Ultrastructure Of Muscle In Typical And Atypical Duchenne Muscular Dystrophy A Comparative Study

Felix Teodoro Oteruelo

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ULTRASTRUCTURE OF MUSCLE IN TYPICAL AND ATYPICAL DUCHENNE MUSCULAR DYSTROPHY. A COMPARATIVE STUDY

by

Félix Teodoro Oteruelo
Department of Pathology

Submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
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To María Pilar Balaguer de Oteruelo, my wife; she made this work possible.
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ABSTRACT

Attempts at separation of nosologically distinct entities from the large group of muscular dystrophies, on the basis of specific ultrastructural changes, have not been very fruitful.

In the course of clinical and biochemical studies of a large number of patients with all forms of muscular dystrophies it became apparent that the clinical features of two young patients were at variance with those of the typical sex-linked Duchenne muscular dystrophy. The disease was not known to occur in their families and manifested itself early in life, but was very slowly progressive. Biochemically, the oxidation of fatty acid by mitochondria from muscle was normal in the atypical and decreased in the typical patients of Duchenne muscular dystrophy.

It seemed, therefore, of interest to study muscle tissue from both groups of patients in order to compare their ultrastructure, and in the hope of detecting meaningful differences and characteristics features for each group.
It became evident that prior to such comparative studies, detailed knowledge of the ultrastructure of normal muscle must be acquired.

The present study supports the clinical and biochemical differences; however, they should not be considered final, as some features may be found in both groups. Other features thought to be present only in one group of patients may be apparently absent in the other, thus reflecting perhaps the limitations of sampling rather than true absence. Therefore, it seems more reasonable at present to depend upon the different patterns of morphological changes for the two groups, rather than on one or two features seemingly characteristic for either group. This and the possible association of the fiber types with any of the changes are problems worth pursuing further.

The different patterns of changes for each group are as follows: the atypical patients of Duchenne muscular dystrophy were identified by the recognition of subsarcolemmal bumps, sarcoplasmic (usually subsarcolemmal) deposits of glycogen, spiral fibers, and the presence of the two first types of myofibrillar degeneration. On the other hand, typical patients of Duchenne muscular dystrophy were recognized by the preferential wasting of central myofibers in a given bundle, the concurrent appearance of several types of degenerative processes (hyaline, granular, vacuolar, etc.) within the same bundle of fibers, rare
cellular infiltration, and by the presence of sarcoplasmic bodies in myoblasts and myotubes, ring fibers, Z-lines streaming, target fibers and the third type of myofibrillar degeneration.

The presence of subsarcolemmal bumps, consisting of myofibrillar proliferation, and of spiral fibers, resulting from local proliferation and periaxial migration of satellite cells within the basement membrane of a myofiber, suggested a failure in the last stages of regeneration. The finding of sarcoplasmic bodies in myoblasts and myotubes was interpreted as an early sign of regression, and, therefore, also suggested a failure of regeneration; they appeared to be originated from a defective linking of thin filaments in the Z-line. The possibilities that the ring fibers might be normal and special components of muscle tissue and the intimate mechanisms of myofibrillar degeneration are discussed in some detail.

Taking into consideration the implications of limited tissue sampling, it seems justified to state that the two atypical patients do not suffer from the classical sex-linked severe recessive Duchenne muscular dystrophy; nor do they suffer from the benign type of Duchenne dystrophy. It is possible that the disease in these two patients represents a "de novo" mutation of a Duchenne-like sex-linked type of muscular dystrophy.
The purpose of this work was to report the results of an ultrastructural study of muscle tissue from five patients with the typical and two patients with an atypical Duchenne muscular dystrophy. Moreover, to establish a baseline for the evaluation of pathological changes, results of ultrastructural studies on normal muscle tissues were reported in some detail. It is not within the scope or province of this thesis to discuss from any point of view myopathies or muscular dystrophies, other than as deemed applicable.
I. INTRODUCTION AND PURPOSE OF THE THESIS

The role of the electron microscope in the diagnosis and elucidation of pathogenetic mechanisms in muscle diseases has been limited because of several factors. Criteria for, and standards of normality for individual muscle types and at various ages are by no means firmly established and the interpretation of pathological changes depends on the technical excellence of tissues for examination. Any deviation from attention to greatest detail in processing and examination of skeletal muscle, may result in artifacts that in turn lead to misinterpretation. Skeletal muscle is subject to an enormous sampling error for electron microscopy. For example, whereas by light microscopy up to eighty per cent of the fibers appear to be abnormal in patients of preclinical phase of Duchenne muscular dystrophy, it is not unusual to observe many completely normal muscle fibers by electron microscopy (98a).

Nevertheless, the application to the study of muscle disorders has been very useful in segregating
specific diseases from entities grouped together not because they were "related", but because formerly a classification was possible only on the grounds of clinical similarities. On the basis of a relatively specific ultrastructural appearance distinct entities have been ultimately separated from the group of the so-called congenital hypotonias. Best examples are the nemaline myopathy (223b) and central core disease (223a), although admittedly the characteristic features of the muscle in the latter were first observed by light microscopy. Similarly, the electron microscope proved of value in the diagnosis and separation of some metabolic myopathies.

On the other hand, attempts at separation of nosologically distinct entities on the basis of specific ultrastructural changes from the large group of muscular dystrophies have not been very fruitful.

Diseases known as muscular dystrophies were grouped together on the basis of the belief that each was a myopathic disease inherent in the muscle itself, and because on light microscopic examination changes affecting the muscle fibers in a characteristic pattern were similar in all these disorders. Weakness of a given muscle group clinically identifiable first constituted the basis for classification. The grouping of these diseases on the above basis is not satisfactory for a
variety of reasons and scientifically not sound. There is a considerable overlapping of "territory" of muscle involvement and different patterns of inheritance have been recorded within one, seemingly the same type of dystrophy. Thus, the application of electron microscopy to the study of the entire group of muscular dystrophies is extremely desirable and some, if only a slight progress has been made in "segregation" on the basis of ultrastructural observations. For example, lipid droplets and structurally abnormal mitochondria were observed in muscle of a woman with very slowly progressive myopathy affecting her shoulder girdle (23a), — features not described in dystrophies to date.

In the course of clinical and biochemical studies of a large number of patients with all forms of muscular dystrophies carried out in the departments of Clinical Neurological Sciences and Biochemistry at the University of Western Ontario, it became apparent that two young patients suffered from a disease clinically resembling and yet not identical with, the classical sex-linked Duchenne muscular dystrophy (100 and 136). The disease was not known to occur in their family and manifested itself early in life, but was only very slowly progressive. Moreover, the level of mitochondrial oxidation of fatty acids was at variance with that established for patients with the classical type of Duchenne dystrophy.
It seemed, therefore, of interest and importance to study muscle tissue from both groups of patients, in order to compare their ultrastructure, and in the hope of detecting meaningful differences, and characteristic features for each group. It became evident that prior to embarking on such comparative studies, detailed knowledge of the ultrastructure of (appropriate) normal muscle must be acquired.

The purpose of this thesis is to report the results of an ultrastructural study of muscle tissue removed by standardized procedures at biopsy from five patients with the typical and two patients with an atypical Duchenne muscular dystrophy, undertaken with the above aims. Moreover, to establish a baseline for the evaluation of pathological changes, results of ultrastructural studies carried out on normal muscle tissues obtained from three patients matched for age will be reported in some detail.

It is not within the scope or province of this thesis to discuss from any point of view myopathies or muscular dystrophies, other than as deemed applicable.
II. REVIEW OF THE LITERATURE

1. Structure and Function of Human Striated Muscle

A). Light Microscopy.

Muscle tissue does not consist of muscle cells alone, as it contains connective tissue, nerves and vascular components. A given muscle is wrapped in a connective tissue sheath, the epimysium. On cross sections it is apparent that extensions of the epimysium into the muscle tissue divide it into bundles or fasciculi; these connective tissue prolongations constitute the perimysium. The endomysium is formed by delicate projections of the perimysium into each bundle and thus between muscle cells. The endomysium appears as a delicate network carrying capillaries and nerve endings. The connective tissue elements of a given muscle, i.e., epimysium, perimysium and endomysium, are all continuous with the connective tissue structures to which the muscle is attached and on which it exerts a pull on contraction.

Longitudinal sections of muscle fibers under the light microscope have a cross-striated appearance. The cross-
striations result from the apparent alternating light and dark bands. Light bands are the so-called I-bands (I stands for isotropy to polarized light) and dark bands are referred to as A-bands (A for anisotropy for polarized light). Each I-band is bisected by a thin dark line called the Z-line (Z stands for intermediate disk: Zwischenscheibe). The Z-lines of adjacent fibers are usually in continuation with one another, thus emphasizing the cross-striated appearance imparted upon the muscle fiber by the dark and light bands. A muscle fiber consists of longitudinally disposed myofibrils embedded in cytoplasmic substance termed sarcoplasm. Nuclei are scattered along the body of the fiber immediately beneath the muscle cell membrane. Muscle fibers measure from 1 to 40 mm in length and from 10 to 40 μ in width; this large cellular mass may explain the presence of several nuclei per fiber. The entire muscle fiber is limited by a poorly resolved thin membrane called the sarcolemma.

B). Ultrastructure.

Three major systems can be discerned by electron microscopy of a striated muscle fiber, each playing an important role in muscle contraction. The myofibrils constitute a system of elongated elements arranged parallel to the long axis of a muscle fiber; they are the contractile elements of the muscle. Mitochondria situated between the myofibrils supply them with adenosine triphosphate (ATP); the more
active the muscle is the more numerous are mitochondria. Finally there are two sets of tubules and vesicles arranged between the myofibrils, that at first glance do not appear to relate to each other. The first type consists of thin transverse tubules seemingly representing invaginations of the plasma membrane. The second set consists of elements of sarcoplasmic reticulum with its interconnecting transverse and longitudinal sacs and tubules.

In addition to the above mentioned constituents, the nucleus (with associated structures) and the satellite cells are the other important components of the morphological and functional unit of a muscle fiber.


The satellite cells, first described by Mauro (149) in the tibialis anticus muscle of the frog, are usually mono-nucleated fusiform cells with long axes oriented parallel to the long axis of the striated muscle fiber. They are enclosed by the basement membrane of the muscle fiber; their cytoplasm does not contain myofilaments (Figure 1). The studies of Katz (114) on the ultrastructure of muscle spindles in the frog showed the presence of similar cells related to a certain type of fibers. The only absolute criterion for identification of the satellite cells is the location as described above. Their situation beneath the basement membrane of the muscle fiber distinguishes them from endothelial cells, pericytes, macrophages,
endomysial fibroblasts and Schwann cells (associated with muscle innervation). The demonstration of two apposing plasma membranes, one belonging to the satellite cell and the other to the myofiber itself, is also necessary for identification of the satellite cell and ascertains the distinction from a multi-nucleated muscle fiber syncytium. This is particularly important as the nuclei of satellite cells are often similar to the myonuclei in the associated muscle fiber; however, the chromatin substance in the former appears less evenly dispersed than in the muscle nuclei. Nucleoli have not been observed in satellite cells. A small Golgi apparatus and a few profiles of endoplasmic reticulum lie in the perinuclear area. Centrioles and cilia have been described in satellite cells of bat muscles (31), but they have not been reported to date in other species. Pinocytotic caveolae are present at the plasma membrane, being more conspicuous at the side facing the muscle fiber; they are also prominent at the apposing plasma membrane of the myofiber.

Red and white striated muscle fibers (to be described below) contain a variable number of satellite cells; none has been observed in mammalian cardiac muscle (161). Estimations of the frequency with which satellite cells occur lack precision because they depend on small electron microscopic samplings. Therefore, nuclei of satellite cells have been reported variously as being 10 per cent, (162), 4.8 to 5.8 per cent, (243), or 12.5 per cent, (32), of the nuclei counted. Allowing a mean content of 80 nuclei per
millimeter length (163), the above figures suggest that there is approximately one satellite cell for each 150 to 225 micra of length of a skeletal muscle fiber. This length or segment of fiber syncytium has been recently designated as the "satellite cell segment"; it is defined as a single satellite cell and its adjacent segment of fiber syncytium, and considered to be the smallest morphological subunit of skeletal muscle (32).

The concept of the satellite cell segment implies that these cells are "reserve myoblasts" capable of mitotic division and that they are dormant cells which may become active under certain circumstances such as regeneration of muscle tissue. The concept that satellite cells represent reserve stem cells has been disputed, however, by several investigators (87 and 206) who propose just the reverse, i.e., that the satellite cell is the outcome of separation of a portion of myonucleus and adjacent sarcoplasm from the myofiber, and that this process of separation could be initiated by stimuli possibly related to degeneration.

The function of the satellite cells and their relation to the muscle fiber in normal and pathological states has not been established with certainty. The fact that satellite cells become active and increase in number under certain conditions suggests that they may be important in the process of repair of the muscle fibers.
b). Sarcolemma.

A membranous sheath separates the muscle fiber from the extracellular environment. Schwann (221) and Bowman (23) independently conceived of this membrane even by light microscopy, and Bowman observed that each muscle fiber was encased in "... a tubular membranaceous sheath of the most exquisite delicacy, investing every fasciculus from end to end, and isolating its fibrillae from all surrounding structures ...". He confirmed Schwann's descriptions of vertebrate and insect muscle, studied the permeability properties of this membrane, and named it "sarcolemma".

The sarcolemma is a complex consisting actually of two components: the plasma membrane and the basement membrane enveloping the fiber (Figure 1). The plasma membrane is an approximately 75 Å thick unit membrane limiting the muscle fiber. It appears as two dense lines separated by a less dense layer, each approximately 20 to 25 Å thick. The plasma membrane is a bimolecular leaflet of lipid (light layer), each chain of lipid molecules being covered on the outer surface by protein (dense lines). The plasma membrane invaginates into the muscle fiber to form the T-system in fish (65) and dragonfly (227). Small, flask-shaped inpocketings, i.e., caveolae or pinocytotic vesicles, resembling those described in the endothelial cells of capillaries and other vascular channels, are present at the plasma membrane; they are particularly
numerous at the muscle cell-satellite cell interface. Their function in muscle probably resembles that in other cells and is concerned with the transcellular transport of metabolites.

The other constituent of the sarcolemma, the basement membrane, is an ill-defined, approximately 800 Å thick layer, external and in close vicinity to the plasma membrane. Generally, it is considered to be composed of mucopolysaccharides, i.e., long-chain polymers of protein-sugar complexes. The resistance of the basement membrane to injury is an important factor in muscle regeneration. Collagen fibrils are attached to the basement membrane.

It is believed that the sarcolemma participates in the conduction of electrical impulses and acts as a directional permeability device between the extra- and intracellular compartments.

In the region of the nerve ending the muscle fiber usually presents a depression of varying shape. The nerve ending adapts its shape to the muscle fiber infoldings; thus its membrane (presynaptic) is at a constant distance from the muscle membrane (postsynaptic). The space between the two membranes is filled with the basement membrane of the myofiber (Figure 1).

c). Nucleus and Nucleolar System.

Each striated muscle cell contains many nuclei characteristically distributed beneath the sarcolemma; the long
axes of the nuclei parallel the long axis of the cell (Figure 1). The nuclear chromatin is usually condensed in areas adjacent to the inner nuclear membrane. The chromatin consists of fine fibrils and granular material representing dispersed chromosomes. Chemical studies have disclosed that the nuclear chromatin contains basic and acidic proteins and deoxyribonucleic acid (DNA). The chromatin is embedded in the nuclear sap or karyoplasm which contains proteins and some DNA; other components such as lipids are present in insignificant amounts. The nuclear sap appears in the form of dispersed particles and delicate filaments. The nucleus is known to synthesize ribonucleic acid (RNA) and protein (157).

The nuclei of muscle cells contain nucleoli. The nucleoli usually are situated near the nuclear membrane and consist of a coiled arrangement of granules of approximately 150 Å in diameter and fine filaments 50 Å in diameter (226). This coiled arrangement of granules and filaments, referred to as nucleolonema is embedded in an amorphous matrix. Nucleoli contain RNA and protein; some protein appears to be synthesized in the nucleolus, and it has been suggested that this synthesis is largely related to nucleolar growth (226). The function of the nucleolus is that of an amplifier of the synthesis of chromosomal RNA (145).
The nucleus of the muscle cell is limited by a double, 75 Å thick membrane perforated by a variable number of pores that measure approximately 100 Å in diameter. The inner and outer nuclear membrane are continuous at the periphery of the pores; the existence of a thin membranous diaphragm closing the pores makes an open communication between nucleus and sarcoplasm through the pores doubtful. The inner membrane is in direct contact with the chromatin material of the nucleus; the sarcoplasmic surface of the outer membrane is usually studded with ribosomes and it is continuous with the profiles of the endoplasmic reticulum (157).

d). Golgi Apparatus.

The Golgi apparatus appears as an aggregation of parallel flat saccules usually situated in the sarcoplasm at a nuclear pole. This complex is less prominent in mature muscle cells than in secretory cells; it is, however, prominent in developing and regenerating muscle cells. The Golgi apparatus is composed of three elements: smooth-surfaced and flattened sacs packed closely together; small microvesicles often appearing at the ends of the saccules and presumably derived from them and large vesicles, some of which may contain electron dense material. Functionally, the Golgi apparatus is the site of accumulation, segregation and storage of secreted protein, thus preventing the produced protein from being in contact with the cytoplasm. Moreover,
it is concerned directly with the formation of glycoproteins. The Golgi apparatus, the granular endoplasmic reticulum, and the nuclear and cellular membranes delineate an extensive system of intracellular channels that connect the extracellular environment with the nucleus and cytoplasm of the cell. The role of the Golgi apparatus in the muscle cell has not been entirely defined; as elsewhere, it may play a role also here in the protein synthesis and transport (138).

e). Mitochondria.

Schwann in 1839 described cellular granules distinct from the nucleus, and from fibrillar material of the muscle cell (221). These granules were later observed between the myofibrils (6). The name bioblast was coined subsequently for these intracytoplasmic granules (4). Other names also have been suggested: sarcosomes, microsomes, plasmosomes, chondriosomes, and so on. Mitochondrion (mitos: thread, condrion: granules), the name elected by Benda (14), has remained the most commonly used. Recently Roizin (208) found the term mitochondria inadequate and has replaced it by pleomorphic metabolosomes. For detailed historical data relevant to mitochondria the reader is referred to the reviews by: Cowdry (36), Newcomer (171), Bennett (16), Rouiller (213), Novikoff (172), and Roizin (208).

Retzius (203) applied the name sarcosome to the granules observed earlier in the intermyofibrillar spaces by Kölliker (124) and others (6 and 221). Almost twenty years
later these granules were recognized as the mitochondria of the muscle cell (202). An attempt at introducing a new classification of sarcoplasmic organelles was made by Harman (82). He chose the word cytochondria and applied it to all the recognizable cytoplasmic bodies. Under the term cytochondria Harman included mitochondria and sarcosomes or particles of Flögel. This differentiation and the usage of the word sarcosome chosen by Harman seems to be unnecessarily confusing since the name sarcosome was initially applied to the granules of Kölliker, later shown to be mitochondria. Furthermore, the description of the particles or granules studied by Flögel (63) corresponds well with that of mitochondria by Retzius and Kölliker. On the other hand, the sarcosomes of Harman are similar in size, distribution and chemical characteristics to the lipid droplets demonstrated often in the sarcoplasm (26).

In summary, the following may be stated with regard to the classification of the cytochondria of muscle as proposed by Harman: Harman's mitochondria are true mitochondria equivalent to the sarcosomes of Retzius (203) and the granules of Kölliker (124); Harman's sarcosomes correspond to the lipid droplets of Kölliker (124) and Bullard (26).

Electron microscopic studies have shown that the mitochondrion is an organelle distributed universally; it is present in unicellular organisms as well as in mammals. It is a round, oval or elongated body limited by an external or outer, and internal membranes; the latter invaginates
into the matrix forming cristae mitochondriales. A filamentous network in the mitochondrial matrix, particulate components associated with the inner membrane, and smooth "patches" covering the particles of the inner membrane, have been described recently (255).

The origin of the mitochondria is still disputed. In the past the "de novo" hypothesis implied that the mitochondria originated as either actively differentiating portions of protoplasm or as metaplastic bodies from other cytoplasmic elements (171). Different suggestions have been made to date as to the possible source of the mitochondria: certain increments of cytoplasmic particles close to the nucleo-cytoplasmic interface (84) that are significant in the chemical exchange across the nuclear membrane (237); the microbodies of de Duve or lysosomes of Novikoff (172 and 213); the pinocytotic vesicles (96); and the cellular membrane, (207). Definite evidence of the role of mitochondria in embryonal development also remains to be established. It is worthy of note that mitochondria appear in embryonic cells prior to the noticeable development of specialized activities of these cells (36).

The significance and complexity of the mitochondrial metabolic properties are reflected in the various names by which mitochondria have been qualified: powerhouse or power plant (138), biochemical machines and transducing systems (77), pleomorphic metabolosomes (208), and so on.
In general terms, three fundamental functions are being attributed to mitochondria: citric acid cycle oxidations, electron transport and oxidative phosphorylation. Extensive literature concerning the metabolic aspects of mitochondria has been reviewed recently by several investigators (77, 132 and 138), and will not be elaborated upon here.

Mitochondria in muscle have the general structural and functional features of the same organelles studied in other tissues. In addition, mitochondria in muscle show some additional characteristics of morphology and distribution according to the type of fiber in which they occur. Moreover, the size, shape and number of mitochondria within a given muscle fiber depend upon the location; thus, perinuclear subsarcolemmal mitochondria differ in appearance from those in the intermyofibrillar position. Details concerning the appearance of mitochondria in the muscle fibers will be reviewed later when the different types of myofibers will be considered.

f). Tubulo-Vesicular System.

Retzius (203) in 1881 confirmed the observations of earlier workers (39 and 238) concerning the structure and localization of the sarcoplasmic reticulum. He suggested the possible participation of the reticulum in the transmission of excitation within the muscle fiber. Subsequently, it was noted that the elements of the reticulum were attached to the inner surface of the sarcolemma (151). The work of Veratti (244), a pupil of Golgi, may be
considered as one of the classics regarding the muscle structure. He studied the mature and immature vertebrate and invertebrate muscle, and observed considerable variations in the arrangement of the sarcoplasmic reticulum from muscle to muscle. He further demonstrated the relation between the reticulum and the sarcolemma on one hand, and the myofibrils on the other. Subsequently, the transverse component of the sarcoplasmic reticulum was described (89) and it was recognized that myofibrils and sarcoplasmic reticulum are two separate, but related entities (15 and 20).

The sarcoplasmic reticulum is a complex network of tubules and sacs performing the functions of rapid "telegraphy, plumbing and supply" within the striated muscle fiber (195). The electrical stimulus travelling along the sarcolemma has to be conveyed rapidly to inner fibrils if all the fibrils are to be excited simultaneously. The myofibrils must have access to energy and rid themselves of waste products. The sarcoplasmic reticulum (or distribution system) resides between the myofibrils and it is formed by tubules running parallel to the myofibrils and transversely around them (Figure 1a). The transverse channels of the sarcoplasmic reticulum are placed at the level of the H-zone and the Z-line of each sarcomere. A special structural configuration of these channels, i.e., the triad, is apparent at the level of Z-line (Figure 2). Two of the elements forming the triad are sacs belonging to the sarco-
plasmic reticulum. The third one, between the two sacs, is a tubule that runs across some muscle fibers at the level of the Z-line and in other muscles at the junction between the A-, and I-bands. This separate tubular network is termed the T-system (transverse system). In some species, the tubules of the T system are formed by deep invaginations of the plasma membrane into the interior of the fiber (65 and 104). There is no continuity between the sarcoplasmic reticulum and the T-system at the levels of the triads. Both systems are separated by a distance of 150 Å bridged by an array of cementing materials (204 and 246). The membranes of the T-tubule and the terminal cisternae of the sarcoplasmic reticulum are brought to close proximity by "dimples" or evaginations formed by the latter membrane (115). The existence of continuity through pore-like openings between the two elements of the triad, is still controversial. There is evidence of molecular transfer between these two systems at the level of the triad (140 and 193). On the other hand, it appears that the area of actual close proximity between the membranes at the level of "dimples" is quite small and therefore unlikely a significant coupling avenue for the volume of molecular transfer between the T-system and the sarcoplasmic reticulum (66). It may be said, nevertheless, that the structural relations of plasma membrane, T-system and sarcoplasmic reticulum explain the rapidity of the response of muscle fibril to stimulation. It is now accepted that
the wave of excitation travels along the sarcolemma, presumably causing contraction by releasing Ca++ (138). This release of Ca++ by the sarcoplasmic reticulum during excitation is being studied intensely at present. There is evidence to show that sarcoplasmic vesicles bind Ca++ very tightly (10 and 38) and that significant amounts of a membrane-bound creatine phosphokinase are present in the sarcoplasmic reticulum (9). The latter finding indicates a possible source of ATP for calcium transport processes during muscle relaxation.

g). Myofibrils.

The various bands, zones and lines described by light microscopy can be interpreted with the help of the electron microscope in terms of two sets of filaments, thin and thick, overlapping with each other.

The sarcomere lies between two Z-lines and is the smallest repeating unit in the contractile system (Figure 3). The thin I-filaments extend on either side of the Z-line. Overlapping with two sets of thin filaments are the thick A-filaments, the center of which is demarcated by M-line. The H-zone is a region at the center of the sarcomere on either side of M-line, where the thin and thick filaments do not overlap. The thick filaments carry projections or cross-bridges on their surface; these projections are absent near the center of the filaments, thus resulting in the appearance of the pseudo-H-zone. Therefore, the
A-band is the region represented by the A-filaments (thick), overlapping with the thin I-filaments, and the I-band is the region where the I-filaments (thin) of two adjacent sarcomeres do not overlap with thick filaments (Figure 3). The arrangement of thin and thick filaments in the A-band is appreciated better on cross sections. Every thick filament is surrounded by six thin filaments in such a fashion that there are always two thin filaments between every pair of thick filaments, every thin filament being surrounded by three thick filaments (Figure 3).

h). Mechano-Chemical Proteins.

Ultracentrifugation studies (250) showed that the "myosin" extracted earlier by Kühne (126) was not a homogenous substance. The discovery of actin (232 and 233) indicated that "myosin" was actually composed of two proteins: actin and myosin. Later, another major protein component was isolated from muscle and named tropomyosin (7). Although the precise role of tropomyosin in muscle contraction is not known, its ubiquitous presence (15 per cent of muscle protein) suggests that it must be of importance in muscle work. Actin, myosin and tropomyosin make up the bulk of total protein of the myofibril. Some other minor components, e.g., actinin, troponin, and M-substance, also have been identified (46 and 147). They appear to have a regulatory function in the process of contraction-relaxation.
Actin is a relatively small globular molecule with a molecular weight of 68,000. It exists in two different forms: globular (G-actin) and fibrous (F-actin). Actin is the main component of the thin myofilaments. G-actin polymerizes into F-actin in the presence of ATP, KCl and Mg++, with the conversion of ATP to adenosine diphosphate (ADP). The ADP is firmly bound to the F-actin in such a fashion that the number of ADP-containing molecules is equal to the number of G-actin units in the F-actin polymer. The complex consisting of ADP and F-actin can be split by reacting with myosin. The fact that actin liberates a phosphate bond during the polymerization suggests its catalytic function. However, actin differs from a normal enzyme in that each G-actin unit splits off under normal conditions only one phosphate bond. Examination of F-actin under the electron microscope shows a rigid filament of a double helical nature (81). The precise structure of this helix has not been defined.

Myosin, with a molecular weight of approximately 500,000, is the principal component of the thick myofilaments. Myosin seems to be composed of two identical large and two small chains. The complete myosin molecule is approximately 1,550 Å long. Under the electron microscope it appears as a bulbous head 150 Å long and 50 Å wide with a long tail 20 Å wide. Controlled tryptic digestion splits the myosin molecule into two particles: light (m.w.: 126,000) and heavy meromyosin (m.w.: 324,000). Light meromyosin
appears to correspond to the tail region and heavy meromyosin to the bulbous head of the myosin molecule. Light meromyosin is of low solubility and aggregates easily forming long fibers. Heavy meromyosin is water soluble and retains the ATPase properties of myosin as well as the affinity for actin. It is known that ATP does not affect the configuration of the myosin molecule, and both actin and myosin must be present for ATP to exert a physical effect. Thus, it may be stated that the actomyosin complex is the primary mechano-chemical transducer system. But what in fact happens to ATP, actin and myosin when they are interlocked and in intimate contact, remains uncertain.

Tropomyosin is an elongated molecule with a molecular weight of 54,000. It appears to be associated with the actin filaments and localized largely at the Z-line. It has been suggested that tropomyosin at the Z-line may be part of a lipid-protein complex (199). A "native tropomyosin", or a factor sensitizing actomyosin to Ca++, has been isolated recently (46). The native tropomyosin differs from other forms (7) in that the latter do not respond to Ca-ions.

Troponin was discovered when native and other tropomyosins were investigated (45). The native tropomyosin could be separated into tropomyosin and a globular protein named troponin. Troponin seems to be a factor that promotes the aggregation of tropomyosin. It has been shown recently that troponin consists of two smaller molecules,
troponin A and B (85), and suggested that troponin B is associated with the Ca++ sensitivity of actomyosin. Troponin appears located along the actin filaments of muscle.

The interaction between actin and myosin would not culminate in a real contraction in living muscle without the aid of other protein factors not well characterized to date. One of these is alpha-actinin believed to act by facilitating the lateral aggregation of F-actin filaments (46). It has been demonstrated in the Z-line, but its definite localization has not been established. Beta-actinin also shows a profound effect on the physico-chemical properties of F-actin. It appears to be an actin-dispersing factor that regulates the length of the polymerizing F-actin and inhibits the formation of a net-like structure (146).

The M-substance is another recently isolated protein; it is apparently localized exclusively at the level of the M-line (147).

Living muscle, when not stimulated, is in a relaxed state and, therefore, soft and stretchable. After death, it becomes brittle and non-extensible, and thus is in a state of rigor. It is now known that rigor is the consequence of disappearance of ATP from the muscle, as actomyosin requires ATP to maintain the relaxed state of muscle. Whether ATP brings about contraction or maintains the muscle in a relaxed state depends upon the presence of Ca++. The proper sequence of events is performed as well as regulated by the above mechano-chemical proteins.
C). Morphogenesis of Contraction.

Consequent to the release of Ca++ from the sarcoplasmic reticulum and in the presence of ATP derived from mitochondria, the muscle contracts. The mechanism of muscle contraction is best explained by the sliding theory (102). It is based upon the observation that in contracted muscle, there is an increase in the extent of overlap of the interdigitating thin and thick filaments without an overall change in the length of these filaments. The sliding phenomenon may be explained by a property residing in the thick myofilament (103). As mentioned above, the myosin molecule consists of a long tail harbouring the light meromyosin and small bulbous head containing the heavy meromyosin with its ATPase and actin-binding ability. It has been shown that the bulbous heads of the myosin molecule are the cross-bridges that interact with the actin-containing thin filaments. Furthermore, the myosin molecules are aggregated in a tail to tail fashion, with their heads pointing in opposite directions. This characteristic arrangement of the myosin molecules gives an opposite polarity in the two halves (on each side of M-line) of the thick filament, and, therefore, the thin filaments slide over each half of thick filament in opposite directions. Other effect of this characteristic arrangement is a smooth, 1,500 Å long, region in the center of thick filaments, the pseudo-H-zone (101) (Figure 3). The thin filaments have also opposite polarity on either side of the Z-line. Thus,
both types of filaments have the required polarity to support the sliding theory. Detailed studies of the cross-bridges in the myosin filament suggest that they are organized in a helical configuration; there are six cross-bridges per turn of helix and each helix is approximately 400 Å long (105).

The original sliding theory has been expanded on subsequently and at present may be summarized as follows: calcium ions released from the sarcoplasmic reticulum react with appropriate sites of the ATP on the myosin cross-bridges and with the reactive sites on the actin filaments, pulling the filaments together (156). The bridges bend inward toward the axis of the myosin filament, thus pulling the actin filaments and shortening the muscle. The cross-bridges now are folded against the myosin filament; the ATPase activity of this filament splits the ATP immediately to ADP, and, therefore, breaks the calcium-linked connections between the myosin cross-bridges and the actin. Energy from other sources causes almost immediate reconstitum of the ADP to ATP with the resulting outward direction of the cross-bridges. The cross-bridges now are ready to bind other calcium ions and effect a further step in pulling upon the actin filaments (105).

Other theories have been advanced to explain the contraction of muscle. Thus, Davies (37) proposed a molecular hypothesis which invokes alternating cycles of shortening and extension of the cross-bridges, and Reedy (201) suggested that the cross-bridges turn inward toward the
center of the sarcomere following attachment to the actin. There have been other attempts at clarifying the complex problem of muscle contraction. Among other, a kinetic model of muscle contraction viewed as a sequence of chemical reactions was proposed by Jöbsis (110); it was suggested that the contractile force in muscle originated from the interactions of electrical double layers on the myofilaments (223); and according to Harrington (83), the core of the thick filament is supposed to be the tension-generating site which will lead to out of phase oscillating movement of the cross-bridges.

Whereas all the various theories explain within certain limits the fundamental processes of muscular contraction, they have not taken into consideration all the newly described structures, for example: the coiling filaments of Garamvölgyi (69), the T-filaments of Hoyle (97), the fibrillin filaments of Guba (79), the \( N_1 \) and \( N_2 \) lines (67), and the proposed submicroscopic structure of the myofibril as a network of preformed longitudinal and transverse elements (54). Therefore, it may be expected that the present day concepts of the mechanism of muscular contraction will be again modified.

D). Types of Muscle Fibers.

Studies on normal and pathological striated muscle tissue have been increasingly clouded by inconsistencies in the
definition of fiber types and lack of correlation between different systems of nomenclature. Older classifications were based on gross appearance of the muscle fibers, and in one of these muscle fibers were distinguished as "protoplasmic" and "protoplasmareich" (123). It was proposed in a similar classification that some fibers are arranged in closely adjacent columns ("Felderstruktur") and other in a distinct rounded fashion ("Fibrillenstruktur") (125). Fibers most numerous in red muscle were called red fibers while those predominating in white muscles were termed white fibers.

On the basis of electron microscopic examination fibers have been classified recently as white, intermediate and red (182), and this classification offered as an alternative to that previously in use in which fiber types had been designated as A, B and C (229). The designation of fibers as A, B and C had led to some confusion, possibly because of similar designations for nerve fibers. Moreover, an attempt at refining the terms of this classification by comparing the A, B and C types as shown by the succinic dehydrogenase reaction with the alpha, beta and alpha-beta types as revealed by the actomyosin adenosine triphosphatase (ATPase) reaction (256), failed. No such "reconciliation" between these two classifications was possible, nor between classifications for various species, and the conclusion was drawn from these comparative studies that it is not possible to achieve a unified classification and nomenclature for all species.
Biochemically, it is customary to speak of white and red myosin obtained from homogenates of red and white muscle respectively, although such homogenates must be derived from muscle containing an unspecific number of different fiber types (228).

Physiologists have been aware for long that muscles can be categorized functionally as fast, intermediate or slow, depending upon the speed of contraction and other characteristics (33).

The heterogeneity of the enzymatic activity in the muscle fibers became apparent with the development and application of histochemical techniques. On the basis of these reactions muscle fibers were grouped into types I and II corresponding largely to red and white type fibers (41). Type I fibers had high oxidative enzyme, and low ATPase and phosphorylase activity; the reverse was true for type II fibers. Subsequently, these two types were subdivided into eight different fiber types as distinguished by a profile of histochemical reactions (209). Recently, type II fibers have been subdivided into types IIA, IIB and IIC on the basis of their susceptibility to preincubation at low pH prior to the calcium ATPase reaction (25).

Further evidence for heterogeneity of the muscle fibers was obtained by studying their subcellular organization. A comparative study on mammalian muscle fibers (72) disclosed differences in the number and location of mitochondria, lipid droplets and other cellular elements; a
morphologically intermediate type of fiber also was identified.

In the present study the classification of fibers into white, red and intermediate types has been adopted and each type delineated as follows:

a). White Fiber.

White fibers possess elongated subsarcolemmal nuclei. These nuclei show, at times, deeply indented outlines that cannot be correlated with contraction of the fiber. Instead, it has been suggested that the invaginated configuration of the muscle cell nuclei might be induced by intracellular ion changes, especially by bivalent cations (64). The nuclei may contain one or two nucleoli. The nuclear chromatin is condensed peripherally in areas adjacent to the limiting nuclear membrane. A small Golgi apparatus and a few small mitochondria may be seen in the perinuclear zone. In the interior of the fiber, slender and elongated mitochondria are present in pairs one on each side of the Z-line; they have few cristae and the matrix is of low density (174). Glycogen granules are numerous between the myofibrils, but only a few lipid droplets are present. Free ribosomes are found exclusively beneath the motor endplate. The motor endplate of the white fiber is large and has numerous, well developed junctional folds (173). The T-system is a regular, well organized but complex component that effects triadic contacts with terminal cisternae of the sarcoplasmic
reticulum at the junction of the A- and I-bands. The sarcoplasmic reticulum shows at the level of the H-zone a compact arrangement of broad and parallel tubules (73). The sarcomeres are slightly shorter and the Z-lines are thinner than those of the red fibers (219). The size and disposition of fibrils in the white muscle fiber is regular and the M-line is well delineated (147) (Figure 4).

b). Red Fiber.

The red fibers have the smallest average diameter of all muscle fibers, and the ratio of sarcoplasm to myofibrils is greater than that in the white fibers. The nuclei resemble those of the white muscle, but they have fewer and shallower indentations. The main characteristics of this type of muscle reside in the mitochondria and the lipid content. Accumulations of large mitochondria are located beneath the sarcolemma and in the perinuclear region, and longitudinal chains of mitochondria are disposed along the myofibrils. Plump and multiform mitochondria are located around the fibrils at the levels of the I-bands; they have numerous cristae and dense matrix (174). In general, mitochondria are larger than those of white muscle fibers. Lipid droplets are numerous; they are located usually near the I-band and in close association with mitochondria (197). Red fibers have multiple nerve terminals (93) which end in small motor endplates with poorly developed junctional folds (173). There is paucity of T-system elements, and
those present are not regularly spaced. The T-system effects triadic contacts with the terminal cisternae of the sarcoplasmic reticulum which in turn form an elaborate network of narrow tubules at the level of the H-zone (73). The sarcomeres are slightly longer and the Z-lines are thicker than those in the white muscle fibers. The size and disposition of the myofibrils is somewhat irregular. The Z-line may show a zigzag pattern and the M-line is either absent or less conspicuous than that in the white fibers (35), (Figure 5).


The intermediate muscle fibers, as the name implies, possess characteristics intermediate between the red and white fibers. They have a wider diameter, and their mitochondria are somewhat smaller and have less prominent cristae than do the red fibers. The motor endplates show fewer well developed junctional folds than those in the white fibers (173). The width of the Z-lines is comparable to that of white fibers (73).

The functional differences between muscle fiber types have not been clarified entirely. Red muscle fibers are considered to be associated with high metabolic activity and high frequency of contraction, as opposed to speed of individual contraction, whereas low metabolic activity and a lower rate of contraction is being associated usually with white muscle fibers. The small size of red muscle fibers
imps shares a high surface-volume ratio that favors exchange of gases, ions and metabolites. The abundance of mitochondria provides a readily available supply of energy, and the close association of lipid with mitochondria suggests high oxidative activity. The network of sarcoplasmic tubules over the H-zone provides a large membranous surface area that may be related to speed of contraction. It has been suggested recently (219) that the variable structural pattern of muscle fibers may be related to two distinct physiological parameters, speed of contraction and resistance to fatigue. However, the exact correlation between fiber type and speed of individual contraction (to be distinguished from frequency of contraction) has not been established. Red fibers have been associated with muscles that have a slow speed of individual contraction, and white fibers with muscles that have a fast speed of individual contraction, but there are some exceptions to this rule.

2. Muscular Dystrophies

A). Historical Considerations.

Isolated cases that are recognized now as examples of this group of diseases were recorded in England in 1830 by Sir Charles Bell (13). Two Italian physicians, Coste and Gioja (34) described two brothers with enlarged muscles and progressive muscular weakness in 1838. Additional series
of cases were reported by Meryon (152), Oppenheim (176), Duchenne (43), and Gowers (75).

In his initial communication Meryon (152) reported the clinical aspects of the disease, the gross condition of the brain, spinal cord and peripheral nerves, and the gross and microscopical appearance of diseased muscles. He emphasized the singular quality of "dead weight" when these patients were lifted, noted the nervous tissue to be free of disease and observed the paleness of, and the presence of oil globules in the skeletal muscle. A description of findings at the autopsy of one of these patients was published by Partridge (185), who reported that the muscles examined had undergone fatty degeneration.

Whereas Duchenne was not the first author to recognize the disease named after him, it was he who gave the first clear account of it as well as a practical instrument for muscle biopsy, i.e., the "histological punch" (Figure 6) which in time replaced the Mideldorff's harpoon (44). The observations of Duchenne enabled him to make contributions to nosology at a time when the distinction between primary muscle disease and neurogenic atrophy of muscle was not appreciated. None of his patients when first seen was less than six years of age, and in order to catalog the sequence of events during the initial years Duchenne had to depend on histories obtained from the parents. He did not recognize the hereditary nature of the disease and cited several of his patients as recovering after treatment.
It is probable that these patients were examples of benign congenital hypotonia rather than muscular dystrophy.

Eleven years later Gowers (75) described the characteristic manner in which the dystrophic patients rise from prone position, i.e., they use their hands and arms on their legs as support in erecting themselves. He stressed the diagnostic importance of this phenomenon, since known as the "Gower's sign". He recognized also the rare and distinct form of dystrophy that involves initially the distal muscles (76).

Within the two decades that followed the appearance of Duchenne's monograph, the clinical features of other forms of dystrophy were recognized. Leyden (134) and Möbius (158) described a form with features similar to those observed by Duchenne, except for a later onset and longer, more benign course. Landouzy and Dejerine (128) described another adult type, the facioscapulohumeral form, and Erb (52) reported a juvenile disease characterized by shoulder girdle wasting. On the basis that there was similarity in the microscopic appearance of the above and other diseases in this group, Erb suggested, that all dystrophies constituted one entity, and proposed the name of progressive muscular dystrophy for all the girdle dystrophies (53). Ingalls and Webber published the first case of Duchenne muscular dystrophy in North America in 1870, and analyzed forty one cases reviewed from literature (106).
The major achievement of Duchenne was not that he was one of the first to describe and characterize a disorder of muscle, but that through his writings he made the medical profession aware of the existence of a whole field of unexplored muscular diseases in man. It is perhaps in tribute to this that a monument in his honour was installed in the Hôpital Salpêtrière in Paris. It depicts a physician leaning over a patient to whom he applies the electrodes of a simple apparatus. Above the bas-relief is a plaque with the following legend:

1806 - 1875
A: Duchenne (de Boulogne)
Électrisation localisée
Physiologie des mouvements
Neuropathologie

B). Definitions and Synonyms.

The muscular dystrophies are defined at present as a group of progressive, genetically determined myopathic diseases. The word dystrophy (Greek, from "dys": disordered and "trephein": to nourish) implies a defect in nutrition of the muscle tissue. The generic name of muscular dystrophy was accepted and has been widely used since its introduction in the literature by Erb (53); however, there is no direct evidence indicating a defect in the
nutrition process of muscle tissue. Myopathy embraces all
the primary diseases of muscle tissue, exclusive of those
that are secondary to alterations of the nervous system.
The concept of myogenic (44) pathogenesis of muscular
dystrophies has been challenged by proponents of neurogenic
theory (30), and the matter has not been settled to date.
On clinical grounds Duchenne proposed the name of pseudo-
hypertrophic muscular paralysis for the disease he described.
He also suggested the name myosclerotic paralysis on the
basis of the pathological findings in the affected muscles.
Other names also had been proposed for this disease:
lipomatosis musculorum luxurians (91), atrophia musculorum
lipomatoso (222), pseudohypertrophic muscular dystrophy
or progressive muscular dystrophy of childhood (241),
Duchenne type rapidly progressive muscular dystrophy of
young boys (231), and severe generalized familial muscular
dystrophy (1). Today, it is known as pseudohypertrophic
muscular dystrophy or simply as Duchenne muscular dystrophy.

C). Classification.

All attempts at classification of muscular dystrophies
have been difficult largely because we are much better ac-
quainted with the clinical aspects of this group of diseases
than with their pathological features. Poore (194) made
one of the first attempts at classification based on the apparent
different distribution of muscle involvement; he concluded
that "... there seems to be no rule as to its mode of distribution, and any attempt at classification would simply be an enumeration of the muscles involved in each case ...". Since that time the muscular dystrophies have been divided clinically into a variety of types according to specific patterns of muscle involvement (257). However, the alterations of the individual muscle fiber are virtually identical in all clinical patterns. The most accepted classification, based upon the studies of Walton and Nattrass (247), is as follows (modified):

1. Duchenne muscular dystrophy
   A. Sex-linked recessive variety
      Severe type
      Benign type
   B. Autosomal recessive variety
2. Limb girdle muscular dystrophy
   A. Autosomal recessive variety
   B. Autosomal dominant variety (rare)
3. Facioscapulohumeral muscular dystrophy
   A. Autosomal dominant variety
   B. Autosomal recessive variety (rare)
4. Distal myopathy
5. Ocular myopathy
6. Congenital muscular dystrophy

As outlined in the Introduction and Purpose of Thesis, the present studies concern patients with the sex-linked recessive variety of the classical Duchenne muscular dystrophy and patients with a variant of the same disease
characterized by defineable biochemical and some clinical features. Therefore, the remaining parts of this review will be limited to the sex-linked recessive variety of the classical Duchenne muscular dystrophy, as the variant represented by the second group has not been reported to date outside our University Center. However, information regarding other forms of dystrophies will be also cited whenever it contributed to the development of our knowledge on Duchenne muscular dystrophy and its variants.

3. Pseudohypertrophic Muscular Dystrophy


The sex-linked recessive Duchenne muscular dystrophy occurs in a severe form, the most common and serious of all the muscular dystrophies, and in a benign pattern. The severe form of the disease is usually apparent soon after birth, involves the muscles of the pelvic girdle, and causes an early waddling gait. The muscular involvement later extends upward to affect the shoulder girdle and arms. It is principally characterized by an enlargement or pseudohypertrophy of the calf muscles and occasionally other muscle groups.

The disease is caused by an X-linked recessive gene and is expressed only in the male who, being XY, has no counter-acting normal gene. Disregarding the extremely unlikely possibilities of the disease appearing in a female carrier
because of absence of the "normal" X chromosome by chromosome deletion (X0 Turner's syndrome) or, excessive inactivation of the normal X chromosome under the provision of the Lyon Hypothesis, a female patient can have the disease only if she is conceived of a diseased man who has attained fertility and has contributed his mutant X-linked gene to the mutant gene of a carrier mother: a most improbable circumstance.

The sex-linked, recessive benign form of Duchenne muscular dystrophy begins later in life, and causes symmetrical weakness and atrophy of the muscles in the pelvic girdle, shoulders and upper arms. There is some pseudo-hypertrophy of the calves and often the deltoids (12). Patients affected by the benign type of dystrophy survive for many decades with only a slightly to moderately reduced normal longevity.

Bonsett (21) has tabulated the results obtained from the study of forty three male patients ranging in age from fifteen months to seventeen years. His results, reproduced in Figure 7, concern the clinical features characterizing the disease, such as posture, gait, foot appearance and ankle mobility, configuration of the calf, the "sliding-through" phenomenon, enlargement of the serratus anterior muscle, and differential wasting of the pectoralis major muscles. The chart presents the clinical manifestations as a continuum showing the development and relation of the different features to age and other data.
Duchenne (43) stressed the importance of deformity in both posture and gait as one of the diagnostic criteria of this disease. Some years later, Gowers (75) described the characteristic manner in which these patients stand up from squatting to erect position. The degree of lordosis found in these patients shows a considerable variation. Remarkably, the dystrophic patient maintains an erect posture during the initial years of life, and only with progression of the disease variable and increasing degrees of lordosis occur. Accentuated spinal deformities may occur during the years spent in the wheelchair.

Histories of dystrophic patients always disclose difficulty in climbing stairs and frequent falling when running, even before abnormalities in posture and gait are detected clinically. The deterioration in posture and gait occurs almost concurrently. The waddling gait of the patients is noticed at about four years of age and the ability to walk usually ceases between seven and eleven years (247).

Concurrent with the postural change is the initiation of the equinovarus deformity of the feet. Posterior displacement of the center of gravity of the body with progressive lordosis necessitates a compensatory shift of weight forward to the balls of the feet, this producing the equinus and varus deformity. The varus component becomes exaggerated later in life as a consequence of passive postural factors rather than of progression of the disease itself (21).
The enlargement and increased firmness of muscles occurs early and affects commonly the calf muscles. In some patients the enlargement has been detected also between the second and sixth years of life in the quadriceps, the gluteal and the lower paravertebral muscles. With the exception of the serratus anterior, enlargement of muscles in the upper half of the body is less consistent (21).

The muscle stretch reflexes do not become abnormal until after the fourth year of life. The superficial abdominal and the cremaster reflexes may be present even terminally.

Another diagnostic feature, noted by Meryon (152), is the impression that the child seems to slide through when being lifted and held under the arms. This phenomenon is usually manifested after the fourth year of life. When present, it indicates atrophy of the thoracic musculature.

B). Biochemistry.

Biochemical changes of muscle tissue, blood and urine have been reported over the years, and the results reviewed by Dreyfus and Schapira (40) who concluded that only two of these changes are of clinical value: the variations in the urinary creatine-creatinine excretion, and the alterations in serum enzyme levels. Hypocreatinuric and hypercreatinuria found in patients with muscular dystrophies were, until recent years considered by some to be the only consistent biochemical abnormalities, and variations in urinary creatine-creatinine excretion helpful
in distinguishing a myopathy from a disease of the nervous system. However, hypocreatininuria and hypercreatininuria may occur in any process of atrophy or destruction of muscle tissue (164), and thus, these changes are not specific for dystrophic muscles.

The results of reports on serum enzyme changes appeared promising as diagnostic criteria. The degree of elevation of serum enzyme levels is said to be proportional to the rate of muscle tissue destruction (242). Thus, the highest levels may be expected in Duchenne muscular dystrophy. In disorders such as ocular dystrophy, where the process of muscle destruction is extremely slow, the serum enzymes are usually normal. Of all the serum enzymes studied to date, the level of serum creatine phosphokinase (CPK) appears important because it is elevated disproportionately when compared to other enzyme levels in muscular dystrophy. The high levels of serum CPK in muscular dystrophies have proved valuable in differentiating these from disorders of the central or peripheral nervous system, as well as in detecting the carrier state in Duchenne dystrophy. The levels of other serum enzymes also have been found elevated: transaminase (both glutamic oxalacetic (GOT), and glutamic pyruvic (GPT)), aldolase, lactic dehydrogenase (LDH) and malic dehydrogenase (MDH) (236). The increased levels of these serum enzymes reinforces the diagnostic value of elevated serum CPK in dystrophic patients or in a carrier of the disease.
Abnormal serum enzyme activity in Duchenne dystrophy has been attributed to a defect of the muscle cell membrane. It has been postulated that there is an escape of enzymes into the blood serum from skeletal muscle and possibly from myocardium, liver and erythrocytes (121). If this concept be true one may expect that small molecules such as amino acids would leak into plasma and in turn cause a generalized aminoaciduria. The latter, however, was not detected in a recent study on the amino acids content of plasma and urine from patients suffering from Duchenne, myotonic, limb girdle and facioscapulohumeral types of muscular dystrophies (8). The absence of aminoaciduria does not necessarily exclude the possibility of a defect in the muscle cell membrane, considering the factors that regulate plasma amino acids concentration; the liver plays a major role in this control mechanism (68 and 142).

In the last decade attention has been focused on the metabolic energy pathways of affected muscle in the muscular dystrophies. There seems to be some indication of failure in glycogenolysis (210), or oxidative phosphorylation that is reduced in the late stages of the disease (175), and some abnormalities of coenzyme Q also have been suggested (58). Reported changes include depletion of glycogen, decreases in lactic acid and glycogenic and respiratory enzymes, an increase in proteolytic enzymes; and the suggested abnormality in the structure of myoglobin (253). The metabolism of lipids in muscle from dystrophic mouse
was recently studied; results indicate an enhancement of lipid synthesis and an impairment of fatty acid oxidation (135).

It has been assumed that an inborn metabolic defect is present in all muscular dystrophies. A whole host of biochemical alterations have been identified in the abnormal muscle fibers, and the interested reader is referred to the review of Perry (192) and Schapira, Dreyfus and Schapira (218). However, it may be possible that these changes represent secondary alterations of diseased muscle rather than having causal significance.

C). Tissue Changes.

Several changes are known to affect the muscle fiber and its environment in muscular dystrophies, but the steps involved in the sequence of these alterations have not been definitely established. Moreover, only few reports regarding morphological features of other organs have been published, and all efforts appear to be concentrated upon muscle; it may well be that muscle tissue in muscular dystrophies is only the target organ.

a). Historical Notes.

The concept of a group of primary dystrophic diseases of muscle and the main histologic features of this class emerged from the work of Erb (53). As indicated above, isolated cases of muscular dystrophy were described by
Bell (15), Coste and Gioja (34), Oppenheim (176) and Partridge (185), but the nature of the disease was not appreciated. Postmortem examination of two cases described by Meryon (152) showed the spinal cord and nerves to be morphologically normal but the muscles were the seat of a granular degeneration. Gross enlargement of the muscles was mentioned only incidentally. This condition aroused great interest in Germany and a further report appeared emphasizing the absence of any changes in the central nervous system and the presence of fatty tissue in muscles in a patient with pseudohypertrophic muscular dystrophy (56). At the same time, muscle biopsies confirmed the presence of abundant adipose tissue in large muscles (78). Biopsies were obtained with the Mideldorff's harpoon; the procedure was in those days particularly hazardous and the ethics of scientific curiosity prompting biopsies were strongly criticized (44). Duchenne obtained biopsies from muscle with the histological punch and examined the hypertrophic muscles sequentially at different stages of the disease. Duchenne sent portions of muscle specimens to Ordoñez (177), and devoted several pages of his monograph to the histopathological reports of Ordoñez. They both noted the increase of connective tissue and fat in the affected muscles and concluded that the disease affected primarily the interstitial muscular tissue, a concept recently reemphasized by other authors (22 and 29). Duchenne and Charcot had an opportunity to examine one of the patients with pseudo-
hypertrophic muscular paralysis at post mortem. Once again, the interstitial changes in muscles and the absence of changes in the nervous system were confirmed (30).

Electrocardiographic changes in patients with muscular dystrophy have been demonstrated recently (5), and the authors consider these changes to be pathognomonic for the Duchenne type of muscular dystrophy; however, there are not many morphological studies of cardiac muscle reported to date, and the changes observed to not appear to be specific of the disease (5 and 191a).

b). Muscle.

The gross appearance of the enlarged gastrocnemii muscles in patients with pseudohypertrophic muscular dystrophy is that of a fatty mass bearing little resemblance to muscle tissue. Other muscles are remarkably small and vary in color from yellow to pinkish-gray. This alteration of color depends on the relative amounts of fat and fibrous tissue that replace the muscle fibers.

The histologic features of dystrophic muscles as observed by light microscopy are reasonably distinctive when compared to other diseases of muscle. However, all forms of dystrophy included in the classification modified from Walton and Nattrass (247) and outlined in page 38 resemble each other at the microscopic level. The histological lesion in Duchenne muscular dystrophy consists of a progressive involvement of individual muscle fibers distributed at random within a given muscle bundle. The most characteristic feature
in its later stages is a progressive atrophy and disappearance of whole fibers, while other fibers are abnormally hypertrophic (53). Early in the disease, particularly in the pseudohypertrophic variety, the phenomenon of gross hypertrophy occurs; the underlying changes causing an apparent enlargement of muscle mass consist of an increase in fat and connective tissue content (186). Individual fibers show a variety of regressive alterations including hyalinization, focal vacuolation, fragmentation of the cytoplasm and shrinkage from the investing sarcolemmal sheath (17). The nuclei of the muscle fiber appear to be prominent and increased in number. This probably reflects the loss of cytoplasmic mass and, therefore, is only relative. These changes vary in severity depending upon the clinical stage of the disease, but it has been estimated that by the time there is clinical evidence of deranged function already more than half of the fibers of the muscle are abnormal (189). The evolution of the pathologic process is not very fast, hence the suggestion that there is a preclinical phase in Duchenne muscular dystrophy. The changes observed in muscle during this preclinical stage consist of a moderate variation in diameter of fibers within the same muscle bundle, a uniform acidophilic hyaline alteration in many fibers, and the presence of basophilic regenerating fibers (188). Histological changes have been also observed in female patients who are carriers of the gene for the Duchenne dystrophy. These changes included hypertrophic fibers with hyaline degeneration (107), focal necrosis and phagocytosis of
muscle fibers, formation of nuclear chains, and abortive regenerative activity (187).

Electron microscopic studies of muscles with pseudo-hypertrophic muscular dystrophy have shown that all the cellular elements are affected in the advanced stages of the disease. The ultrastructural changes reported to date have been variable and non-specific. They include the demonstration of focal myofibrillar alterations in the early stages of the disease with associated loss of mitochondria and smudging of Z-line (148). Zones of sarcoplasm containing only disorganized filaments and altered mitochondria have been described often (129). Occasionally, muscle fibers show splitting (113); the significance of this phenomenon is not well understood, and some authors believe that it represents merely a normal reaction of the fiber to injury (2). The sarcomeres are shortened and show an increased osmiophilia (113). Necrosis of the muscle cell is manifested by supercontracted, indistinct sarcomeres with dissolution of the component myofilaments. The necrotic process is believed to commence in the subsarcolemmal region (186), or in more central areas (180). Although there seems to be some biochemical evidence of membrane damage, no alterations have been observed in the sarcolemma (62) with exception of one report on the advanced stages of the dystrophic process (154). Other studies in advanced stages of the disease did not disclose any changes in the sarcolemma or nuclei (217). The nuclei of muscle fibers have been reported to be normal; in several
studies they were observed to be located centrally, forming a row, or a chain. The possible significance of this phenomenon is not clear; it may reflect only a degenerative process, as with the disappearance of the myofilaments the nuclei assume a more central position. Dilatation and vacuolation of the sarcoplasmic reticulum was considered to be the earliest alteration of the muscle fiber in muscular dystrophy (24); subsequent reports, however, have offered evidence that the sarcoplasmic reticulum becomes swollen only in advanced stages of degeneration (217) and may be secondary to mitochondrial (62), or myofibrillar (154) changes. The reports on the status of mitochondria and myofilaments vary considerably (24 and 27). Among other changes ring fibers also have been observed in dystrophic muscles (88 and 181). They are accepted at present as true structures and not as artifacts of fixation or of biopsy procedure. Ring fibers or the Ringbinden of the German school, consist of a peripheral subsarcolemmal myofibrillar band encircling a central core of longitudinal myofibrils.

It is known that following injury normal muscle regenerates (196); the morphogenesis of regeneration is still under discussion (206). Particular attention has been paid lately to the repair mechanisms of muscle in muscular dystrophy. It has been postulated that the regenerative component of the lesion is intrinsically abnormal (148), as abnormally formed myofibrils associated with ribosomal increase were observed by electron microscopy. Alterations
of growth and differentiation of muscle cells were suggested on the basis of tissue culture studies of dystrophic muscle as compared with normal controls (112). The changes described included retarded development of myotubes and the presence of bizarre cell forms. Preliminary ultrastructural studies in carriers of Duchenne muscular dystrophy (116) showed features resembling those reported in patients suffering from the disease, including local changes of the Z-lines and myofilaments.

Many disorders of muscle have been described in animals, but there are only two that may prove valuable as models for the study of muscular dystrophies in man. These are the house-mouse strain 129 with muscular dystrophy (153) and the Rottnest quokka, Setonix brachyurus, with vitamin E responsive myopathy (111). Similarly the study of induced muscle lesions has provided useful information (196).

c). Nervous System.

The integrity of the nervous system plays a role in the normal development and maturation of muscle fibers. To date, there is no direct evidence of a relation between the functions of the nervous system and muscle tissue in muscular dystrophies. However, there are a few scattered and perhaps unrelated reports that are worth mentioning. Several cases of Duchenne muscular dystrophy were examined at autopsy; no consistent pathological changes were found in the nervous system (42), and the occasional accompanying
mental retardation was considered to be incidental. On the other hand, abnormalities of cerebral development, microscopic heterotopia and pachygyria, and the observation that one third of the patients with Duchenne muscular dystrophy are mentally subnormal, had been stressed by Rosman and Kakulas (211). The same authors suggested subsequently a close parallelism between cerebral defects and myopathic changes and proposed that both were genetically determined alterations that appear early in gestational life (212). Furthermore, it has been found that several children with evidence of brain dysfunction developed a predominance of white or fast muscle fibers (60). However, the metabolism of muscle in muscular dystrophy resembles that of red muscle, suggesting to some investigators that in dystrophic muscle there may be a regression of metabolism to that of the slow or less mature muscles (80). Finally, some investigators are of the firm belief that the dystrophic process results from a chronic dysfunction of motoneurons (165). Particular attention is being paid at present to the poorly understood influence of trophic factors of peripheral nervous system upon muscle tissue. The trophic effect of the neuron is considered as a biological control system in which closed circuit endocrine factors are being transported along axons and across junctions to a target organ. Whereas there are probably many trophic factors serving different functions, the most explored have been the trophic relations of the neurons to their axons and to muscle (109). It is known that the
type of a given muscle may be changed by "switching" its innervation with that of a different muscle type; moreover, in this instance the change occurs without functioning of morphologically demonstrable synaptic contacts between the implanted nerve and the muscle (61). Furthermore, the atrophic process following denervation of muscle may be retarded, apparently, by the injection of trophic brain proteolipids (216). A possibly fundamental experiment providing evidence on the role of neurotrophic factors in dystrophic muscle has been reported recently (215). Thus, minced dystrophic muscle transplanted into regenerating muscles of normal mice was capable of developing tension approximately equivalent to that of normal regenerated muscle; alternatively, normal minced muscle transplanted into dystrophic mice showed no functional regeneration.

It is tempting to correlate these experimental findings with observations concerning a possible primary abnormality of the nerve supply to dystrophic muscle of man as suggested recently (120, 165 and 167). However, it still remains a debatable issue whether any of the experimental conditions may be equated to one or more forms of human muscular dystrophy.
III. ORIGINAL OBSERVATIONS.

1. The Patients.

A group of seven patients was selected for the present study. The two first unrelated male patients have a disease with features resembling those of Duchenne muscular dystrophy. The other five patients suffer from the classical, sex-linked recessive, Duchenne muscular dystrophy, and belong in two different sibships. Pertinent clinical data of patients are summarized in Table I on page 55.

Patient 1., D.G., is a white male child born in 1964. The parents and a three year old sister are healthy. There is no known occurrence of muscular dystrophy in the family with the possible exception of a paternal aunt who may have been dystrophic. The patient was first examined at the age of four years. At that time he was slow to walk, had some difficulty in climbing stairs, and was tired early in the evening. He has had several colds but none of the childhood illnesses. The patient has been examined again at six years of age. He used his hands and arms on his legs as support in erecting himself from the squatting position, and when lifted his shoulders rose in the typical
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fashion of the dystrophic child. The muscles of the lower extremities were weak with the exception of the gastrocnemius muscles which were strong and had a pseudohypertrophic appearance. The volume of the other muscles appeared normal. The condition of the patient showed only very slight deterioration during the two years between examinations.

Patient 2., R.G., is a white boy born in 1963. The parents, one sister and one brother are living and not affected by muscular dystrophy. There is no known history of muscle diseases in this family. The patient was examined first at the age of five years. At that time, it became evident that he was walking late, had some difficulties climbing stairs, and stumbled. In 1969, at the age of six years, he was operated for lengthening of the left Achilles tendon. He was reexamined at seven years of age, and at that time he showed the typical Gower's sign. When the patient was lifted his shoulders rose indicating muscle weakness; however, the shoulder girdle had a normal appearance. The muscles of the lower extremities were weak with the exception of the gastrocnemii which were both strong. A pseudohypertrophic appearance was more evident in the right than in the left calf. The child was of normal intelligence. The course of the disease in this patient has been only slowly progressive during the last three years of this study.
Patient 3., D.W., is a white male child born in 1959. The parents and two sisters are healthy; however, there is evidence of muscular dystrophy in this family. A maternal uncle of the patient died at seventeen years of age of muscular dystrophy. The patient is related through a maternal aunt to the next two patients, T.S. and R.S. He sat and walked at the normal age and was not known to have had any serious illness; however, it was noticed at the age of three years that he started having difficulties in rising from the floor. The patient was examined initially at the age of nine years. It was evident at that time that he had difficulties climbing stairs and that his arms appeared thin. He was reexamined at the age of eleven years; at that time he walked with the typical waddling gait of the dystrophic patients, and required the Gower's manoeuvre to erect himself. When lifted under the arms his shoulders rose demonstrating weakness of the shoulder girdle. The pseudohypertrophic appearance of the calf muscles was evident. The patient appeared to be of normal intelligence. The disease has a moderately progressive course.

Patient 4., T.S., is a white boy born in 1959. This is a brother of the next patient (R.S.) and a maternal cousin of D.W. (patient 3); a maternal uncle died of muscular dystrophy. The remaining members of the family, i.e., parents, two brothers and two sisters, are not affected by the disease. The patient developed normally until the age of two years. He
was examined first at the age of eleven years. He showed some facial weakness, could not raise his arms above the head and had profound weakness of the shoulder and pelvic girdles. On the other hand, he still maintained good strength of the gastrocnemius muscles which were pseudo-hypertrophic. The patient has been confined to a wheelchair for the last two years. He appeared to be of normal intelligence. The course of the disease is progressive.

Patient 5., R.S., is a white boy born in 1957. He is related to the two preceding patients; he is the brother of T.S. and a maternal cousin of D.W. The patient has been affected with progressive weakness since the age of three years. He could not run well and fell often. At the time of examination, at thirteen years of age, the patient was weak and obese. The weakness of the orbicularis oculi was the only myopathic characteristic of his face. The shoulder and pelvic girdles were profoundly weak. He could not raise his arms above the head. The gastrocnemius muscles seemed to be quite strong. The patient appeared to be of normal intelligence. The disease in this patient has a progressive course.

Patient 6., M.D., is a white boy born in 1959. He is a brother of the next patient (P.D.). The remaining members of the family, i.e., parents, two sisters and one brother, are not affected by the disease. The early development
of the patient was apparently normal until the age of three years. Since that time physical and mental deterioration have been progressive. At the time of study the patient was eleven years old and the only evidence of a myopathic facies was some weakness of the orbicularis oculi. He was not able to raise his arms above the head. The shoulder and pelvic girdles were very weak. The gastrocnemius muscles were strong and had a pseudohypertrophic appearance. The patient is mentally retarded. The disease has a progressive course.

Patient 7., P.D., is a white male child born in 1958. He is a younger brother of the patient described above (M.D.). The muscular weakness in this patient was apparently noticed since birth. At the age of eight years he was not able to stand or walk and was using a wheelchair. He was examined at the age of twelve years and was found to have extremely weak shoulder and pelvic girdles. The gastrocnemius muscles were quite strong and had a pseudohypertrophic appearance. The intellectual development of this patient has been always slow.

The facts that there were no known histories of muscle disease in the families of patients 1 and 2 (D.G. and R.G.), that both were very young patients, that during the three years of study the disease in both patients was only slowly-, or almost non-progressive, and that all the other clinical
signs and symptoms, appeared to be of lesser magnitude than those of five other patients, suggested that clinically these two patients do not suffer from either the severe or benign form of the classical, sex-linked recessive, Duchenne muscular dystrophy (100). Moreover, biochemical data also support the clinically observed differences between the two groups of patients. Thus, the oxidation of fatty acids, particularly palmitic acid, by mitochondria obtained from muscle tissues of the two patients was within normal range, whereas the oxidation in the group of the other five patients with the classical sex-linked recessive Duchenne disease was reduced (136).

From here on patients 1 and 2 will be referred to as having an atypical or pseudo-Duchenne muscular dystrophy, and patients 3 to 7 will be referred to as having the typical or classical Duchenne muscular dystrophy.


The material utilized for this study consisted of specimens of tissue obtained at biopsy of vastus lateralis and gastrocnemius muscles from patients affected with the typical muscular dystrophy (patients 3 to 7) and two patients with some features of the disease at variance with the typical Duchenne dystrophy (patients 1 and 2), all admitted to Victoria Hospital, London, Ontario, for investigation. The specimens of muscle tissue were
obtained by employing special isometric clamps (Figure 8). Portions of the removed muscle were fixed in 6.25 per cent glutaraldehyde for electron microscopy processing, and in 10 per cent formalin for light microscopy. Additional unfixed tissue was immediately deep-frozen for isolation of mitochondria and study of fatty acid oxidation (the results of this biochemical study are not the subject of this thesis and were reported elsewhere (136)).

Tissues for light microscopy were processed in tetrahydrofuran (86), and embedded in paraffin for cross and longitudinal sectioning. They were cut at three micra and stained with hemalum-phloxine-saffron (HPS), Masson's trichrome, periodic acid Schiff (PAS), and phosphotungstic acid hematoxylin (PTAH) stains.

Tissues for electron microscopy were fixed for 90 minutes at 4°C in 6.25 per cent buffered glutaraldehyde (214). Following washing in 0.1 M phosphate buffer (pH 7.4), small pieces of tissues (1 to 2 cubic millimeters) were postfixed for 90 minutes at 4°C in a solution of 1 per cent osmium tetroxide in Veronal buffer (pH 7.4) containing 0.045 grams of sucrose per milliliter (28), and for an additional 30 minutes at room temperature. Dehydration of tissues was commenced in 50 per cent ethanol and completed in four successive changes of ascending concentrations of ethanol. Tissues were embedded in Epon-812 (139), the ratio of dodecenyl succinic anhydride to nadic methyl anhydride being 6 to 4, or 5 to 5. Cross and longitudinal
orientation of the tissues in embedding medium was achieved by inserting the specimens in gelatin ring-tubes, according to the method described elsewhere (178). To facilitate penetration and avoid air trapping in tissues, all the blocks were kept for 10 to 12 hours in a vacuum chamber prior to polymerization. The blocks were cut with glass or diamond knives on MT-2 Porter-Blum or Reichert ultramicrotomes. Thick sections were cut at 1 micron, stained with toluidine blue (240), paraphenylenediamine (55), and basic fuchsin-methylene blue stains (98), and studied by light microscopy. Thin sections were picked up on formvar coated or uncoated 100-, and 300-mesh copper grids, stained with uranyl acetate (249) and lead citrate (205), and examined with Philips EM-200, or EM-300 electron microscopes operated at accelerating voltages of 60 Kv, 80 K v or 100 Kv.

For control purposes tissues from gastrocnemius and vastus lateralis obtained at surgical procedures from three male patients approximately matched for age and not known to have metabolic diseases, myopathies or neuropathies, were processed and examined in identical fashion.

Fifteen blocks of tissue embedded for electron microscopy, were utilized from each of the twenty muscle biopsies in the present study (two muscle biopsies from each of the seven patients and three controls); thus, a total of approximately three hundred blocks of tissue were studied during a two year period.
3. Results.

Both groups of patients, the classical and the atypical Duchenne types show some muscle changes that are similar to those found in most conditions associated with muscle degeneration (189). Other morphological alterations observed seemed of more specific nature, but occurred in both groups of patients. However, some of these changes were more common in one, and other alterations in the second group of patients; whenever possible quantitative differences will be indicated.

A). Gross Examination.

At the time of biopsy, the appearance of muscle tissue in the two patients with atypical Duchenne dystrophy was not strikingly changed. It was normal in colour or had a more reddish tinge, and contracted upon stimulation. On sectioning, the tissue seemed to be slightly more fibrous in texture than normal muscle. On the other hand, muscles of patients with the typical Duchenne dystrophy contracted poorly or not at all, appeared pale and were definitely fibrous in texture.

B). Light Microscopic Examination.

A characteristic change of muscle tissue from the two
patients with atypical disease (patients 1 and 2) is the feature of "subsarcolemmal bumps" (Figure 9). In longitudinal sections, the bumps appear to be composed of a sarcoplasmic mass located in a subsarcolemmal position and projecting above the level of the fiber. Sections from plastic embedded material stained with toluidine blue show the homogenous quality of the bumps, and the apparent close proximity of these subsarcolemmal bumps to small vessels. Often, nuclei are observed in the bumps, but no changes are present in the immediately subjacent sarcomeres (Figure 9). However, localized changes of the sarcomeres are apparent in adjacent muscle fibers (Figure 10). They consist of stretched sarcomeres (Figure 9). However, localized changes of the sarcomeres are apparent in adjacent muscle fibers (Figure 10). They consist of stretched sarcomeres accompanied by derangement in the structure of the bands. Central nuclei at times are associated with the alterations of sarcomeres. Other muscle fibers have a normal architecture (Figure 10).

Cross sections of similar fields from muscles of patients with the pseudo-Duchenne dystrophy show different size of the muscle fibers (Figure 11). A clear, peripheral zone is often observed around muscle fibers of small diameter. Increased connective tissue filling the spaces between the muscle fibers is demonstrated best in the trichrome stained sections (Figure 11). Hyalinized fibers are observed either isolated (Figure 12) or in groups (Figure 13). They
have central nuclei, sarcoplasmic vacuoles and are closely related to hypertrophic fibers.

Other deposits of homogenous material are at times present in a subsarcolemmal position in the muscles of the atypical patients. They are strongly PAS-positive (Figure 14) and stain dark blue with toluidine blue (Figure 15).

A peculiar spiral arrangement of the myofibrils is observed in the muscle fibers (Figures 16 - 19), being much more common in patients with the atypical than classical Duchenne disease. On cross section the "spiral fibers" are arranged concentrically or spirally around the longitudinal axis of the fiber. This organization of the myofibrils is well demonstrated with the PTAH stain (Figure 17) and gives a positive PAS reaction (Figure 18). Thinner sections stained with toluidine blue show the elongated appearance of the nuclei, following the spiral pattern and with an unusual orientation with respect to the sarcoplasm; the nuclei in the spiral fibers are perpendicular to the longitudinal axis of the muscle fiber (Figure 19).

Low power microscopy of muscle tissue from the group of patients affected by typical Duchenne muscular dystrophy shows almost complete disappearance of myofibers from the muscle bundles. It seems that within a given bundle the first fibers affected are those situated centrally; and the few, small remaining fibers are all located at the periphery of the bundles (Figure 20). Similar tissue sections stained with HPS show well the degree of replacement
of myofibers by connective tissue (Figure 21). The size of individual myofibers varies considerably; some hypertrophic fibers contain masses of dark and granular material, other fibers are vacuolated and show several central nuclei (Figure 22). Muscle fibers of normal size show at times subsarcolemmal accumulations of a pale homogenous material of a non-descript nature (Figure 23).

In addition, muscles from the patients with the typical Duchenne dystrophy show localized areas of deranged sarcomeres often present in association with dark homogeneous masses of irregular contour and resembling the Z-line substance. They are observed either in central (Figure 24) or peripheral position (Figure 25). The dark masses extend over several sarcomeres, and those found at the periphery of the fiber are associated with outward protrusions of the sarcoplasm (Figure 25). A similar disorganization of myofibrillar structure is observed in the subsarcolemmal region of otherwise normal muscle fibers (Figure 26). Here disorganization is not focal but rather extending along the sarcolemma over large areas, encircling normally arranged sarcomeres in the interior of the fiber (Figure 26). Other alterations of the fibrils consist of hyalinization, which is localized to a few sarcomeres or extends throughout the muscle fiber, and of subsarcolemmal deposits of a PAS-positive material (Figure 27). The disorganization of the myofibrils results at times in curious patterns resembling those of the ring fibers (Figure 28).
Cross sections of muscle tissue from typical Duchenne muscular dystrophy show various and different patterns of myofibrillar derangement; myofibrils are oriented in a perpendicular fashion to the longitudinal axis of the fibers and in others, stained with PTAH or PAS, features resembling those of the spiral fibers are present (Figures 29 - 31). Fibers with a bizarre shape, also resembling the spiral fibers, are observed occasionally. Cross sections stained with PTAH and HPS, show the haphazardous arrangement of the myofibrils (Figure 32) and the complex pattern of distribution of the sarcoplasmic nuclei (Figure 33). Finally, fibers with a peripheral subsarcolemmal ring of myofibrils forming sarcomeres which are surrounding a central core of well organized myofibrils (Figure 34) are often found in the muscles of the patients with the classical Duchenne muscular dystrophy; because of their appearance these fibers are named "ring fibers".

C). Electron Microscopic Examination.

a). Normal Muscle.

In the normal striated muscle controls the M-line is situated in the center, in the middle of the A-band and the H-zone; it is resolved into filaments that will be referred to as M-filaments. The M-filaments are perpendicular to the longitudinal axis of the sarcomere and, therefore, parallel to the Z-lines; they seem to be in continuation with similar filaments of the adjacent myofibrils
(Figure 35). Their close relation to the myosin filaments becomes evident under higher magnification (Figures 36 and 37). The M-filaments are more osmiophilic than the actin filaments and of about the same, 50 to 60 Å, diameter. A dark, homogenous substance fills the network formed at the M-line by the myosin and M-filaments.

Deep invaginations of the plasma membrane into the muscle fiber are observed at times (Figures 38 - 42). They closely resemble similar structures described by Franzini-Armstrong and Porter (65) in muscle tissue of certain fishes. The invaginations observed show at least two variations in distribution within the subsarcolemmal space. Those of the first type (Figures 38 - 40) penetrate straight into the subsarcolemmal space and appear to branch out into different planes (Figure 40). The invaginations of the second type (Figures 41 and 42), also within the subsarcolemmal space, follow a complex pattern; occasionally, their association with the specialized terminal cisternae of the sarcoplasmic reticulum is noted (Figure 42).

Satellite cells are observed in their characteristic position beneath the basement membrane of the sarcolemmal complex (Figures 43 - 48). There appear to be two different types of satellite cells (Figures 43 and 45), the difference consisting largely in the degree of density of their cytoplasm. One type of satellite cell (Figure 43) corresponds to the cells described by Mauro (149); it is small in size and has a clear and scanty cytoplasm with few
organelles, pinocytotic vesicles, and a nucleolus-free nucleus. The second type of satellite cells (Figure 45) has a more electron dense cytoplasm. It seems to be filled with a dark and homogenous substance of finely fibrillar or finely granular nature. There are no myelin figures or other features of degeneration in the dark satellite cells or in the adjacent sarcoplasm. Filaments are present in the cytoplasm of some satellite cells (Figure 46). They are arranged in bundles with a random orientation and closely related to free ribosomes or to elements of the rough endoplasmic reticulum. Figures suggestive of cell division are observed occasionally in the satellite cells of the first or clear type (Figure 43). Cilia are recognized in close vicinity to the satellite cells beneath the basement membrane; high magnification discloses the microtubular doublets and the dense linear "stalk" connecting them to the ciliary membrane (Figure 44). Occasionally, centrioles are present in a perinuclear zone in the cytoplasm of the satellite cells (Figures 47 and 48).

b). Regenerating Muscle.

Cells with all the typical features of active myoblasts are observed in groups (Figure 49), or singly (Figure 50); they are usually related to vessels, and are in close vicinity to atrophying muscle fibers. Myoblasts are found more often in the atypical than in the classical cases of Duchenne muscular dystrophy. In a given group, they appear to be
at different stages of differentiation (Figure 49). Thus, the content of myofilaments and other organelles varies from cell to cell; cells with a clear cytoplasm containing only a few filaments are in immediate vicinity of cells that are almost filled with myofilaments. No well defined sarcomeres are observed in the very young myoblasts. Thin filaments, structures resembling triads, and mitochondria are the organelles seen earliest in the clear myoblasts. Accumulations of a dark substance resembling the Z-line material are observed in all myoblasts and young myocytes; they are particularly evident in the early stage of the myoblast, before any organized sarcomere may be detected (Figure 50). The dark substance appears closely apposed to the inner surface of the plasma membrane; bundles of thin filaments radiating into the cytoplasm are attached to this dark substance on the opposite side, facing the cytoplasm. Mitochondria of various sizes, primitive triads and pinocytotic vesicles are observed also in the cytoplasm of the myoblasts.

Cells with features of mature myotubes in regeneration are often observed in patients with the classical or typical Duchenne muscular dystrophy in muscle considered to represent advanced stages of regeneration (Figures 51 - 59). Zones containing a finely granular material, ribosomes and myofilaments are noted in a subsarcolemmal position (Figure 52). In other areas, often perinuclear in location, a peculiar arrangement of the myofilaments is observed (Figure 53). Myofilaments forming incomplete sarcomeres are organized in a fashion radiating from a common center; this center
consists of an osmiophilic substance similar to the Z-line material. The sarcoplasm in other cells contains compact masses of sarcomeric components (Figure 54). Under high power these "sarcoplasmic masses" are shown to be composed of actin and myosin filaments and Z-line material, but neither triadic nor other sarcotubular elements are discerned. However, an increased number of triads are observed in the area immediately surrounding the sarcoplasmic masses. There are also variations in the haphazard formation of sarcoplasmic accumulations of compact sarcomeric components (Figures 55 - 59). Thus, a central core, apparently composed of Z-line material, may be surrounded by a lighter halo consisting of thin filaments and of granular material that is less dense than the central core. An outer zone composed of deranged sarcomeres is always present (Figure 55). As sarcomeres from adjacent areas become involved, disorganization of the sarcotubular system follows (Figure 56). The central core of the sarcoplasmic masses is seen at times as a dark and compact structure; it has also a lighter halo and an outer zone of haphazardly arranged sarcomeres (Figure 57). Occasionally, a satellite cell is present beneath the basement membrane of the fibers containing these sarcoplasmic masses (Figure 57). At some stages the sarcoplasmic masses show certain similarities to the cytoplasmic bodies described by King Engel (118). Figures interpreted as further stages of development of the sarcoplasmic masses show a central
core of granular nature surrounded by a dark mid-zone and an outer area formed by radiating filaments (Figure 58). Finally, similar masses are found composed largely of a very osmiophilic material with a central core of a granular substance having a crystalline appearance (Figure 59).

c). Degenerating Muscle.

Regenerative features often coexist with degenerative processes. Three different types of changes of muscle fiber are found that are considered degenerative in nature but do not appear related to each other (Figures 60 - 62). In the first type, fibrils seem fragmented without any apparent pattern and some fibrils extend only over a few sarcomeres. The different bands and lines have a normal appearance. Adjacent areas within the same fiber show small and scattered fibrillar fragments less than a sarcomere in length. The interfibrillar space is occupied by a granular material morphologically consistent with glycogen; the few mitochondria present are small and the sarcotubular system is difficult to identify. The sarcolemma of such fibers has a normal appearance.

In the second type, fibrils also seem fragmented but the sarcomeres are tightly contracted. The density of the Z-line contrasts with the absence of the I-band and there is some difficulty in recognizing actin filaments. Longitudinal splitting of the myofibrils is often observed. The sarcoplasm in this second type of degeneration consists
of a finely granular and filamentous material; the mitochondria are small and their matrix is dense. The sarcolemma has a crenated appearance as might be expected from the supercontracted state of the myofibrils (Figure 61). These two types of degeneration of muscle tissue (Figures 60 and 61) are observed as isolated instances among regenerating or normal myofibers. They are found more often in the patients with atypical than in typical Duchenne muscular dystrophy.

The third type of degeneration of muscle fibers (Figures 62 - 69) is found more commonly in the typical than atypical Duchenne muscular dystrophy. Such fibers are observed at times in the neighbourhood of apparently normal fibers and those in the process of regeneration (Figure 62). The plasma membrane component of the sarcolemma in these fibers is not always easily identifiable; however, the basement membrane is visible in all instances. This type of myofibrillar degeneration appears to affect the fiber either in a generalized fashion (Figure 63), or progressively through zones that appear to be in different stages of degeneration (Figure 64). The sarcomeres are stretched, i.e., the distance between the Z-lines in a given sarcomere is increased. The Z-lines are thin and irregular having sharp peaks; they are often out of alignment and synchronism with Z-lines of adjacent myofibers (Figure 65). Osmiophilic masses are observed in some fibers; they consist of closely packed myofilaments, mitochondria and other
organelles (Figure 66). Pools of a finely granular material and remnants of cellular organelles are embedded within the osmiophilic masses without any membrane separating the latter from the former (Figure 66). Mitochondria in fibers with the third type of degeneration vary; they may have a clear and swollen matrix (Figure 63), or they may be small, having a dark matrix and disintegrating cristae (Figure 67). Nuclei and remnants of other sarcoplasmic organelles may be observed surrounded by a dispersed and finely granular substance (Figure 67). Occasionally, laminated structures of uncertain origin are seen in myotubes limited only by the basement membrane (Figures 68 and 69).

A close comparative study of degenerating and necrotic myocells in controls (Figure 70) and patients (Figure 71) does not disclose significant differences.

Vacuolation of the myofibers is not a prominent finding, and encountered more often in the muscles of patients with the typical muscular dystrophy (Figures 72 and 73). In these patients the vacuoles are observed in any location within the muscle fibers, and are either subsarcolemmal (Figure 72) or more central in position (Figures 22 and 73). In general, they are round to oval, of varying size and filled with a fine granular or hyaline material. Vacuoles are always membrane-bound. Cellular debris is found in the interior of the vacuoles. The vacuolation found in the muscles of patients with the atypical type of muscular
dystrophy is localized largely to the subsarcolemmal and perinuclear area (Figures 74 and 75). Occasionally, vacuoles are seen to be formed from or within distensions of the outer membrane of the nuclear envelope (Figure 75). The swelling occurring between the nuclear membrane apparently causes fragmentation and separation of the nuclear pores. A membranous debris of uncertain origin (Figure 75), or a finely granular material (Figure 74) are observed at times in these vacuoles. Intracellular fluid is present in association with the vacuoles only occasionally, but a moderate swelling of the T-system and sarcoplasmic reticulum accompanies the presence of vacuoles quite often.

The subsarcolemmal accumulations of dark homogeneous substance observed by light microscopy (Figures 14 and 15) appear to correspond to masses of osmiophilic granular material morphologically consistent with glycogen granules (Figure 76). Another finding consists of a membranous or crystalloidal structure attached to the inner surface of the plasma membrane (Figure 76). It is composed of alternating layers of membranes and small granules.

Streaming of the Z-lines is a common feature in the muscle of patients with the typical Duchenne dystrophy but is observed only rarely in the atypical group. The alteration of Z-line structure may be focal (Figure 77), more extensive, or even generalized (Figure 78). In the focal form (Figure 77), interpreted as an early lesion, the Z-lines are widened and irregular, spreading into the adjacent I-, and A-bands. As the process advances, an
entire sarcomere may be affected and its filaments obscured by the dense material. It appears that the larger lesions result from the extension and coalescence of smaller adjacent lesions (Figure 78). The dense material originates probably from the Z-lines and obscures the double array of thick and thin filaments (Figure 79). The thick filaments are no longer recognizable in some portions of the lesion, although thin filaments may be still present and intermingled with the dense Z-line substance. Other sarcoplasmic organelles, such as mitochondria and elements of the T-system, and glycogen, are found in the immediate vicinity of zones undergoing myofibrillary degeneration (Figure 79); however, all organelles appear displaced and decreased in number.

Various changes are observed in mitochondria and these concern their size and contents (Figures 80 - 86). Occasionally, muscle fibers of small diameter show thin and elongated mitochondria; they appear to have one or two cristae arranged centrally, and very osmiophilic matrix (Figure 80). Mitochondria found between overstretched myofibrils are often swollen, lack identifiable matrix and their cristae are thin and distorted (Figure 81). Crystalloid structures, possibly originating in mitochondria, are seen in atrophic muscle cells showing also changes of necrosis (Figure 82). Muscle fibers of normal appearance may contain mitochondria with changes of either matrix or cristae (Figures 83 - 86). Mitochondria that
seem to be dividing are observed in the perinuclear area; their matrix is dense and contains small, osmiophilic round bodies (Figure 83). Other mitochondria contain myelin figures (Figures 84 and 85) and dark cristae are arranged in a parallel fashion (Figure 85). Finally, some mitochondria are increased in size and contain numerous cristae (Figure 86); the cristae are parallel, closely packed and embedded in a matrix occasionally containing small osmiophilic bodies.

d). Other Observations.

In addition to the above electron microscopic alterations other changes were observed; these were mentioned in the Section on Light Microscopy as subsarcolemmal bumps (Figure 9), spiral fibers (Figure 19) and ring fibers (Figure 34). The ultrastructure of these three features will be described here rather than in the Sections on regeneration or degeneration, because some of these changes may represent one, the other or both processes. Moreover, these changes may represent different stages or expressions of processes that may be characteristic of one or both groups of muscular dystrophy under discussion.

The subsarcolemmal bumps (Figure 9) show by electron microscopy their perinuclear position clearly. In areas of bumps the nucleus is surrounded by an accumulation of mitochondria and the bumps are formed by disorganized myofilaments coursing in all directions. Occasionally,
satellite cells are observed in close association with the periphery of the bumps (Figure 87). Higher magnification of the subsarcolemmal bumps shows that the myofilaments within these are forming primitive sarcomeres; thin actin filaments are associated with blebs of Z-line material; elements of the T-system and sarcoplasmic reticulum are present between the early sarcomeres (Figure 88). In addition, early forms of subsarcolemmal bumps are repeatedly found in perinuclear positions in seemingly unaltered muscle fibers (Figures 89 - 92). They consist largely of thin actin filaments, mitochondria, polyribosomes and glycogen (Figure 89). Forms that are interpreted as more advanced bumps consist of primitive sarcomeres with no apparent relation to the adjacent sarcomeres within the same muscle fiber (Figure 90). Higher magnification shows them to be composed of Z-line material, actin and myosin filaments, triadic elements, glycogen and polyribosomes (Figure 91). The periphery of these forms of subsarcolemmal bumps contain mitochondria and ribosomes associated with filaments; they are embedded in a pool of dark granules morphologically consistent with glycogen (Figure 92).

Electron microscopy of the spiral fibers described by light microscopy in patients with the atypical Duchenne muscular dystrophy (Figure 19) indicates that they may be "initiated" by local activation, division and migration of the satellite cells beneath the basement membrane of the
muscle fiber (Figures 93 - 96). Satellite cells are observed in close association with cells that contain in their cytoplasm myofilaments and primitive sarcomeres (Figure 93). The latter cells are interpreted as daughter cells resulting from division of the satellite cells. The daughter and the satellite cells are enclosed by the same basement membrane of the muscle fiber (Figure 94). The three different cells: muscle cell, satellite cell and its daughter cell, each shows clearly its own plasma membrane (Figure 95). The plasma membranes separating the muscle cell and the adjacent daughter cell at times appear discontinuous, suggesting stages of fusion of the cytoplasm of the two cells. In such instances, glycogen granules occupy the areas of interruption extending on both sides of the fragmented cellular membranes into the bodies of both cells (Figure 96). The spiral arrangement of these fibers is evident at low magnification by electron microscopy; degeneration of sarcoplasm is observed simultaneously in some areas located centrally. Elements of the T-system, sarcoplasmic reticulum and other organelles are present but arranged in a haphazardous fashion between the sarcomeres.

Ring fibers are seen often in the patients of the typical Duchenne muscular dystrophy (Figure 34). Electron microscopy shows that a central core of apparently normal myofibrils is surrounded by an outer band of muscle fibrils oriented in a ring fashion perpendicularly to the central core (Figure 97). High magnification of the zone
between the central and ring parts shows that the two are not separated by membranes or other structures (Figure 98). No alterations are found in the T-system or sarcoplasmic reticulum in the ring fibers. However, careful search of the subsarcolemmal portion of the ring fibers discloses features consistent with regenerative changes (Figure 99). These consist of accumulations of mitochondria, elements of the T-system, Z-line substance, disorganized filaments, ribosomes and glycogen. The characteristic invaginations of the sarcolemma suggesting the vicinity of a neuromuscular junction are observed at times in the ring fibers, and satellite and Schwann cells are associated with these junc-tional areas (Figure 100). The Schwann cell of the junc-tional complex (Figure 101) is recognized by the presence of a basement membrane.

Muscle fibers occasionally contain two groups of myofibrils oriented in "reverse" to those of ring fibers (Figure 102), i.e., the peripheral myofibrils are arranged in a longitudinal fashion along the axis of the muscle fiber, whereas those in center are oriented in a transverse plane with respect to the long axis of the fiber. However, the arrangement in these fibers was never as orderly and the separation into the two parts as clear-cut as was that in the typical ring fibers.
IV. DISCUSSION

In 1868 Duchenne (44) described a muscular disorder that he named pseudohypertrophic muscular paralysis and that became known later as Duchenne muscular dystrophy. It is interesting to note that Duchenne did not recognize the hereditary nature of the disease, although he compared his findings with those of Wernich (252) and Heller (91) in young male siblings affected by a progressive hypertrophic muscular disease. Today, Duchenne muscular dystrophy has been recognized as being transmitted by a sex-linked recessive gene (248), and most of the cases described by Duchenne would not conform to the criteria required presently (155) for the clinical diagnosis of the disease under study; for example, he mentioned that several of his patients recovered after physiotherapy. Thus, it is apparent that not all aspects of the disease we know at present as Duchenne muscular dystrophy were described over one hundred years ago by Duchenne himself.
1. General Considerations.

In the present study the general features of degenerative aspects of muscle were found to be similar in both groups of patients, the classical or typical Duchenne dystrophy and the pseudo-Duchenne or atypical cases. However, even at the level of light microscopy morphological features have been observed apparently characteristic for one or the other group. It has been accepted that gastrocnemii muscles are the last to be involved in the progressively advancing disease and this feature was confirmed in the present study. The age of the patients is an important factor as it parallels the involvement of muscles. However, the rate of progression and degree of involvement by the disease was found to be different in the two groups; thus, the muscles of patients affected by the classical Duchenne dystrophy appeared to deteriorate more rapidly than those in the atypical cases. Deterioration was maximal in the vastus lateralis muscle of the oldest patients (R.S., classical Duchenne dystrophy) and minimal in the youngest patient (D.G., pseudo-Duchenne dystrophy).

The fact that muscle tissue was obtained from different patients at different times, may raise the question of validity of comparison of samplings of the same muscle from patient to patient. To minimize this problem, arrangements were made ensuring that the same surgeon obtained
the tissues at biopsies of all patients using his standard and consistent procedures, and site of a given muscle.

It is well known that electron microscopic studies suffer from the inherent limitations in the size of tissue sampling; this factor is particularly restricting with respect to assessing a pathological process in muscle tissue. These limitations could not be eliminated in the present study just as they cannot be eliminated in any other similar study. Efforts were made, however, to examine as many blocks processed for electron microscopy as possible and feasible in the time allotted.

The intense study of normal muscle was thought necessary to set the baseline for comparative purposes not only for the evaluation of muscle tissues of patients in the present study but in further work planned to be extended to study of other forms of muscular dystrophies and other myopathies.

2. Normal Aspects of Muscle Tissue

Observations concerning the M-filaments, the T-system and the satellite cells were carried out largely on specimens utilized as controls.

A). M-Filaments.

A structure in the middle of the A-band, i.e., the M-line, which differed in light transmission from other
parts of the sarcomere was observed by Dobbie (39), as it is still at the limit of resolution by light microscopy. However, its detailed structure and relation to other components of the myofibril were not revealed until the introduction of glutaraldehyde as a fixative for electron microscopy. Franzini-Armstrong and Porter (65) described thin filaments in cross sections of the M-line and suggested that they might be the S-filaments of Huxley and Hanson (102). Subsequently, Knappeis and Carlsen (122) suggested that the filaments shown by Franzini-Armstrong and Porter may have been, in fact, actin filaments drawn into the M-line by shortening of the sarcomere, and for frog muscles proposed instead a complicated model of bridges and filaments forming the M-line. A new protein, the M-substance, believed to constitute the M-filaments, has been isolated recently from chicken skeletal muscle (147). In another experiment the M-filament component was extracted from myofibrils without any apparent alteration of the structural integrity of the myosin filament (127).

On the basis of the present studies it seems that the main function of the M-filaments within the M-line is to keep the myosin filaments in position in the longitudinal as well as in the transverse directions. This special arrangement of M-filaments and myosin filaments at the M-line may provide "guidance" for the actin filaments when they enter the M-line region in the phase of shortening of the sarcomere. The central area of thick filaments (the pseudo-
H-zone; Figure 3) does not have cross-bridges, thus during the process of contraction of the sarcomere the tips of the actin filaments enter the pseudo-H-zone and become free. The network formed by the myosin and the M-filaments may provide the required support to the actin filaments, and, therefore, prevent their possible bending, tangling or fragmentation.

In several respects, the function of the M-line is similar to that of the Z-line (170), as their framework also tends to keep the filaments in register. However, there are differences in the structural arrangement of the M- and Z-lines that must be reflected in their function. The Z-filaments are in continuity with the actin filaments (170); this implies that a force acting on the fiber is carried by the Z-filaments, whereas the M-filaments do not participate in this process.

B). T-System.

On the basis of his studies on the canine myocardium Lindner (137) suggested that the T-system might be a prolongation of the plasma membrane into the muscle fiber. The same relation was demonstrated in cardiac muscle of sheep by Simpson and Oertelis (225) and in fish muscle (65). Ezerman and Ishikawa (57) studied tissue cultures of chicken skeletal muscle and confirmed that the tubules of the T-system were formed by invaginations of the plasma
membrane. It would, thus, appear that the T-system is open to the extracellular space. Some evidence has been provided that in muscle fibers from certain animals the content of the T-system, indeed, is exchangeable with outside solutions (104 and 183). For example, ferritin injected into the space between skeletal muscle fibers of frog penetrated into the transverse tubules. Despite intensive research by numerous investigations, no such direct opening has been demonstrated in muscle fibers of man.

Present studies show that similar features of the T-system may be found in human striated muscle. The plasma membrane, i.e., the inner component of the sarcolemma, invaginates into the sarcoplasm forming the tubules of the T-system (Figures 38 - 41). Two different types of tubular invaginations were observed in the present study; one was short, branching out and close to the subsarcolemmal zone, whereas the other was longer and with a more complicated course and divisions. The precise significance of these two types of tubules is not known; it may be postulated that they play a role in the immediate, almost-instantaneous conduction of nerve impulses to all the sarcomeres of the myofiber. These tubules may be also a reflection of "myofiber growth"; as the fiber increases in diameter by subsarcolemmal addition of sarcomeres, new tubular invaginations are being formed.
The majority of the tubules of the T-system are arranged in a perpendicular fashion in relation to the myofilaments; however, it was not unusual to find triads and tubules of the T-system oriented longitudinally and parallel to the myofilaments (Figures 41 and 42). Therefore, the "T" of T-system should stand for tubular rather than transversal.


Satellite cells were often observed in normal muscle. Occasionally, two satellite cells adjacent to each other were observed in the same field (Figure 43); they appeared to have cytoplasm of similar characteristics. When satellite cells appeared in pairs in an area of a myofiber, cilia were present in the same space occupied by the satellite cells; between the two components of the sarcolemma. The number of peripheral doublets within the cilia varied, presumably owing to the level of the cross section. Cilia have been observed in differentiating myoblasts from hearts of embryonic chicks (199), and it was suggested that they were related to the stage of cellular differentiation. The same interpretation may be applied to cilia observed in the satellite cells. The formation of cilia, mediated by the transformation of mitotic centrioles, may be associated with the cessation of cellular division and beginning of differentiation. Centrioles were observed
occasionally in the cytoplasm of satellite cells (Figures 47 and 48), whereas additional free centrioles were not present in the ciliated cells.

It was observed as early as 1898, that cilia were formed upon transformation of centrioles into basal bodies with subsequent growth of the cilia shaft, and suggested that this transformation might be part of the control mechanism for the regulation of mitosis and differentiation (92 and 133). The transformation of centrioles into basal bodies may be the result, and not necessarily the cause, of the cessation of cell division. However, it appears from subsequent investigations in striated muscle differentiation (19, 198 and 200), and on the basis of present observations on satellite cells that mitotic activity and the presence of cilia are mutually exclusive activities. Thus, the restriction of nonmotile cilia to differentiating nondividing cells may constitute evidence supporting the control mechanism hypothesis of Henneguy (92) and von Lenhossek (133). If these assumptions be correct, nonmotile cilia should be a common finding in the dormant satellite cells of striated muscle tissue. The fact that satellite cells are not numerous in normal muscle tissue and that there is lack of studies employing serial sectioning may explain why the presence of these structures has not been reported in satellite cells to date.
3. Regeneration.

The concept of "budding" or "continuous" regeneration of muscle fibers in mammals has been seriously questioned recently. It was believed in the past that in all or most instances the continuous (or budding) type was the only form of regeneration (206 and 235). At present, it is largely accepted that the regeneration of muscle occurs in a "discontinuous" way, essentially representing a reversion to the original ontogenetic process of muscle tissue (3 and 196). However, the discontinuous regeneration and the embryonic development of muscle, cannot be considered identical. It has been shown that in the discontinuous regeneration the presence of the basement membrane tube to protect and guide the fusing myoblasts is necessary (196), but obviously there are no preformed tubes of basement membrane "waiting" for the myoblasts in the embryo.

A). Early Stages.

Based on the present observations (Figures 49 and 53) it seems reasonable to suggest that the basement membrane tube is not necessary for the myoblasts to divide, fuse and form muscle cells. There is increasing evidence that satellite cells divide. Half of the resulting daughter cells are incorporated into the fibers and the other half divides subsequently; this process of satellite cell
replication has been described recently as the source of nuclei in muscles of growing normal rats (160), and in certain abnormal conditions of muscle tissue (181). It is feasible to postulate that dividing satellite cells or myoblasts become free (as depicted in Figure 49), and fuse subsequently into multinucleated and primitive sarco-
blasts. Thus, it is possible that two different processes are involved in regeneration, depending upon circumstances including the nature of injury. In the process of repair or maintenance under conditions of normal environment, such as muscle growth (160), or subcellular and highly localized damage, there may be activation of a few satellite cells without the need for their migration. The second process is that of regeneration, and this may occur in extensive injuries to muscle or in prolonged abnormal conditions of striated muscle tissue. In these conditions the damage is extensive, the cellular environment may be abnormal and the architectural relations are lost. Thus, the normal configuration of myofibers and other organelles are deranged, myofibrils are disrupted, and the sarcolemmal components fragmented. Phagocytic cells gain entry into the sarcoplasm, and satellite cells presumably escape into the interstitial space, where they may begin dividing and forming myoblasts. Groups of myoblasts at different stages of differentiation and without any apparent relation to basement membrane tubes that may remain from damaged myofibers, were observed in the present study often in the
interstitial spaces. At times, these groups of myoblasts were in proximity to muscle cells having all the features of recently developed cells (Figure 49).

The myofilaments appear in the cytoplasm of the myoblast early in the development. They do not arise "de novo" with the filaments arranged in the precise order in which they are found in a mature myofiber as it was believed to be the case until recently (1). It is currently assumed that sarcomeres form at random throughout the cytoplasm and that eventually they become aligned forming the mature myofibrils (143 and 150). Cells with all the appearance of young myoblasts have been observed often in the present study. These cells showed patchy accumulations of dark material resembling the Z-line substance on the internal surface of the cellular membrane with thin filaments attached to this dark substance. Thus, a very primitive unit is being formed, probably by an association of an actin and a myosin filament and their subsequent attachment to the Z-line material. The repetition of this attachment will form a primitive sarcomere.

Triads were observed apparently "floating" free in the cytoplasm of early myoblasts. It is possible that the cellular membrane invaginates close to the primitive sarcomeres, establishes contact with the free triads, and thus an early stage of the sarcotubular system is being formed. The sarcoplasmic reticulum component of the triads often contained dark and granular substance. Vesicles containing
similar material were observed embedded in the hyaloplasm of these cells, and mitochondria were observed with morphological features suggestive of division. The formation of myofilaments and sarcomeres appears to be a highly organized process rather than a random phenomenon as suggested by earlier workers (143 and 196).

B). "Cytoplasmic Bodies".

In the sarcoplasm of mature myoblasts or myotubes, masses of sarcomeric elements were observed occasionally that resembled the "cytoplasmic bodies" originally described by King Engel (118) and later related to structural anomalies of the Z-line (169). The structure of the Z-line and its abnormalities have been of interest to investigators for quite a while. Rod-like structures were found to originate in the Z-line of muscle in the so-called rod or nemaline myopathy (74), in myopathies of late onset (179), and in normal myocardium of cats (59). Other alterations of the Z-line, such as streaming and disintegration, have been described in neurogenic atrophy of muscle (190) and in chronic myopathies (47).

The structures under consideration have been observed in all circumstances in striated muscle (skeletal, myocardial); therefore, the term "sarcoplasmic bodies", rather than cytoplasmic bodies is more appropriate and has been employed in the present study. They were found often in
myotubes of the classical cases of Duchenne muscular dystrophy. The fact that these structures were observed in regenerating cells appears to indicate a defect in the process of regeneration in muscles from Duchenne dystrophy patients. Sarcoplasmic bodies consisted of a central core of Z-line material with radiating myofilaments. Myofilaments appeared to be attaching to certain Z-lines in all three dimensions rather than in the two dimensions necessary to form a sarcomere. Early forms of sarcoplasmic bodies (Figures 53 - 55) showed the presence of the Z-line material (tropomyosin?) in the center which in turn was surrounded by a tangle of myofilaments, but no other organelles were evident. However, the periphery showed an increased number of triads and elements of the sarcoplasmic reticulum. Subsequent stages involved possibly the gradual formation of a compact and osmiophilic mass with a granular, crystal-like center (Figure 59). It is possible that a non-specific change of structure in the Z-line is responsible for the disorganized aggregation of myofilaments. The alteration does not seem to be specific, as similar changes have been observed in normal and diseased muscles. The fact that the sarcoplasmic bodies were observed largely in adult myotubes might be explained alternatively by a failure of these cells to become innervated. Myoblasts differentiate and eventually form myotubes; satellite cells may be already present (Figure 57), but in order to become functional they have to be innervated. It is conceivable
that failure of innervation may induce regressive changes in these cells and thus, the formation of sarcoplasmic bodies may represent one of the initial regressive changes.

4. Degeneration.

Most of the recent investigations in dystrophic muscle have been concerned with the detection of early lesions, since these are likely to be of great value in the study of the pathogenesis of the muscular dystrophies. Present study provides evidence that necrosis and degeneration, are the two main features of dystrophic muscle. The close relation of these two processes, observed even in myoblasts, was discussed above. Other changes considered to be indicative of either degeneration, or non-specific necrotic alterations, were found and will be discussed below.

A). General Changes.

The microscopic appearance of a dystrophic muscle is thought to reflect the duration and stage of the dystrophic process rather than the particular clinical syndrome. Since some changes may be observed in different diseases of muscle tissue, these changes are non-specific in nature. Other changes may be more important as they may be characteristically present or prominent in one or the other form of dystrophies, nevertheless, the study of the non-specific
changes may be helpful in the understanding of the different possible patterns of muscle reactions to injury.

In the atypical cases, there were occasional rounded myofibers, increased acidophilia of myofibers and a slightly increased number of subsarcolemmal nuclei. In the advanced cases of Duchenne dystrophy there was a considerable variation in size and shape of myofibers. Some myofibers appeared hyalinized; other were vacuolated or showed granular degeneration. Extensive fatty and fibrous replacement of muscle occurred in patients in the late stages of muscular dystrophy. It was concluded on the basis of a similar study (186) that it was impossible to distinguish on histologic grounds alone between the various types of non-myotonic muscular dystrophies. There is some controversy also regarding the necrosis of myofibers that occurs in human muscular dystrophy. It was suggested that necrosis of muscle fibers was rare (1); other workers reported that focal necrosis did occur (186) and confirmed later this finding in a different series of patients (187). Hyaline necrosis, and granular and vacuolar degeneration have been observed in the present study in different stages of disease; however, there were few inflammatory cells related to the necrotic areas in the muscles studied. There was no difference in appearance of necrotic dystrophic muscle and necrotic muscle in control patients (Figures 70 and 71).
Other changes, considered also nonspecific, were evident by electron microscopy and will be discussed under the following headings.

B). Sarcomeres.

Three different types of degeneration of the sarcomeres have been observed. In the first type, the myofibrils were fragmented without any apparent pattern, sarcomeres were of a normal length and the different bands and lines had a normal appearance (Figure 60). In the second type, myofibrils were also fragmented, the sarcomeres were contracted and the density of the Z-line contrasted with absence of the I-band (Figure 61). The sarcomeres in muscle fibers with the third type of degeneration had an overstretched appearance (Figures 62 - 69). The I-band seemed to be affected first. The sarcoplasm between the sarcomeres appeared to be increased and contained aggregations of mitochondria and increased amount of glycogen granules.

The first two types were found often in the atypical cases and the third type was observed in the patients of the classical or typical Duchenne dystrophy. The two first types of degeneration may represent the different expression of myofibrillary degeneration in the two different types of muscle fibers (white and red). It was not possible, however, to correlate the incidence of these types of degeneration with the types of myofibers. There are no
descriptions of similar findings in the literature, and thus, comparative studies are not possible.

Similar findings to those described here as the third type of degeneration have been reported repeatedly in man (24 and 217) and in experimental animals (196). The increases in mitochondria and glycogen may be only apparent and a direct consequence of the myofibrillar degeneration; as the sarcomeres disintegrate the organelles become more conspicuous, but must not be necessarily increased in number. Sarcomeres in a perinuclear location also seemed to be reduced to small and patchy masses of fibrillar material (Figure 67); at times, remnants of the sarcoplasm within sarcolemmal tubes were of uncertain origin (Figure 69). The significance of, and possible relation between the three types of degeneration are all not known.

C. Vacuolation.

Vacuolation of varying size may occur in muscle fibers in many muscular disorders, and are a very prominent feature in periodic paralysis (51). The vacuoles are bounded by a single membrane and may lie in the subsarcolemmal space (Figures 72 and 74), in the perinuclear area (Figure 75), and between myofibrills (Figure 73). Some contain slightly electron dense granular or filamentous material, tubules or vesicles, whereas other are empty. Other "clear" spaces, not bound by a membrane (Figure 66), were also observed in
muscle. It is difficult to state how these vacuoles arise; they may be derived from any component of the muscle fiber such as the mitochondria, sarcoplasmic reticulum, T-system and the Golgi apparatus. Vacuoles are thought to arise in the muscle fiber as a result of degradation of the various sarcoplasmic components and are a non-specific finding of many disorders (51 and 143). That vacuolation is not a specific change is suggested by the presence of vacuoles in other abnormal conditions of muscle; thus, vacuoles were observed in primary hypokalemic (51) and hyperkalemic periodic paralysis (168), in chloroquine myopathy (70), and in acid maltase deficiency (50). We have observed them in human muscular dystrophy (180) and in a myopathy of late onset (99). The vacuolation in muscle may be secondary to other more basic changes and in some instances dependent upon the nature of the disease process; for example, in chloroquine neuromyopathy, a reversible process, the vacuolation disappears when the administration of chloroquine is discontinued (103). In essence, the vacuolation of muscle appears to be a non-specific consequence of chronicity of a given muscle disease.

D). Z-lines and Target Fibers.

Loss of myofilaments in the sarcomere is apparent initially in the I-bands; consequently, the Z-line becomes
irregular and seems to extend into the I-, and A-bands. This change of the Z-line is referred to generally as "streaming of the Z-line" (Figure 77). The change has been reported in various pathological conditions of muscle (49). It is possible that the lesion enlarges by confluence of adjacent streaming Z-lines. If the streaming of the Z-lines continues concurrently with the loss of myofilaments, large areas of the myofiber will be occupied by an electron dense material (Figure 78) replacing the sarcomeres in zones involved (Figure 79). Fibers showing these changes are known as target fibers; they were originally described by King Engel (117) and interpreted as a feature of denervation. Target fibers are found in other disorders of muscle and are considered to be of non-specific nature. The ultrastructure of the target fibers has been reviewed recently (239) and suggestion was made that they are the result of an intramuscular degenerative process presumably resulting from a chronic and incomplete denervation.

The chemical composition of the Z-line has not been identified entirely to date. There is morphologic evidence of the presence of tropomyosin in the Z-lines (103), but by immunochemical methods this protein was identified in the I-bands rather than in the Z-lines (191). Alternatively, the Z-line could contain proteins similar to those present in the I-band, i.e., actin or actin and tropomyosin. However, extraction of actin from muscle fibers leaves some residual material in the Z-line (48), and selective
extraction of the Z-line leaves I-band filaments behind (234).

Streaming and disintegration of Z-lines is a step in sarcomere and myofibril degeneration, concurrent with vacuolation, accumulation of glycogen and other changes. The alteration of the Z-lines are a common finding and often is associated with, or perhaps conditioned by, a decrease in mitochondrial population in the affected muscle fibers. It is also possible that the streaming of the Z-lines may be the result of an altered (qualitative or quantitative) protein synthesis.

E). Mitochondria.

The ultrastructural observations made in this study suggest that mitochondrial alterations, although probably non-specific, may represent a significant and early structural manifestation of the disorder in muscle. One of the changes observed was swelling of the mitochondria. The cause of mitochondrial swelling has not been elucidated; alterations in the pH, osmotic effects and the action of swelling agents have been suggested as possible etiologies (132). Mitochondrial swelling, vacuolation and electron dense inclusions have been described in cultures of skeletal muscle of chick embryo (130). These features, commonly regarded as evidence of degeneration of these organelles,
were interpreted by the above authors as steps in the continuous turnover of mitochondria; thus, mitochondria showing the changes presumably were no longer able to function normally and this necessitated their replacement.

The rate of mitochondrial turnover may be accelerated as a compensatory mechanism to the other regressive changes in the muscle fiber. Comparable changes have been encountered in many other situation, for example, in the mitochondria of ischemic muscle tissue prior to myofibrillar alterations (230). It appears unlikely, therefore, that defective or non-energized mitochondria represent the initial factor in the pathogenesis of the dystrophic disorders. However, three different myopathies have been recently described under the heading of "mitochondrial myopathies" or "myopathies with primary mitochondrial defects". In one, the mitochondria showed a loosely coupled state of oxidative phosphorylation (141); it was suggested that a structural defect in the organization of the mitochondrial membrane rather than a true enzymatic defect was responsible for the metabolic disturbance. A different myopathy characterized by the presence of giant mitochondria with various inclusions was described subsequently (224); the mitochondrial changes were viewed as an expression of an anomaly selectively related to the membrane subunits concerned with fatty acid metabolism. In a third type, known as pleoconial myopathy, an increase
in number, but not size, of mitochondria was observed (224); the mitochondria contained round inclusions. No suggestion was offered regarding the morphogenesis of all the mitochondrial changes in this myopathy. Mitochondrial changes similar to those described in the three mitochondrial myopathies were observed in a single patient with a myopathy of late onset (179).

Fatty acid oxidation by mitochondria of muscle was markedly reduced in Duchenne dystrophy but normal in the atypical cases included in the present study (136). These biochemical differences of mitochondria could not be correlated with definite morphological counterparts as obvious mitochondrial differences were not observed in muscles of the two groups of patients included in the present study.

5. Other Findings.

A). "Subsarcolemmal Bumps".

The "subsarcolemmal bumps" are interpreted as localized attempts at formation of sarcomeres in a subsarcolemmal region. This localized process of regeneration was observed both in early and advanced stages. In almost all instances, the bumps were related to satellite cells and muscle nuclei. It is difficult to offer meaningful suggestions regarding
these structures at present, with the exception of the possibility that the bumps may represent local responses to cellular injury. The response may be mediated by the adjacent satellite cells, and the initial stimulus may originate either in the myonucleus, the sarcolemma, or the tubular system. The fact that these bumps were found largely in the atypical cases of muscular dystrophy supports the above expressed view that they may represent a focal regeneration in response to localized cellular injury, since the muscular involvement in the atypical dystrophic patients was of lesser magnitude than in patients with the classical Duchenne disease. To our knowledge, similar changes have not been reported to date. Nevertheless, so-called "humps" were described by Mastaglia, Papadimitriou and Kakulas (148). However, the humps were formed by nuclei projecting above the level of sarcolemma of small fibers which were interpreted as regenerating. The small humps contained only the nuclei without evidence of filaments, mitochondria or formation of sarcomeres.

B). "Spiral Fibers" and Ring Fibers.

"Spiral fibers" were observed more often in the atypical cases than in patients with the classical Duchenne dystrophy; the reverse is true for ring fibers.
The spiral fibers were interpreted as the result of local activation, division, and migration of satellite cells around the myofibers, and may represent localized and "misguided" regenerating phenomena. An alternative interpretation would be that the spiral fibers are the earliest morphological expression of denervation of muscle fibers. It is known that following denervation muscle fibers undergo an immediate but transient hypertrophy, (94). Immediately following denervation, the satellite cells may become activated and form a temporary hypertrophic fiber; this fiber, however, lacking the stimulus of innervation, eventually will atrophy and degenerate. It is perhaps at this time of atrophy and degeneration of the denervated fiber that the remaining satellite cells may initiate the discontinuous type of regeneration. It appears that the sarcolemma enveloping the myofiber with its corresponding satellite cells may be the key factor in the "holding" or "release" mechanism of these cells. Whether it is the plasma membrane, the basement membrane, or both is not known. No electron microscopic studies were reported in the literature regarding the herein described spiral fibers. The only comparable work published is that on the light microscopic appearance of the "snake coil fibers" (94, 95 and 119) observed in experimental denervation. No suggestion has been offered regarding the
pathogenesis of the snake coil fibers. It would seem on the basis of comparison of light microscopic appearance that the snake coil fibers and the spiral fibers are not identical; moreover, lack of ultrastructural studies of snake coil fibers makes any meaningful comparison difficult.

Finally, spiral fibers may represent the initial steps in the formation of ring fibers. As mentioned above, the spiral fibers were observed more often in the atypical cases of Duchenne dystrophy: the disease in these patients was only slowly progressive and the morphological evidence obtained indicated also that the tissue changes progressed slowly. Thus, it may be that some of the changes observed in the patients with the atypical Duchenne dystrophy represent early expressions of changes present in the classical Duchenne cases, and that the spiral fibers may in time become ring fibers.

The ring fibers were described in the past century independently by Bataillon (11) and Erb (53). Erb described them in the muscles of a boy with pseudohyper- trophic muscular dystrophy. The term "Ringbinden" was introduced by Heidenhain (90) to indicate the characteristics of today's ring fibers. These fibers also have been described in several skeletal muscles of normal ageing adults (254), and in a variety of ageing domestic (144) and experimental animals (251). They have been observed in several types of muscular dystrophy, but most often in
dystrophia myotonica (88, 129, 220 and 254). The suggestion of Adams et al., (1) that ring fibers are artifacts of fixation has not been generally accepted. It is evident that the ring fibers are single cells rather than conglomerates of two or more cells, as no membrane separates the central filaments from the peripheral ring.

At present, the ring fibers are considered by the majority of investigators not to be artifacts but degenerating fibers. The ring portion of the fiber is said to represent disrupted peripheral myofibrils contracted around the intact central fibrils (18 and 88). In contrast to the above view, present studies indicate that ring fibers are actively regenerating rather than degenerating fibers. This interpretation is based on the observation that the peripheral zone between the sarcolemma and the ringed portion of these fibers showed the characteristic features of muscle regeneration. Junctional folds were observed in the plasma membrane of ring fibers, and cells with all the appearances of Schwann cells were associated with these folds, both features indicating that at least some ring fibers were innervated.

Nothing is known, at present, of the possible role of ring fibers in disease. The possibility that they may be normal components of muscle tissue better observed in cases of muscle degeneration when other myofibers disappear,
should be considered, too. This would imply that for unknown reasons the ring fibers are more resistant to degeneration than are the other myofibers, and it could be interpreted that they form part of a normal feedback or regulatory mechanism. The fact that ring fibers have been found in normal muscle (144, 159 and 254) seems to support the above contention.

In some fibers the myofibrils were oriented in a fashion reversed to that of ring fibers, i.e., the peripheral myofibrils were longitudinal and thus parallel to the axis of the muscle fiber, whereas the central portion was formed by myofibrils oriented in a transverse plane with regard to the long axis of the fiber. However, the organization in both the central and peripheral portion was never as clear-cut as it was in the typical ring fibers. Similar changes have not been reported in the literature; therefore, comparative studies are not possible. The significance of these "reversed" ring fibers, is unknown.

6. Comparison between the Two Types.

Comparative analysis of morphological observations made on muscle of the two groups of patients does show patterns of changes that appear to be characteristic for one or the other group. The atypical patients of Duchenne muscular dystrophy may be identified morphologically by the presence of subsarcolemmal bumps, sarcoplasmic (usually
subsarcolemmal) deposits of glycogen, spiral fibers, and the presence of the two first types of myofibrillar degeneration. On the other hand, the classical or typical patients of Duchenne muscular dystrophy were recognized by the preferential atrophy of central myofibers in a given bundle, the concurrent appearance of several types of degenerative processes (granular, vacuolar and hyaline) within the same bundle of fibers, rare cellular infiltration, and by the presence of sarcoplasmic bodies in myoblasts and myotubes, ring fibers, Z-lines streaming, target fibers and the third type of myofibrillar degeneration.

This separation is, however, not absolute, as some features may be found in both groups. Other features thought to be present characteristically in one group of patients may be absent in the other only apparently and reflect the limitations of sampling rather than true absence. It would, therefore, seem more reasonable at present to depend more upon the "constellation" of given morphological observations as outlined above, rather than on one or two features seemingly characteristic for either group.

It is not possible to state on the basis of the present study whether any particular type of muscle (white, red or intermediate) was affected more often or exclusively in any of the two groups of patients, or whether any of
the changes observed was associated with either of the
types. This is a problem worth pursuing further.

Taking into consideration all the factors discussed
including the important implications of limitation of
tissue sampling, it seems justified to state that the results
of the present morphological studies strengthen the vali-
dity of clinical and biochemical data indicating that the
two young boys, D.G. and R.G. (patients 1 and 2, Table I),
do not suffer from the classical sex-linked severe recessive
Duchenne muscular dystrophy. This raises several questions.
Is it possible that their disease is of the so-called
benign type variety of this disease? This may be
excluded in view of the fact that there was no family
history of similar disease, and because the benign type
of the disease begins much later in life, manifesting
itself for the first time even as late as in the fourth
decade. Nothing is known of the oxidation of fatty acids
by muscle mitochondria in the benign type, and to the
best of the author's knowledge, ultrastructural studies
on muscle have not been reported. Is it possible that the
disease in these two boys represents a "de novo" mutation
of a Duchenne-like, sex-linked type of muscular dystrophy?
This is a matter of semantics and terminology with some,
but with other investigators a serious questioning of the
very basis for such a proposal; "Duchenne-like" would not
imply, however, that in reality this is a different disease,
different from the true (=genetic) point-of-view and not merely a different expression of the same (=genetic) disease.

The present study points to the great need of co-operative efforts between various scientific disciplines necessary in unravelling the great grey mass of myopathies lumped together as the so-called muscular dystrophies. It is the clinician, biochemist, geneticist, pathologist and other members of a team who together may be able to identify individual disease entities in this group. The pathologist's contribution lies not only in the area of confirming differences between the various types of "dystrophies" and thus, facilitating the diagnosis by observing features characteristic for one or the other type. Moreover, by careful ultrastructural studies of all components of the muscle, he may at times offer the first clue of the basic alteration in a given disease. However, the task of the morphologist in examining the muscle tissues obtained either at biopsy or autopsy is not easy. Muscle tissue must be handled with special care and artifacts of surgical procedure, and tissue processing for examination, as well as autolytic changes must be kept in mind at all times when interpreting the morphology. The greatest difficulty — as mentioned above — is inherent in the limitations of sampling. The validity of changes observed in a minute segment of a large mass of tissue should not be extrapolated to the latter, although some times this does apply.
With all the enormous accumulation of literature on the ultrastructure of muscle in health and disease that accumulated during the past decade, we are still unable to put together the "jig-saw" type of knowledge into a meaningful whole. Each electron microscopic study in this field provides a piece of information necessary for the ultimate understanding of muscle diseases in general, and of the so-called muscular dystrophies, in particular.
V. SUMMARY AND CONCLUSIONS

During the study of several patients with muscular dystrophy of the Duchenne type, two patients were found whose clinical features were at variance with those of the typical Duchenne patients. The two atypical patients did not have any family history of the disease. Biochemically, the oxidation of fatty acid by mitochondria from muscle was normal in the atypical and decreased in the typical patients of Duchenne muscular dystrophy.

The present study was undertaken to test whether the two groups, typical and atypical patients of Duchenne muscular dystrophy, show structural changes or patterns of changes characteristic for one or the other group of patients. An attempt at the description of these changes in terms of early morphogenesis has been made.

The present study supports the clinical and biochemical differences; however, they should not be considered as final, as some features may be found in both groups. Other features thought to be present
only in one group of patients may be apparently absent in the other, thus reflecting perhaps the limitations of sampling rather than true absence. Therefore, it seems more reasonable at present to depend upon the different patterns of morphological changes for the two groups, rather than on one or two features seemingly characteristic for either group. This and the possible association of the fiber types with any of the changes are problems worth pursuing further.

On the basis of the present observations, the following conclusions may be made:

Taking into consideration the implications of limited tissue sampling, it seems justified to state that the two atypical patients do not suffer from the classical sex-linked severe recessive Duchenne muscular dystrophy; nor do they suffer from the benign type of Duchenne dystrophy. It is possible that the disease in these two patients represents a "de novo" mutation of a Duchenne-like, sex-linked type of muscular dystrophy.

The different patterns of changes for each group are as follows: the atypical patients of Duchenne muscular dystrophy were identified by the recognition of subsarcolemmal bumps, sarcoplasmic (usually subsarcolemmal) deposits of glycogen, spiral fibers, and
the presence of the two first types of myofibrillar
degeneration. On the other hand, typical patients
of Duchenne muscular dystrophy were recognized by the
preferential wasting of central myofibers in a given
bundle, the concurrent appearance of several types of
degenerative processes (hyaline, granular, vacuolar,
etc.) within the same bundle of fibers, rare cellular
infiltration, and by the presence of sarcoplasmic
bodies in myoblasts and myotubes, ring fibers, Z-lines
streaming, target fibers and the third type of myo-
fibrillar degeneration.

A failure in the last stages of regeneration was
suggested by the presence of subsarcolemmal bumps and
spiral fibers in the muscles of the atypical patients.
Whether this is owing to a defect of final innervation
or to a derangement of the satellite cells is not
known at present.

The sarcoplasmic bodies, observed in myoblasts
and myotubes, appeared to originate from a defective
linking of thin myofilaments in the Z-lines; thus,
forming a tridimensional mass instead of a bidimen-
sional sarcomere.

The three types of myofibrillar degeneration may
be a reflection of the differences in the molecular
mechanisms of derangement of myofibrils in the two
groups of patients.
Ring fibers were not degenerating; instead, the presence of sarcomeric elements at the periphery, junctional folds in the sarcolemma and Schwann cells in association with the junctional zones indicate that ring fibers are innervated and growing cells. The possibilities that they may be normal and special components of muscle tissue are discussed.

The study of normal muscle tissue showed:
M-filaments as a network maintaining the myosin filaments in position and guiding the actin filaments; two types of plasma membrane invaginations forming the tubules of the T-system; and two types of satellite cells containing cilia, centrioles and filaments.
1. Adams, R.D., Denny-Brown, D. and Pearson, C.M.
   "Diseases of Muscle. A Study in Pathology".

2. Adams, R.D.
   "Pathological Reactions of the Skeletal
   Muscle Fibre in Man".
   In: "Disorders of Voluntary Muscle",

3. Aloisi, M.
   "Patterns of Muscle Regeneration".
   In: "Regeneration of Striated Muscle, and
   Myogenesis", A. Mauro, S.A. Shafiq and
   A.T. Milhorat, eds. Excerpta Medica,
   Amsterdam, 180-193, 1970.

4. Altmann, H.W.
   "Über die Strukter der Muskeln der Insekten".
   In: "Handbuch der Allgemeinen Pathologie",

5. de los Arcos, E., Sánchez Cascos, A. and de Rábago, P.
   "Estudio Electrocardiográfico de la Distrofia
   Muscular Progresiva Tipo Duchenne".

6. Aubert, D. "Ueber die Eigenthumliche Struktur der
   Thoraxmuskeln der Insekten".
7. Bailey, K.
"Tropomyosin: A New Asymmetric Protein Component of the Muscle Fibril".

8. Bank, W.J., Rowland, L.P. and Ipsen, J.
"Amino Acids of Plasma and Urine in Diseases of Muscle".

9. Baskin, R.J. and Deamer, D.W.
"A Membrane-Bound Creatine Phosphokinase in Fragmented Sarcoplasmic Reticulum".

10. Baskin, R.J.
"Ultrastructure and Calcium Transport in Crustacean Muscle Microsomes".

11. Bataillon, E.
"Recherches Anatomiques et Experimentales sur la Metamorphose des Amphibiens Anoures".

"Eine Neue X-Chromosomale Muskeldystrophie".

13. Bell, C.
"The Muscular Dystrophies".
Cited by: Gowers, W.R. (1903)

14. Benda, C.
"Die Mitochondria"

15. Bennett, H.S. and Porter, K.R.
"An Electron Microscope Study of Sectioned Breast Muscle of the Domestic Fowl".
16. Bennett, H.S.
"The Cytology of Striped Muscle".
Yale University Press, New Haven.

17. Benson, E.S. and Burke, M.D.
"Diseases of Muscle"
In: "Concepts of Disease", J.G. Brunson and

18. Berthrong, M. and Griffith, P.
"Ring Forms in Skeletal Muscle".

"Mitosis and the Processes of Differentiation
of Myogenic Cells in Vitro".

20. von Boga, L.
"Beiträge zur Kenntnis des Muskelgewebes von
Trichopterenlarven. Eine Mikroskopische
Studie des Feineren Baues des Insektenkörpers".

"Studies of Pseudohypertrophic Muscular
Dystrophy".

22. Bourne, G.H. and Golarz, M.N.
"Human Muscular Dystrophy as an Aberration of
the Connective Tissue".

23. Bowman, W.
"On the Minute Structure and Movements of
Voluntary Muscle".

"Myopathy Associated with Abnormal Lipid Metabolism
in Skeletal Muscle".
24. van Breemen, V.L.
"Ultrastructure of Human Muscle. II. Observa-
tions on Dystrophic Striated Muscle Fibers".

"Muscle Fiber Types: How Many and What Kind?".

26. Bullard, H.H.
"On the Interstitial Granules and Fat Droplets
of Striated Muscle".

27. Cameron, C.H.S.
"Electron Histochemistry of Normal and
Dystrophic Muscle".

28. Caufield, J.B.
"Effects of Varying the Vehicle for OsO_4 in
Tissue Fixation".

29. Cazzato, G.
"Considerations about a Possible Role Played
by Connective Tissue Proliferation and Vascular
Disturbances in the Pathogenesis of Progressive
Muscular Dystrophy".

30. Charcot, J.M.
"Progressive Muskeldystrophie".
In: Progressive Muskeldystrophie, Myotonie,
Myasthenie. Kuhn, E. ed. Spring-Verlag,
Berlin. 13-19, 1869.

31. Church, J.C.T.
"Satellite Cells and Myogenesis; a Study in
the Fruit-Bat Web".

32. Church, J.C.T.
"A Model for Myogenesis Using the Concept
of the Satellite Cell Segment".
In: Regeneration of Striated Muscle, and Myo-
genesis. 118-121, 1970. A. Mauro, S.A. Shafiq
33. Close, R.
"Dynamic Properties of Fast and Slow Skeletal Muscles of Mammals".

34. Coste, M.A. and Gioja, L.
Annali Clin. dell'Ospedale degli Incurabili di Napoli, 1838.
Abstracted by: Schmidt, C.C.

35. Couteaux, R.
"Structural Aspects of the Striated Muscle".

36. Cowdry, E.V.
"The Reactions of Mitochondria to Cellular Injury".

37. Davies, R.E.
"A Molecular Theory of Muscle Contraction: Calcium-dependent Contractions with Hydrogen Bond Formation plus ATP-dependent Extensions of Part of the Myosin-Actin Cross-bridges".

38. Deamer, D. W. and Baskin, R.J.
"Ultrastructure of Sarcoplasmic Reticulum Preparations".

39. Dobie, W.M.
"Observations on the Minute Structure and Mode of Contraction of Voluntary Muscular Fibre".

40. Dreyfus, J.C. and Schapira, G.
"Biochemistry of Hereditary Myopathies".
41. Dubowitz, V. and Pearse, A.G.E.
"A Comparative Histochemical Study of Oxidative Enzyme and Phosphorylase Activity in Skeletal Muscle".

42. Dubowitz, V. and Crome, L.
"The Central Nervous System in Duchenne Muscular Dystrophy".

43. Duchenne, G.B.A.
"De L'Électrisation Localisée et de son Application à la Pathologie et à la Thérapeutique".
J. B. Bailliére, Paris. 2nd ed. 1861.

44. Duchenne, G.B.A.
"Recherches sur la Paralysie Musculaire Pseudo-Hypertrophique, ou Paralysie Myosclérosique".

45. Ebashi, S. and Kodama, A.
"Interaction of Troponin with F-Actin in the Presence of Tropomyosin".

46. Ebashi, S.
"Structural Proteins and their Interaction".

47. Engel, A.G. and Gómez, M.R.
"Congenital Myopathy Associated with Multi-focal Degeneration of Muscle Fibers".

49. Engel, A.G.
"Ultrastructural Reactions in Muscle Disease".

50. Engel, A.G.
"Acid Maltase Deficiency in Adults: Studies
in Four Cases of a Syndrome which May Mimic Mus-
cular Dystrophy or other Myopathies".

51. Engel, A.G.
"Evolution and Content of Vacuoles in Primary
Hypokalemic Periodic Paralysis".

52. Erb, W.
"Ueber die "Juvenile Form" der Progressiven
Muskelatrophie. Beziehungen zur Sogenannten
Pseudo-Hypertrophie der Muskeln".

53. Erb, W.
"Dystrophia Muscularis Progressiva. Klinische
und Pathologisch-anatomische Studien".

54. Ernst, E., Kovacs, K., Metzger-Torok, G. and Trombitas, C.
"Transversal Structure of the Striated Fibril".
Acta Biochim. Biophys. Acad. Sci. Hung. 4:

55. Estable-Puig, J.P., Bauer, W.C. and Blumberg, J.M.
"Paraphenylenediamine Staining of Osmium-Fixed,
Plastic-Embedded Tissue for Light and Phase
Microscopy".

56. Eulenburg, A. and Cohnheim, T.
"Ergebnisse der Anatomischen Untersuchung
eines Falles von Sogenannter Muskelhypertrophie".
57. Ezerman, E.B. and Ishikawa, H.  
"Differentiation of the Sarcoplasmic Reticulum and T System in Developing Chick Skeletal Muscle in Vitro".  

58. Farley, T.M., Scholler, J. and Folkers, K.  
"Research on Coenzyme Q and Muscular Dystrophy".  

59. Fawcett, D.W.  
"The Sporadic Occurrence in Cardiac Muscle of Anomalous Z Bands Exhibiting a Periodic Structure Suggestive of Tropomyosin".  

60. Fenichel, M.  
"Cerebral Influence on Muscle Fiber Typing. The Effect of Fetal Immobilization".  

61. Fex, S. and Sonesson, B.  
"Histochemical Observations after Implantation of a "Fast" Nerve into an Innervated Mammalian "Slow" Skeletal Muscle".  

62. Fisher, E.R., Cohn, R.E. and Danowski, T.S.  
"Ultrastructural Observations of Skeletal Muscle in Myopathy and Neuropathy with Special Reference to Muscular Dystrophy".  

63. Flögel, J.H.L.  
"Über die quergestreiften Muskeln der Milben".  

64. Franke, W.W. and Schinko, W.  
"Nuclear Shape in Muscle Cells".  
65. Franzini-Armstrong, C. and Porter, K.R.
"Sarcolemmal Invaginations Constituting the T System in Fish Muscle Fibers".

66. Franzini-Armstrong, C.
"Studies of the Triad. I. Structure of the Junction in Frog Twitch Fibers".

67. Franzini-Armstrong, C.
"Details of the I Band Structure as Revealed by the Localization of Ferritin".

68. Freeman, S. and Svec, M.
"Effect of Complete Hepatectomy upon Plasma Concentration and Urinary Excretion of 18 Amino Acids".

69. Garamvölgyi, N.
"Observations Préliminaires sur la Structure de la Strie Z dans le Muscle Alaïre de l'Abeille".

70. Garcin, R., Rondot, P. and Fardeau, M.
"Sur les Accidents Neuro-Musculaires et en Particulier sur une "Myopathie Vacuolaire".
Observés au Cours d'un Traitement Prolongé par la Chloroquine: Amélioration Rapide après Arret du Médicament".

71. Gardner-Medwin, D.
"Mutation Rate in Duchenne Type of Muscular Dystrophy".

72. Gauthier, G.F. and Padykula, H.A.
"Cytological Studies of Fiber Types in Skeletal Muscle. A Comparative Study of the Mammalian Diaphragm".
73. Gauthier, G.F.
"On the Relationship of Ultrastructural and
Cytochemical Features to Color in Mammalian Skeletal Muscle".

74. Gonatas, N.K.
"The Fine Structure of the Rodlike Bodies in
Nemaline Myopathy and their Relation to
the Z-Discs".

75. Gowers, W.R.
"Clinical Lectures on Pseudo-Hypertrophic Paralysis".

76. Gowers, W.R.
"A Manual of Diseases of the Nervous System".
Philadelphia.

77. Green, D.E. and Baum, H.
"Energy and the Mitochondrion".

78. Griesinger, W.
"Ueber Muskelhypertrophie".
Arch. Heilkunde. 6: 1-13, 1865.

79. Guba, F.
"Ultrastructure of Myofibrils in Selective
Extraction of Proteins".

80. Gutmann, E.
"Basic Muscle Type Differentiation".
In: Exploratory Concepts in Muscular Dystrophy
Ed. A.T. Milhorat, Excerpta Medica Foundation,
New York.

81. Hanson, J. and Lowy, J.
"The Structure of F-Actin and of Actin Filaments
Isolated from Muscle".
82. Harman, J.W.
"Relation of Mitochondria to Enzymic Processes in Muscle".

83. Harrington, W.F.
"A Mechnochemical Mechanism for Muscle Contraction".

84. Hartmann, J.F.
"Electron Microscopy of Motor Nerve Cells Following Section of Axones".

85. Hartshorne, D.J., Theiner, M. and Mueller, H.
"Studies on Troponin".

86. Haust, M.D.
"Tetrahydrofuran (THF) for Routine Dehydration, Clearing and Infiltration".

87. Hay, E.D.
"Regeneration of Muscle in the Amputated Amphibian Limb".

88. Hayward, M. and Mair, W.G.P.
"The Ultrastructure of Ring Fibres in Dystrophic Muscle".

89. Heidenhain, M.
"Die Kontraktile Substanz".
90. Heidenhain, M.
"Über Progressive Veränderungen der Muskulatur bei Myotonias Atrophica".

91. Heller, H.
"Klinische Beobachtungen über die Bisher als Muskelhypertrophie Bezeichnete Lipomatosis Musculorum Progressiva".

92. Henneguy, L.F.
"Sur les Rapports des Cils Vibratiles avec les Centrosomes".

93. Hess, A.
"Vertebrate Slow Muscle Fibers".

94. Hikida, R.S. and Bock, W.J.
"Effect of Denervation on Pigeon Slow Skeletal Muscle".

95. Hogenhuis, L.A.H. and King Engle, W.
"Histochemistry and Cytochemistry of Experimentally Denervated Guinea Pig Muscle".

96. Holter, H.
"Problems of Pinocytosis, with Special Regard to Amoebae".

97. Hoyle, G.
"A Discussion on the Ultrastructure of Striated Muscle".
98. Huber, J.D., Parker, B. and Odland, G.D.  
"A Basic Fuchsirn and Alkalized Methylene  
Blue Rapid Stain for Epoxy-Embedded Tissue".  

98a. Hudgson, P., Pearce, G.W. and Walton, J.N.  
"Pre-Clinical Muscular Dystrophy: Histopatho-  
logical Changes Observed on Muscle Biopsy".  

"Unusual Generalized Myopathy of Late Onset.  
Clinical and Morphological Study".  
In: "Actualités de Pathologie Neuro-Musculaire".  
G. Serratrice and H. Roux eds., L'Expansion  

and Strickland, K.P.  
"Muscular Dystrophy with Clinical Resemblance  
to the Duchenne Type: Ultrastructural and  
Biochemical Studies".  
In: "Muscle Diseases", B. Kakulas, J.N. Walton,  

101. Huxley, A.F.  
"Muscle".  

102. Huxley, H.E. and Hanson, J.  
"Changes in the Cross-Striations of Muscle  
during Contraction and Stretch and their  
Structural Interpretation".  

103. Huxley, H.E.  
"Electron Microscope Studies on the Structure  
of Natural and Synthetic Protein Filaments  
from Striated Muscle".  
104. Huxley, H.E.

105. Huxley, H.E.

106. Ingalls, W. and Webber, S.G.

107. Ionasescu, V., Vuia, O., Luca, N., Popa, P., Anutei, V. and Anutei, E.

108. Itabashi, H.H. and Kokmen, E.

109. Järlfors, U. and Smith, D.S.

110. Jöbsis, F.F.

111. Kakulas, B.A. and Adams, R.D.
"Normal and Abnormal Muscle in Tissue Culture".

113. Kakulas, B.A.
"Research in Muscular Dystrophy".

114. Katz, B.
"The Terminations of the Afferent Nerve Fibre
in the Muscle Spindle of the Frog".

115. Kelly, D.E.
"The Fine Structure of Skeletal Muscle
Triad Junctions".

116. Ketelsen, U.P., Freund-Mölbert, E. and Bekmann, R.
"Feinstrukturelle Untersuchungen der Muskulatur bei Duchenne-Muskeldystrophie zur
Identifizierung von Konduktorinnen. Die
Myofibrilläre Degeneration".

117. King Engel, W.
"Muscle Target Fibers, a Newly Recognized
Sign of Denervation".

118. King Engel, W.
"The Essentiality of Histo- and Cytochemical
Studies of Skeletal Muscle in the Investigation
of Neuromuscular Disease".

119. King Engel, W., Brooke, M.H. and Nelson, P.G.
"Histochemical Studies of Denervated or Tenotomized Cat Muscle: Illustrating Difficulties
in Relating Experimental Animal Conditions
to Human Neuromuscular Diseases".
120. King Engel, W.
"Selective and Nonselective Susceptibility of Muscle Fiber Types. A New Approach to Human Neuromuscular Diseases".

121. Kleine, T.O.
"Die Progressive Muskeldystrophie Erb. Ein Generalisiertes Enzymverlustsyndrom?".

122. Knappeis, G.G. and Carlsen, F.
"The Ultrastructure of the M Line in Skeletal Muscle".

123. Knoll, P.
"Über Protoplasmaarme und Protoplasmareiche Musculatur".

124. Kölliker, A.
"Einige Bemerkungen über die Endigungen der Hautnerven und den Bau der Muskeln".

125. Krüger, P.
"Über einen möglichen Zusammenhang zwischen Struktur, Funktion and chemischer Beschaffenheit der Muskeln".

126. Kühne, W.
"Untersuchungen über Bewegungen and Veränderungen der Contraktilen Substanzen".

127. Kundrat, E. and Pepe, F.A.
"The M Band. Studies with Fluorescent Antibody Staining".
128. Landouzy, L. and Dejerine, J.

129. Lapresle, J. and Fardeau, M.

130. Larson, P.F., Jenkinson, M. and Hudgson, P.

131. Lehninger, A.L.

132. Lehninger, A.L.

133. von Lennhosek, M.

134. Leyden, E.

135. Lin, C.H.
    "Fatty Acid Oxidation by Skeletal Muscle
Mitochondria in Duchenne Muscular Dystrophy".

137. Lindner, E.
    "Die Submikroskopische Morphologie der
Herzmuskels".

138. Loewy, A.G. and Siekevitz, P.
    "Cell Structure and Function".

139. Luft, J.H.
    "Improvements in Epoxy Resin Embedding Methods".

140. Luft, J.H.
    "Ruthenium Red Staining of the Striated Muscle
Cell Membrane and the Myotendinal Junction".

141. Luft, R., Ikkos, D., Palmieri, G., Ernester, L. and Afzelius, B.
    "A Case of Severe Hypermetabolism of Non-
Thyroid Origin with a Defect in the Maintenance
of Mitochondrial Respiratory Control: A
Correlated Clinical, Biochemical and Mor-
phological Study".

142. Lund, C.C. and McMenamy, R.H.
    "Effect of Infusion of a Protein Hydrolysate
on Blood Cell Amino Acid Distributions".

143. Mair, W.G.P. and Tomé, F.M.S.
    "Atlas of the Ultrastructure of Diseased
Human Muscle".
144. Malan, E.

"Étude d'Histologie Comparée sur quelque Modification Particulieres des Fibres du Tensor Tympani Dues a la Severance".

145. Marinozzi, V.

"Cytochimie Ultrastructurale du Nucleole. RNA et Proteines Intranucleolaires".

146. Maruyama, K.

"A New Protein-Factor Hindering Network Formation of F-Actin in Solution".

147. Masaki, T., Takaiti, O. and Ebashi, S.

"M-Substance, a New Protein Constituting the M-Line of Myofibrils".


"Regeneration of Muscle in Duchenne Muscular Dystrophy: An Electron Microscope Study".

149. Mauro, A.

"Satellite Cell of Skeletal Muscle Fibers".


"Regeneration of Striated Muscle, and Myogenesis".
Excerpta Medica, Amsterdam, 1970.

151. Melland, K.H.

"A Simplified View of the Histology of the Striped Muscle-Fibre".

152. Meryon, E.

"On Granular and Fatty Degeneration of the Voluntary Muscles".
153. Michelson, A.M., Russel, E.S. and Harman, P.J.
"Dystrophia Muscularis: a Hereditary Primary
Myopathy in the House Mouse".

"Changes in Muscle Structure in Dystrophic
Patients, Carriers and Normal Siblings Seen
by Electron Microscopy; Correlation with Levels
of Serum Creatinephosphokinase (CPK)".

155. Milhorat, A.T.
"The Problem of Muscle Disease: Muscular
Dystrophy".
In: "Exploratory Concepts in Muscular Dystrophy
and Related Disorders", A.T. Milhorat, ed.,
Excerpta Medica, Amsterdam. 5-12, 1967.

156. Miller, A. and Tregear, R.T.
"Evidence concerning Crossbridge Attachment
during Muscle Contraction".

157. Mitchison, J.M.
"Some Functions of the Nucleus".

158. Möbius, P.J.
"Ueber die Hereditaren Nervenkrankheiten".

159. Mori, M.
"Striated Anular Fibers in Ocular Muscle".

160. Moss, F.P. and Leblond, C.P.
"Satellite Cells as the Source of Nuclei
in Muscles of Growing Rats".
161. Muir, A.R.
"Further Observations on the Cellular Structure of Cardiac Muscle".

162. Muir, A.R., Kanji, A.H.M. and Allbrook, D.
"The Structure of the Satellite Cells in Skeletal Muscle".

163. Muir, A.R.
"The Structure and Distribution of Satellite Cells".

164. Munsat, T.L. and Pearson, C.M.
"The Differential Diagnosis of Neuromuscular Weakness in Infancy and Childhood. Part I. Non-Dystrophic Disorders".

165. McComas, A.J., Sica, R.E.P. and Currie, S.
"Muscular Dystrophy: Evidence for a Neural Factor".

166. McComas, A.J., Sica, R.E.P. and Brown, J.C.
"Myasthenia Gravis: Evidence for a "Central" Defect".

167. McComas, A.J., Sica, R.E.P. and Campbell, M.J.
""Sick" Motoneurones. A Unifying Concept of Muscle Disease".

168. Macdonald, R.D., Newcastle, N.B. and Humphrey, J.G.
"The Myopathy of Hyperkalemic Periodic Paralysis: An Electron Microscopic Study".
169. MacDonald, R.D. and Engel, A.G.
"The Cytoplasmic Body: Another Structural Anomaly of the Z-Disk".

170. MacDonald, R.D. and Engel, A.G.
"Observations on Organization of Z-Disk Components and on Rod-Bodies of Z-Disk Origin".

171. Newcomer, E.H.
"Mitochondria in Plants".
Botan. Rev. 6: 85-147, 1940.

172. Novikoff, A.B.
"Mitochondria (Chondriosomes)".
In: The Cell. Brachet, J. and Mirsky, A.E.

173. Ogata, T. and Murata, F.
"Fine Structure of Motor Endplate in Red, White and Intermediate Fibers of Mammalian Fast Muscle".

174. Ogata, T. and Murata, F.
"Cytological Features of Three Fiber Types in Human Striated Muscle".

175. Olson, E., Vignos, P.J., Woodlock, J. and Perry, T.
"Oxidative Phosphorylation of Skeletal Muscle in Human Muscular Dystrophy".

176. Oppenheim, F.T.
"The Muscular Dystrophies".
Cited by: Gowers, W.R. (1903)

177. Ordoñez, L.M.
178. Oteruelo, F.T.
"Some Ultrastructural Features of Brain in Phenylketonuria, and their Possible Relation to Mental Retardation".

179. Oteruelo, F.T., Hudson, A.J. and Haust, M.D.
"Unusual Generalized Myopathy of Late Onset. Clinical and Morphological Studies".

180. Oteruelo, F.T., Hudson, A.J. and Haust, M.D.
"Comparative Ultrastructural Studies of Muscle in Para-Duchenne and Duchenne Types of Muscular Dystrophy".

181. Oteruelo, F.T. and Hudson, A.J.
"Spiral Fibers" and Ring Fibers in Duchenne Muscular Dystrophy".

182. Padykula, H.A. and Gauthier, G.F.
"Morphological and Cytochemical Characteristics of Fiber Types in Normal Mammalian Skeletal Muscle".

183. Page, S.
"The Organization of the Sarcoplasmic Reticulum in Frog Muscle".

184. Palade, G.E.
"The Cytoplasm: Intracellular Membrane Systems".
185. Partridge, L.C.  
"Fatty Degeneration of Muscle".  

186. Pearce, G.W. and Walton, J.N.  
"Progressive Muscular Dystrophy: the Histopathological Changes in Skeletal Muscle Obtained by Biopsy".  

187. Pearce, G.W., Pearce, J.M.S. and Walton, J.N.  
"The Duchenne Type of Muscular Dystrophy: Histopathological Studies of the Carrier State".  

188. Pearson, C.M.  
"Histopathological Features of Muscle in the Preclinical Stages of Muscular Dystrophy".  

189. Pearson, C.M.  
"Muscular Dystrophy. Review and Recent Observations".  

190. Pellegrino, C. and Franzini-Armstrong, C.  
"An Electron Microscope Study of Denervation Atrophy in Red and White Skeletal Muscle Fibers".  

191. Pepe, F.A.  
"Some aspects of the Structural Organization of the Myofibril as Revealed by Antibody-Staining Methods".  

191a. Perloff, J.K., de Leon, A.C. and O'Doherty, D.  
"The Cardiomyopathy of Progressive Muscular Dystrophy".  

192. Perry, S.V.  
"Development and Specialization in Muscle and the Biochemistry of the Dystrophies".  
193. Philpott, C.W. and Goldstein, M.A.  
"Sarcoplasmic Reticulum of Striated Muscle: Localization of Potential Calcium Binding Sites".  

194. Poore, C.T.  
"Pseudo-Hypertrophic Muscular Paralysis, with an Analysis of Cases".  

"The Sarcoplasmic Reticulum".  

196. Price, H.M., Howes, E.L. and Blumberg, J.M.  
"Ultrastructural Alterations in Skeletal Muscle Fibers Injured by Cold. II. Cells of the Sarcolemmal Tube: Observations on "Discontinuous" Regeneration and Myofibril Formation".  

197. Prineas, J. and Ng, R.C.Y.  
"Ultrastructural Features of Intracellular Lipid in Normal Human Muscle".  

198. Przybylski, R.J. and Blumberg, J.M.  
"Ultrastructural Aspects of Myogenesis in the Chick".  

199. Rash, J.E., Shay, J.W. and Bieseie, J.J.  
"Urea Extraction of Z Bands, Intercalated Disks and Desmosomes".  

200. Rash, J.E., Shay, J.W. and Bieseie, J.J.  
"Cilia in Cardiac Differentiation".  
201. Reedy, M.K., Holmes, K.C. and Tregear, R.T.
"Induced Changes in Orientation of the Cross-Bridges of Glycerinated Insect Flight Muscle".

202. Reguad, C. and Favre, M.
"Granulations Interstitielles et Mitochondries des Fibres Musculaires Striees".

203. Retzius, G.
"Zur Kenntniss der quergestreiften Muskelfaser".

204. Revel, J.P.
"The Sarcoplasmic Reticulum of the Bat Cricothyroid Muscle".

205. Reynolds, E.S.
"The Use of Lead Citrate at High pH as an ElectronOpaque Stain in Electron Microscopy".

206. Reznik, M.
"Origin of Myoblasts during Skeletal Muscle Regeneration. Electron Microscopic Observations".

207. Robertson, J.D.
"The Ultrastructure of Cell Membranes and their Derivatives".

208. Roizin, L.
"Some Basic Principles of Molecular Pathology.
3. Ultracellular Organelles as Structural, Metabolic and Pathogenetic Gradients".
209. Romanul, F.C.A.


211. Rosman, N.P. and Kakulas, B.A.

212. Rosman, N.P.

213. Rouiller, C.

214. Sabatini, D.D., Bensch, K. and Barrnett, R.J.

215. Salafsky, B.

216. Sarnels, A.J. and Gorevic, P.
"Evidence Suggesting a Retardation of Denervation Atrophy upon Injection of Trophic Brain Proteolipids". Life Sc. 7: 1169-1179, 1968.
217. Santa, T.
"Fine Structure of the Human Skeletal Muscle in Myopathy".

218. Schapira, G., Dreyfus, J.C. and Schapira, F.
"Biochemistry of Striated Muscle During Human Muscular Diseases".

219. Schiaffino, S., Hanzlikova, V. and Pierobon, S.
"Relations between Structure and Function in Rat Skeletal Muscle Fibers".

220. Schröder, J.M. and Adams, R.D.
"The Ultrastructural Morphology of the Muscle Fiber in Myotonic Dystrophy".

221. Schwann, T.
"Mikroskopische Untersuchungen über die Ubereinstimmung in der Struktur und dem Wachsthum der Thiere und Pflanzen".

222. Seidel, H.L.
"Die Atrophia Musculorum Lipomatosa".

223. Shear, D.B.
"Electrostatic Forces in Muscle Contraction".

223a. Shy, G.M. and Magee, K.R.
"A New Congenital Non-Progressive Myopathy".

223b. Shy, G.M., King Engel, W., Somers, J.E. and Wanko, T.
"Nemaline Myopathy: New Congenital Myopathy".
224. Shy, G.M., Gonatas, N.K. and Pérez, M.
"Two Childhood Myopathies with Abnormal Mitochondria. I. Megaconial Myopathy. II. Pleoconial Myopathy".

225. Simpson, F.O. and Oertelis, S.J.
"The Fine Structure of Sheep Myocardial Cells; Sarcolemmal Invaginations and the Transverse Tubular System".

226. Sirlin, J.L. and Jacob, J.
"Function, Development and Evolution of the Nucleolus".

227. Smith, D.S. and Aldrich, H.C.
"Membrane Systems of Freeze-Etched Striated Muscle".

"Studies on Myosin from Red and White Skeletal Muscles of the Rabbit. I. Adenosine Triphosphatase Activity".

"Histochemical Classification of Individual Skeletal Muscle Fibers of the Rat".

230. Stenger, R.J., Spiro, D., Scully, R.E. and Shannon, J.M.
"Ultrastructural and Physiologic Alterations in Ischemic Skeletal Muscle".

231. Stevenson, A.C.
"Muscular Dystrophy in Northern Ireland. I. An Account of the Condition in Fifty-One Families".
232. Straub, F.B.
   "Actin. I".

233. Straub, F.B.
   "Actin. II".

234. Stromer, M.H., Hartshorne, D.J. and Rice, R.V.
   "Removal and Reconstitution of Z-Line Material in a Striated Muscle".

235. Studitsky, A.N., Zhenevskaya, R.F. and Rumyantseva, O.N.
   "Fundamentals of the Technique of Restoration of Muscle by Means of Transplantation of Minced Muscle Tissue".

236. Swaiman, K.F. and Sandler, B.
   "The Use of Serum Creatine Phosphokinase and other Serum Enzymes in the Diagnosis of Progressive Muscular Dystrophy".

237. Tewari, H.B. and Bourne, G.H.
   "Histochemical Evidence of Metabolic Cycles in Spinal Ganglion Cells of Rat".
   J. Histochem. Cytochem. 10: 42-64, 1962.

238. Thin, G.
   "On the Structure of Muscular Fibre".

239. Tomonaga, M. and Sluga, E.
   "Zur Ultrastruktur der Target-Fasern".

240. Trump, B.F., Smuckler, E.A. and Benditt, E.P.
   "A Method for Staining Epoxy Sections for Light Microscopy".
241. Tyler, F.H. and Wintrobe, M.M.
"Studies in Disorders of Muscle. I. The Problem of Progressive Muscular Dystrophy".

242. Vassella, F., Richterich, R. and Rossi, E.
"The Diagnostic Value of Serum Creatine Kinase in Neuromuscular and Muscular Disease".

243. Venable, J.H. and Lorenz, M.D.
"Trial Analysis of the Cytokinetics of a Rapidly Growing Skeletal Muscle".

244. Veratti, E.
"Ricerche sulla Fine Struttura della Fibra Muscolare Striata".

245. Vogel, F.S. and Kemper, L.
"Structural and Functional Characteristics of Deoxyribonucleic Acid-Rich Mitochondria of the Common Meadow Mushroom, Agaricus campestris. II. Extracellular Cultures".

246. Walker, S.M., Schrödt, G.R. and Edge, M.B.
"The Density Attached to the Inside Surface of the Apposed Sarcoplasmic Reticular Membrane in Vertebrate Cardiac and Skeletal Muscle Fibres".

247. Walton, J.N. and Nattrass, F.J.
"On the Classification, Natural History and Treatment of the Myopathies".

248. Walton, J.N.
"Inheritance of Muscular Dystrophy: Further Observations".
249. Watson, M.L.
   "Staining of Tissue Sections for Electron Microscopy with Heavy Metals".

250. Weber, H.H.
   "Die Muskeleiweisskörper und der Feinbau des Skeletmuskels".

251. Weiss, P. and James, R.
   "Aberrant (Circular) Myofibrils in Amphibian Larvae: An Example of Orthogonal Tissue Structure".

252. Wernich, M.A.
   "Fall von Muskelhypertrophie".

   "The Myoglobin Molecule and its Possible Structural Alterations in Diseased States of Skeletal and Cardiac Muscle".

254. Wohlfahrt, G.
   "Dystrophia Myotonica and Myotonia Congenita. Histopathologic Studies with Special Reference to Changes in the Muscles".

255. Wrigglesworth, J.M., Packer, L. and Branton, D.
   "Organization of Mitochondrial Structure as Revealed by Freeze-Etching".

256. Yellin, H. and Guth, L.
   "The Histochemical Classification of Muscle Fibers".

257. Zundel, W.S. and Tyler, F.H.
   "The Muscular Dystrophies".
Figure 1. Drawing representing schematically a longitudinally sectioned muscle fibril. It shows the relations between the main components of a muscle cell. The nucleus (N) is subsarcolemmal in position in contrast to the satellite cell (SC) that is placed within the sarcolemma. The sarcolemma is composed by the plasma membrane (P) and the basement membrane (B). The motor axon (A) of the neuromuscular junction is accompanied by a Schwann cell (S); it terminates in a depression on the surface of the muscle fiber. Synaptic vesicles (V) containing acetylcholine are clustered in the axoplasm of the ending close to the muscle fiber. The sarcolemma underlying the nerve ending shows junctional folds (J). The nerve ending is separated from the muscle surface by the synaptic cleft (C), this space is filled with the basement membrane of the myofiber.
Figure 1a. Schematic representation of a portion of the structural elements of skeletal muscle tissue. It shows the relation between the myofibrils, the T-system, the terminal cisternae and the sarcotubules. (From: D. W. Fawcett and S. McNutt; J. Cell Biol. 25: 209, 1965. The Rockefeller University Press).
Figure 2. Schematic representation of the fine structure of the triad. A tubule of the T-system is shown in the middle between two terminal cisternae of the sarco-plasmic reticulum. The triadic junctions with their gaps bridged by "dimples" projecting from the apposed membranes of the terminal cisternae and presumed adhesive materials are seen along the upper and lower surfaces of the T-tubule. The flocculent material usually seen in the terminal cisternae has been omitted from the figure. The horizontal plane of section outlined corresponds to the approximate thickness of the specimens studied by electron microscopy. (From: D. E. Kelly; J. Ultrastruct. Res. 29: 37, 1969. Academic Press).
Figure 3. Organization of the sarcomere and schematic representation of overlapping thick and thin filaments. A: A low-magnification electron micrograph showing the different bands, lines and zones of the sarcomere. B: Interpretation of the different components (zones, lines and bands) in terms of overlapping actin and myosin filaments. C: High-magnification electron micrograph showing details of filament structure. D: Cross sections of the sarcomere at different levels. (From: A. G. Loewy and P. Siekevitz; "Cell Structure and Function", 2nd ed., 1969, (modified after H. E. Huxley), Holt, Rinehart and Winston).

Figure 5: Schematic representation of a red or slow, I, C, aerobic or mitochondrial muscle fiber. It has more sarcoplasm in relation to the fibrils than the white fiber (Figure 4). Mitochondria and lipid droplets are numerous, and closely related to the I-band. The Z-line is thicker than in white fibers. M: mitochondria, LD: lipid droplets. (From: T. L. Lentz, "Cell Fine Structure", 1971, W. B. Saunders).
Figure 6. Reproduction of the original drawing by Duchenne of the histological punch designed by himself. The photograph shows the closed shaft of the instrument and an enlargement of the tip to show the cavity that accommodates the piece of removed muscle tissue. Apparently there is a typographical error in the original text: it says that figure 25 shows a "closed" shaft; it is evident that the shaft is open.
Fig. 24. Tige fermée de l'emporte-pièce histologique. — Fig. 25. Sa tige fermée et une portion de son manche. — Fig. 26 et 27. Sa tige grossie trois fois, afin de montrer la cavité qui reçoit le morceau de muscle enlevé par l'instrument.
Fig. 24. Tige fermée de l'emporte-pièce histologique. — Fig. 25. Sa tige fermée et une portion de son manche. — Fig. 26 et 27. Sa tige grossie trois fois, afin de montrer la cavité qui reçoit le morceau de muscle enlevé par l'instrument.
Figure 7. The chart shows the relation of some pathological events and clinical features of Duchenne muscular dystrophy. (From: C. A. Bonsett, "Studies of Pseudohypertrophic Muscular Dystrophy", 1969, Charles C. Thomas).
Figure 8. Isometric clamps utilized in the present study to obtain the specimens of muscle tissue. The muscle is clamped before final dissection and remains in the clamps for the first 20 to 30 minutes of fixation in the glutaraldehyde solution.
Figure 9. D.G. Gastrocnemius. Two subsarcolemmal bumps project above the level of the muscle fiber (arrows). Note the close proximity of the bumps to the vessel (between them). Plastic embedded tissue; toluidine blue stain; x 2000.

Figure 10. R.G. Gastrocnemius. Muscle nuclei are centrally located, occasionally associated with stretched sarcomeres and derangement of band structure. Plastic embedded tissue; toluidine blue stain; x 500.

Figure 11. D. G. Vastus lateralis. Variation in size of muscle fibers and increase of intercellular connective tissue. Note clear zones around the small fibers. Paraffin embedded tissue; Masson's trichrome stain; x 600.

Figure 12. R.G. Gastrocnemius. An isolated hyalinized muscle fiber (arrow) is surrounded by interstitial cells. Plastic embedded tissue; toluidine blue stain; x 600.

Figure 13. R. G. Vastus lateralis. Increased number of nuclei surround a group of muscle fibers showing hyalinization (arrow), vacuolation and central nuclei. Paraffin embedded tissue; PAS stain; x 600.
Figure 14. **R.G. Gastrocnemius.** Deposits of an amorphous substance, peripherally arranged and strongly PAS-positive (arrows). Paraffin embedded tissue; PAS stain; x 600.

Figure 15. **D.G. Gastrocnemius.** Longitudinal section shows subsarcolemmal location of deposits of dark and amorphous material. Plastic embedded tissue; toluidine blue stain; x 2000.

Figure 16. **D.G. Vastus lateralis.** Cross section shows an early stage of the spiral arrangement of muscle fibers. Paraffin embedded tissue; PAS stain; x 600.

Figure 17. **R.G. Gastrocnemius.** A mature spiral fiber shows the spiralling internal arrangement of the sarcoplasmic substance. Paraffin embedded tissue; PTAH stain; x 500.

Figure 18. **R.G. Gastrocnemius.** Same field as in Figure 17, stained differently, showing the characteristic disposition of nuclei and myofibrils in the sarcoplasm. Paraffin embedded tissue; PAS stain; x 500.

Figure 19. **D.G. Vastus lateralis.** Cross section shows the spiral arrangement of one fiber. Note the arcuate alignment of prominent nuclei delineating the spiral arrangement. Plastic embedded tissue; toluidine blue stain; x 20000.
Figure 20. D.W. Vastus lateralis. Almost complete disappearance of myofibers. The center of the bundle appears empty; the few remaining fibers are situated at the periphery of the bundles. Paraffin embedded tissue; PAS stain; x 100.

Figure 21. M.D. Gastrocnemius. The degree of replacement of muscle fibers by fatty and fibrous tissue is evident. Only a few atrophic fibers remain. Compare with Figure 11. Paraffin embedded tissue; HPS stain; x 300.

Figure 22. T.S. Vastus lateralis. Fibers with a hypertrophic appearance contain masses of a dark and granular material, vacuoles and central nuclei. Plastic embedded tissue; toluidine blue stain; x 1000.

Figure 23. R.S. Gastrocnemius. Cross section shows muscle fibers of normal size with sub-sarcolemmal accumulation of a pale and homogenous material (arrow). Plastic embedded tissue; toluidine blue stain; x 1000.
Figure 24. T.S. Vastus lateralis. Centrally located dark masses of a homogenous substance with an irregular contour. Note clear zones in close apposition to the dark masses. Plastic embedded tissue; toluidine blue stain; x 2000.

Figure 25. T.S. Vastus lateralis. Dark masses, resembling Z-line material, projecting beyond the level of the muscle fiber. Plastic embedded tissue; toluidine blue stain; x 2000.

Figure 26. T.S. Gastrocnemius. Longitudinal section shows subsarcolemmal disorganization of myofibrillar structure, several sarcomeres deep. Note apparent perpendicular orientation of sarcomeres in these zones. Plastic embedded tissue; toluidine blue stain; x 1000.

Figure 27. T.S. Vastus lateralis. Muscle fibers show different degrees of hyalinization, varying from extensive zones to rather localized areas of only a few sarcomeres (arrow-heads). The changes in some fibers bear resemblance to Nageotte's bands although they were not reflected in electron microscopic examination. Paraffin embedded tissue; PAS stain; x 400.

Figure 28. P.D. Gastrocnemius. Longitudinal section shows curious patterns resembling those of the ring fibers. Paraffin embedded tissue; PAS stain; x 400.
Figure 29. R.S. Gastrocnemius. Cross section shows myofibrils oriented in a perpendicular fashion (arrow) with regard to the longitudinal axis of the fiber. Paraffin embedded tissue; PTAH stain; x 2000.

Figure 30. R.S. Gastrocnemius. Myofibrils are perpendicularly oriented in the cross section (arrow) with regard to the longitudinal axis of the fiber. Other are in a pattern resembling the spiral fibers. Paraffin embedded tissue; PTAH stain; x 2000.

Figure 31. T.S. Gastrocnemius. A small, atrophic muscle fiber showing a spiralling of the internal nuclei and sarcoplasmic substance. Paraffin embedded tissue; PAS stain; x 500.

Figure 32. T.S. Vastus lateralis. Cross section of muscle fibers with a complex appearance shows the haphazardous internal arrangement of the myofibrils. Paraffin embedded tissue; PTAH stain; x 6000.

Figure 33. T.S. Vastus lateralis. Cross section of this complex muscle fiber shows nuclei arranged in accordance with the orientation of the myofibrils. Paraffin embedded tissue; HPS stain; x 600.

Figure 34. R.S. Gastrocnemius. Ring fibers consist of a peripheral subsarcolemmal ring of well organized sarcomeres encircling a central core of longitudinally arranged myofibrils. Paraffin embedded tissue; Masson's trichrome stain; x 2000.
Figure 35. Control. Gastrocnemius. Ultrastructure of normal muscle. Tubules of the T-system (arrow-head) course parallel to the Z-line establishing contacts with the terminal cisternae (black arrow) of the sarcoplasmic reticulum through a specialized surface. Note "dimples" (white arrow) between the two apposed membranes of the triad and finely granular substance filling the terminal cisternae. The M-filaments (double arrow) are evident at the M-line; they seem to be continuous from one sarcomere to the next. M: mitochondria; G: glycogen. x 34000.

Figure 36. Control. Gastrocnemius. The M-filaments, horizontal in the micrograph, appear closely related to the vertical myosin filaments. Note a flocculent substance filling the network formed at the M-line by the myosin and M-filaments. x 98000.

Figure 37. Control. Vastus lateralis. Tangential section showing the M-filaments probably in at least two different planes. x 51000.
Figure 38. Control. Vastus lateralis. First type of plasma membrane invagination. They appear to connect the extracellular space with the T-system. x 62000.

Figure 39. Control. Gastrocnemius. First type of plasma membrane invagination. Note that the basement membrane does not penetrate with the tubule. x 31600.

Figure 40. Control. Gastrocnemius. First type of plasma membrane invagination. It is shorter than those in Figures 38 and 39 and seem to branch out. x 78800.

Figure 41. Control. Gastrocnemius. Second type of plasma membrane invaginations. They are more complex and appear to extend deeper into the sarcoplasm before they form triads. Note the plasma membrane opening (white arrow) and triads (black arrows). x 24000.

Figure 42. D.G. Vastus lateralis. Second type of plasma membrane invaginations. Note plasma membrane opening (arrow-head), complex progress within the subsarcolemmal space and triads (arrows). x 16900.
Figure 43. Control. Vastus lateralis. Two adjacent clear type of satellite cells and a cilium (arrow). $x$ 28700.

Figure 44. Control. Vastus lateralis. High magnification of cilium seen in Figure 43. Plane of section passes close to the tip of the cilium and only four microtubule doublets are seen. Two of the doublets show the dense linear "stalk" connecting them to the ciliary membrane (arrows). $x$ 57400.
Figure 45. Control. Vastus lateralis. Dark type of satellite cell shows osmiophilic cytoplasmic contents. x 16600.

Figure 46. Control. Gastrocnemius. Bundles of filaments in the cytoplasm of a satellite cell. They course in all directions and are closely related to free ribosomes or to elements of rough endoplasmic reticulum. x 49200.

Figure 47. Control. Gastrocnemius. A transverse section of one of the components of a centriole in the cytoplasm of a satellite cell. x 78800.

Figure 48. Control. Vastus lateralis. Tangential section of a centriole embedded in the cytoplasm of a satellite cell. They were always in a perinuclear position. x 82500.
Figure 49. D.G. Vastus lateralis. Group of six myoblasts at different stages of differentiation. Note differences in cytoplasmic contents. × 9000.

Figure 50. D.G. Gastrocnemius. A single myoblast close to a vessel (black arrow). Accretions of a dark substance in the internal surface of the cellular membrane (white arrow) resemble Z-line material. Filaments appear attached to the inner surface of the dark accretions. Note primitive triads and absence of sarcomeres. M: mitochondria in division (?). × 14700.
Figure 51. M.D. Vastus lateralis. A myotube in regeneration (Figure 52) shows already signs of early structural derangement in the sarcomeres (Figure 53), mitochondria and other organelles. x 7650.

Figure 52. M.D. Vastus lateralis. Subsarcolemmal area in the myotube shown in Figure 51. Myofilaments and sarcomeres are being formed in these zones as indicated by the presence of disorganized filaments in association with ribosomes and glycogen. x 7000.

Figure 53. M.D. Vastus lateralis. Higher magnification of a perinuclear zone in the myotube shown in Figure 51. Incomplete sarcomeres are organized in a radial fashion with a common central origin consistent with Z-line substance. x 10400.
Figure 54. M.D. Vastus lateralis. A myotube showing two sarcoplasmic bodies. These masses are composed of streaming Z-line material and deranged myofilaments. x 5000.

Figure 55. T.S. Gastrocnemius. Cross section of a mature myotube. The mass in its sarcoplasm shows a central core of a dark substance, possibly Z-line material, surrounded by an accumulation of filaments. Note resemblance of this mass with the so-called cytoplasmic bodies. x 6850.
Figure 56.  M.D. Vastus lateralis. The sarcoplasmic body is surrounded by an area of deranged sarcomeres, triads, elements of the sarcoplasmic reticulum and mitochondria. X 7000.

Figure 57.  M.D. Vastus lateralis. The central large core of the sarcoplasmic body surrounded by a light halo consisting of a finely granular material and myofilaments. Note a satellite cell (SC) beneath the basement membrane of the muscle cell. X 8000.
Figure 58. T.S. Vastus lateralis. The core of the sarcoplasmic body has a finely granular, moderately electron-lucent center. x 6400.

Figure 59. D.W. Gastrocnemius. Interpreted as one of the last stages in the development of the sarcoplasmic bodies were forms similar to the one shown in this micrograph. The center of the core of the sarcoplasmic body consists of a granular electron-dense substance with a crystalline appearance. x 14800.
Figure 60. R.S. Gastrocnemius. This micrograph shows the first of the three different types of derangement of muscle fiber structure. Fibrils are fragmented without any apparent pattern; some fibrils extend over few sarcomeres; other fragments are less than a sarcomere in length. x 4360.

Figure 61. R.S. Gastrocnemius. The main feature of muscle fibers undergoing the second type of degeneration is their contracted state. The I-band cannot be recognized. Note small mitochondria and crenated appearance of the sarcolemma. x 6380.

Figure 62. P.D. Gastrocnemius. Three muscle cells are shown in this micrograph. One is apparently normal (left), the cell in the center is at the early myotube stage, and the fiber to the right is undergoing the third type of degeneration. x 9150.
Figure 63. R.G. Gastrocnemius. One of the features of the third type of degeneration was the apparent stretching of sarcomeres. All the sarcomeres in the fiber shown in this micrograph are overstretched. Note swollen mitochondria. x 4750.

Figure 64. P.D. Gastrocnemius. Subsarcolemmal zone with overstretched fibrils. Adjacent myofibrils have normal sarcomeres and show vacuolation. Normal muscle fiber is seen at top left corner of micrograph. x 4150.

Figure 65. M.D. Gastrocnemius. Z-lines, out of alignment with each other, appear thin and wavy. Glycogen granules and vacuoles are apparent between the myofilaments. x 12400.

Figure 66. P.D. Vastus lateralis. Pool of a granular material of a non-descript nature with remnants of cellular organelles is surrounded by closely packed myofilaments, mitochondria and other organelles. x 6650.
Figure 67. T.S. Gastrocnemius. This micrograph depicts a muscle fiber considered to be in last stages of degeneration. A mass of sarcoplasmic debris is present to the left. Note two nuclei and degenerating mitochondria. x 10000.

Figure 68. R.S. Vastus lateralis. A sarcolemmal tube at top of microphotograph. The tube is devoid of organelles and filaments; only the basement membrane remains. It contains a finely granular non-descript substance. x 18050.

Figure 69. R.S. Vastus lateralis. High magnification of a structure contained in the sarcolemmal tube shown in Figure 68. It is laminated or crystalloid in nature and of uncertain origin. x 40000.
Figure 70. Control. Vastus lateralis. Muscle cell with a necrotic appearance. Normal myofibers are seen above and below the degenerating myocell. x 7630.

Figure 71. P.D. Gastrocnemius. Necrotic muscle cell in the vicinity of a vessel (bottom). x 9250.
Figure 72. P.D. Gastrocnemius. Vacuole in the subsarcolemmal space. It is enveloped by a membrane and contains a flocculent material and membranous debris. x 6360.

Figure 73. T.S. Vastus lateralis. Micrograph of a vacuole corresponding to those seen in Figure 22 (light microscopy). Note finger-like projections of the peripheral sarcoplasm into the vacuole. x 4250.

Figure 74. R.G. Gastrocnemius. Membrane-bound vacuole in a subsarcolemmal and perinuclear location. x 11610.

Figure 75. D.G. Vastus lateralis. Distentions of the outer membrane of the nuclear envelope are associated with enlarged (arrow-head) and separated nuclear pores (arrow). The space between the nuclear membranes contains a finely granular substance and membranous debris of uncertain origin. x 13650.
Figure 76. D.G. Gastrocnemius. Accumulations of glycogen granules in subsarcolemmal and interfibrillar spaces. A laminated or crystallloid structure is in close apposition to the inner surface of the plasma membrane. x 23700.

Figure 77. D.W. Gastrocnemius. Widened and irregular Z-lines spread into the adjacent I- and A-bands in a localized manner. x 15480.

Figure 78. R.S. Vastus lateralis. Several myofibrils are involved in a generalized fashion with changes of the Z-lines. It seems that this large lesions of the myofibrils arise from the extension and coalescence of smaller adjacent lesions similar to those shown in Figure 77. x 6240.

Figure 79. R.S. Vastus lateralis. High magnification of an area from Figure 78 showing that the osmiophilic zones appear to consist of Z-line substance and actin filaments. x 47700.
Figure 80. R.G. Vastus lateralis. Thin and elongated mitochondria in a small muscle fiber. They show one or two cristae centrally arranged in a very osmiophilic matrix. x 6380.

Figure 81. R.S. Gastrocnemius. Swollen mitochondria with rarefied matrix and with thin and distorted cristae are seen between overstretched myofibrils. x 22500.
Figure 82. P.D. Gastrocnemius. A crystalloid structure, possibly originated from mitochondria, is seen in an atrophic muscle cell. x 26750.

Figure 83. M.D. Gastrocnemius. Mitochondria in division (?) in the perinuclear space. They show dense matrix with osmiophilic and round bodies. x 23000.

Figure 84. R.G. Vastus lateralis. Mitochondria show dense matrix, fragmented membranes and myelin figures. x 12750.

Figure 85. D.W. Gastrocnemius. Dark and osmiophilic closely packed cristae and myelin figures. x 14810.

Figure 86. T.S. Gastrocnemius. A large mitochondrion contains numerous, closely packed cristae. x 20500.
Figure 87. D.G. Gastrocnemius. Subsarcolemmal bump similar to those seen in Figure 9. It is formed by a local proliferation of myofilaments. Accumulation of mitochondria (M), nucleus (N) and satellite cell (SC) surround the bump. x 31800.

Figure 88. D.G. Gastrocnemius. High magnification of the zone transitional between the subsarcolemmal bump and the adjacent body of the fiber. It shows the haphazardous arrangement of the myofilaments, the apparent attempt at forming sarcomeres and the presence of primitive triads. x 63800.
Figure 89. **R.G. Gastrocnemius.** An early form of subsarcolemmal bump is perinuclear in position. It does not project above the level of the sarcolemma and consists of thin filaments, mitochondria, ribosomes and glycogen granules. x 12900.

Figure 90. **D.G. Gastrocnemius.** Some bumps show primitive sarcomeres without any apparent relation to the adjacent sarcomeres. x 7500.

Figure 91. **D.G. Gastrocnemius.** High magnification of Figure 90 showing that this bump consists of Z-line material, actin and myosin filaments, triadic elements and glycogen granules. x 22050.

Figure 92. **R.G. Gastrocnemius.** Subsarcolemmal zone adjacent to one of the early bumps. The zone contains mitochondria and filaments associated with ribosomes, all embedded in a matrix rich in glycogen granules. x 13050.
Figure 93. D.G. Vastus lateralis. Peripheral zone of the spiral fiber shown in Figure 19. The main body of the muscle fiber (MB), the satellite cell (SC) and the daughter cell (DC) are all enveloped by the same basement membrane. Note differences in the cytoplasm of the three cells. x 1040.

Figure 94. D.G. Vastus lateralis. High magnification of the upper left half of Figure 93. Each cell has a distinct plasma membrane and all have a common basement membrane (arrow). x 16190.

Figure 95. D.G. Vastus lateralis. Absence of basement membrane between the cells similar to those depicted in Figure 93. There are organized myofilaments in the cytoplasm of the daughter cell (arrow). x 13250.

Figure 96. D.G. Vastus lateralis. The plasma membrane separating the muscle cell (MC) and the adjacent daughter cell (DC) appears interrupted (arrows). The same granular material is present at both sides of the fragmented membrane. x 15710.
Figure 97. R.S. Gastrocnemius. Electron micrograph of a ring fiber similar to that shown in Figure 34. The normal longitudinal orientation of the central core of fibrils and the circular outer band of muscle fibrils oriented in a perpendicular fashion are apparent. x 2025.
Figure 98. R.S. Gastrocnemius. High magnification of the transitional zone of a ring fiber to show that both components, the central core (bottom) and the ring part (top), are not separated by membranes or other structures. x 39600.

Figure 99. R.S. Gastrocnemius. Subsarcolemmal zone and the subjacent ring portion of a ring fiber. The subsarcolemmal zone shows mitochondria, Z-line substance, elements of the T-system and other organelles characteristic of regeneration. x 13600.
Figure 100. P.D. Gastrocnemius. Portion of a ring fiber showing a junctional zone and a satellite or Schwann cell (see Figure 101). x 4330.

Figure 101. P.D. Gastrocnemius. Higher magnification of Figure 100 shows a basement membrane between the cell on left and the myofiber, thus identifying the cell as a Schwann cell which belongs to the junctional complex. x 11610.

Figure 102. T.S. Gastrocnemius. The muscle fiber in this micrograph shows other arrangement of the myofibrils. It consists of a peripheral band of longitudinally (normal) oriented fibrils, whereas the center is occupied by perpendicularly oriented fibrils. x 14050.
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