A HISTOLOGICAL STUDY OF SKELETAL MUSCLE IN ACUTE ISCHEMIA *

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The death of cells is a complicated process which may be assumed to proceed in an orderly manner depending upon the initiating cause.[1] In the progress of studies upon voluntary or skeletal muscle it became necessary to make correlation between cell death and its early morphological manifestation. Muscle is of exceptional value, owing to its contractility, as a mechanism in which the morphological alterations constituting necrosis may be studied conveniently. The present investigation undertakes, therefore, to define the early histopathological alterations accompanying the necrosis of ischemic voluntary muscle and to correlate these changes with the times of loss of viability and contractility in this tissue.

Earlier workers,[2-8] because of interest in the pathogenesis of Volkmann's contracture, have devoted most of their attention to the chronic lesions arising from ischemia of voluntary muscle, and have given little attention to the initial phases or changes that occur during the first 24 hours. It has been strongly indicated [7] that the changes peculiar to Volkmann's contracture commence during the first hours of ischemia and are irreversible soon thereafter; however, no record of a detailed study of that initial period has been found. Those who have studied Zenker's degeneration [9-13] have similarly paid little attention to the very early changes and applied themselves to the later manifestations of the lesion or confined their early studies to artificial systems *in vitro*. Indeed, many of these workers have indicated that vascular occlusion causes no change in voluntary muscle even after 18 hours. In studies of skeletal muscle regeneration,[12,14] the phenomena analyzed closely are mainly those that occur after a lapse of 1 or more days following injury or repair. Consequently, despite their importance, the initial lesions and their pathogenesis remain to be described.

METHODS

A state of complete, acute ischemia was induced in the hind limbs of rabbits weighing 2000 to 3500 gm. and in albino rats of the Sprague-Dawley strain with an average weight of 250 gm. In the rabbit the

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ischemia was produced in two ways: by ligation of vessels and by application of tourniquets. The animals were operated upon under amytal anesthesia, occasionally supplemented with ether; the common iliac, common femoral, the inferior epigastric arteries and conspicuous branches of the common femoral artery were doubly ligated, under sterile precautions. Ligation of these particular vessels theoretically should preclude intervention of the collateral circulation, such as was described for the hind limb of the normal rabbit,[15] and the efficacy of these ligations was empirically indicated by one observer.[7] By this method a more complete ischemia of certain muscles was obtained than by selective ligation of the arteries to the particular muscles, because even with careful occlusion of the main vessels, a muscle may retain some effective vascularization from its connection with periosteum.[16] In 12 rabbits, including a few weighing 1500 gm., a tourniquet was applied tightly around one thigh so as to occlude arterial blood flow for the desired interval; these animals were also under amytal narcosis. The duration of the experiment was measured from the initial ligation of the common iliac artery, or the application of the tourniquet, until the muscle was removed for fixation. On the other hand, in rats ischemia was induced only by application of the tourniquet to the thigh under nembutal anesthesia. The duration was limited to 12 hours or less because the animal may either mutilate the occluded limb or bite off the tourniquet.

After the required time interval the animals, with further anesthesia when necessary, were prepared for operation. The muscles of both hind limbs were exposed and tested *in situ* for contractility with a faradic current, applied directly to the muscle through metal electrodes, which derived current from a DuBois-Reymond coil, arranged for faradization by a Neef's hammer. The strength of the current was gauged so as to produce a vigorous contraction of the normal limb muscles and was then used to test the contralateral counterpart in the ischemic limb. Contractions were graded as absent, weak, or tetanic, relative to those in the normal limb.

For histological studies the tissues were placed in 10 per cent neutral formalin, absolute ethyl alcohol, and freshly prepared Zenker's solution, at 37° C. Tissues were embedded in paraffin, cut longitudinally, approximately 8 μ in thickness, and stained with hematoxylin and eosin, and Mallory's phosphotungstic acid hematoxylin. The numerical incidence of abnormal muscle fibers was calculated approximately, by a count of 100 fibers in each slide, and represented as a percentage. In all instances comparisons were made between ischemic muscle and the contralateral normal muscle of the same animal. In addition,

teased unfixed fragments of ischemic and normal muscle from rabbits and rats were examined. Several samples of human muscle, taken at necropsy within an hour of death, were also studied.

Experimental Observations

Numerous normal muscles, fixed immediately in either formalin or Zenker's fluid, afforded a picture with which the alterations in ischemic muscle might be contrasted. Certain peculiarities of normal, rapidly fixed skeletal muscle were conspicuous. Of these the most salient was the prominence of longitudinal striations, which contributed largely to the pattern of the fascicles; the fibrils composing the striations were wavy or loosely undulant, usually giving the particular group of fibers a watered-silk appearance (Figs. 1 and 2). In association with this the individual fibers were compactly arranged and so closely approximated that, except for an occasional linear arrangement of nuclei, they frequently could not be distinguished and resembled a syncytium. Because of this I have designated these compact structural forms as "syncytoid." In contrast to the prominence of the longitudinal striations and fibrils, cross striations were inconspicuous. In fact, the definition of the longitudinal and cross striations was reciprocal; the distinctness of the one was proportional to the vagueness of the other.

It may be said, therefore, that the normal mammalian skeletal muscle (rabbit, rat, man) is syncytoid, with conspicuous longitudinal striations and vague cross striations. This differs from the pattern usually regarded as normal, in which the structure of the cross striation is accentuated.

When ischemia was of sufficient duration this pattern was altered in a significant manner. The syncytoid structure was lost early, owing to a departure of the fibers from their collective compact arrangement. They showed widespread individualization and separation by clear structureless spaces (Figs. 3 and 5). The individualized fibers appeared compact and cylindrical. The longitudinal striations in such fibers were vague or absent, and when present were no longer undulant. In contrast the cross striations (Fig. 4) were very manifest and became a dominant feature. With shorter periods of ischemia the finer structure of these cross striations did not differ from that in normal muscle. Following longer periods of ischemia a new type of cross striation appeared with increasing frequency. This differed from the normal, owing to the inordinately great bulk of the anisotropic segment, which was excessively wide and broad in contrast with the narrow isotropic segments (Fig. 6). Eventually the enlarged anisotropic segments assumed the appearance of a series of adherent disks, a feature enhanced

by the splitting away or cracking off of these segments (Fig. 7). The longer the period of ischemia the more widespread and well developed were these changes, particularly those affecting the transverse striations (Fig. 8).

The degree to which changes occurred differed considerably in the various muscles of the ischemic limb (Table I). In the series of rabbits studied the proximal thigh muscles were usually least affected, whereas the flexors and extensors of the leg were always involved to a degree dependent upon the duration of the ischemia. Of the various muscles examined the tibialis anticus reflected most constantly the duration of the ischemia, whereas in the other muscles no regularly reproducible pattern of behavior was demonstrable. In view of the constancy in

TABLE I

The Percentage of Ischemic Muscles Showing Abnormal Fibers in the Rabbit Series

Muscle	No. of muscles examined	No. with abnormal fibers	No. expected with abnormal fibers	Percentage*
Gracilis	11	4	11	36
Adductor magnus	11	2	11	18
Quadriceps	10	3	10	30
Gastrocnemius	20	11	14	78
Tibialis anticus	25	19	19	100

* The percentage is based on the number of muscles involved compared with the number expected to be involved from the duration of ischemia.

gradation of response of the leg muscles, especially the tibialis anticus muscle, this muscle was selected to present the development of the morphological changes due to ischemia, although similar alterations occurred in other muscles, but less frequently.

An alteration in structure was first observed in the muscles of animals subjected to ischemia for at least 4 hours. This was characterized by individualization of fibers, regression of longitudinal striation, and enhancement of cross striation. After 6 hours of ischemia there was, in addition, a change in the type of cross striation to that of abnormal anisotropic disks as described above. The number of fibers composed of such disks increased with the duration of ischemia (Table II), until after 18 to 24 hours nearly all were affected, with considerable fragmentation and splitting off of individual anisotropic disks. The onset of depression of contractility regularly preceded the observed morphological changes to a significant extent. The contractions were vermicular and weak after 2 hours, and entirely absent after 4 hours of ischemia.

On the other hand, in the rat (Table III) these changes were first

seen at the second hour and were well developed, with disk formation by the fourth hour. Thereafter the progression toward more extensive numerical involvement was identical with that in the rabbit. The contractions were unobtainable or weak following an ischemia of 2 hours and could not be elicited after longer periods. When this absence of

TABLE II

The Time of Appearance of Individualization of Fibers and Abnormal Disks, Correlated with Disappearance of Contractility, in the Tibialis Anticus of 25 Rabbits

Duration of ischemia	No. of animals	Individualization		Abnormal anisotropic disks		Contractible muscles
		No. of animals affected	Fibers	No. of animals affected	Fibers	
(*hours*)			(*per cent*)		(*per cent*)	
2	4	0	0	0	0	4
4	4	4	60	2	10	1
6	4	4	100	4	42	0
12	4	4	100	4	60	0
18	2	2	100	2	70	0
24	4	4	100	4	90	0
48–96	3	3	100	3	90	0

contractile response was correlated with the type of lesion it was apparent that it first became absolute at about the time of, or just prior to, the appearance of the abnormal cross striations. The association was that loss of function preceded the formation of the disks, whereas the presence of disks invariably indicated a loss or depression of function dependent upon the extent of the change.

TABLE III

The Time and Extent of Occurrence of Individualization of Muscle Fibers and Abnormal Cross Striations in Ischemic Tibialis Anticus of 14 Rats

Duration of ischemia	No. of animals	Individualization of fibers	Abnormal anisotropic disks	Contractible muscles
(*hours*)				
1	3	0	0	3
2	4	4	0	2
4	3	3	3	0
8	4	4	4	0

In a series of 14 rats in which the blood supply was permitted to return to the limb after varying periods of ischemia, this relationship was apparent. The muscles deprived of blood for an interval less than 12 hours contained a few small areas of normal syncytoid tissue, commingled sparsely with the numerous abnormal fibers composed of disks. These muscles were only weakly contractile. The muscle ischemic for 12 hours never regained its function and was composed completely of abnormal fibers. It is noteworthy that even after an ischemia of 3

hours the return of blood flow did not prevent the further extensive development of abnormal fibers.

During the early stages of cytoplasmic change no alteration in the nucleus was detectable. Normally the nucleus is of a diffuse, hazy, bluish gray (hematoxylin and eosin) with a few discrete, ill defined clumps of chromatin. After continuous ischemia for 12 or more hours the haziness vanished and the chromatin clumps were more conspicuous and numerous, leaving a clear structureless nucleoplasm. After 24 hours the nuclear membrane faded until eventually it completely disappeared through karyolysis with disruption of the entire structure.

Discussion

In freshly fixed rabbit, rat, and human skeletal muscle a compactness of structure which imparted a syncytium-like appearance was observed. This structural arrangement of closely adherent fibers in skeletal muscle has led certain observers [17-19] to consider it as syncytial, like cardiac muscle tissue. However, I am not inclined to accept the reasoning that either a syncytium-like appearance or difficulty in teasing the fibers apart warrants the designation of this tissue as a true syncytium, because the ease and completeness with which the fibers separate in the earliest stage of ischemia strongly indicate that the union between adjacent fibers is not intimate nor tenacious. However, owing to its constancy in normal muscle, this syncytium-like structure may be regarded as an important histological feature of healthy muscle, which has been designated as "syncytoid." This feature, together with the scarcity and vagueness of cross striations which Leser [2] observed and the accentuation of longitudinal striations, affords a picture strikingly different from that usually drawn by histologists, who accentuate the cross striations. Schäfer [20] did remark, however, that longitudinal striation is seen better in proportion as cross striation is less marked. Millar [21] advocated that for the better demonstration of the structure of skeletal muscle it is preferable to use tissue which has been cooled overnight at ice box temperature, because otherwise the cross striations are vague and interfered with by longitudinal striation. Mallory [22] emphatically stated that to demonstrate the "myoglia fibers" autopsy tissue is practically useless and urged the use of muscle tissue obtained at operation. This inverse proportion of longitudinal and cross striations, with the enhancement of one at the expense of the other, tends to substantiate Carey's [23] concept of the entire muscle fiber as a single morphological and functional unit composed of an undifferentiated "neuromyoplasm." Certainly the reciprocal evanescence of both structures does not support either as the sole permanent structural unit.

The considerable importance attached to the mere presence of cross striations as an indication that a particular fiber or group of fibers is normal requires modification. The finding that, under the conditions of acute and chronic ischemia, the cross striations are rendered conspicuous and coarse, and are altered in type, suggests not only that cross striations do not always represent viable muscle but that they may, if of a certain type, actually signalize nonviable, degenerated fibers. This type of peculiar discoid cross striation was first described by Bowman,[24] although he believed it to be a normal feature; such cross striations have been referred to as Bowman's disks. They have been demonstrated by Leser [2] and photographed by Clark,[14] under circumstances which leave no doubt of their degenerative nature; Clark named them "conchoidal plates," and found them in degenerated muscle grafts 7 and 11 days old. Clarke [8] observed them in muscle tissue from cases of clinical and experimental Volkmann's contracture, of 70, 97, and 300 days' duration. The significance of the disks has usually been overlooked; for example, among Griffiths' [7] illustrations of ischemic contracture one shows these peculiar cross striations which he simply referred to as "preservation of striations," as have other observers including Wells.[10] In an illustration of Fishback and Fishback [9] where an absence of striations is indicated, these disks are conspicuous.

I have used the tibialis anticus to determine the time and rate of appearance of these changes. This muscle invariably underwent massive necrosis when ischemia was sufficiently prolonged. Although Wilson [6] indicated that the quadriceps was most frequently involved in his cases, he based his figures on scattered foci of necrosis and excluded muscles with extensive necrosis, so that his criteria were different. When necrosis occurred in other muscles in my series the rate of appearance and character of the lesions paralleled closely those found in the tibialis anticus.

In numerous sections of normal muscle from a variety of species, including man, rabbit, and rat, I have been unable to find Bowman's disks. On the contrary, they are a constant feature in all of my examples of *ischemic* muscle, with a constancy of time of appearance and rate of development. Since they are coincident in time of appearance with biochemical irreversibility in ischemic muscle,[25] they may represent the morphological manifestation of structural irreversibility consequent upon the establishment of static biochemical equilibrium or death due to ischemia. According to Schoenheimer,[26] in order to maintain structure against its tendency to collapse energy must be expended continuously. Under circumstances in which the oxidation, phosphorylation, and synthetic processes are known to cease, *e.g.*, ischemia,

their cessation is closely followed by such structural collapse, which is seen, in this instance, as a peculiar aggregation of the cytoplasmic constituents.

It is averred by Clark [14] that the formation of these disks is induced by formaldehyde used as a fixative, which cross links such chain molecules of myosin as may be in a more parallel arrangement. Against this it may be cited that Bowman [24] first described these structures 53 years before formaldehyde was introduced into cytological technic.[27] Furthermore, I have demonstrated them with fixatives other than formaldehyde, *e.g.*, Zenker's fluid and alcohol, and in freshly teased ischemic muscle. Moreover, Fischer [28] found that 40 per cent formaldehyde has only slight power of precipitation of proteins and a 4 per cent solution none whatever; on the contrary it renders protein solutions incoagulable by many coagulating agents. It may be pointed out in addition that if formalin alone produced this alteration it should be seen in the normal formaldehyde-fixed muscle, where it was never found. Nevertheless, it is not improbable that the fixative acts upon some fundamentally altered protein, which, since it comprises most of the muscle protein, is most likely myosin. The manner of action postulated by Gustavson [29] is that formaldehyde effects methylene cross links between peptide chains and micellar units; this view is shared by Theis and Ottens [30] and by Wilson.[31] Since this conclusion is reached from study of the collagen type of protein molecule and not the myosin-keratin type, and since the detailed structure of protein molecules is still largely unknown,[32] as is also the exact mode of union of formaldehyde with them, it would be hazardous to attribute such a change in the protein of ischemic muscle solely by analogy to altered or new chemical linkages. Furthermore, with the recognized importance of "coacervats" [33] in biological phenomena, their state in all systems reaching the equilibrium of death requires consideration. That alteration in colloidal state may constitute the basis of these changes is indicated by Bechhold,[34] who may be quoted:

> "*With the occurrence of death protoplasm gelatinizes,* Brownian movement of the smaller particles ceases, and the structure of the gel appears in the ultramicroscope as a conglomeration of many reflecting platelets. It makes a substantial difference whether the protoplasm slowly dies or is suddenly killed by a fixative (alcohol, formalin, etc.). In the first instance there is a precipitation (flocculation), whereas, in the latter there is a *stiffening;* this difference may be readily recognized under the ultramicroscope."

Whatever may be the nature of the underlying change, its importance as a morphological manifestation appears to be that it represents a terminal structural event in the process of cell death. Under restricted

conditions of ischemia it is possible to enumerate this series of events as cessation of the processes of oxidation and phosphorylation, depletion of biochemical reserves, loss of contractility, biochemical irreversibility, and finally irreversible structural change or degeneration.

Summary and Conclusions

1. Skeletal muscles of rabbits and rats, rendered ischemic for periods of from 1 to 96 hours by ligation of vessels and application of tourniquets, were studied histologically and compared with normal contralateral muscles.

2. The characteristics of normal, rapidly fixed muscles are a syncytoid structure with vague cross striations and a conspicuousness of undulant longitudinal striations. Nuclei are deeply basophilic, with a fine chromatic network.

3. With ischemia of 2 to 4 hours' duration the fibers are individualized, longitudinal striations disappear, and cross striations become a conspicuous cytological feature. After longer periods of ischemia abnormal anisotropic disks, Bowman's disks or conchoidal plates, appear and involve the muscle fibers in increasing numbers up to 18 hours of ischemia, at which time they are nearly ubiquitous. They are true degenerative forms and not artefacts caused by fixation and sectioning.

4. Weakness or absence of contractility precedes and accompanies the appearance of these disks and is correlated with their presence and extent of involvement, so that they serve as a clear indication of nonviable fibers and constitute a morphological manifestation of cell death in skeletal muscle.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 89

FIG. 1. Normal, formalin-fixed skeletal muscle of the rabbit. The fibers are closely approximated and appear syncytial. Hematoxylin and eosin stain. × 70.

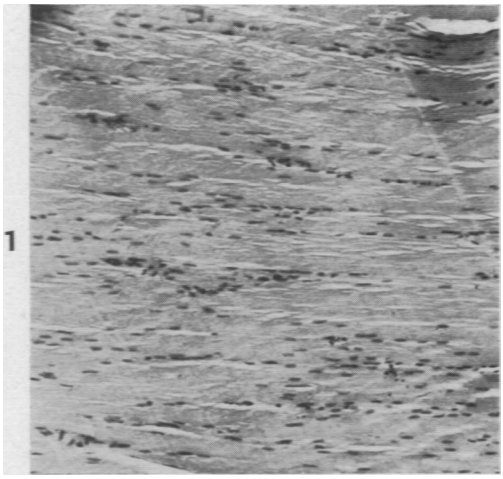
FIG. 2. Normal tibialis anticus muscle from the rabbit. The longitudinal striations are strongly evident. Cross striations are indistinct and fine. Fibers are not separate. Hematoxylin and eosin stain. × 300.

FIG. 3. Tibialis anticus of a rabbit after 4 hours of ischemia. The muscle fibers are individualized and separate. Hematoxylin and eosin stain. × 70.

FIG. 4. High power photomicrograph of the same muscle seen in Figure 3. The longitudinal striations are not seen clearly, but the cross striations stand out prominently, with well marked isotropic bands. Hematoxylin and eosin stain. × 300.

FIG. 5. Tibialis anticus of a rabbit after 6 hours of ischemia. The individualization is further accentuated. Contours of the fibers appear more cylindrical. Hematoxylin and eosin stain. × 70.

FIG. 6. High power view of the same muscle seen in Figure 5. The new type of broad anisotropic disk and the splitting off of these disks are notable features. Hematoxylin and eosin stain. × 300.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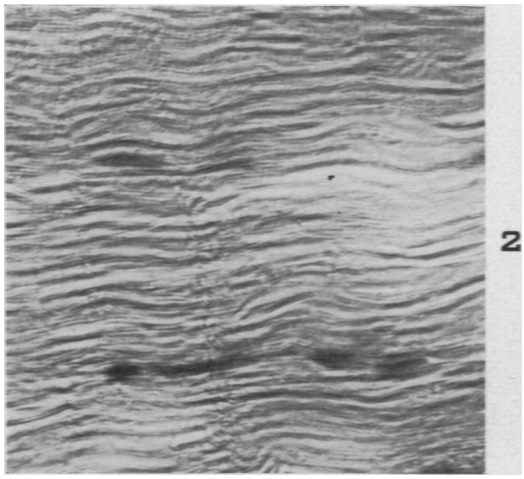
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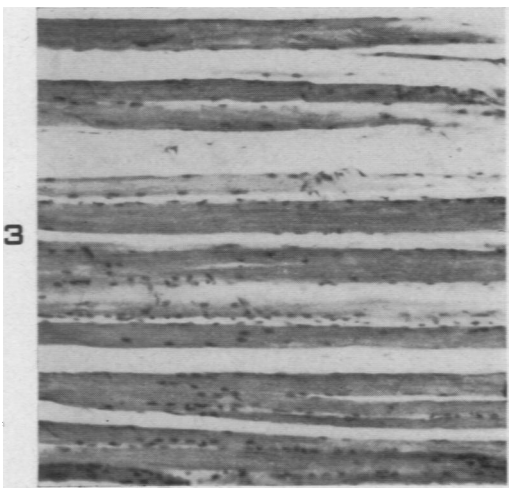
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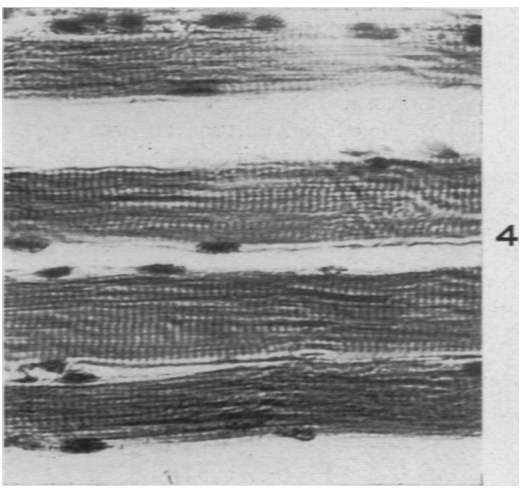
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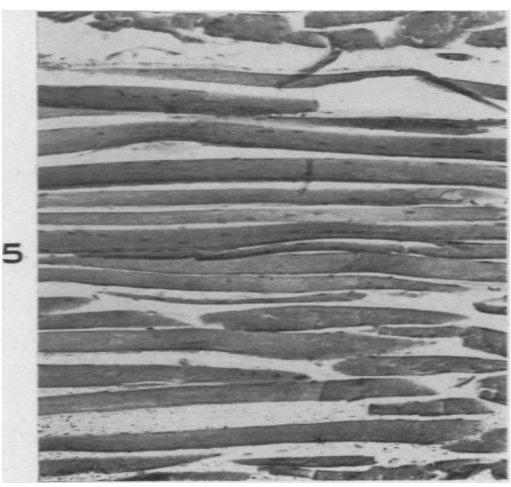
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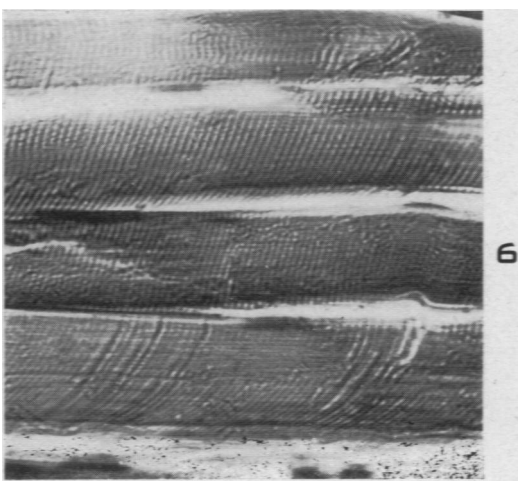
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Harman Skeletal Muscle in Ischemia

PLATE 90

FIG. 7. Tibialis anticus of a rabbit after 96 hours of ischemia. The fibers are broad, individualized and have developed multiple transverse cracks. Hematoxylin and eosin stain. × 70.

FIG. 8. High power view of the same muscle seen in Figure 7. Here the splitting off and isolation of disks, responsible for the transverse cracking, are seen. Hematoxylin and eosin stain. × 300.

FIG. 9. Zenker-fixed tibialis anticus of a rabbit after 96 hours of ischemia. The fixative accentuates the Bowman's disks. Hematoxylin and eosin stain. × 300.

FIG. 10. Extensor muscle (tibialis anticus) of a rat after 12 hours of ischemia. The alterations are similar to those in the rabbit. Tourniquet was released shortly before the muscle was excised. Hematoxylin and eosin stain. × 300.

FIG. 11. Tibialis anticus of a rat after 8 hours of ischemia. The fragmentation, disk formation, and cracking are evident. Hematoxylin and eosin stain. × 300.

FIG. 12. Normal human pectoralis major muscle, taken at autopsy within an hour of death. The pattern is identical with that of normal rabbit and rat muscle. The transverse striations are more conspicuous, however, in autopsy tissue. Hematoxylin and eosin stain. × 300.

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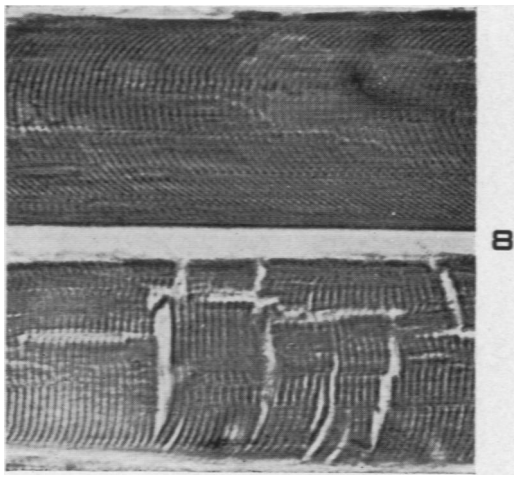

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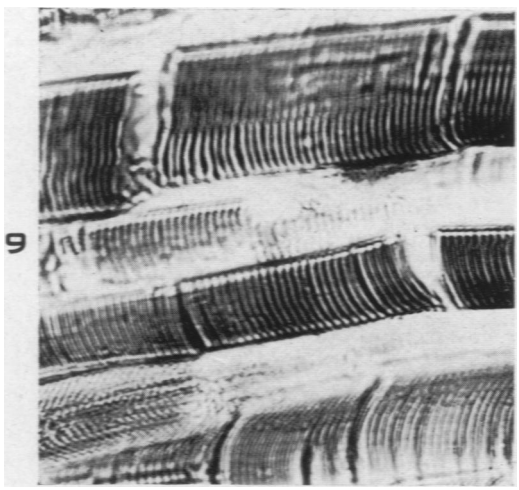

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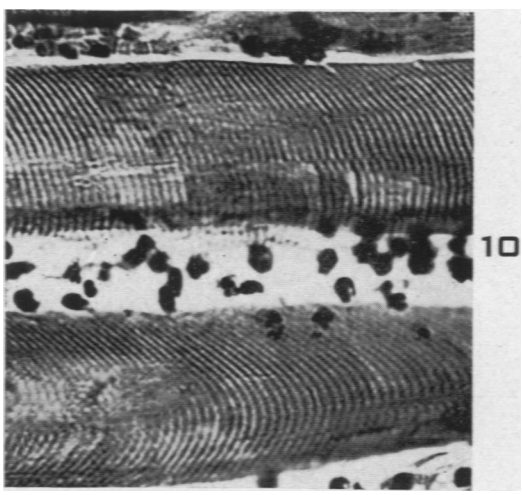

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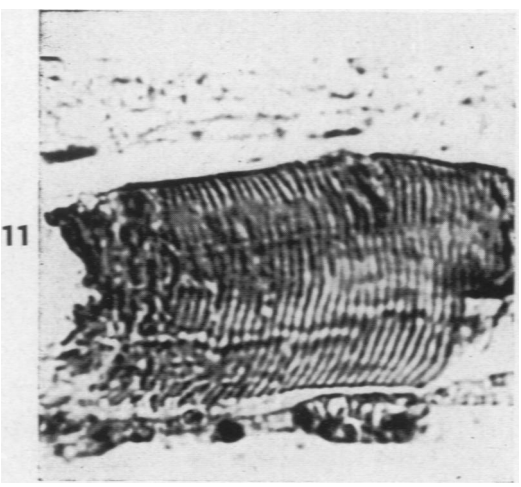

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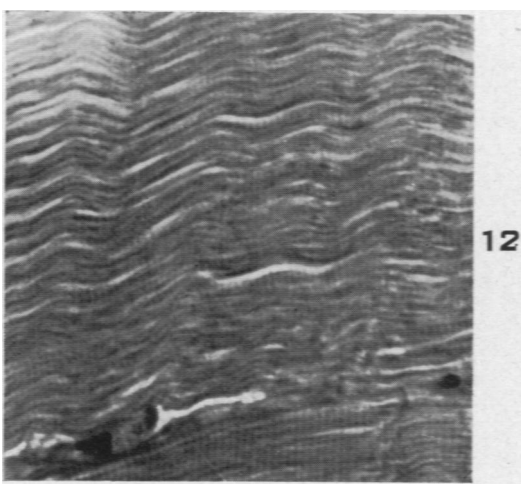

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