% uranyl acetate. After removal of excess stain, the negatively stained intermediate filaments were examined in an RCA EMU-4 electron microscope operated at 100 kV.

Other procedures

Protein concentrations were measured by the biuret method (Gornall *et al.*, 1949) as modified by Robson *et al.* (1968) or by the Folin–Lowry procedure (Lowry *et al.*, 1951). Urea solutions used in the present study were prepared fresh as needed and passed through a column of Amberlite MB-3 (Mallinckrodt, St. Louis, MO, U.S.A.) ion-exchange resin to remove cyanate and heavy-metal-ion contamination. Quantification of the percentage desmin in fractions obtained at selected steps during the isolation and purification procedures was accomplished by densitometry of cylindrical SDS/polyacrylamide gels that had been run on the appropriate samples by the method of Weber & Osborn (1969) as modified by Suzuki *et al.* (1976). Gels were scanned with a Zeiss PMQ II spectrophotometer equipped with a t.l.c. attachment modified to accept cylindrical gels. Stained gels were scanned at 550 nm, and peak areas were measured with a Spectra-Physics System I computing integrator (Spectra-Physics, Santa Clara, CA, U.S.A.) that was connected directly to the output of the spectrophotometer.

Results

Preparation of crude desmin

The major objective of the present study was to devise a satisfactory method for preparing purified desmin from skeletal muscle of mature mammalian animals. A very small amount of material migrating on SDS/polyacrylamide gels with a mobility corresponding to a mol.wt. of 55 000 was observed in preliminary experiments in which we were preparing fractions enriched in Z-line material. Results of those studies, plus those reported by Small & Sobieszek (1977) on isolation of avian smooth-muscle skeleton (desmin) formed the rationale for preparation of crude desmin as outlined in the purification scheme in the Materials and methods section. Steps I–IV consist of preparation of washed myofibrils from ground porcine skeletal muscle. Unambiguous identification of a polypeptide migrating with a mobility corresponding to an approximate mol.wt. of 55 000 in the homogenized muscle sample (lane b, Fig. 1) is difficult, although a very faint band (between two others of ~54 000 and 57 000) can occasionally be observed at this position, and desmin can be unambiguously identified in two-dimensional gels of muscle homogenates (results not shown; also see Izant & Lazarides, 1977). Approx. 40% of the protein in the original homogenate is removed during preparation of the washed myofibrils. As expected, this material consists of a heterogeneous mixture of proteins, corresponding primarily to sarcoplasmic proteins plus a small amount of myofibrillar proteins (a few myofibrils not sedimented at the low centrifugal forces used) (lane c, Fig. 1). The two bands corresponding to molecular weights of approx. 54 000 and 57 000 are removed during preparation of the washed myofibrils. Desmin is, at best, faintly visible in the gel of the washed myofibril (lane d, Fig. 1), but can be seen at heavier loads. Extraction of the myofibrils with the high-ionic-strength solutions (500 mM-NaCl/5 mM-EDTA/5 mM-ATP/1 mM-cysteine/40 mM-imidazole/HCl, pH 7.1) in steps V and VI removed most of the myosin and much of the actin and tropomyosin (compare high-ionic-strength extract of the myofibril in lane f with the residue remaining after the two high-ionic-strength washes on lane e in Fig. 1). A polypeptide migrating with a mol.wt. of 55 000 is, for the first time, clearly visible (at this protein load) in the gel of the residue after the high-ionic-strength extraction (lane e, Fig. 1). Extraction of this material with KI (Steps VII and VIII in purification scheme) removes much of the remaining thin-filament proteins as expected, together with much of the α -actinin (lane h, Fig. 1). Two washes with water were used (Steps IX and X), to remove the KI. The residue remaining after the KI treatment and the two water washes consists primarily of significant

Fig. 1. SDS/polyacrylamide-gel electrophoresis of fractions obtained at selected steps during preparation of crude desmin as outlined in the purification scheme in the Materials and methods section

a, standards (myosin heavy chain, α -actinin, bovine serum albumin, avian smooth-muscle desmin, actin and carbonic anhydrase); b, the whole-muscle homogenate (Step I before centrifugation); c, protein removed in standard-salt-solution wash (supernatant resulting from Step I); d, washed myofibrils (sediment resulting from Step IV); e, residue remaining after second extraction with high-ionic-strength solution (sediment resulting from Step VI); f, protein removed in first extraction with high-ionic-strength solution (supernatant resulting from Step V); g, residue remaining after two extractions with KI solution and two water washes (sediment resulting from Step X); h, protein removed in first extraction with KI solution (supernatant resulting from Step VII); i, protein dissolved in the acetic acid extract and precipitated with $(\text{NH}_4)_2\text{SO}_4$ (sediment resulting from Step XII); j, residue remaining after extraction with acetic acid (sediment resulting from Step XI); k, protein dissolved in 6M-urea solution (supernatant resulting from Step XIII=crude desmin); and l, residue remaining after extraction with 6M-urea solution (sediment resulting from Step XIII). The arrow denotes location of mammalian desmin. The amount of protein loaded was 30 μg in b and c, 20 μg in d-h, and 15 μg in i-l. In the Laemmli (1970) gel-electrophoretic system, avian smooth-muscle desmin migrates with a mobility corresponding to a chain weight of 53 000 daltons, whereas mammalian desmin migrates with a mobility corresponding to a chain weight of 55 000 daltons (O'Shea *et al.*, 1979).

amounts of poorly resolved high-molecular-weight components, residual myosin and α -actinin, and prominent amounts of desmin and actin (lane g, Fig. 1). In this residue, desmin constitutes approx. 5% of

the protein present (Table 1). Extraction and solubilization of the desmin in 1M-acetic acid (Step XI) resulted in a significant increase in proportion of desmin (lane i, Fig. 1). In particular, the relative proportions of actin and desmin were reversed after the acetic acid extraction, a result in accordance with that of Small & Sobieszek (1977) in studies on the smooth-muscle system. A small amount of desmin was left behind (i.e. not extracted) during the acetic acid-solubilization step (see lane j, Fig. 1). The protein extracted by acetic acid was precipitated by adjustment of the pH to 5.5. $(\text{NH}_4)_2\text{SO}_4$ was added to ensure quantitative precipitation of the desmin (Step XII). The sediment was then dissolved in a 6M-urea-containing solution (Step XIII), clarified, and represents the 'crude desmin'. A rather large amount of high-molecular-weight material, plus significant amounts of actin and α -actinin, and several other minor contaminants were present with desmin in the crude desmin extract (lane k, Fig. 1).

Chromatographic purification of desmin

Chromatography of crude desmin on hydroxyapatite in the presence of urea resulted in marked improvement in degree of purity (Fig. 2). Approx. 25% of the material loaded did not bind to the column (fraction 1, Fig. 2). It consisted primarily of three to four components having very large subunit mol.wts. of 200 000 and over, and species corresponding in size to 141 000, 117 000, 58 000 (possibly vimentin), 42 000 (co-migrated with actin) and 35 000. Fraction 2 also was heterogeneous in composition, having primarily 55 000-dalton (desmin), 42 000-dalton and two to three smaller components. Fraction 3 (Fig. 2) contained most of the desmin that had been present in the crude desmin, together with several impurities, most notably those having subunit mol.wts. of 39 000 and 50 000. The 50 000-dalton component is probably a breakdown product of desmin, because it increases in amount during prolonged storage. The trailing edge (fraction 4, Fig. 2) of the desmin peak consists primarily of desmin, the 50 000- and 39 000-dalton components, and several components (each present in very small amounts) larger than desmin. The remainder of the trailing desmin peak, together with more tightly adsorbed material, was eluted as a sharp boundary with 200 mM-phosphate. Besides desmin, it contained material migrating at approx. 35 000 daltons and several components migrating above desmin.

Chromatography of the hydroxyapatite-purified desmin (fraction 3, Fig. 2) on DEAE-Sephacel CL-6B in the presence of urea resulted in the profile shown in Fig. 3. Fraction 1 contained the 39 000-dalton contaminant. Desmin was eluted as a symmetrical peak between about 145 and 160 mM-NaCl. The trailing edge (fraction 3, Fig. 3) con-

Fig. 2. Elution profile of crude desmin from a hydroxyapatite column

A sample of 480 mg of crude desmin in 70 ml was loaded on the column and eluted as described in the Materials and methods section. The broken line (----) represents the linear phosphate gradient. Tightly bound protein (fraction 5) was eluted with 200 mM-phosphate (added at the arrow). The inset shows an SDS/polyacrylamide slab gel of the original crude desmin (CD) and the pooled fractions (1, 2, 3, 4 and 5) represented by the vertical lines (---) in the elution profile. The amount of protein loaded was 15 µg of crude desmin, fractions 1, 2 and 4, and 20 µg of fractions 3 and 5. The extreme right-hand lane corresponds to the standards myosin heavy chain, α-actinin, bovine serum albumin, avian smooth-muscle desmin, actin and carbonic anhydrase. Fraction 3 is the hydroxyapatite-purified desmin.

tained more of the 50 000-dalton breakdown product than did the first portion of the peak (fraction 2).

The DEAE-Sepharose-purified desmin (fraction 2, Fig. 3) was examined electrophoretically with both light (only one band visible; results not shown) and heavy protein loads. It was routinely over 97% (Table 1) pure as measured by densitometry of stained SDS/polyacrylamide gels. The major contaminant present in the purified desmin was the 50 000-dalton polypeptide that usually accounted for 1–3% of the total protein. No actin was detectable. Typical yields of recovered purified desmin ranged from 20 to 30 mg from preparations initiated with 600 g of ground muscle.

A summary of the purification procedure, based on quantitative densitometry of SDS/polyacrylamide cylindrical gels of selected fractions, is given in Table 1. The starting point for the summary was the washed myofibrils (as opposed to homogenized muscle), because desmin could not be reliably measured even at heavy protein loads, nor identified apart from other proteins, in earlier fractions. Desmin makes up approx. 0.35% of the total protein in myofibrils and 18% of the protein recovered from the acetic acid extract. Approx. 14% recovery was achieved by the overall procedure. Some losses were incurred during the early stages of the preparation, primarily due to the large number of

Table 1. *Purification of desmin from mammalian skeletal muscle*
Results are from a representative preparation, 600 g (wet weight) of porcine skeletal muscle being used.

Purification step	Total protein (mg)	Purity (%) [*]	Total desmin (mg) [†]	Recovery [‡] (%)	Purification (fold)
Washed myofibrils (sediment at end of Step IV in the purification scheme)	50 000	0.35	175	100	1
Residue after extractions with KI and water (sediment at end of Step X in the purification scheme)	2800	5.3	148	85	15
Acetic acid extract after precipitation with (NH ₄) ₂ SO ₄ (sediment at end of Step XII in the purification scheme)	480	18	86	49	51
Hydroxyapatite-purified desmin (fraction 3, Fig. 2)	48	88	42	24	251
DEAE-Sepharose-purified desmin (fraction 2, Fig. 3)	25	97	24	14	277

^{*} Calculated by quantification of SDS/polyacrylamide gels as described in the Materials and methods section.

[†] Total protein × purity.

[‡] Based on desmin present in washed myofibrils (≡100%).

Fig. 3. *Elution profile of hydroxyapatite-purified desmin from a DEAE-Sepharose CL-6B column*

A sample of 48 mg of hydroxyapatite-purified desmin (fraction 3, Fig. 2) in 35 ml was loaded on the column and eluted as described in the Materials and methods section. The broken line (---) represents the NaCl gradient. The inset shows an SDS/polyacrylamide slab gel of the hydroxyapatite-purified desmin (HPT) and the pooled fractions (1, 2 and 3) represented by the vertical lines (---) in the elution profile. The amount of protein loaded was 20 µg of hydroxyapatite-purified desmin, fraction 2 and fraction 3, and 10 µg of fraction 1. The right-hand lane corresponds to the standards myosin heavy chain, α-actinin, bovine serum albumin, avian smooth-muscle desmin, actin, gizzard tropomyosin doublet (TM; 39 000 and 36 000 mol.wt.) and carbonic anhydrase. Fraction 2 is the DEAE-Sepharose-purified desmin.

homogenization, extraction and centrifugation steps needed, and owing to incomplete extraction of desmin by acetic acid. Losses also occurred during the chromatography, primarily because of discrimination used in selecting the pooled fractions and deemed necessary to achieve an acceptable final degree of purity. Overall, approx. 250–300-fold purification was achieved during isolation of desmin from the myofibrils.

Two-dimensional gel analysis of desmin and actin

Lazarides and associates (Izant & Lazarides, 1977; Lazarides & Balzer, 1978) have reported that chicken muscle desmin is resolved into two isoelectric variants (α and β) and that rat or guinea-pig muscle desmin consists primarily of one variant when crude actomyosin extracted residues or myofibrils are analysed in a two-dimensional gel-electrophoretic system. We have examined the extracts and residues resulting from preparation of crude porcine skeletal-muscle desmin (Fig. 4) as

outlined in the purification scheme in the Materials and methods section and fractions obtained during the chromatographic purification of the desmin (Fig. 5) by this technique.

Porcine skeletal actin (α -actin) and gizzard actin (primarily γ -actin) (Rubenstein & Spudich, 1977; Zechel & Weber, 1978) were used to correctly identify positions of the desmin and actin variants. Two-dimensional gel electrophoresis of an 8 M-urea extract of washed myofibrils (Fig. 4a) shows one major spot corresponding to a mol.wt. of 55 000. A smaller, minor spot at the same molecular weight, but on the acidic side of the major desmin variant, was also usually present. α -Actin is the actin variant present (verified by co-migration with actin standards; results not shown). The two (α and β) subunits of tropomyosin can also be identified in this sample. The actin removed in the actomyosin extracted with the 0.5 M-NaCl-containing solutions (Steps V and VI; lane f, Fig. 1) was the α -actin variant (results not shown), as was the actin (Figs. 4b and 4c) removed with the KI extractions (Steps

Fig. 4. Two-dimensional gel electrophoresis of fractions obtained during preparation of crude desmin as outlined in the purification scheme in the Materials and methods section

The directions of migration in both the first dimension (isoelectric focusing) and second dimension (electrophoresis) are indicated. The acidic end of the pH gradient in the isoelectric-focusing dimension is to the left. Spots corresponding to the isoelectric variants of actin, desmin and tropomyosin (TM) are labelled. Samples were as follows: (a) 8 M-urea extract of washed myofibrils prepared from mature porcine skeletal muscle (sediment at end of Step IV); (b) protein removed in first extraction with KI solution (supernatant resulting from Step VII); (c) KI extract shown in (b) with purified porcine skeletal (α) and chicken gizzard (γ) actins added as standards; (d) 8 M-urea extract of residue remaining after two extractions with KI solutions and two water washes (sediment resulting from Step X). High-molecular-weight polypeptides such as myosin heavy chains do not enter the isoelectric-focusing gel and are not resolved.

EXPLANATION OF PLATE 1

Electron micrograph of negatively stained 10 nm filaments formed from purified porcine skeletal-muscle desmin by dialysis against 100 mM-NaCl/1 mM-MgCl₂/10 mM-imidazole/HCl, pH 7.0
The horizontal bar represents 200 nm.

Fig. 5. Two-dimensional gel electrophoresis of fractions obtained during the chromatographic purification of desmin. The acidic end of the pH gradient in the isoelectric-focusing dimension is to the left. Spots corresponding to the isoelectric variants of desmin and actin are labelled. Samples were as follows: (a) hydroxyapatite-purified desmin (fraction 3, Fig. 2); (b) hydroxyapatite-purified desmin shown in (a) with purified porcine skeletal and chicken gizzard actins added as standards; (c) DEAE-Sephacel-purified desmin (fraction 2, Fig. 3); (d) DEAE-Sephacel-purified desmin shown in (c) with purified porcine skeletal and chicken gizzard actins added as standards.

VII and VIII; lane h, Fig. 1). The residual α -actin and most of the desmin (Fig. 4d) remaining after the KI (and two water washes) migrated essentially as they had in the original washed myofibril (Fig. 4a), except that the amount of desmin relative to actin has increased as expected (and see lane g, Fig. 1). The very minor, more acidic (relative to major desmin variant and just slightly more acidic than α -actin) spot at 55 000 daltons is again present.

Fig. 5 shows the results of two-dimensional gel electrophoresis of desmin fractions obtained during chromatography. The actin in the crude desmin (results not shown) and the very small amount of residual actin in the hydroxyapatite-purified desmin (Figs. 5a and 5b) migrated entirely as the α -variant. The migration of the final DEAE-Sephacel-purified desmin (Fig. 5c) was essentially the same as that seen in the original myofibril (Fig. 4a), with one major desmin variant and one minor spot migrating at its acidic side. The absence of additional spots in the sample of purified desmin (Fig. 5c), and the fact that the isoelectric position of desmin relative to the actin standards (Fig. 5d) did not change during the preparation indicate that the purification procedure has not resulted in significant charge modification. A number of additional, more acidic (relative to desmin) spots were obtained at a mol.wt. of 55 000

in samples that had been dissolved and chromatographed in urea at room temperature.

Reconstitution of 10 nm filaments from purified desmin

The purified desmin remained in solution after removal of urea by extensive dialysis against 1 mM-dithioerythritol/10 mM-Tris/acetate, pH 8.5, and clarification at 134 000 g (r_{av} , 5.9 cm) for 1 h. Samples of the desmin (1 mg/ml) were then dialysed overnight against 100 mM-NaCl/1 mM-MgCl₂/10 mM-imidazole/HCl, pH 7.0, at 2°C. Examination under the electron microscope of the resulting transparent viscous suspension after negative staining demonstrated that the desmin has assembled into filaments having an average diameter of about 10 nm (Plate 1). There was some variation in filament diameter [range 9–11.5 nm; $10.4 \text{ nm} \pm 0.16$ (mean $\pm$ s.e.m.)] and some instances of side-to-side aggregation of filaments. It was difficult to measure lengths of the filaments, but many individual filaments could be observed that were several micrometres long. More than 90% of the total protein under these solvent conditions was sedimented by centrifugation at 12 000 g (r_{av} , 7.1 cm) for 30 min (determined by measurement of protein remaining in supernatant).

Discussion

The studies described herein demonstrate that the small amount of desmin known to be present in skeletal-muscle cells (Izant & Lazarides, 1977; Fellini *et al.*, 1978) can be purified to near homogeneity and free of electrophoretically detectable quantities of actin. For reasons outlined previously (O'Shea *et al.*, 1979), it is unlikely that any significant amount of the desmin prepared from mature mammalian skeletal muscle by our procedure originated from contaminating vascular smooth-muscle cells. The purification scheme developed consists of three major phases.

(1) Preparation of high-ionic-strength (NaCl) and KI-extracted residues from well-washed myofibrils. This phase both decreases the amounts of any contaminating myofibrillar and cytoskeletal proteins from contaminating cell types and permits considerable enrichment of desmin, which, as for smooth-muscle desmin (Cooke, 1976; Schollmeyer *et al.*, 1976; Small & Sobieszek, 1977), has limited solubility in both low- and high-ionic-strength buffers. We reported recently (Huiatt *et al.*, 1980) that the use of KI for removal of additional actin after high-ionic-strength extraction (Small & Sobieszek, 1977; Lazarides & Hubbard, 1976) was unnecessary for purification of desmin from smooth muscle provided that the protein was subjected to purification by chromatography. The amount of desmin in skeletal muscle, however, was so small that the additional enrichment of desmin resulting from the KI extraction was a useful step. It also served to differentiate desmin from contamination by a putative Z-line protein having a chain weight of 55 000, which is soluble in KI and remains soluble in non-denaturing solvents (Ohashi & Maruyama, 1979).

(2) Solubilization of desmin in 1M-acetic acid. This step, which is from the smooth-muscle procedure of Small & Sobieszek (1977), also permitted the relative enhancement of desmin relative to other proteins such as actin. Without this step, the two chromatographic columns were not sufficient for preparation of homogeneous skeletal-muscle desmin. Repeated cycles of solubilization of desmin by 1M-acetic acid and subsequent precipitation of desmin by neutralization by the method of Small & Sobieszek (1977), however, did not give clean or satisfactory separation of desmin from actin and other components. Fellini *et al.* (1978), Hubbard & Lazarides (1979) and Johnson & Yun (1980), who used acetic acid-solubilization steps in their studies on smooth-muscle desmin, have experienced similar problems.

(3) Chromatographic purification of acetic acid-solubilized desmin in the presence of urea. Others [Small & Sobieszek (1977) for smooth-muscle

skeleton/desmin; Stigbrand *et al.* (1979a,b) for 10nm-filament protein from Purkinje cells] have used gel-permeation chromatography in the presence of SDS for preparation of intermediate-filament proteins. We wished to avoid SDS for routine preparation because of uncertainties in getting 100% removal of the detergent. The chromatographic purification steps used herein, which were adapted from our previous studies on smooth-muscle desmin (Huiatt *et al.*, 1980), gave very reproducible results. The hydroxyapatite column, in particular, was quite effective in removing most contaminants from desmin. There was no detectable actin in the desmin after the second chromatographic column as judged by electrophoresis done by the Laemmli (1970) or Weber & Osborn (1969) methods.

The yield of purified desmin obtained herein (6mg/100g of ground muscle) contrasts with the 29-fold higher yield (150–200mg of purified desmin/100g of ground muscle) obtained from gizzard (Huiatt *et al.*, 1980). This, however, reflects the much higher concentration of desmin in smooth muscle [desmin makes up about 8% of 'washed' avian smooth-muscle myofibrils (Huiatt *et al.*, 1980) compared with about 0.35% of skeletal-muscle myofibrils]. Comparison of desmin yields with other work on skeletal muscle cannot be done because, insofar as we know, this is the first report on purified skeletal-muscle desmin.

Examination of the purified desmin by two-dimensional gel electrophoresis demonstrated the presence of one major desmin variant, a result in concordance with that of Lazarides & Balzer (1978), who identified only one major variant in two-dimensional gel electrophoresis of mammalian skeletal myofibrils as opposed to two major (α and β) variants in avian muscle desmin. A minor, slightly more acidic spot, however, was also routinely present. This acidic spot could be detected even in the early stages of the procedure if the gels were overloaded; thus, it did not arise from the procedure itself. Lazarides and associates (Gard *et al.*, 1979; O'Connor *et al.*, 1979) have recently identified a minor, more acidic (relative to α -desmin of avian muscle or to major desmin variant of mammalian cells) isoelectric variant of desmin. We are not certain our minor, more acidic spot is identical with theirs. Our one- and two-dimensional gels demonstrated clearly that we have no fibroblastic intermediate filament protein (termed 'vimentin' by Franke *et al.*, 1978) in our purified skeletal-muscle desmin [vimentin has a slightly higher molecular weight and focuses at a considerably more acidic position relative to desmin (Gard *et al.*, 1979; Tuszynski *et al.*, 1979)]. There is general agreement (Bennett *et al.*, 1979; Gard & Lazarides, 1980) that, during myogenesis, presumptive myoblasts synthesize vimentin and that desmin synthesis is

initiated at the onset of fusion. There is disagreement, however; as to how long vimentin continues to be synthesized and present. Bennett *et al.* (1979) found that vimentin, although present in myoblasts and early myotubes, disappears within 10 to 14 days in culture. Gard & Lazarides (1980), however, reported that vimentin synthesis continues through 20 days in culture, and Granger & Lazarides (1979) reported that they could identify some vimentin in adult (age not given) avian skeletal-muscle myofibrils. We could not detect vimentin in our purified porcine skeletal-muscle desmin by two-dimensional gels even when it was grossly overloaded with protein. Nor could we unambiguously identify any significant amount of vimentin in two-dimensional gels of well-washed adult porcine skeletal myofibrils. Overall our results suggest that vimentin plays a minor role (if any) in adult mammalian skeletal-muscle cells. Our results are more in accord with those of Bennett *et al.* (1979), although it is noteworthy that our studies were done with adult mammalian skeletal muscle and that Granger & Lazarides (1979) stated that the ratio of vimentin to desmin is greater in muscles of young chickens than in older chickens. We also were unable to detect any 68 000-dalton neurofilament protein in our purified desmin. Wang *et al.* (1980) has suggested that this component is also a component of chicken skeletal-muscle myofibrils.

Although it has been reported previously that skeletal muscle contains essentially one actin variant (α), it seemed conceivable that a different, but very minor, variant might co-purify with desmin and become more easily detected during the preparation. Our results, however, demonstrated that the actin solubilized by acetic acid and even the last trace of detectable actin occasionally still present in the hydroxyapatite-purified desmin were the α -actin variant.

The only known role for desmin is that it is a major constituent of 10 nm filaments. By using this as a functional test, we demonstrated formation of 10 nm filaments from highly purified skeletal-muscle desmin under near-physiological conditions of pH and ionic strength. This strongly suggests that our protein has adopted a native structure after removal of urea (also see discussion in Huiatt *et al.*, 1980). The synthetic filaments look similar to native 10 nm filaments from smooth muscle (Cooke, 1976; Small & Sobieszek, 1977; Huiatt *et al.*, 1980) and to synthetic filaments prepared from highly purified avian smooth-muscle desmin (Huiatt *et al.*, 1980).

It remains puzzling that antibodies to smooth-muscle desmin (Lazarides & Hubbard, 1976; Campbell *et al.*, 1979; F. Richardson, M. Stromer, T. Huiatt, & R. Robson, unpublished work) or to mammalian skeletal-muscle desmin (F. Richardson, J. O'Shea, R. Robson, M. Stromer & P. Bechtel

unpublished work) bind to, or at the periphery of, skeletal-muscle Z-lines, but that 10 nm filaments have never been unambiguously identified in normal adult skeletal-muscle cells (see discussion in Bennett *et al.*, 1979). They have been observed in cells of the working myocardium (Virágh & Challice, 1969; Rash *et al.*, 1970; Ferrans & Roberts, 1973; Behrendt, 1977) and in heart Purkinje fibres (Oliphant & Loewen, 1976; Eriksson & Thornell, 1979; Stigbrand *et al.*, 1979a,b). Lack of identification of 10 nm filaments in adult skeletal-muscle cells may simply reflect the small number of such filaments present, but it is conceivable that desmin is present, but not in the form of 10 nm filaments. That properties of the skeletal-muscle protein (such as amino acid composition and peptide 'maps') show considerable homology with those of smooth-muscle desmins (O'Shea *et al.*, 1979) and that the highly purified skeletal-muscle protein will self-assemble into 10 nm filaments argues, albeit indirectly, for their presence in skeletal-muscle cells.

It has been proposed that desmin plays an important role in organizing myofibrils in striated muscle cells (Granger & Lazarides, 1978; Lazarides, 1980), presumably through its attachment to Z-lines of adjacent myofibrils. Yet nothing is known regarding the components of Z-lines and myofibrils that are capable of interacting with desmin. Ability to prepare highly purified fractions of skeletal-muscle desmin now makes this problem amenable to biochemical investigation.

We are grateful to Ms. Mary Bremner for expert technical assistance and to Ms. Joan Andersen for typing of the manuscript. This research was supported, in part, by grants from the Muscular Dystrophy Association (MDA) and the National Institutes of Health (no. HL-15679), by an MDA Fellowship to J. M. O'S., and by a grant-in-aid from the American Heart Association (no. 79-948) with funds contributed in part by the Iowa Affiliate. This is Journal Paper no. J-9851 of the Iowa Agriculture and Home Economics Experiment Station, Ames; projects nos. 2361 and 2127.

References

- Allen, R. E., Stromer, M. H., Goll, D. E. & Robson, R. M. (1978) *J. Cell Biol.* **76**, 98–104
- Bechtel, P. J. (1979) *J. Biol. Chem.* **254**, 1755–1758
- Behrendt, H. (1977) *Cell Tissue Res.* **180**, 303–315
- Bennett, G. S., Fellini, S. A., Croop, J. M., Otto, J. J., Bryan, J. & Holtzer, H. (1978a) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4364–4368
- Bennett, G. S., Fellini, S. A. & Holtzer, H. (1978b) *Differentiation* **12**, 71–82
- Bennett, G. S., Fellini, S. A., Toyama, Y. & Holtzer, H. (1979) *J. Cell Biol.* **82**, 577–584

- Campbell, G. R., Chamley-Campbell, J., Gröschel-Stewart, U., Small, J. V. & Anderson, P. (1979) *J. Cell Sci.* **37**, 303–322
- Cooke, P. (1976) *J. Cell Biol.* **68**, 539–556
- Dahl, D. & Bignami, A. (1975) *Biochim. Biophys. Acta* **386**, 41–51
- Davies, P. J., Wallach, D., Willingham, M. C., Pastan, I., Yamaguchi, M. & Robson, R. M. (1978) *J. Biol. Chem.* **253**, 4036–4042
- Davison, P. F., Hong, B.-S. & Cooke, P. (1977) *Exp. Cell Res.* **109**, 471–474
- Dayton, W. R., Goll, D. E., Zeece, M. G., Robson, R. M. & Reville, W. J. (1976) *Biochemistry* **15**, 2150–2158
- Eriksson, A. & Thornell, L.-E. (1979) *J. Cell Biol.* **80**, 231–247
- Fellini, S. A., Bennett, G. S., Toyama, Y. & Holtzer, H. (1978) *Differentiation* **12**, 59–69
- Ferrans, V. J. & Roberts, W. C. (1973) *J. Mol. Cell. Cardiol.* **5**, 247–257
- Franke, W. W., Schmid, E., Osborn, M. & Weber, K. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5034–5038
- Franke, W. W., Schmid, E., Bretkreutz, D., Lüder, M., Boukamp, P., Fusenig, N. E., Osborn, M. & Weber, K. (1979) *Differentiation* **14**, 35–50
- Gard, D. L. & Lazarides, E. (1980) *Cell* **19**, 263–275
- Gard, D. L., Bell, P. B. & Lazarides, E. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3894–3898
- Gilbert, D. (1978) *Nature (London)* **272**, 577–578
- Goldman, R. & Knipe, D. (1973) *Cold Spring Harbor Symp. Quant. Biol.* **37**, 523–534
- Goldman, R. D., Milsted, A., Schloss, J. A., Starger, J. & Yerna, M.-J. (1979) *Annu. Rev. Physiol.* **41**, 703–722
- Gornall, A. G., Bardawill, C. J. & David, M. M. (1949) *J. Biol. Chem.* **177**, 751–766
- Granger, B. L. & Lazarides, E. (1978) *Cell* **15**, 1253–1268
- Granger, B. L. & Lazarides, E. (1979) *Cell* **18**, 1053–1063
- Hubbard, B. D. & Lazarides, E. (1979) *J. Cell Biol.* **80**, 166–182
- Huiatt, T. W., Robson, R. M., Arakawa, N. & Stromer, M. H. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 1607
- Huiatt, T. W., Robson, R. M., Arakawa, N. & Stromer, M. H. (1980) *J. Biol. Chem.* **255**, 6981–6989
- Hynes, R. O. & Destree, A. T. (1978) *Cell* **13**, 151–163
- Ishikawa, H., Bischoff, R. & Holtzer, H. (1968) *J. Cell Biol.* **38**, 538–555
- Izant, J. G. & Lazarides, E. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1450–1454
- Johnson, P. & Yun, J. S. (1980) *Int. J. Biochem.* **11**, 143–154
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Lazarides, E. (1978) *Exp. Cell Res.* **112**, 265–273
- Lazarides, E. (1980) *Nature (London)* **283**, 249–256
- Lazarides, E. & Balzer, D. R., Jr. (1978) *Cell* **14**, 429–438
- Lazarides, E. & Granger, B. L. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3683–3687
- Lazarides, E. & Hubbard, B. D. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4344–4348
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- O'Connor, C. M., Balzer, D. R., Jr. & Lazarides, E. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 819–823
- O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007–4021
- Ohashi, K. & Maruyama, K. (1979) *J. Biochem. (Tokyo)* **85**, 1103–1105
- Oliphant, L. W. & Loewen, R. D. (1976) *J. Mol. Cell. Cardiol.* **8**, 679–688
- O'Shea, J. M., Robson, R. M., Huiatt, T. W., Hartzer, M. K. & Stromer, M. H. (1979) *Biochem. Biophys. Res. Commun.* **89**, 972–980
- Rash, J. E., Bieseke, J. J. & Gey, G. O. (1970) *J. Ultrastruct. Res.* **33**, 408–435
- Robson, R. M. & Zeece, M. G. (1973) *Biochim. Biophys. Acta* **295**, 208–224
- Robson, R. M., Goll, D. E. & Temple, M. J. (1968) *Anal. Biochem.* **24**, 339–341
- Robson, R. M., Goll, D. E., Arakawa, N. & Stromer, M. H. (1970) *Biochim. Biophys. Acta* **200**, 296–318
- Rubenstein, P. A. & Spudich, J. A. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 120–123
- Schlaepfer, W. W. & Lynch, R. G. (1977) *J. Cell Biol.* **74**, 241–250
- Schollmeyer, J. E., Furcht, L. T., Goll, D. E., Robson, R. M. & Stromer, M. H. (1976) in *Cell Motility* (Goldman, R., Pollard, T. & Rosenbaum, J., eds.), vol. 3, Book A, pp. 361–388, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- Singh, I., Goll, D. E., Robson, R. M. & Stromer, M. H. (1977) *Biochim. Biophys. Acta* **491**, 29–45
- Small, J. V. & Sobieszek, A. (1977) *J. Cell Sci.* **23**, 243–268
- Spudich, J. A. & Watt, S. (1971) *J. Biol. Chem.* **246**, 4866–4871
- Steinert, P. M., Idler, W. W. & Zimmerman, S. B. (1976) *J. Mol. Biol.* **108**, 547–567
- Steinert, P. M., Zimmerman, S. B., Starger, J. M. & Goldman, R. D. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 6098–6101
- Stigbrand, T., Eriksson, A. & Thornell, L.-E. (1979a) *Biochim. Biophys. Acta* **577**, 52–60
- Stigbrand, T., Eriksson, A. & Thornell, L.-E. (1979b) *Acta Chem. Scand. B* **33**, 620–621
- Suzuki, A., Goll, D. E., Singh, I., Allen, R. E., Robson, R. M. & Stromer, M. H. (1976) *J. Biol. Chem.* **251**, 6860–6870
- Taussky, H. H. & Shorr, E. (1953) *J. Biol. Chem.* **202**, 675–685
- Tuszynski, G. P., Frank, E. D., Damsky, C. H., Buck, C. A. & Warren, L. (1979) *J. Biol. Chem.* **254**, 6138–6143
- Uehara, Y., Campbell, G. R. & Burnstock, G. (1971) *J. Cell Biol.* **50**, 484–497
- Virágh, S. & Challice, C. E. (1969) *J. Ultrastruct. Res.* **28**, 321–334
- Wang, C., Asai, D. J. & Lazarides, E. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1541–1545
- Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412
- Yamaguchi, M., Robson, R. M., Stromer, M. H. & Dahl, D. S. (1978) *Nature (London)* **271**, 265–267
- Zechel, K. & Weber, K. (1978) *Eur. J. Biochem.* **89**, 105–112
- Zeece, M. G., Robson, R. M. & Bechtel, P. J. (1979) *Biochim. Biophys. Acta* **581**, 365–370