AN ELECTRON MICROSCOPE STUDY OF DENERVATION ATROPHY IN RED AND WHITE SKELETAL MUSCLE FIBERS

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ABSTRACT

A study, mainly by electron microscopy, has been made on two leg muscles of rat, in the course of atrophy experimentally induced by total denervation. As a preliminary the chief distinctive features of the soleus, chosen as a representative of pure red muscle, and of the gastrocnemius, representative of pure white muscle, are described. Two major phases of atrophy, somewhat overlapping in time, were observed. In the first, a degenerative autolytic process takes place in areas of the fiber, with loss of striation. It can be detected as early as the 7th day, but the maximum is observed at the 14th day, and accounts for a gross weight loss of 50 per cent. The first alteration appears in the Z lines; disorder in the disposition of filaments then follows. The process occurs very rapidly, leaving large areas in the cell in which one can detect only ground substance, glycogen, rare randomly disposed vesicular elements, and some mitochondria. Several lysosomes and masses of lipoproteins, which assume the configuration of concentric lamellae, show up in these fibers. Subsequently large parts of the waste sarcoplasm are discarded into the intercellular spaces. In the second major phase the so called "simple" atrophy takes place. The process starts early, but its effects are more detectable after 1 month. In this period, single myofibrils undergo different degrees of reduction in diameter, while the spatial disposition of primary and secondary filaments inside the fibrils remains normal. The appearance of the fibrils in longitudinal sections suggests that the process takes place by the detachment of filaments from the periphery of the fibrils and by their subsequent breakdown in the interfibrillary spaces. The sarcoplasmic reticulum is still well preserved, and relatively overdeveloped. Mitochondria disappear in parallel with the contractile material.

INTRODUCTION

The effect on muscle of a deficiency in the motor nerve supply has been of primary interest in human pathology. Erb (18), Kopits (41), Chor (13), Bowden and Gutmann (7), Adams *et al.* (1), and Altschul (2, 3) all studied alterations in muscle histology in diseases which lead to the destruction of motoneurons or to lesion of the peripheral nerves. However, in human material the denervation is mostly incomplete and the time at which the alteration takes place is not always known. A clear correlation between the alterations observed and the lack of innervation is not possible in such material. For the experimental study of atrophy it is therefore useful to employ laboratory animals.

The method of choice in most studies has been to cut the nerve supplying one or a group of muscles and to study the alterations occurring in the respective muscles at various time intervals after the operation.

The most evident macroscopic change in a

muscle after denervation is a weight loss, which is directly related to the mean diminution in fiber diameter (Gutmann, 25). The first histological alterations in a muscle after nerve section appear in the motor end-plate after 2 or 3 days (Gutmann, 24; Chor, 13). Reger (64), in an electron microscope study, demonstrated that a recession of the terminal axon endings from the synaptic groove occurs within 48 hours following nerve section. A similar phenomenon was observed by Birks *et al.* (6) in a frog muscle. The authors described, at 3 days after nerve section, changes in the fine structure of the axon terminal and at 1 week a complete disappearance of the terminal. During the 1st week after nerve section no alteration in the muscle fiber were demonstrated by the light microscope. In 2 or more weeks, a detectable reduction in fiber size was noted. Although cross- and longitudinal striations are maintained even for a very long time (Richer and Ellenbeck, 65; Chor *et al.*, 15; Tower, 71), the striations fade and are difficult to detect (Krauss, 42; Chor, 13). A reduction in the number of mitochondria (Willard and Grau, 76; Nachmias and Padykula, 57) and a relative increase in the number of nuclei (Morpurgo, 56; Willard and Grau, 76; Wohlfart, 78) were also demonstrated. The first alterations and the course of events which lead to the diminution in fiber diameter and to the loss of contractile material are not clear. The observations of Wechsler and Hager (74, 75), in an electron microscope study of rather advanced stages of denervation atrophy, indicate that the fading of the cross-striations mentioned above is due to a diminution in diameter of the single fibrils. This leads to a masking of the bands by the relatively increased sarcoplasmic material which surrounds the fibrils. Only in very late stages (5 to 9 months) did Wechsler and Hager observe degenerative processes that destroyed the whole fibril.

Recent advances in electron microscopy have made possible a more thorough and detailed investigation of the fine structure of the normal skeletal muscle fiber, particularly of the myofibrils (Huxley, 36) and of the sarcoplasmic reticulum (Porter and Palade, 62). In view of the detailed information now available on normal muscle ultrastructure, a study of muscle after nerve section seemed likely to yield new information on the atrophy induced by denervation. Such a study would also provide information on the early stages of the process. Two types of fibers, the so called "red" and "white" (Kühne, 47), are present in skeletal muscle; thus it was considered of interest to study the process of atrophy induced by denervation in both kinds of fibers. The soleus muscle is composed of red fibers, and the medial head of the gastrocnemius of white fibers: we chose these two muscles for our study. A comparison of the normal structures of the two muscles is interesting in correlation with the difference in the mode of function of red and white fibers. This topic will be dealt with in the discussion.

MATERIALS AND METHODS

Adult albino rats weighing 100 to 300 gm were used for the experiments. The whole right leg was denervated by removing 1 cm of the sciatic nerve high in the thigh. At intervals of 8, 15, 30, and 60 days after the operation, the soleus and gastrocnemius muscles were carefully dissected from both legs, and their weight on the operated and contralateral sides was recorded. The site of the operation was exposed, in order to ascertain that no nerve regeneration had occurred. In late stages the proximal stump of the nerve was also excited electrically to show that no conduction of the excitation to the muscle was present.

For light microscopy, the muscles were fixed in 10 per cent acrolein in xylene overnight at 0°C (Feder, 19 and personal communication). After dehydration in several changes of 1:1 methanol:ethylene glycol monomethyl ether at 0°C, they were transferred for 24 hr. each to 100 per cent ethanol and 100 per cent *n*-propanol at −20°C, then infiltrated 3 to 4 days in a graded series of *n*-propanol and methacrylate mixture at room temperature. They were subsequently embedded in hydroxyethyl methacrylate containing 5 per cent Carbowax 200 and 0.3 per cent α-α'-azodiisobutyronitrile, according to the procedure suggested by Feder (personal communication). Polymerization occurred overnight at 60°C. With this resin embedding sections could be cut at 1 micron, allowing good resolution of the fibrillar pattern in cross-sections. Sections were cut with a dry glass knife on a Porter-Blum microtome; they were then transferred to a drop of water over a glass slide, which spread them, and subsequently air dried. After overnight exposure to a saturated solution of dimedone in water, to block the free aldehyde groups, the sections were stained by the conventional PAS method.

For electron microscopy the entire muscle was carefully dissected and fixed in cold 1 per cent OsO_4 buffered with veronal-acetate, according to Palade (59). The superficial layer of the muscle was subsequently dissected in 70 per cent alcohol. Embedding

was carried out in Epon 812 (Luft, 52). Sections were cut on a Porter-Blum microtome with a Dupont diamond knife, stained by Karnovsky's procedure (39), and observed in a Siemens Elmiskop I.

Adjacent 0.5 micron sections were observed in phase contrast, after dissolution of the Epon with sodium methoxide (Mayor *et al.*, 54).

white muscle, this stain demonstrates an even distribution of glycogen in the sarcoplasm. The interfibrillar areas seem also fairly uniform and the fibrils are round and disposed in a regular pattern (Fig. 3).

The same stain applied to the soleus, on the other hand, does not show uniform intensity

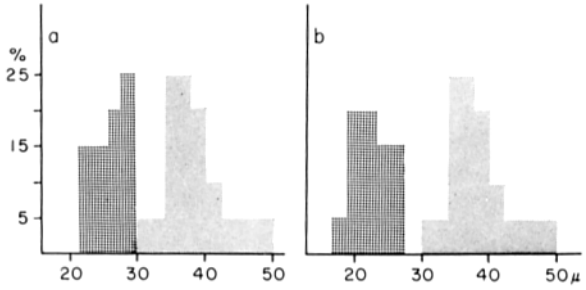


FIGURE 1

Changes in the distribution of muscle fiber diameters in the gastrocnemius at 2 weeks (*a*) and 2 months (*b*) after denervation, as compared with the normal muscle. The atrophying fibers are represented by the larger dots and the normal fibers by the smaller dots. Abscissa, fiber diameter in microns; ordinate, number of fibers in individual groups as percentage.

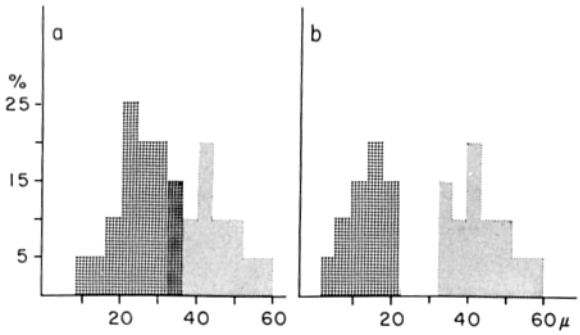


FIGURE 2

Similar comparison for the soleus.

RESULTS

1. *Comparison between Soleus and Gastrocnemius Fibers*

a) LIGHT MICROSCOPY

In a light microscope comparison the diameter of the fibers was shown to be larger in the soleus (from 32 to 60 microns) than in the gastrocnemius (from 30 to 48 microns). (See Figs. 1 and 2.)

In order to demonstrate the glycogen distribution and, indirectly, the disposition of the fibrils in the fiber, we used PAS staining on transverse sections of both muscles. In the gastrocnemius, a

throughout the fiber. The sarcoplasmic spaces are more variable in width, with the result that the fibrils appear to have an irregular shape and occasionally seem to merge, at times, forming ribbon-like structures (Fig. 5).

b) ELECTRON MICROSCOPY

Neither great variation in diameter nor confluence of fibrils is found in electron micrographs of the soleus. On the contrary, these show that the fibers contain more or less regularly round fibrils, completely separated from one another by sarcoplasmic spaces of variable width (Fig. 6). The ribbon-like appearance of the cross-section of fibrils in the

light microscope is due to the fact that the sarcoplasmic spaces are sometimes so narrow that they cannot be easily detected, particularly if the section is not perfectly transverse. The large mitochondria present in the soleus fibers contribute to the irregularity in the distribution of the fibrils.

In the white muscle gastrocnemius, as demonstrated by the electron micrograph in Fig. 4, the sarcoplasmic spaces are denser, narrower, and less variable in width. The regularity in the dimensions of the interfibrillar spaces contributes to the formation of a regular pattern in the distribution of the fibrils in cross-section (Fig. 3) though the shape of the fibrils is not regular (Fig. 4).

In longitudinal sections, the fibrils of the gastrocnemius are closely enveloped by the sarcoplasmic reticulum. No space is left between the small mitochondria and the contractile material (Fig. 7). In the soleus, the fibrils are farther apart, so that some sarcoplasmic material lies between them and around the mitochondria (Fig. 8). Both the soleus and the gastrocnemius have two triads (Porter and Palade, 62) per sarcomere. An interesting difference is to be noted in the amount of sarcoplasmic reticulum: the "cisternae" at the A band level are much more developed in the white fibers of the gastrocnemius than in the red fibers of the soleus (Figs. 7 and 8).

The glycogen granules (heavily stained by the lead used in our preparations) are regularly distributed in the white fibers, but mainly arranged in clusters in the red ones, thus perhaps explaining the uneven PAS staining of cross-sections of the soleus fibers.

Both kinds of fibers present oval-shaped nuclei, at the periphery, with a smooth membrane and a homogeneous content.

2. *Muscle Modifications after Nerve Section*

a) Weight of the Muscle and Diameter of the Fibers

After nerve section the weight of the group of muscles consisting of the gastrocnemius, plantaris, and soleus was determined. Compared with the normal contralateral muscles, the denervated muscles had decreased in weight approximately by one-third at the 1st week, one-half at the 2nd week, and five-ninths at the 4th week. These data, however, are only a rough indication of the changes in the muscle tissue. In optical cross-sections the fiber diameter is decreased (Figs. 1, 2, 13, and 17) with a more irregular distribution than normal. As shown in Figs. 1 and 2, at the 15th day the diminution in fiber diameter is more evident in the gastrocnemius, but after 2 months it is approximately the same in the two kinds of muscle. All fibers are affected, but at different

Figure 3

Light micrograph of a transverse section of normal rat gastrocnemius, PAS stained. The picture shows a typical "white" or "phasic" fiber. Note the even distribution of glycogen and the regular pattern formed by the fibrils. *c*, capillary. × 1100.

Figure 4

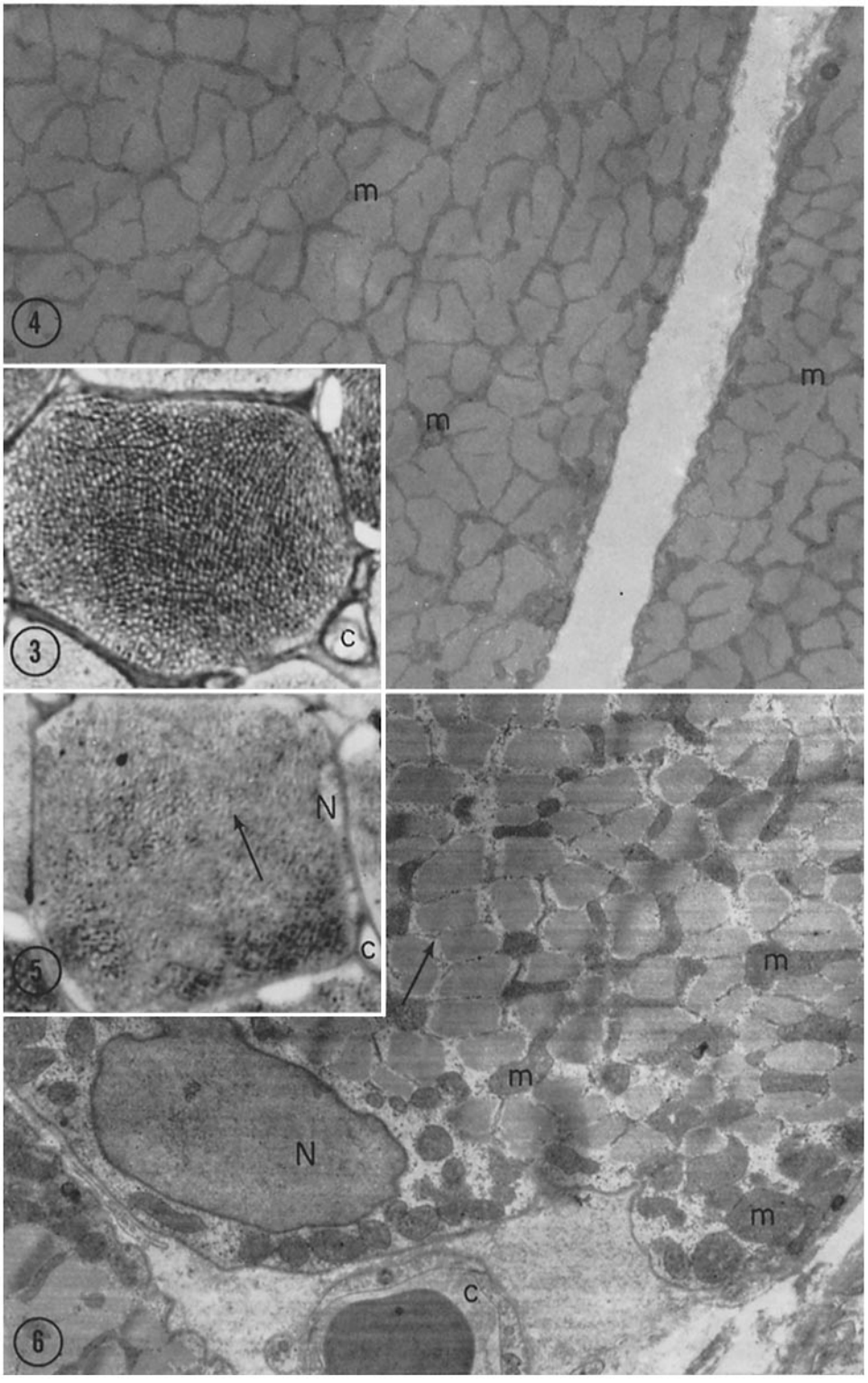
Low power electron micrograph of a transverse section of normal rat gastrocnemius The interfibrillar spaces are very narrow and contain small mitochondria (*m*). × 7500.

Figure 5

Transverse section of a "red" or "tonic" fiber from a normal rat soleus. Light micrograph of a section stained with PAS. The distribution of glycogen is irregular and there is a suggestion of ribbon-like fibrils, owing to the fact that the section is not perfectly transverse. Arrow points to one such fibril which might correspond to the group of fibrils shown in Fig. 6. *N*, nucleus; *c*, capillary. × 1100.

Figure 6

This electron micrograph of a transverse section through a soleus fiber demonstrates that the fibrils are round. They sometimes come so close to one another (arrow) that they may appear confluent in a light micrograph. See arrow in Fig. 5. The sarcoplasmic spaces vary in width and the mitochondria are large (*m*). *N*, nucleus; *c*, capillary. × 7500.


m
m
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times and to a different degree. While the fibers preserving a larger diameter are more or less round, the smaller ones are irregularly shaped (Fig. 17). The spaces around the fibrils are irregular in both red and white muscles (Fig. 17). Nuclei in the denervated fibers seem to be more numerous, and sometimes have a central location.

The glycogen content, which is already slightly different from one fiber to another in the normal tissue, shows a great variability at 15 days after denervation (Fig. 13). At 1 and 2 months the distribution is again more regular, a reduction having apparently occurred in the most heavily loaded fibers (Fig. 17).

b) Electron Microscopic Observations

1. FIRST TWO WEEKS: Transverse sections for electron microscopy fail generally to show that the fibrils depart from the normal in their form and distribution at the 1st week of atrophy. Occasionally, it is possible to detect at the periphery of the fibers areas showing disarrangement of the contractile material or its disappearance. Longitudinal sections depict mainly fibers in which all components have the normal structure, though in some white fibers the sarcolemma is not so closely in contact with the fibrils as it usually is, and presents an irregularly scalloped surface (Fig. 9). It thus seems that, although a reduction of material inside the fibers has already taken place, it is difficult to detect a reduction in diameter of the single fibrils. In the red fibers, even in normal ones, the sarcolemma is not closely apposed to the fibrils, so that a similar modification, if present, is difficult to detect.

Inside the fibers the fibrils are, at this stage, still closely packed and the reticulum is well preserved at all levels. The presence of small lysosomes is to be noted between the fibrils.

During the first 2 weeks after nerve section, a distinct reduction of the number of fibrils within the fibers takes place. This contributes to the loss of weight of the whole muscle, which is, as already mentioned, considerable during this first period of atrophy. The reduction is caused by the appearance of areas of disorganization of the contractile material affecting several contiguous fibrils at a time. Such areas are present in the white fibers mainly at the periphery, but in the red fibers they can be found inside also.

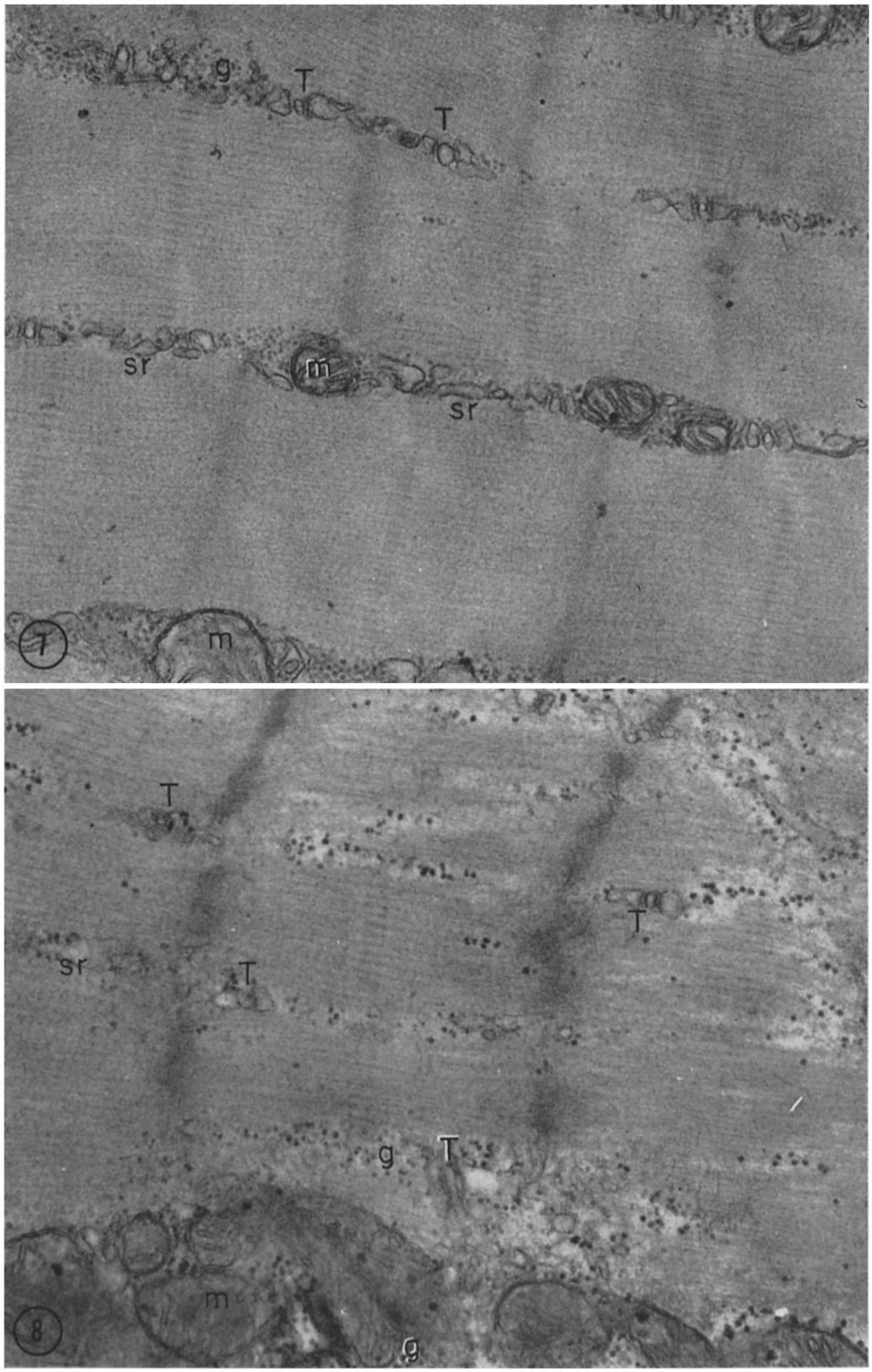
The alteration shows first at the Z lines: initially they lose their straight line configuration across the fibrils, become bent, and sometimes reach even the H band (Fig. 11). Subsequently they no longer show any filamentous structure and their material is irregularly spread over a large area of the sarcoplasm.

In areas adjacent to the more irregular Z lines, the filaments lose their parallel arrangement over the whole depth of the fibril and begin to spread into the interfibrillar spaces, where they subsequently disappear (Fig. 12). The alteration spreads transversely in the fiber, along the Z line, from one fibril to the next. A single fibril may show such regions of disorganization at several points along its length, with normal sarcomeres in between. No signs of alterations of the sarcoplasmic reticulum, except a possible dislocation of its elements, are noticeable at the beginning of these fibril changes. Later, in the areas where the Z line is completely disorganized and the filaments are undergoing a process of disintegration, the reticulum breaks down into isolated vesicles.

The process just described starts in the 1st week, when a few fibers show the initial stages. Later it spreads rapidly, so that at a fortnight after denervation a few fibers show the late stages of the process, and most of them only the consequence of it. In the latter case the fibers show large areas, particularly at the periphery, containing just glycogen, some mitochondria, and remnants of the sarcoplasmic reticulum. These remnants take the form of isolated vesicles and of tubules, whose derivation from the intermediate elements of the triad is ascertained by comparison with the ones occasionally seen in normal muscle (Fig. 15). The sarcolemma, together with the basement membrane, is indented in these undifferentiated zones. This is the beginning of the process by which the fiber discards the useless material in the interfiber spaces: the indentations become deeper, reaching the first layer of preserved fibrils, and parts of the

FIGURES 7 AND 8

Comparison between longitudinal sections of gastrocnemius (Fig. 7) and soleus (Fig. 8) fibers. Both fibers present two triads (*T*) per sarcomere, but the cisternae of the sarcoplasmic reticulum (*sr*) are more extensively developed in the gastrocnemius. Mitochondria (*m*) are larger in the soleus fiber. *g*, glycogen. × 40,000.


g
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g

sarcoplasm are pinched off. The fibers at this stage show a variable content of glycogen, so that fibers lying near one another may show a marked difference in density (Fig. 14). This is confirmed by optical microscopy of PAS-stained sections (Fig. 13). Also, large areas of concentric membranes, closely packed, are often encountered (Fig. 20), as well as large lysosomes, filled with dense material. The peripheral fibrils sometimes appear abruptly interrupted when reaching the degenerated region. The inner fibrils, on the other hand, show good preservation and alignment of the bands, although the interfibrillar sarcoplasmic spaces are slightly enlarged.

We occasionally found fibers in which the process of degeneration went so far as to produce an extreme reduction of contractile material across the whole fiber. In this case the contractile material completely disappears, except for a few dense patches, remnants of the Z line, and some scattered filaments. Very few small mitochondria are left, which, however, do not show any sign of degeneration; the reticulum is present in the form of sparse, round vesicles. The sarcolemma is lined by numerous pinocytotic vesicles. Some small lysosomes are present, as well as glycogen granules. Even those fibers completely lacking in contractile material do not show any tendency toward a transformation into fibroblasts, so that, at least at this stage, the possibility of a metaplasia, as proposed by Tower (71) and Altschul (2), is definitely excluded. This fact is also confirmed by the light microscope work of Sunderland and Ray (69), who reported an extreme reduction in fiber diameter, but no metaplasia.

In general, only restricted areas in the fiber are affected by the degenerative process. The observation of semithin sections by light microscopy showed that a single fiber may be affected several times along its length, but the disarrangement of the contractile material does not occur in more than two or three sarcomeres at a time. This explains why it is very difficult to detect the areas of disarrangement in transverse sections.

II. LATER STAGE OF ATROPHY: At 1 month most of the fibers are greatly reduced in diameter; in the smallest ones the changes from the normal structure are most prominent. Large degeneration areas are no longer encountered, but empty peripheral areas deriving from the preceding degeneration of the contractile material are still present. The sarcolemma is deeply indented in these areas, and the fiber presents long, irregular protrusions devoid of contractile material. The basement membrane closely follows the sarcolemma in its invaginations. Inside the fibers, the fibrils are reduced in number and diameter. Normal gastrocnemius fibers contain from 1200 to 2900 fibrils, and normal soleus fibers from 1900 to 3000.

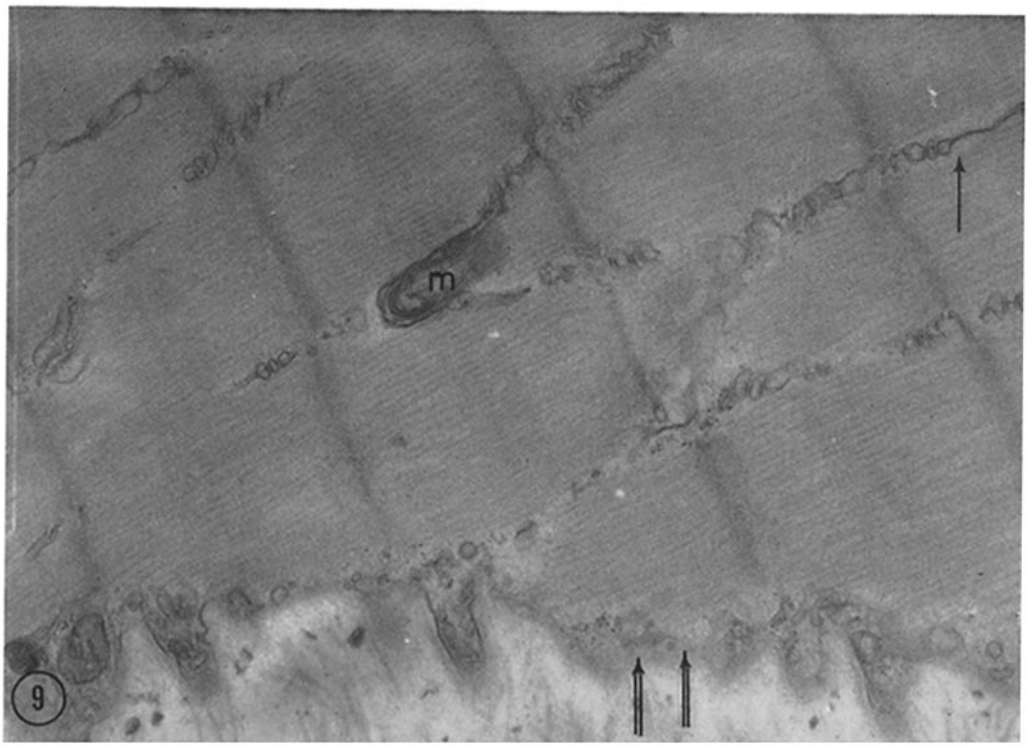
Two months after nerve section, the number of fibrils per fiber is reduced to a minimum of a few hundreds in both kinds of muscle. In both the soleus and the gastrocnemius, after denervation, the shape of the fibrils is irregular and the diameter of the fibrils is variable. The latter is occasionally reduced from the normal 1 micron value to 0.2 micron, as illustrated in Fig. 16. The filaments in the center of the fibrils show the normal spatial disposition (Huxley, 36). It could be also clearly ascertained that the Z line maintains its peculiar square pattern (Knappeis and Carlsen, 40). The decrease in size of the fibrils is the result of a process that can already be detected at the end of the 1st week (Fig. 9), but which is more apparent in the late stages because of the slightly enlarged interfibrillar spaces. This process consists in a disarrangement of the filaments at the periphery of the fibrils and their subsequent detachment from the body of these structures (Fig.

FIGURE 9

Longitudinal section through the periphery of a gastrocnemius fiber, 1 week after section. A slight enlargement of the sarcoplasmic spaces (single arrow) and scalloping of the sarcolemma (double arrows) suggest that some reduction in the contractile material has taken place. A mitochondrion (*m*) is undergoing degeneration and a small lysosome (*l*) is present. × 19,000.

FIGURE 10

Periphery of a gastrocnemius fiber, 2 months after nerve section, showing leakage of filaments from the periphery of a fibril and enlargement of the interfibrillar spaces (arrow). Triads (*T*) are still present. × 62,000.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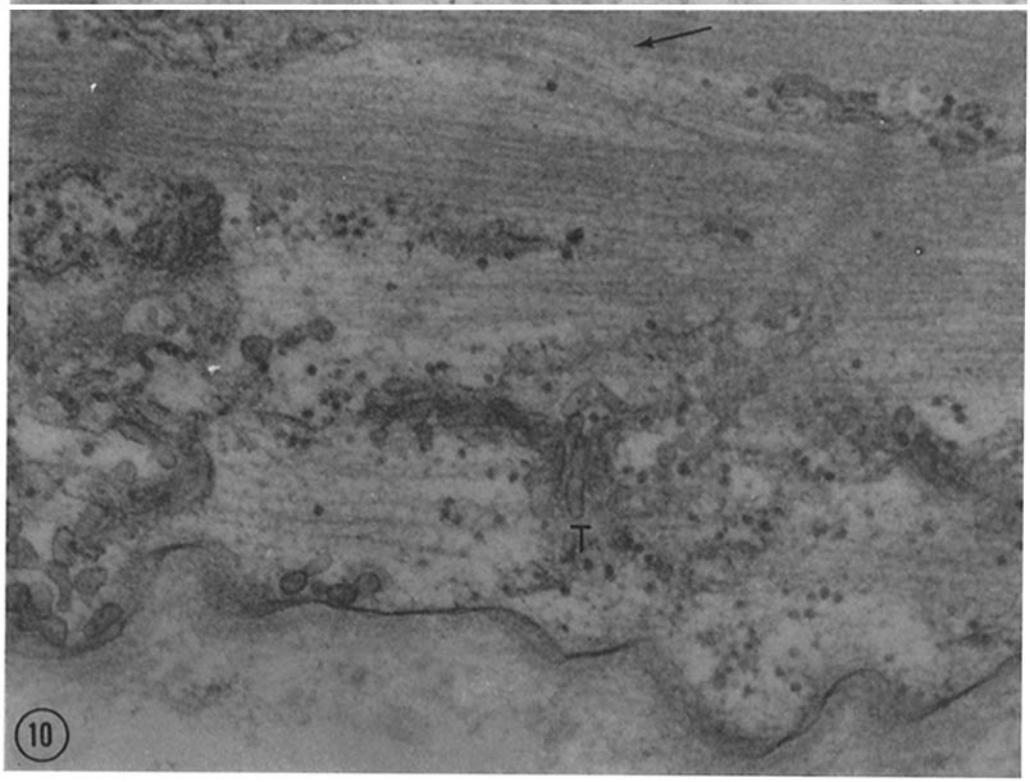

T
10

10). Since no traces of filaments free in the interfibrillar spaces were ever found, we must assume that a breakdown takes place immediately. The loss of filaments is not regular along the length of the single fibrils, so that their course is slightly irregular in longitudinal sections. The interfibrillar spaces are but slightly enlarged, even when the loss of material from the fibrils is fairly large. The overall reduction of the fiber diameter is thus due to elimination of the filaments, with eventual breakdown, and to rather close packing of the reduced fibrils. The numerous small lysosomes, which appear at random among the fibrils, might possibly be used for the subsequent digestion of the filaments' breakdown products (Fig. 18). The reticulum is somewhat disordered and apparently overdeveloped, particularly in the most reduced fibers. It is already possible to note some "pentads," *i.e.*, structures composed of two intermediary vesicles, running parallel around a fibril at a short distance from one another. They are separated and lined on both sides by sacs resembling the "terminal sacs" of the triad (Fig. 18). The pentads, as well as the overcrowding of the sarcoplasmic sacs, are probably an expression of the loss of contractile material not followed by a general reduction in the structural elements of the sarcoplasmic reticulum. At the same time in both muscles, but more evidently in the gastrocnemius, there is, throughout the fiber, an overproduction of tubules deriving from the intermediary vesicles of the triad.

The nucleo-cytoplasmic ratio is much higher at this stage (Fig. 19) and the nuclei are often found in long, longitudinal rows, occasionally located centrally in the fibers. The outlines of the nuclei are extremely irregular, showing deep indentations. The chromatin distribution is irregular, with dense areas at the periphery, and the nucleoli are enlarged.

Mitochondria are still present between the fibrils. They present the usual dense matrix and the cristae, which, however, are not so closely packed as in the normal mitochondrion. Moreover, the over-all population of mitochondria appears reduced, since large clumps are no longer encountered at the periphery of the fibers.

At 2 and 3 months after denervation, the fibers in both the soleus and the gastrocnemius are very much reduced in diameter. In both there is a marked tendency of the nuclei to be localized centrally.

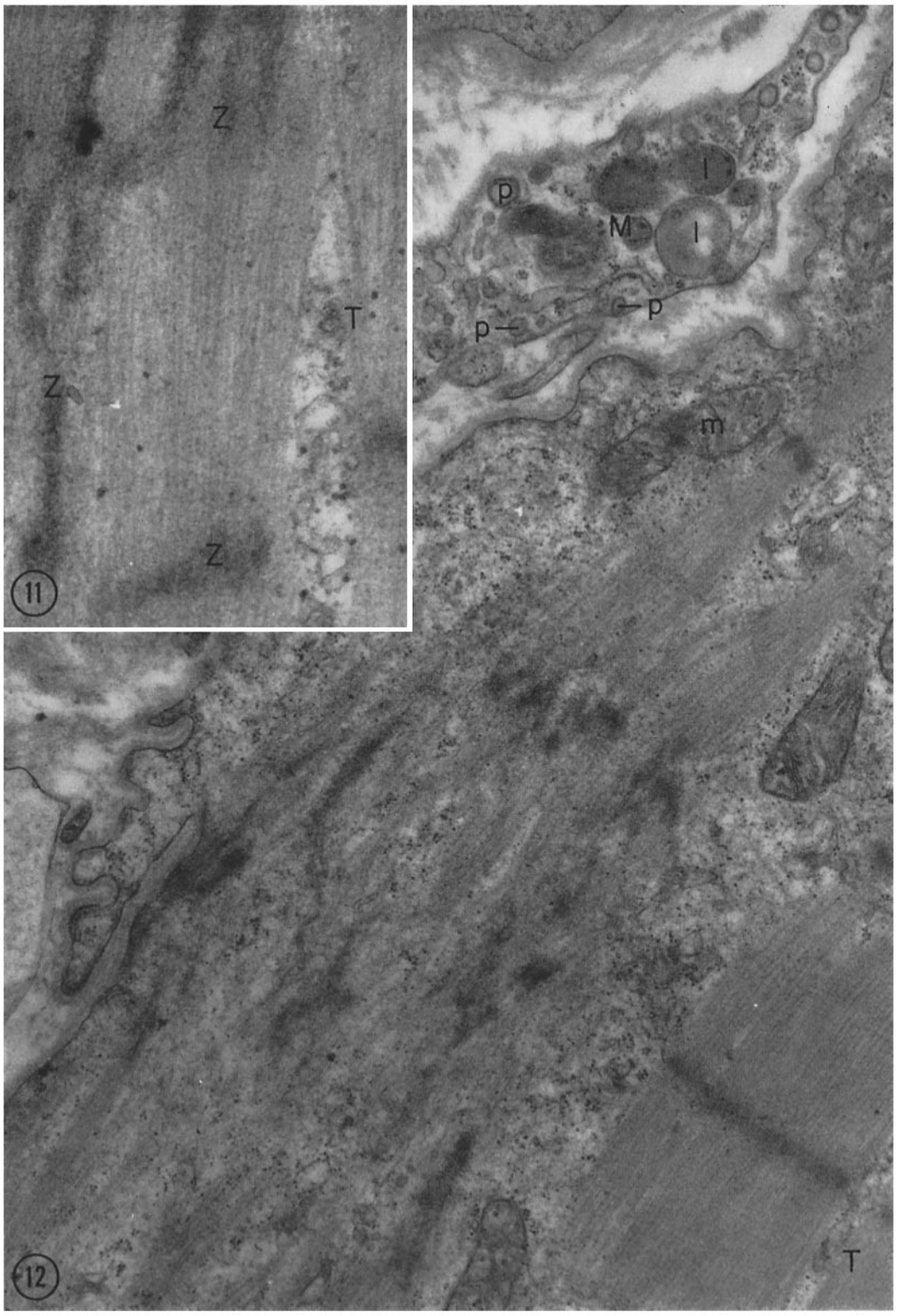
The peripheral undifferentiated areas produced by the degenerative process in the first stages are completely eliminated at this stage, so that the sarcolemma is very close to the peripheral fibrils (Fig. 19), though not so close as in the normal tissue. Inside the fibers, also, the spaces between the fibrils are reduced in size to almost the normal value. The fiber as a whole appears extremely reduced in diameter because of the loss in contractile material. As a result of the localized areas of degeneration which occurred in the early stages, the fiber shows along its length sudden variations in the number of fibrils. The irregular diminution of the diameter of the single fibrils, due to leakage of peripheral filaments into the sarcoplasmic spaces, is emphasized by the irregular course of the fibrils along the fiber and by an exaggeration of the previously noticed overcrowding of the sarcoplasmic reticulum (Fig. 18). Terminal sacs and intermediary elements of the triad join together more often to form "pentads." At the same time, in the gastrocnemius, the cisternae at the A band level appear preferentially reduced and in the form of isolated vesicles, so that they resemble the ones found normally in the red muscle soleus. Owing to this fact and to the slight enlargement of the interfibrillar spaces, at this late stage of atrophy the differences between red and white fibers, described in the normal tissue, are lost.

Both kinds of muscle show an overproduction of

FIGURES 11 AND 12

Insert (Fig. 11) shows the beginning of the alteration illustrated in Fig. 12. The Z line (*Z*) is wavy and Z line material is present at the A band level. The filaments are still rather regularly disposed and a triad (*T*) is preserved near by. × 58,000.

The periphery of a soleus fiber (Fig. 12), 1 week after denervation, shows a large area of degeneration in which the Z lines, as well as the filaments, are undergoing disorganization. The reticulum has disappeared. Mitochondria (*m*), well preserved fibrils, and triads (*T*) are present at the periphery of such an area. In the interfibrillar spaces, a macrophage (*M*) is present which shows some peculiar pinocytotic vesicles (*p*) and dense lysosomes (*l*). × 26,000.


Z
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small tubules deriving from the intermediate system of the triad. These, in the form of small tubules, lie preferentially at the level of the triad from which they derive, but frequently run also in the interfibrillar spaces, sometimes reaching the H band. They are so well developed at times that they join together to form a large labyrinthine structure at the A-I junction (Fig. 18).

The reduction of mitochondrial content is parallel to the diminution of contractile material, so that neither clusters of mitochondria nor areas devoid of them are ever encountered. Several small lysosomes are present between the fibrils undergoing the process of reduction.

Glycogen granules are still present among these structural components of the fibers; they never aggregate into the large clusters present in the normal tissue, so that we may state that their absolute content in the fibers is certainly reduced.

The nuclei are even more deeply and irregularly indented than in the earlier stages, and the nucleoli are very evident (Fig. 19). The Golgi apparatus is often noticeable in the perinuclear space, as in the normal tissue. Some lysosomes are still encountered.

The fibers composing the muscle spindle undergo a process of reduction similar to that described in the muscle fibers. In a transverse section of a spindle from a soleus, 2 weeks after denervation, the fibers were reduced in diameter and presented an irregular outline. Several close lines, of the same density and dimensions as the basement membrane, were visible near the atrophying fibers. They represent the basement membrane left behind by the retracting nerve endings. Actually no nerve can be seen to reach the spindle fibers, whereas in the normal tissue they do so in great numbers.

Frequently, at all stages of the atrophy process, some elongated macrophages are encountered in the spaces between the fibers. They contain numerous pinocytotic vesicles presenting an increased density around the cell membrane, which are similar to the differentiated sites of protein uptake described by Roth and Porter (66) in rat liver cells and mosquito oocytes. Also, several lysosomes are present in these cells (Fig. 12).

The interfibrillar spaces present a great abundance of collagen fibers. The increase is possibly relative.

Occasionally an infiltration of fat cells in the atrophic muscle, particularly the soleus, has been observed. This alteration during the course of atrophy has already been described by Krauss (42), Chor *et al.* (15), Altschul (2), and Adams *et al.* (1). It can, however, be considered secondary to the atrophic process since its appearance is variable in different animals.

DISCUSSION

1. *Ultrastructural Elements of Rat Soleus and Gastrocnemius Muscles*

As a result of our observations, it is clear that in the rat a morphological difference exists between the fibers composing a red muscle, the soleus, and a white one, the medial head of the gastrocnemius. The same muscles have been found by Eccles *et al.* (17) to have, in the cat, a different physiological behavior: the soleus is a purely tonic muscle,

FIGURE 13

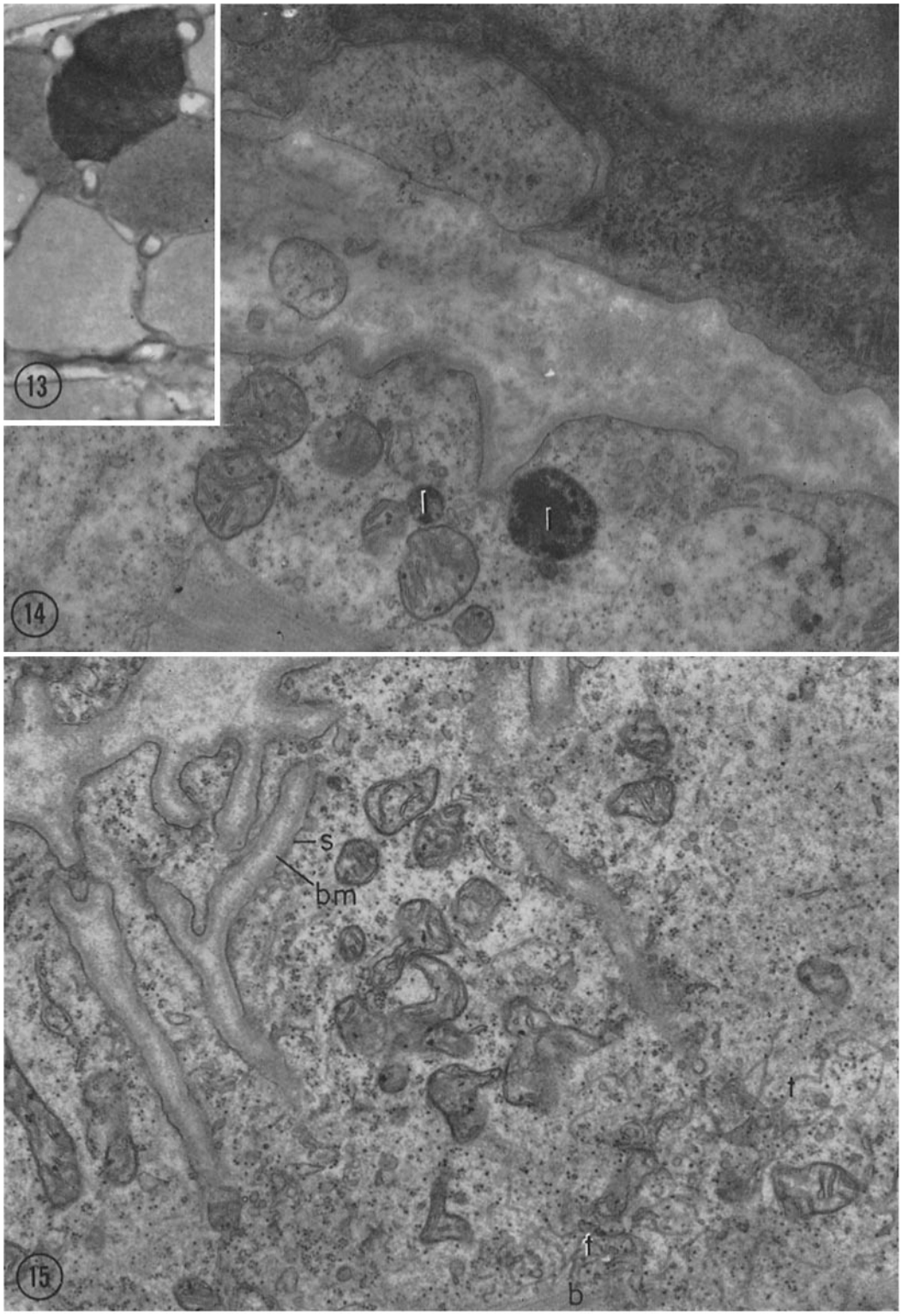
Light micrograph of a PAS-stained transverse section of soleus, 2 weeks after denervation. The stain demonstrates a large difference in glycogen content in the different fibers. × 800.

FIGURE 14

Two adjacent fibers of soleus, 2 weeks after denervation, show peripheral areas devoid of differentiated structures, except for a few mitochondria. Two lysosomes are present (*l*). The large difference in density is mainly due to difference in glycogen content. × 28,000.

FIGURE 15

The periphery of a gastrocnemius fiber, 2 weeks after nerve section, shows a large undifferentiated area, typical of fibers at this stage of atrophy. The sarcolemma (*s*) followed by the basement membrane (*bm*) is deeply invaginated and almost reaches the preserved fibrils (*f*, at the bottom of the picture). Several tubules (*t*), which represent derivatives of the intermediary tubule of the triad, can be observed. × 28,000.


13
14
15
s
bm
f
f
b

whereas the gastrocnemius is phasic; this means that the soleus has a time of contraction and relaxation which is double that of the gastrocnemius (Wills, 77). We observed that the tonic muscle, as compared with the phasic muscle, has larger and more irregular sarcoplasmic spaces and a less developed sarcoplasmic reticulum at the A band level; mitochondria are larger and more irregularly distributed. It is to be noted that both kinds of fiber contain distinct and more or less round fibrils. Our results contrast with the work of Krüger (43) and Krüger and Gunther (44, 45), who described, in frogs, salamanders, rats, and cats, among others, the tonic and phasic muscles as being composed, respectively, of "field" and "fibrillar" structure fibers. According to the authors, the former are composed of ribbon-like fibrils, the latter of smaller round ones. It is interesting to note that, as clearly shown by Peachey and Huxley (60), the classification of Krüger and Gunther applies to the frog muscles. In these the differences in structure are related to a definite difference in the mode of innervation and contraction of the two kinds of fibers; *i.e.*, the "field" structure fibers are innervated by small diameter nerve fibers and present no propagated action potential (Kuffler and Vaughan Williams, 46). This is in turn followed by a slow, long lasting contraction. The "fibrillar" structure fibers, on the other hand, are innervated by large neurons and respond with a twitch to the propagated action potential. Tonic and phasic fibers in the rat are physiologically as well as morphologically related to this second group of twitch fibers in the frog, since both are twitch fibers innervated by large motoneurons (Buller *et al.*, 8, 9) and present a "fibrillar" structure.

2. *Muscle Atrophy*

In our study the process of atrophy leads, in the course of 2 or 3 months, to the formation of fibers at times extremely reduced in diameter, but still maintaining a close array of fibrils. These, in turn, though reduced in diameter, preserve inside a good alignment of thick and thin filaments, such as is described by Huxley (36). The sarcoplasmic reticulum shows a relative increase in amount. Tubules deriving from the intermediary tubule of the triad are overdeveloped, in comparison with the elements of the sarcoplasmic reticulum. Mitochondria are present between the fibrils and show a normal structure. Large clusters of them are no longer detectable at the periphery of the fibers.

We can describe two modes in the process of atrophy. The first one consists in a degeneration of the fiber components in relatively large areas. It is responsible, in part at least, for the fast reduction in muscle volume which occurs in the first 15 days after nerve section, in agreement with the data of Gutmann (25). The contractile material is first broken down and hydrolyzed, in a process which suggests an enzymatic attack at the level of the Z lines. Subsequently, the sarcoplasmic reticulum and the mitochondria disappear in the areas devoid of contractile material. The process leaves large areas in the fibers that contain no differentiated structures except for a few randomly disposed vesicles, remnants of the sarcoplasmic reticulum, and glycogen granules. Large bundles of possibly lipoproteinic material, which may represent the result of the degeneration of the membranous structures of the fibers, accumulate in some sites.

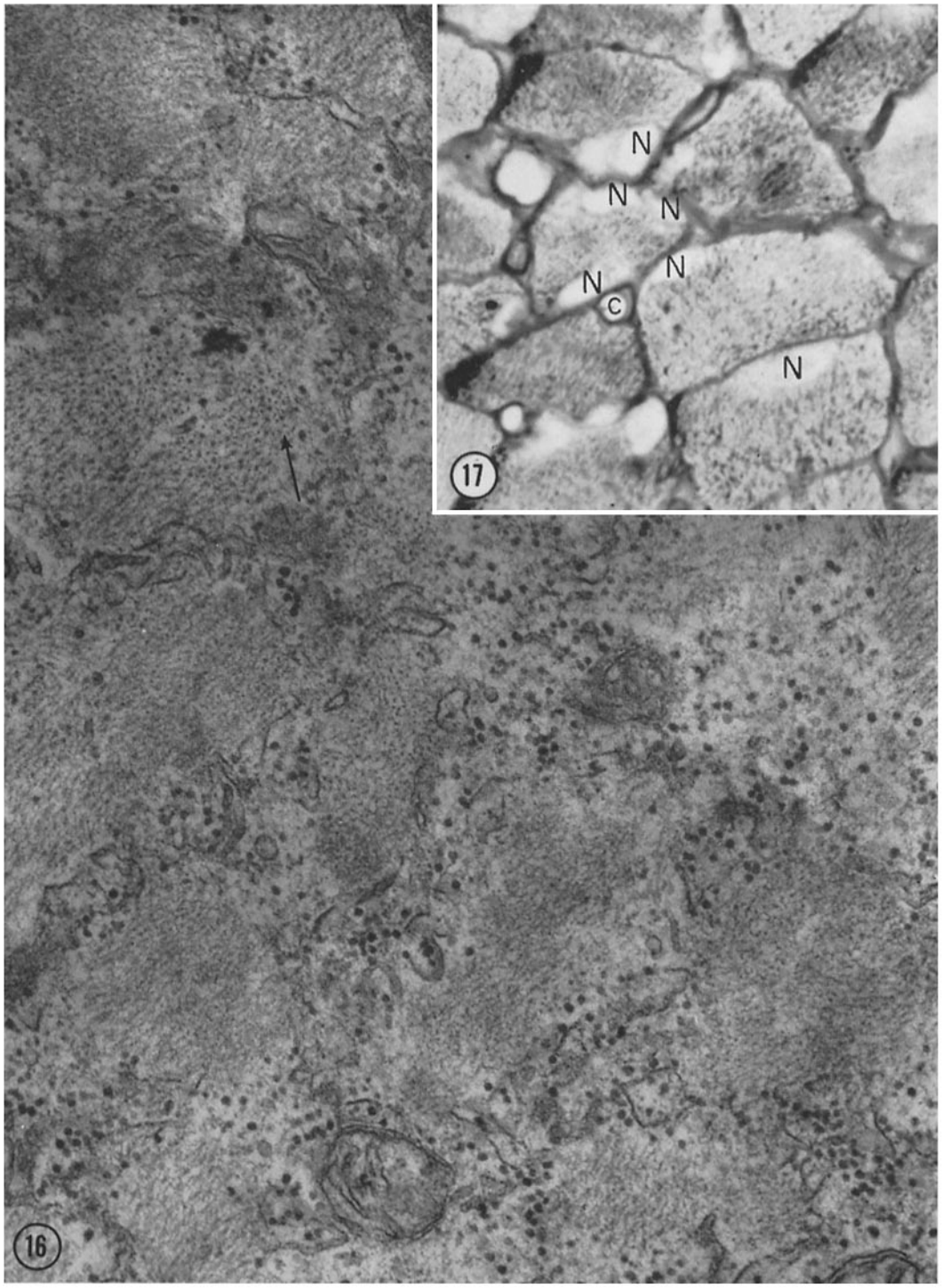
Of particular interest is the presence of large lysosomes in the fibers undergoing the process of degeneration, particularly toward the end of the

FIGURE 16

Transverse section of a gastrocnemius fiber, 1 month after denervation. The fibrils are reduced in diameter through a loss of filaments at the periphery, while in the center the regular pattern of thin and thick filaments can be observed (arrow). The sarcoplasmic spaces are slightly enlarged, but the sarcoplasmic reticulum is mostly well preserved. × 58,400.

FIGURE 17

Light micrograph of a PAS-stained transverse section of a gastrocnemius, 1 month after denervation. The reduction in fiber diameter is evident. The fibers have an irregular shape and a relatively increased number of nuclei (*N*); the pattern of the fibrils is disordered. *c*, capillary. × 1070.


N
N
N
N
N
C
N
17
16

first 2 weeks after nerve section. Though the presence of lysosomal enzymes has been shown by Pellegrino *et al.* (61) and by Tappel *et al.* (70) in normal muscle, no lysosomes have ever been detected by electron microscopy in normal fibers. During the process of atrophy lysosomes are formed, and they appear larger and more heavily loaded with material, as the rate of degeneration increases in the fibers. A clear correlation can thus be established between the reduction of the muscle components and the appearance of these organelles. Pellegrino *et al.* (61) have observed a definitive increase of the activity of beta-glucuronidase and acid phosphatase, two lysosomal enzymes, during the course of atrophy of the tibialis anterior of the rat. They were not able to localize the enzymes in the tissue. The present observations suggest that this increase of lysosomal enzymes is due in part to the appearance of lysosomes inside the fibers. Moreover, macrophages containing lysosomes are occasionally present around the atrophying fibers. The presence of these and of several particularly differentiated pinocytotic vesicles in the macrophages suggest that these cells are taking up the material discarded by the atrophying fibers in the intercellular spaces. It is interesting to note that in vitamin E dystrophy of the rabbit (Zalkin *et al.*, 80) and in genetic muscular dystrophy of the mouse and chicken (Tappel *et al.*, 70) large increases in lysosomal enzymes were found.

The described first stage in the atrophy process can be considered "degenerative" on the basis of the classical definition (Mönckeberg, 55) of "degeneration" as a loss in the typical properties of a cell, including its structure. This classical concept can be extended to include modifications at the intracellular level. Our electron microscopic observations confirm the isolated histological report by Chor and Dolkart (14) that an occasional destruction of the cross-striation occurs early in the denervated muscle. Tower (72), on the contrary, holds the more classical view that a simple atrophy, with preservation of the cross-striations, follows denervation.

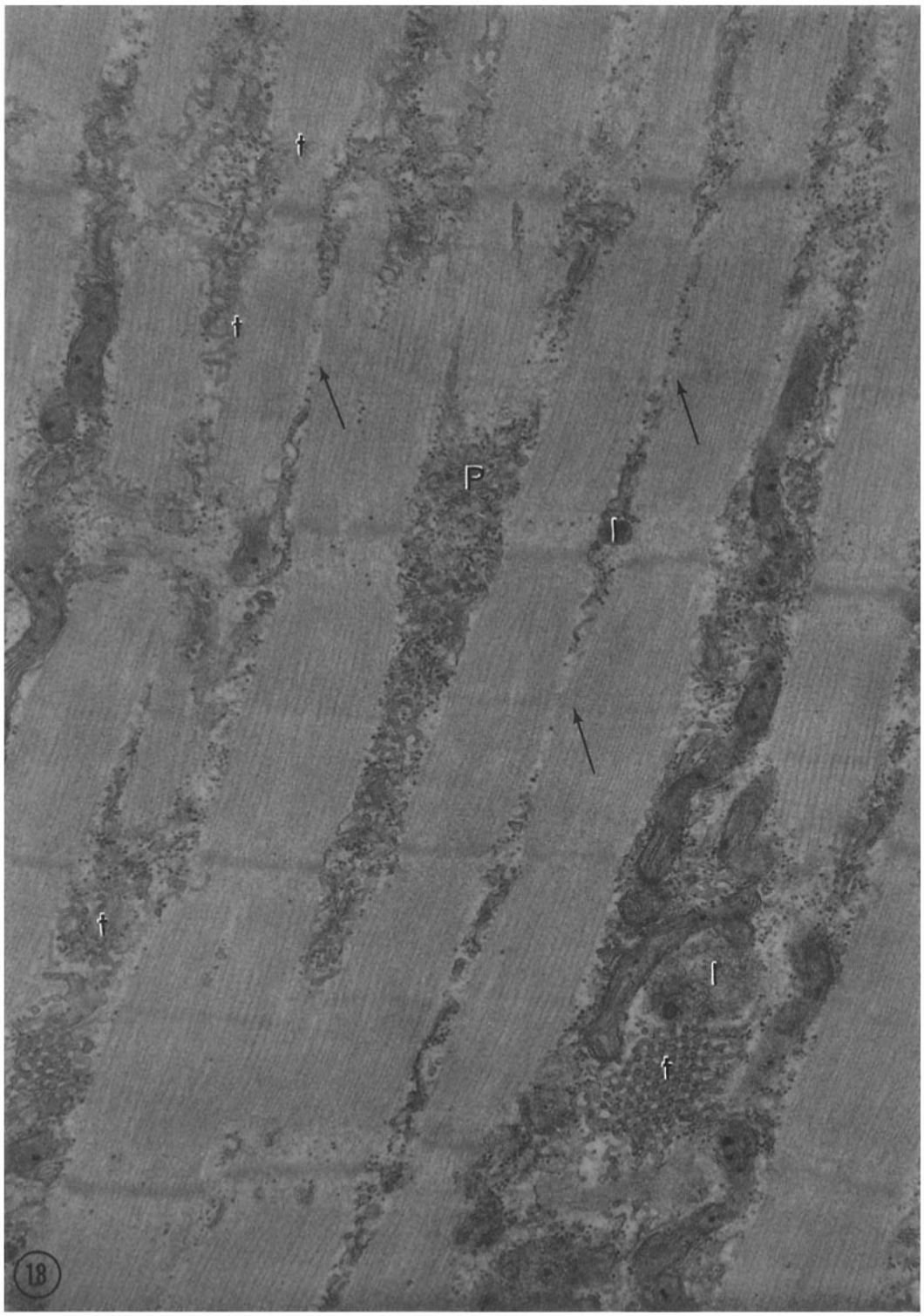
Two weeks after denervation, large areas devoid of contractile structure are present at the periphery of most cells. These areas are subsequently reduced by a retraction of the sarcolemma, which presents deep indentations that reach the preserved fibrils. The indentations eventually pinch off sarcoplasmic portions. Seldom have we observed the spreading of the destructive process over a whole fiber, leading to complete destruction of its contractile material.

The second mode of atrophy accounts for the reduction in the dimensions of the single fibrils, through a leakage of peripheral filaments, a few at a time, into the interfibrillar spaces. The phenomenon is present in all fibers during the entire process of atrophy. It starts in the very early stages, though it does not involve all the fibers to the same degree. As a result of the decrease in the number of filaments, the interfibrillar spaces are larger. The amount of contractile material that disappears is larger than the increase in the size of the interfibrillar spaces. Thus the volume of the whole fiber appears reduced. Here again, the filaments are destroyed *in situ*, while small lysosomes become apparent among the fibrils. Our results here are in agreement with the ones recently published by Wechsler and Hager (74, 75). These authors observed that in rather advanced stages of neuromuscular atrophy the reduction of the fibril diameter was due to a loss of filaments at the periphery, with a complete preservation of the normal pattern inside the fibrils.

There is a good correlation between our electron microscopic observations and the published data of biochemical analyses on the denervated muscle. The decrease in total protein content (Hoagland, 33; Fischer, 20, 21), as well as in the content of contractile material—myosin (Crepak, 16; Fischer, 20; Fischer and Ramsey, 22)—can be related to both the early degenerative process and the continuous loss of filaments from the fibrils. Some of the properties of the proteins were

FIGURE 18

Longitudinal section of a gastrocnemius fiber 2 months after nerve section. The cisternae of the sarcoplasmic reticulum at the A band level have mostly disappeared (arrows). A pentad is marked with *P*. Tubules (*t*), derivatives of the intermediary vesicle of the triad, are numerous, particulary at the I band level. They form, in places, a labyrinthine structure. Lysosomes (*l*) are present among the fibrils. The latter are reduced in diameter, while the sarcoplasmic spaces are slightly enlarged. × 33,000.


18

found to be altered after denervation: they can be digested more easily (Chen *et al.*, 12); the viscosity of actomyosin decreases (Fischer, 20), as well as the ATPase activity of the myosin. Various authors (Malakhov, 53; Padieu, 58; Schapira *et al.*, 68) have observed also a decrease in the uptake of labeled amino acids in the proteins of atrophic muscles: this indicates a decrease in protein synthesis. The same fact is confirmed by Zåk and Gutmann (79), who showed the inability of denervated muscle to increase its protein content after stimulation, in contrast with the normal muscle.

While the contractile material is slowly reduced, a corresponding reduction of the elements of the sarcoplasmic reticulum does not occur. On the contrary, the reticulum looks overdeveloped and slightly disordered owing to the slight enlargement of the interfibrillar spaces and to the selective disappearance of the filaments. As an expression of this phenomenon, "pentads" are often encountered in fibers very much reduced in volume. It is interesting to note, moreover, that both the soleus and the gastrocnemius present a definite overdevelopment of tubules deriving from the intermediary vesicle of the triad. These tubules, occasionally observed in the normal tissue, and probably concerned with the contraction cycle (Franzini and Porter, 23), are so largely developed as to be, at times, confluent in a labyrinthine structure. In fact, the atrophying fibers, even when reduced in volume, are the site of an intense and irregular contractile activity, which consists in fibrillation. This phenomenon, observed earlier by Langley and Kato (48, 49), has been the object of several physiological studies. It is typical of denervation atrophy, whereas it is not present in other kinds of atrophy (Josefsson and Thesleff, 38; Chor and Dolkart, 14), begins in the 1st week after denervation. Fibrillations are caused by action potentials which start from one point of the fiber and spread over it (Li *et al.*, 51). The focus is considered by Hayes and Woosley (28) and by Jarchs *et al.* (37) to coincide with the denervated motor end-plate.

In the white muscle gastrocnemius the cisternae of the sarcoplasmic reticulum, which normally form a continuous pattern at the A band level, in the late stage of atrophy frequently appear interrupted as isolated vesicles, particularly in areas where a more active reduction of the contractile material has taken place. As a result of this interruption, the gastrocnemius is no longer readily distinguishable from the red muscle soleus. This fact is probably responsible for the similar appearance of the two muscles during atrophy, when stained with gold chloride, as shown by Krüger (43). Though losing the specific differentiation into a red and a white kind, the fibers are still different from the embryonic ones during development (Hibbs, 30; Ruska and Edwards, 67; Randall, 63; Bergman, 4) and from the fibers undergoing a dedifferentiation process (Hay, 27). In both these cases, the fibers present large undifferentiated area and a poorly developed reticulum, whereas during atrophy the reticulum is mostly preserved and the fibrils tend to maintain their closely packed arrangement.

At 1 to 3 months the nucleoli are largely increased in diameter and similar to the ones found in embryonal (Bergman, 4) and regenerating muscle (Hay, 27). This would suggest that the fibers are ready for a rapid proliferation. In fact, they have been shown to be able to make a rapid recovery (Gutmann and Young, 26; Gutmann, 25).

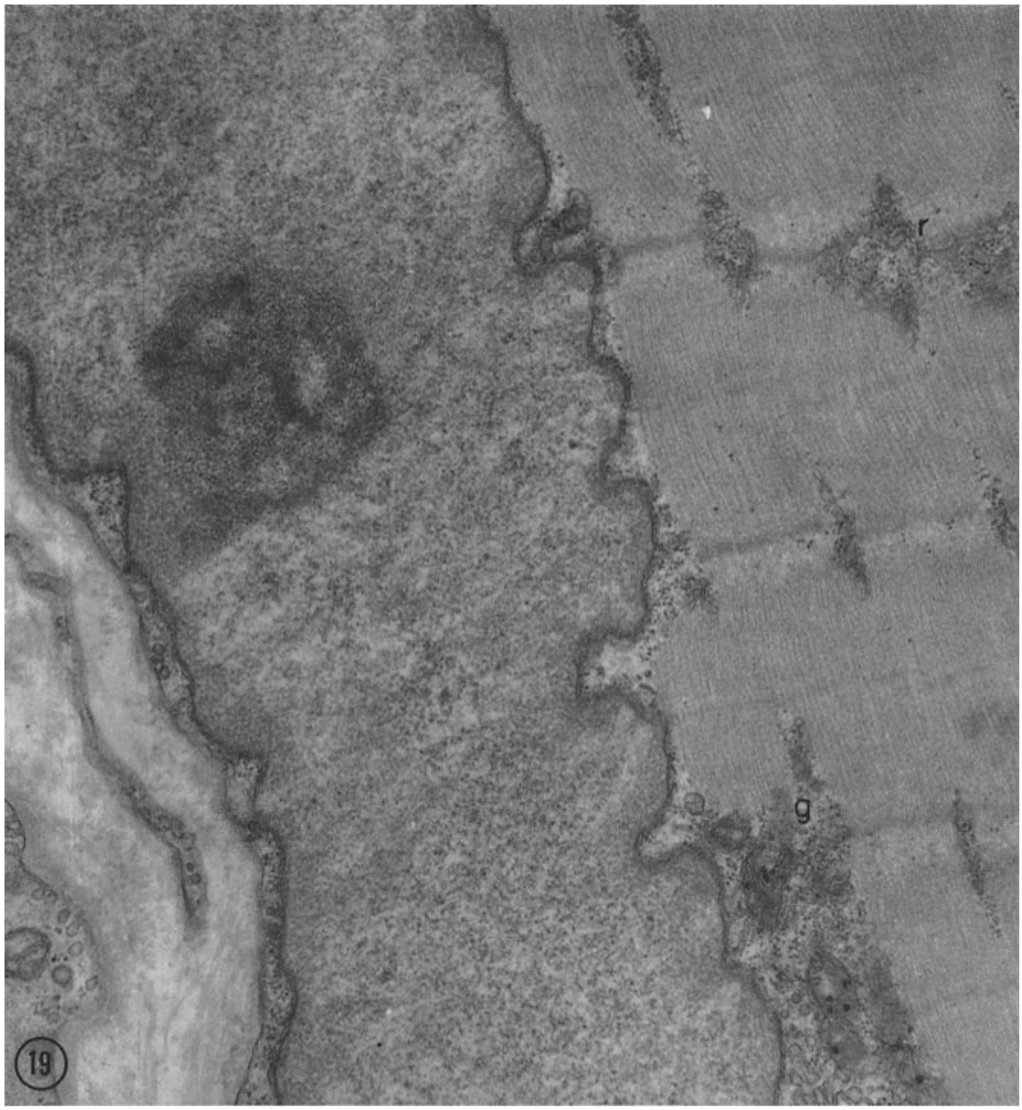
It is to be noted that, in our observations, the basement membrane closely follows the sarcolemma, where the latter invaginates. Birks *et al.* (5) had observed, on the contrary, that in atrophy the white frog muscle shows a dissociation of the

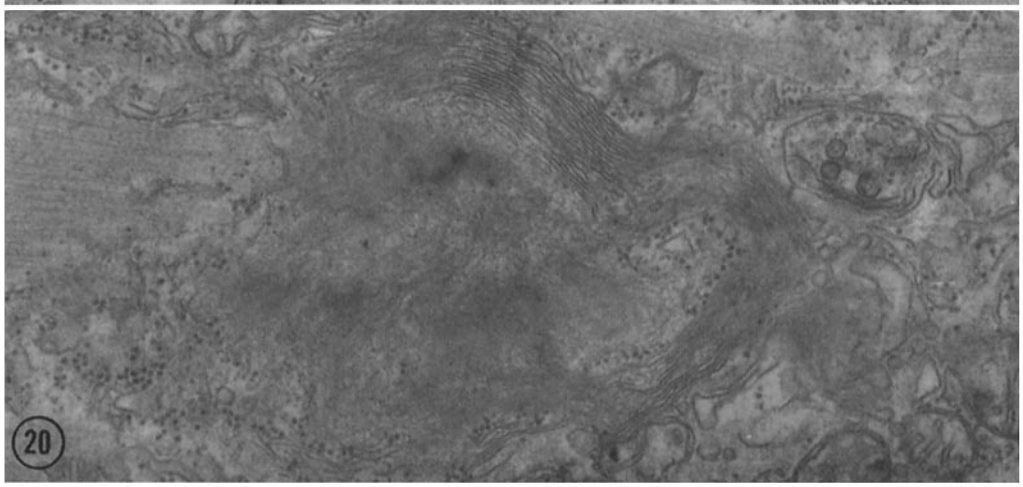
FIGURE 19

A soleus fiber 3 months after denervation. The fiber is extremely reduced in diameter (no more than five fibrils were present in the plane of section). The sarcolemma is closely apposed to the fibrils and the interfibrillar spaces are narrow. The nucleus is large and presents a scalloped surface. The nucleolus is very evident. Reticulum (*r*), mitochondria, and glycogen (*g*) are preserved. × 27,400.

FIGURE 20

A gastrocnemius fiber, 1 month after denervation, presents a large condensation of lipoprotein material among the fibrils. × 58,700.


r
g
19


20

"membrane complex system" due to retraction of the sarcolemma not followed by the basement membrane.

As regards the mitochondria, no noticeable modifications of their structure and number are detectable in the first stages, except in the areas of degeneration. Later, they undergo a diminution in number roughly proportional to the reduction in contractile material in the fiber. It is very rare to find intermediate steps in mitochondrial degeneration. Rare mitochondria present a disorganization in the disposition of the cristae, suggesting the mechanism of their disappearance. Mitochondria are involved in the atrophy process only in a secondary way, as compared with the contractile material, their disappearance being probably due to the lessened energy requirement. The morphological demonstration of their disappearance is in accordance with the biochemical data of the literature. Humoller *et al.* (35) and Hearn (29) demonstrated a decrease in cytochrome oxidase; Humoller *et al.* (34), Hoagland (33), and Hines and Knowlton (31) demonstrated a decrease in succinodehydrogenase. Both are typical mitochondrial enzymes. The diminution of the activity of the same enzymes have been shown, histochemically, by Nachmias and Padykula (57).

Biochemical data on glycogen content in atrophying muscles are extremely controversial. Both an increase (Chandelon, 11; Levine *et al.*, 50) and a decrease (Vay, 73; Cedrangolo, 10; Hines and Knowlton, 32; Humoller *et al.*, 34) have been observed. In our results, a large difference in the glycogen content of different fibers is observed in the early stages. Later, all fibers show a clearly diminished glycogen content. The present study and the one published by Wechsler and Hager (74) confirm the earlier optical microscope observations showing that the main process at work in the muscle fibers after denervation is the regular loss of contractile material, leading to a reduction in diameter of the fiber. The reduction of the fibrils can lead eventually even to the formation of fibers completely lacking any differentiated contractile structure. Denervation atrophy can, therefore, be considered an atrophy with a loss of differentiation, rather than a "simple" atrophy, as usually described.

The electron microscopic observations demonstrate that several distinct processes contribute to this general trend. One of these can be described as "degenerative," applying to the ultrastructural level the long established criteria for the definition of this kind of lesion. It consists in a rapid destruction of whole parts of myofibrils in a confined area of the cell, and it is present in the first 2 weeks after the section of the nerve. Its contribution to the process is limited in time. As a consequence of the disappearance of the contractile material and of its substitution by large areas of undifferentiated cytoplasm, the elimination of the latter in blocks takes place. This is an active process, in which the sarcolemma is particularly involved.

The main process by which the progressive destruction of the contractile material is carried out is the slow but continuous peripheral detachment of filaments from the fibrils, followed by their breakdown. It can be detected early and it operates until the later stages. It can, therefore, be considered the fundamental expression of this type of atrophy.

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