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An Electron Microscopic Study of Muscle in Werdnig-Hoffmann's Disease

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Summary. An ultrastructural study of the quadriceps muscle of a $6^1/_2$ month old infant with Werdnig-Hoffman's disease has been made. The diagnosis was established on the basis of clinical and laboratory findings, the electromyographic examination and the light microscopic post-mortem study of the spinal cord and different muscles of the extremities.

The fine structural changes of denervation observed in the different components (myofibrils, nucleus, sarcolemmal sheath) of the muscle fibers proper are comparable with those previously described by other authors. The most striking feature in this study is the relative high number of cells which fulfil the criteria given by Mauro (1961) and which are designated satellite cells.

Cells lying free in the interstitial spaces or in proximity of capillaries and showing great morphological resemblance to "pericytes" are thought to be precursors of the satellite cells. The findings further suggest that these satellite cells may assume myoblast-like properties and that regeneration or restoration could be brought about by activity of these cells.

No sufficient evidence can be given that the presence of satellite cells and the submicroscopic changes of the contractile component of the muscle fibres in Werdnig-Hoffmann's disease are due to or represent arrest of maturation.

Introduction

Relatively few studies have been made on the ultrastructure of striated voluntary muscle of human beings with neurogenic muscular atrophy. To our knowledge, observations dealing with the fine structure of muscle in Werdnig-Hoffmann's disease and related conditions, e.g. Kugelberg-Welander syndrome, atrophy of Aran-Duchenne type, have been published respectively by Shafiq *et al.* (1967a), Hausmanowa-Petrusewicz *et al.* (1968), Hughes and Brownell (1969), Roth *et al.* (1965), and Wechsler and Hager (1962). Furthermore, in none of these studies mention has been made of the occurrence of so-called satellite cells in voluntary muscle undergoing denervation.

The present communication deals with the fine structural appearances in the muscle of a six-and-a-half-month-old infant with the clinical features of Werdnig-Hoffmann's disease. Special reference is made to the impressive number of satellite cells observed by us and the problem of their origin and significance will be discussed.

Material and Methods

Muscle tissue was removed from the right quadriceps muscle 40 minutes post-mortem. The specimen was minced in an ice-cold 2 per cent veronal-acetate buffered osmiumtetroxide solution and fixed for $1^1/_2$ hour. This was followed by dehydration in a graded series of ethanol and embedding in Epon 812. Thin sections were stained with aqueous uranyl acetate and lead citrate and examined with a Philips EM 300.

Clinical Details[1]

The patient was admitted for the first time when five months old and was noted to be unable to lift his head and to make spontaneous movements of the arms and legs. Family history was non-contributory. His mother had stated normal fetal movements during her pregnancy.

Physical examination revealed a well-developed and well-nourished boy, showing weakness and diminished tone of the muscles of the extremities. The stretch and tendon reflexes were absent. Some movement of the feet and hands was still possible. No sensory defects could be demonstrated. Neither fibrillations nor fasciculations were seen. He cried rather weakly. His intelligence appeared to be normal. Electromyography gave evidence of lower motor neuron involvement.

Lactic dehydrogenase activity was 190 mU/ml[2]; the levels of SGOT and SGPT were 33[2] and 5 U[2] (Wroblewsky) respectively. Creatine phosphokinase was 9.0 mU/ml[2]. There was no evidence of a primary myopathy.

The child manifested repeatedly respiratory complications and died in the sixth week after admission.

Observations

The principal findings of the necropsy and histological examination included purulent bronchitis, early bronchopneumonia and patchy atelectasis of both lower lobes of the lungs. There was moderate fatty metamorphosis of the liver. The brain weighed 900 g and coronal sectioning showed slight edema. Histologically, the sections from many spinal cord segments revealed a reduced number of neurons in the anterior grey horns. In addition, some of the neurons still present displayed degenerative changes such as vacuolisation of the cytoplasm or pycnosis of their nuclei. There was no inflammatory infiltrate.

Light Microscopy of the Skeletal Muscle

Transverse and longitudinal sections show typical neurogenic lesions with small or large clusters of atrophic fibers adjacent to groups of normal and/or slightly hypertrophic fibers (Figs. 1 and 2). Uniformly atrophic fibers juxtaposed to a few larger fibers can also be found within the same muscle bundle. Data about the range of the diameters of cross-sectioned fibers are given in Fig. 3. A moderate number of peri- respectively endomysial collagen fibers and fat cells can be present. Neither neutrophil leucocytes nor degenerative changes are observed.

Electron Microscopy

At low magnification, the presence of small, denervated fibers surrounded by or dispersed among normal fibers is a submicroscopic finding common to all the thin sections examined (Fig. 4).

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1 We wish to thank Dr. M. C. B. Loonen (Department of Pediatrics, University of Nimegen), who provided the clinical data.

2 Within normal limits of our laboratory.

Figs. 1 and 2. Light micrographs of a transverse respectively longitudinal section of the right quadriceps muscle. They show that the majority of the muscle fibres are atrophied. Thin epon section; toluidine blue stain. ×210

Fig. 3. Histogram demonstrating the range of the diameters of cross-sectioned muscle fibers of the patient and of an infant at the same age without muscle disease. 1013 fibers were counted in each case

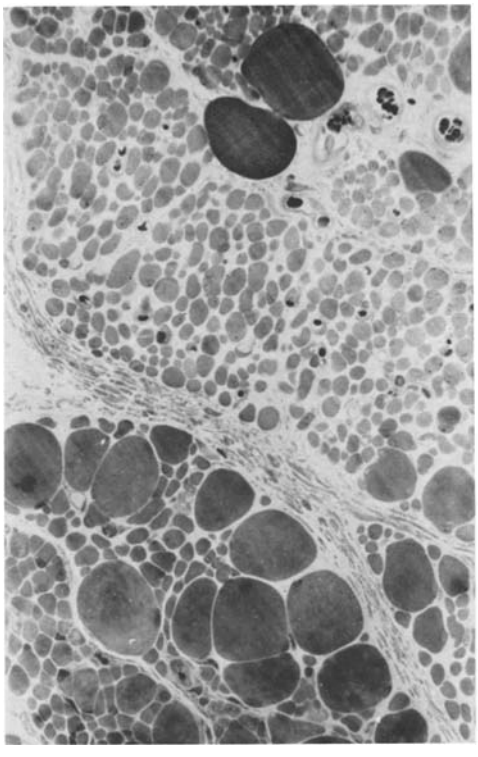

Fig. 1

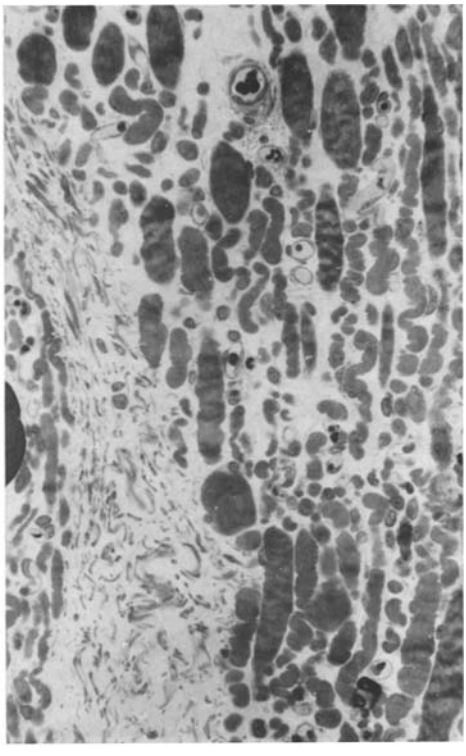

Fig. 2

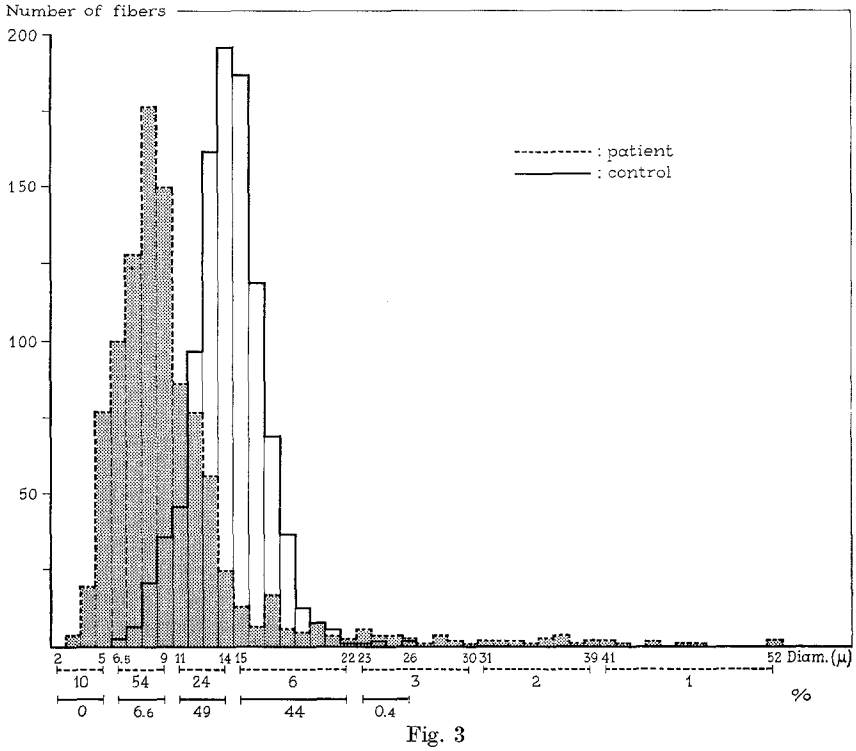

Fig. 3

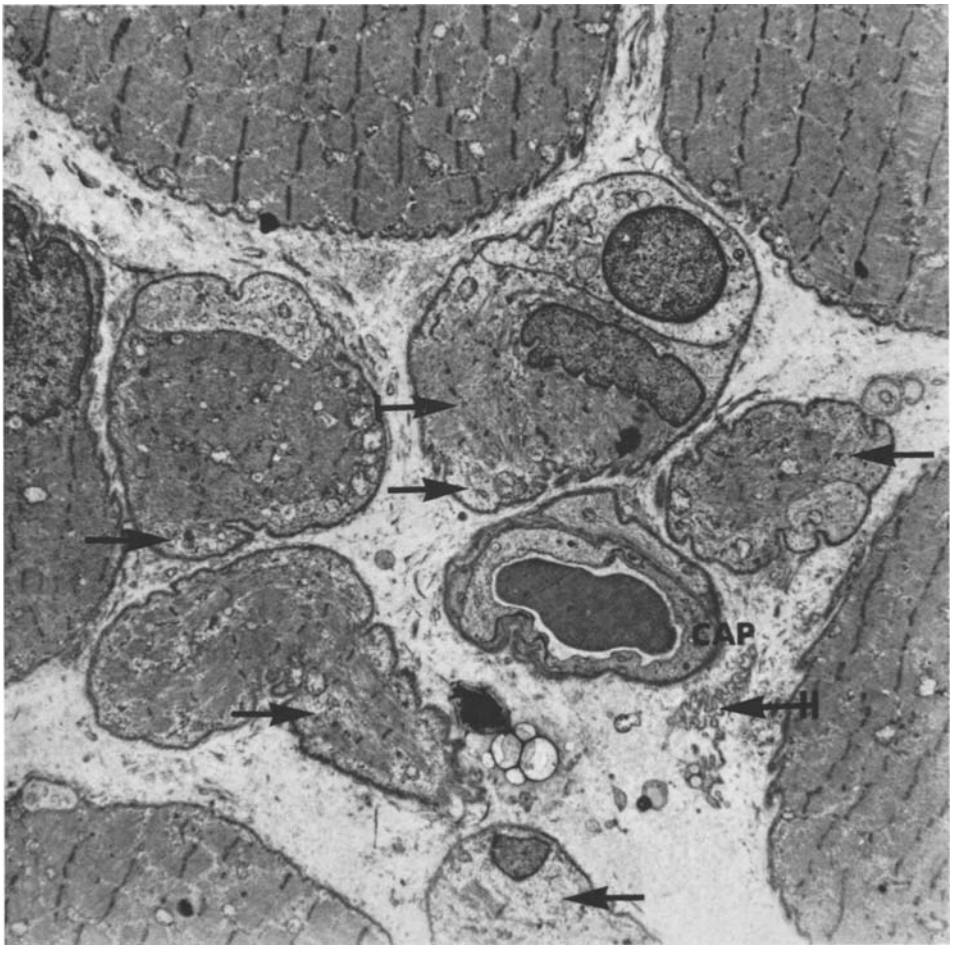

Fig. 4. The center of the picture shows muscle fibres in various stages of denervation. In these atrophied fibres myofibrils have disappeared (→) and the surviving filaments are disorientated (↦). Basement membrane material of a sarcolemmal sheath lies free in the interstitial space (↦). *Cap* capillary. ×4200

Longitudinal or slightly oblique sections of these abnormal fibers show a most striking change, namely diminution of the width of the myofibrils due to loss of the constituent myofilaments. Both lateral and central fibrils are involved in this process. The loss of filaments is patchy in distribution or can even be limited to

Fig. 5. Part of a muscle fibre with focal loss of myofilaments. Remnants of Z-bands are visible as irregular densities. Some mitochondria (*M*) are swollen. *S* sarcolemma. ×13700

Fig. 6. Local accumulation of organelles of lysosomal nature. Part of them contain osmiophilic lamellated material (→). In between the lysosomes glycogen particles can be detected. ×17400

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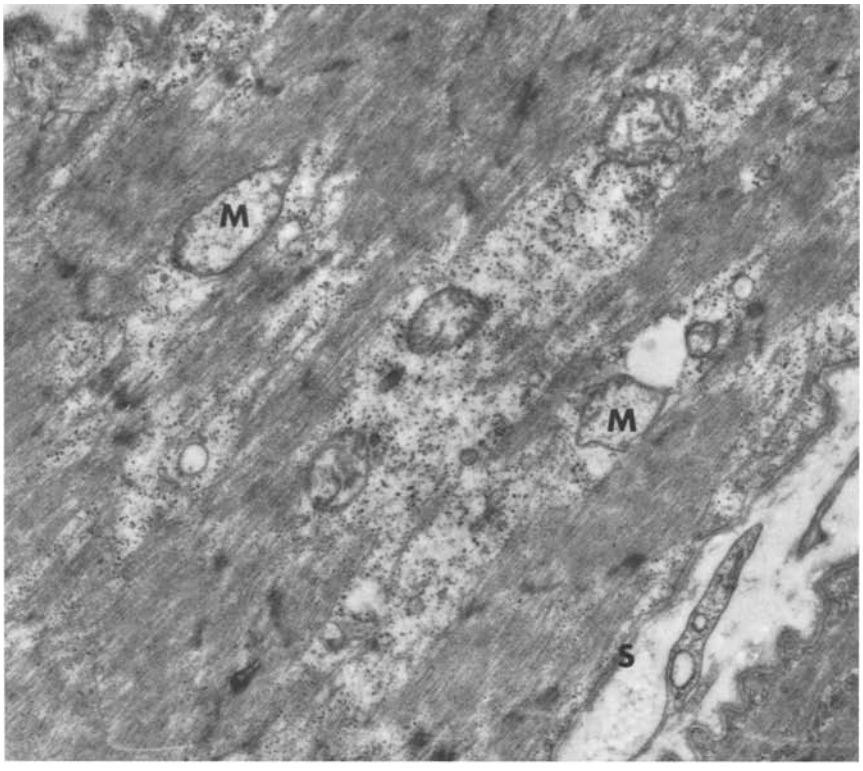

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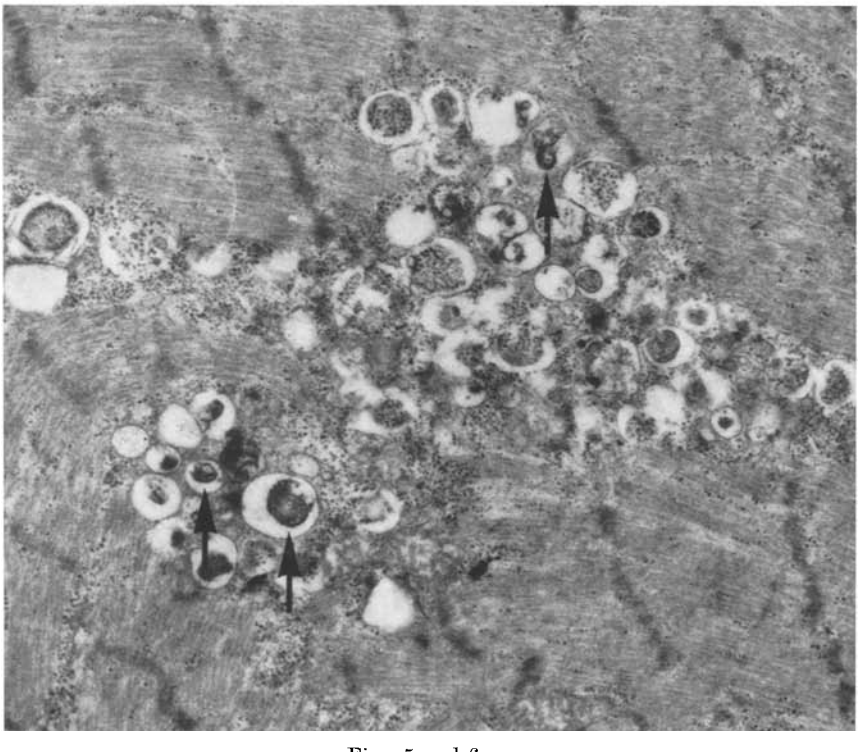


Figs. 5 and 6

one sarcomere. In addition, numerous filaments appear to be frayed and disorganized (Fig. 5). The spaces between the surviving fibrils or filaments are widened to varying degrees and contain in the clear sarcoplasmic background some glycogen particles, a few mitochondria and, less frequently, secondary lysosomes which are partly filled with osmiophilic material (Fig. 6). The overall substructure, i.e. intact sarcomeres with visible A, I, Z and M bands, appears to be well preserved in many fibres or parts of them. However, disorganisation and blurring of the band structure can be encountered as well. In that case, deformed Z-lines or remnants of these lines lie scattered throughout the sarcoplasm and A and I bands can no longer be discerned. In some fibers a more or less well circumscribed dense core of fine fibrillar material lacking evident periodic pattern and presumably derived from Z-lines, is present. An intermediate light zone (halo) of radially oriented myofilaments is immediately adjacent to the dense core (Fig. 7).

The nuclei, which sometimes are located deep into the abnormal muscle fibres, display a very irregular form with complex infoldings or outpouchings of their membrane. In some instances, a few closely apposed nuclei or possibly separate masses of the same segmented nucleus can be detected (Fig. 8). One or two well developed round or oval nucleoli with dense outer and light inner regions are usually situated in the center of the nucleoplasm.

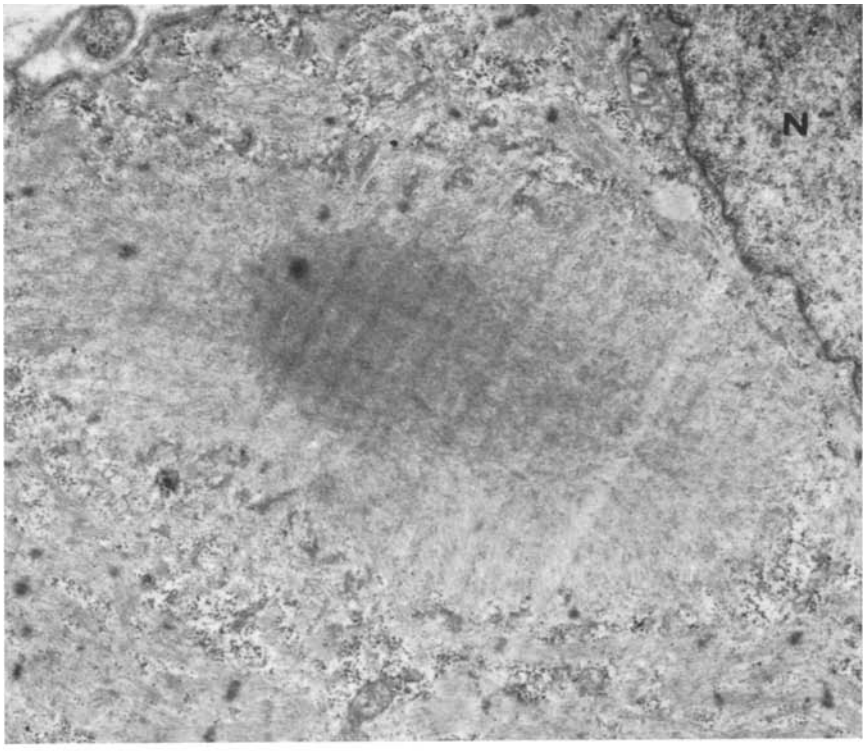
An accentuated tortuous folding or saw-tooth appearance is visible over varying distances of the sarcolemmal sheath. The two components of this sheath, namely the inner dense sarcolemma and the outer less dense basement membrane, remain mostly closely applied to each other. In some places, however, the inner and the outer layer become dissociated and even loose scalloped basement membrane material can be encountered in the interstitial spaces (Figs. 4 and 13c).

The outstanding and interesting finding in our material is the relative large number of cells which fulfil the criteria given by Mauro (1961) and which are designated "satellite cells" (Fig. 9). They occur both in very atrophic muscle fibres and, less frequently, in normal or hypertrophic fibres. The preliminary results of counts on low-powered electron micrographs taken at random of non-serial sections from 13 different tissue blocks are given in the table. The separate

Table

Nuclei		
Total number	of muscle fibers	of sat. cells
94	55	39 (41%)
Muscle fibers		
Total number	without sat. cell	with sat. cell
95	49	46 (48%)

cells are situated between the basement membrane and the sarcolemma of the associated muscle fibres. They have their own plasma membrane which in places shows half-desmosome-like structures (Fig. 9C). The two cell membranes run relatively straight or show slight interdigitation, and are separated from each



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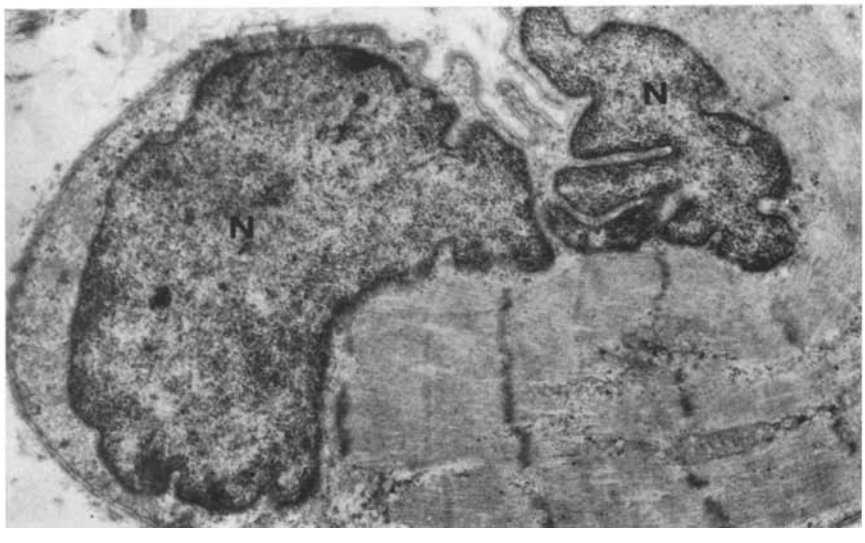

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Fig. 7. A cytoplasmic body (?) occupies the center of this picture. The dense core is surrounded by a halo of more or less radially arranged thin filaments. The latter filaments are continuous or seem to merge with myofilaments. *N* nucleus. ×10400

Fig. 8. Electron micrograph which shows either the separate parts of a divided sarcolemmal nucleus (*N*) or the result of section of a nucleus with a very irregular outline due to contraction of the muscle fibre. ×12200

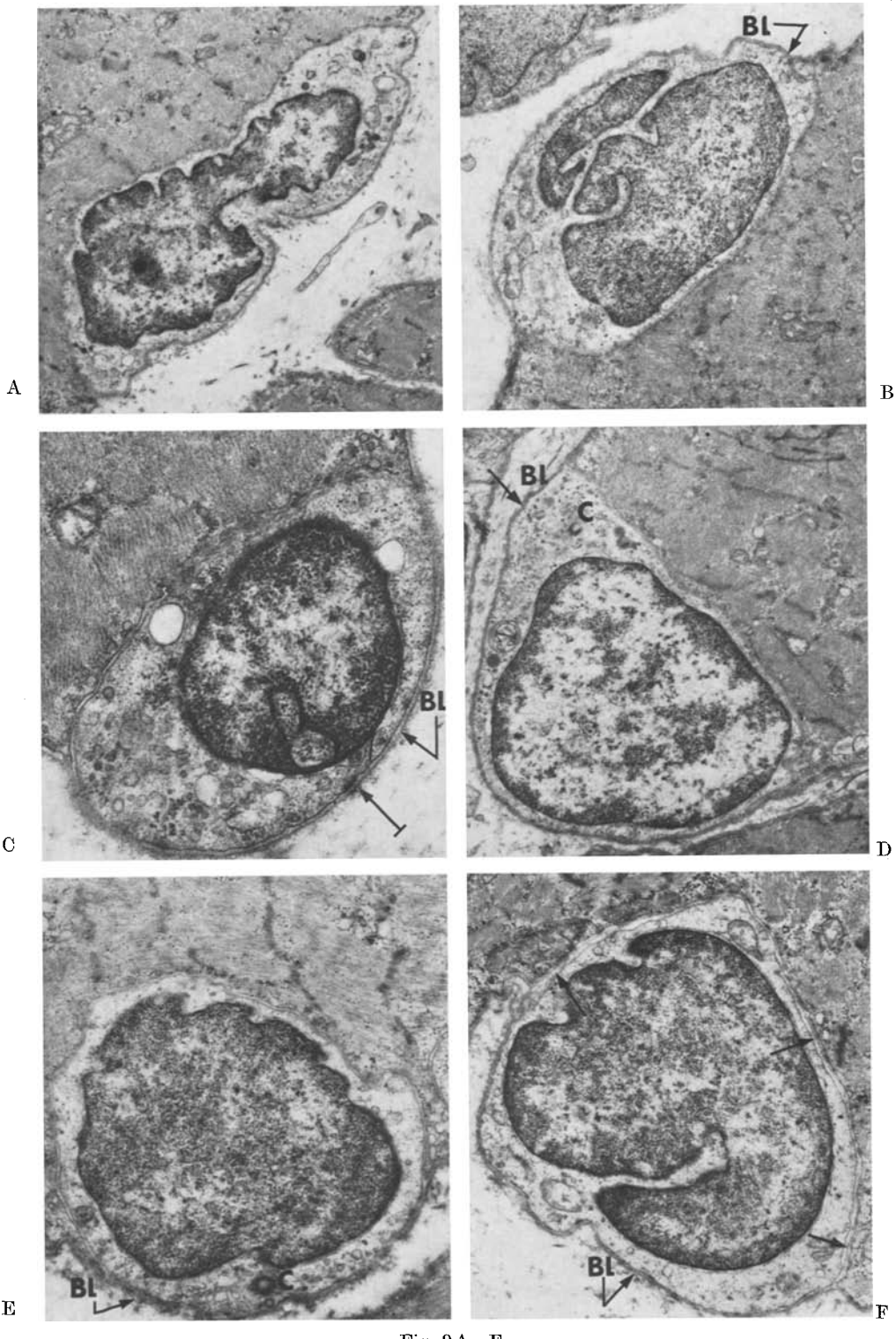
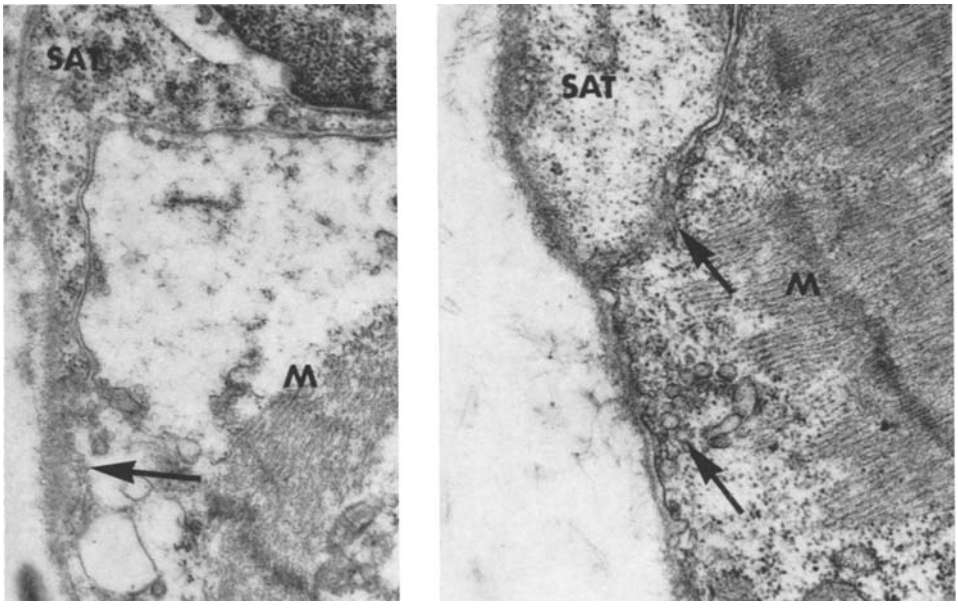


Fig. 9A—F



A B

Fig. 10A and B. Micrographs to demonstrate possible confluence between satellite cell (*SAT*) and muscle fibre (*M*) plasmalemmae and the accumulation of small vacuoles in these areas (→). A ×22000; B ×30000

other by a space of about 250 Å. Basement membrane material can extend into this interspace at one or both peripheral edges of the cell (Fig. 9F). Laterally the plasma membranes sometimes appear blurred out or have disappeared and a cluster of small vesicles is seen in the immediately adjacent sarcoplasm (Fig. 10A and B). The relatively large nucleus with prominent amounts of chromatin mostly displays a very irregular form with an infolded nuclear membrane and pseudo-inclusions (Fig. 9C). The cytoplasm is clear and contains solitary or grouped dense granules considered to be ribosomes. In addition, some mitochondria, a few irregularly dispersed profiles of smooth-surfaced endoplasmic reticulum and especially centrioles are present (Fig. 9D and E). Contractile elements are barely discernible in the cytoplasm. As can be seen in Fig. 9B, protruding of the satellite cells can really distort the external surface of the associated muscle fibre. Extreme examples of this situation are illustrated in Figs. 11 and 12, which show

Fig. 9A–F. These micrographs demonstrate six different satellite cells lying beneath the basement laminae (*BL*) and in apposition to the muscle fibre plasmalemmae. The relative large nuclei show an irregular outline and a complex infolding of the nucleoplasm. Centrioles (*C*) are present. At the peripheral edges basement lamina can be seen interposed between the satellite cell and the muscle fibre (Fig. 9F→). In Fig. 9C structures resembling half-desmosomes (↦) seem to be present at the plasma membrane of the satellite cell. A×6000; B×9000; C×20400; D 7700; E×11800; F×9400

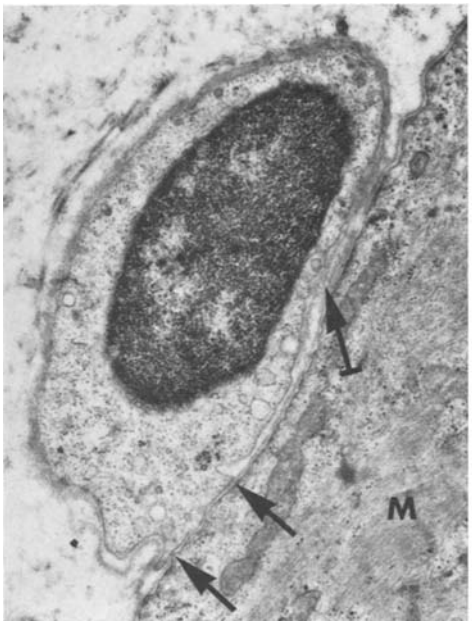


Fig. 11

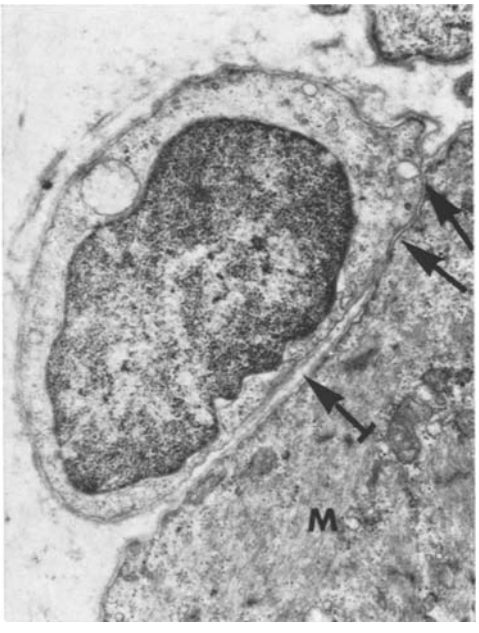


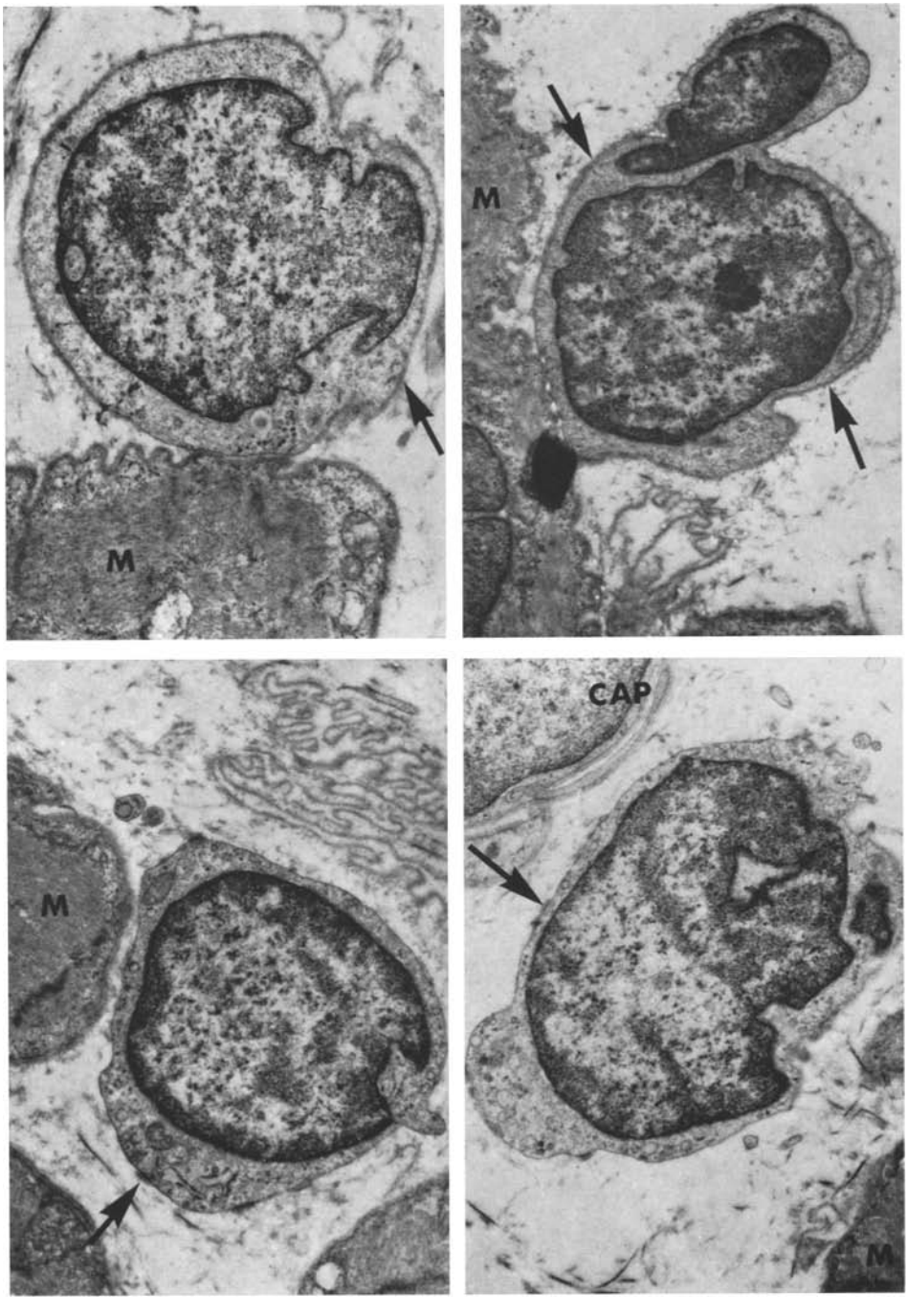
Fig. 12

Figs. 11 and 12. Satellite cells, almost completely surrounded by a proper basement membrane, lie in close apposition to a muscle fibre (*M*). Only in places (→) these cells contact the sarcolemma. The basement laminae run parallel or seem to merge with each other (↦). Fig. 11: ×13000; Fig. 12: ×12600

satellite cells almost completely surrounded by a basement membrane whereas their plasma membrane makes contact with the sarcolemma only over short distances. Parts of the two basement membranes run parallel to each other, but fusion between both seems to occur as well.

Finally, attention must be paid to the presence of round or slightly oval cells lying free in the interstitial space (Fig. 13), and preferably situated in proximity of a capillary and/or a muscle fibre (Fig. 14). These cells are partly or completely surrounded by a basement membrane (Fig. 13A and B). In places, half-desmosome-like structures can be detected and many caveolae are situated along the inner side of the plasma membrane. The small rim of cytoplasm contains a few mitochondria, ribosomes, centrioles and almost no granular endoplasmic reticulum. The huge nucleus is marked by an irregular outline and many indentations of the nucleoplasm.

Fig. 13A–D. Four cells lying free in the interstitial space and in proximity of a muscle fibre (*M*). In places a basement membrane can be detected (→) on their perimeter. Compare cytological characteristics of these cells with those of the satellite cells (Figs. 9, 11 and 12). Loose scalloped basement membrane material is seen in the upper right corner of Fig. 13C. *Cap* partly visible capillary. A×8500; B×8000; C×9200; D×7700


A
M
B
M
C
M
CAP
D
M

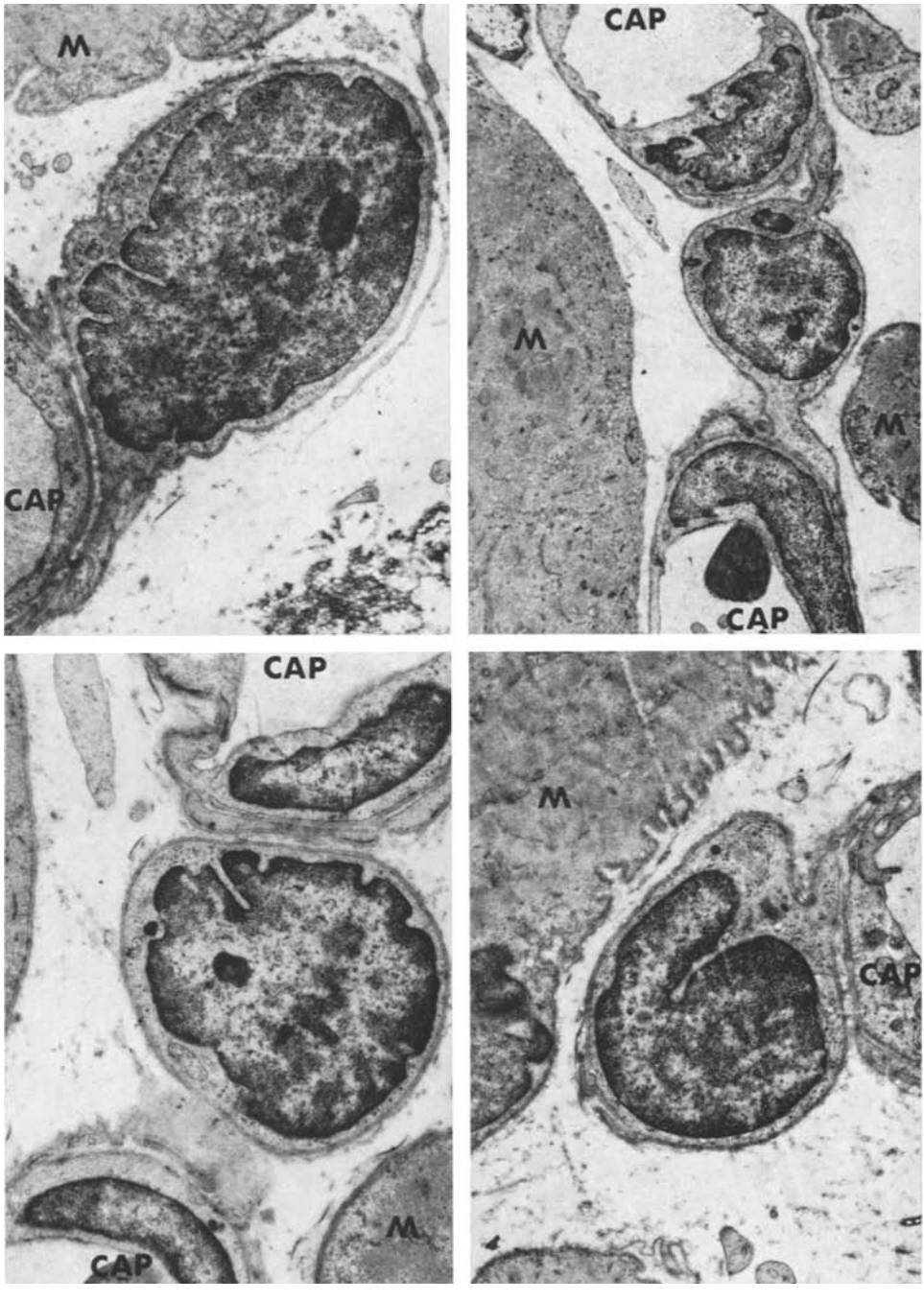


Fig. 14 A–D. These cells show great resemblance to those depicted in Fig. 13. In addition, they are located in proximity of a capillary (*Cap*) or close to a muscle fibre (*M*). They can be classified as "pericytes". A ×10400; B ×4600; C ×4300; D ×6800

Discussion

Most of our findings pertinent to the myofibrils and the sarcolemma complex are comparable with those reported by Shafiq *et al.* (1967a) and Hausmanowa-Petrusewicz *et al.* (1968), and with the observations recently published by Hughes and Brownell (1969), as far as they deal with the fine structure of muscle in Werdnig-Hoffmann's disease. The focal degenerative changes in our material (Fig. 7) can be compared with the lesion found by Shafiq *et al.* (1967a; Fig. 17) in various types of muscular atrophy, except his four cases of Werdnig-Hoffmanns disease, and resemble also the structural anomaly of the Z-disk recently described by MacDonald and Engel (1969; Fig. 7). In general, the fine structural alteration encountered so far in the contractile components of muscle tissue, bear no specific character and have no great significance in the differential diagnosis of muscular atrophies. From the literature it is obvious, that no unanimous answer is given to the controversial question whether conspicuous changes or division occur in the sarcolemmal nuclei of human muscle undergoing denervation. In this context, the frequent occurrence in our material of deeply infolded nuclei, respectively closely apposed nuclear portions underneath the sarcolemma, are in contrast to the findings of Roth *et al.* (1965) and Hausmanowa-Petrusewicz *et al.* (1968), but confirm the observations of Shafiq *et al.* (1967a) and Hughes and Brownell (1969). The question, however, whether these pictures represent true mitotic respectively amitotic divisions of the nuclei, as assumed by the last mentioned authors, or simply an effect of the plane of section of a severely contracted muscle fiber, could not be settled by us. Neither by light- nor by electron microscopy were mitoses observed by us.

The unique feature in our study is the frequency with which we encountered satellite cells. To the best of our knowledge, these cells have been described for the first time by Mauro (1961) in the sartorius muscle of the adult rat and in frog muscles. It is assumed now, that these cells are present in all vertebrate skeletal muscle (Muir *et al.*, 1965). Yet, only little information is available about the number of satellite cells in normal mature human voluntary muscle and about the relationship, if any, between their frequency and the age. This is probably due to the fact that by light microscopy the nuclei of the satellite cells are indistinguishable from those of the muscle fibers proper. Counts by Muir *et al.* (1965) on still maturing pectoralis major muscle from mice 3 days after birth showed that about one-fifth of the muscle nuclei belong to satellite cells. In his study on satellite cells in human gastrocnemius muscle in various fetal stages of development, Ishikawa (1966) states that as development proceeds, generally the number of satellite cells diminished in sections. Satellite cells are found very frequently in developing muscles, but rarely in mature muscles. Exact figues, however, are not given by the author.

When discussing our results with reference to the large number of satellite cells, we can take into account the fact, first, that the muscle tissue is obtained from a young patient and secondly, that most of these cells are found in fibres with marked atrophy. It may be reasonable to assume, that decrease in size of the fibre itself can lead to a relative increase of the satellite cells which normally are present. Thirdly, the findings described in the last paragraph of the results are equally of considerable interest when trying to explain the increase in number of the satellite cells and to gain insight into their origin. The cells depicted in Fig. 13 and 14 display an undifferentiated cytoplasmic matrix and seem to be of an unspecific nature. However, the irregular, deeply infolded nucleus, the presence of a basement membrane, which completely or partly surrounds the cell body, and their location in near proximity of a capillary or a muscle fiber

are remarkable. Sometimes their own basement membrane seems to merge with the basement membrane of the muscle fibre. It is unlikely that the cells just mentioned, are fibroblasts neither blood elements nor Schwann cells extending out from axon terminals. They rather resemble so-called pericytes or represent mesenchymal, interstitial elements with multiple capabilities of differentiation, whereby a myogenic property should be taken into account. We believe that these cells can be accepted as satellite cell precursors which, as suggested by Figs. 11 and 12, ultimately can move beneath the basement membrane of the muscle fibers. From this stage onward, the cells may be regarded as satellite cells since the criteria given by Mauro (1961) are then fulfiled. In addition, mitotic division seems possible as indicated by the presence of centrioles. This capability of mitosis has otherwise been stated by Shafiq *et al.* (1968).

With regard to the role of satellite cells, in particular the question of their differentiation into myoblasts under certain circumstances, it is interesting to note that the possibility of continuity or fusion of the cytoplasm of satellite cells with the sarcoplasm has been denied by Muir *et al.* (1965) and Ishikawa (1966). Some of our observations (Fig. 10 A and B), however, and the studies on hypertrophic human deltoid muscle (Reger-Craig, 1968) and on regenerating muscles (Allbrook *et al.*, 1966) as well as on developing muscle tissue (Shafiq *et al.*, 1968) favor the view that continuity is possible. Furthermore, some other data from the literature are worth mentioning in relation to the significance of the satellite cells. The cells described by Muir *et al.* (1965; Fig. 11) in differentiating muscle from postnatal mice resemble satellite cells in their shape, size and relation to basement membrane and are seen to contain a few (the first ?) myofibrils. Besides myoblasts, an increase in the number of satellite cells was noted by Shafiq *et al.* (1965) at the wound area of injured muscle of mice and in regenerating muscle of patients with Duchenne-type muscular dystrophy and polymyositis (Shafiq *et al.*, 1967 b). These authors state that in their general structure and peripheral position, the satellite cells are comparable to early myoblasts. Reger and Craig (1968) pointed out that the morphology of the satellite cells observed and their relationship with the muscle fibers are similar to fusing myoblasts which previously have been described for embryonic muscle. However, it must be stated here, that as yet no sufficient structural evidence for fusion of myoblasts has been reported. Recently Teravainen (1970) demonstrated an increase and subsequent decrease of satellite cells in extraocular muscles of the rat after slight compression injury.

The data mentioned above and our own findings present suggestive structural evidence that in pathological circumstances satellite cells, which in contrast to the previous examinations on atrophic muscle fibers (Shafiq *et al.*, 1967 a; Hausmanowa-Petrusewicz *et al.*, 1968; Hughes and Brownell, 1969), come in prominence in our study, can assume myoblast-like properties. Our findings are of value in this concern and lend further support to the concept that satellite cells give rise to new myoblasts during restoration respectively regeneration ("discontinous type") of striated muscle.

Finally, we want to dwell on the question if arrest of maturation could be a feature that must be taken into account when discussing the findings in Werdnig-Hoffmann's disease, particularly the relative high number of satellite cells.

It must be pointed out that loss of myofilaments, focal degenerative changes, basement membrane alterations, blurring of the band structure etc. constitute findings which can hardly be reconciled to this hypothesis. However, some of the small fibres encountered lack these changes and give rise to speculation about immaturity. These fibers are marked by an irregular nucleus with a distinct nucleolus, and by the accumulation of glycogen particles and ribosomes between the oriented myofibrils. They are covered by a basement membrane, but are not closely associated with each other. Such fibres are reminiscent of the formed muscle fiber according to Hay (1963) or of the muscle fibres described by Ishikawa (1966, Fig. 7 and 10) in human fetus. It must be stated, however, that fibres as described above, are rarely encountered in our material. Moreover, a satellite cell is not always found in association with such fibres.

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