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Ultrastructural Study of *Uromastyx aegyptia* Skeletal Muscles

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ABSTRACT. The present study was concerned with the ultrastructure of the mandibular externus of the jaw and the tail skeletal muscles of *U. aegyptia*. Results obtained revealed differences in general morphology between the two muscles. The sarcoplasmic reticulum, T-system, and large deposits of glycogen granules, were encountered in the jaw muscle. However, the SR are not well developed. In contrast, indistinct glycogen granules, sarcoplasmic reticulum and T-system are the main features of the tail muscles.

Furthermore, the jaw muscle demonstrated a typical sarcomere with parallel striation but no indication of M-line. Results showed that although the properties and details of the fine structure of the two muscles studied varied, the fundamental structure was typical vertebrate skeletal muscle. Experiments on physiological and biochemical aspects should be obtained to arrive to a final conclusion about the type of muscle.

Introduction

The muscle fibers in different animals are by no means all identical in structure and function, even though they appear to generate force by the same basic mechanism. Huddart[1] reported that despite the variability of fiber diameter and arrangement of fibers within the muscle, the basic myofibrillar machinery is almost surprising uniform, particularly in the case of length of the contractile subcomponents. The study of Goldspink and Ward[2] showed that the vertebrate skeletal muscles are not composed of a homogeneous population of fibers. The above authors stated that the skeletal muscles in different animals or in different parts of the same animal are specialized for particular functions[3,4].

From the physiological point of view, vertebrate skeletal muscles can be divided into two types, phasic (fast, twitch) and tonic (slow) fibers[4]. Histological and histochemical studies have made it possible to distinguish three broad categories of skeletal muscles, oxidative, glycolytic and oxidative/glycolytic[3,5-9].

Detailed ultrastructural studies of vertebrate muscles[1,10-16] and insect flight muscle[17] have revealed the principal features of fast and slow acting muscle. For example, in the garter snake segmental muscle, Hess[12] found that the twitch fibers (fast) have abundant sarcoplasmic reticulum (SR), regular T-tubules (T-system) order triads in the A-band margins and focal innervation. On the other hand, the slow fibers have little SR, the T-system is totally absent and innervation is multiterminal.

The present study was undertaken to determine the general pattern of fine structure of the jaw and tail skeletal muscles of the lizard *Uromastyx aegyptia,* assuming the jaw to be phasic (fast) and the tail to be tonic (slow).

Material and Methods

The muscles used in this investigation were (i) the adductor mandibular externus of the jaw, and (ii) tail muscles of *Uromastyx aegyptia.* They were dissected and prefixed in 4% glutaraldehyde in cacodylate buffer at pH 7.3 for two hours at room temperature. Subsequently, pieces of muscle were rinsed in the cacodylate buffer and post-fixed in 2% buffered osmium tetroxide for two hours. Sections were cut by LKB ultramicrotome, stained in 2% ethanolic uranyl acetate, then by lead citrate[18] and viewed by JEOL 100 cx EM.

Thick sections (0.5-1.5 μ) were stained for light microscopy by 1% toluidine blue for 1 minute.

Results and Discussion

Examination of light and TEM micrographs of transverse sections of the adductor mandibular externus muscle of *Uromastyx aegyptia* showed that the shape and size of each individual fiber and myofibrillar profiles vary considerably (Fig. 1). Collagen fibrils, blood vessels and nerves were encountered in the endomysium in contact with the muscle fibers (Fig. 2).

The sarcolemma which surrounds each muscle fiber consists of a typical cell membrane (plasmalemma) and a layer of amorphous material of moderate electron density (external lamina) to which a little reticular connective tissue is adherent (Fig. 2,3). Pinocytotic vesicles are clearly shown at the plasmalemma (Fig. 3). Nuclei lie in a medial interfibrillar position, as clearly shown in Fig. 4. This arrangement represents the normal position of these nuclei in almost all muscle cells examined. It has been reported that the position of nuclei in most fast acting muscle is at the periphery close to the cell membrane[19].

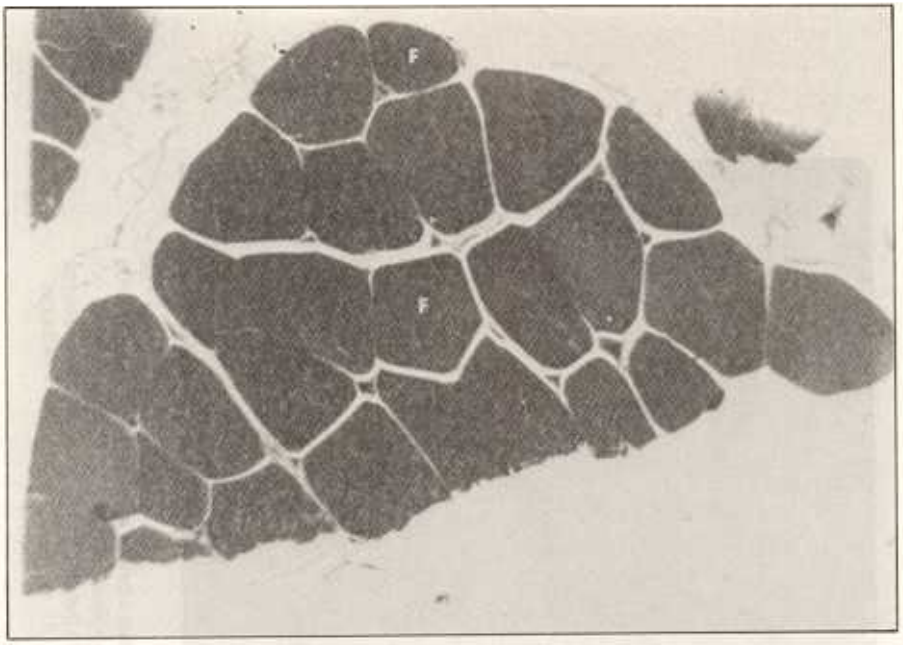

FIG. 1. A photomicrograph of a transverse section through jaw muscle of *Uromastyx aegyptia;* stained with toludine blue. Note the different profiles of muscle fibers (F). × 500

In longitudinal sections (Fig. 5,6) the myofibrils are usually in almost perfect parallel register as demonstrated by the relatively straight rows of z-lines. The sarcomere exhibits the band patterns typical of striated skeletal muscle. Similar results have been reported in various skeletal muscle[12,16,17,20]. There is a light H-band in the middle of the A-band, within which one can observe no indication of the M-line. The latter is thought to represent the cross-link between the myosin filaments in the center of the A-band[1].

Mitochondria are few in the jaw muscle fibers (Fig. 7,8). They are very small but with clearly defined outer and inner membranes and contained electron dense bodies and cristae in different profiles in a moderately electron-dense matrix (Fig. 3). Some of the mitochondria are rod-like and localized near the Z-line (Fig. 3). The profiles of the majority of mitochondria range from circular to oval (Fig. 7,8). The very low number of mitochondria in these muscles suggests that the energy needed for contraction is mostly derived from the glycolytic pathway rather than the oxidative phosphorylation pathways[2,4,7].

The longitudinal section of the muscle fibers demonstrated large deposits of glycogen (Fig. 3,5,7), which are localized in the I-band (Fig. 3,5) as well as between the myofibrils (Fig. 5,7,8). In addition, small glycogen granules can be observed within the myofibrils and large amounts of glycogen are situated in close contact to the outer mitochondrial membrane is also present (Fig. 3,7).

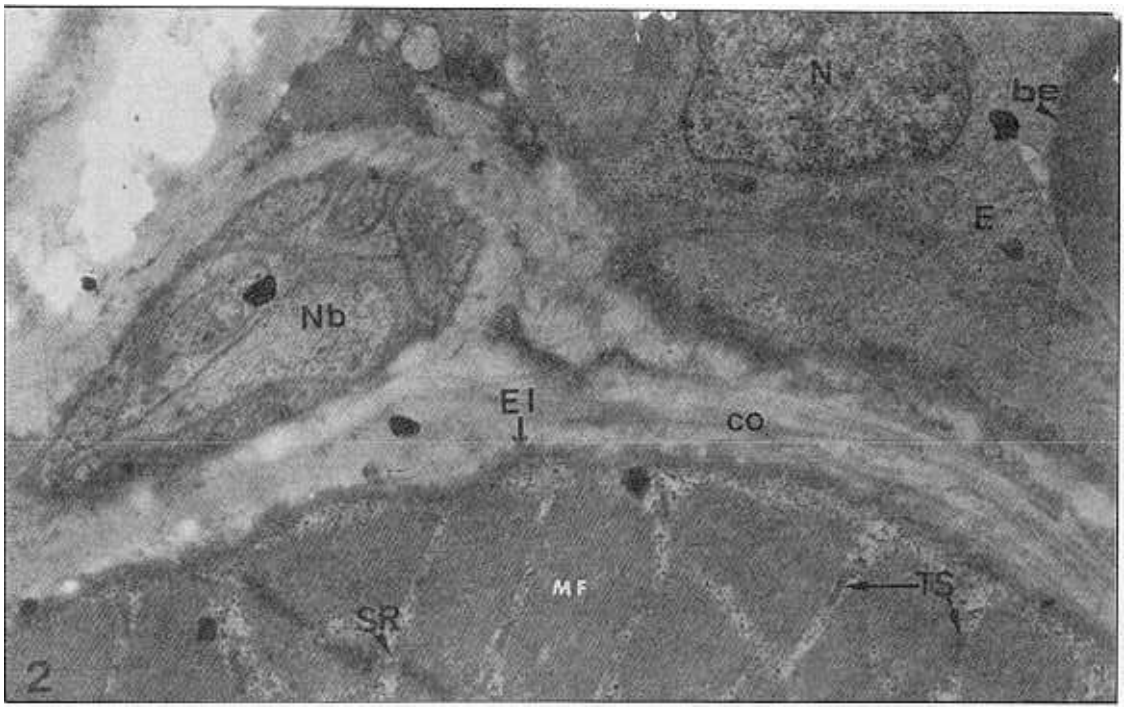

FIG. 2. A longitudinal section of *U. aegyptia* jaw muscle showing a part of myofibrils (MF) which is surrounded by a typical cell membrane and a layer of amorphous material of moderate electron density. El: external lamina; co: collagen fibrils; Nb; Nerve bundle; N: nucleus; E: endothelium; be: blood erythrocyte, TS: T-system; Z: Z-line. × 14850

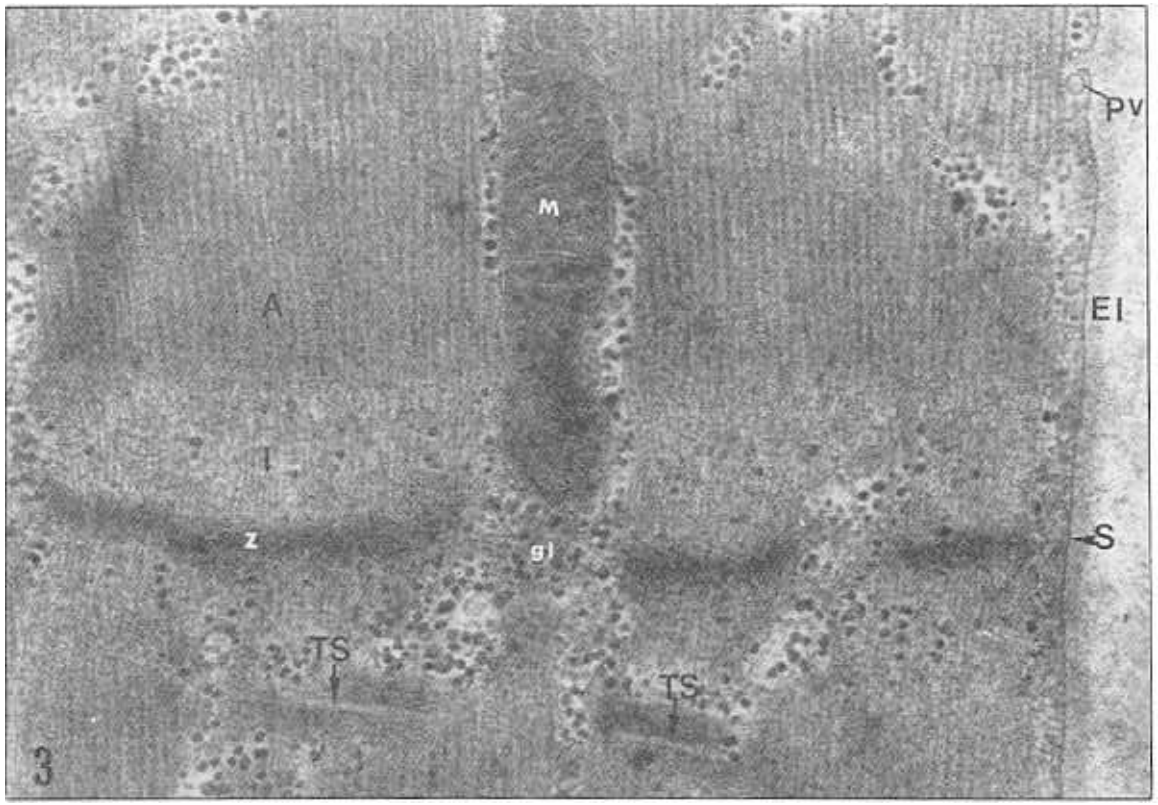

FIG. 3. Longitudinal section of *U. aegyptia* jaw muscle showing the sarcolemma (S), external lamina (El), and pinocytotic vesicle (pv). Note the position of T-systems (TS), mitochondrion (M) surrounded by glycogen granules (gl); A: A-band; Z: Z-line. × 36800

FIG. 4. Low magnification electron micrograph of longitudinal section of *U. aegyptia* jaw muscle showing the position of the nuclei (N); Ne: nucleolus; MF: myofibrils. × 6930

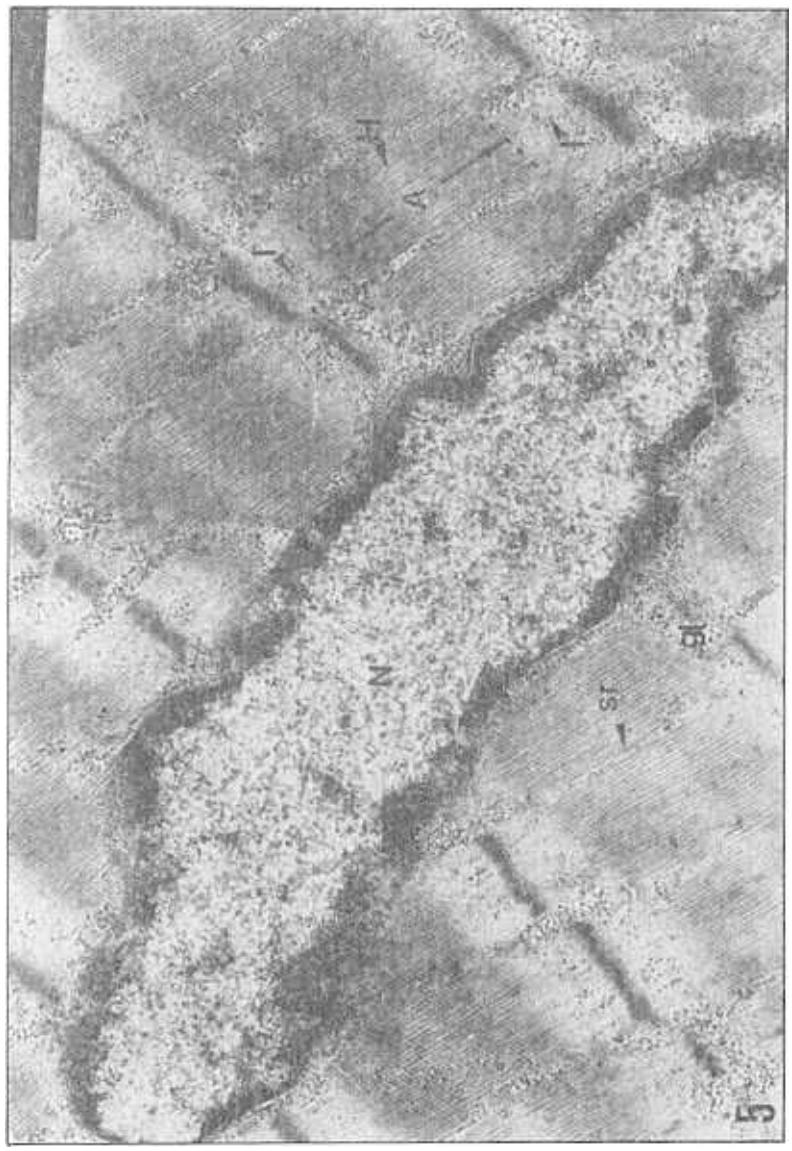

FIG. 5. Electron micrograph of longitudinal section of *U. aegyptia* jaw muscle. SR: Sarcoplasmic reticulum; gl: glycogen granules; N: nucleus; Z: Z-line; I: I-band, H: H-zone. × 18260

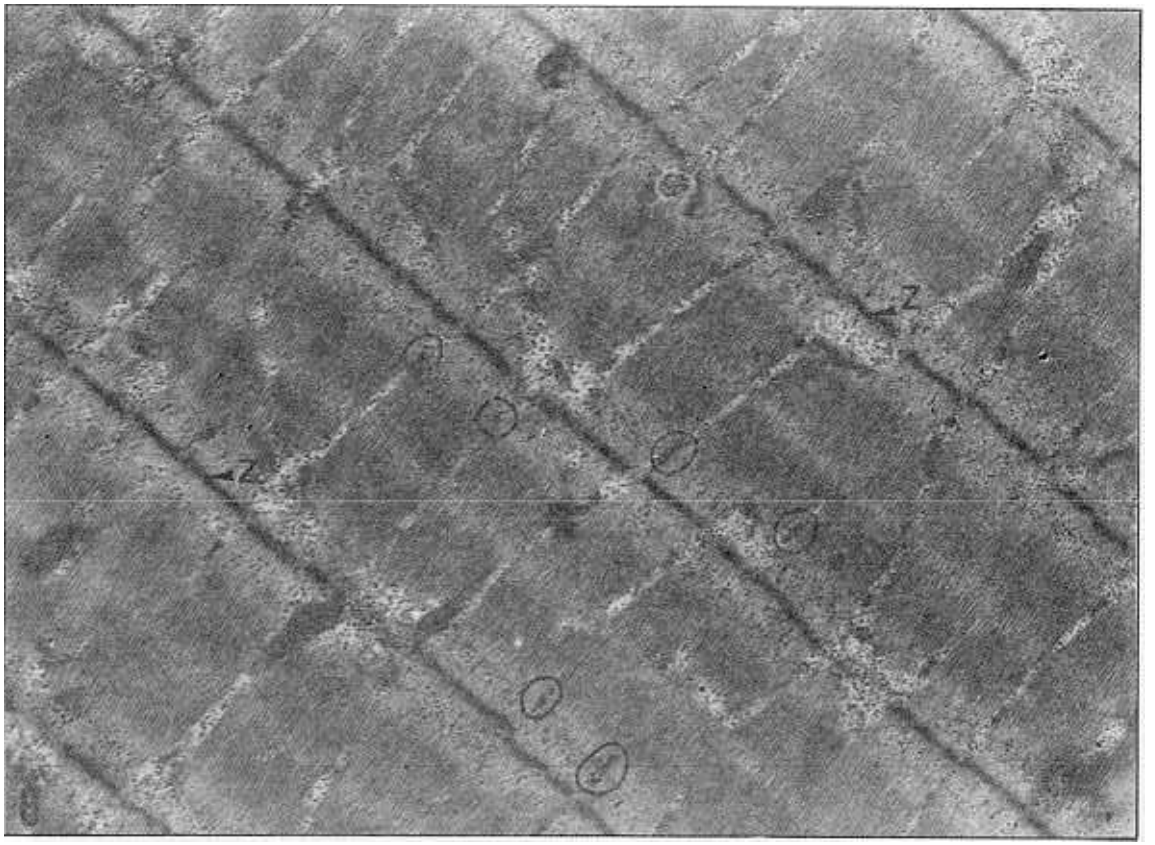

FIG. 6. A longitudinal section of *U. aegyptia* jaw muscle showing that the myofibrils are in almost perfect register as demonstrated by the straight rows of Z-line (Z). Note the position of T-system (encircled areas). × 15180

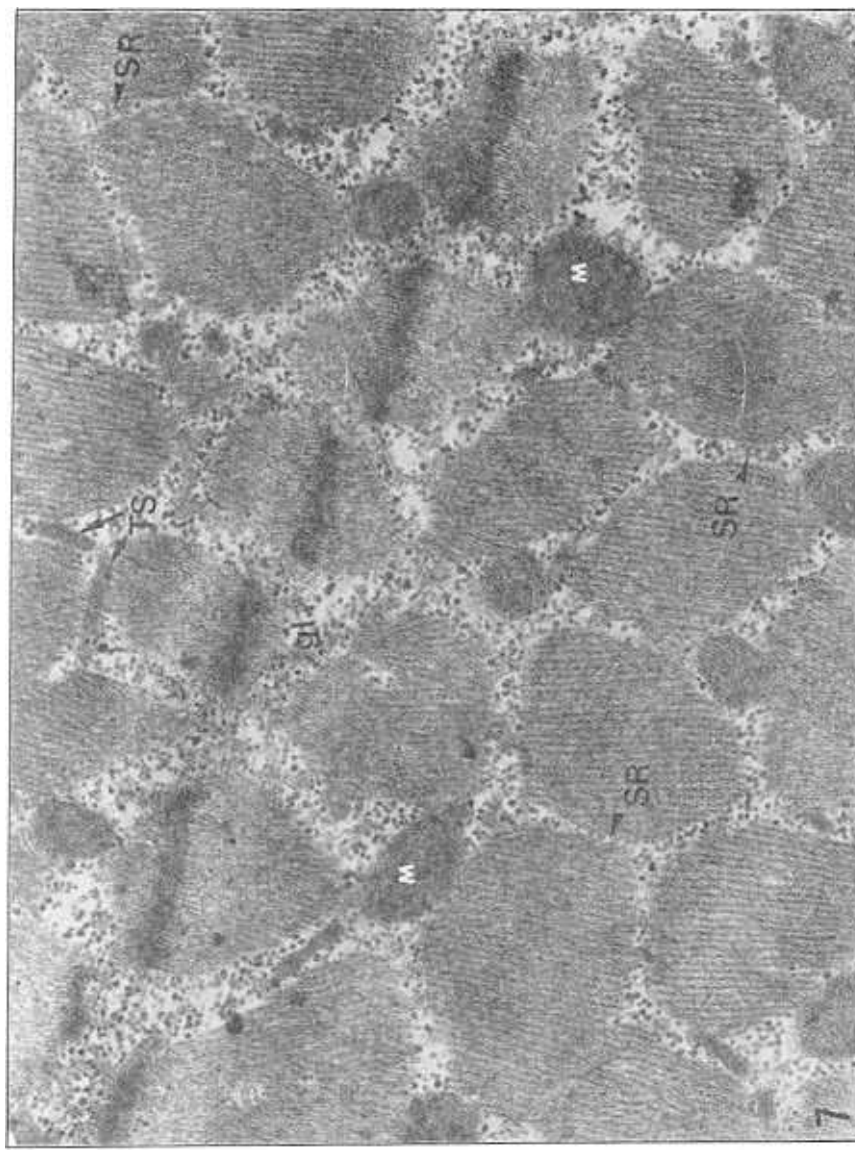

FIG. 7. An electron micrograph section of *U. aegyptia* jaw muscle. M: Mitochondria; SR: Sarcoplasmic reticulum; TS: T-system, gl: Glycogen granules. × 19920

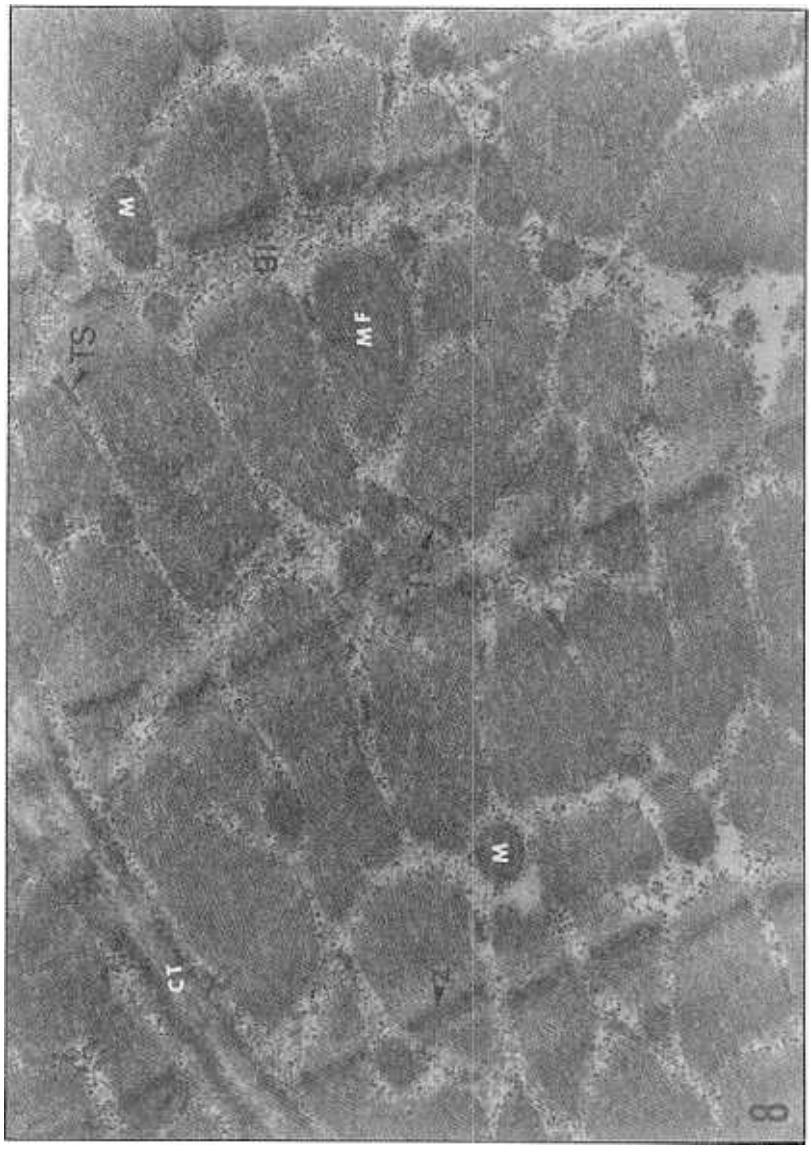

FIG. 8. Oblique section of *U. aegyptia* jaw muscle showing a large deposit of glycogen granules (gl). Note the shape of mitochondria (M); MF: Myofibrils; CT: Connective tissue; TS: T-system; Z: Z-line. × 13200

Sarcoplasmic reticulum can be seen as small vesicles between the myofibrils (Fig. 2,5,7), but they are not well developed. The sarcoplasmic reticulum is known to be the site of release and accumulation of Ca^{2+} which is involved in the initiation of muscle contraction and relaxation[21,22]. The T-system was clearly seen in the region of A/I bands (Fig. 3,6,7). The association between T-system and sarcoplasmic reticulum in the form of triads or dyads is believed to be the pathway of depolarization conduction from the T-system to the cell membrane. The depolarization of the cell membrane leads to Ca^{2+} discharge from the sarcoplasmic reticulum, which initiates contraction by the contractile protein[1,22-26]. The association between sarcoplasmic reticulum and T-system in *U. aegyptia* jaw muscles is characterised by an electron-opaque material found within the T-system (Fig. 7,8). This suggests that the electron-opaque material found within the T-system may reflect yet unknown functions[16,27]. The paucity of sarcoplasmic reticulum in the muscle often leads to incomplete separation of adjacent myofibrils. This situation is in great contrast to that of fast acting muscle[1,12]. However, it is possible that the sarcoplasmic reticulum in *U. aegyptia* jaw muscle was masked by the large amount of glycogen granules, which accumulate in the space between the myofibrils.

In the tail muscle of *U. aegyptia,* the myofibrils are farther apart (Fig. 9). In contrast to the almost perfect register described for the myofibril arrangement in the jaw muscle, the tail myofibrils have a wavy appearance (Fig. 9,10). The interlacing muscle fibers of the tail run in different directions, longitudinally, transversely and obliquely (Fig. 11). This arrangement is believed to give maximal mobility and physical control of the tail. In longitudinal section (Fig. 9,10), one can observe the typical pattern of skeletal muscle with clearly defined M-lines and Z-lines. The latter show no parallel register as described for the jaw muscle.

Mitochondria were also few and lay between myofibrils without any special order relative to the striated pattern in the tail muscles (Fig. 9,11). In addition, the tail muscle showed indistinct glycogen granules, T-system and sarcoplasmic reticulum (Fig. 12).

From the results discussed above, on the basis of the fine structure of the two muscle types considered in this study, it is very difficult to assign to the commonly known muscle divisions, fast and slow acting muscles. Characteristics of various muscle types have been reported[1,12] (see also the introduction). According to the different fine structure described, one can suggest that the two muscles subserve different functions in the animal. Schiaffino *et al.*[28] studied the relation between structure and function in rat skeletal muscle. They suggested that the structure of rat muscle fibers is an expression of two main functional parameters, speed of contraction and resistance to fatigue. Due to the structural needs of different muscle functions, the above authors concluded that the classification of rat muscle, and skeletal muscle in general, should be integrated into a more complex multiple system which takes into account the interaction of several variables. It is worth mentioning that Penny and Goldspink[29] reported that energy of activation calculated for a range of tempera-

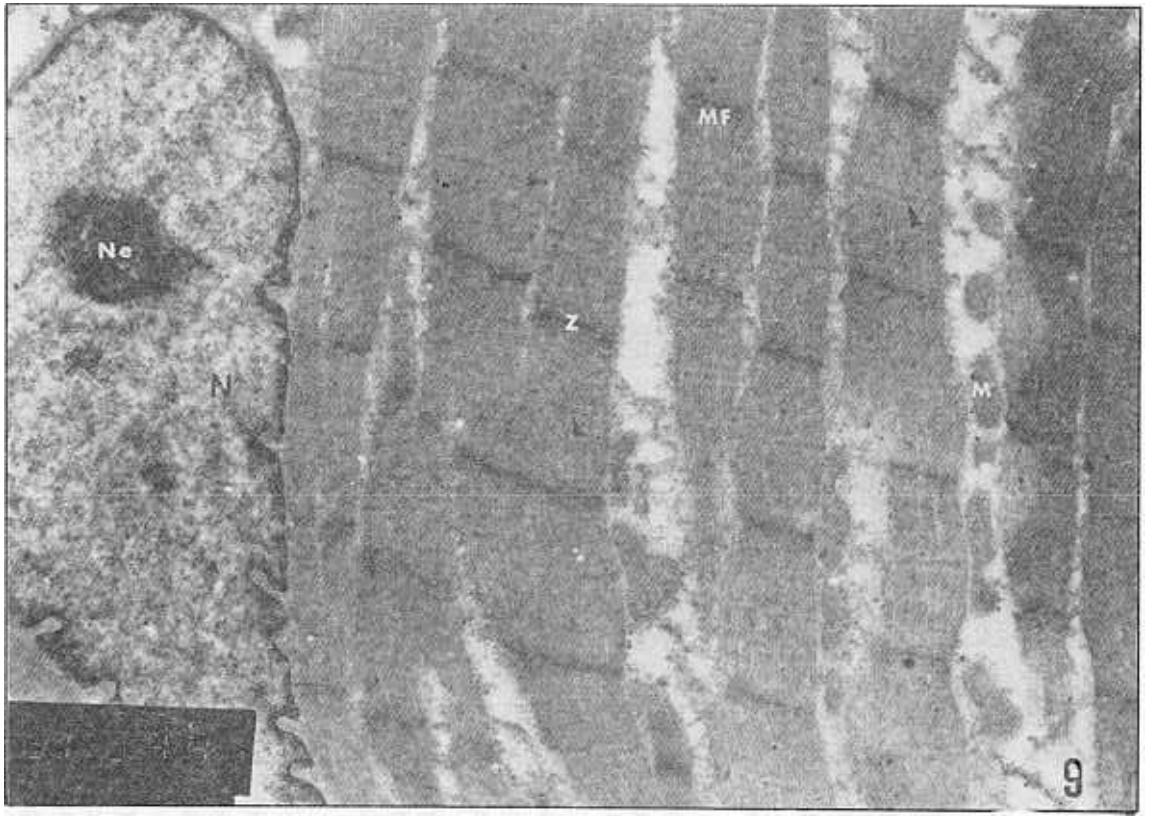

FIG. 9. A longitudinal section through muscle fiber of *U. aegyptia* tail. Note that the myofibrils are further apart but showing no perfect parallel register. M-line is clearly seen (arrow head). MF: myofibrils; Z: Z-line; M; mitochondria; N: Nucleus; Ne: Nucleolus. × 13300

FIG. 10. Longitudinal section through muscle fibers of *U. aegyptia* tail to demonstrate the wavy appearance of the myofibrils (MF). Z: Z-line. × 16500

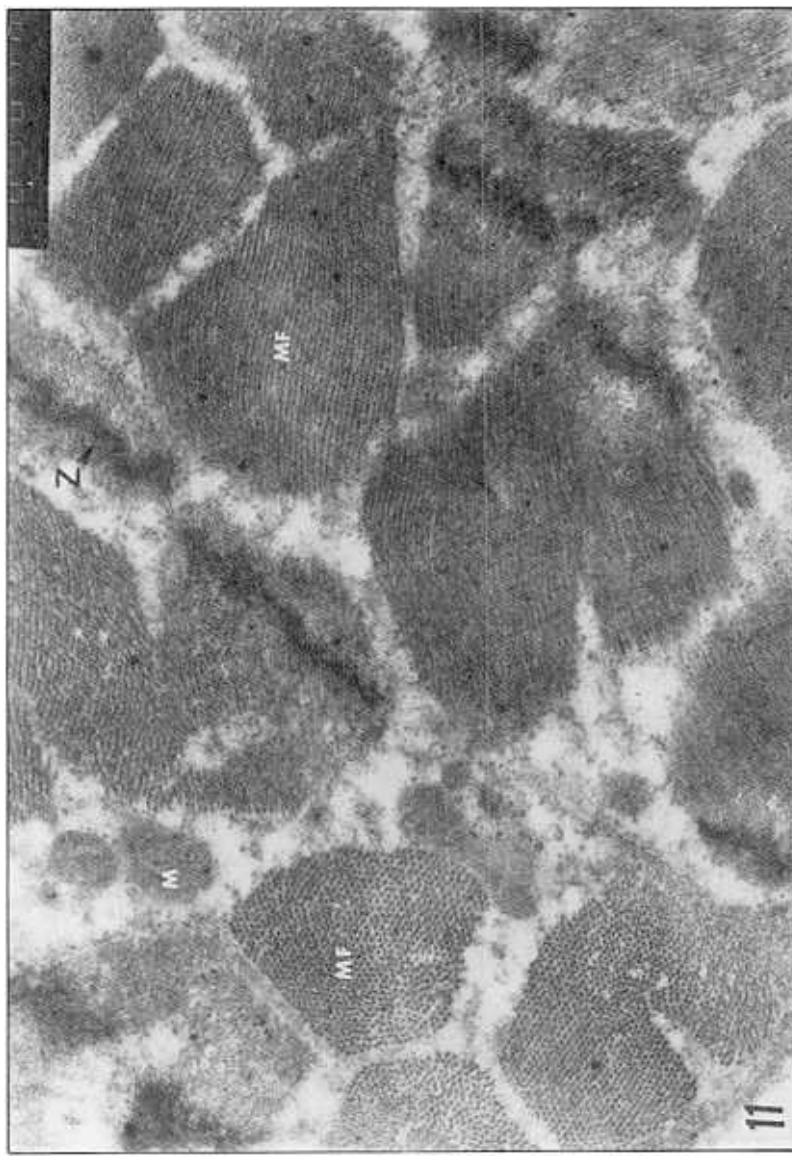

FIG. 11. An electron micrograph of the muscle fiber of *U. aegyptia* showing the myofibrils (MF) radiating in different directions. M: Mitochondria; Z: Z-line. × 18260

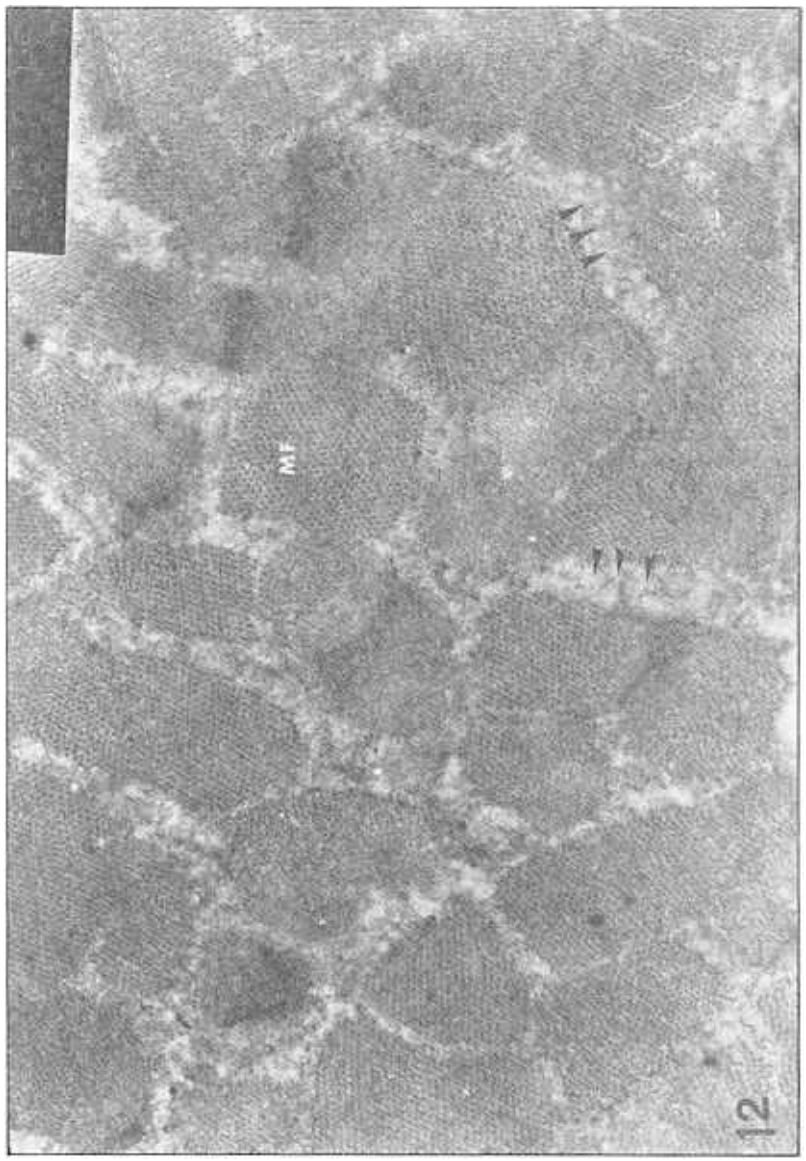

FIG. 12. A transverse section through muscle fibers of *U. aegyptia* tail. Note the large interspace (arrow heads) between the myofibrils (MF) with indistinct sarcoplasmic reticulum and T-system × 20000

tures indicated that the contractile apparatus of the related species *U. microlepis* is designed to work at a relatively high physiological temperature.

To reach a final conclusion about the type of muscle studied in the present investigation, further histochemical and biochemical research on the muscles is needed to supplement the ultrastructural research reported here.

References

[1] **Huddart, H.,** *The Comparative Structure and Function of Muscle,* Pergamon Press, Oxford (1975).
[2] **Goldspink, G.** and **Ward, P.S.,** Changes in rodent muscle fibre types during post-natal growth, under-nutrition and exercise, *J. Physiol.* **296**:453-469 (1979).
[3] **Goldspink, G.,** Development and Specialization of Skeletal Muscle, *Society for Experimental Biology. Seminar Series,* 7:19-35 (1980).
[4] **Squire, J.M.,** *The Structural Basis of Muscular Contraction,* Plenum Press, New York (1981).
[5] **Lannergrew, J.,** Fat in twitch and slow muscle fibres, *Acta Physiol. Scand.* **63**:193-194 (1965).
[6] **Lannergrew, J.** and **Smith, R.S.,** Type of muscle fibres in toad skeletal muscle, *Acta Physiol. Scand.* **68**:363-374 (1966).
[7] **Dubotwitz, V.** and **Brooke, M.,** *Muscle Biopsy: A Modern Approach,* Saunders, Philadelphia (1973).
[8] **Hoyle, G.,** Diversity of striated muscles, *Am. Zool.* **7**:432-449 (1967).
[9] **Hoyle, G.,** Comparative aspects of muscles, *Ann. Rev. Physiol.* **31**:43-84 (1969).
[10] **Fawcett, D.W.** and **Revel, J.P.,** The sarcoplasmic reticulum of a fast-acting fish muscle, *J. Biophys. Biochem. Cytol.* **10** (Suppl.): 89-109 (1961).
[11] **Revel, S.P.,** The sarcoplasmic reticulum of the bat cricothyroid muscle, *J. Cell Biol.* **12**:571-588 (1962).
[12] **Hess, A.,** The sarcoplasmic reticulum, the T-system, and the motor terminals of slow and twitch muscle fibres in the garter snake, *J. Cell Biol.* **26**:467-476 (1965).
[13] **Buller, B., Eccles, A.J.C.** and **Eccles, R.M.,** Differentiation of fast and slow muscles in the cat hindlimb, *J. Physiol.* **150**:399-416 (1960).
[14] **Porter, K.R.,** The sarcoplasmic reticulum. Its recent history and present status, *J. Biophys. Biochem. Cytol.* **10**(4):219-226 (1961).
[15] **Grinyer, I.** and **George, J.C.,** An electron microscopic study of the pigeon breast muscle, *Can. J. Zool.* **47**:517-523 (1969).
[16] **Page, S.G.,** Structure and some contractile properties of fast and slow muscles of the chicken, *J. Physiol.* **205**:131-145 (1969).
[17] **Al-Robai, A.A.S.,** *Studies on developmental changes in fine structure and metabolism of flight muscle of Locusta migratoria* L., Ph.D. Thesis, University of Durham, p. 239 (1981).
[18] **Reynolds, E.S.,** The use of lead citrate at high pH as an electron-opaque stain in electron microscopy, *J. Cell Biol.* **17**:208-212 (1963).
[19] **Goldspink, G.,** Development of muscle, *in:* **Goldspink, G. (ed.),** *Differentiation and Growth of Cell in Vertebrate Tissues,* Chapman and Hall, pp. 69-99 (1974).
[20] **Peachey, L.D.** and **Huxley, A.F.,** Structural identification of twitch and slow striated muscle fibers of the frog, *J. Cell. Biol.* **13**:177-180 (1962).
[21] **Ebashi, S.** and **Endo, M.,** Calcium ion and muscle contraction, *Prog. Biophys. Mol. Biol.* **18**:123-183 (1968).
[22] **Sandow, A.,** Skeletal muscle, *Ann. Rev. Physiol.* **32**:87-138 (1970).
[23] **Sandow, A.,** Excitation-contraction coupling in skeletal muscle, *Pharmac. Rev.* **17**:265-320 (1965).
[24] **Sandow, A.,** Electrochemical transforms and the mechanism of excitation-contraction coupling, *J. mechanochem. Cell Motility* **2**:193-207 (1973).

[25] **Ebashi, S.,** Excitation-contraction coupling, *Ann. Rev. Physiol.* **38:**293-313 (1976).
[26] **Endo, M.,** Calcium release from the sarcoplasmic reticulum, *Physiol. Rev.* **57:**71-108 (1977).
[27] **Franzini-Armstrong, C.,** Membrane particles and transmission at the triad, *Federation Proc.* **34:**1382-1389 (1975).
[28] **Schiaffino, S., Hanzlikova, V.** and **Pierobon, S.,** Relations between structure and function in rat skeletal muscle fibers, *J. Cell Biol.* **47:**107-119 (1970).
[29] **Penny, R.K.** and **Goldspink, G.,** Adaptation of the contractile proteins of the desert lizard *Uromostyx microlepis*, *J. Univ. Kuwait (Sci.)* **6:** 159-167 (1979).

التركيب الدقيق للعضلات الهيكلية للضب المصري

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يهتم هذا البحث بالتركيب الدقيق للعضلتين الفكية الخارجية والذيلية في الضب المصري . وقد أظهرت النتائج اختلافات في الشكل المورفولوجي العام بين العضلتين . فقد وجد في العضلة الفكية الخارجية : الشبكة الإندوبلازمية (غير متطورة) ، وجهاز (T) ، ووفرة حبيبات النشا الحيواني . وعلى النقيض فإن هذه المكونات غير متميزة في العضلات الذيلية .

بالإضافة إلى ذلك ، فإن العضلة الفكية أظهرت قطعاً عضلية مثالية ذات تخطيط متوازٍ ولكن خط (M) غير واضح . ولقد أظهرت النتائج اختلاف صفات وتفاصيل التركيب الدقيق للعضلتين المدروستين ، إلا أن التركيب الأساسي لهما مشابه لتركيب العضلات الهيكلية للفقاريات . ويجب عمل دراسات بيوكيميائية وفسيولوجية للوصول إلى معرفة نوع العضلة .