Ultrastructural Organisation and the Sites of Calcium Localisation in the Lamprey Striated Muscle

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Abstract. The present paper examines the ultrastructure of the sarcoplasmic recitulum (SR) and the T system in the striated muscle of the lamprey. The pyroantimonate method was used to visualise the sites of intracellular calcium localisation. Characteristic for the muscle studied are the presence of numerous intricately shaped invaginations on the surface membrane of muscle fibres and peripheral contacts between SR cisternae and the sarcolemma. In addition to calcium localised in the terminal cisternae of SR and N-bands of the I-disk, as typical of vertebrate muscles, a great amount of calcium is present in the subsarcolemmal region, corresponding to the area of invaginations, and in longitudinal elements of SR.

Key words: Lamprey muscle — Sarcoplasmic reticulum — Intracellular calcium localisation

Introduction

The piston apparatus of the lamprey has a complex anatomy; it is composed of numerous striated muscles (Fürbringer 1875; Krause 1923) of which m. longitudinalis linguae has been most intensively studied with regard to both its function and morphology (Itina and Skorobovichuk 1967; Skorobovichuk and Itina 1968; Fominykh 1970). This muscle consists of uniform fibres with a diameter of 40—60 μm. Each muscle fibre receives single motor innervation

localised in the proximal part of the muscle; however, sensitivity to acetylcholine (Ach) is observed over the entire surface membrane of the fibre.

Ach was shown to activate the contractile mechanism in lamprey muscle more effectively than KCl. It was suggested that Ach not only depolarises the muscle fibres but also activates the calcium channels at the surface and T membranes, and releases Ca^{2+} bound to T-tubule membranes (Nasledov et al. 1980; Skorobovichuk and Nasledov 1978; Samosudova et al. 1984). This would suggest the presence of certain structures responsible for functional specificity of lamprey muscle. We examined the ultrastructure of those elements of m. longitudinalis linguae which are involved in electromechanical coupling.

It is well known that in all types of muscles the contraction-relaxation cycle is controlled by changes in the concentration of Ca ions in the myoplasm (for a review see Zachar 1971). Mechanical activity of known vertebrate striated muscles is regulated primarily by the release of Ca ions from the terminal cisternae of SR and a subsequent uptake of these ions. However, this mechanism shows peculiar features in different muscle types. Nothing is known about Ca^{2+} translocation during contraction-relaxation cycle in relatively specific muscles of the cyclostomes. On the basis of physiological experiments it may be expected that Ca translocation would differ depending on whether contractions have been elicited by membrane depolarisation or by a neurotransmitter (Ach). The present paper describes the first stage of current investigations on this topic, and is aimed at establishing the sites of Ca ions stores within the lamprey muscle at rest. The results showed that calcium localisation in the muscles of this primitive vertebrate species considerably differs from what has been known in this respect of higher developed vertebrates; some resemblance to invertebrate locomotor muscles could be stated.

Materials and Methods

Experiments were performed on bundles of 10—20 muscle fibres dissected from m. longitudinalis linguae of the lamprey *Lampetra fluviatilis*. The fibres were slightly stretched and fixed for 1 hour at 4°C in 2.5 % glutaraldehyde in 0.1 mmol/l phosphate buffer, pH 7.2—7.4, then washed and postfixed in 1 % veronal-acetate buffered OsO_4 for an additional hour. After dehydration in an alcohol series, the fibres were embedded in Epon. The sections were contrasted with uranyl acetate with subsequent exposure to lead citrate.

For histochemical demonstration of calcium muscle bundles were placed for 1.0—1.5 hours in a cold solution containing: 2 % potassium pyroantimonate ($K_2H_2Sb_2O_7 . 4H_2O$), 1 % OsO_4 and 3 % sucrose, and 0.01 N acetic acid, pH 7.0—7.1. According to Shiida et al. (1970), when the pyroantimonate method is used for calcium ions determination, pH should be 6.9—7.2, whereas sodium ions can be detected with pH above 7.2. In order to prevent nonspecific K-pyroantimonate precipitate formation during dehydration, the tissues after fixation were rinsed for 10—15 min in Na-cacodylate buffer (0.1 mmol/l) at pH 7.2, and rapidly dehydrated in alcohol and propylene oxide. The tissues

were then embedded in an Epon-Araldite mixture. Sections were prepared on an LKB ultratome and stained with uranyl acetate and lead citrate. On several instances unstained sections were used.

The physiological solution contained (in mmol/l): Na^+ 121; K^+ 2.5; Ca^{2+} 1.8; HCO_3 2.4. Prior to fixation, some muscle bundles were treated for 5 min in tenfold Ca^{2+} (18 mmol/l) solution and then fixed using the above technique. In control tests,muscle bundels were incubated, for 30 min prior to fixation in cold calcium-free solution containing EDTA and Mg^{2+} (1 or 4 mmol/l).

Results

Organisation of the T-system and the sarcoplasmic reticulum

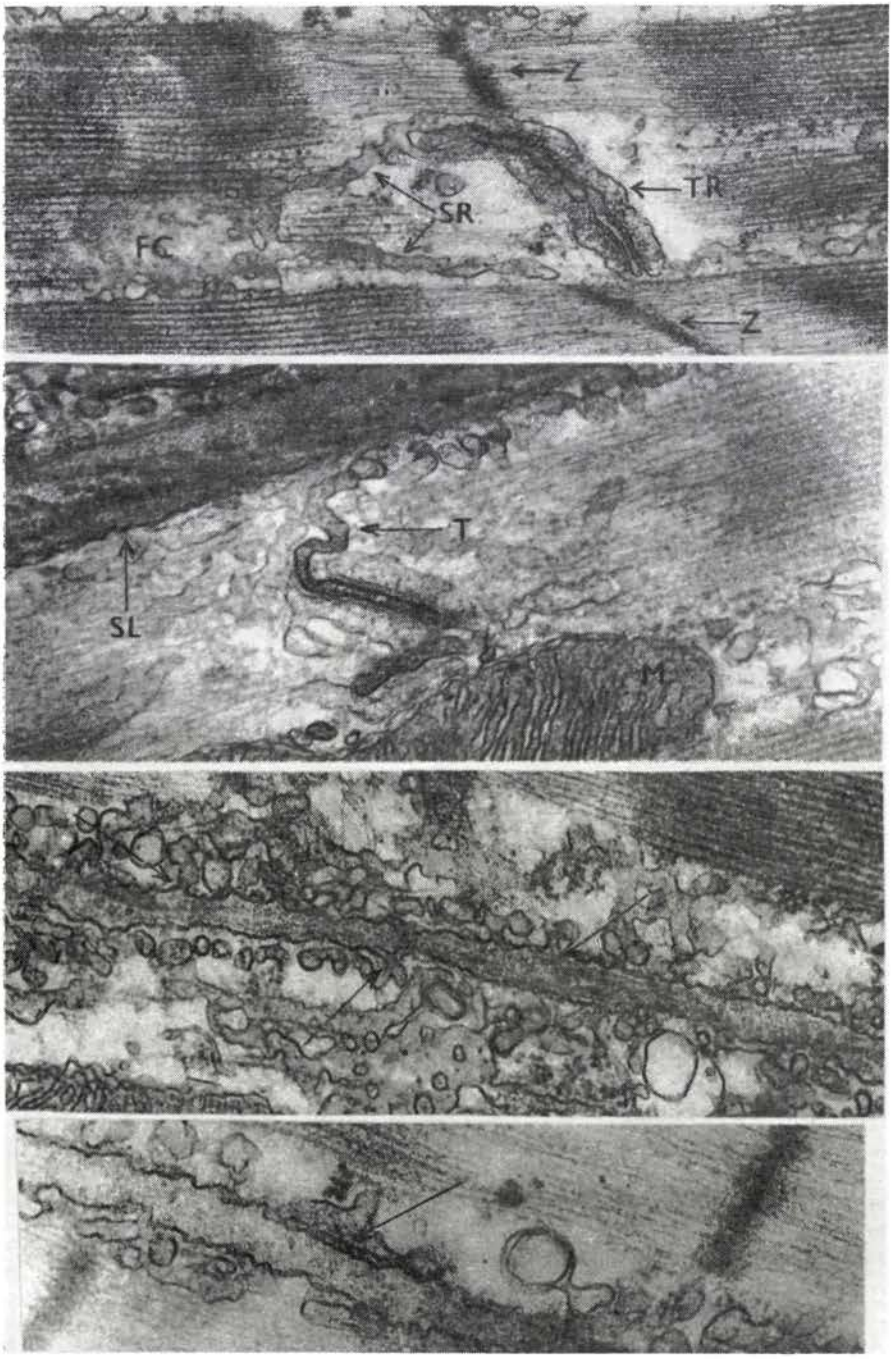
M. longitudinalis linguae is a well organised striated muscle. The sarcoplasm between the myofibrils was found to be occupied by elements of the sarcoplasmic reticulum developed at all parts of the sarcomere. At the A-disc level, individual myofibrils were surrounded by SR, the latter fusing there and forming a fenestrated collar.

From the site of fusion, SR tubules were running towards the Z-line, where they became dilated, and transformed into terminal cisternae filled up with electron-dense material. At these points SR was in contact with the tubules of the T-system to form triads, rarely pentads (Fig. 1). T-tubules were oriented predominantly perpendicularly to the long axis of the fibre, but they could be also found to extend along the fibre. We happened to observe one such T-tubule pursuing its course along the fibre over 6 sarcomeres and forming longitudinal triads around each of them. T-tubules may ramify thereby producing pentads. In some instances we observed T-tubules mouthing on the fibre surface (Fig. 2).

A specific feature of the muscle ultrastructure was the presence of multiple invaginations of the surface membrane. In addition to invaginations in form of vesicles or caveolae, as common in muscle fibres of other vertebrates, there were many more complexly shaped and extended invaginations including straight or variously bent tubules up to 0.2 μm long. We failed to observe any confinement of these invaginations to a definite level of the sarcomere (Fig. 3). In fibres of the muscle studied numerous peripheral junctions connecting SR cisternae to the sarcolemma were observed (Fig. 4).

Localisation of Ca accumulating structures

Several investigators have shown that Ca is the major cation in precipitates which form following muscle tissue fixation in osmium pyroantimonate (Yarom and Meiri 1971; Garfield et al. 1972; Sugi et al. 1982). However, the pyroantimonate method can be used to reveal not only Ca^{2+} but also Mg^{2+} and Na^+. Klein et al. (1972) have shown that the method is more sensitive to Ca^{2+} than to Mg^{2+} or Na^+. The method is sensitive to Ca^{2+}, Mg^{2+} and Na^+ at concentra-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tions of 10^{-6}, 10^{-5} and 10^{-2} mol/l respectively. The formation of precipitates is linearly proportional to the cation concentration in the medium. A direct evidence for calcium being the main cation of precipitates, formed in muscle tissue during the fixation with osmium-proantimonate was obtained also by X-ray microanalysis (Yarom and Chandler 1974). In the present work this fixation technique was used to visualise the major sites of Ca storage in the lamprey muscle (Samosudova 1983). As shown in Fig. 5, precipitates were observed in sites, typical of other vertebrate skeletal muscles, namely in the terminal cisternae of SR, N-bands of the I-discs, and within the nuclei. However, in the lamprey muscle some additional sites of Ca storage were observed, namely in the subsarcolemmal region (Fig. 6). The depth of precipitation was about 0.2 μm, which corresponded to the depth of multiple invaginations. On unstained transverse sections (Fig. 7), the precipitate delineated distinctly individual myofibrils. This distribution pattern suggests that precipitation is most likely to occur in the longitudinal elements of SR. Mitochondrial membranes were usually free of precipitates (Fig. 5).

Incubation of muscle bundles in physiological solution with an increased Ca ions concentration for 5 min prior to fixation resulted in an increase in the amount of precipitates in muscle fibres, especially in myofibrillar structures. Myofibrils became "hairy". The increase in density of precipitates in the I-disc revealed the periodicity of troponin. Precipitates were also observed in the centre of the A-disc, mainly in the H-zone (Fig. 8). After the treatment of the preparation with EDTA and Mg^{2+}, precipitates disappered almost entirely (Fig. 9). Glycogen particles which are seen in Fig. 9 differ from precipitates, in that they are larger in size, of less density, and especially, in that they show a typical granular structure (Wanson and Drochmans 1968).

Fig. 1. Longitudinal section of the lamprey muscle fibre. The organization of the sarcotubular system. Z — Z-line; TR — triad; SR — sarcoplasmic reticulum; FC — fusion of sarcotubules into a fenestrated collar. × 33,000.

Fig. 2. Connection of a T-tubule to the fibre surface seen on the longitudinal section. T — tubule of the T-system; SL — sarcolemma; M — mitochondria. × 51,000.

Fig. 3. Longitudinal section. Numerous invaginations in the sarcolemma (arrows) of two neighbouring fibres. × 36,000.

Fig. 4. Longitudinal section. The arrow points to the contact between the sarcolemma and SR cisternae. × 53,000.

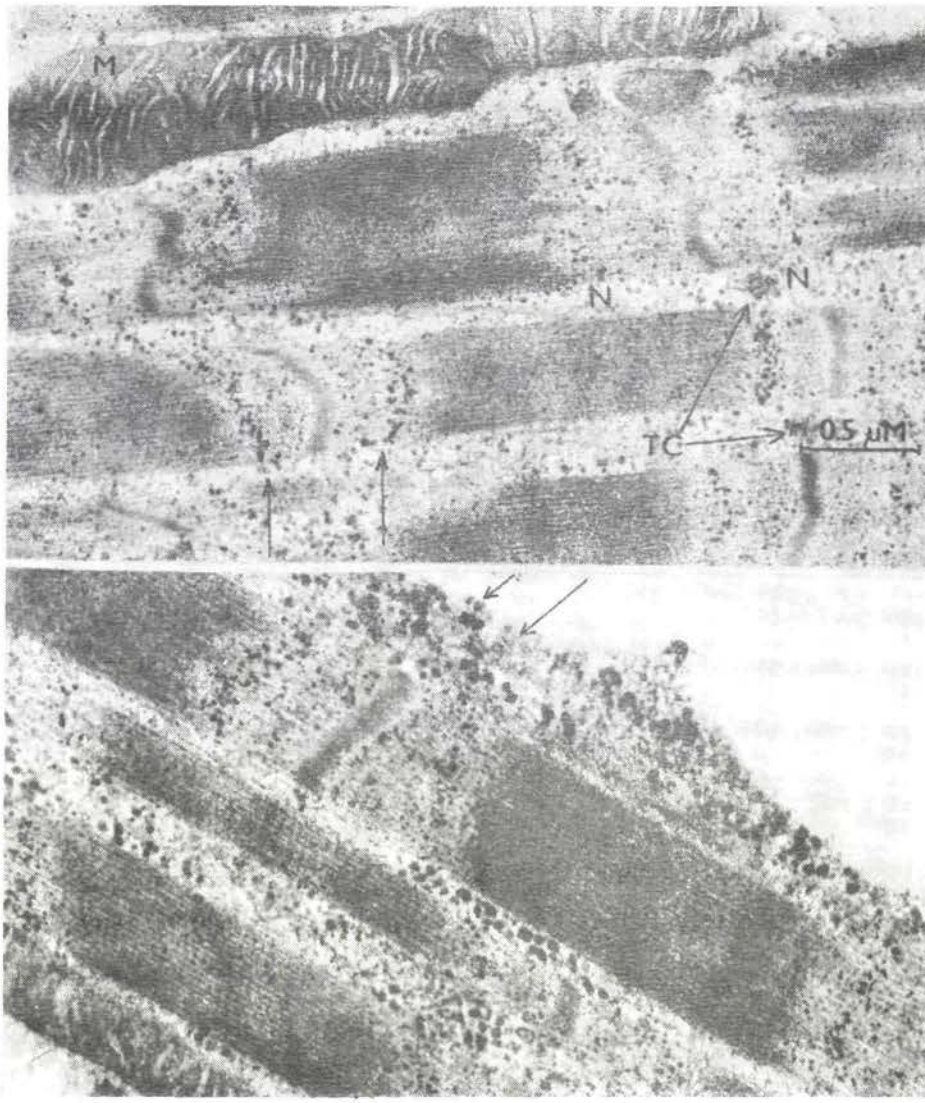

Fig. 5. The distribution of calcium pyroantimonate precipitates within the muscle fibre. TC — terminal cisternae; Z — line; M — precipitate-free mitochondria. × 32,000.

Fig. 6. The distribution of calcium pyroantimonate precipitates in the subsarcolemmal region (arrows) and among the myofibrils. × 35,000.

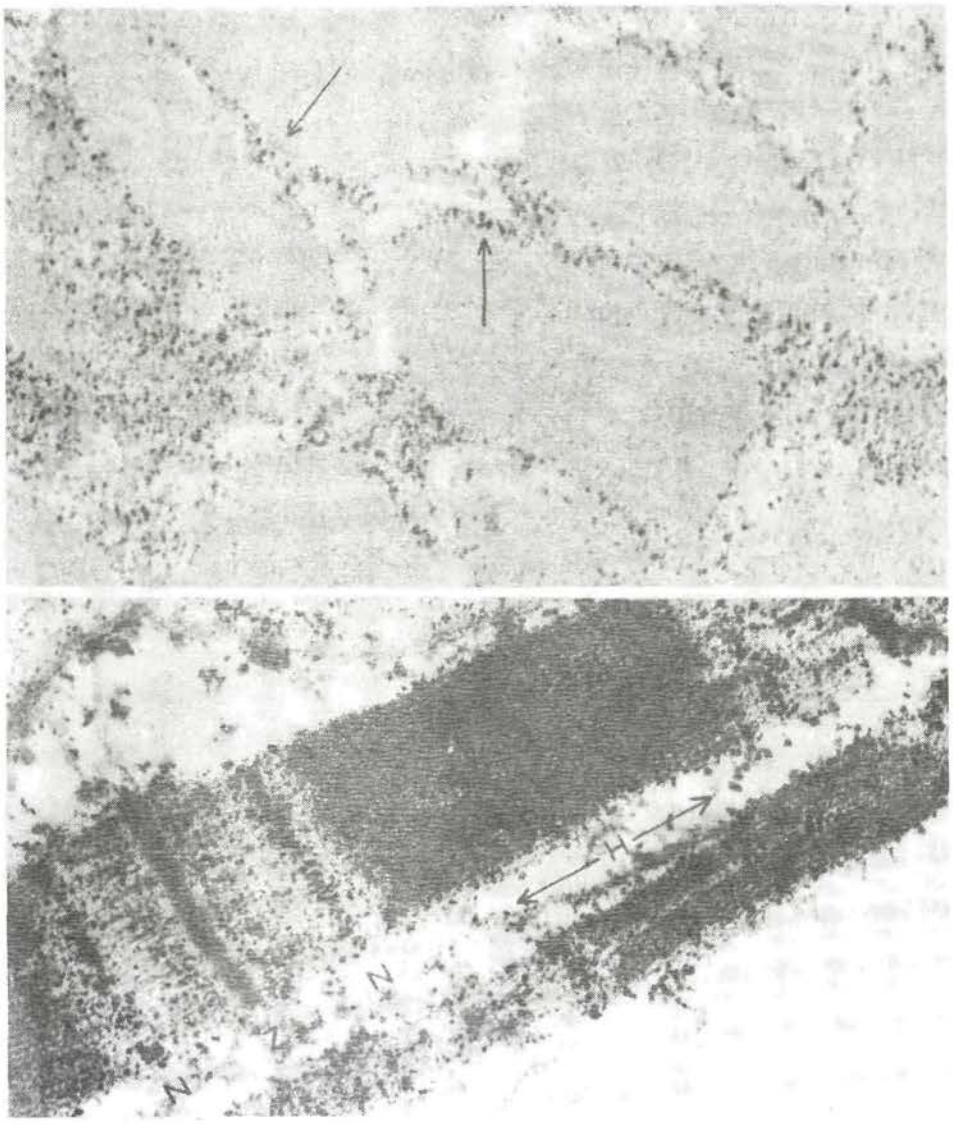

Fig. 7. Transverse section, unstained. Individual myofibrils are delineated with the precipitates of calcium pyroantimonate (arrows). × 35,000.

Fig. 8. Longitudinal section. Prior to the fixation, the muscle was incubated in 10-fold calcium solution for 5 min. Note the increase in precipitates in the H-zone (H) and in N-discs (N). Troponin periodicity in I-disc is obvious. × 35,000.

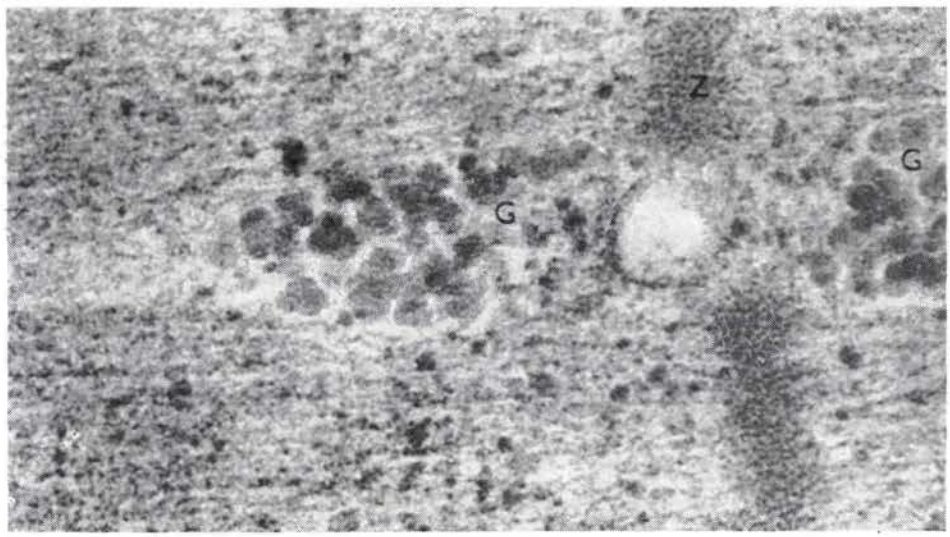

Fig. 9. A muscle fibre following 5 min treatment in calcium-free solution with 1 mmol/l EGTA and 4 mmol/l $MgCl_2$. Precipitates are practically absent. GL — glycogen granules. × 140,000.

Discussion

Electron microscopic investigation of the sarcotubular system of a twitch striated muscle of a primitive vertebrate, lamprey, showed that the muscle has a well developed SR including its typical elements characteristic of phasic skeletal muscles in vertebrates: longitudinal elements, coupling at the level of A-disc, terminal cisternae which come into contact with tubules of the T-system thereby forming triads and pentads at the level of the Z-bands. A regular distribution of T-tubules in the muscle was observed. However, in addition to features of the sarcotubular system, typical of twitch fibres a number of peculiar characteristics could be observed, included deep and abundant sarcolemmal invaginations of various shapes.

In the subsarcolemmal region of vertebrate skeletal muscles usually there are caveolae of more or less constant size concentrated primarily at the Z-line (Dulhunty and Franzini-Armstrong 1975; Zampighi et al. 1975). It has been assumed that the tubules of the T-system join the sarcolemma via these caveolae (Luff and Atwood 1971; Zampighi et al. 1975). In the lamprey just as in fish (Franzini-Armstrong and Porter 1964) T-tubules open directly into the extracellular space. Moreover, sarcolemmal invaginations of the lamprey muscle have a more complex shape and are localised at any level of the sarcomere, often in immediate vicinity of the longitudinal SR. Fibres with such sarcolemmal

invaginations resemble immature muscle fibres of higher vertebrates at the period of the T-system formation during the development of connections between the surface membrane and SR (Ezerman and Ishikawa 1967; Schiaffino and Margreth 1969). In the lamprey muscle, ramification of T-tubules were observed in both transversal and logitudinal directions, forming longitudinally oriented triads; this is also typical of vertebrate muscles during the early postnatal ontogenesis.

Peripheral contacts between the subsarcolemmal cisternae of SR and surface membrane which are extremely rare in the skeletal muscles of other vertebrates (Schiaffino and Margreth 1969; Spray et al. 1974) and more common in smooth or cardiac muscles (Simpson and Rayns 1968; Somlyo et al. 1971), were often observed in the lamprey muscle. Thus the organisation of the sarcotubular system suggests a certain primitiveness of the striated twitch muscle of the lamprey.

The pyroantimonate method used in the present work to demonstrate Ca allows to detect Ca activated in the contraction-relaxation cycle, since pyroantimonate binds either to free or to slightly bound calcium through the substitution of Ca^{2+} for K^+ (Samosudova 1983). A comparison between the Ca distribution patterns in the fibre obtained in the present work and those observed in other vertebrates has shown that in this respect lamprey muscles significantly differ from the "classical" pattern. In addition to precipitates contained in the terminal cisternae of SR and in the I-discs, the presence of precipitates distinctly delineating individual myofibrils and penetrating into the subsarcolemmal region was clearly observed in the muscle at rest. Obviously, precipitates delineating individual myofibrils could be related to longitudinal elements of SR, and precipitates in the subsarcolemmal region were assumed to relate to the sarcolemma, invagination membranes and membranes of submembrane vesicles as well as to the peripheral cisternae of SR. This was confirmed by the fact that the depth of the precipitates under the surface membrane corresponded to the depth of invaginations (about 0.2 μm). Calcium accumulation in similar subsarcolemmal structures and participation of these ions in activation of contraction has been described in vertebrate smooth muscles (Popescu 1974).

The lamprey muscle although singificantly differing from other vertebrate muscles, shows a similar Ca localisation as observed in invertebrate muscles in which contracture is triggered by both intracellular and extracellular calcium. The lamprey muscle is similar to that of crustaceans (Uhrík and Zacharová 1983; Samosudova et al. 1981) since after the reaction had been completed the elements of SR become filled with precipitates; similarities also exist with the molluscan muscles since a considerable amount of calcium is bound to the subsarcolemmal membrane structures.

It can be concluded that skeletal musçles of the lamprey have features

characteristic of both the vertebrate and invertebrate muscles. This conclusion may be applied not only to the muscle system but to other lamprey systems as well, and primarily to the nervous system of the lamprey (Rovainen 1979).

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