THE RECOVERY OF SKELETAL MUSCLE FIBERS FROM ACUTE ISCHEMIA AS DETERMINED BY HISTOLOGIC AND CHEMICAL METHODS*

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In a previous study of the histologic changes induced in skeletal muscle by acute ischemia,[1] it was found that death of muscle fibers occurred in an orderly, progressive manner up to 12 hours, at which time the extent of necrosis is considerable. Subsequently it was shown[2] that, even following brief intervals of ischemia, further extension of the necrosis was not prevented when a vascular occlusion was released. The duration of ischemia was perpetuated by both stasis and capillary damage, thereby increasing significantly the extent of necrosis. Although the increase in necrotic fibers was marked, many appeared normal by histologic standards after as long as 6 hours of complete ischemia. The fate of such intact fibers is of paramount importance; their number and their ability to regain contractility, among other factors, may determine the degree of functional recovery of the muscle as a whole.

It has been tacitly assumed[3,4] that, with the initiation of ischemia in skeletal muscle, there is a *pari passu* depletion of energy reserves, onset of autolysis, and incidence of death in all the muscle fibers. Depletion of energy reserves is presumed to be a criterion of cell death[5] and in some instances of autolysis.[6] While current studies of chemical events appear to substantiate such a view, the behavior of the histologic pattern is not so easily reconciled with it, unless there are within many fibers alterations more subtle than are detectable by microscopic examination. Because of this discrepancy it is relevant to ascertain the ability of such fibers to recover from ischemia and to determine the factors responsible for cell death or irreversible changes in fibers; it is particularly important to decide whether the essential irreversible event is structural or chemical. If chemical alteration alone is the determinant, the histologic picture may be equivocal in the evaluation of viability; whereas if the histologic structure represents a reliable criterion of viability, its exact significance deserves closer consideration. This relationship of histologic structure to viability and chemical change is, therefore, the object of the present study.

* Read by title at the Forty-Fifth Annual Meeting of The American Association of Pathologists and Bacteriologists, Philadelphia, March 12 and 13, 1948.

Received for publication, July 15, 1948.

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Experimental Procedures

Ischemia was produced in one hind extremity of male albino rabbits, weighing 2000 to 3000 gm. and of Sprague-Dawley male rats, weighing 250 to 300 gm., by a technic previously described.[2] After varied periods of ischemia the tourniquet was released and the extensor muscles were excised during nembutal narcosis from both the ischemic and normal legs, following recovery for 0, 3, and 24 hours. Immediately before excision the muscles were tested for contractibility by faradic current.[1] The excised muscles were fixed in 10 per cent formalin, at 37° C., embedded in paraffin, and sectioned 8 μ thick. The muscles were sampled to allow representative examination of all parts; sections were stained with hematoxylin and eosin. In each section the normal and necrotic fibers were identified, and 100 consecutive fibers were counted differentially, so that an average percentage of normal and of diseased fibers was calculated for each muscle. The contralateral muscle from the normal limb served as a control in each animal.

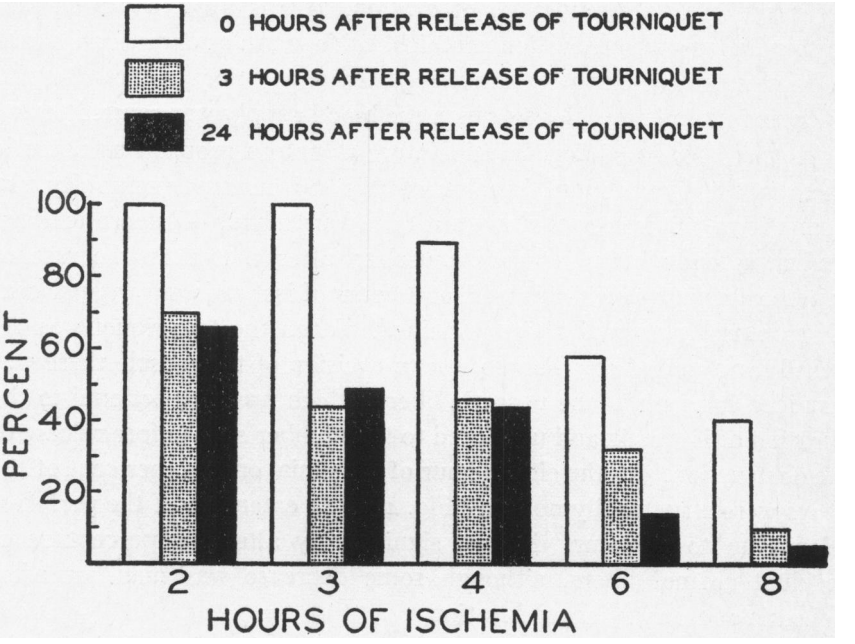
In a second series of male rats the tourniquet was applied to one thigh for 4 hours and a recovery period of 24 hours permitted, after which the animals were narcotized with nembutal and the extensor muscles of the legs exposed. The distal tendinous attachment of the tibialis anticus muscle was carefully sectioned and the muscle freed by gentle dissection to its proximal fleshy attachment. Contractility was tested by a faradic current and sufficient time for restitution then allowed, after which the muscle was rapidly excised and plunged into liquid air. The frozen muscle was then pulverized in a chilled bronze chamber. The chamber consisted of a central well into which was fitted a closely adapted solid cylinder for crushing the tissue, with a surrounding moat for containing the chilling liquid, usually liquid air. Portions of crushed tissue were distributed to tared 50 cc. flasks containing 5 cc. of cooled 10 per cent trichloracetic acid and zinc sulfate solutions, respectively. After reweighing the flasks the tissues were examined for several components. The fraction contained in trichloracetic acid was exhaustively extracted and the extract analyzed quantitatively for adenosine triphosphate, phosphocreatine, and acid-soluble phosphorus by the scheme of LePage.[7] Glycogen was estimated on the extracted residue by the method of Good, Kramer, and Somogyi,[8] with determination of sugar by adaption of Hoffman's[9] technic to a spectrophotometric procedure. The fraction extracted by zinc sulfate solution was used for determination of lactic acid by Winnick's microdiffusion method.[10]

The analytic figures were corrected for increases in volume due to edema by simultaneous excision of the digitorum longus muscles from

the ischemic and normal legs. These muscles were weighed in tared crystal dishes at once and were reweighed after drying for 12 hours at 110° C. Corrected values might have been expressed by MacFarlane and Spooner's formula[11] or, as in this instance, interpreted directly for 100 gm. of dried tissue. It is assumed that there is no significant loss of tissue protein from the damaged muscle.

Results

With complete unrelieved ischemia the histologic pattern changed from the normal syncytoid[1] structure at the second hour, with disappearance of the normally conspicuous longitudinal striations and accentuation of the transverse striations, which were much less conspicuous in



Text-Figure 1. The percentage of histologically normal fibers after different periods of ischemia and intervals of release for 65 rabbits.

the rapidly fixed muscle. Further ischemia was associated with the appearance of Bowman's discoid degeneration (Fig. 1) at about the fourth hour; the number of fibers affected by this change reached a maximum of nearly 90 per cent by the twelfth hour of ischemia. If the ischemia was relieved at any time after the second hour, there was a further extension of degenerated fibers over the next 3 hours, when the number reached a maximum and did not increase even after 24 hours of recovery (Text-Fig. 1). It was apparent, however, that, in contradistinction to the muscles with unrelieved ischemia, those with release of

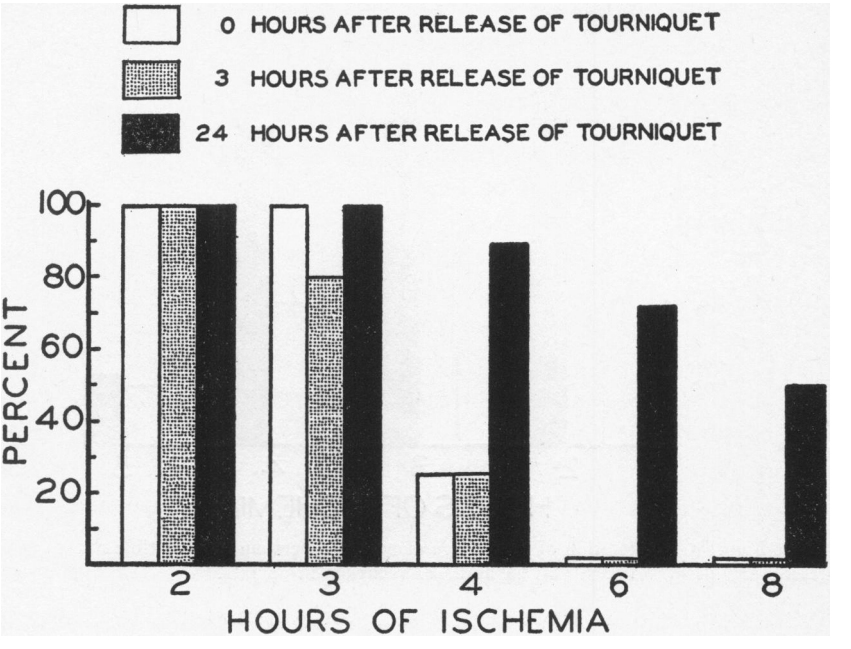
occlusion contained fibers with several types of necrosis. Many of the fibers conformed in appearance with Zenker's hyaline degeneration; they were large, homogeneous, and contained a structureless, faintly acidophilic cytoplasm (Fig. 2). Others were composed of a granular, disorganized, deeply acidophilic cytoplasm and might be designated as showing granular necrosis (Fig. 3) in accordance with Fishback's description.[12] The percentage of these different forms of necrosis in the muscles released from ischemia was variable and unpredictable; occasionally the Bowman's type was predominant, and at other times either the Zenker or the Fishback form was most prevalent. It was not possible to discern the transformation of one form to another, except that certain fibers appeared to progress through either the discoid or waxy degeneration to a granular disintegration. It was clear, however, that neither Zenker's degeneration nor Fishback's granular degeneration was to be seen in muscles with unrelieved ischemia; they occurred exclusively in ischemic muscles to which the blood supply was readmitted.

All three forms of degeneration were therefore grouped as necrotic fibers in the fiber counts, because of this independent variability. In Text-Figure 1 it is seen that not until the fourth hour did necrotic fibers appear in completely ischemic muscles; after that time the rate of appearance was progressive until at 8 hours of ischemia only 33 per cent of structurally normal fibers remained. When the tourniquet was released for a period of 3 hours prior to excision of the muscle there was a sudden increase in the necrotic fibers, which was proportional to the duration of ischemia and unrelated to the number seen before release of the obstruction. By the eighth hour of ischemia, only 10 per cent of the fibers were structurally normal (Fig. 4). The extension of the period of release up to 24 hours did not significantly alter the percentage of residual normal fibers, although some decrease was manifest after ischemia of 6 and 8 hours' duration.

It is apparent that release of the occlusion, rather than obviating the necrosis, favors its extension. The return of blood flow is associated with a sudden increase in necrotic fibers, which rapidly reach a maximum with little subsequent progression. The connection between the inflow of blood, the appearance of the two forms (Zenker's and Fishback's types) of degeneration, and the sudden increase in necrotic fibers seem correlative. These phenomena may well be, but in such an instance one would expect that after ischemia for 2 and 3 hours with subsequent release, such muscles would contain necrotic fibers solely of these types, especially as Bowman's disks are absent before release, yet all forms

are usually well represented. Even if the inflow of blood is instrumental in the increase of necrosis, other factors cannot be excluded.

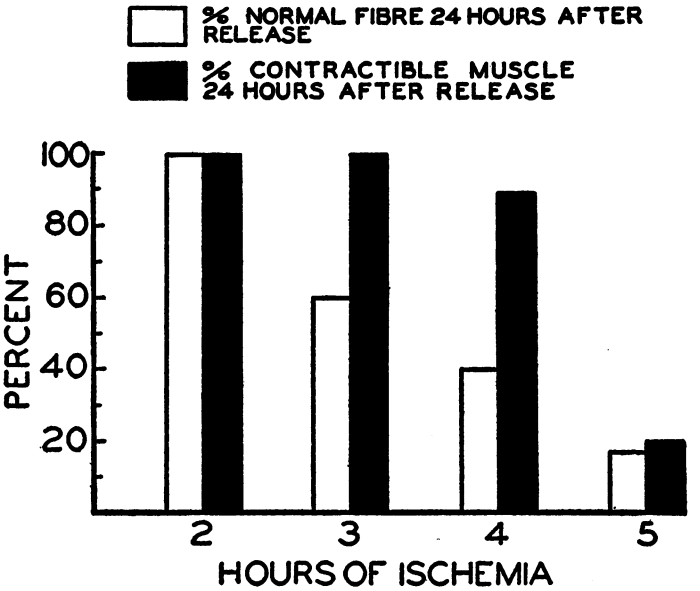
When the contractility of muscles was assessed after various periods of ischemia, it was found that nearly all had lost this property by 4 hours, and all had become non-contractible by six hours (Text-Fig. 2). By 3 hours after release of vascular occlusion the state of the muscle was little altered, whereas by 24 hours after release a considerable number of muscles had regained contractibility to greater or less degree. Up to 90 per cent had recovered marked ability to contract after 4 hours of ischemia, and 50 per cent were again contractible, at least in part, after 8 hours of ischemia. As might be expected from the greater percentage of surviving fibers after shorter durations of ischemia, the muscles sub-



Text-Figure 2. The percentage of contractible muscles after various periods of ischemia and intervals of release for 70 rabbits.

jected to briefer periods of ischemia recovered a greater strength of contraction. The survival of histologically normal fibers after different durations of ischemia was paralleled not only in the recovery of contractibility, but also in the vigor of the contraction, which bore an approximate proportion to the extent of fiber survival. In Text-Figure 3 it is demonstrated that the same relationship between contractibility of muscles and recovery of normal fibers also applies to ischemic rat muscle, which follows a similar trend.

To elucidate more exactly the interrelationship of fiber survival and ability of muscles to contract, the muscles were divided into the contractible and non-contractible groups. In each of these the percentage of histologically normal surviving muscle fibers was determined and the average assessed for each period of ischemia for both rabbit and rat muscle. The results are shown in Text-Figure 4. In all contractible muscles the normal fibers comprised a considerable part of the muscle mass, even after 6 hours of ischemia; the low level of 10 per cent was reached only after 8 hours of vascular occlusion. On the other hand, among the non-contractible muscles the average percentage of normal

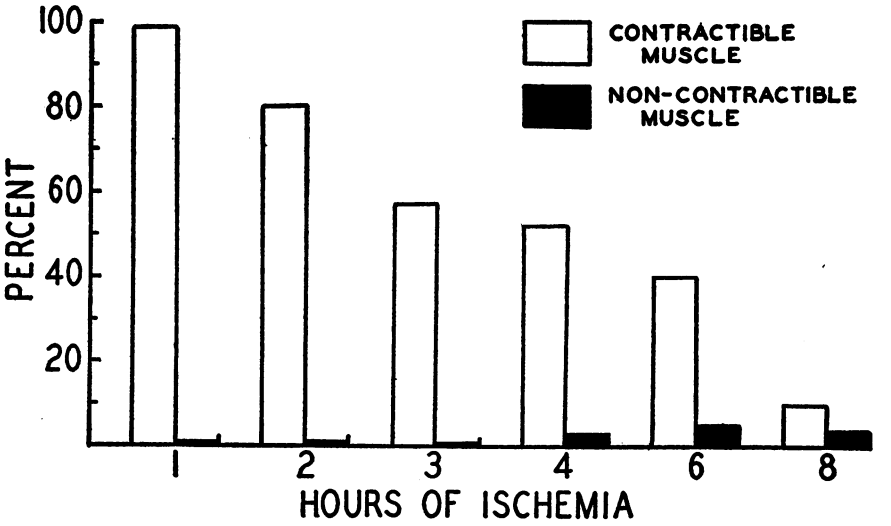


Text-Figure 3. Correlation of percentage of normal fibers and contractible muscles after different periods of ischemia and 24 hours of recovery in 60 rats.

fibers never exceeded 4 per cent; the majority of muscles in this group contained no normal fibers whatsoever. It again became evident that the ability to respond as well as the strength of response to electrical stimulation is intimately related to the presence and number of these histologically normal fibers.

The maximum degeneration of fibers was accomplished within 3 hours after release of the occlusion, whereas contractibility failed to return before about 24 hours subsequent to release despite the presence of many histologically normal fibers. In such instances there existed a form of structurally normal but physiologically inactive fiber. Chemical analyses on ischemic muscle from several rabbits and rats immediately after termination of 4 hours of ischemia revealed that there was

complete depletion of various energy reserves at such time. Such was the condition, also, after a further 3 hours of recovery. However, after a recovery period of at least 24 hours following an ischemia of 4 hours, the restitution of high energy compounds was considerable; adenosine triphosphate, glycogen, and phosphocreatine reaccumulated to between one-quarter and one-third of that found in the contralateral normal muscle (Table I). Whereas in normal muscle such might signify a marked depletion, in this ischemic contractible muscle in which the percentage of histologically normal fibers was 40 per cent or lower (Text-Fig. 3), the re-gain of essential metabolites fairly approximated that of the



Text-Figure 4. Correlation of contractibility and normal fiber percentage at 24 hours after release from different periods of ischemia in 65 rabbits and 60 rats.

surviving contractible tissue. The reaccumulation of these compounds essential to the contractility of muscle accorded well with the structural survival of muscle fibers. Although there was recovery of oxidative metabolic function and resynthesis of both phosphorylated compounds and glycogen, the injured muscles contained a significantly greater amount of lactic acid, indicating the presence of some glycolysis. It is true, however, that these muscles were heavily infiltrated with polymorphonuclear leukocytes to which such an accumulation of lactic acid might be due in part. There also was considerable stasis and sluggishness of circulation which might contribute to the slow removal of the products of contraction induced by the faradic test for contractibility.

The interval required for recovery adequate to permit contraction appears so markedly a function of time that the results of an additional

TABLE I

Results of Chemical Analyses on Ischemic Rat Muscle *

Animal no.	Tissue weight	Water	Acid-soluble phosphorus	Phospho-creatine	Adenosine triphosphate†	Lactic acid	Glycogen‡
	gm.	%	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*
1 Nor.	0.6579	69.6	136.8	243	321	19.5	430.6
1 Isc.	0.7726	82.1	31.2	72	63	23.8	37.6
2 Nor.	0.6212	74.7	123.4	236	296	16.9	414.2
2 Isc.	0.8064	82.2	29.6	62	79	39.5	48.1
3 Nor.	0.8785	71.3	117.5	194	326	20.1	386.7
3 Isc.	0.9729	79.6	28.4	41	120	27.6	56.2
4 Nor.	0.8853	62.4	128.7	233	341	17.8	448.2
4 Isc.	0.9945	89.9	32.4	56	96	37.6	16.2
5 Nor.	0.4064	58.9	121.4	221	352	18.7	426.1
5 Isc.	0.7751	71.2	37.5	49	102	28.4	61.0
6 Nor.	0.3359	67.8	156.0	254	301	22.4	451.0
6 Isc.	0.6383	79.0	22.9	59	51	29.7	
7 Nor.	0.4214	66.8	139.2	216	314	13.6	433.7
7 Isc.	0.5177	77.2	44.1	42	78	23.2	
8 Nor.	0.4133	66.0	119.5	201	329	17.4	424.6
8 Isc.	0.5989	76.5	16.8	21	91		47.2
9 Nor.	0.6081	67.2	124.2	214	311	18.5	419.5
9 Isc.	0.8449	74.6	26.7	68	84	29.6	29.2
10 Nor.	0.6544	75.8	136.6	229	324	22.0	401.2
10 Isc.	0.8216	80.1	31.2	36	81	28.7	102.1
11 Nor.	0.7569	66.3	134.0	231	317	20.6	443.2
11 Isc.	0.8455	77.2	39.7	44	76	34.2	57.1
12 Nor.	0.6423	67.1	127.2	217	312	23.1	432.0
12 Isc.	0.8735	75.4	41.2	31	106	31.2	42.1
Mean: Nor.			130.3	224.0	328.5	19.2	425.9
Isc.			31.0	48.4	85.6	30.3	53.4
Stand.) Nor.			±10.6	±16.2	±17.2	±2.7	±18.2
dev.) Isc.			± 7.7	±14.5	±17.8	±4.9	±19.7
Stand.) Nor.			± 2.2	± 3.3	± 3.5	±0.5	± 3.7
error) Isc.			± 1.5	± 2.9	± 3.6	±1.1	± 4.6

* All rats were subjected for 4 hours to total ischemia of one hind limb and then allowed to recover for a further 24 hours, at termination of which time the chemical analyses were performed on the tibialis anticus muscle. Prior to excision for chemical analysis, the ischemic muscles were tested faradically and were found to be contractible in all animals.

† Calculated from pentose and easily hydrolyzable phosphate (7 min. at 100° C. in HCl).

‡ Glycogen is expressed as glucose.

experiment designed and performed on 25 rats are pertinent. The animals, after 4 hours of ischemia and under nembutal narcosis, were prepared by exposure of the tibialis anticus muscle and attachment of the distal tendon of this muscle to the lever of a kymograph. Tests by direct faradic stimulus elicited no response until at least 21 hours had

elapsed subsequent to relief of ischemia. The factors responsible for this particular time interval of recuperation are not clarified by these experiments and further investigation along different paths will be necessary.

Discussion

The interpretation of structurally normal muscle fibers, which survive the period of anoxia, as viable cells is substantiated by the recovery of contractibility and resynthesis of energy reserves by muscles containing a significant number of such fibers. Moreover, the ability to contract and the degree of contraction are closely allied to the proportion of histologically normal fibers which persist. Further support of this is offered by the fact that in such muscles as become non-contractible, normal fibers are entirely lacking or present only in small numbers, whereas in contractible muscles these fibers comprise a major element of the tissue. From this it may be deduced that the structural condition of the fibers is an important factor in their ability to recover from ischemia. Fibers which maintain structural integrity throughout the period of vascular occlusion and the first few hours thereafter are with few exceptions capable of regaining function; it is tantamount to saying that structurally intact fibers are viable fibers, although in the early phases of recovery they contain no energy reserves and are still non-contractible.

The view that such fibers, which contain no energy reserves and are non-contractible, are still viable requires elucidation. Definitions of viability depend in one respect upon criteria which are consistent with non-viability or death. If the content of energy-rich compounds and the presence of irritability are accepted as decisive characteristics of living tissue, it might appear that their absence signifies death of the tissue. Upon closer scrutiny it is not entirely logical to conclude that because these attributes signify viability therefore their lack indicates death. It consequently becomes apparent that the state of non-viability and the state of simple depletion of energy reserves may not always be synonymous, although they are usually concomitant. All tissues which suffer exhaustion of high-energy supplies do not *ipso facto* die, even when the duration of depletion is prolonged. Many tissues, muscle particularly, recover their anabolic capacity and regain function. The decision of the incidence of death is related, therefore, not so much to the loss of essential metabolites and function as to the inability of the tissue to regain these within an appropriate length of time. The irreversibility of the loss is the essential factor rather than the loss itself.

In muscle the gap between loss of energy reserves and its irreversibil-

ity is very marked, and for many fibers may represent several hours. If such loss itself represents death or "biochemical death" as it has been called, then all fibers should die simultaneously about the time of exhaustion of these reserves and the cessation of expenditure of energy. We have found this to be untrue for muscle; the fibers commence to die at the time of complete exhaustion of the energy reserve and gradually are destroyed in proportion to the duration of ischemia. The state of the energy reserves, therefore, is indecisive as an indication of the condition of the muscle fibers. This is more accurately reflected in the structural picture of the fibers provided that adequate time is allowed for full development of necrosis, when the histologically normal and abnormal fibers may be accurately separated. Since the structure of the fiber is more closely related to its state of health than is the immediate energy content, it is probable that the decisive determinant of irreversibility is not the condition of energy expenditure but the structural integrity. Although it is acceptable to agree with Schoenheimer[13] that energy must be expended continuously to maintain structure against its tendency to collapse, it may be emphasized that the critical event in the tendency toward irreversibility is the collapse of structure rather than the preliminary breakdown of the energy cycle. The series of events leading to ischemic necrosis may then be enumerated as anoxia, glycolytic depletion of energy reserves, cessation of energy exchange, and structural collapse, which last decides the biochemical irreversibility.

It has been proposed by MacFarlane and Spooner,[11] on the basis of indirect evidence, that ischemic muscle fails to resynthesize phosphorylated adenosine compounds because it is unable to re-aminate inosinic acid. But we have found on direct chemical analysis a considerable resynthesis of adenosine triphosphate and phosphocreatine, as well as a marked ability to contract, in muscles recovering from ischemia. The reason for this discrepancy is not readily apparent. It is pertinent, however, that in intact muscle the inosinic acid formed during contraction is rapidly re-aminated,[14] and that this re-amination occurs only under aerobic conditions.[15] The relative anoxia induced by the stasis which follows protracted ischemia[2] may for a time prevent this re-amination, in view of its sensitivity to lack of oxygen. When stasis diminishes and the circulation improves, re-amination takes place. The evidence of our experiments substantiates this view and receives support from the observations of Bollman and Flock[3] that in instances in which fatal shock is avoided the return of acid-soluble phosphates to the ischemic muscle is appreciable after several days, and that after 2 weeks the animals regain functional activity of the ischemic leg.

The observation that following long periods of ischemia many fibers are capable of oxidative resynthesis finds its corollary in the work of Bielschowsky and Green,[16] who postulated that ischemic muscle might be capable of resynthesizing adenosine triphosphate and were able to achieve this *in vitro* with the enzyme systems of both ischemic and normal rat muscle. Our studies have confirmed their results *in vivo* and established the validity of their view that the re-accumulation of the phosphorylated compounds is mainly a matter of adequate recovery time. The enzyme system is present and intact so that other factors may be responsible for the slow rebuilding of the energy reserves. The nature of these factors is not clarified by the present experiments, although there is some indication from the persistent high level of lactic acid that important elements may be stasis and a high state of aerobic glycolysis.[17] The significance of stasis in the perpetuation of ischemia has been discussed in a previous study[2] and is further suggested in the present analysis by the occurrence of Bowman's discoid necrosis in muscles recovering from 2 to 3 hours of ischemia. These forms usually are not found before the fourth hour of ischemia, so that their occurrence in these muscles under the circumstances of recovery indicates some persistent ischemia. In view of the previous vascular experiments[2] the cause of the relative ischemia may be attributed to capillary stasis. It is probable that this persistent stasis and anoxia preclude the accumulation of biochemical reserves and account for the slow restitution of contractility.

Summary

The correlation of histologic structure, irritability, and biochemical alterations in skeletal muscles recovering from acute ischemia reveals that histologic evaluation permits a more accurate index of viability of fibers than do the biochemical methods utilized.

Cellular death is not so much dependent upon depletion of biochemical reserves as upon the irreversibility of the depletion. This irreversibility is determined by the integrity of the cell structure.

The slow functional recovery of ischemic skeletal muscle is associated with a prolonged relative ischemia due to stasis, which may delay biochemical restitution in the residual normal fibers.

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DESCRIPTION OF PLATES

PLATE 112

FIG. 1. Muscle fibers from skeletal muscle ischemic for 6 hours followed by a recovery period of 3 hours are composed of Bowman's anisotropic disks. Nuclei are pyknotic. Hematoxylin and eosin stain. × 500.

FIG. 2. Zenker's hyaline change is manifest in several fibers removed 3 hours after release from an ischemia of 6 hours' duration. The structureless homogeneity of the cytoplasm and pyknosis of nuclei are evident. Hematoxylin and eosin stain. × 500.

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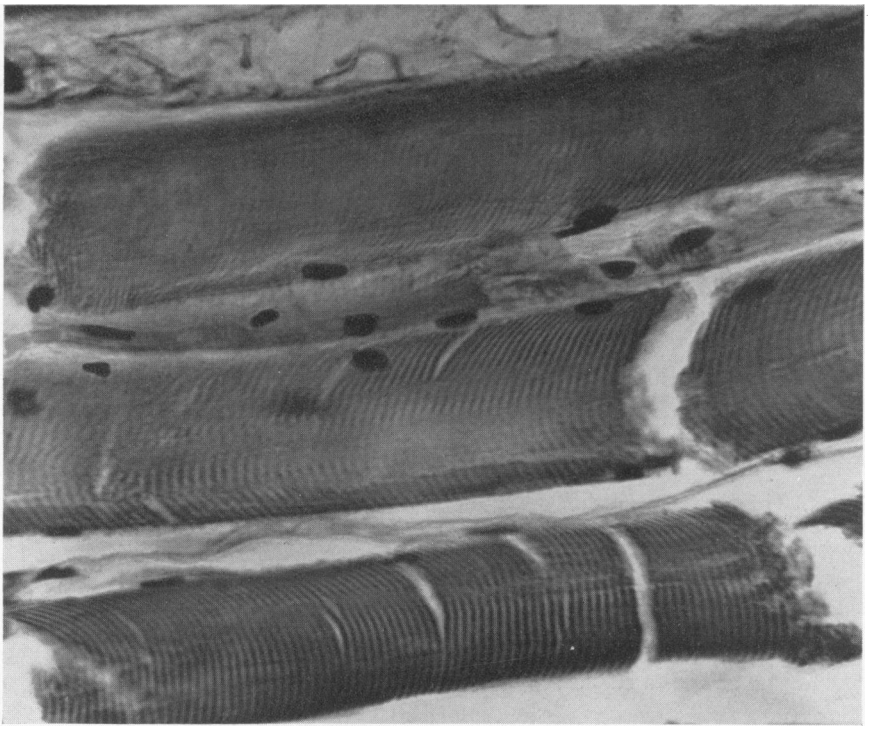

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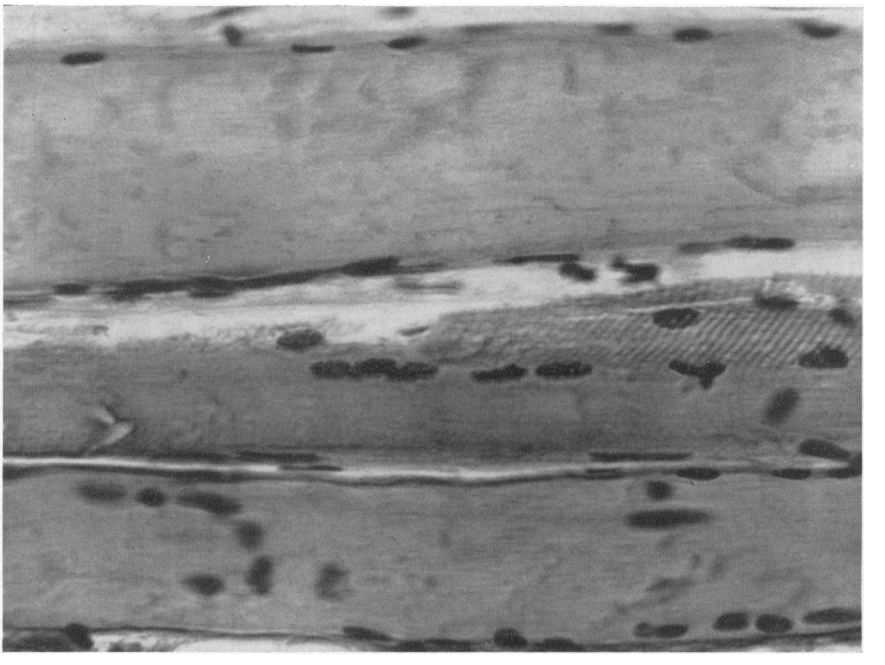


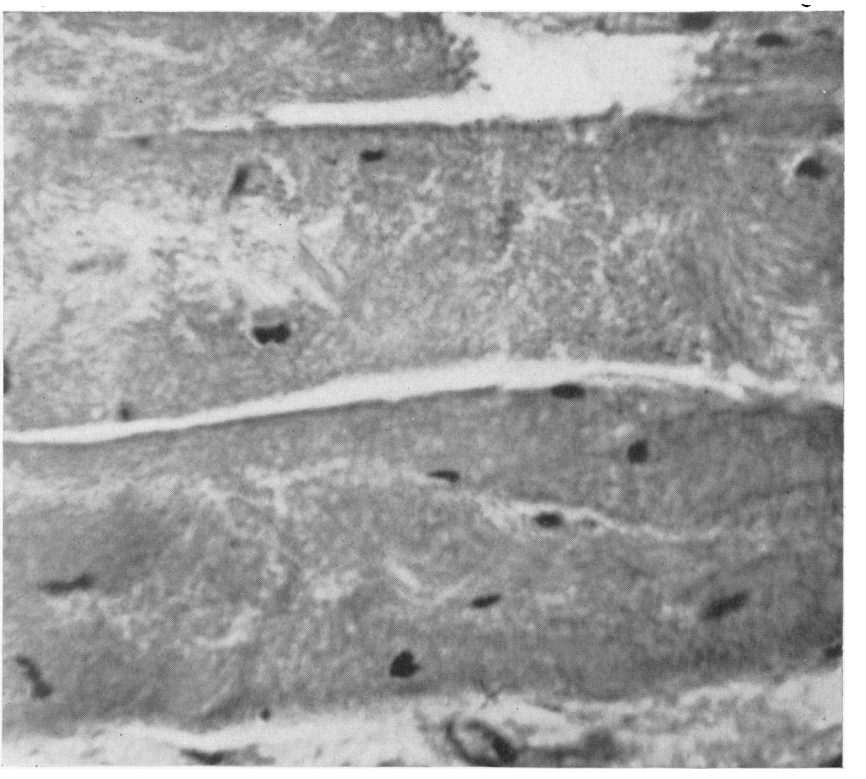
Harman and Gwinn Recovery of Muscle from Ischemia

PLATE 113

FIG. 3. The fibers have disintegrated into masses of cytoplasmic granules, a form of granular or "acute molecular" degeneration. The muscle had been released for 3 hours, after 6 hours of ischemia. Hematoxylin and eosin stain. × 500.

FIG. 4. The tissue is an admixture of normal fibers and others in the process of granular or hyaline necrosis. Both edema and acute inflammatory cells are conspicuous. The ischemia lasted 8 hours and was released for 3 hours. Hematoxylin and eosin stain. × 400.

3



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