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Original article

Ultrastructural demonstration of glucose-6-phosphatase activity and glycogen in skeletal muscles of newborn piglets with the splayleg syndrome

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Summary — The ultrastructural localization of glucose-6-phosphatase activity and glycogen were investigated in the longissimus dorsi and biceps femoris muscles of normal and splaylegged newborn piglets. Significant differences were ascertained in the distribution of the reaction product of glucose-6-phosphatase activity between the two groups of animals. A fine precipitate was found in the sarcoplasmic reticulum and in the perinuclear cisternae of normal piglet muscles. In splaylegged muscles, variable deposits of coarse reaction product were observed within the extremely dilated cisternae of sarcoplasmic reticulum at their periphery. Moreover, both longitudinal and transversal ultrathin sections of these muscles showed a reduced number of myofibrils and an increased accumulation of glycogen (especially within the large extramyofibrillar spaces) in comparison with muscles of normal piglets.

splayleg / muscle ultrastructure / glucose-6-phosphatase / glycogen / pig

Résumé — Démonstration ultrastructurale de l'activité glucose-6-phosphatase et du glycogène dans les muscles squelettiques de porcelets nouveau-nés atteints du syndrome d'abduction des membres. *La localisation ultrastructurale de l'activité glucose-6-phosphatase et du glycogène a été analysée dans des muscles* (longissimus dorsi *et* biceps femoris) *de porcelets nouveau-nés normaux ou atteints du syndrome. Des différences significatives ont été mises en évidence dans la distribution du produit de réaction de la glucose-6-phosphatase entre les deux groupes d'animaux. Un fin précipité a été observé dans le réticulum sarcoplasmique et l'espace périnucléaire dans les muscles de porcelet témoin. Dans les muscles anormaux, des dépôts variables et grossiers du produit de réaction ont été localisés dans les vésicules très dilatées du réticulum sarcoplasmique. De plus, les*

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coupes ultrafines longitudinales et transversales de ces muscles montrent un nombre réduit de myofibrilles et une accumulation de glycogène (spécialement dans les larges espaces extramyofibrillaires) par rapport aux muscles de porcelets normaux.

abduction des membres / ultrastructure musculaire / glucose-6-phosphatase / glycogène / porc

INTRODUCTION

Splayleg is a congenital developmental disorder which influences the locomotion and posture of newborn pigs. The aetiology of splayleg is unknown. It probably has a polygenic basis with a varying degree of penetrance. Histological and histochemical alterations of splaylegged muscles suggest that it is caused by the retardation of muscle differentiation. The forelimb muscles, which complete their developmental process earlier than the hind limb muscles, are less affected by muscle weakness (Thurley et al, 1967; Swatland, 1974; Lax, 1971; Ward, 1978; Hanzlíková, 1980; Stein, 1993). The immaturity of the muscle fibres is not caused by insufficient motor innervation since the neuromuscular transmission and morphological and biochemical characteristics of the motor endplates are not altered in splaylegged muscles (Hník and Vejsada, 1979; Hanzlíková, 1980; Tuček and Hanzlíková, 1984; Ohnishi et al, 1989). Morphological findings of Zelená and Jirmanová (1979) and Jirmanová (1983) suggest that splayleg might represent a congenital form of glucocorticoid myopathy. Increased hormone levels in stress-susceptible pregnant sows could influence the pathological development of fetal muscles. On the other hand, Ducatelle et al (1986) found some differences between splaylegged piglets and piglets with experimentally-induced glucocorticoid myopathy.

A deficiency in myofibrils, termed 'myofibrillar hypoplasia' (MFH), is the most characteristic microscopic feature of splayleg disease. MFH ranges from a slight reduction of myofibrillar content to severe myofibrillar deficiency, vacuolization, focal degeneration and necrosis. Besides the limb muscles, the longissimus dorsi muscle is also severely affected. The sarcoplasm is partly filled with glycogen instead of contractile elements (Thurley et al, 1967; Thurley and Done, 1969; Ward, 1978; Zelená and Jirmanová, 1979; Bradley et al, 1980). MFH, however, has also been found in many clinically normal newborn piglets (Kaman et al, 1977; Cox et al, 1979; Ducatelle et al, 1986). The term MFH should not therefore be used as a synonym for porcine splayleg. Regardless of this discrepancy in terminology, it is clear that MFH is causally associated with splayleg, and its manifestation is probably dependent on the distribution of MFH-free muscle fibre within the muscle fascicles (Cox et al, 1979).

Glycogen metabolism seems to be altered in the splayleg syndrome. A higher accumulation of glycogen was histochemically demonstrated in the muscle fibres of affected piglets in comparison with normal ones (Hanzlíková, 1980). Large extramyofibrillar spaces accompanying the myofibrillar degeneration revealed a poorly structured cytoplasm with a 'watery' appearance (Bergmann, 1976). The spaces were filled with granules which were probably a mixture or ribosomes and accumulated glycogen (Deutsch and Done, 1971).

Glucose-6-phosphatase (G-6-Pase) is considered to be a key enzyme required for the utilization of glycogen reserves (Banks et al, 1976). In the present study we demonstrate its activity and glycogen distribution at the ultrastructural level in skeletal muscles of newborn splaylegged and normal piglets.

MATERIAL AND METHODS

Muscle samples were obtained from four normal (without visible phenotypical manifestation of splayleg) and six splaylegged newborn littermate piglets of Belgian Landrace. Small sections of the longissimus dorsi and biceps femoris muscles were removed on the first day after birth from animals anaesthetized with Nembutal.

Routine procedure was used for electron microscopical examination (ie, fixation in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2–7.4, postfixation in 2% OsO_4 in the same buffer, dehydration with an ascending alcohol series and embedding in Durcupan ACM). Ultrathin sections were contrasted with uranyl acetate and lead citrate.

Glycogen was localized in the ultrathin durcupan sections according to Thiéry (1967) using periodic acid (20 min), thiosemicarbazide (2 h) and silver proteinate (30 min). The ultrathin sections could be contrasted or left without contrasting.

G-6-Pase activity is sensitive to the fixation process. Very short treatment with glutaraldehyde is recommended for successful ultrastructural demonstration of G-6-Pase (Berteloot and Hugon, 1975; Kanamura, 1975). For this reason, cryostat sections (40 μm) were fixed in cooled 3% glutaraldehyde for 1 min, thoroughly washed in distilled water and incubated in the medium described by Wachstein and Meisel (1956) for 30, 45 and 60 min at room temperature. For the control sections, substrate-free medium was used for the incubation. Adult rat liver acted as the test organ. After the reaction, the sections were postfixed in 1% OsO_4, dehydrated in an alcohol series and embedded in Durcupan ACM. Ultrathin sections were used without any further contrasting.

All the ultrathin sections were prepared using an LKB Nova III ultramicrotome and observed with a JEOL JEM CX II 100 electron microscope.

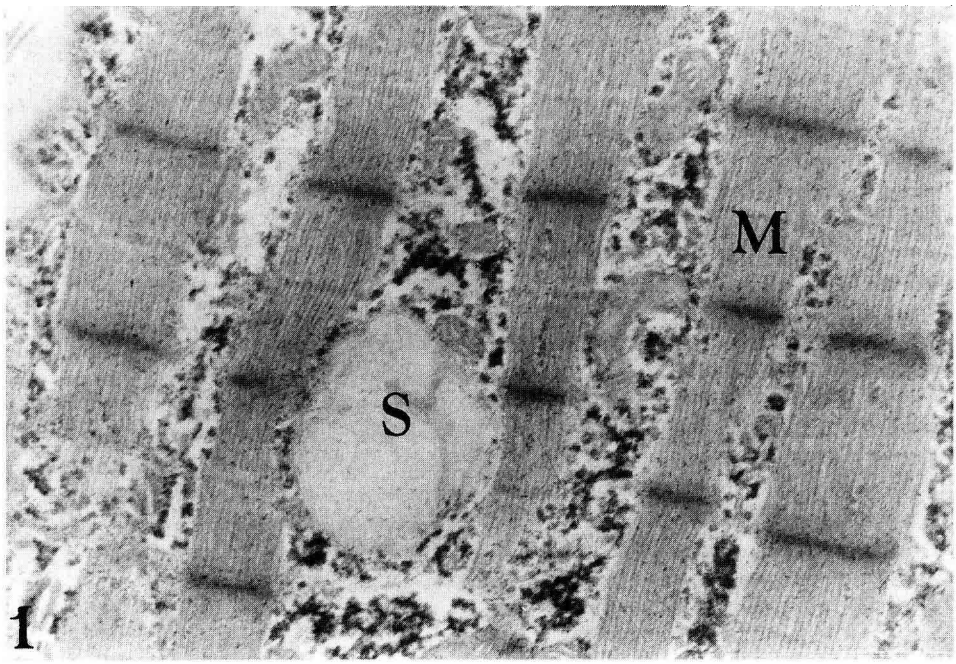
RESULTS

The muscle fibres of normal piglets were in close contact with each other. They were filled with myofibrils showing a regular cross striation. Extramyofibrillar spaces were rare. Nuclei were located peripherally, and in dense chromatin reflected a relatively quiescent mitotic state. Mitochondria were short and ovoid with simple straight cristae and were often located near the sarcolemma. The fine sarcoplasmic reticulum was visible among the myofibrils as well as small groups of glycogen particles and a small number of lipid droplets. Electron microscopic analysis of both longitudinal and transverse ultrathin sections of splaylegged muscle fibres demonstrated a reduced number of myofibrils. Mitochondria, lysosomes and lipid droplets were present within the sarcoplasm. The cisternae of the sarcoplasmic reticulum were enormously dilated and the T-tubules were small and irregular. Large extramyofibrillar spaces were optically empty or filled with glycogen particles (figs 1, 2).

As mentioned above, high glycogen accumulation is one of the characteristic symptoms of the splayleg syndrome. Glycogen, demonstrated by Thiéry's method (1967), was represented by simple, solitary round particles known as β-granules.

In the muscles of the normal animals, the glycogen was dispersed among the myofibrils and appeared near the nuclei as a fine perinuclear rim (fig 3). The splaylegged muscles showed glycogen particles dispersed in the spaces among the myofibrils. They also filled the vast areas at the muscle fibre periphery where a clump (fig 4) or chain-like (fig 5) distribution was sometimes observed.

Significant differences were found in the ultrastructural localizations of the G-6-Pase activity. It was regularly distributed in the cisternae of the sarcoplasmic reticulum near the Golgi apparatus and in the perinuclear spaces of the muscle fibres of normal piglets (figs 6, 7). In splaylegged muscles, variable concentrations of reaction product were demonstrated within the extremely dilated cisternae of the sarcoplasmic reticulum near the muscle fibre borders (fig 8). This was also sometimes observed in the glycogen occupied area.



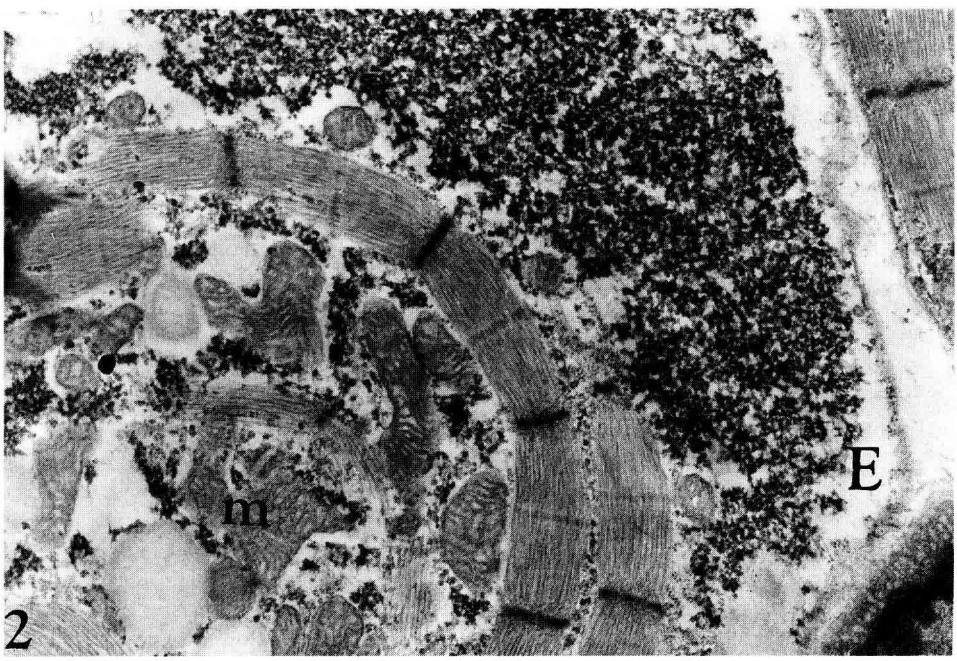
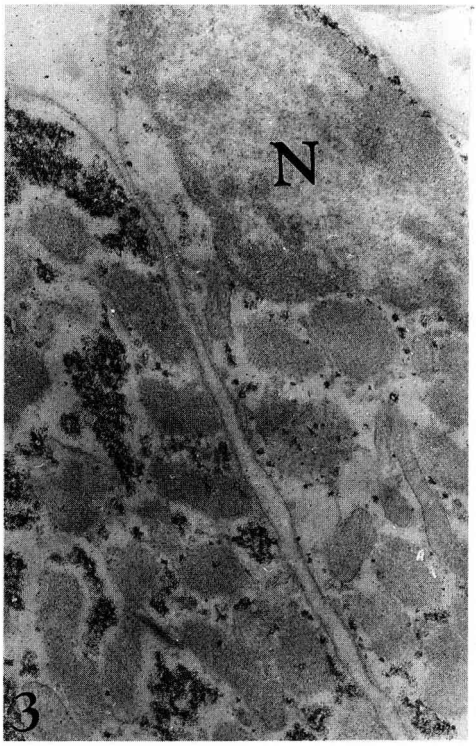


Fig 1. Routine electron microscopy: the biceps femoris muscle of newborn piglet with splayleg. Myofibrilar content is slightly reduced, glycogen particles are dispersed among myofibrils. Note the greatly dilated cisternae of the sarcoplasmic reticulum (x 32 000).

Fig 2. Routine electron microscopy: the longissimus dorsi muscle of newborn piglet with splayleg. Cell organelles, lipid droplets and glycogen are dispersed among myofibrils. Large extramyofibrillar space is filled with glycogen particles (x 16 000).



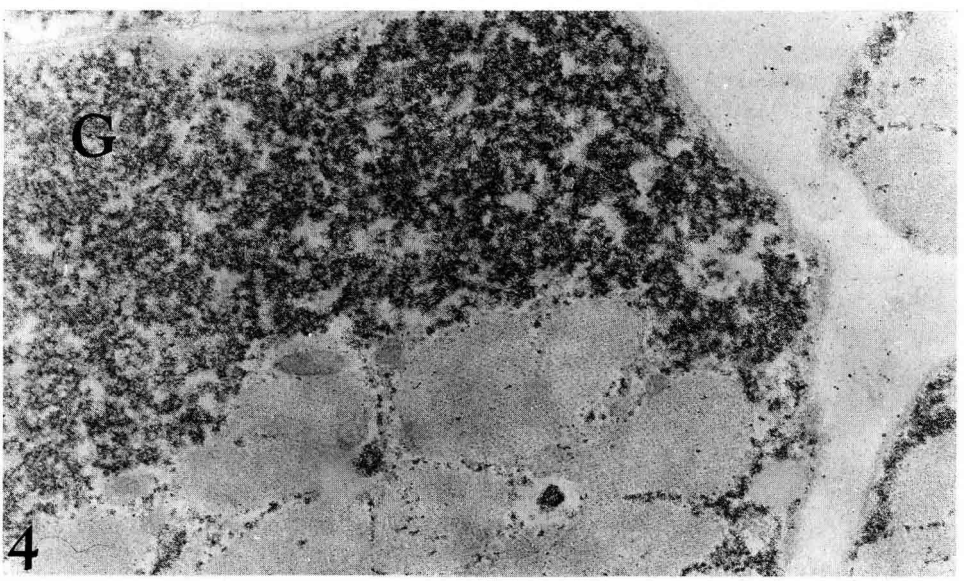
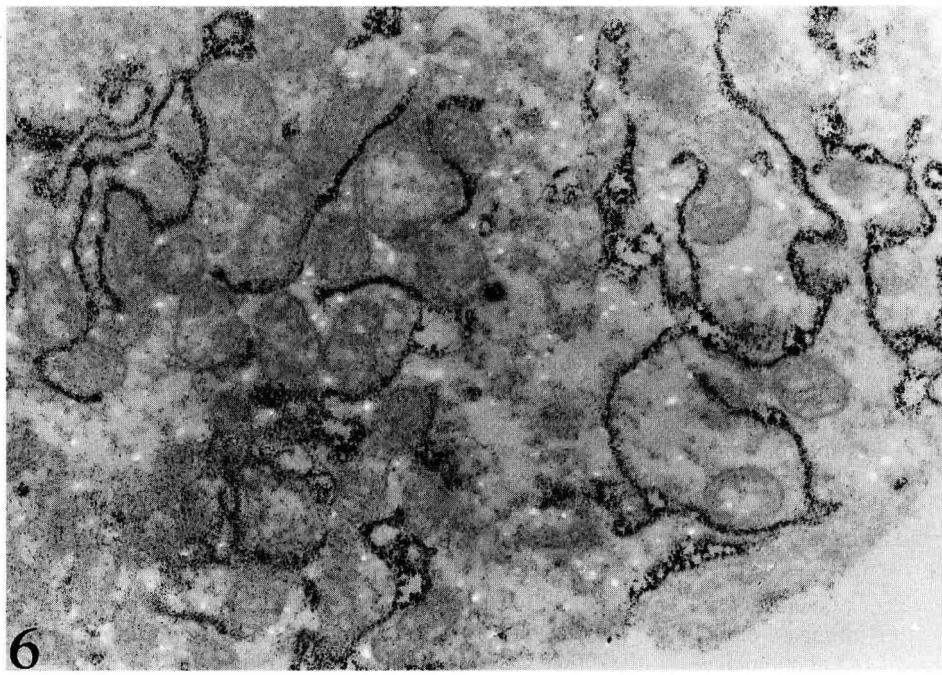
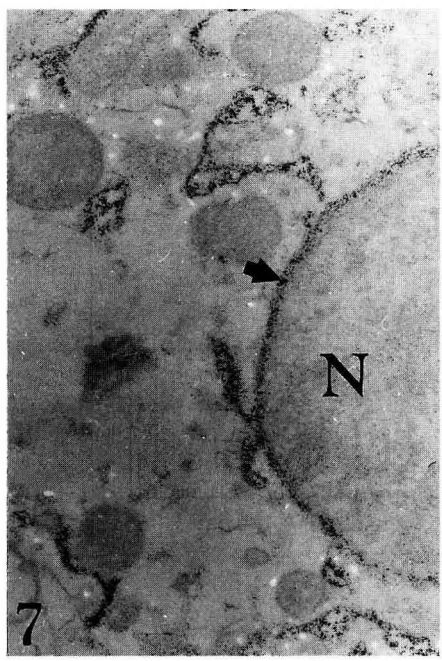


Fig 3. Thiéry's method without any contrasting: the biceps femoris muscle of normal newborn piglet. Small quantities of glycogen particles are present among the myofibrils and around the nucleus forming a fine rim (x 23 000).

Fig 4. Thiéry's method without any contrasting: the longissimus dorsi muscle of newborn piglet with splayleg. The large extramyofibrillar space is filled with glycogen particles (compare fig 2) (x 23 000).

Fig 5. Thiéry's method without any contrasting: the longissimus dorsi muscle of newborn piglet with splayleg. Glycogen particles are visible as a chain-like structure (arrow) at higher magnification (x 52 000).





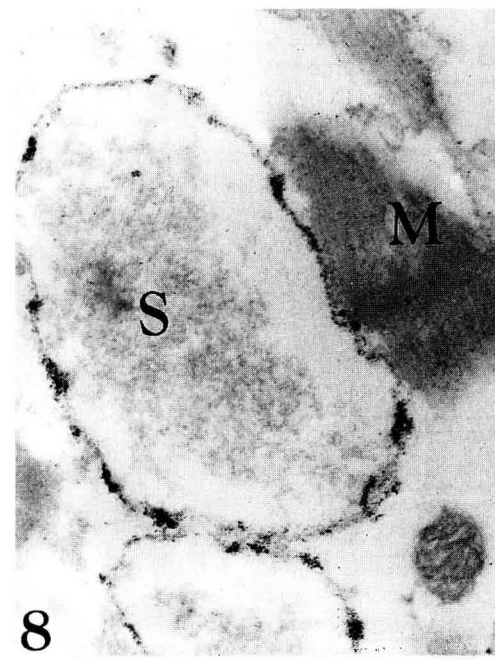


Fig 6. G-6-Pase reaction without any contrasting: the biceps femoris muscle of normal newborn piglet. G-6-Pase activity is present in the sacroplasmic reticulum cisternae (x 30 000).
Fig 7. G-6-Pase reaction without any contrasting: the biceps femoris muscle of normal newborn piglet. G-6-Pase activity is also visible in the perinuclear space (arrow) (x 30 000).
Fig 8. G-6-Pase reaction without any contrasting: the longissimus dorsi muscle of newborn piglet with splayleg. G-6-Pase activity is demonstrated at the periphery of the dilated sarcoplasmic reticulum (x 62 000).

No substantial differences in the fibre ultrastructure, glycogen distribution G-6-Pase activity localization were found between the longissimus dorsi and the biceps brachii muscles of splaylegged piglets.

DISCUSSION

Glycogen particles are frequently found in normal fetal skeletal muscle fibres (Kelly and Zacks, 1969). They serve as a major metabolic fuel for increasing muscular activity after birth because of low fatty acid oxidation capacity and glycolysis at this time (Curtis, 1970; Schiaffino and Hanzlíková, 1972).

Ultrastructural analysis of newborn splaylegged muscles clearly demonstrates an increased accumulation of glycogen compared with the muscles of normal piglets. Glycogen granules have been previously observed in large extramyofibrillar spaces (Deutsch and Done, 1971; Bergmann, 1976; Hanzlíková, 1980). Our results agree well with these findings. Glycogen levels were increased in the affected muscles. Large deposits of glycogen were found especially within the extramyofibrillar spaces. Since glycogen depletion mainly takes place during the first few days after birth (Schiaffino and Hanzlíková, 1972) a fast lysosomal metabolism of glycogen could be considered a normal phenomenon in newborn pig muscles. In fact, we observed very few secondary lysosomes and residual bodies. This suggests a low level of glycogen store mobilization in the splaylegged muscles. This finding is in discrepancy with the results of Zelená and Jirmanová (1979), who described the present of autophagolysosomes with digested glycogen.

Several enzyme activities have been demonstrated using histochemical techniques in the muscles of splaylegged piglets (Hanzlíková, 1980). Such evidence is not observed, however, on the ultrastructural level. To obtain new information at this level we demonstrated the activity of G-6-Pase, the key enzyme in glycogen metabolism. Dense and coarse aggregates of reaction product were found at the periphery of the extremely dilated cisternae of the sarcoplasmic reticulum in the muscle fibres of splaylegged piglets. In contrast, the muscle fibres of normal animals showed a uniform distribution of a fine reaction precipitate mainly inside the sarcoplasmic reticulum. An anomalous distribution of G-6-Pase activity in the splaylegged muscles could be at least partially responsible for the slower utilization of, and higher glycogen reserves in, affected animals. Demonstration of, other important enzymes in glycogen metabolism is necessary to explain completely the elevated level of glycogen storage in the skeletal muscles of splaylegged piglets.

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