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MUSCLE ULTRASTRUCTURE IN NEWBORN PIGLETS WITH SPLAYLEG SYNDROME*

[1]) J. BUČEK, [2]) Z. LUKÁŠ, [3]) L. PIVNÍK, [3]) J. KAMAN

[1]) II. Department of Pathology, Children's Hospital, J. E. P. University, 662 63 Brno [2]) Children's Hospital, 662 63 Brno [3]) Department of Pathological Morphology and Parasitology, University of Veterinary Science, 612 42 Brno.

Dedicated to the 60th birthday of Prof. MVDr. Miloslav Zendulka

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Abstract

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Muscle samples of the m. longissimus dorsi and m. biceps brachii from 6 splaylegged newborn piglets (12–48 hours post partum) were compared with similar muscles of the control animals of the same age. In most muscle fibres the sarcolemma was only partially filled with myofibrils, the rest – the extramyofibrillar space (EMS) showed metachromatic staining with toluidin blue in the semithin sections. In the EMS we found glycogen particles showing positive Thiéry reaction, they were arranged in rosettes. This reaction and amylase digestion have shown that, in addition to glycogen, the EMS also contains ribosomes and polysomes which are usually located in the subsarcolemmatic areas. Polysomes were also found in the close proximity of the fine granular fibrous material inside the glycogen masses. They are probably bundles of maturated actin fibres. Glycogen is highly soluble in the aldehyde fixatives. Myofibrils were changed only exceptionally. The only variation was the finding of streaming Z lines. A decrease in number of myofibrils and alteration in their arrangement were observed in the proximity of the altered Z lines. These changes, however, were also recorded in control animals. No changes were found in the nuclear membrane of the karyoplasm or organelles suggestive of the splayleg syndrome.

Myofibrillar hypoplasia, muscle fibre development, neuromuscular unit.

Although the splayleg syndrome of newborn piglets has recently been subject of extensive histological, biochemical and ultrastructural studies, its etiopathogenesis remains unclear. This also reflects in various names used in connection with the disease: myofibrillar hypoplasia, myofibrillar degeneration, myofibrillar retardation. Ultrastructural studies have described some positive findings that could be characteristic of the splayleg syndrome. The findings involve above all, changes in the nucleus and nuclear envelope (Zelená et al. 1978) and myofibrillar changes (Bergmann 1976; Zelená and Jirmanová 1979). By comparison of ultrastructural findings in muscles of splaylegged and healthy piglets we aimed at assessing the diagnostic significance of the changes reported.

Materials and Methods

We studied muscle samples from 6 splaylegged piglets. As controls we used 3 healthy piglets from a herd affected with splayleg syndrome. Other 3 control piglets came from a herd free of splayleg. The muscle excisions were performed on the m. longissimus dorsi and biceps femoris. The samplings were carried out 12 to 48 hours after birth. The excised material was cut in order to obtain

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blocks up to 2 mm² and immediately fixed in 1,6 % glutaraldehyde in phosphate buffer according to Schulze and Karlsson (1965). The duration of the fixation in the glutaraldehyde solution was 60—120 min. Following the threefold rinsing of the blocks in the above mentioned buffer the material was osmificated (1% OsO_4 in the above mentioned phosphate buffer). Already in the course of dehydration some of the blocks were contrasted with uranyl-acetate. The blocks were embedded in Epon-Araldite and cut in Ultratone IV LKB. Semi-thin sections were stained with toluidin blue. Thin sections were contrasted with uranyl acetate and lead citrate and photographed in the Tesla BS 500 microscope. Glycogen in the thin sections was detected by using diastase digestion and positive detection of glycogen was performed by the method described by Thiéry (1967).

Results

Evaluation of the semi-thin sections obtained from both affected and intact piglets shows that there are no significant morphological differences between these two groups. The myofibrils are arranged in unsplit bundles and located nearer to the sarcolemma. They generally fill only a part of the cytoplasm. The remaining part of it, known as extramyofibrillar space (EMS), gives metachromatic reaction with toluidin blue.

Only sporadically the myofibrils fill the major part of the cytoplasm or fill it completely. In the cytoplasm of a number of muscle cells, lipid vacuoles can be detected. The EMS is stained with the toluidine blue.

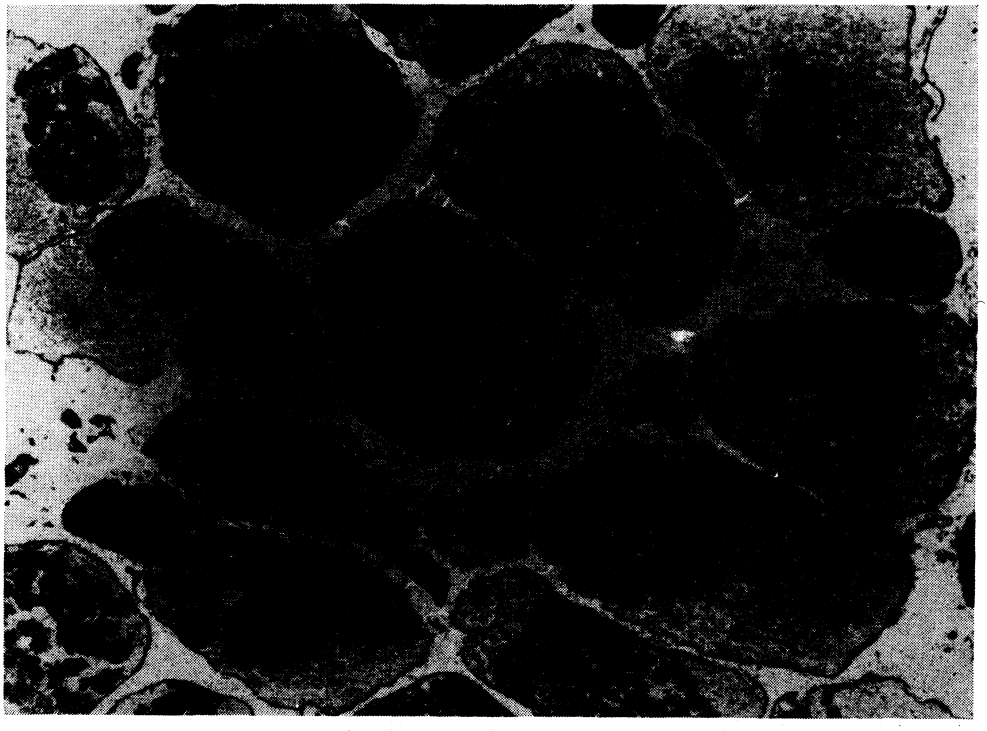


Fig. 1

Muscle cells in cross section. Myofibrillar bundles filling only a part of the muscle cell. The remaining part of the cytoplasma is filled with granular material. (g) × 3000.

In accordance with participation of myofibrils, mitochondria and lipid vacuoles and extension of the extramyofibrillar space, three types of muscle cells can be described:

1. Cells the cytoplasm of which is filled with a small number of myofibrils. The extensive EMS is mainly filled with particles of about 4—40 nm in diameter (Figs 1, 2, 4). Their electronoptic density is markedly varying.

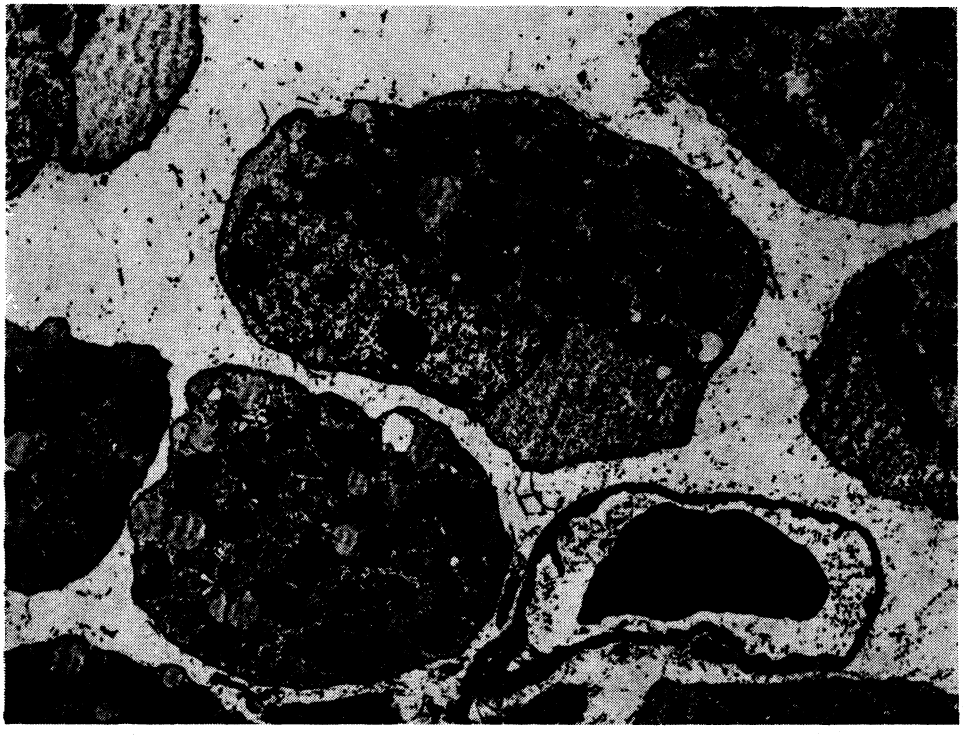
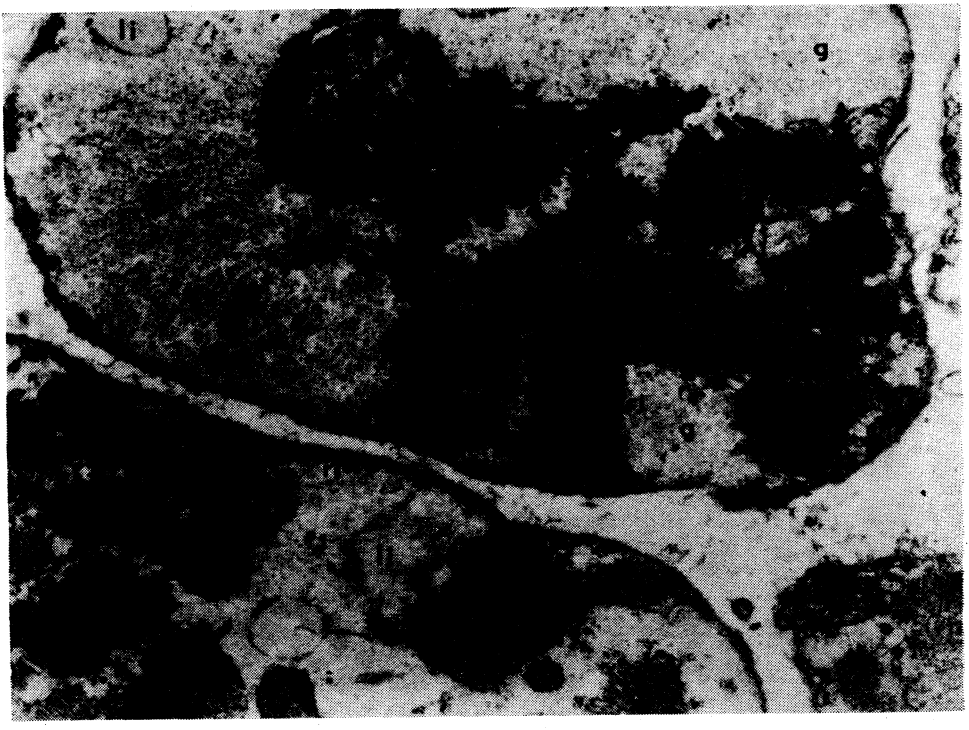


Fig. 2.
Muscle cells in cross section. Sporadic cells are completely filled with myofibrillar bundles. Numerous lipid vacuoles in the muscle cells. × 4500.

2. Cells which also have a clearly developed EMS filled with the above described granular material, but there is a higher number of mitochondria and also higher frequency of lipid vacuoles (Fig. 2) among the myofibrils.
3. The less frequent cells with cytoplasm almost completely filled with myofibrillar bundles and other organelles.

The cytoplasmic membrane of the muscle cells was surrounded by a complete basal membrane of about 30—50 nm width. In comparison with the maturated state, the diameter of the basal membrane was less than a half of it.

The fine and coarse granular material filling the major part of EMS was analysed in greater detail. We could see smaller isolated particles of about 4—10 nm in diameter and also bigger ones. The bigger particles of about 35—40 nm in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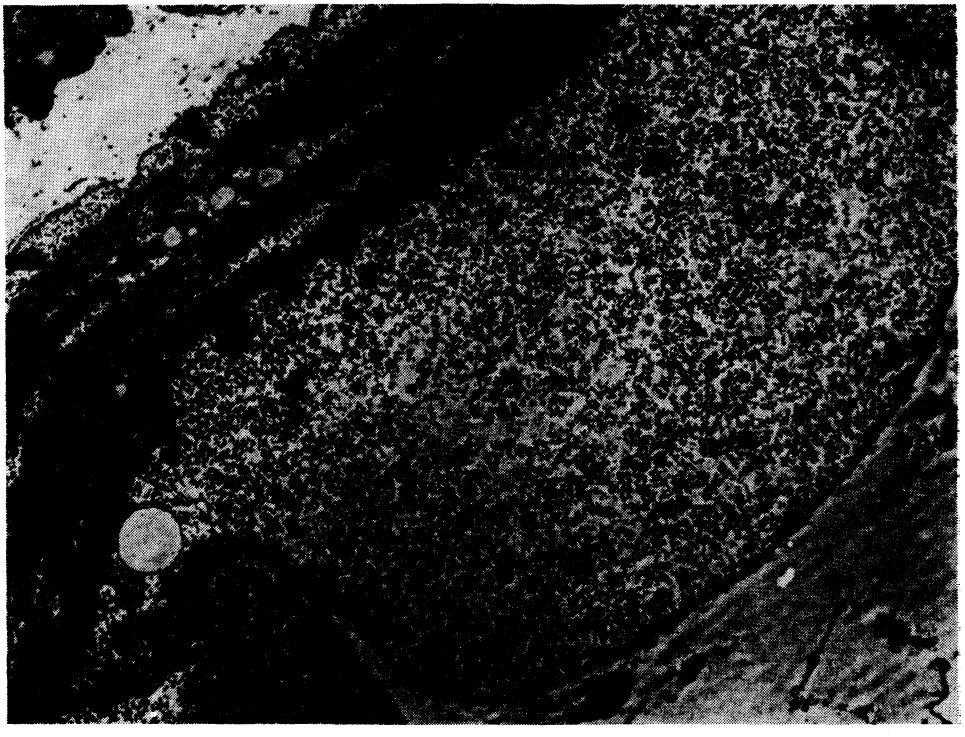


Fig. 3.

Partial amylase digestion of glycogen. Glycogen granules (g) are shadowy. Isolated ribosomes (ri) and polysomes are more numerous near the cytoplasmic membrane and areas adjoined to myofibrils, above all in the region of actin fibres (a). The Golgi apparatus (ga) with high participation of coated vesicles. Lipid vacuoles (li). x 16 000.

diameter were formed by aggregation of large number of smaller particles of about 4—6 nm in diameter. The small particles correspond to gamma particles and the big ones to beta particles of glycogen. The negative assessment of glycogen particles is carried out by amylase digestion (Figs 3, 9, 10). The digestion indicates that the major part of the granular material filling the EMS is glycogen. Positive glycogen findings in the thin section were confirmed by using the reaction described by Thiéry (1967). The results of the reaction show that the basic glycogen structure in muscle fibres consists of particles of about 4—6 nm in diameter forming larger particles. Moreover, glycogen proves not to be limited to the EMS, but numerous granules can be found even in the intermyofibrillar space and a small part also in the proximity of the Golgi apparatus (Figs 5, 6, 7).

Even after the amylase digestion in the EMS, isolated and also aggregated particles of high density can be detected. Their size is 10—15 nm. They are located subsarcolemmatically, but they can be also detected among the glycogen particles in the EMS. They are also abundant in the proximity of mitochondria of some lipid particles. They were also more frequent at the site where the glycogen-filled EMS adjoins the myofibrils (Figs 3, 8, 10). Their size, impossibility of digestion by diastase and a strong electron-optical contrast indicate that they are isolated ribosomes and polysomes.

The density of glycogen particles, especially in the EMS, was generally lower than that revealed by routine electron microscopy, even when the material was sufficiently osmificated and contrasted by uranium and lead salts. Some of the EMS, probably as a consequence of the glycogen elution during the aldehyde fixation and sample rinsing, were partly empty (Figs 4, 5).

The Golgi apparatus was located perinuclearly. Most pronounced was its vesicular part with high proportion of coated vesicles. The coated vesicles were more frequently found subsarcolemmatically, occasionally in contact with the cytoplasmic membrane.

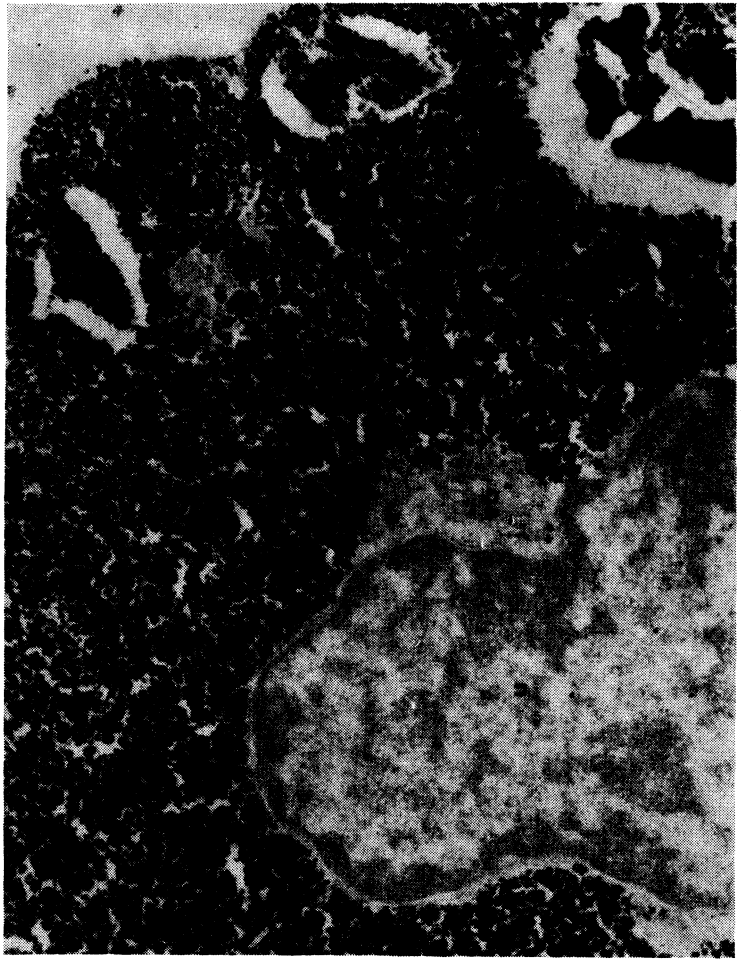
Some tubules of the smooth reticulum were also located mainly subsarcolemmatically (Fig.3). The short tubules of the granular endoplasmic reticulum were most frequent among the myofibrillar bundles.

The polysomes were located subsarcolemmatically, in close proximity of myofibrils and among them, especially near accumulated granular-fibrous material of medium electron-density. This material was often associated with myofibrils and was considered as actin fibres (Figs. 3, 9, 10).

In most cases myofibrils filled only a part of the muscle cells. The diameter of the myofibrillar bundles varied. Occasionally we found irregularly formed

Fig. 4.

Longitudinal section through a muscle cell. The EMS is filled with granular material (g). × 6000.



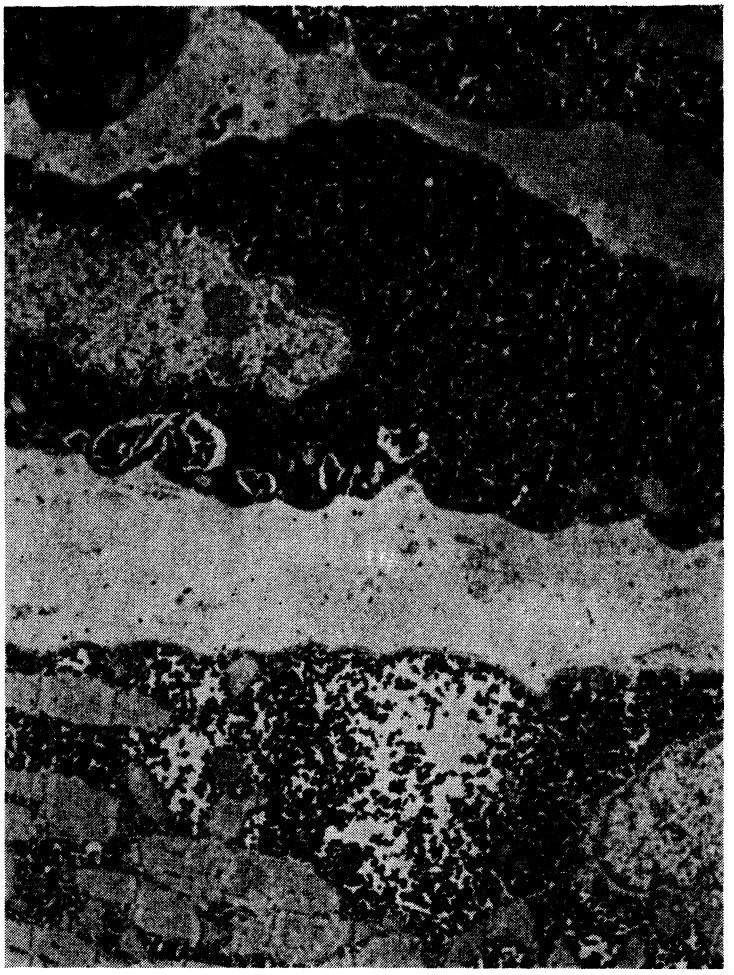


Fig. 5
Projection of glycogen in the EMS by using the method by Thiéry.

Z lines, named as streaming Z lines (Fig. 8), and adjacent areas with reduction of myofibrils and their irregular arrangement.

However, streaming of Z lines, decreased numbers of myofibrils and changes in their arrangement in the proximity of streaming Z lines were also recorded in some control animals without splayleg.

Lysosomes were recorded only occasionally, phagolysosomes and areas of focal cytoplasmic degradation were absent.

In some of the muscle cells mitochondria were numerous. The cells with higher number of mitochondria had higher numbers of myofibrils and very often lipid vacuoles.

Within 12—48 hours after birth rather high numbers of lipid vacuoles were present in the muscle cells. They were located among the bundles of myofibrils and also in the EMS among the glycogen particles. As to formation of the nuclear membrane, chromatin, nucleoli and other cytoplasmic organelles, no variations were recorded.

Discussion

The amylase digestion and demonstration of the glycogen particles by using the Thiéry (1967) method have shown that the EMS was filled with glycogen particles of about 4—6 nm in diameter, mostly aggregated into larger formations. The smaller particles (4—6 nm) correspond to the glycogen gamma particles. The presence of polysaccharides in the EMS was accounted for by the metachromatic staining of the EMS in the semi-thick sections (Bradley et al. 1980; Ward and Bradley 1980). According to our experience the EMS glycogen was more easily soluble in aldehyde aqueous fixatives and thus it was probably more water-soluble. Apart from the higher solubility, the EMS glycogen in newborn piglets does not produce so clear electron-optical contrast, which was probably due to its partial elution.

Deutsch and Done (1971) described ribosomes in the EMS and Bradley et al. (1980) report that the contrast particles in the EMS in newborn piglets consist of polysaccharides, especially glycogen. The use of amylase digestion enabled detection of isolated ribosomal particles and polysomes in the EMS, especially in the subsarcolemmatic location, occasionally round the lipid vacuoles and more often round the accumulated finely granular fibrous material in the EMS. This material is of medium electron-optical density and corresponds to bundles of actin fibres. Similar material can be found in association with myofibrils. Its occurrence in the EMS is not considered as pathological and we presume that it indicates maturation of muscle fibres of this area.

Fig. 6.
Thiéry method. Beta particles prevailing in the EMS. They had been formed by aggregation of small granules (4—6 nm). × 18 000.

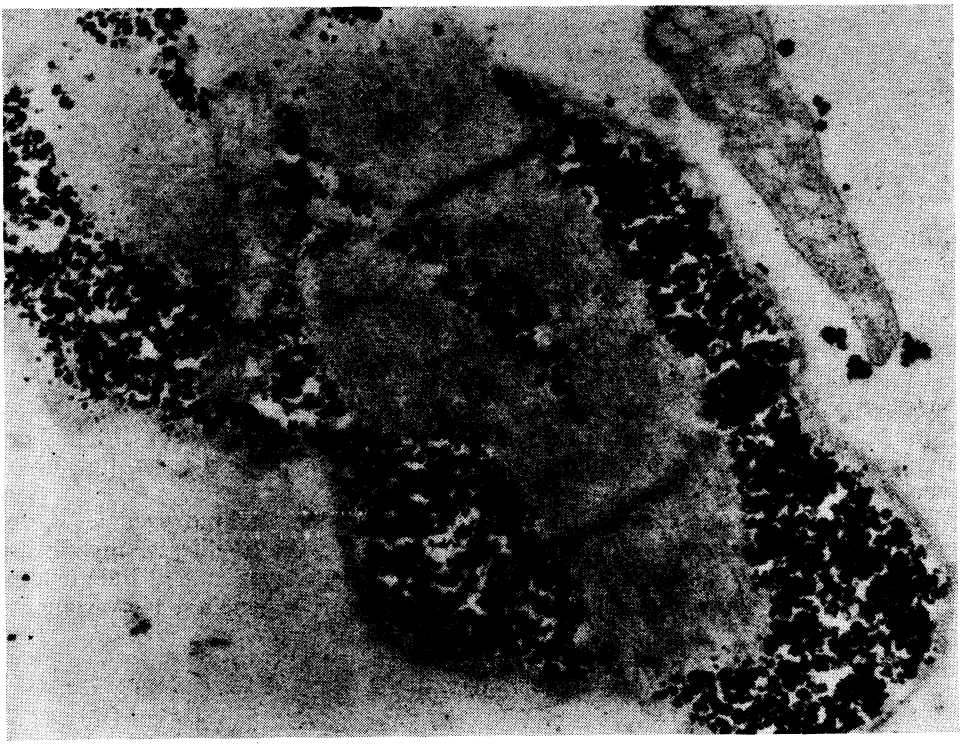


Fig. 7.

Thiéry method demonstrating glycogen particles in the EMS around in the intermyofibrillar space.

Alterations in the nuclear membranes in the affected animals, as described by Zelená et al. (1978), have been observed in our material neither in splaylegged nor in healthy animals.

Within one week after birth the number of lipid vacuoles in the muscles of the newborn piglets increases (Bradley et al. 1980; Moody et al. 1978; Schlotke and Koch 1978). They were also detected in our material and were frequent in the muscle cells with higher numbers of mitochondria. The above-mentioned relation cannot be considered as absolute and we think that it is not sufficient for ultrastructural type-classification of muscle fibres (Bradley et al. 1980).

In myofibrils we occasionally observed irregular formation of Z lines, so called streaming, as described by Zelená and Jirmanová (1979), in connection with reduction and disorganization of myofibrils in their close proximity. The findings of alterations in myofibrils were very scarce and, moreover, they were detected in some of the control animals. For this reason, these ultrastructural findings cannot be considered as unambiguously suggestive of the splayleg syndrome. The streaming Z lines were also found in the human skeletal muscles of healthy volunteers (Reske-Nielsen 1974). No extensive disintegration of myofibrils (myofibrillar degeneration) with the subsequent cytoplasmic reaction with numerous phagolysosomes was recorded. It cannot be excluded that the negative feature of our findings could be a consequence of a short postnatal investigation interval (12—48 hours).

The comparison of muscle ultrastructure of healthy and splaylegged newborn piglets in our investigation did not reveal any significant diagnostic ultrastructural differences, in contrast to some other authors as Bergmann (1976), Zelená et al. (1978), Zelená and Jirmanová (1979). Any interpretation of ultrastructural changes is difficult without knowledge of the physiological maturation process. Therefore the solution can be seen, above all, in quantitative evaluation of muscle changes on the basis of the physiological maturation determined. Some authors have recently attempted at such evaluation (Zelená and Jirmanová 1979; Cox et al. 1979).

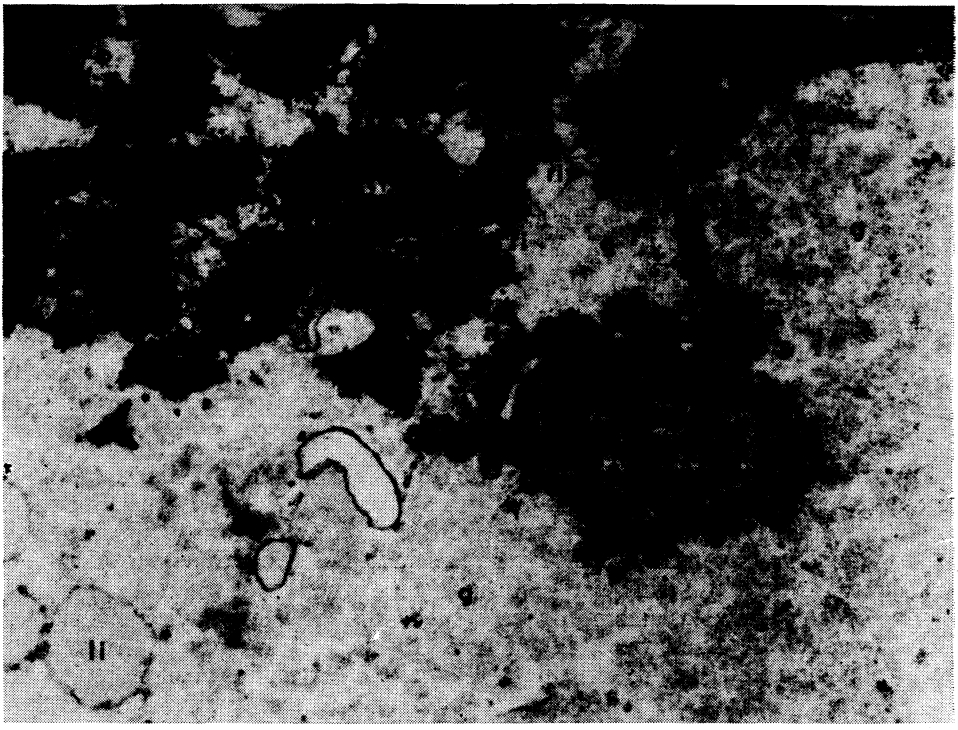
Technical cooperation of Dr. Ivan Straka is gratefully appreciated.

Ultrastruktura svalů novorozených selat postižených syndromem svalové slabosti končetin

Vzorky m. longissimus dorsi a m. biceps brachii šesti nemocných novorozených selat (12—48 hod. post partum) byly porovnány se stejnými svaly stejně starých kontrolních zvířat. Většina svalových vláken měla sarkoplasmu jen zčásti vyplně-

Fig. 8.

Irregular formation of Z lines and failure in myofibrillar arrangement in the affected areas. × 30 000



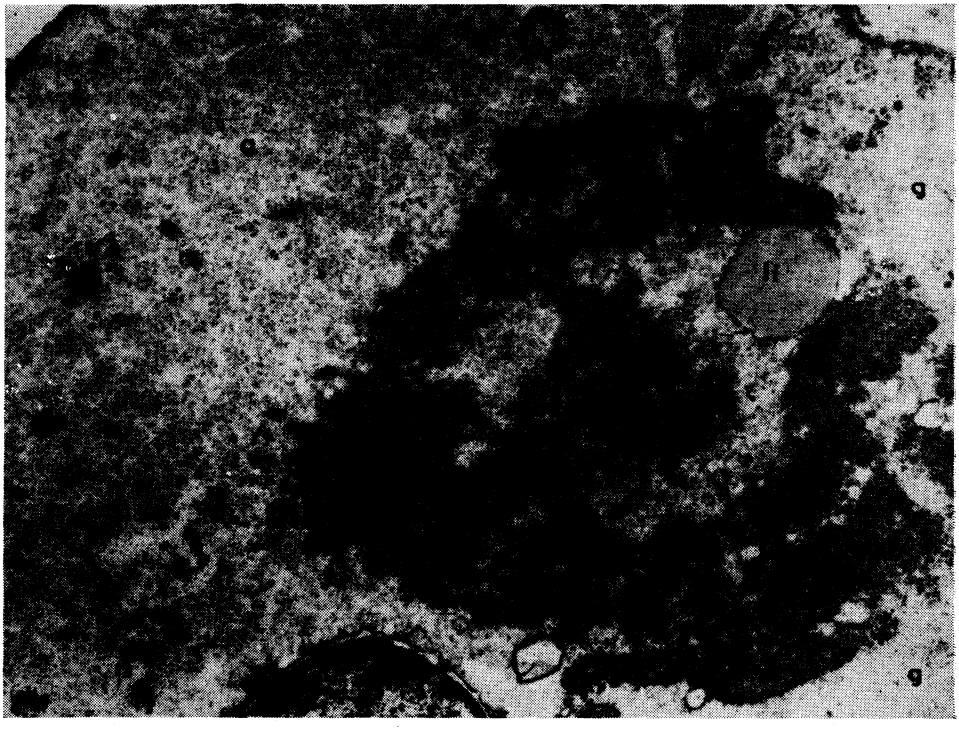


Fig. 9.

Partial amylase digestion. The ribosomal particles (ri) are located under the sarcolemma, near myofibrils. Focuses of accumulated actin-like fine granular fibrous material (a). Glycogen (g) is shadowy. Lipid vacuoles (li) × 16 000.

nou myofibrilami, zbývající prostor vykazuje v polosilných řezech metachromatické zbarvení toluidinovou modří. V tomto prostoru byly nalezeny glykogenové částice, skýtající pozitivní Thiéryho reakci, uspořádané do roset. Podle této reakce a digesce amylázou bylo prokázáno, že vedle glykogenu se tam vyskytují i ribosomy a polysomy které jsou situovány nejčastěji do subsarkolemmatických oblastí. Polysomy, se též nacházejí v těsné blízkosti jemně granulárně vláknitého materiálu uvnitř glykogenových mas. Jde pravděpodobně o nakupení polysomů kolem maturujících vláken aktinu. Glykogen je značně rozpustný v aldehydových fixativech. Myofibrily byly alterovány jen výjimečně. Jedinou zřetelnější odchylou byl nález „streaming Z-lines". V okolí změněných Z lines je snížený počet myofibril a alterovaná jejich organizace. Uvedené změny byly však nalezeny i u kontrolních zvířat. Nebyly nalezeny změny jaderné membrány karyoplasmy či organel, které by byly pro syndrom SSK příznačné.

Ультраструктура мыщ новорожденных поросят с синдромом расскольвания конечностей

Образцы m. longissimus dorsi и m. biceps brachii шести поворожденных (12—48 час. post partum), пораженных синдромом расскольвания конечностей поросят сравнивались с теми же мышцами контрольных животных одинакового возраста. У большинства мышечных волокон саркоплазма только частью была заполнена миофибриллами, остающееся пространство на полутолстых разрезах отличается метахроматической окраской толуидиновым синим. Вэтом пространстве были обнаружены сформированные в розеты гликогеновые частицы, дающие положительную Thiéry — реакцию. Согласно этой реакции и путем дигестии амилазой установлено, что кроме гликогена здесь находятся также рибосомы и полисомы, которые наиболее часто локализованы в субсарколемматические области. Полисомы находятся также в близком соседстве с тонким гранулярно-волокнистым материалом внутри гликогеновой массы. Это, очевидно, объясняется накоплением полисом вокруг созревающих волокон актина. Гликоген легко растворим в альдегидных фиксативах. Изменения у миофибрилл наблъдались лишь исключительно. Было обнаружено единственное резко выраженное видоизменение — »streaming Z-lines«. В соседстве измененных Z-lines наблюдаются уменьшение

Fig. 10.

Partial amylase digestion. Glycogen (g) is not dense. Ribosomes (ri) and polysomes are multiplied near the nuclear membrane and in the region where the EMS is bordering on the myofibrils, and in the vicinity of actin fibres (a), mitochondria, Golgi apparatus and occasionally even in the regions bordering upon the lipid vacuoles (li). × 18 000.

количества миофибрилл и изменения в их организации. Однако указанные изменения были обнаружены также у контрольных животных. Не были обнаружены изменения в ядерной мембране кариоплазмы и органелл, свойственные синдрому расскольза ния конечностей.

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