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ULTRASTRUCTURAL CHANGES IN HEREDITARY MUSCULAR HYPERTROPHY IN CATTLE*

By

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KING, WM ALLAN and PARVATHI K. BASRUR: *Ultrastructural changes in hereditary muscular hypertrophy in cattle.* Acta vet. scand. 1979, *20*, 245—257. — Biopsies of skeletal muscle collected from 24 animals classified as "double muscled" were examined by light and electron microscopy. The muscle samples exhibited degenerative changes including the presence of vacuolations and lamellated structures, fragmentation of myofibers, accumulation of glycogen granules, disruption of neuromuscular junctions and disorganization of the sarcolemma. In the light of the excessive fragility of the erythrocyte membranes noted previously, the alteration in the sarcolemma suggests that a generalized cell membrane defect may be the most consistent feature of the "double muscling syndrome" in cattle.

ultrastructure; hereditary muscular hypertrophy; cattle.

Hereditary muscular hypertrophy (double muscling) in beef cattle is characterized by excessive muscle development and reduced subcutaneous fat and connective tissue elements (*Vissac* 1968, *Oliver & Cartwright* 1969, *Rollins et al.* 1972, *Kieffer et al.* 1972). While this trait has been recognized to be an inherited condition in beef cattle for many years (*Oliver & Cartwright*), the lack of a reliable method to identify the carriers of this trait leaves the mode of inheritance obscure (*Kieffer et al.*). The variability in "double muscled" phenotype has been attributed in the past to an autosomal gene which is incompletely dominant or incompletely penetrant (*Oliver & Cartwright*). More recently, a diallelic mode of inheritance involving two recessive genes has been proposed for this trait (*Sopena & Blanco* 1972).

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When fully expressed, the syndrome is characterized by bulging muscles of the shoulder and thigh, prominent intramuscular grooves and round rump region (*Oliver & Cartwright, Rollins et al., Kieffer et al.*). Since the double muscled trait is known to cause difficult parturition, increased mortality of the young, impaired fertility in adults and susceptibility to disease and environmental stresses (*Oliver & Cartwright, Kieffer et al.*), the need for recognizing the carriers is becoming increasingly clear. In the studies described here, histological and ultrastructural features of double muscled animals are compared with those of normal animals. This investigation was undertaken in the hope that the characterization of the overtly affected animals may provide a basis for the eventual recognition of the carriers of this trait.

MATERIALS AND METHODS

Biopsy specimens of skeletal muscle were collected from 24 animals representing three breeds of beef cattle. The animals were classified by gross morphology and breeding records into normal and double muscled. The muscle samples examined included longissimus dorsi, triceps femoris, sternohyoideus, semitendiosus, vastus lateralis, biceps femoris and cutaneous trunci.

Tissues were cut into small pieces and fixed in 2.5 % glutaraldehyde buffered with 0.2 M phosphate buffer (pH 7.3). The fixed tissues were washed in 6.5 % sucrose buffer and stored in buffer at 4°C until postfixation in 1.0 % osmium tetroxide. After one postfixation, the tissues were dehydrated in a graded series of acetone and embedded in epon.

Histological examinations were carried out on 1 μ sections stained with 1.0 % toludine blue (*King* 1975). Ultrathin sections cut with glass knives and mounted on copper grids were stained with 2.5 % uranyl acetate for 2 min and with 0.25 % lead citrate for 40 s prior to examination in a JEM-6A at 80 kV.

RESULTS

Histological sections of muscle samples from double muscled animals exhibited a wide variation in the size of the individual muscle fibers compared to the corresponding sections from normal animals (Figs. 1—2). The histopathological changes noted in muscle samples of double muscled animals included vacuoliza-

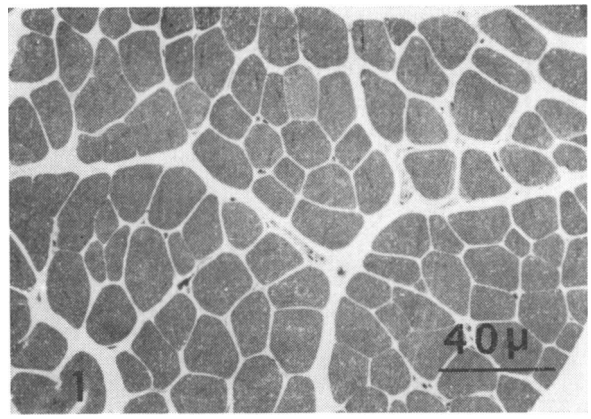


Figure 1. A cross section of vastus lateralis of a normal Piemontese showing the size and shape of individual muscle fibers.

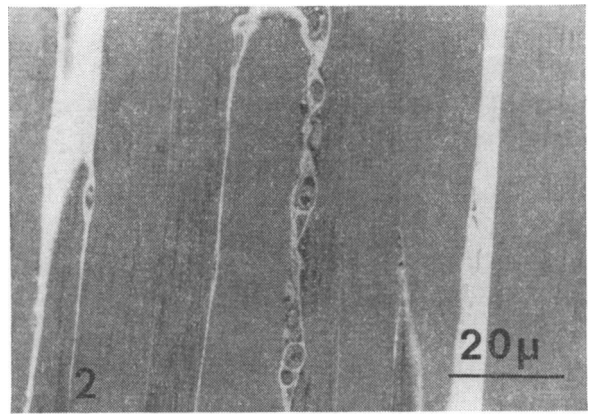


Figure 2. Longitudinal sections of semitendinosus of a normal Angus showing vascular and neural supply.

tion and extreme variation in the diameter of individual muscle fibers, distortions of cross striations, coagulation of the contractile elements and a shift of the peripherally located nuclei towards the center of the muscle fibers (Figs. 3—4).

The ultrastructural features of the double muscled animals revealed a variety of abnormalities as compared to controls (Fig. 5). The most consistent changes included the presence of vacuolations and "lamellated" structures beneath the sarcolemma and between myofibrils and localized degeneration of myofibrils (Fig. 6). Mitochondria were generally hypertrophied and contained

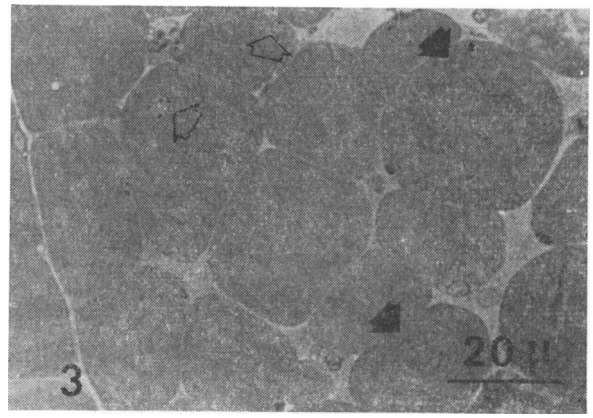


Figure 3. Cross sections of cutaneous trunci from an affected Angus showing variable size and shape of myofibers. Note the circular pattern in intact muscle cells (solid arrows) indicative of degeneration. The myonuclei (open arrows) are prominent (and dissociated in some samples), and edematous changes are detectable.

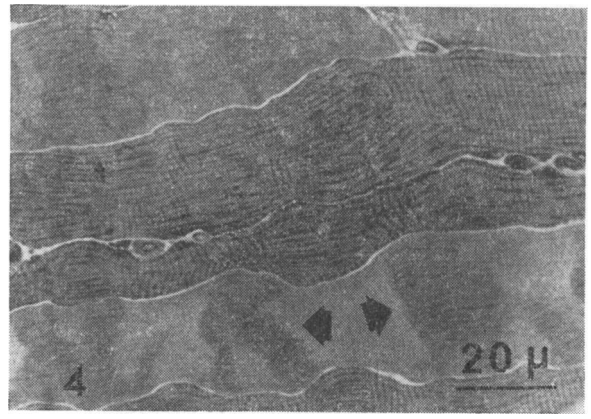


Figure 4. A longitudinal section of triceps femoris from an affected Angus showing disorientation of contractile elements, super contraction rings (arrows), focal swelling and loss of cross banding patterns.

distorted cristae and electron dense bodies (Fig. 7). Some lamellated structures were noted also in the intercellular space and in the connective tissue (Fig. 8).

The myofibrillar structure appeared disrupted and the transverse tubular system was disoriented. The disruption of the cross striation pattern was variable at the electron microscopic level in

that some regions were only slightly out of register while others were so disoriented that there was no discernible pattern. The accumulation of glyogen granules between contractile elements and at the periphery of the muscle fibre obliterating the transverse tubular system was evident in the vicinity of the nucleus and mitochondrial aggregations and in areas of myofibrillar degeneration (Fig. 9). The neuromuscular junction was generally difficult to locate and those detected had an abundance of connective tissue elements (Fig. 10) as compared to control samples (Fig. 11). Distinct neurolemma-sarcolemma contact was not detected in areas which, on other criteria, appeared to be myoneural junctions. The sarcolemma in some regions exhibited a serrated appearance and was often accompanied by separation of packages of sarcoplasm with vacuoles in each projection (Fig. 12).

The structural alterations were consistent in the animals which on the basis of breeding histories and gross appearance were homozygous double muscled animals. Although similar features were detected in all three breeds there was some degree of variation in the severity of degenerative changes.

DISCUSSION

Histometric and histochemical studies have indicated that the hypertrophy of muscle in bovine double muscling syndrome results from the hypertrophy and hyperplasia of white (glycolytic) fibers. The increase in white fiber population has been attributed to an increased rate of postnatal conversion of alpha-white fibers (*Holmes & Ashmore* 1972, *Hendricks et al.* 1973). The variation in the size of muscle fibers and the apparent increase in the white fibers noted in this study support the postulation that both hypertrophy and hyperplasia contribute to the increase in the size of individual muscle bundles.

Increase in white fiber numbers has been suggested to lead to an increase in glycolytic activity (*Holmes & Ashmore* 1972). Deviation from normal metabolism in double muscled animals may be reflected by the accumulation of large amounts of glycogen in the muscle fibers. Assays of blood lactic acid revealed a higher level in double muscled cattle compared to normal and that it increased more drastically during exercise than in similarly treated controls (*Holmes et al.* 1972 a). Electrophoresis of plasma lactate dehydrogenase (LHD) has indicated that the levels of

enzyme in affected cattle are higher than that of normal animals although the proportion of each isoenzyme was similar to that of controls (*King* 1975). In cases where the double muscled condition was highly exaggerated, heavy exercise has been known to cause paralytic myoglobinuria (*Holmes et al.* 1972 b). The histological changes noted in muscle tissue, the damages to the myofibrils and transverse tubular system accompanied by the presence of lysosomes and "lamellated" structures are similar to that noted in myoglobinuria (*Jubb & Kennedy* 1970). It would appear that normal exercise leads to active glycolysis and to the production of excessive amounts of lactic acid. The inability of these animals to adequately remove such large amounts of lactic acid from the muscle leads to the accumulation of lactic acid which in turn causes the distortion of the structural components of the muscle.

Communication between nerve and muscle is known to be a prerequisite for the regulation of growth and metabolism of muscle fibers. Structural alterations of cell membranes noted in double muscled animals may interfere with the chemical communications between nerve and muscle. It is conceivable that the abnormal neuromuscular contact leads to the overproduction of muscle fiber in the fetus and to the shift in muscle metabolism. Alteration of nerve-muscle communication is further suggested by the observation that 12—22 % of the terminal axons of double muscled animals innervate two myofibers whereas in normal animals terminal axons generally innervate single myofibers. Occasionally terminal axons were observed to innervate three myofibers in double muscled animals while this occurred very rarely in normal animals (*Swatland* 1973).

Increased sensitivity of erythrocytes to osmotic shock has been reported to be a consistent feature of this condition (*King et al.* 1976). The ultrastructural appearance of the sarcolemma and the interrupted contact between neurolemma and sarcolemma indicate that the alteration of the cell membrane manifested by increased lysis of erythrocytes may extend to the sarcolemma and/or neurolemma. Thus, it would appear that the hereditary muscular hypertrophy (double muscling) in cattle is associated with a generalized cell membrane defect.

Figure 5. Electron micrographs of semitendinosus from a normal Piemontese showing cross striations and variations in size of mitochondria (M), sarcoplasmic reticulum and transverse tubular system (arrows) characteristic of normal mammalian muscle.

Figure 6. Electron micrograph of triceps femoris from an affected Angus showing localized degeneration of the myofibrils extending over several sarcomers. Note the accumulation of glycogen and disorientation of the transverse tubular system (arrows).

Figure 7. Electron micrograph of a section of biceps femoris from an affected Piemontese showing lamellated structures (arrows) within, and in the vicinity of hypertrophied mitochondria.

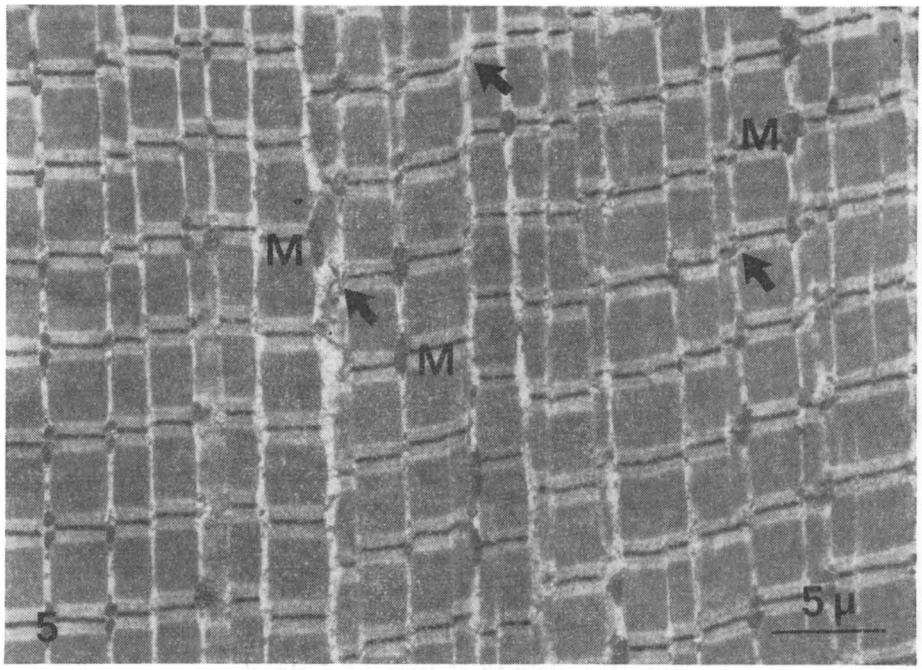
Figure 8. Electron micrograph of triceps femoris from an affected Angus showing electron dense lamellated bodies (arrows) in the intercellular space. Some of the lamellated structures may be remnants of nerve elements. The glycogen granules accumulated under the sarcolemma and between myofibrillar bundles are striking in the muscle fibers in the vicinity of lamellated structures.

Figure 9. Electron micrograph of a section of triceps femoris from an affected Angus showing glycogen granules, mitochondria (M) and membrane bound bodies (arrows) and vacuoles in the region normally occupied by contractile elements.

Figure 10. Electron micrograph of biceps femoris from an affected Charolais showing abnormal neuromuscular region. Note the indentation of the sarcolemma into the sarcoplasm reminiscent of junctional folds.

Figure 11. Electron micrograph of vastus lateralis from a normal Piemontese showing characteristic neuromuscular junction. Note Teloglial cell nucleus (TN), myonuclei (MN) and junctional folds (arrows).

Figure 12. Electron micrograph of biceps femoris from an affected Piemontese showing projections of the sarcolemma into the intercellular space. Note that the sarcolemma and the connective tissue investment around it is intact although the intercellular space is filled with granular material and some traces of collagen bundles. The vacuoles (arrows) probably replacing mitochondria are numerous.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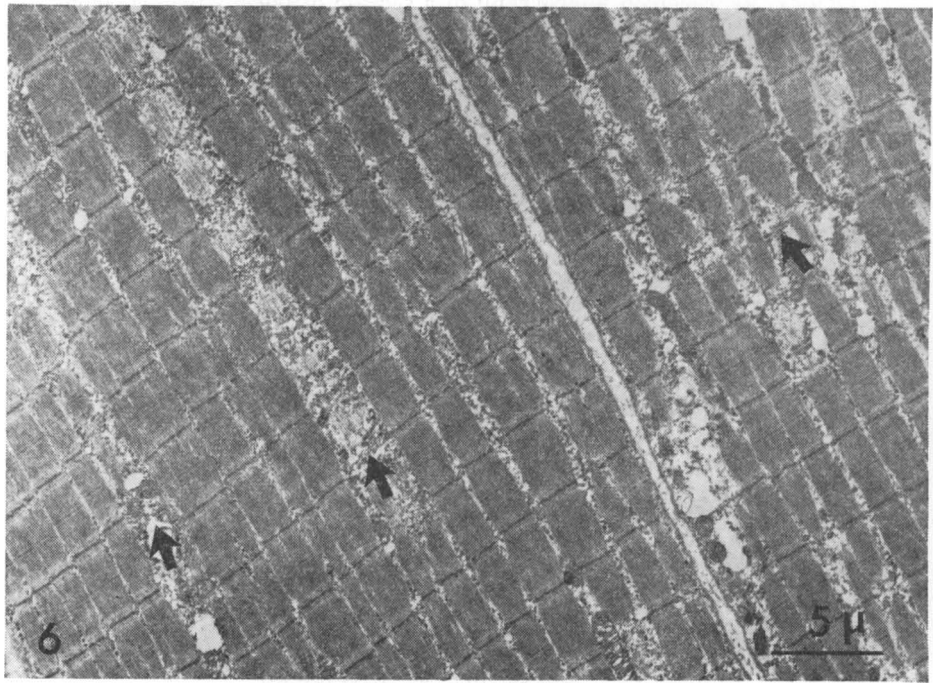
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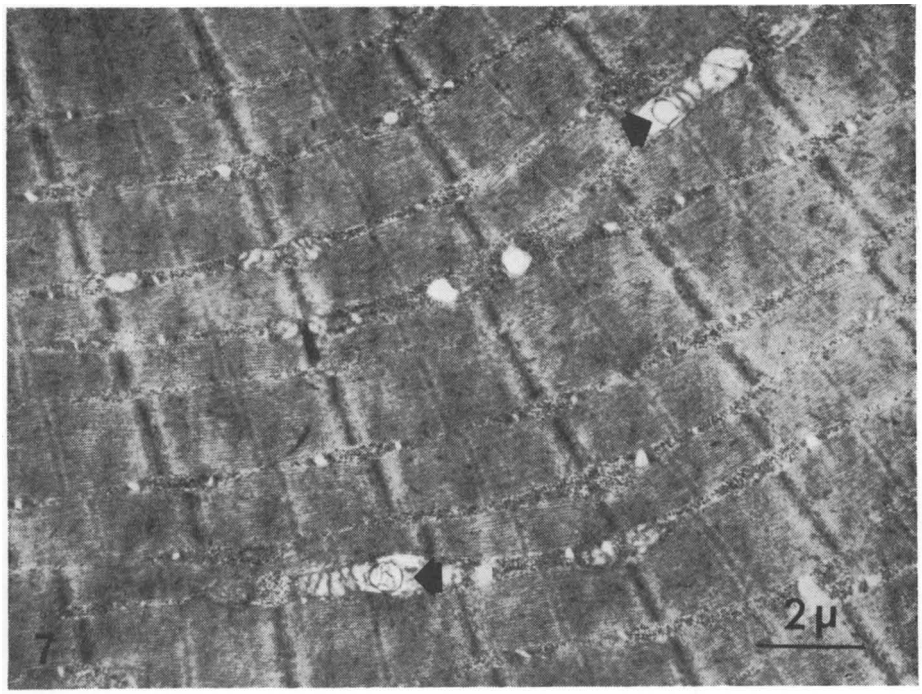
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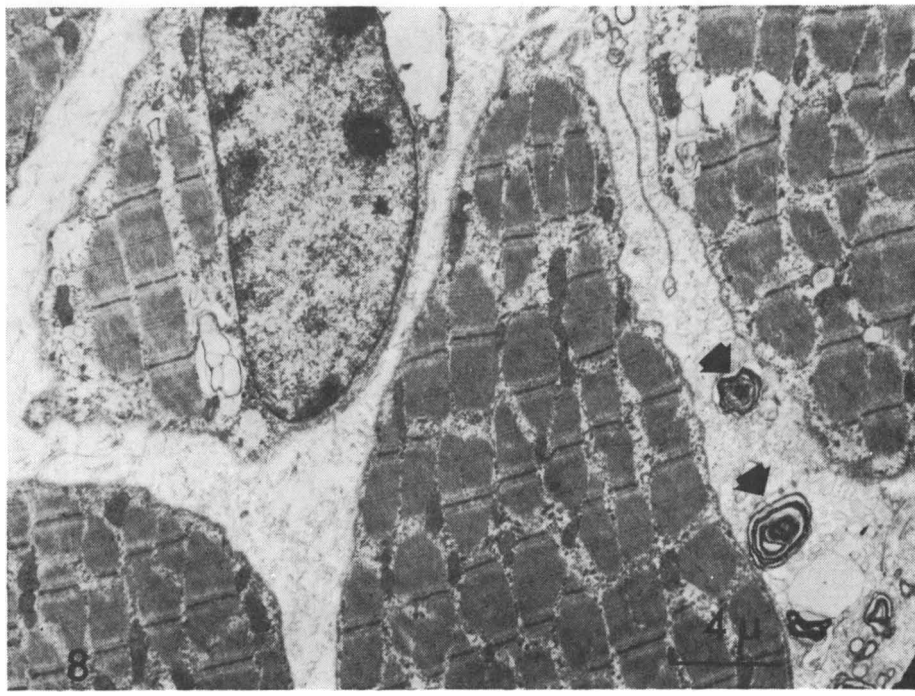
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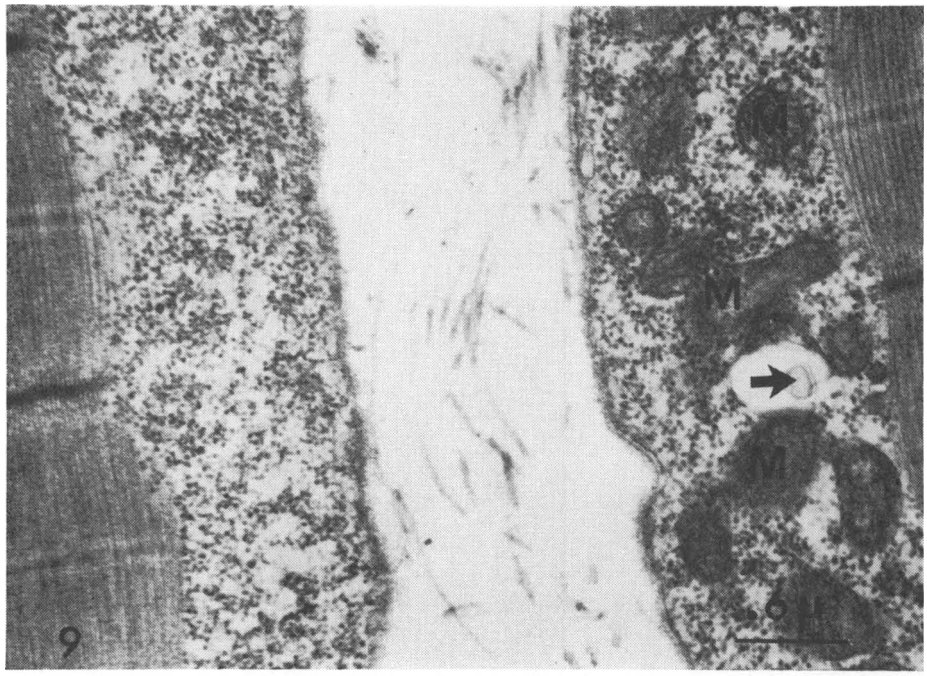
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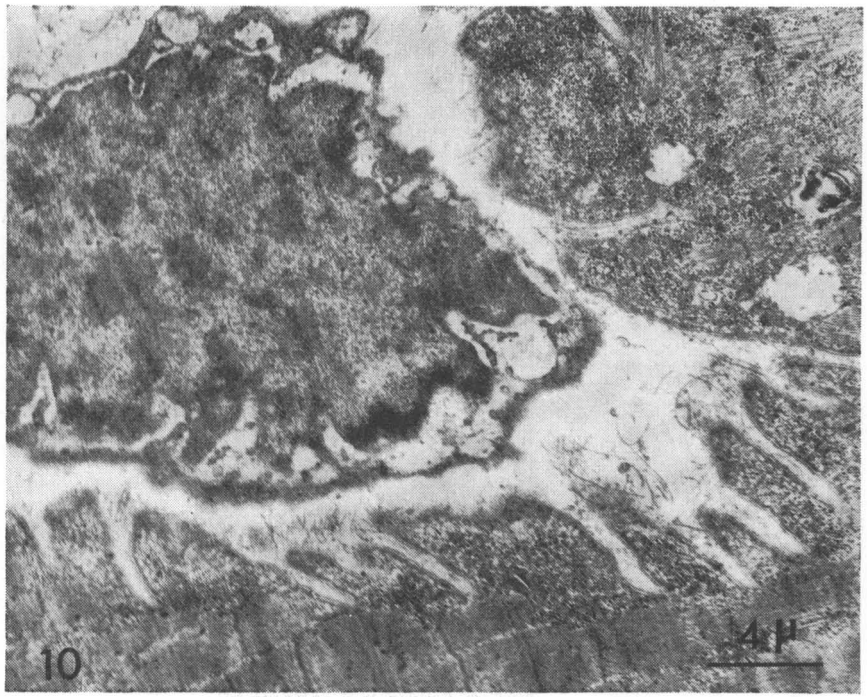
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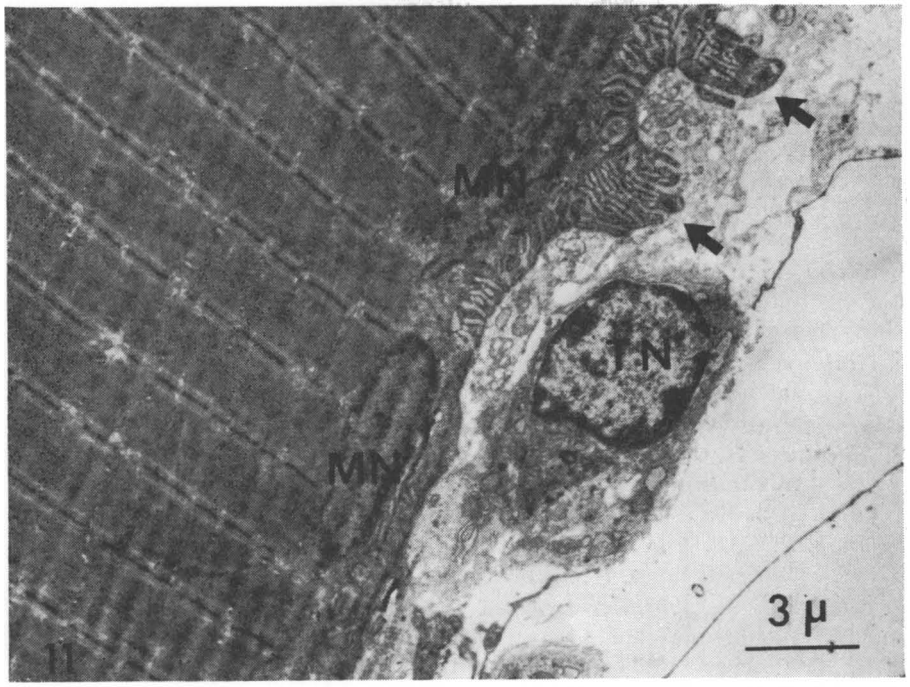

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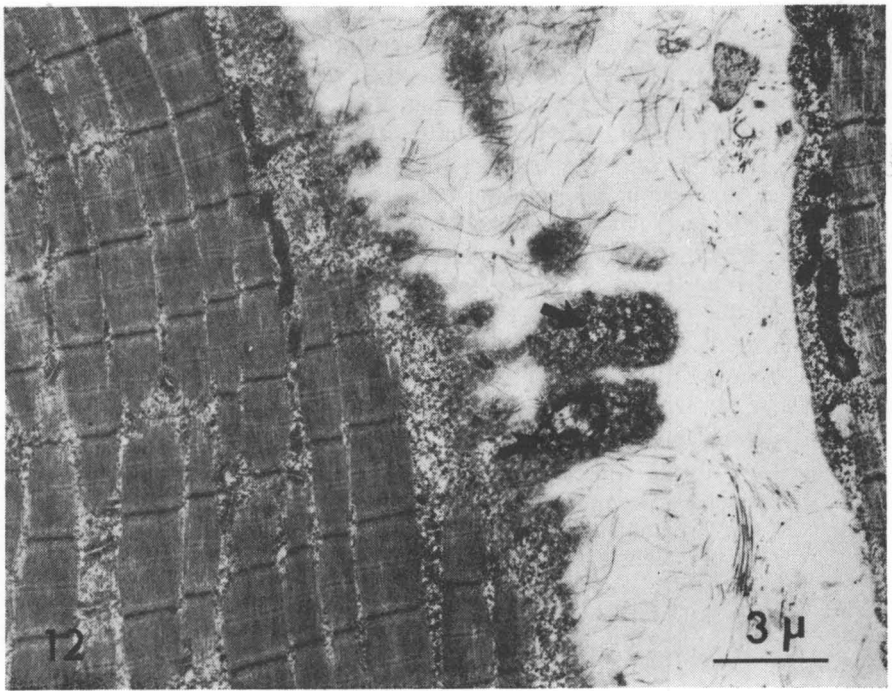

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SAMMANFATTNING

Ultrastrukturella förändringar vid hereditär muskelhypertrofi hos nötkreatur.

Skelettmuskelbiopsier från 24 djur klassificerade som "double muscled" undersöktes med ljus- och elektronmikroskop. Muskelproverna visade degenerativa förändringar omfattande vakuolisering och uppträdande av lamellartade figurer, ansamling av glykogenkorn, uppsplittring av muskelfibrerna, isärsprängning av nerv-muskelförbindelser samt desorganiserat sarcolemma. Med tanke på den extrema känslighet som tidigare observerats i erythrocytmembranet antyder förändringarna i sarcolemmat att en generell membrandefekt kan vara det mest genomgående draget i "the double muscling syndrome" hos nötkreatur.

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