and rabbit spermatozoa, on the other hand, reflects a true species difference or is due to methodological differences. The structural localization of retinol in human spermatozoa was not studied. In rabbit and bull spermatozoa, most of the vitamin A has been postulated to be bound to the acrosome and/or the plasma membrane overlying the acrosome[4,6].

When the hydrolysis of the seminal plasma was omitted the vitamin A concentration was approximately half of that obtained after hydrolysis. When hydrolysis of the spermatozoa was omitted only 5-20% of the retinol was recovered. This may indicate that the vitamin A in the seminal fluid occurs as retinyl esters rather than free retinol and that the vitamin is intimately bound to sperm structures and/or occurs exclusively as a conjugated compound (e.g. retinyl ester).

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Myosin heavy chains in fast skeletal muscle of chick embryo

L. Dalla Libera[1]

C.N.R. Unit for Muscle Biology and Physiopathology, Institute of General Pathology, University of Padova, Via Loredan 16, I-35100 Padova (Italy), 20 March 1981

Summary. The peptide map obtained by electrophoresis after digestion of purified myosin heavy chains from pectoralis muscle of embryonic chicken with the *Staphylococcus aureus* V8 protease, produces a peptide pattern very similar but not identical to that of adult fast myosin. In fact, some components that are present in a small amount in the map of slow adult myosin are visible in the embryonic pattern.

The data available in the literature about the nature of the myosin(s) in embryonic muscle tissues are still very controversial. In fact, while some authors suggested that in embryonic tissues only 1 type of myosin exists, which closely resembles that of fast skeletal muscle[2-6], it has been demonstrated that developing rat skeletal muscle in embryos or in tissue culture contains a distinct embryonic form of myosin[7-9]. Similar results were obtained in rabbit[10-12] and in chick embryo[13]. In contrast, evidence obtained by means of immunofluorescence studies has indicated on the one hand that early myotubes of the chick can synthesize slow, fast and cardiac myosins[14-17], but, on the other hand, that fetal diaphragm contains only slow-type myosin in addition to fast-type moysin[18]. The discrepancy between these different results may be due to the material examined and the techniques used. In this study, in order to analyze myosin types in immature skeletal muscle more precisely, myosin was purified from the pectoralis muscles of embryonic chickens and the composition of heavy chains was investigated by means of proteolytic mapping of the electrophoretically purified heavy chain portion of the molecule[13].

Materials and methods. The fast twitch pectoralis of 14-day-old embryo Leghorn chicks was studied. For comparative studies myosin was also isolated from the pectoralis, anterior latissimus dorsi (ALD) and the ventricles of adult chicken. Myosin was purified according to the previously reported procedure[19], as specifically detailed by Dalla Libera et al.[20]. Digestion of electrophoretically purified myosin heavy chains was performed according to Rushbrook and Stracher[13], as specifically detailed by Carraro et al.[21], using the V8 protease from *Staphylococcus aureus* as the proteolytic agent (Miles). The following modifications were applied: the thickness of the slab gel was 1 mm; the stacking and the separating gels were 1.6 and 8.5 cm long, respectively. The electrophoretic run was started at 22 mA; 1 h after the tracking dye front had passed in the separating gel, the current was raised to 35 mA. The run was stopped as the tracking dye front reached the bottom of the slab. The amount of protein and protease loaded on each gel is indicated in the legends of the figures. The slabs were stained with Coomassie blue[22]. Densitometric tracings of

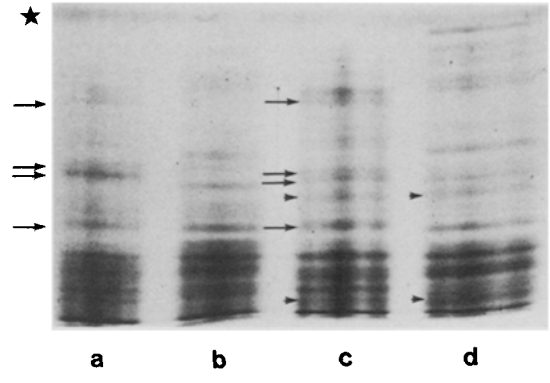

Figure 1. One-dimensional peptide mapping with *Staphylococcus aureus* V8 protease of electrophoretically purified myosin heavy chains from chicken muscles. 20 µg of protein per lane; the *S. aureus* V8 protease/myosin ratio was 1/20. *a* Adult fast pectoralis; *b* adult ventricles; *c* 14-day-old embryonic pectoralis; *d* adult slow ALD. The star locates the *S. aureus* V8 protease band. The bands indicated with arrows are common to embryonic and adult fast maps; the bands indicated by arrowheads are common to embryonic and slow maps. Only the region of the slab under the V8 band is shown.

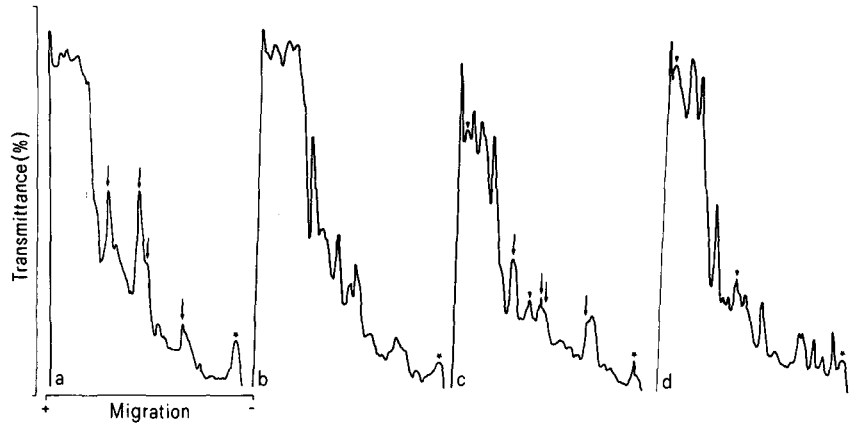

Figure 2. Densitometric scans of the proteolytic patterns shown in figure 1. Key and symbols are as in figure 1.

the stained gels were obtained by scanning at 578 nm in a Perkin Elmer spectrofluorimeter MPF 2A equipped with the TLC apparatus.

Results and discussion. Mapping of the digested heavy chains of adult fast, slow and cardiac myosins produced patterns with distinct differences (figs 1, 2a, b, d), although some similarities could be observed between slow and cardiac digests (figs 1, 2a, c), as previously shown[22]. In contrast, fast embryonic myosin heavy chains, besides possessing several peptides typical of adult fast myosin (arrows in figs 1 and 2) displayed some bands common to slow adult myosin (arrowheads in figs 1 and 2). As first reported by Rushbrook[13], the digestion patterns of the myosin heavy chains are highly reproducible under fixed experimental conditions (length of the stacking gel, current applied, protease amount): compare e.g. panel b in figures 1 and 2 with panel a in figures 3 and 4. The molecular weights of the peptides obtained with a fixed quantity of protease were almost insensitive to the amount of protein loaded (from 5 to 35 µg); only the intensity of the bands varied and more myosin remained undigested as the protein loading increased (results not shown). The digestion experiments carried out on electrophoretically copurified myosin heavy chains of adult slow and embryonic types confirmed, more precisely, the presence in embryonic myosin of bands common to slow adult myosin (arrows in figs 3 and 4). However, some peptides which are predominant in the slow digestion pattern are virtually absent in the embryonic myosin pattern (arrowheads in figs 3 and 4).

The evidence that the peptide map of the heavy chains of embryonic fast muscle myosin has the general features of that of the heavy chains of fast white fibers, containing, however, definite differences from the latter, was obtained by Rushbrook[13]. In the present work it was possible, on the one hand, to confirm these results with the same techniques and, on the other, to demonstrate for the first time, by means of coelectrophoresis experiments with adult slow myosin heavy chains, the presence of peptides typical of adult slow myosin in embryonic pectoralis muscle.

Immunocytochemical studies carried out directly on the fast embryonic tissues of the chick have shown the presence of antigenic determinants of adult fast, slow and cardiac heavy chains[14,15]. Moreover the presence of fast and slow

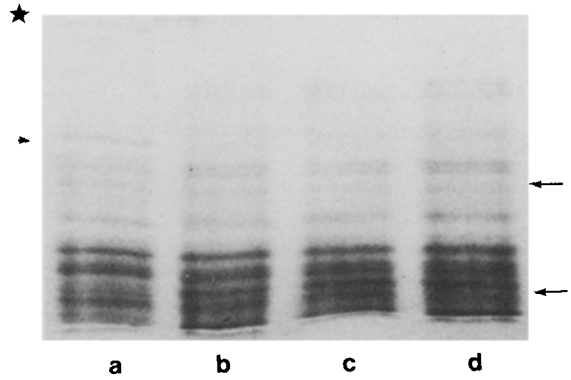

Figure 3. One-dimensional peptide mapping with *S. aureus* V8 protease of electrophoretically purified myosin heavy chains from chicken muscles. 20 µg of protein per lane. The *S. aureus* protease/myosin ratio was 1/20. *a* Adult slow (100%); *b* adult slow (25%) + embryonic fast (75%); *c* adult slow (12.5%) + embryonic fast (87.5%); *d* embryonic fast (100%). The star locates the *S. aureus* V8 protease band. Arrows indicate bands common to embryonic and slow maps; arrowheads indicate bands prominent in slow digest not present in embryonic digest. Only the region of the slab under the V8 band is shown.

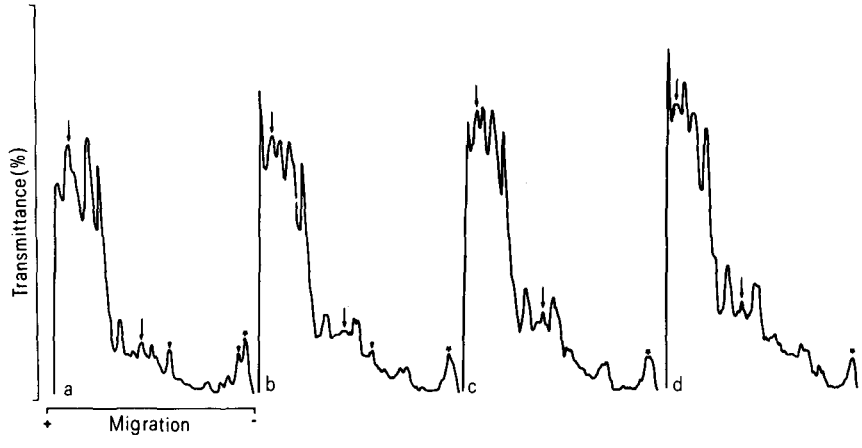

Figure 4. Densitometric scans of the proteolytic patterns shown in figure 3. Key and symbols are as in figure 3.

myosin light chains has been demonstrated in embryonic pectoralis muscle myosin[16,17]. This heterogeneity may be explained either by the presence of different myosin isoenzymes or by the existence of molecules characteristic of embryonic tissue. The structure of the embryonic tissue molecules could be composed of fast and slow adult light chains associated with special heavy chains. The heavy chains may be made up of stretches of sequences similar to adult fast heavy chains interspersed with sequence-stretches typical of adult slow heavy chains. The fact that in the map of embryonic heavy chains only a few components peculiar to the slow myosin digest are present (one would have expected all the slow myosin components if myosin in embryonic tissue was made by a mixture of fast and slow isoenzymes) supports the hypothesis of the existence of a hybrid myosin.

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Photodynamic studies on acid ribonuclease from pea cotyledons

P.S. Phillips and Carol A. Phillips

Caterham College, Harestone Valley, Caterham, Surrey CR3 6YA (England), 5 March 1981

Summary. In crude extracts, pea cotyledon acid ribonuclease is not inactivated by photodynamic treatment, but after 150-fold purification it is markedly inactivated when illuminated in the presence of rose bengal at pH 7.1. Data suggests that histidine photo-oxidation reduces catalytic activity.

Only 5 amino acids, cysteine, histidine, methionine, tryptophan and tyrosine are modified by photodynamic treatment[1]. By careful control of the reaction conditions and selection of an appropriate oxidizing dye, photo-oxidation may be limited to a single amino acid species[2]. It is therefore possible to determine the importance of the amino acids which are susceptible to dye-sensitized photo-oxidation for the activity of the enzyme. For the great majority of enzymes, most of the amino acid residues are involved in the maintenance of catalytic activity, and modification of any amino acids species leads to loss of activity.

It has been demonstrated[3] that a small fragment of pea cotyledon acid ribonuclease (EC. 2.7.7.16), with a mol. wt of 3100 retains some catalytic activity. The retention of activity in such a small fragment suggests that a large part of the amino acid sequence may not be directly involved in maintaining the integrity of the active site. Acid ribonuclease is therefore an interesting subject for study by photodynamic techniques.

Experimental. Pea seeds (*Pisum sativum* L., cv Feltham First) were surface sterilized in Na hypochlorite solution, washed in running tap water for 4 h, planted in moist vermiculite and allowed to germinate at 22–25 °C. The seedlings were harvested after 5 days. The cotyledons were homogenized in 0.2 M Na acetate buffer, (pH 5.4) with a pestle and mortar at 3 °C. The homogenate was centrifuged at 34000 × g for 15 min at 4 °C. Differential precipitation of acid ribonuclease with $(NH_4)_2\,SO_4$, followed by gel filtration on columns of Sephadex G-50 (fine) results in a purification of about 150-fold (2.1 ± 0.2 µg RNA hydrolyzed/mg protein/min in crude extracts; 312 ± 105 µg RNA hydrolyzed/mg protein/min in partially purified extracts). During irradiation the samples were maintained at 1–5 °C. Aliquots were removed at 15-min intervals for assay of acid ribonuclease[4]. Control samples containing dye were kept in darkness. Aliquots from control samples were taken at zero time and after 60 min. For experiments in which irradiation was performed under alkaline conditions, enzyme samples were prepared in 0.05 M Tris-HCl buffer. The pH was adjusted to 5.4 after irradiation. Samples of enzyme preparations were irradiated with visible light from an Osram 1500-W tungsten bulb (120 mm distance from sample) in the presence of dye.

Results and discussion. Pea cotyledon acid ribonuclease, in crude supernatants, is completely insensitive to photodynamic action over a range of pH (5.6–8.1). In contrast, acid phosphatase in the same crude supernatants is extensively inactivated by photodynamic action. The figure (a and b) shows the effects of irradiation of the partially purified enzyme preparation in the presence of rose bengal at pH 5.6 and 7.1. It is clear that at pH 5.6 irradiation in the presence of rose bengal and crystal violet, has no effect on the activity of acid ribonuclease. Irradiation, at pH 8.1, in the presence of rose bengal has no effect on the enzyme activity. In contrast, irradiation of the enzyme at pH 7.1