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Ultrastructural Features of the Muscle Fibers in Congenital Hypothyroidism

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Summary. Muscles of three patients (2 years, 2 years and 7 months, and 11 years of age) affected by congenital hypothyroidism due to dysgenesis of the thyroid gland were studied by electron microscope. The biopsies were performed on the quadriceps muscle. Several alterations were found in the ultrastructure of the muscle fibers. The contractile material underwent atrophy by two recessive processes: by reduction and by degeneration. The former, which affected mainly the white fibers, led to the progressive detachment of myofilaments from the periphery of the myofibrils while they maintainad a normal arrangement in the center. Consequently, their diameter decreased and their interfibrillar spaces enlarged. The latter enlargment, involving the red fibers, caused areas of dedifferentiation involving either the contractile apparatus or the other cell organelles. Modifications of the sarcolemma and basement membrane were seen in the fibers affected by the atrophying processes of the contractile material. Changes of the ultrastructure of the sarcoplasmic reticulum and of the T system were observed with numerous morphological abnormalities of the mitochondria. In addition, accumulation of glycogen and striking changes of the satellite cells were found. The ultrastructural findings are reviewed and discussed in relation to the literature on hypothyroid myopathy.

Introduction

It is well experienced that congenital hypothyroidism causes a myopathy characterized by muscular weakness and slow contraction and relaxation of tendon reflex (Mc. Ardle, 1969). Although the several clinical descriptions of the hypothyroidism in children, few informations are available about the histology of the muscle fibers in this syndrome. The literature reports the modifications of the muscle fibers in the hypothyroidism in adults. Thereby, these informations are few and debated so that it has been concluded that the muscle biopsies miss to point out specific and constant lesions underlying the pathological phenomena of the hypothyroidism (Adams, 1969).

The aim of the present work is to approach the problem studying the ultrastructure of the muscle fibers in three children affected by congenital hypothyroidism due to dysgenesis of the thyroid gland. It is important to note that these patients had received an inadequate substitutive hormonal therapy before the biopsies were carried out.

Materials and Methods

Specimens of the quadriceps muscle, obtained by biopsies, have been examined by both light and electron microscopy.

Small blocks of tissue were fixed in cold phosphate-buffered glutaraldehyde 2.5% (pH 7.3) for 2 hours, washed in 0.1 M phosphate buffer for 6–10 hours and post-fixed in 1%

osmium tetroxide in 0.1 M cacodylate buffer (pH 7.3) (1 hour). Finally they were dehydrated and embedded in Epon 812. Thin sections cut with diamond knife on a Porter-Blum MTl microtome, were stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) and observed with Siemens Elmiskop I microscope. Thick sections (1.5—2 μ) stained with a 1% toluidine blue—1% methylene blue mixture were used for light microscopy.

Case Reports

Case 1. B.M., a boy, was admitted to the Children's Hospital of the Perugia University at the age of 2 years because of congential hypothyroidism (No. 392/1968). This diagnosis has been done at the age of 11 months. Since then the boy has been treated discontinuously with 50 mg of dried thyroid pro die. Family anamnesis was negative. Our physical examination revealed thick, dry and pale skin; coarse and dry hair; wide apart eyes; the anterior fontanelle still open; batrachial abdomen with a small umbilical hernia. A generalized muscular hypotonia, hypertrophy of the gastrocnemia and slow relaxation time of the tendon reflex were noted. His mental age was estimated at 1 year, equivalent to an I. Q. of 50.

Laboratory Investigations. Haemoglobin and blood count were normal; serum cholesterol was 456 mg-%. Creatine kinase was 2.6 μmole/min/l. Serum creatine kinase electrophoretic pattern on cellulose acetate, according to Cao et al. (Cao *et al.*) showed the muscle isoenzyme alone. Serum P.B.I. was 2.8 per 100 ml. Electronencephalogram: the recording was carried out during the III sleep stage (sleep induced by 6 mg of Chlorpromazine and 1 mg of Diazepan). Background activity was represented by polymorphic 3-4C/S high voltage waves with superimposed fast rhythm; syncronous and asyncronous sharp waves occurred in all the derivations Radio-iodine (^{131}I) uptake was not observed in normal thyroid tissue but it was present in ectopic sublingual thyroid tissue (values of 3.8% at 24 hours). Diagnosis: congenital hypothyroidism by hypoplasia of an ectopic thyroid gland.

Case 2. A.P., a girl was referred to the Children's Hospital of the Perugia University at the age of 2 years and 7 months for congenital hypothyroidism (No. 1004/1967). The diagnosis was established at the age of 2 years. The girl has been treated discontinuously with 50 mg of dried thyroid pro die. Family anamnesis was negative. Our physical examination revealed a poorly developed and ill-fed child; pale skin; anterior fontanelle still open; wide apart eyes; snub nose; short neck; batrachial abdomen with a small umbilical hernia. A slight muscular hypotonia was checked as well a retarded mental function.

Laboratory Investigations. Haemoglobin and blood count were normal as well the values of Ca and P serum, the urea nitrogen, the blood sugar. Creatine kinase was 3.2 μmoles/min/l. Serum creatine kinase electrophoretic pattern method according Cao et al. (Cao *et al.*) showed the muscle isoenzyme alone. Serum PBI was 3.2% ml. Electronencephalogram (recorded during sleep induced by 6 mg of Chlorpromazine and 1 mg Diazepan): the background activity of I sleep stage was characterized by 4–6 c/s polymorphic waves with a fast superimposed rhythm. In this stage, also diffused and synchronous biphasic high voltage spikes occurred. Centroencephalic spike dysrhythmia was found. Radio-iodine (^{131}I) uptake was present only in ectopic sublingual thyroid tissue (24% at 24 hours).

Diagnosis: congenital hypothyroidism due to hypoplasia of an ectopic thyroid gland.

Case 3. I.A., 11 years old boy was admitted in the Children's Hospital University of Perugia (No. 274/1968), in consequence of a previous diagnosis of congenital hypothyroidism. The diagnosis has been done at the age of 7 months on the basis of retarded motor development and stunted growth. The boy was treated with 50 mg of dried thyroid pro die and later on discontinuously with 100 mg untill the age of 11 years. Family anamnesis was negative. In our physical examination the skin appeared thick, dry and pale, flattened nose, short neck, batrachial abdomen. A generalized muscular hypotonia without weakness was noticed. The mental age was estimated at about 8 years equivalent an I. Q. of 72.

Laboratory Investigations. Haemoglobin and blood count were normal. Cholesterol was 340 mg-%; creatine kinase 1 μmoles/min/l. P.B.I. 4% ml. Electronencephalogram (recorded during the sleep induced by 6 mg chlorpromazine and 1 mg of Diazepan). Polymorphic middle and low voltage 4-6 c/s waves occurred in the back regions of the two hemispheres. Fast and slow voltage rhythm was recorded in the frontal and central region. ^{131}I uptake was present in an ectopic sublingual thyroid (2% at 24 hours). Diagnosis: congenital hypothyroidism due to hypoplasia of an ectopic thyroid gland.

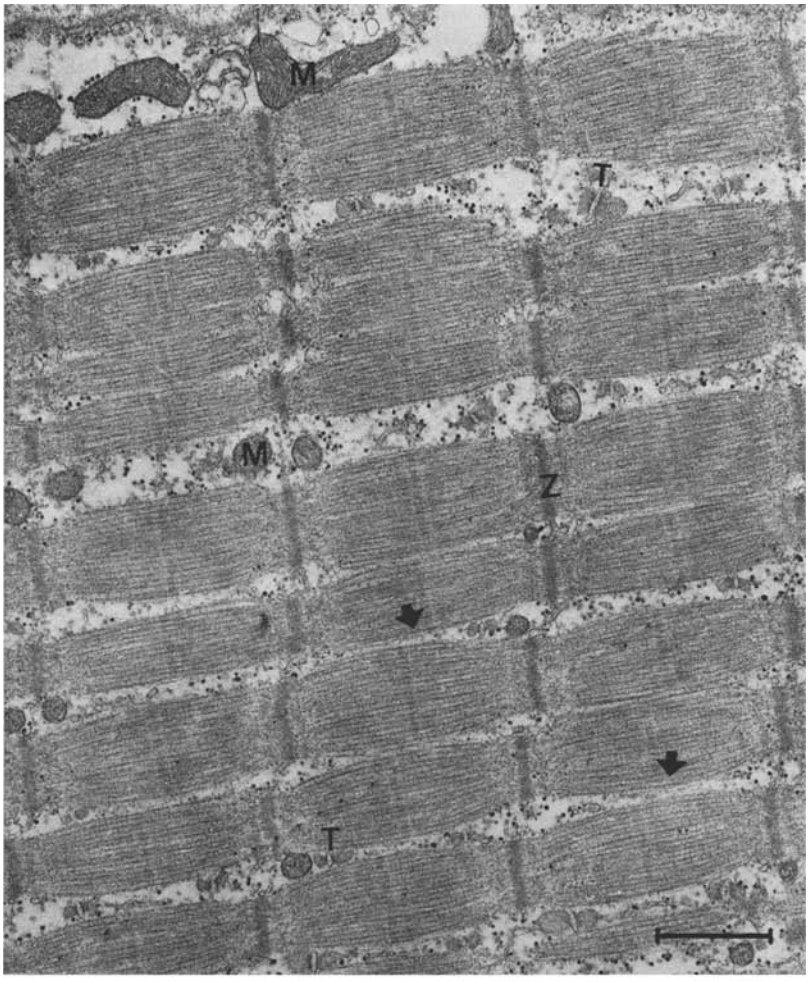
Results

We like to emphasize that the modifications found in the muscle fibers do not seem to be dependent of the development of the syndrome in that they are similar in all the cases studied. Therefore, we will refer the reaction of the cell organelles to the pathological condition, leaving out the description of every single case.

a) Light Microscopy. In longitudinal section the muscle fibers run irregularly showing marked ondulations and surrounded by amorphous material. The structure of the myofibrils appears to be normal; occasionally some fibers show enlarged interfibrillar spaces containing small vacuoles and intensely stained material. The nuclei are generally located at the periphery of the fibers.

b) Electron Microscopy. The quadriceps muscle, as well as most skeletal muscles, has a mixed content of fibers. The types mainly represented are the red fibers (or type I) and white ones (or type II). The formers present numerous and large mitochondria which separate the fibrils each other by their longitudinal branches and form rows in the interfibrillar spaces. Besides, masses of these organelles are located in the perinuclear region and at the fiber periphery. In the white fibers, on the contrary, the mitochondria are smaller, less numerous and only occasionally form masses beneath the sarcolemma. The fibrils, consequently, are well packed together with narrow interfibrillar spaces. Other morphological differences characterize the two types of fibers: the sarcoplasmic reticulum and T-system arrangement, the fibrils diameter, the M band structure, etc.

In the congenital hypothyroidism the muscle fibers undergo atrophic processes which differ according the fiber type especially as far as concerns the response of the contractile material and the mitochondria. The muscle fibers run irregularly and are surrounded by abundant, amorphous, electrondense material in



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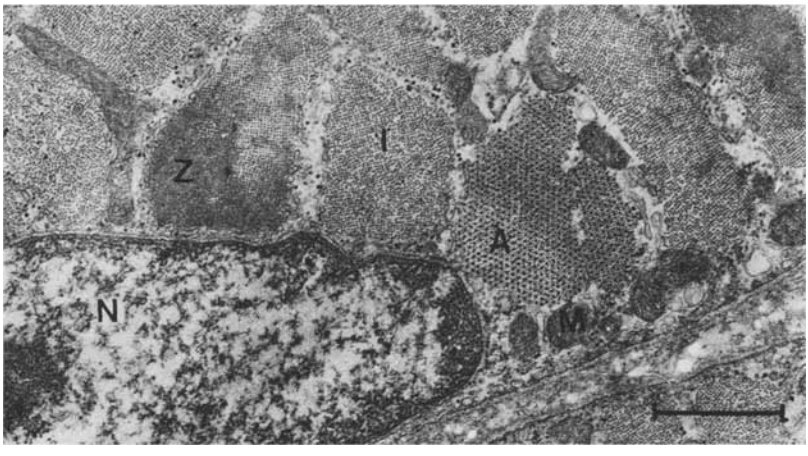

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Figs. 1 and 2

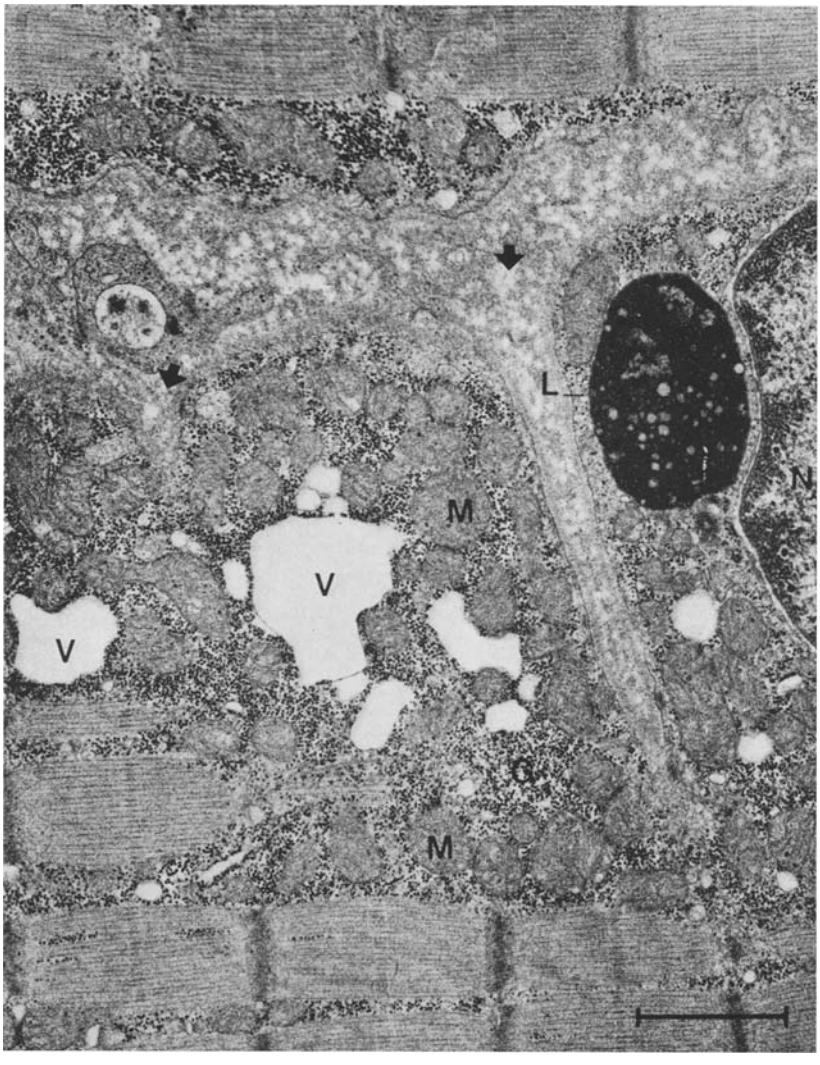


Fig. 3. Longitudinal section of 2 fibers. The sarcolemma and the basement membrane penetrate into the fiber forming marked invaginations (arrows). Finely granular electron dense material fills the spaces between the fibers. *N* nucleus, *L* lysosomes, *G* glycogen granules, *V* vacuoles, *M* mitochondria. × 20000

Fig. 1. Longitudinal section of a white fiber. The fibrils, with a normal spatial disposition, display enlarged interfibrillar spaces due to the detachement of myofilaments (arrows). Small mitochondria (*M*) and elements of the sarcotubular system (triad *T*) are visible in the interfibrillar spaces. *Z* Z-line. × 15000

Fig. 2. The insert shows a cross section of the same fiber type. The fibrils are reduced in diameter by the loss of filaments at the periphery while in the center maintain a regular arrangement. *Z* Z-line, *A* A band, *I* I band, *M* mitochondria, *N* nucleus. × 18000

which collagen fibers and fibroblasts are seen. The white fibers show a marked increase in width of the interfibrillar spaces caused by the reduction of the fibril diameter. Such reduction, evident in both longitudinal and cross sections, is due to the peripheral detachment of myofilaments from the fibrils. The filaments spread and breack down in the interfibrillar spaces while the fibrils maintain their normal spatial disposition and do not differ for any other aspect from the normal (Figs. 1, 2). Small mitochondria, elements of the sarcotubular system (sarcoplasmic reticulum and T-system) and glycogen granules take the place of the contractile material whether in the widened intermyofibrillar spaces or beneath the sarcolemma, mainly at the fiber periphery. The sarcolemma, followed by the thickened basement membrane, invaginates into the fiber, giving rise to marked indentations (Figs. 1, 3). By the regressive process the fibers reduce the amount of the contractile material and, subsequently, their volume.

In the red fibers the contractile material is slightly affected by the regressive process and by second one, known as "degenerative process". The fibrils appear well organized and substantially do not seen to decrease in diameter as much as they do in the white fibers (Figs. 3, 4). The degenerative process starts with a lesion of the Z-line material which becomes bent and spreads into the sarcomere. Consequently the arrangement of the filaments is disturbed, causing areas of disorganization in the fibrils. In these areas the sarcoplasmic reticulum and the T-system are dislocated as well as the mitochondria (Figs. 5–7). Entire sarcomeres are affected especially at the fiber periphery. We have to point out that in the muscles examined, the degeneration process injures slightly the fibers, interesting only small and few regions and is less widespread than the regressive one. The glycogen granules are scattered irregularly throughout the lesion. Corresponding with the affected zones the sarcolemma and the basement membrane invaginate into the fiber. Sometimes areas of sarcoplasm seem to be segregate and, eventually, poinched off from the fibers by invagination movement of the sarcolemma (Figs. 8, 9). This has been observed in the denervation and castration atrophy and referred as a defense mechanism by which the fibers lose the affected material in consequence of a damaging cause. Both the atrophic processes of the contractile material, have been observed in a variety of experimental atrophies and human dystrophies (Gori *et al.*, 1967; Engel, 1968; Pellegrino and Franzini-Armstrong, 1969).

The basement membrane of the muscle fibers of the hypothyroid children appears constantly thickened and fuses with the electron dense, finely filamentous material which fills up abundantly the interfibers spaces (Figs. 2, 3, 7–9). Generally the relationship between the sarcolemma and the basement membrane is maintained around the whole fiber so that the latter followes the sarcolemmal invaginations into the fibers. In some regions, on the other hand, the basement membrane forms complex folds projecting in the space between the fibers. These folds appear as convolute branches and are present mainly in fibers with a reduced diameter. Therefore, is seems likely that the basement membrane folds form in fibers in which the volume is decreased because of the atrophy process, leaving the basement membrane alone.

As regards the mitochondria, these organelles show numerous and peculiar changes. As already mentioned, the mitochondria of the white fibers are normally

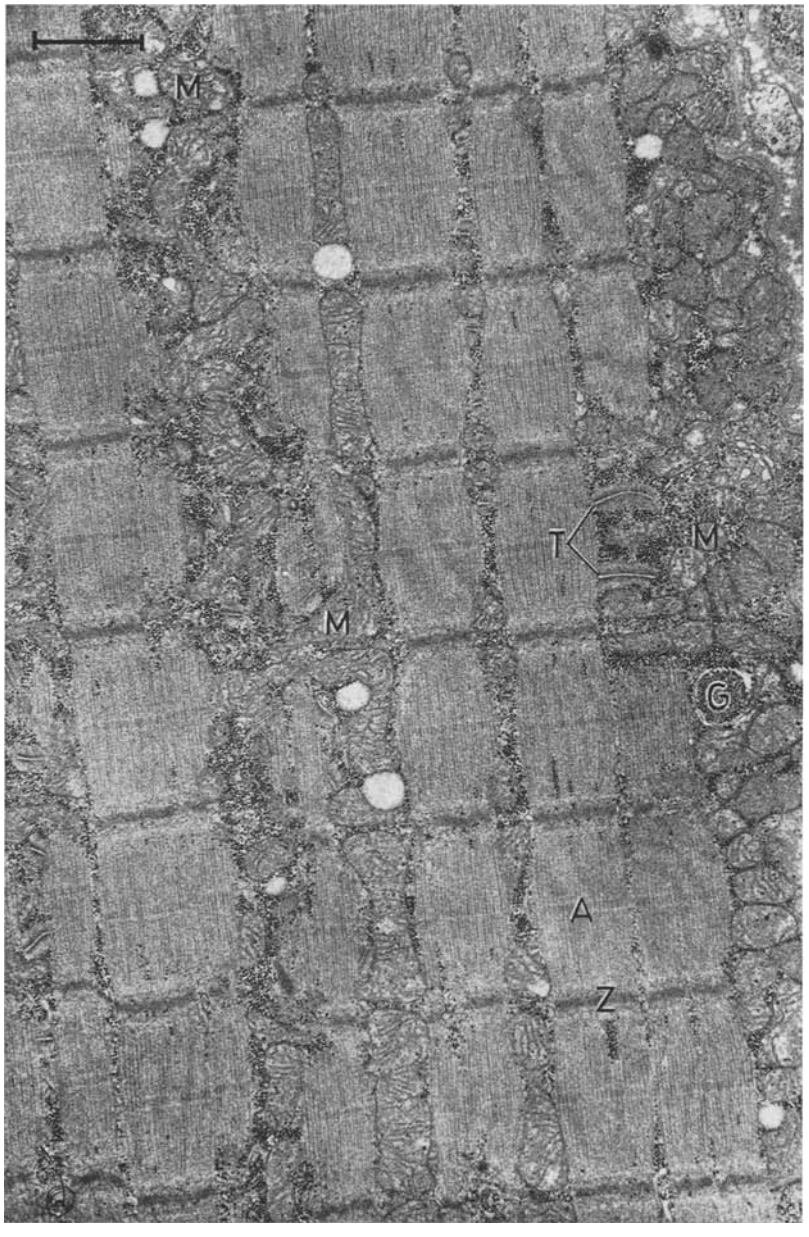
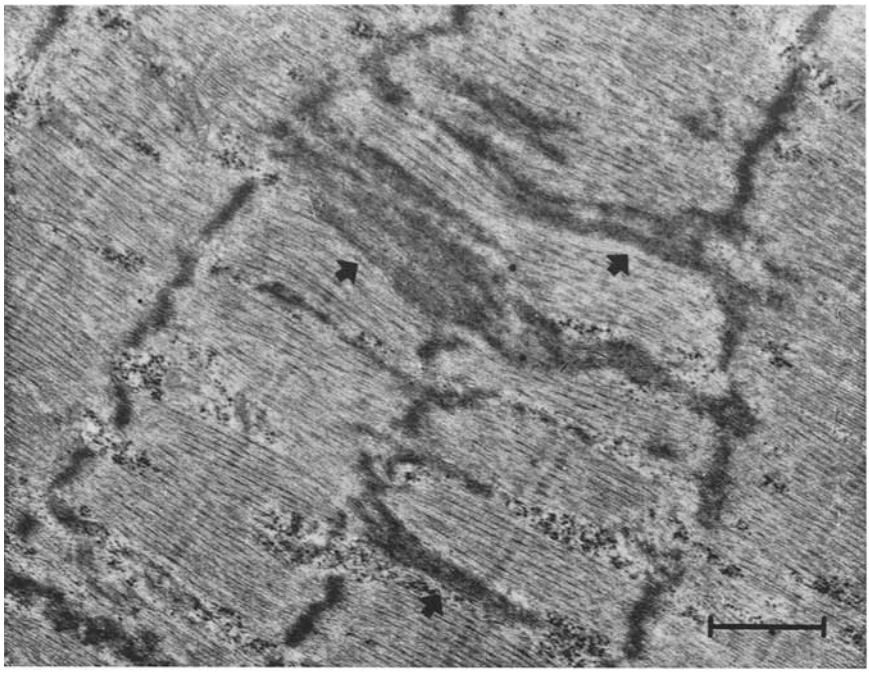


Fig. 4. Longitudinal section of a red fiber. The fibrils are well packed together. Large and numerous mitochondria (*M*) are located in the interfibrillar spaces and beneath the sarcolemma. *T* triad, *G* glycogen granules, A A band, *Z* Z-line. × 14000

few and small. In the hypothyroid myopathy they present a very electron dense matrix and short cristae. Often the membrane is altered and they are vacuolized. Their size seems to be normal while their number appears increased: masses of



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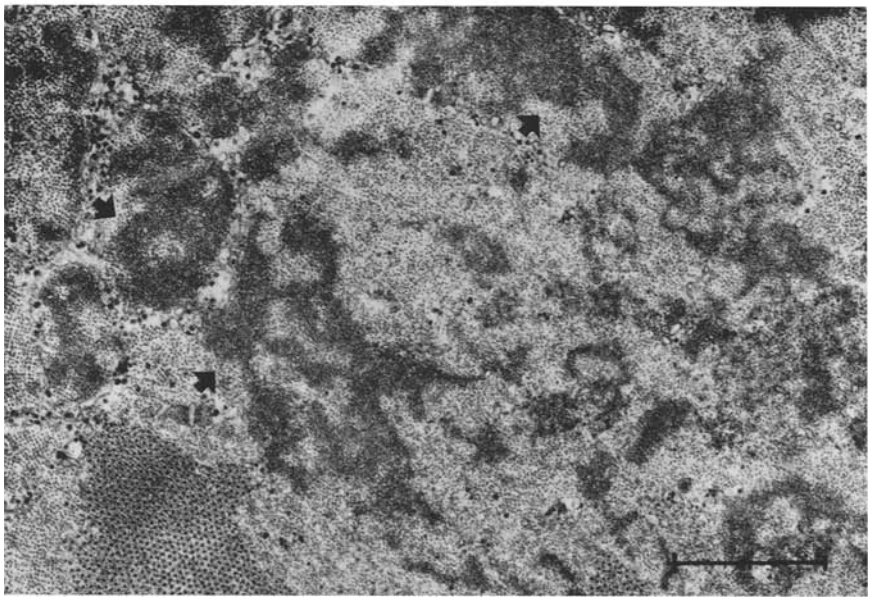

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Fig. 5. Longitudinal section of a red fiber. An early stage of alteration of the Z-line structure with blurring of Z-line material in the sarcomere (arrows). × 15000

Fig. 6. The cross section shows the alteration of the Z-line structure (arrows). Its material and the contractile apparatus are undergoing disorganization so that the fibrils fuse together. Mitochondria have disappeared. × 20000

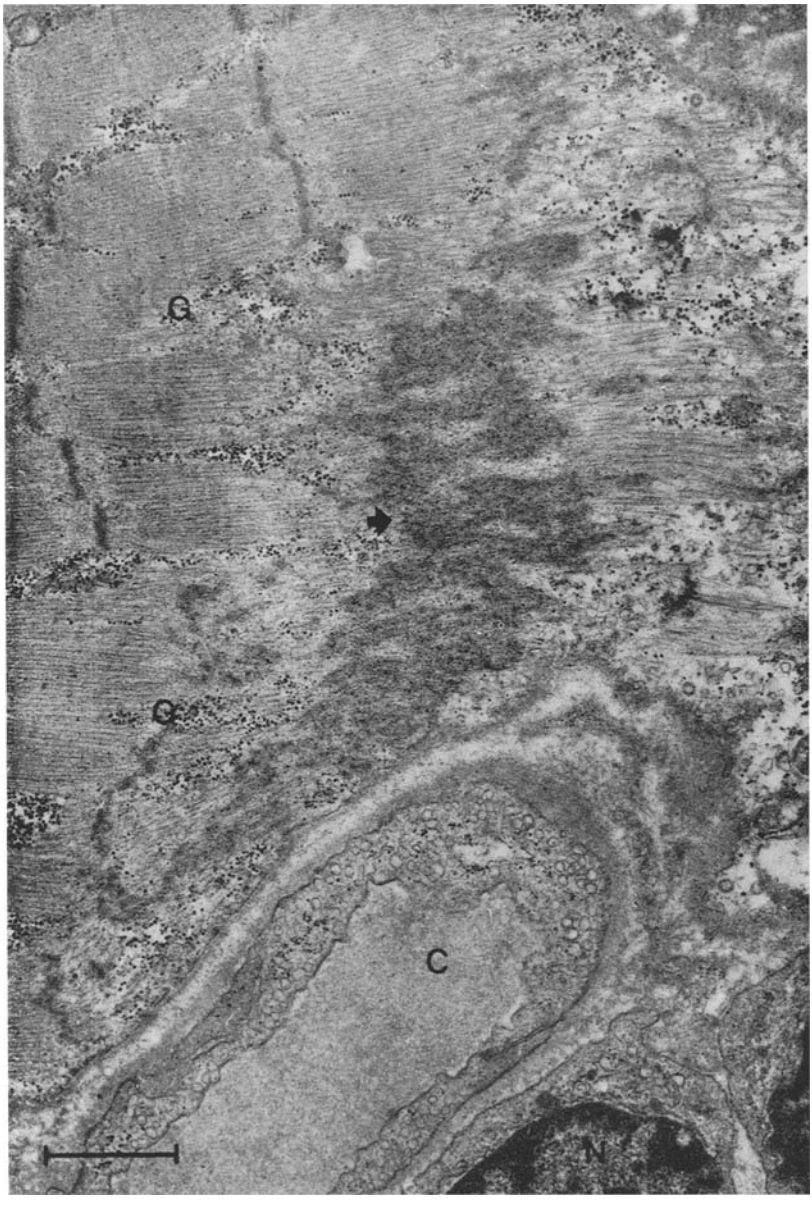

Fig. 7. Longitudinal section through the periphery of a fiber. A large area of degeneration with desorder of the Z-line material and disarray of the filaments. *G* glycogen granules, *C* capillary, *N* nucleus. × 18000

these organelles can be found beneath the sarcolemma. The red fibers mitochondria are more severely injured. They are larger, occupy regularly the interfibrillar spaces and show well developed cristae. In our muscles the mitochondria alter whether their shape or their volume. The cristae are hyperdeveloped,

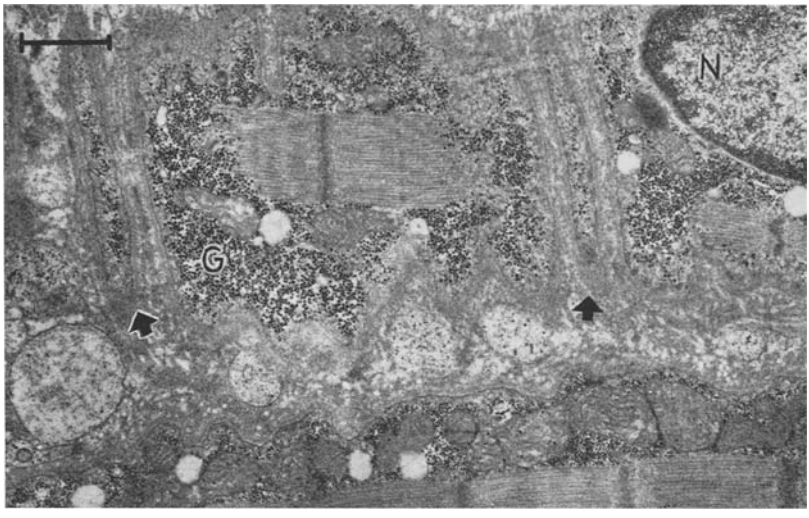

Fig. 8. In this longitudinal section the sarcolemmal invaginations go deeply into the fiber to divide it in blocks (arrows). The basement membrane follows the sarcolemma. *N* nucleus, *G* glycogen granules. × 12000

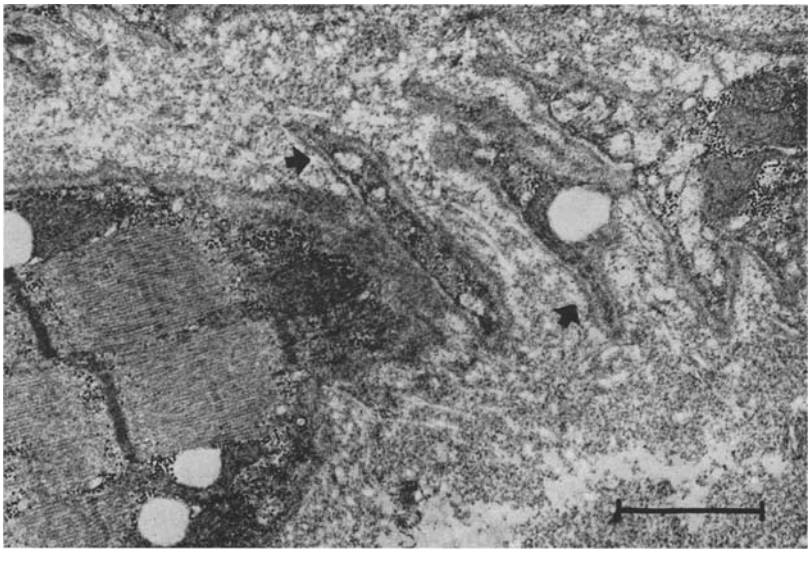

Fig. 9. Areas of sarcoplasm pinched off from the fiber (arrows). Finely granular material is seen around the fiber. × 15000

more numerous and often show an atypical arrangement, forming complex networks simulating an honeycomb structure. Otherwise some mitochondria present fragmented cristae; the matrix is fairly electron dense, the membrane altered. Several mitochondria are swollen and larger than normal: frequently a large vacuole, containing none or glycogen granules, occupies the whole mitochondria (Fig. 10).

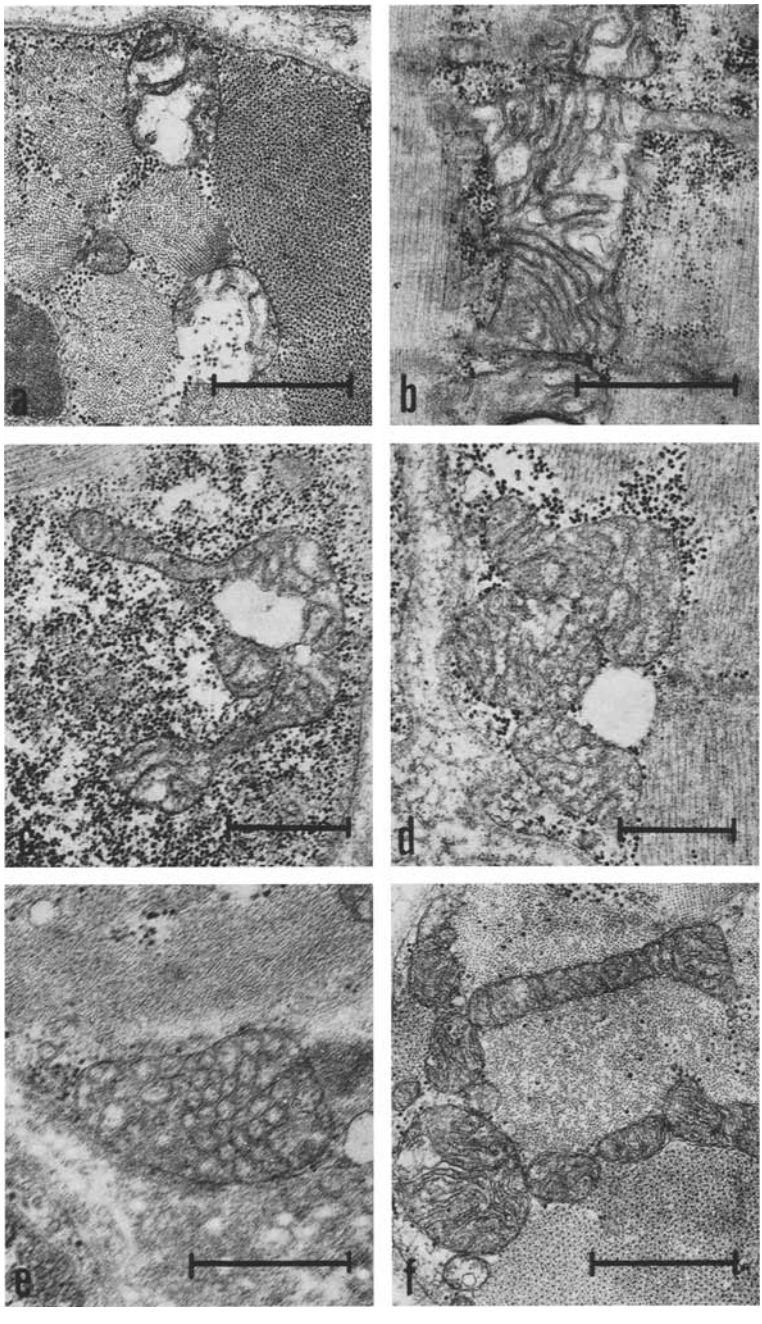

Fig. 10a–f. The mitochondria display different aspects of alteration. Scale 1 μ

As regards other cell organelles their response to the pathological stimulus is the same in both red and white fibers. The nuclei are deeply indented so that in many tangent sections they appear divided in several lobes separated by the

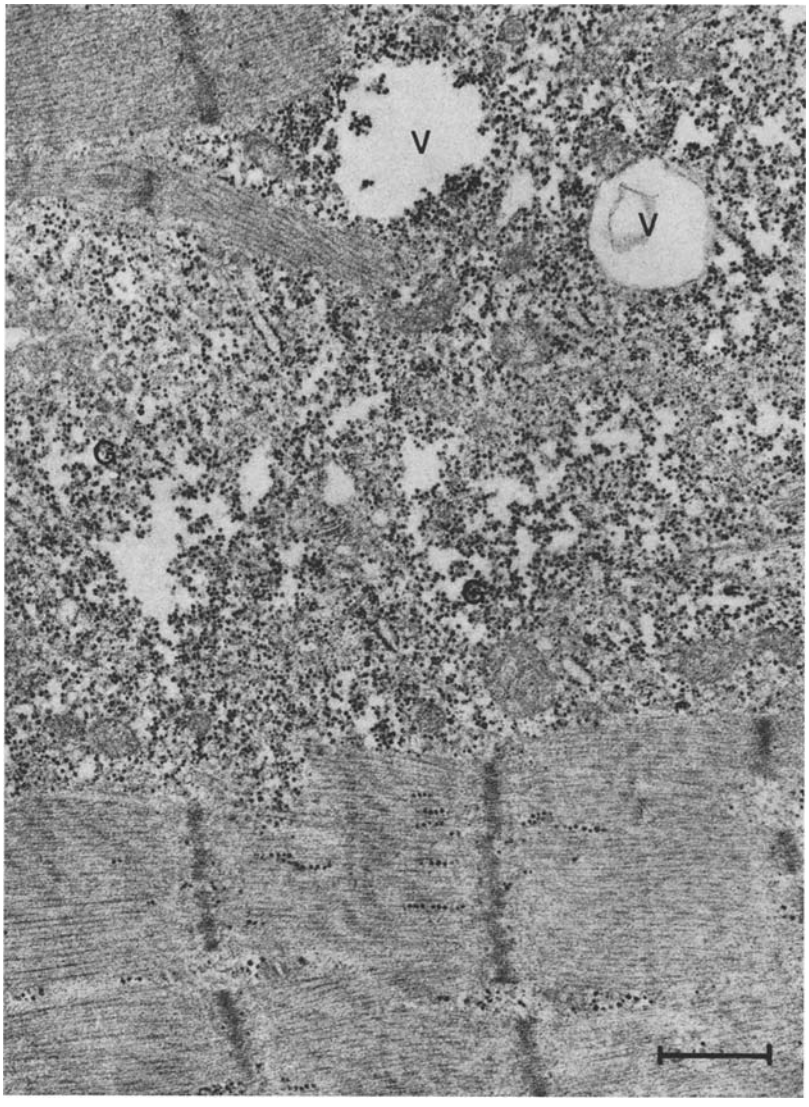

Fig. 11. Longitudinal section of a fiber. Large deposit of glycogen granules (*G*) between the fibrils. *V* vacuoles. × 14000

sarcoplasm. Generally they are located at the fiber periphery and show clear signs of atrophy. Lysosomes are present whether in the perinuclear regions or among the mitochondrial masses at the fiber periphery (Fig. 3). They are more numerous and of a greater size than in normal muscle fibers. The sarcoplasmic reticulum and the T-system are well preserved except that, sometimes, their elements are dilated and dislocated. Numerous vacuoles, varying in size, are easily seen between the fibrils and beneath the sarcolemma. The smaller ones are enlarged sarcoplasmic reticulum cisternae; others are autophagic vacuoles or secondary

lysosomes (De Duve and Wattiaux, 1966; Hruban *et al.*, 1963). The glycogen granules are abundant and occupy the same location as in normal fibers. Moreover, extensive amounts of glycogen granules are quite common feature beneath the sarcolemma, in the perinuclear region and between the fibrils (Fig. 11). It seems likely that the glycogen content of the fibers increases in the congenital hypothyroidism myopathy. Peculiar aspect and modifications are shown by the satellite cells. These cells are normally small, located between muscle fibers and the basement membrane of the muscle fibers themselves. In longitudinal section, they are spindle-shaped along the muscle fiber with a large nucleus. In cross section the cytoplasm forms a tiny "ring" around the nucleus. In our muscles, the satellite cells are numerous, their cytoplasm is more abundant than normal and shows numerous ribosomes, whether free or aggregates to form polysomes, and mitochondria. The rough endoplasmic reticulum cisternae are often enlarged and filled by a fairly electrondense material. The most interesting feature is represented by the presence of bundles of filaments in the cytoplasm of most cells.

Discussion

It is accepted that the muscle fibers, as regards the ultrastructure, responde quite uniformely to a great variety of diseases. The present ultrastructural study on muscles of patients affected by congenital hypothyroidism seems to confirm the rule, bringing out modifications of almost all the cell components. Modifications, which, on the other hand, failed to be stressed by the light microscope.

As regards the alterations of the contractile material, these develop mainly by two regressive processes already known from the experimental muscle pathology. Such processes, one reductive the other degenerative, differ because of whether their characteristics or the fiber type interested. While the reductive is obviously very slow, interesting few filaments progressively in each fibrils and is especially represented in the white fibers, the latter is accompanied by the dedifferentiation of all fiber components and involves large areas of the fibers, mainly of the red ones. Both processes lead to reduce the amount of the contractile material and consequently the volume of the fibers themselves (Pellegrino and Franzini, 1963; Gori *et al.*, 1965; Gori *et al.*, 1967; Gori, 1967; Gori, 1969; Pellegrino and Franzini-Armstrong, 1969, for a rev.). As regards the muscles observed, while the reductive process is more widespread and generalized to most of the fibers, the degenerative one, whatever present, is limited to small areas and to few fibers. Besides, it seems of interest to note the selective response of the contracile material in the two types of fibers to the same pathological cause: the white fibers undergo atrophy more constantly then the red ones, which only sporadically are affected by the degenerative and/or the reductive process. It seems likely that this peculiarity may be related to the morphological and metabolical differences which characterize the two fiber types. Although the contractile material is seriously damaged, we did not find necrosis of the fibers as reported in muscles of patients affected by myxoedema or other similar thyroid disorders. In these cases, otherwise, the contractile apparatus maintained a normal ultrastructure (Astrom *et al.*, 1961; Norris and Panner, 1966;

Pearce and Aziz, 1969). Such contrasting finding might referred whether to the time in which the lack of the thyroid hormone started or to the age of the patients. It known that the effects of the hormone want are strictly conditioned by the age (Wilkins, 1967).

As regards the sarcoplasmic reticulum and T-system there is no evidence of a serious damaging of these structures with the exception of an occasional displacement. It is well known the role of the sarcoplasmic reticulum in the glycogen synthesis (Margreth *et al.*, 1963; Andersson-Cedergren and Muscatello, 1963). We observed glycogen accumulation whether in the widened intermyofibrillar spaces or beneath the sarcolemma at the fiber periphery. On the basis of purely morphological data we may suggest that the glycogen excesses are not due to a damaged synthesis but more likely to an altered glycolithic pathway. We found small membrane-bound vacuoles filled with glycogen, altered mitochondria containing glycogen. The finding reminds the glycogen sequestrations characteristic of type II glycogenosis in which the glycogen accumulation was related to the deficiency of a lysosomal enzyme with a limiting function of the glycogen accumulation (Baudhuin *et al.*, 1964; Smith, 1967). The several ultrastructural lesions of the mitochondria suggest disorders of the oxidative metabolism. Hyperplasia of the cristae, probable increase in number of these organelles and degenerative features are constantly detected. Similar aspects were reported in the myxoedema and in the thyreotoxic myopathy and were inferred to the influence of the thyroid hormone on the oxidative metabolism (Norris and Panner, 1966; Engel, 1966; Hudgson and Pearce, 1969). Increase on both number and size of these organelles was reported in rat muscle after thyroidectomy and interpreted as a "compensatory" effect of the thyroxine hormone lack (Gustafsson *et al.*, 1965). Though we do not have quantitative data on the increase in number of the mitochondria, we observed that the fibers appeared richer of these organelles than the normal fibers.

In most fibers the sarcolemma was modified: invaginations were distributed along the fiber length and at the fiber apices. Similar features have been found in many experimental muscle atrophies and in the thyreotoxic myopathy. (Pellegrino and Franzini, 1963; Gori *et al.*, 1965; Engel, 1966; Gori *et al.*, 1967; Gori, 1967, 1969). In the latter they have been suggested to be significant for the pathogenesis of the syndrome together with the degenerative changes of the mitochondria, because of the relationship between the sarcolemma-impulse conduction and the mitochondria-oxidative metabolism (Engel, 1966). The basement membrane appears constantly thickened and often loses its relationship with the sarcolemma, forming deep folds in the interfiber spaces. This modification seems to be characteristic of the denervation atrophy and suggested to be a result of the atrophy of the muscle fibers, i.e. of the reduction of the volume (Birks *et al.*, 1959; Gori, 1969; Miledi and Slater, 1969; Gori, 1970). Signs of the denervation (namely a segmental demyelination) have been observed in cases of myxoedema and referred to a peripheral neuropathy (Pearce and Aziz, 1969).

It is of interest to stress the modifications of the satellite cells which are suggested to be "dormant myoblasts" playing an important role in the regeneration of the muscle fibers (Mauro, 1961; Laguens, 1963; Muir, 1965; Shafiq *et al.*, 1967). We observed many satellite cells with an increased amounts of cytoplasm

and of ribosomes. The most striking finding was the presence of small bundles of filaments irregularly arranged. This led to hypothesize that the satellite cells are losing their "dormant" characteristic and are developing in myotubes. It seems to be confirmed, however, a possible regeneration of the muscle fibers, according to the data reported for the myxoedema and for the thyreotoxic myopathy. (Norris and Panner, 1966; Pearce and Aziz, 1969).

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