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Segmental Fibre Breakdown and Defects of the Plasmalemma in Diseased Human Muscles*

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Summary. Thick round fibres common in cross sections of muscle biopsies from patients with muscular dystrophy are due to contracted and swollen segments of otherwise normal muscle fibres. This contracture leads to segmental fibre breakdown, which is identical with Zenker's waxy degeneration.

In biopsies from 90 patients suspected of neuromuscular disease, segmental contracture was seen in all or nearly all patients with infantile muscular dystrophy, necrotic myopathy or acute alcoholic myopathy. It was present in half of the patients with polymyositis or myotonic dystrophy. In restricted forms of muscular dystrophy it was rare as it was in neurogenic atrophy. In 9 clinically normal patients it was absent.

In electron micrographs of the initial stage sarcomeres were moderately shortened, the sarcoplasmic reticulum was distended and the mitochondria were normal. In the plasmalemma holes were found, through which glycogen granules were lost into the interstitial tissue. In later stages myofibrils were overcontracted and homogenized; in large areas the plasmalemma was absent.

Based on these findings a hypothesis for the development of waxy degeneration is proposed: locally defects of the plasmamembrane cause segmental contracture, glycogen granules and water soluble enzymes are lost through holes in the plasma membrane, and finally the affected fibre segment becomes necrotic.

Key words: Diseased human muscle — Fibre breakdown — Contracture — Plasma membrane.

Introduction

In cross sections of dystrophic muscles round fibres thicker than ever seen in normal muscle are a common feature. These fibres have been considered to be hypertrophic (Pearson, 1965; Adams, 1960, 1969) or hyaline (Bradley *et al.*, 1972). In fact, the increase in fibre diameter is due to segmental contracture and swelling; adjacent segments of the same fibre may be of normal diameter (Schmalbruch, 1973). In stained or unstained sections of muscle fibres embedded in epoxy resin, contracted segments are easily recognized even if the fibre is not swollen, because they appear dense. In paraffin or frozen sections they can be missed because staining with haematoxylin and eosin may be normal or nearly so. Segmental contracture leads to segmental fibre breakdown which is identical with Zenker's waxy degeneration (Zenker, 1864). This process has been studied by electron microscopy in the Duchenne type of muscular dystrophy by Cullen and Fulthorpe (1975).

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In the study presented muscle biopsies of patients with different neuromuscular disorders were examined with respect to segmental contracture by light and electron microscopy. Changes in the sarcolemma and sarcoplasmic reticulum are described, which may elucidate the mode of evolution of these lesions. For comparison I have produced contracture knots in rat muscle by transection of fibres.

Materials and Methods

Muscle biopsies from 90 patients aged between 2 months and 75 years were studied. The diagnoses were based on clinical, electrophysiological, histological and histochemical findings and are listed in Table 1.

Table 1

Clinical and histological diagnose	Number of patients	Age (years)	Number of patients with segmental contracture
Primary Myopathy			
Duchenne type	7	2—9	7
Clinically similar but in girls	2	8—9	2
Benign (Becker) from of Duchenne dystrophy	1	17	—
Congenital dystrophy	1	0.5	1
Myotubular myopathy	1	0.5	1
Gycogen storage disease (?)	1	1	—
Nemaline myopathy	1	17	—
Restricted types of dystrophy	19	17—70	3
Necrotizing myopathy	2	51—52	2
Myotonia congenita	1	47	—
Myotonic dystrophy	11	17—53	4
Polymyositis and dermatomyositis	7	8—55	4
Alcoholic mypoathy	3	28—51	2[a]
Thyroid myopathy	2	21—37	—
Acremogaly	1	31	—
Carcinomatous myopathy	1	75	—
Neurogenic atrophy			
Anterior horn cell affections			
type Werdnig Hoffmann	4	0.2—5	2
type Charcot-Marie-Tooth or Kugelberg-Welander	3	8—34	—
motor neuron disease (ALS)	3	46—60	1
Peripheral neuropathy	6	20—51	1[b]
Myasthenia gravis	1	43	—
Polymyalgia rheumatica	3	35—51	—
Normal	9	19—59	—
Unclassified	1	47	1[c]

[a] With myoglobinuria.

[b] Diabetic neuropathy with complete loss of myelinated axons in the sural nerve.

[c] Isolated weakness of one anterior tibial muscle. Root compression or sequelae of poliomyelitis or of anterior tibial syndrome.

Biopsies measuring 3 cm by 0.5 cm by 0.5 cm were taken under local anaesthesia (in small children under general anaesthesia) from the brachial biceps or quadriceps femoris muscle or from both. In 3 patients the anterior tibial muscle was investigated. Fibre bundles were stretched to 120 to 150% equilibrium length, half were frozen and processed for histology and histochemistry and the other half fixed for 24 hrs in 2.5% glutaraldehyde (4—10° C) and for 90 min in 1% osmium tetroxide (20° C) (both in 0.1 m phosphate buffer, pH 7.5) and embedded in Epon 812. Thin sections were stained with lead citrate and studied in a Jeol 5Y or Siemens 102 electron microscopy. The latter was equipped with a specimen tilting device. For light microscopy 3 μm sections were cut with dry glass knifes, stained with *p*-phenylenediamine (Holländer and Vaaland, 1968) and photographed with phase contrast optics. The decision whether contracted segments were present or not (Table 1) was based on Epon-sections alone.

Contractured segments (i.e. retraction clots) were produced in rat muscles: under Halothane® anaesthesia superficial fibres of the anterior tibial muscle were transversely cut with a razor blade. After 20 min the muscle was fixed *in situ* by perfusion with 2.5% glutaraldehyde (Schmalbruch, 1971), the cut fibres were isolated, postfixed and embedded as described.

Results

Segmental contracture of muscle fibres and segmental fibre breakdown occurred in all muscles from children with muscular dystrophy, from patients with necrotizing myopathy and from 2 of 3 patients with acute alcoholic myopathy. In polymyositis and myotonic dystrophy it was seen in about half of the patients. In restricted forms of muscular dystrophy and in other slowly progressing myopathies it was rare. In neuropathies contracted segments were seen in 2 of 4 children with Werdnig-Hoffmann disease, in 1 patient with motor neurone disease and in 1 with severe diabetic neuropathy. In 1 unclassified patient who complained about weakness of 1 anterior tibial muscle, the biopsy showed loss of normal muscle fibres, many contractured fibres and numerous foci of regeneration (Table 1).

In 12 patients with normal muscle histology contracted segments were absent. In 3 polymyalgia rheumatica was diagnosed, in 9 the complaints could not be related to a neuromuscular disorder.

The incidence of contracted segments in a given muscle varied between different Epon-blocks. They were frequently seen in dystrophic muscles from children, in alcoholic myopathy with myoglobinuria and in polymyositis. In restricted forms of muscular dystrophy and in neuropathy they were rare.

The Morphology of Contractured Segments in Human Muscle

Light Microscopy. The mildest change detectable on longitudinal sections was localized shortening of a few sarcomeres close to the fibre periphery. Overstretched sarcomeres were absent, but occurred when the number of shortened sarcomeres and the degree of shortening increased. Finally myofibrils were disrupted, and contracted segments condensed to homogeneous clumps. In some cases banding patterns with a 0.5 μm spacing were distinguishable within these clumps. The gaps between them were invaded by phagocytes. The sarcolemma appeared to remain intact. Occasionally a narrow rim of regular and normal spaced sarcomeres occurred in the periphery of these fibres which indicated regeneration. Probably new muscle fibres (satellite elements) were formed by satellite cells in the periphery of a necrotic fibre beneath the basal lamina (James, 1973; Schmalbruch, 1975) (Fig. 1).

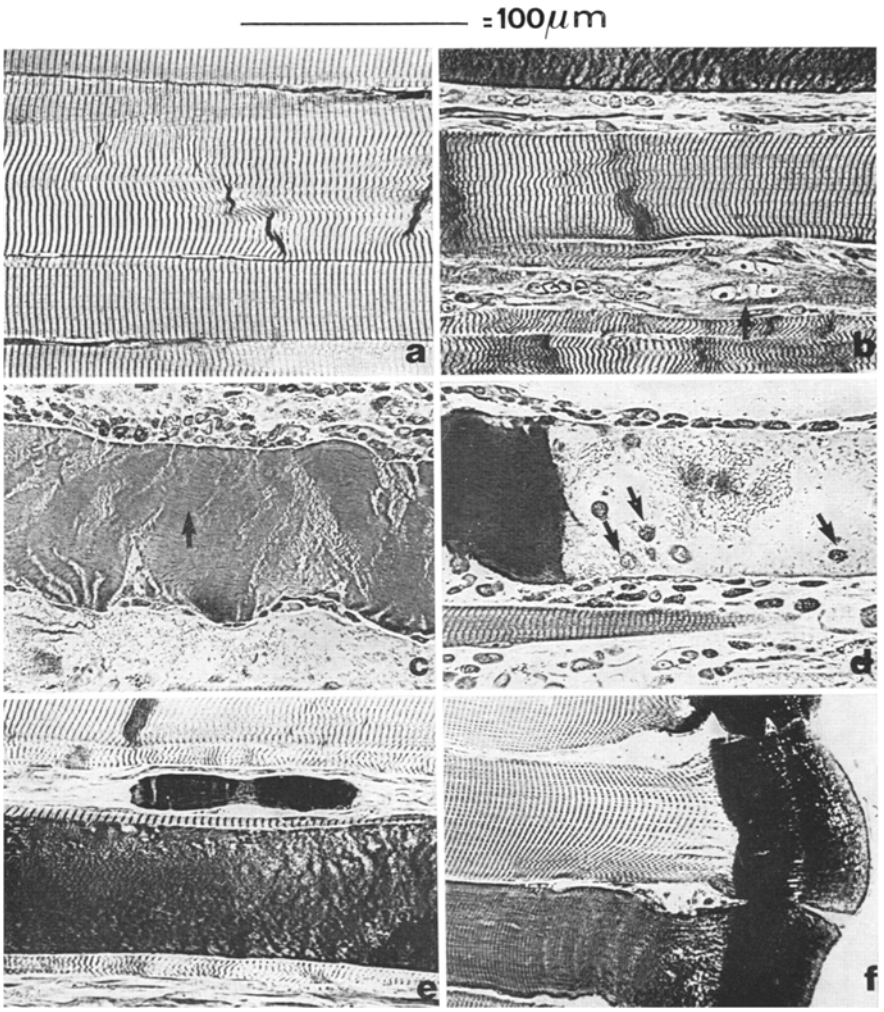

Fig. 1. Biopsies from 2 girls of 8 and 9 years with muscular dystrophy (b—e) and a female aged 20 years with myotonic dystrophy (a). Epon-sections 3 μm thick, stained with *p*-phenylenediamine. Phase contrast. (a, b) Localized shortening of sarcomeres. In (b) alsolong contracted segment (top) and vesicular nuclei belonging to myoblasts or myotubes (arrow). (c, d) Contracture knots (waxy degeneration) with interruption of myofibrils. In (c) narrow cross banding (arrow) In (d) sarcolemma containing phagocytes (arrows) and cell debris. Note the fibre with normal sarcomere spacing below. (e) Regenerated thin fibres in the periphery of a swollen contracted fibre. (f) Rat muscle fibres 20 min after transection. Close to the cut (right) contracture knots similar to those in human biopsies (c—e). Adjacent sarcomeres are overstretched; more distant parts of the fibre appear normal (middle) or dense (below). In dense segments sarcomeres are shortened

Electron Microscopy. In the early stage of contracture myofibrils appeared normal except that sarcomeres were moderately shortened. Distorted Z-lines ("streaming") could not be observed. The sarcoplasmic reticulum was swollen and transformed to empty vesicles; mitochondria appeared to be normal (Fig. 2).

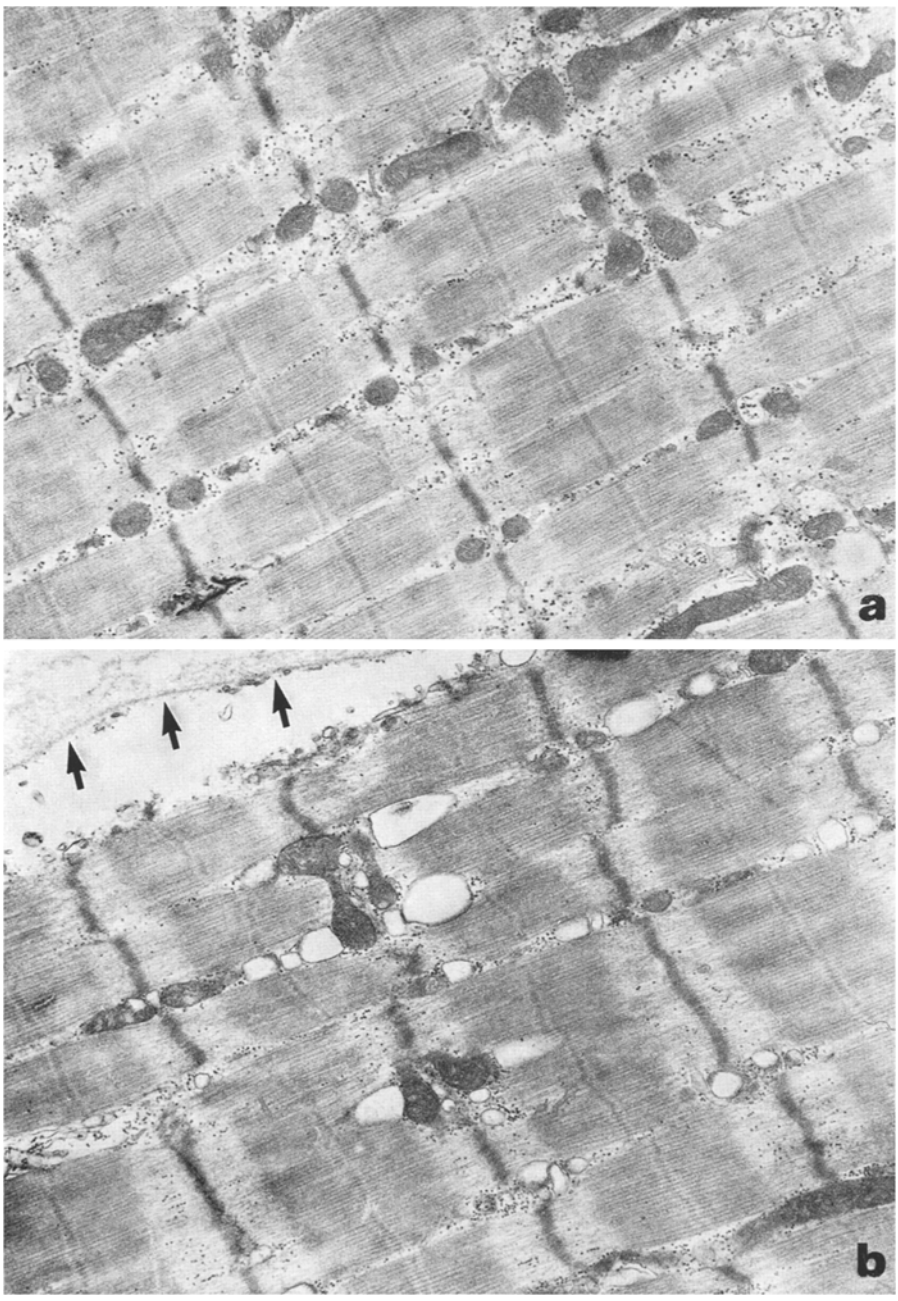

Fig. 2a and b. Boy aged 9 years with Duchenne type muscular dystrophy. Electron micrographs 14500:1. Both were taken from the same section. In (a) normal fibre, in (b) fibre with commencing segmental contracture. Sarcomeres are not yet shortened, but less well ordered than in (a) Mitochondria are normal. The sarcoplasmic reticulum is distended and the plasmalemma is defective and disintegrated into small vesicles. The basal lamina (arrows) is intact

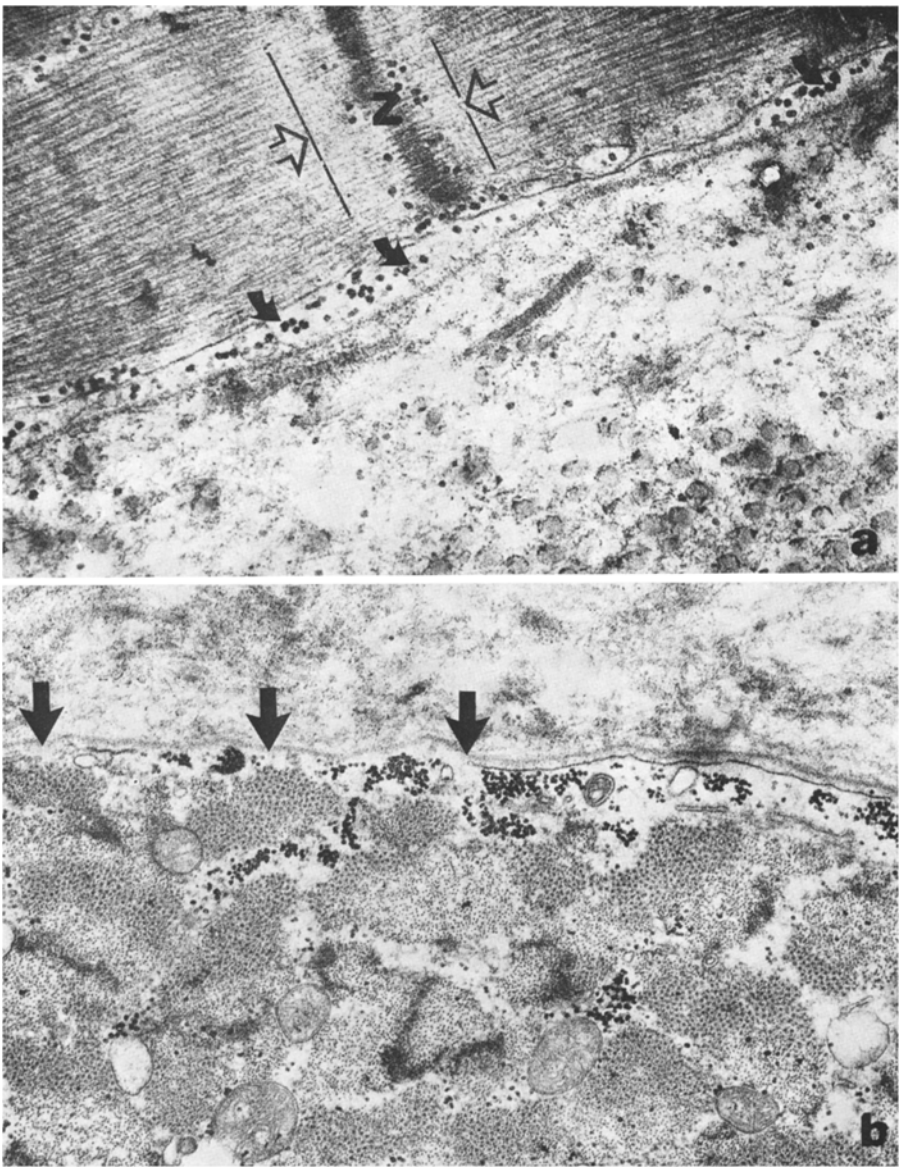

Fig. 3a and b. Boys aged 2 and 9 years with Duchenne type muscular dystrophy. Electron micrographs 40000:1 and 26000:1. (a) Longitudinal section. Dissociation of plasmalemma and basal lamina. Glycogen granules (arrows) in the newly formed extracellular space. Below collagen fibrils. Myofibrils are moderatley shortened, the end of the A-Band is indicated by open arrows, (*Z* Z-line). (b) Cross section. Defect (arrows) of the plasmalemma. The basal lamina is intact

In sections passing through the sarcolemma adjacent to shortened sarcomeres sometimes its two leaflets—plasmalemma and basal lamina—were detached from each other (Fig. 3). The split was up to 2 μm wide and contained glycogen granules.

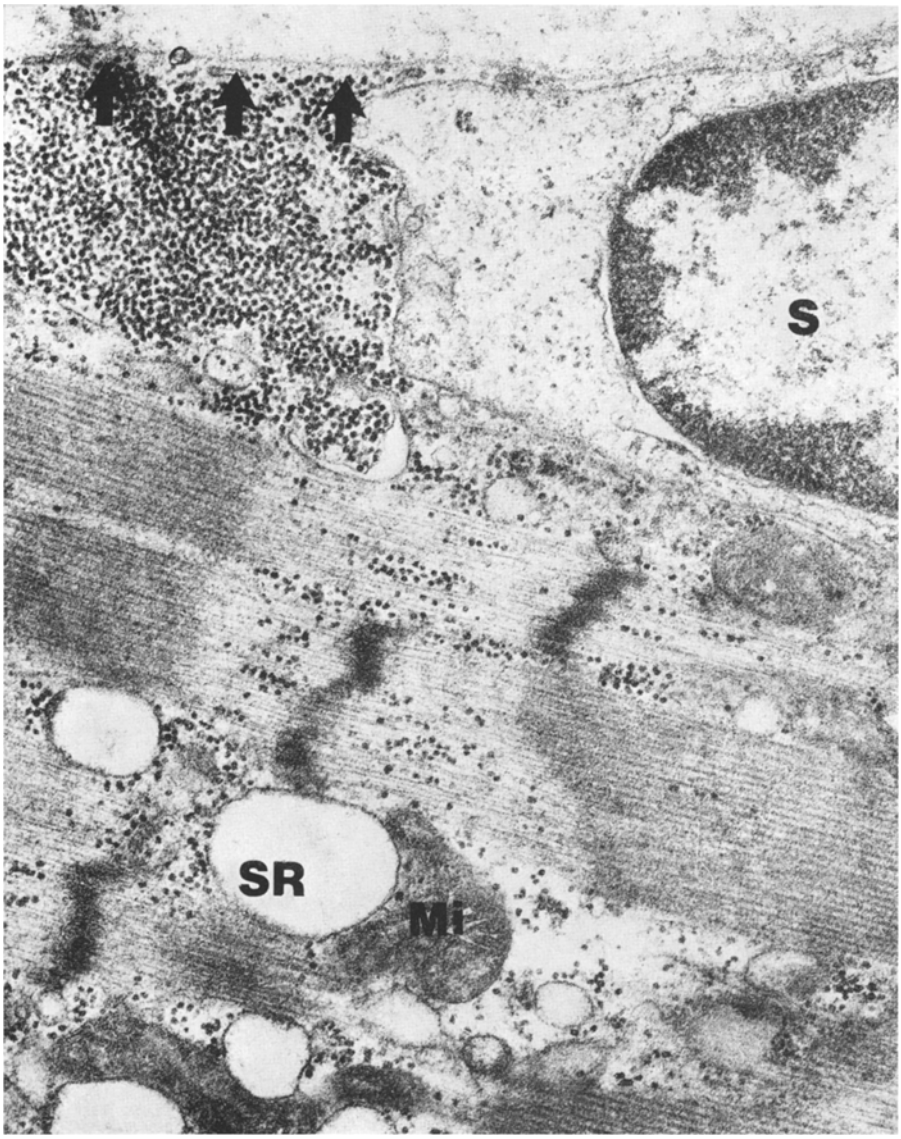

Fig. 4. Boy aged 2 years with Duchenne type muscular dystrophy. Electron micrograph 37000:1. Satellite cell (*S*) adjacent to a fibre segment in the initial stage of contracture. The plasma membrane of the satellite cell is intact, but the plasmalemma of the muscle fibre is absent and the fibre is bordered by a basal lamina alone (arrows). Note swelling of the sarcoplasmic reticulum (*SR*) and normal appearance of mitochondria (*Mi*)

In some places the plasmalemma was interrupted, extracellular glycogen granules were found mostly close to these holes in the plasmalemma. The defects in the plasmalemma were not simulated by oblique sectioning. This was ascertained by tilting the section in the electron microscope by $\pm 20°$.

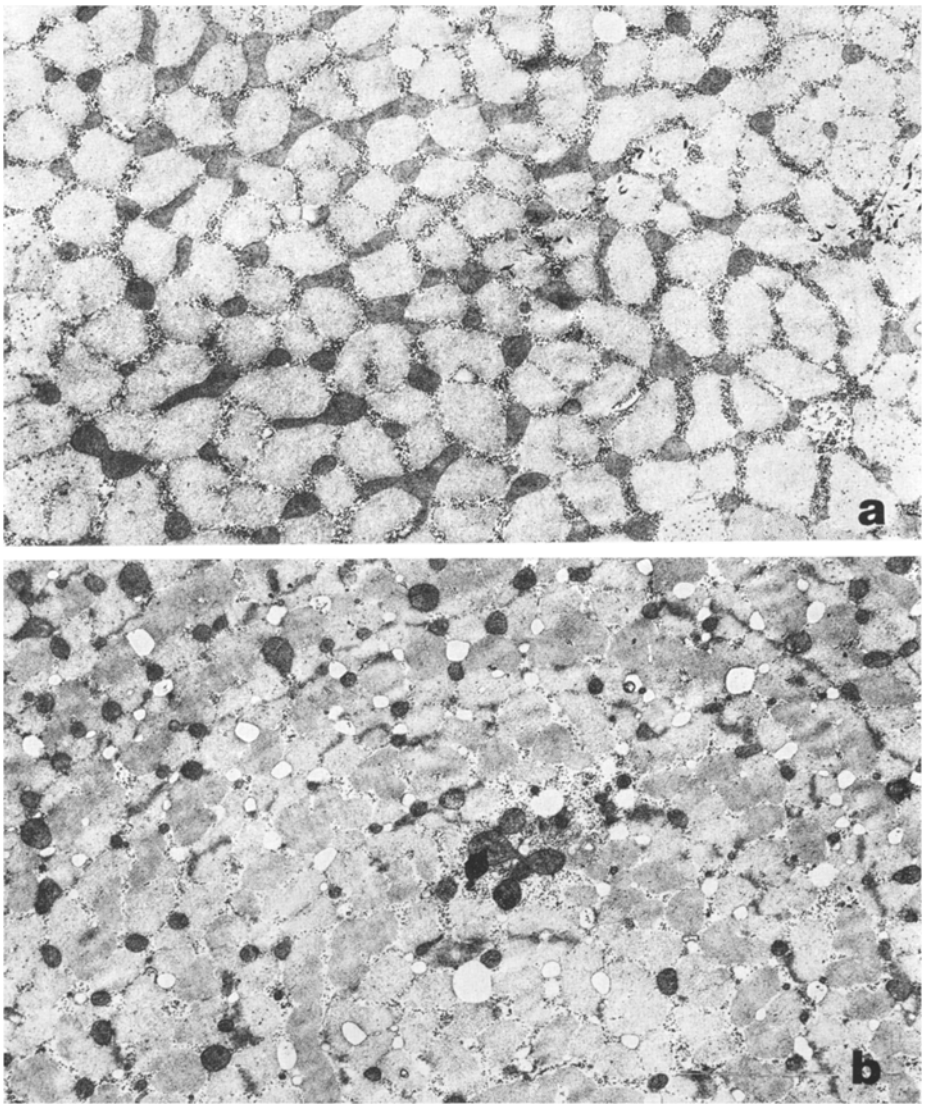

Fig. 5a and b. Boy aged 9 years with Duchene type muscular dystrophy. Electron micrographs 6000:1. (a) Cross section. Normal fibre. (b) Cross section. Fibre in an early stage of segmental contracture. Note generalized swelling of the reticulum and normal mitochondria

When larger segments of the fibre were contracted and overstretched, larger defects in the plasma-membrane occurred. The basal lamina was always retained. Swelling of the sarcoplasmic reticulum was pronounced. In several fibres satellite cells were found within a contractured segment. The plasmalemma of the muscle fibre was interrupted but the cell membrane of the satellite cell was intact (Fig. 4). In fibres with clot formation the plasmalemma was almost absent; the vesicles of the sarcoplasmic reticulum had now disappeared.

On cross sections fibres in the initial stage of segmental contracture were easily identified by their circular shape and by the prominent swelling of the sarcoplasmic reticulum (Fig. 5). These changes differed from fixation artefacts in that mitochondria were of normal size and density. Myofibrils displayed a regular pattern of thick and thin filaments. Glycogen granules were seen outside the basal lamina in the interstitial space (Fig. 6). In later stages of segmental contracture the mass of myofilaments became more dense and so homogeneous that the two sets of myofilaments could not be distinguished any more. The swollen sarcoplasmic reticulum disappeared. Boundaries of former myofibrils were marked by strands of glycogen. In these fibres mitochondria were either absent or swollen and fragmented. The contrast of all membranes was low and nuclei looked pyknotic. Cross sections through gaps between clumps of myofibrils showed basement membrane tubes with cell debris. These changes indicated definite necrosis of the contracted segment.

Experimentally Evoked Segmental Contracture

20 min after transection of the fibres 3 zones could be distinguished: the first 50—100 μm adjacent to the cut were dense and homogeneous. In electron micrographs dark cross bands with 0.5 μm spacing indicated the site of sarcomeres. Mitochondria were shrunken and arranged in longitudinal strands. The next 20—50 μm appeared light. Sarcomeres were overstretched and out of register. The sarcoplasmic reticulum was swollen and could be seen as vacuoles in the light microscope. Mitochondria were well preserved. The third 100—200 μm long zone was of normal density. The sarcoplasmic reticulum was swollen, the arrangement of the sarcomeres was fairly regular. More distant from the cut the fibres looked normal. In some fibres the first 2 zones were as described but over the next 100 to 500 μm sarcomeres were evenly shortened to 1.5 to 2.0 μm and the fibre appeared dense in the light microscope (Fig. 1).

Discussion

It might be argued that contracted segments are artefacts because they can be produced by squeezing (Buchthal and Kaiser, 1951) or fixation (Field, 1960). Glutaraldehyde itself does not cause contracture (Huxley, 1968); in rat muscle phosphate buffer gives rise to contracture at 37° C but not at 20° C or below (own observations). The reason why I do not consider (see also Cullen and Fulthrope, 1975) improper handling of the biopsy as the cause of segmental contracture in man are as follows:

1. It did not occur in biopsies taken under identical conditions from patients classified as normal (Table 1).

2. It occurred randomly and not in groups of fibres or only in the periphery of the specimen as in squeezed muscles (Schmalbruch, 1973).

3. Some fibres contained phagocytes (Fig. 1).

4. Some contracted segments showed closely attached regenerating fibres with normal sarcomere spacing (Fig. 1).

5. Acute shortening of fibre segments by 60—80 % would have distorted neighbouring fibres, especially in dystrophic muscles in which connective tissue

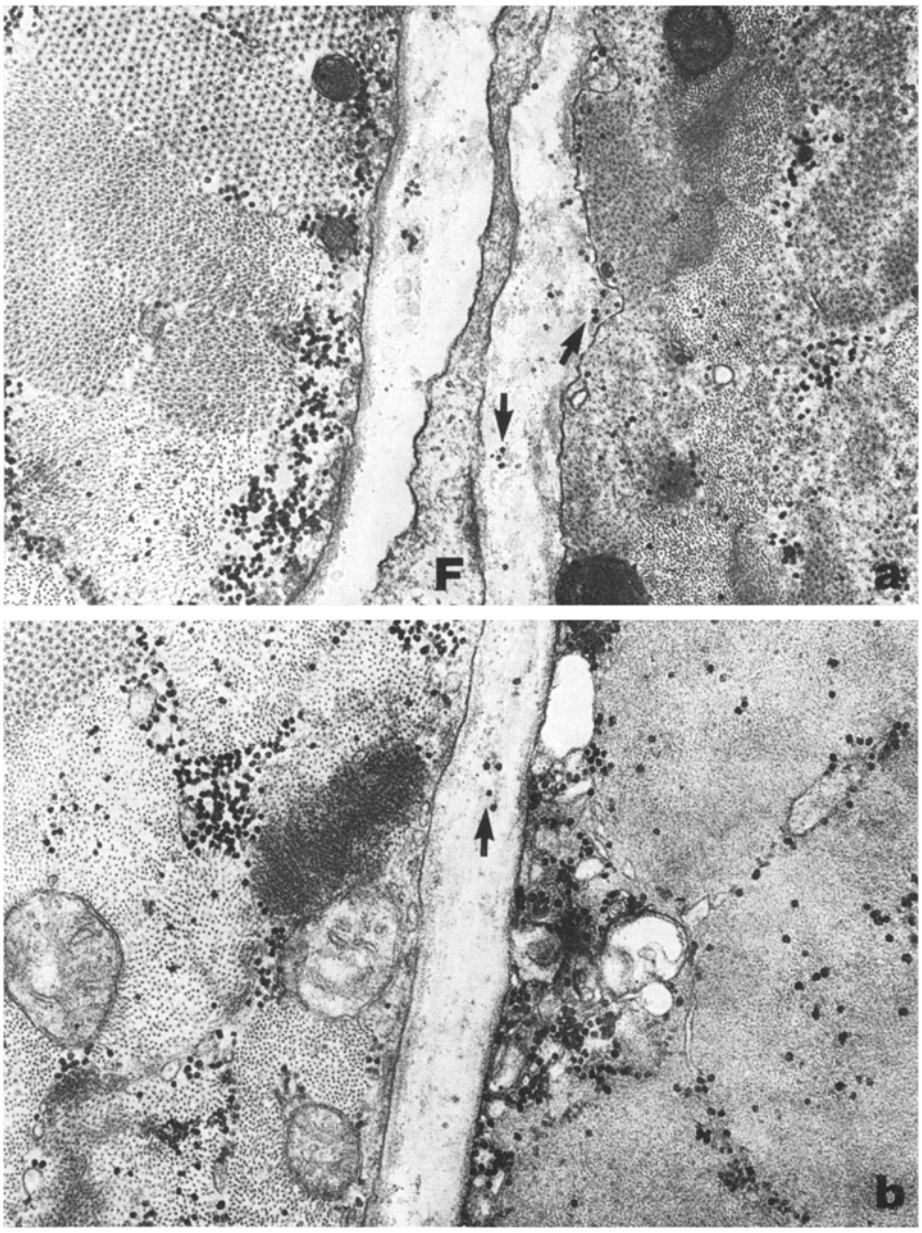

Fig. 6a and b. Boy aged 8 years with Duchenne type muscular dystrophy. Electron micrographs 40000:1. Cross sections. Normal fibres (left) as compared to contractured fibres (right) in an early (a) and late stage (b) of segmental contracture. In the right fibre in (a) thin and thick myofilaments can still be distinguished though the hexagonal array is lost. In the right fibre in (b) myofilaments appear homogenized. Note extracellular glycogen granules (arrows). (*F* Fibrocyte)

is increased. This makes the muscle less compliant and binds fibres more closely to each other than normal.

6. Identical changes have been shown by Zenker (1864) in unfixed fibres from patients with typhoid fever in which so many fibres were affected that the muscle had been ruptured *in vivo*.

It is unlikely that defects in the plasmalemma and swelling of sarcoplasmic reticulum (Figs. 2—5) were due to improper fixation, because mitochondria, which are most sensitive to fixation artefacts, appeared normal. Detachment of basal lamina and plasmalemma and displacement of glycogen into the extracellular space (Figs. 3 and 6) have not been observed in normal muscle.

Defects of the plasmamembrane are common in necrotic cells (David, 1967; Mair and Tomé, 1972). In Duchenne type muscular dystrophy and in genetic muscular dystrophy of chickens and mice there is evidence that the plasmalemma is leaky even in muscle fibres still functioning. The internal potassium concentration is lower than normal (Horvath *et al.*, 1955), already in the preclinical stage (Blahd *et al.*, 1953). The excitability is increased and the refractory period decreased, which indicates that the outer cell membrane is abnormally permeable to ions (Farmer *et al.*, 1959). In cell membranes, phosphoglycerids and cholesterol are present in abnormal relative proportions (Owens and Hughes, 1970; Hughes, 1972; Kunze *et al.*, 1973). This causes an increase in the microviscosity of isolated cell membranes and probably the increased potassium efflux from dystrophic muscle fibres (Sha'afi *et al.*, 1975).

Swelling of the reticulum occurred in contracted segments of human muscle fibres and in contracted segments of injured rat muscles; it was observed as well when contracture had been evoked by a high external potassium concentration (Schmalbruch, 1964). The sarcoplasmic reticulum was distended in parts of the fibre in which sarcomeres were not yet shortened (Fig. 2). This indicates that swelling of the reticulum precedes shortening of sarcomeres whether depolarisation of the membrane is caused by a high extracellular potassium or loss of internal potassium through a leaky plasmalemma.

Based on the findings presented the following hypothesis for the development of segmental contracture and waxy degeneration is proposed: Locally the plasmalemma becomes so leaky that myofibrils contracture. Irreversible rigor develops unless the membrane is restored; through holes in the plasmalemma glycogen granules and probably also water soluble enzymes are lost, which gives rise to a high serum enzyme concentration. Finally the affected fibre segment becomes necrotic. Probably the defect in Duchenne type muscular dystrophy manifests itself morphologically in the plasma membrane. A few months after birth before clinical signs can be detected the serum enzyme concentration is increased; at the same time hyaline (i.e. contracted) fibres occur as the only histological sign of abnormality (Bradley *et al.*, 1972).

Though prominent in Duchenne type muscular dystrophy segmental fibre breakdown occurred in a variety of other neuromuscular disorders as well (Table 1). The incidence in histological sections may depend on the rate of cell death and phagocytosis rather than on differences in the cellular mechanism of fibre breakdown.

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Note Added in Proof

Defects of the plasma membrane of muscle fibres were observed by B. Mokri and A. G. Engel in Duchenne type muscular dystrophy (27th Annual Meeting of the American Academy of Neurology, May 1—3, 1975), Abstract in Neurology (Minneap.) **25**, 375 (1975).